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## REVIEW



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## LETTER TO THE EDITOR

REVIEW

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# Potential of Natural Therapeutics Against SARS-CoV-2: Phenolic Compounds and Terpenes

## SARS-CoV-2'ye Karşı Doğal Terapötiklerin Potansiyeli: Fenolik Bileşikler ve Terpenler

Duygu YILMAZ AYDIN[1], Selahattin GÜRÜ[2]

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**ABSTRACT**

Coronavirus disease-2019 caused by severe-coronavirus acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) which emerged in China in late 2019 has created an unprecedented global health crisis affecting every sector of human life and causing great damage to the world economy. SARS-CoV-2 is a viral respiratory tract virus that not only causes upper respiratory tract infection but also causes pneumonia and therefore mortality in some patients. There is currently no proven drug for the treatment of SARS-CoV-2. Many chemical and natural active compounds have been testing by the researchers for the treatment. These herbal-based antivirals have been the subject of many studies as they are less toxic and less likely to develop resistance by infectious microorganisms. It has been reported in many studies that natural therapeutics inhibit viral replication. In this review, phenolic compounds and terpenes, which are natural therapeutics known to have antiviral activity, have been evaluated for their potential in the treatment of SARS-CoV-2.

**Keywords:** Phenolic, terpene, secondary metabolite, SARS-CoV-2

**ÖZ**

2019 yılının sonlarında Çin'de ortaya çıkan şiddetli, koronavirüs akut solunum yolu sendromu-koronavirüs-2'nin (SARS-CoV-2) neden olduğu koronavirüs hastalığı-2019, insan yaşamının hemen hemen her sektörünü etkileyen ve dünya ekonomisine büyük zarar veren benzeri görülmemiş bir küresel sağlık krizi meydana getirmiştir. SARS-CoV-2, yalnızca üst solunum yolu enfeksiyonuna neden olmakla kalmayıp aynı zamanda alt solunum yolu mukozası tutulumu da yapabilen ve bu sebeple pnömoniye neden olarak bazı hastalarda ölüme yol açan viral bir solunum yolu virüsüdür. Şu anda SARS-CoV-2 tedavisi için kanıtlanmış bir ilaç olmaması ile birlikte, tedavi için birçok kimyasal ve doğal aktif bileşik araştırmacılar tarafından test edilmiştir. Bu bitkisel bazlı antiviraller, daha az toksik oldukları ve enfeksiyöz mikroorganizmalar tarafından direnç geliştirilmesi daha düşük olasılıklı olduğu için birçok araştırmanın konusu olmuştur. Doğal terapötiklerin viral replikasyonu engellediği de birçok çalışmada bildirilmiştir. Bu derlemede, antiviral aktiviteye sahip olduğu bilinen doğal terapötikler olan fenolik bileşikler ve terpenler, SARS-CoV-2 tedavisinde kullanım potansiyelleri açısından ele alınmıştır.

**Anahtar Kelimeler:** Fenolik, terpen, ikincil metabolit, SARS-CoV-2

## INTRODUCTION

Coronaviruses (CoV) are the family of viruses that caused severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and Middle East respiratory syndrome (MERS-CoV) outbreaks in recent years and came up with a new species of SARS-CoV-2, which was first detected in Wuhan, China at the end of 2019. CoV is an enveloped group of viruses that carry single-stranded ribonucleic acid (RNA) as genetic material in groups of viruses, capable of infecting humans and a wide variety of animal species. Viruses are simple organisms and consist of genetic material and a protein coat called capsid. Some virus species have an envelope consisting of phospholipids and glycoproteins outside the capsid. Viruses cannot reproduce or spread without

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invading a host cell. When the virus encounters host cells, typically epithelial cells in the nose, throat and lungs, it enters the cell by binding to receptors on the membrane of these cells. After it enters the cell, it opens its coating and begins to reproduce using the cell's mechanisms. The spike (S) protein of SARS-CoV-2 is viral attachment to host angiotensin-converting enzyme 2 (ACE2) which is a receptor to get into the host cells. Transmembrane protease serine 2 (TMPRSS2) receptor is also crucial viral gateways in oral, lung, and intestinal epithelial cells of SARS-CoV-2 invasion[1]. 3-chymotrypsin- like protease ($3CL^{pro}$), papain like protease ($PL^{pro}$), RNA-dependent RNA polymerase, and S proteins must be major target of SARS-CoV-2 drugs[2]. SARS-CoV-2 has similar genomic sequence with SARS-CoV[3]. However, the rate of transmission and spread of SARS-CoV-2 infection is quite fast compared to other viral infections encountered so far[4]. SARS-CoV-2 binds to ACE2 receptor with a higher affinity in comparison to SARS-CoV[5]. Some vaccines are developed for SARS-CoV-2. The most prominent vaccine developers are Pfizer and BioNTech, Tüseb-Tübitak, Sanofi-GSK, SinoVac, AstraZeneca and the University of Oxford, Johnson & Johnson and Moderna. They use different strategies for vaccine development and delivery. The used types of vaccines are inactivated pathogen vaccines, subunit vaccines, deoxyribonucleic acid vaccines and mRNA (messenger RNA) vaccines and virus-like particle vaccines[6]. The Pfizer-BioNTech and Moderna vaccines consist of synthetically produced messenger RNAs (mRNAs) that encode a stabilized form of the S protein formulated in a lipid nanoparticle. In an interim analysis of the 2-dose regimen of the Pfizer-BioNTech coronavirus disease-2019 (COVID-19) vaccine, it was observed to provide 95% protection against symptomatic disease[7]. The studies also showed that Pfizer/BioNTech mRNA vaccine (BNT162b2) was effective in different types of variants[8].

Symptoms of COVID-19 infection can be asymptomatic depending on the immune response of the host and comorbid diseases, as well as mild, moderate, severe or critical. In mild patients, there are no symptoms of pneumonia on imaging, and radiological findings of pneumonia, fever and respiratory symptoms are observed in moderate cases. In critical cases, respiratory failure (severe respiratory tract infection, acute respiratory distress syndrome), septic shock and/or multi-organ dysfunction/failure, myocarditis, arrhythmias, cardiogenic shock, metabolic acidosis, coagulation problems, endocrinopathies, acute kidney injury and hepatic dysfunction etc. are observed[9,10]. Reports also show that 30-60% of patients with COVID-19 suffer from neurological complications[11]. COVID-19 caused high anxiety level in people working different sectors[12]. In clinical practice, approximately 20% of COVID-19 patients have abnormal coagulation function and coagulation disorders occur in almost all critically ill patients[13]. Respiratory failure seen in the severe disease picture in COVID-19 is often in the form of hypoxemic respiratory failure. Advanced age, presence of comorbid diseases (cardiovascular disease, diabetes mellitus, chronic respiratory disease, hypertension, cancer), and male gender are risk factors for the development of severe disease[14]. The symptoms may differ depending on the immune responses of patients. It is extremely important to activate the immune response and combat viral infection by increasing the body's combat mechanism, thereby controlling CoV infections[15]. If the virus infects the body, our strong immune system is one of the most effective methods of avoiding the effects of the infected virus. The immune system fulfills the function of defending the human body against disease-causing microorganisms. The best step you can take to keep your immune system strong and healthy naturally is choosing a healthy lifestyle. The immune system against diseases should be strengthened with food and other natural product supplements[16,17].

Plants have been used in the treatment of various diseases since ancient times. According to World Health Organization, about 80% of the world's population use medicinal herbs to meet their health needs[18]. Plants are able to synthesize diverse classes of chemical compounds, named secondary metabolites. The concept of secondary metabolites was first defined in 1891 by biochemist Albrecht Kossel, the Nobel Prize winner in physiology or medicine[19]. The chemical composition of the herbs provides a better understanding of the herb's medicinal value. Secondary metabolites help plants adapt to environmental conditions, defend, protect, survive and regulate their relationships with the ecosystem. They protect the plant against herbivore; bacterial and fungal pathogen attacks and increases their competitiveness with other plants in the same environment. They also protect the plant against abiotic stress factors such as temperature changes, water, light, ultraviolet and mineral substances[20]. Though the functions of secondary products in the plant differ, those with cytotoxic effects against microbial pathogens are used as "antimicrobial agents" in medicine. It is neurotoxic on the central nervous system against herbivores and they are used as anti-depressants, sedatives, muscle relaxants or anesthetic drugs[21]. Some secondary plant metabolites have shown strong antiviral activity against various viral strains such as CoV, human immunodeficiency virus (HIV), influenza virus, SARS[22-25]. When discovering new drugs from both synthetic and natural sources, *in silico* virtual screening studies should be the first step then *in vitro, in vivo* and clinical studies should be carried out. It has been shown that plant secondary metabolites are probably one of the most significant drugs against SARS-CoV-2 by silico analysis[26-30]. The search for natural agents that inhibit different viruses is important to develop a plant-based drug for SARS-CoV-2. The aim of this study is to report previously researched secondary metabolites (phenolics, and terpenes/terpenoids of

plants with antiviral properties that could potentially be used in SARS-CoV-2) and to contribute to the public health by antiviral natural therapeutics (Figure 1).

## PHENOLICS

Phenolic compounds are secondary metabolites abundant in plants. There are various phenolic compounds in different qualities and amounts in all vegetables and fruits[31]. Plant phenolics are thought to play a key role as defense compounds in situations where environmental stresses may cause enhanced production of free radicals and other oxidative species in plants[32]. These compounds also play an important role in the human diet. They are important in terms of their antimicrobial and antioxidative effects and causing enzyme inhibition. Polyphenols comprise a wide range of polyhydroxylated compounds (phenolic acids, cinnamic acids, lignans, coumarins, flavonoids, tannins, among others) and for this reason is divided into classes and subclasses. Flavonoids are low molecular weight secondary metabolites in plants that have positive effects on human health. They are the most prevalent phenolic compounds in the human diet. Flavonoids fall into various classes and in general, six basic classes of flavonoids are reported. These are flavones, flavonones, flavonols, isoflavonoids, anthocyanins and proanthocyanidin. Flavonoids are in aglycon or glycoside structures. The predominant form of flavonoids in foods is the form of glycoside. Absorption of this form from the intestines is more difficult than the lean form. Flavonoid glycosides are separated from the sugar part before entering the intestine, and aglycones can pass freely through cell membranes[33,34]. Phenolic therapeutics are used for the treatment of various disease types[35,36].

## EFFECTS ON IMMUNE SYSTEM

The interaction of phenols with the immune system has complex effects on the prevention of the disease, the treatment of the disease and the immune system. When free radicals are more than the antioxidant capacity of our body, oxidative damage occurs in our cells. Phenols reduce oxidative stress by scavenging free radicals and inflammatory prooxidants such as hydrogen peroxide[37]. There is a close relationship between inflammation and oxidative stress. Especially high free radical production by macrophages at the infection site causes oxidative stress. SARS-CoV or SARS-CoV-2-related complications are mostly caused by severe inflammation caused by viral replication. Patients in critical care units with severe COVID-19 had elevated plasma levels of various cytokines, including granulocyte-colony stimulating factor, interferon (IFN) gamma-induced protein 10, and macrophage inflammatory proteins[38]. Polyphenols support immunity against foreign pathogens in a variety of ways. Polyphenol receptors identify and facilitate cellular uptake of polyphenols, which subsequently activate signaling pathways to generate immunological responses in different immune cells. Polyphenols interact with the intestinal immune system, leading to both protective and deleterious reactions in the host. For example, resveratrol has the ability to improve human immunity and antioxidative systems. Resveratrol has been displayed to directly target central cell parts of adaptive immunity, like macrophages, large lymphocytes, and dendritic cells. In animal experiments, resveratrol showed an immunomodulatory effect by diminishing the expression of

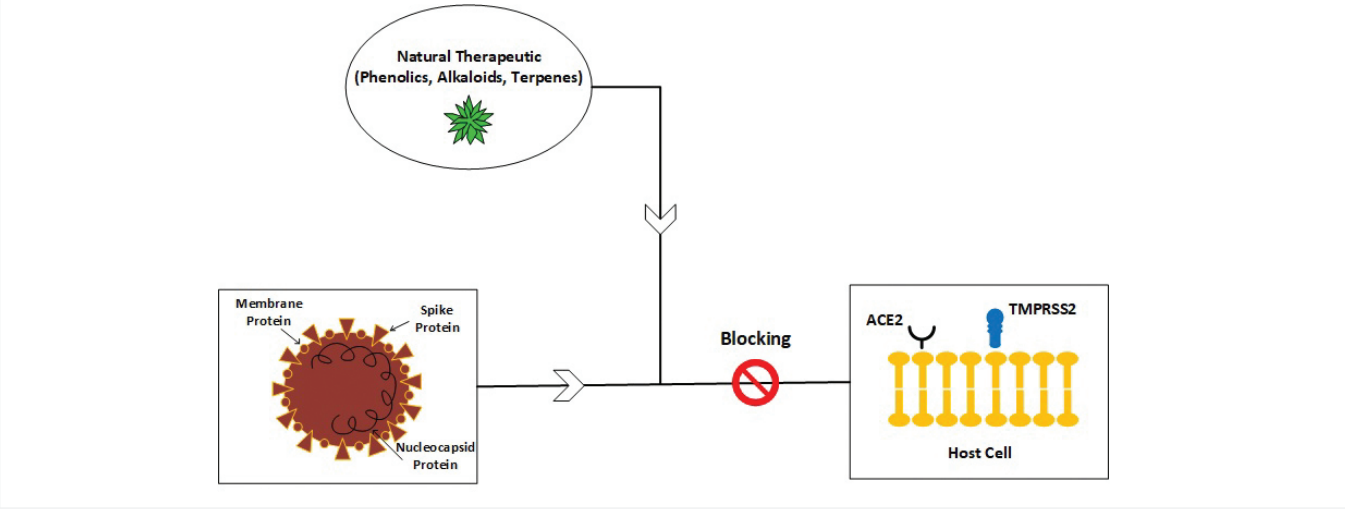


**Figure 1.** Schematic illustration of SARS-CoV-2 that can bind to a cellular receptor (ACE2) of a host cell with its spike proteins and application of natural therapeutics as anti-COVID-19

*COVID-19: Coronavirus disease-2019, SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, ACE2: Angiotensin-converting enzyme 2, TMPRSS2: Transmembrane protease serine 2*

activating CD28 and CD80 receptors on immune cells and enhancing the production of the immunosuppressive cytokine IL-10[39].

## ANTIVIRAL EFFECTS

There are also many studies in the literature that show antiviral potential of phenolics. Natural polyphenol compounds such as quercetin[40], myricetin[41], apigenin[42] and resveratrol[43] have shown antiviral effect against CoVs. Theaflavin, a polyphenolic compound in black tea, have exhibited broad-spectrum antiviral activity against different viruses such as influenza A and B viruses and hepatitis C virus (HCV)[44,45]. Theaflavin has also been shown to have potential inhibitory effect against SARS-CoV-2 that targets RNA-dependent RNA polymerase (RdRp) which is a significant enzyme that catalyzes the replication of RNA from RNA templates[46]. Stilbenes have antiviral activity against HIV and HCV[47,48]. Flavonoids interfere for NLRP3 inflammasome-associated disorders[49]. SARS CoVs activate the NLRP3 inflammasome in lipopolysaccharide-primed macrophages and cause NLRP3 inflammasome activation[50]. Some flavonoids, such as luteolin[51], myricetin[50], apigenin[52], quercetin[53], kaempferol[54], baicalin[55] and wogonoside[56] , inhibit NLRP3 inflammasome activation. Myricetin has been shown to act as a SARS-CoV inhibitor[41]. Isorhamnetin, apigenin, kaempferol, formononetin and penduletin show antiviral protective efficacy against enterovirus 71 (EV71) infection[57]. Apigenin has also been shown to be active against herpes simplex virus-1 (HSV-1), poliovirus type 2 and HCV[58,59] Apigenin is also anti-adenoviruses and hepatitis B virus (HBV)[60]. Emodin was found to block the interaction of SARS CoV S protein and ACE2. Therefore, it may have therapeutic potential in the treatment of SARS CoVs[61]. Resveratrol has been shown to significantly prevent MERS-CoV infection[62]. Kaempferol, a flavonol, exhibits inhibitory effect against Murine Norovirus and Feline Calicivirus[63]. Kaempferol 3-O-α-L-rhamnopyranoside, extracted from *Zanthoxylum piperitum,* has been shown to have antiviral activity against Influenza A virus[64]. Studies have revealed that quercetin, a natural flavonoid, also display strong antiviral activity against a range of infections caused by HSV, Influenza, HBV, Murine Coronavirus and Dengue viruse in cell culture and mouse models[65-67]. In addition, quercetin was found to inhibit H1N1 and H7N9 viruses *in silico* analysis[68,69]. Quercetin, rosmarinic acid, and hesperitin have also shown good binding affinity with SARS-CoV-2 viral protein targets *in silico* virtual screening[70]. Due to caffeic acid, p-coumaric acid, kaempferol and mainly quercetin, which are the phenolic compounds detected with ethanol of *Origanum vulgar*e, the plant shows an inhibitory effect against Alphaarterivirus equid which causes the equine viral arteritis (EVA) diseases[71]. A study has shown that baicalein, a flavonoid extracted from the roots of *S. baicalensis,* inhibits the activity of SARS-CoV-2 $3CL^{pro}$ *in vitro*. Baicalein has been shown to have anti-SARS-CoV-2 activity by molecular docking analysis[72]. Papyriflavonol A, a flavonol isolated from *Broussonetia papyrifera,* has potent SARS-CoV $PL^{pro}$ inhibitory activity[73]. Antiviral activity of the myricetin derivatives and methoxyflavones obtained from *Marcetia taxifolia* have been evaluated against HBV, HSV and Poliovirus. The methoxyflavones have shown antiviral effect against all the evaluated viruses without cytotoxic effects[74]. Phenolic acids have been reported to show antiviral activity against HSV-1 in a study[75]. Rutin is a very impressive therapeutic as anti-inflammatory and antiviral. Rutin has shown the highest activity as SARS-CoV-2 protease inhibitory in the molecular docking simulation study. Therefore, *in vivo* and docking studies of rutin can be hopeful for SARS-CoV-2 potential[76,77]. Luteolin has been found to have inhibitory activity against EV71, coxsackievirus A1 and SARS CoV[78]. The studies show that flavonoids and polyphenols have antiviral effects against many diseases and can be potentially used against SARS-CoV-2 (Table 1, Figure 2).

## TERPENES

Terpenes are a group of compounds commonly found in the plants and are the largest group of secondary metabolites composed of five carbon isoprene subunits. Terpenes are simple hydrocarbons while terpenoids are modified category of terpenes[82]. Terpenes are the major components of essential oils in most herbs and flowers. Terpenoids are a class of modified terpenes with different functional groups. Terpenoids are classified into monoterpenes, diterpenes, sesterpenes, triterpenes and sesquiterpenos according to the units of isoprene. Terpenoids are used in the treatment of many diseases due to their biological activity[83].

## EFFECTS ON IMMUNE SYSTEM

Terpenes have strong effects on the immune system. The effects of naturally occurring triterpenoid compounds such as glycyrrhizic acid, ursolic acid, oleanolic acid and nomilin were studied on the immune system using Balb/c mice[84]. It has been observed that intraperitoneal treatments with five doses of terpenoid compounds increase the total white blood cell count. The results demonstrated the immunomodulatory activity of the naturally occurring triterpenoids used in the study. Terpenes also show anti-inflammatory activities. In a study, rats were treated for 11 days with the standard drug sulfasalazine (500 mg/kg po), geraniol (250 mg/kg po), or a combination of the standard drug and geraniol[85]. It was observed that it significantly reduced the total antioxidant capacity and reduced high nitric oxide (NO) and lipid peroxide levels. In a study, D-limonene was orally administered to rats at a dose of 10 mg/kg[86]. According to the results of the study, D-limonene showed important anti-inflammatory effects *in vivo* and *in vitro*, and its effects included protection at

**Table 1. Antiviral phenolic compounds, target pathogens and mechanism of action**

| Compound (References) | Class | Target virus | Mechanism of action |
|---|---|---|---|
| Emodin[61] | Anthraquinone | SARS CoV | Interferes with S protein-ACE2 interaction |
| Theaflavin[44-46] | Biflavonoid | Influenza A and B viruses, HCV SARS-CoV-2 | Binding to RNA-dependent RNA polymerase |
| Formononetin[57] | Isoflavone | EV 71 | - |
| Apigenin[57-60,79] | Flavone | EV71, HSV-1, poliovirus, HCV ADV and HBV7<br>SARS-CoV | Inhibits SARS-CoV$^{pro}$ activity |
| Luteolin[78] | Flavone | EV71, Coxsackievirus A1<br>SARS-CoV | Binds with S2 subunit and preventing entry |
| Isorhamnetin[57] | Flavanol | EV 71 | Reduces viral genomic RNA replication |
| Penduletin[57] | Flavonol | EV 71 | Reduces viral genomic RNA replication |
| Myricetin[41,74] | Flavonol | SARS-CoV, HBV, HSV and poliovirus | Inhibits nsP13 by affecting the ATPase activity |
| Kaempferol[63] | Flavonol | Murine Norovirus and Feline Calicivirus<br>SARS-CoV | Inhibits 3a ion channel of CoVs |
| Quercetin[40,65-69] | Flavonol | HSV, influenza, HBV, Murine coronavirus, Dengue viruses, H1N1, H7N9 | - |
| Quercetin[80] | Flavonol | SARS-CoV-2 | Inhibits of ACE2 |
| Papyriflavonol A[73] | Flavonol | SARS-CoV | Inhibits SARS-3CL$^{pro}$ activity |
| Rutin[76] | Flavonol | SARS-CoV-2 | Binds to the active site of the SARS-CoV-2 3CL$^{pro}$ |
| Resveratrol[62,81] | Stilbene | MERS-CoV<br>SARS-CoV-2 | Expression of nucleocapsid protein regulates ACE2 expression |

ADV: Adenoviruses, SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, COVID-19: Coronavirus disease-2019, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HSV: Herpes simplex virus, MERS: Middle East respiratory syndrome, ACE2: Angiotensin-converting enzyme 2, S: Spike

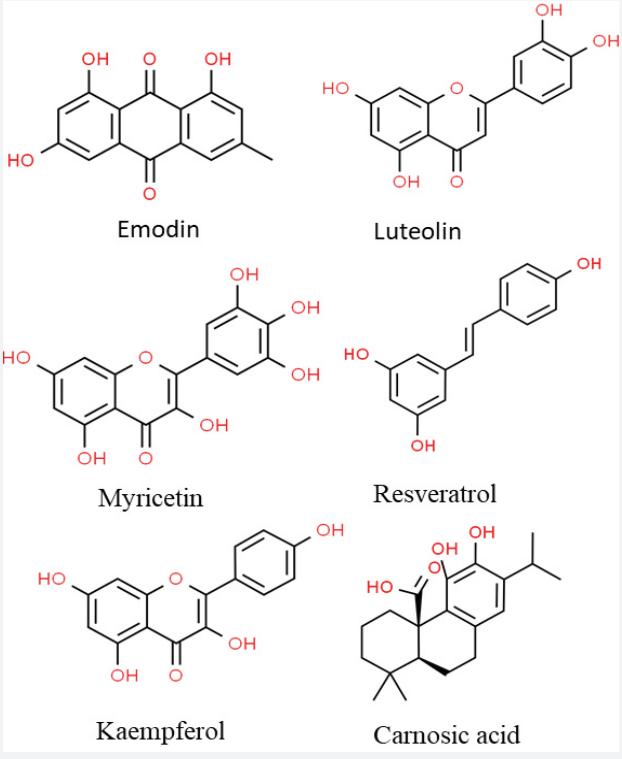


**Figure 2.** Structures of some phenolic compounds

the epithelial barrier and reduction of cytokines. Nuclear transcription factor-kappa B plays an important role in the regulation of immune and inflammatory responses. Labdane diterpenoids show anti-inflammatory effect by inhibiting NF-κB[87]. Tanshinones, a class of abietane diterpene, can reduce inflammation and increase immune responses[88]. Experimental studies have shown that terpenes are able to decrease pro-inflammatory cytokines [tumor necrosis factor (TNF)-α and β, IL-1, IL-1β, IL-6, IL-17, IFN-γ] and enhance anti-inflammatory cytokines (IL-4, IL-10, TGF-β1)[89]. Emodinol, a triterpene, decreases the levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in the serum of monosodium urate crystal-treated mice and provides a reduction in anti-gouty arthritis activity by improving inflammatory response[90]. In another study, glycyrrhizin (a kind of triterpenoid) was found to provide SARS-CoV-2 inhibition by down-regulating proinflammatory cytokines and preventing the formation of intracellular reactive oxygen species[91].

## ANTIVIRAL EFFECTS

There are many studies showing the antiviral properties of terpenes. Glycyrrhizin has shown antiviral effect against SARS, HBV and HIV[92-94]. It also has potential to inhibit SARS CoV-2. Glycyrrhizin has been shown to bind S-RBD and SARS-CoV-2 S-protein attachment with H-ACE2 receptor[95].

1,8-Cineole, a terpene oxide, has been shown to interfere with the binding between RNA and infectious bronchitis virus (IBV) N-protein. So, it exhibits that 1,8-Cineole has anti-IBV properties[96]. (-)-α-pinene and (-)-β-pinene, which are the kinds of terpenoid, have also been shown to possess anti-IBV properties[97]. Triterpenoid saponins are active components isolated from *Bupleurum falcatum*, including saikosaponin A, B, C, and D. Saikosaponin B2 has been found to effectively inhibit HCV by neutralizing virus particles and preventing viral binding[98]. Saikosaponin B2 also has exhibited significant inhibition effect against human coronavirus 229E infection and it has been found that it has potent anticoronaviral activity[99]. Saikosaponin D has been found to have the ability to strongly inhibit EV-71[100]. Terpenoids may interfere with essential amino acid in the enzymatic cavity for inhibiting viral protease enzyme. Some terponoids, including thymoquinone, salvinorin A, bilobalide, citral, menthol, ginkgolide A, noscapine, forscolin, and beta selinene, have been shown to have inhibitory effect against COVID-19 protease molecular insertion by molecular docking method[101]. Isoborneol, an oxygenated monoterpene, has been shown to have a potent antiviral effect against HSV-1 and exactly inhibited glycosylation of viral proteins[102]. A study has shown that limonene, a cyclic monoterpene, is effective in reducing the epithelial expression of ACE2. It has also potential to reduce the mRNA levels of TMPRSS2[103]. Terpenes from *Marrubium vulgare* have been found to interfere with the replication of the HSV-1 and show antiviral effect against HSV-1[104]. Putranjivain A, a diterpen obtained from *Euphorbia jolkini*, has been shown to have an antiviral effect against HSV-2[105]. Moronic acid, extracted from the *Rhus javanica,* has potential to inhibit HSV-1[106]. Andrographolide, a diterpenoid lactone, has been shown to inhibit the replication process of the CKV[107]. Betulinic acid and platanic acid, which are the pentacyclic triterpenoid compounds isolated from *Syzigium claviforum*, have been found to inhibit HIV[108]. Oleanolic acid, a pentacyclic triterpenoid, have also shown anti-HIV activity (Table 2, Figure 3)[109].

## CLINICAL TRIALS

There are only a few clinical trials regarding the application of phenolic compounds and terpenes in SARS-CoV-2. A clinical trial covers the administration of zinc and resveratrol (a stilbene, a type of natural phenol) or double placebo for a period of 5 days in 60 ambulatory SARS-CoV-2 positive volunteers (range of 18-75 age) and monitoring for a 14-day period[117]. The aim of this study is to minimize viral load and severity of resulting COVID-19 disease. Combination therapy contains 50 mg of zinc picolinate for five days and 2 mg of Resveratrol for five days. The stage of this study is still phase 2. Another clinical trial has been conducted with the use of Epigallocatechin-3-gallate, a phenol found in green and black tea plants, in 524 volunteer healthcare worker participants[118].

**Table 2. Antiviral terpene compounds, target pathogens and mechanism of action**

| Compound (References) | Class | Target virus | Mechanism of action |
|---|---|---|---|
| Putranjivain A[105] | Diterpene | HSV-2 | Inhibits of viral attachment and cell penetration |
| Tanshinones[110] | Diterpene | SARS-CoV-2 | Inhibits SARS-CoV $3CL^{pro}$ and $PL^{pro}$ |
| Andrographolide[107] | Diterpenoid | CHIKV | Inhibits viral genome replication |
| Sugiol[111] | Diterpenoid | SARS-CoV-2 | Inhibits $M^{pro}$ |
| Ginkgolide A[101] | Diterpenoid | SARS-CoV-2 | Binds to $3CL^{pro}$ |
| Isoborneol[102] | Monoterpene | HSV-1 | Inhibits glycosylation of viral polypeptides |
| Limonene[103] | Monoterpene | SARS-CoV-2 | Downregulates ACE2 expression |
| 1,8-Cineole[96] | Monoterpene | IBV | Binds between RNA and IBV N-protein |
| (-)-α-pinene and (-)-β-pinene[97] | Monoterpene | IBV | Inhibit of viral replication |
| Geraniol[112] | Monoterpene | SARS-CoV-2 | Inhibits of ACE2, spike glycoprotein |
| α-Cadinol[111] | Sesquiterpenoid | SARS-CoV-2 | Inhibits $M^{pro}$ |
| Saikosaponin B2[98,99] | Terpenoid | HCV, HCoV-229E | Neutralizate of virus particles and inhibits viral entry/fusion |
| Saikosaponin C[113] | Terpenoid | HBV | Inhibits DNA expression |
| Glycyrrhizin[92-94] | Triterpenoid | HBV and HIV SARS | Inhibits replication of the SARS-associated virus |
| Moronic acid[106] | Triterpenoid | HSV-1 | - |
| Betulinic acid[108,114] | Triterpenoid | HIV, HBV | Inhibits of HIV and HBV replication |
| Oleanolic acid[109] | Triterpenoid | HIV | - |
| Platanic acid[108] | Triterpenoid | HIV | Inhibits of HIV replication |
| Celastrol[115,116] | Triterpenoid | SARS-CoV<br>Hepatitis C virus | Inhibits $3CL^{pro}$ inhibits HCV RNA and protein synthesis |

COVID-19: Coronavirus disease-2019, SARS-CoV-2: Severe acute respiratory syndrome-Coronavirus-2

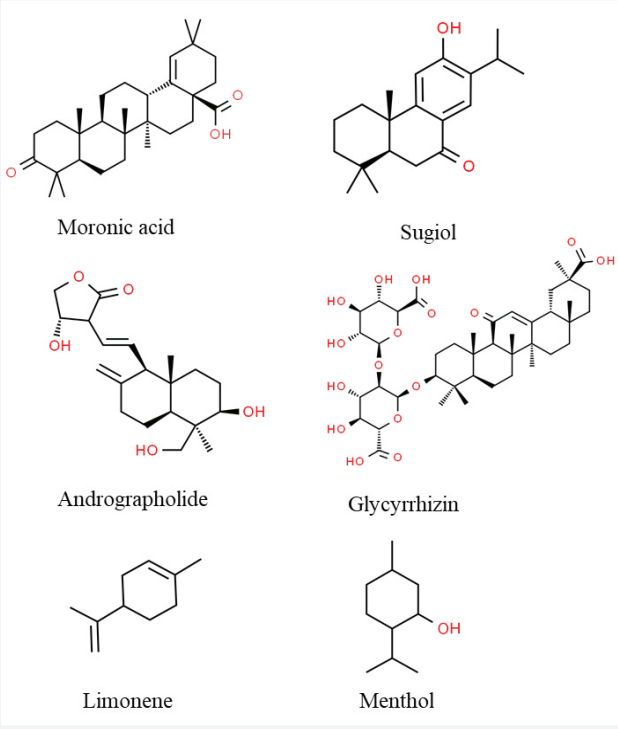


**Figure 3.** Structures of some terpene compounds

The total dose of EGCG per patient was 750 mg per day, 3 capsules per day for 40 days. Participants also took the same dose of starch as a placebo. The purpose of this clinical trial was to determine the efficacy of Previfenon® (EGCG) in preventing COVID-19, enhancing systemic immunity, and reducing the frequency and intensity of selected symptoms when used as pre-exposure chemoprophylaxis to SARS-CoV-2. The stage of this study is still phase 2. Combination of curcumin (a terpene), quercetin (a flavonoid) and vitamin D is used in an ongoing clinical trial in phase 2 to investigate for early COVID-19 symptoms improvement and viral clearance in outpatients[119]. There are 100 participants who are 18 years old and older, tested positive for SARS-CoV-2 by RT-PCR and exhibit typical symptoms of COVID-19 disease. Soft capsule of the investigational treatment contains 42 mg curcumin, 65 mg quercetin and 90 units Vitamin D. Four capsules per day for 14 days are taken. Quercetin (flavonoid) is administrated on 80 participants in a clinical trial to investigate the effectiveness of phytotherapy in the treatment of SARS-CoV-2[120]. Participants will receive one tablet times three per day from quercetix and placebo groups. This study is still phase 1. Combination therapy of quercetin, bromelain, zinc and vitamin C on the clinical outcomes of patients infected with COVID-19 was studied on 60 participants[121]. A daily dose of drugs included quercetin (500 mg), bromelain (500 mg), zinc (50 mg), vitamin C (1000 mg) by proven COVID-19 cases intervention. The stage of this clinic trial is phase 4.

The biggest problem with the use of natural products in the treatment of diseases is their low solubility and bioavailability, which causes problems in clinical studies. Bioavailability issues can be evaluated before starting high-budget clinical trials. The ways to improve drug delivery, bio distribution, biodegradability and bioavailability of plant-based secondary metabolites such as phenolic compounds and terpenes should be sought. Nano carrier systems can be useful as a solution for these problems. Natural therapeutics administered regularly in low doses can reduce the entry of the virus into cells and thus stop the progression of the infection.

## CONCLUSION

The whole world faced a major health crisis with the SARS-CoV-2 pandemic, which caused many human deaths and adversely affected many industries. The fact that it is so widespread and fatal raises the need for improvement of treatment as soon as possible. However, the reliable and certified drug has not yet been developed for the SARS-CoV-2. The use of natural therapeutics has begun with the history of humanity and a significant number of effective plants derived drugs have been developed. They are effective in enhancing the immune response of the host against viral pathogens; therefore, it is considered as a protective and complementary treatment opportunity. Secondary metabolites of plants such as phenolic compounds and terpenes could be highly promising complementary therapeutic agents for the disease. The studies show that secondary metabolites exhibit antiviral activity against different viruses so they can be highly promising therapeutics for the SARS-CoV-2. Natural therapeutics must be subjected to *in vitro* and experimental trials to determine safe and therapeutic levels before conducting clinical trials in humans. This study reveals the antiviral properties of some natural therapeutics for new drug development to overcome these and future pandemic situations. It is thought that the information provided in this study will be useful in the process of developing safe, effective anti-CoV therapeutic agents from compounds derived from natural products.

### Ethics

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: D.Y.A., S.G.

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

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# The Effective Method of Monitoring Visceral Organ Fatty Infiltration Changes After Bariatric Surgery: Ideal IQ Sequence

## Bariatrik Cerrahi Sonrası Viseral Organ Yağ İnfiltrasyon Değişikliklerini İzlemenin Etkili Yöntemi: İdeal IQ Sekansı

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### ABSTRACT

**Aim:** The aim of this study is to demonstrate the efficiency of non-invasive imaging method-MR proton density fat fraction (PDFF); ideal IQ sequence- on detecting the effects of bariatric surgery on liver and pancreatic fatty infiltration.

**Materials and Methods:** Thirty-nine patients (25 females, 14 males) who underwent bariatric surgery between May 2016 and April 2017 were analyzed retrospectively in this study. Body mass index (BMI) and body weight (BW) values of all patients were noted one week before and one month after bariatric surgery, and meanwhile an unenhanced upper abdominal MR imaging was performed. Liver fat fraction (LFF), pancreas fat fraction (PFF), liver volume (LV) and craniocaudal length of liver (LL) were measured with MR-PDFF and T2 weighted images. Changes in all parameters after the surgery were recorded and the correlation of these changes with the change in LFF was analyzed.

**Results:** At the end of first month of bariatric surgery, a significant decrease on mean values of LFF and PFF has been observed along with a decrease of LV, LL, BW and BMI ($p<0.0001$). A moderate positive linear correlation was observed between LFF and PFF, LV, LL ($r=0.69$, 0.61, 0.49; respectively) while a weak positive linear correlation was noticed between LFF and BMI, BW ($r=0.34$, 0.21; respectively).

**Conclusion:** Ideal IQ sequence enables quantitative analysis of fatty infiltration of the liver and pancreas and thus may be used as a non-invasive tool to monitor the positive effects of the bariatric surgery on fatty infiltration of these visceral organs in the postoperative period.

**Keywords:** Bariatric surgery, magnetic resonance imaging, non-alcoholic fatty liver, pancreatic disease, proton-density fat fraction

### ÖZ

**Amaç:** Bariatrik cerrahinin karaciğer ve pankreas yağ infiltrasyonu üzerindeki etkilerinin saptanmasında non-invaziv görüntüleme yönteminin-MR proton dansite yağ fraksiyonu (PDFF); ideal IQ sekansı-etkinliğini göstermeyi amaçladık.

**Gereç ve Yöntem:** Bu çalışmada Mayıs 2016 ile Nisan 2017 tarihleri arasında obezite cerrahisi geçiren 39 hasta (25 kadın, 14 erkek) retrospektif olarak incelendi. Tüm hastaların vücut kitle indeksi (VKİ) ve vücut ağırlığı (VA) değerleri bariatrik cerrahiden bir hafta önce ve bir ay sonra kaydedildi ve bu esnada kontrastsız üst abdomen MR görüntülemesi yapıldı. MR-PDFF ve T2 ağırlıklı görüntülerle karaciğer yağ fraksiyonu (KYF), pankreas yağ fraksiyonu (PYF), karaciğer hacmi (KV) ve karaciğer kraniyokaudal uzunluğu (KKU) ölçüldü. Ameliyat sonrası tüm parametrelerdeki değişiklikler kaydedildi ve bu değişikliklerin KYF'deki değişiklik ile korelasyonu analiz edildi.

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**Bulgular:** Bariatrik cerrahinin 1. ayının sonunda KYF ve PYF ortalama değerlerinde anlamlı azalma ile birlikte KV, KKU, VA ve VKİ'de azalma gözlendi (p<0,0001). KYF ile PYF, KV, KKU arasında orta derecede pozitif bir doğrusal korelasyon gözlenirken (sırasıyla r=0,69, 0,61, 0,49), KYF ile VKİ, VA arasında (sırasıyla r=0,34, 0,21) zayıf bir pozitif doğrusal korelasyon fark edildi.

**Sonuç:** İdeal IQ sekansı, karaciğer ve pankreasın yağ infiltrasyonunun kantitatif analizini sağlar ve bu nedenle postoperatif dönemde bariatrik cerrahinin bu viseral organların yağ infiltrasyonu üzerindeki olumlu etkilerini izlemek için non-invaziv bir araç olarak kullanılabilir.

**Anahtar Kelimeler:** Bariatrik cerrahi, manyetik rezonans görüntüleme, alkolsüz yağlı karaciğer, pankreas hastalığı, proton dansite yağ fraksiyonu

## INTRODUCTION

Obesity may result in various health problems such as coronary artery disease, cerebrovascular disease, and metabolic syndrome, in addition to fatty liver and pancreas diseases[1]. Non-alcoholic fatty liver disease (NAFLD) is present in 70-95% of obese patients. This condition may range from simple steatosis, or from a non-alcoholic steatohepatitis (NASH), to cirrhosis and even to hepatocellular carcinoma[2,3].

Pancreas is the other visceral organ in which ectopic fat accumulation secondary to obesity is frequently observed. Non-alcoholic fatty pancreas disease (NAFPD) is pancreatic fatty infiltration due to obesity. Just as NAFLD progresses to non-alcoholic steatohepatitis (NASH) over time, it is possible for NAFPD to progress to non-alcoholic steoatopancreatitis due to the failure of the metabolic condition over time, and as a result, pancreatitis and pancreatic cancer may develop[4-8].

Quantitative evaluation of fatty infiltration in the liver and pancreatic parenchyma is of great importance for correct diagnosis and treatment. The gold standard method used for this purpose is liver biopsy[9,10]. However, this procedure is invasive and has the disadvantage of complications such as hemorrhage and infection, and evaluation of only a limited section of the liver, which may necessitate new diagnostic methods. Recent studies have shown that various magnetic resonance (MR) imaging (MRI) techniques provide accurate information about the amount of fat in the liver parenchyma similar to liver biopsy. These techniques, besides being reported to be non-invasive, have also the advantage to allow the monitoring of fatty liver disease[11-15]. There is no clear consensus yet on diagnostic methods that can determine the amount of pancreatic fat at an optimum level. However, reviewing the literature, MRI with its non-invasive nature and high sensitivity, is reported to be the most preferred diagnostic imaging method used for this purpose, today. Furthermore, a novel MR-proton density fat fraction (PDFF) method also allows high accuracy quantification of pancreatic fat infiltration[16-18].

Fatty liver disease is frequently observed in patients who are candidates for bariatric surgery, and 25% of these patients have NASH and 1-3% have cirrhosis[19]. NAFLD and secondary parenchymal damage due to fatty infiltration has been shown to be resolved and even completely returned to normal after bariatric surgery in up to 80-92% of the patients[20,21].

Although there are accumulating data on the effects of bariatric surgery on NAFLD in the literature, there is still a necessity for further studies regarding the effects of bariatric surgery on pancreatic fatty infiltration. In this context, the aim of this study was to demonstrate the effects of bariatric surgery on the liver and pancreatic parenchyma from the early postoperative period with a non-invasive imaging method, ideal IQ sequence (MR-PDFF).

## MATERIALS AND METHODS

This retrospective study was approved by the Recep Tayyip Erdoğan University Institutional Review Board in our institution (decision number: 2019/113, approval date: 09.09.2019) and written informed consent was obtained from all patients.

### Patient Selection

A total of 55 patients scheduled for bariatric surgery due to morbid obesity between May 2016 and April 2017 were included in this study. Sixteen patients were excluded from the study for various reasons. The study was conducted with remaining 39 patients who underwent bariatric surgery (35 laparoscopic sleeve gastrectomy, 4 laparoscopic Roux-Y-gastric bypass). The patient flow diagram is shown at Figure 1. All patients were examined with unenhanced upper abdominal MRI a week before and a month after surgical intervention.

### Assessment

The patients were evaluated by the multi-disciplinary bariatric team, including two surgeons, endocrinologist, endoscopist, anesthesiologist, psychiatrist and specialized nurses, prior to surgical treatment. All of the bariatric operations were performed by two general surgeons (M.K.Ç. and S.K.). Patients were not administered a diet programme before the surgery. But, after bariatric surgery, all patients were included in the diet program determined by the bariatric team.

### Inclusion and Exclusion Criteria

Inclusion criteria were body mass index (BMI) of 40 kg/m$^2$ or BMI ≥35 kg/m$^2$ and having related comorbidities such as diabetes, hypertension, coronary artery disease, and chronic lung diseases. Patients with any contraindications for MR examination (presence of pacemaker or any metallic implant

not compatible with MR and claustrophobia) or known diffuse non-NAFLD liver disease (such as chronic hepatitis B or C, Wilson disease and hemochromatosis) were excluded from the study.

## Radiological Measurements

All measurements were performed by a single radiologist (Y.M.) with approximately 8 years of experience in abdominal imaging. On the ideal IQ sequences obtained before and after the surgery in the axial plane, the fat fraction values were measured on the fat fraction maps three times and the mean value was recorded using the 3-cm and 1.5-diameter circular region of interest (ROI) from the liver segment 4A and the pancreas body, respectively. Also, liver volume measurements were made by outlining liver boundaries on each slice at ideal IQ sequence and the final volume was calculated automatically. Liver craniocaudal length (LL) was measured in a coronal T2 W images from the hepatic dome to the lower border of the liver passing through the mid-hepatic point. Also, BMI and BW measurements were performed at preoperative and postoperative periods. BMI was calculated as weight/height$^2$.

## Magnetic Resonance Imaging

All MRIs were obtained at 3T scanner (GE MR750, GE Healthcare, Waukesha, WI, USA) utilizing a multi-channel torso phased-array coil placed over the liver with all subjects in the supine position. Ideal-IQ sequence was used to determine MRI-PDFF. The Ideal-IQ sequence parameters were as follows: repetition time (TR), 5.8 or 6.7 ms; field of view (FOV), 34 cm; NEX, 0.5; matrix, 128×128; bandwidth, 90.91 or 83.33 kHz; flip angle, 3 degree; a slice thickness, 8.0 mm; and acquisition time, 23 s. At our hospital, Ideal-IQ sequence is included in routine MRI protocols for upper abdominal examinations because fatty liver can be easily and quantitatively evaluated by using this sequence. Liver craniocaudal (LL) measurements were performed on coronal single-shot fast spin-echo T2 WI (coronal SSFSE T2WI: TR, 1200-2000 ms; TE, 30-50 ms; matrix, 256×256; FOV, 40 cm; slice thickness, 6.0 mm; NEX, 2; bandwidth 83.33). The images were processed automatically by using the software (AW server 2.0 software; GE, USA) provided by the manufacturer to create water, fat, in phase (IP), out-of phase, R2 star (R2*), and fat fraction maps.

## Statistical Analysis

Data analyses were conducted using SPSS 22.0 Statistical Software (SPSS Inc., Chicago, IL, USA). Descriptive statistics, including the means and ranges, were calculated for the age, body weight, BMI, liver fat-fraction, pancreas fat-fraction, craniocaudal length of liver and liver volume for patients. Normal distributions were verified using the Kolmogorov-Smirnov test. The Paired Sample t-test was used to analyze these parameters. Correlation between liver fat-fraction and body weight, BMI, craniocaudal length of liver, liver volume and pancreas fat-fraction was analyzed using the Pearson's correlation analysis. A p value less than 0.05 indicated statistical significance.

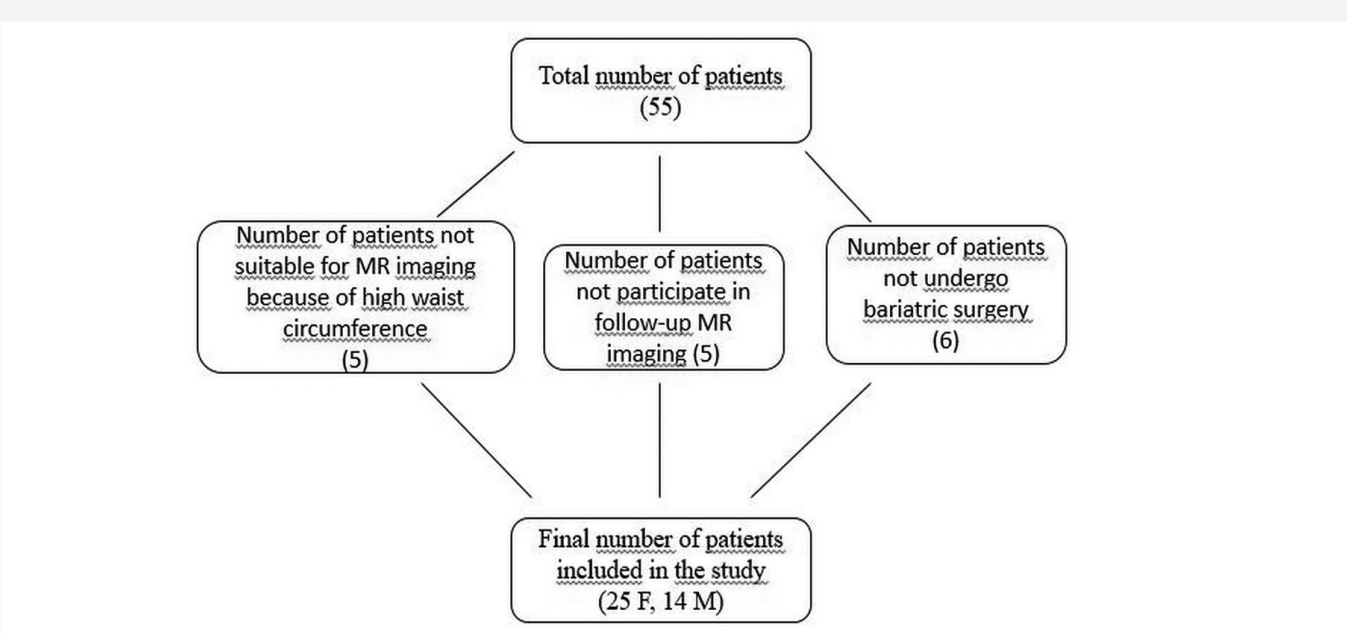


**Figure 1.** The patient flow diagram is shown

*MR: Magnetic resonance, F: Female, M: Male*

## RESULTS

The present study included 25 female (64.1%) and 14 male (35.9%) patients with a mean age of 41.2±12.5 years.

There was a statistically significant change between preoperative and postoperative values of BW, BMI, LFF, PFF, LL and LV (Table 1).

In the preoperative period, 33 (84.6%) of 39 patients had NAFLD, while 6 patients (15.4%) had no fatty infiltration of liver. Fatty infiltration of liver completely resolved in 26 (66.7%) of 39 patients in the postoperative period, which was statistically significant (p<0.001). In the preoperative period, 16 patients (41%) had NAFPD, and after bariatric surgery, complete resolution of fatty infiltration was observed in 12 (75%) of patients, which was statistically significant (p<0.001). There was a statistically significant decrease in the mean LFF and PFF values in the early postoperative period (p<0.001) (Figure 2). Also, the mean LL and LV values decreased significantly (p<0.001) (Figure 3).

According to the correlation analysis (Table 2), there was a moderate positive linear correlation between LFF and PFF, LL, LV (p<0.001) (Figure 4). A weak positive linear correlation was observed between LFF and BMI (p<0.001), BW (p<0.005).

## DISCUSSION

Our study has shown that significant remission is observed in the fatty infiltration of the liver and pancreas in the early period after bariatric surgery, and these positive changes can be detected accurately and non-invasively in a very short time with the ideal IQ sequence.

NAFLD and NAFPD are the diseases that develop due to obesity and, if not treated, can lead to serious health problems in a wide spectrum from infectious processes to malignancy in the liver and pancreas parenchyma[2]. Today, the increasing

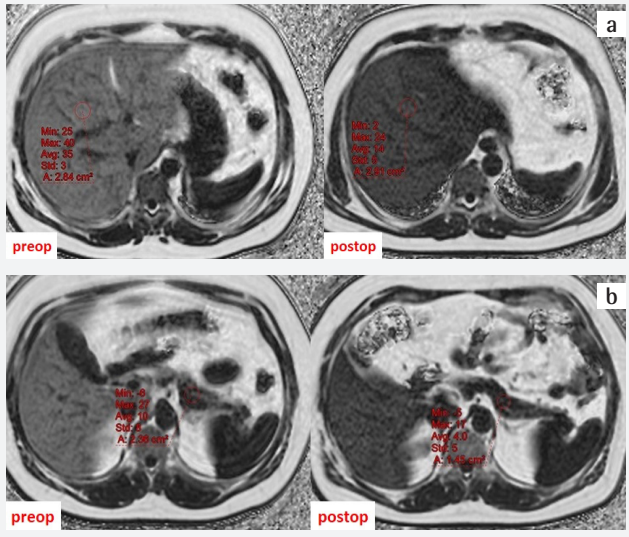


**Figure 2.** a, b) A 35-year-old male patient undergoing bariatric surgery had a reduction in LFF after surgery by 60% (preop LFF=35%, postop LFF=14%). In the same patient, the pancreatic fat fraction also showed a 60% reduction after surgery. Preop PFF=10%, postop PFF=4%)

*LFF: Liver fat fraction*

**Table 1. Results of variables measured at preoperative and postoperative periods**

| Parameters | Preoperative | Postoperative | p value |
|---|---|---|---|
| BW (kg) | 126.4±20.1 (82-175) | 111.5±17.8 (76-155) | <0.001 |
| BMI (kg/m$^2$) | 46.0±5.2 (35-65) | 40.5±4.9 (33-59) | <0.001 |
| LFF (%) | 16.8±10.6 (5-40) | 7.1±4.8 (2-20) | <0.001 |
| PFF (%) | 9.4±4.7 (3-24) | 5.0±4.1 (0-19) | <0.001 |
| LL (mm) | 214.4±22.9 (168-267) | 193.1±20.3 (156-245) | <0.001 |
| LV (cm$^3$) | 2611.7±551.8 (1715-3843) | 1949.7±380.9 (1181-2887) | <0.001 |

BMI: Body mass index, BW: Body weight, LFF: Liver fat fraction, LL: Liver length, LV: Liver volume, PFF: Pancreas fat fraction

**Table 2. The correlation analysis between LFF changes and other variables after the bariatric surgery**

| Parameters | r | $R^2$ | p value |
|---|---|---|---|
| PFF (%) | 0.69 | 0.33 | <0.001 |
| LL (mm) | 0.61 | 0.37 | <0.001 |
| LV (cm$^3$) | 0.49 | 0.24 | <0.001 |
| BMI (kg/m$^2$) | 0.34 | 0.11 | <0.001 |
| BW (kg) | 0.21 | 0.04 | <0.001 |

BMI: Body mass index, BW: Body weight, LFF: Liver fat fraction, LL: Liver length, LV: Liver volume, PFF: Pancreas fat fraction

frequency of obesity and obesity-related diseases has made the importance of bariatric surgery even more evident. Therefore, demonstrating and monitoring the positive effects of bariatric surgery about the course of NAFLD and NAFPD has also gained great importance. At this point, we aimed to investigate the effects of bariatric surgery on liver and pancreatic fatty infiltration non-invasively by using a novel MRI sequence; ideal IQ (MR-PDFF).

Liver biopsy is the gold standard method used in the determination of the fatty infiltration of the liver and its harmful effects on the liver parenchyma[9,22]. However, this invasive method has disadvantages such as having preprocedural complications including bleeding, allowing sampling only from a specific liver area, operator dependency, and high cost[23]. In addition, this method requires performing a liver biopsy before or during surgery, and will expose the patient to these risks several times in the follow-up period. For this reason, non-invasive radiological imaging methods are used instead of liver biopsy to detect fatty infiltration in visceral organs. In the current study, it was aimed to investigate the effects of bariatric surgery both on liver and pancreatic fatty infiltration with MR-PDFF, which is accepted as the most effective and accurate method in the evaluation of fatty infiltration in recent years. Ideal IQ sequence provides volumetric whole-liver and pancreatic coverage in a single breath-hold and generates estimated T2* and triglyceride fat fraction maps in a non-invasive manner. It was found that the MR-PDFF method showed a high correlation with magnetic resonance spectroscopy (MRS) and liver biopsy in the evaluation of fatty liver[24-26]. Among the advantages of this method, unlike MRS, it does not require special experience for evaluation, and it can be easily measured from any level of the liver parenchyma in a shorter time without the need for postprocessing procedures. As in our study, the amount of fat both in the pancreas and in the liver parenchyma can be quantitatively calculated in seconds with a single sequence.

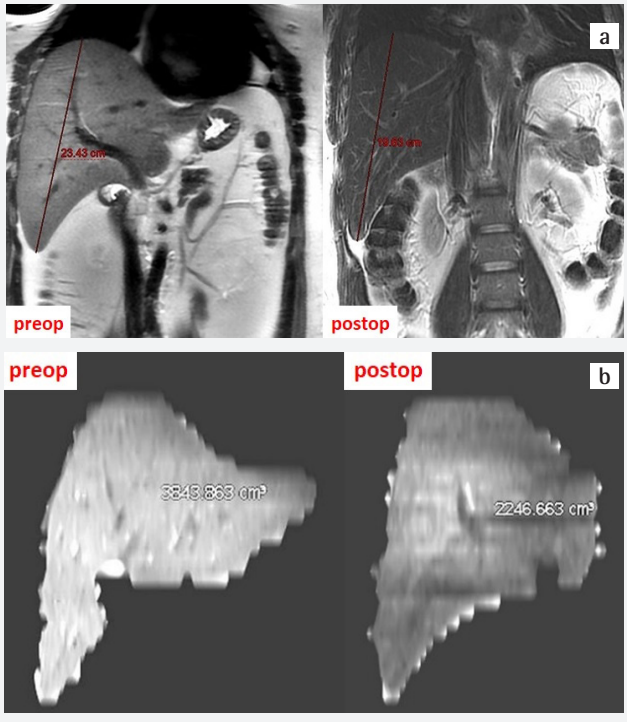


**Figure 3 a, b).** The patient had a 16% reduction in the craniocaudal LL in the postoperative period (preop LL=234.3 mm, postop LL=196.5 mm). Also in the same patient, there was a 41% reduction in liver volume after bariatric surgery (preop liver volume=3843 $cm^3$, postop liver volume=2246 $cm^3$)

*LL: Length of the liver*

One of the most important findings in the current study is that fatty infiltration of the liver decreased significantly at the end of first month after bariatric surgery. In 66.7% of the patients participating in our study, after bariatric surgery, LFF values decreased below 6.5% and NAFLD was completely regressed. Unlike other publications in the literature[27,28], in this study, we found that LFF values regressed to normal values in more patients in the early postoperative period. It is thought that two main factors play a role in determining such a significant decrease in LFF of the patients after bariatric surgery. The first reason is that the basal LFF (16.8±10.6%) and BMI (46.0±5.2 $kg/m^2$) values of the patients who participated in the study were relatively higher than the basal values in other studies. The second reason is that, unlike other studies, our patients were not given very low calorie diet therapy before surgical treatment. In other studies, it was found that patients both showed weight loss and decreased LFF values after an average

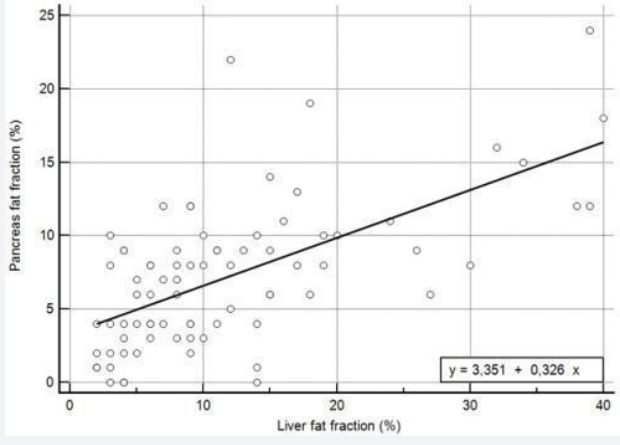


**Figure 4.** Correlation analyses showed a significant moderate positive linear correlation between LFFs and PFFs (r=0.69; R2=0.33, p<0.001)

*LFF: Liver fat fraction, PFF: Pancreas fat fraction*

of two weeks of low-calorie diet before surgery. This was considered as the reason for less weight loss and decrease in LFF compared to our study. It is thought that not applying a calorie diet to our patients before the surgical treatment causes them to lose weight more quickly and effectively after surgery. However, in order to demonstrate this more clearly, it is considered that future studies will be conducted in which patients who are treated and not treated with diet are evaluated comparatively.

There are limited numbers of publications investigating the effects of bariatric surgery on pancreatic fat infiltration[29,30]. Similar to our study, in most of these publications, a significant decrease in pancreatic FF values was detected after bariatric surgery. In the study of Covarrubias et al.[30], a mean decrease of 0.4% in PDFF values at the 1st month and 5.7% at the end of the 6th month was found after bariatric surgery. In another study by Hui et al.[29], it was stated that there was a 1.6% decrease in PDFF rates at the end of 6 months and 4.5% at the end of one year. Although studies have found a decrease in pancreatic FF values at different rates, it is understood that this decrease continues to increase with time. In our study, it was found that 41% of the patients had pre-operative NAFPD, while 75% of the patients had total regression after surgery. Moreover, it was found that there was a 4.4% decrease in pancreatic PDFF rates in the early postoperative period (1 month after surgery). The higher rate of decrease in FF values in our study was evaluated primarily due to the inclusion of more patients.

Today, the time course of postoperative fat fraction changes in visceral organs is not clearly known. However, most studies report that the positive effects of bariatric surgery on fatty infiltration in visceral organs as well as on anthropometric measurements such as BMI and BW continue long term. Accordingly, it was found that LFF reached normal values in almost all patients at the end of the 12th month. On the other hand, it has been reported in most publications that the most significant reduction in liver and pancreatic fat fraction occurs in the early postoperative period (usually at the end of the 1st month)[27,30,31]. In our study, in parallel with the publications in the literature, a statistically significant decrease was found in the liver and pancreatic fat fraction values of all patients in the first month after surgery. In the study of Luo et al.[27], it is understood that the highest decrease in liver PDFF rates occurred in the 1st month after surgery. In addition, while there was no significant change in liver volume in the following imagings, it was determined that the decrease in liver FF values continued, albeit to a lesser extent. Similarly, in another study conducted by Pooler et al.[31], it was observed that the highest decrease in liver FF rates was detected in the first month postoperative controls (5.6%). On subsequent follow-up imaging, the decrease in liver FF values (2.8% at the end of the 3rd month and 1.6% at the end of the 6-10th month) continued at decreasing rates. On the other hand, it was understood that the fatty infiltration completely resolved in 66.7% of our patients with NAFLD and in 75% of our patients with NAFPD after the bariatric surgery. This was determined as an evidence that the positive effects of bariatric surgery were most evident in the early period.

### Study Limitations

Our study has some limitations. Firstly, although relatively few patients were involved in this study, it is among the studies with highest number of patients on the same topic. Secondly, liver biopsy, which is the gold standard method in the diagnosis of fat infiltration, was not performed in the patients in our study because of its invasiveness and difficulty after treatment. Thirdly, since bariatric surgery is a new treatment method that has been started to be used in our center, the long-term effects of the treatment have not been investigated due to the fact that patients did not adapt adequately to follow-up imaging. Fourthly, sleeve gastrectomy was mainly performed on our patients, and other bariatric surgical methods were applied in a limited number of cases. Therefore, the effects of surgical methods on visceral organ fatty infiltration have not been compared. However, it is planned to conduct studies comparing the effects of different bariatric surgery methods on liver and pancreatic fat in the future. Compared to similar studies in the literature, our study is a rare study that includes a larger number of patients and examines the early changes in both liver and pancreatic fatty infiltration caused by bariatric surgery using a non-invasive method. Lastly, to our knowledge, this is the first study on this topic from Eastern Mediterranean region.

## CONCLUSION

Ideal IQ sequence is a non-invasive effective method that allows the monitorization of the positive effects of bariatric surgery on liver and pancreatic fatty infiltration.

### Ethics

**Ethics Committee Approval:** This retrospective study was approved by the Recep Tayyip Erdoğan University Institutional Review Board in our institution (decision number: 2019/113, approval date: 09.09.2019).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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

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

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# The Validation and Reliability Study of Turkish Version of Revised Urinary Incontinence Scale

## Revised Urinary Incontinence Scale'in Türkçe Geçerlilik ve Güvenilirlik Çalışması

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### ABSTRACT

**Aim:** This study aims to verify the validity and reliability of Turkish Version of Revised Urinary Incontinence Scale (RUIS).

**Materials and Methods:** The scale was translated from English into Turkish by three academicians. The first Turkish version of the scale was created by combining the translated texts. This version was translated back into English, and the authors examined language compatibility. The content validity of the scale was examined through the Davis method. The item content validity indices were in the range of 0.78-1, and the scale content validity indices was determined as 0.89. Fifty-six volunteers (83.9% female, 16.1% male) with a mean age of 59.2±14 years were applied the Turkish versions of RUIS, International Consultation on Incontinence Questionnaire Short Form and Urogenital Distress Inventory.

**Results:** The Cronbach alpha coefficient was calculated as 0.810. There was no floor and ceiling effect on the scale. The Kaiser-Meyer-Olkin value of the scale was 0.762, the Bartlett sphericity test chi-square value was 94.583, the p value was 0.0001, and the 'anti-image' correlation values were in the range of 0.709-0.884. The explained variance rate of the scale which preserved its single sub-dimensional structure was calculated as 57.943%, and the eigenvalue was 2.897. We determined that the Scale-Turkish version had an excellent ($r>0.80$, $p<0.05$) agreement with the other two measurement tools. We determined that the scale met the model goodness of fit values in confirmatory factor analyses.

**Conclusion:** RUIS was adapted to the Turkish language, its validity and reliability were ensured, and it was presented to researchers.

**Keywords:** Urinary incontinence, scale, Turkish language, validity, reliability

### ÖZ

**Amaç:** Bu çalışmanın amacı, Revised Urinary Incontinence Scale'in (RUIS) Türkçe geçerliliğini ve güvenilirliğini sağlamaktır.

**Gereç ve Yöntem:** Ölçek, İngilizceden Türkçeye üç akademisyen tarafından çevrildi. Çeviri metinleri, birleştirilerek ölçeğin ilk Türkçe versiyonu oluşturuldu. Bu versiyon, ölçeğin orijinal dili olan İngilizceye tekrar çevrilerek dil uyumu incelendi. Ölçeğin kapsam geçerliliği, Davis yöntemi ile irdelendi. Madde kapsam geçerlilik indeksleri (KGİ) 0,78-1 aralığında, ölçek KGİ ise 0,89 olarak belirlendi. Yaş ortalaması 59,2±14 yıl olan 56 gönüllüye (%83,9 kadın, %16,1 erkek) RUIS, International Consultation on Incontinence Questionnaire Short Form ve Urogenital Distress Inventory'nin Türkçe versiyonları uygulandı.

**Bulgular:** Cronbach alfa katsayısı 0,810 olarak hesaplandı. Ölçekte taban ve tavan etkisi oluşmadı. Ölçeğin Kaiser-Meyer-Olkin değeri 0,762, Bartlett küresellik testi ki-kare değeri 94,583, p değeri 0,0001 ve 'anti-image' korelasyon değerleri 0,709-0,884 aralığındaydı. Tek alt boyutlu yapısını koruyan ölçeğin açıklanan varyans oranı %57,943 ve öz değeri 2,897 olarak hesaplandı. Ölçeğin Türkçe versiyonunun, diğer iki ölçüm aracı ile mükemmel düzeyde ($r>0,80$; $p<0,05$) uyum gösterdiği tespit edildi. Ölçeğin doğrulayıcı faktör analizlerinde de model uyum iyiliği değerlerini karşıladığı görüldü.

**Sonuç:** RUIS'in Türkçeye uyarlandığı, geçerliliğinin, güvenilirliğinin sağlandığı görüldü ve araştırmacıların kullanımına sunuldu.

**Anahtar Kelimeler:** İdrar kaçırma, ölçek, Türkçe, geçerlilik, güvenilirlik

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## INTRODUCTION

Urinary incontinence (UI) is a health problem that can be seen in all age groups worldwide. UI was defined as 'the complaint of any involuntary leakage of urine' by the International Continence Society (ICS) in 2002, and this terminology was adopted in the joint statement of ICS and the International Urogynecology Association (IUGA) in 2010[1,2]. Current terminology studies on UI continue increasingly[3]. While the general prevalence of UI, which has many subtypes, mainly stress, urgency, and mixed type, varies between 25% and 45% in women and between 1% and 39% in men, its incidence increases with age[4].

Rates such as 37.3% in the Middle East and North Africa, 32.2% in Europe and Central Asia, 14.2% in South Asia and 28.8% in Latin America show that UI varies according to the ethnicity, geography, and development level of the countries[5]. In our country, the prevalence is between 14.6% and 49.5%[6-9]. The fact that the prevalence rates were quite different among studies was attributed to the diversity in the questionnaires used by the authors[5].

When evaluating UI, it is necessary to compare before/after treatment or analyze patient complaints in a standard way in clinical studies. During these evaluations, inquiry forms are used to quantify the data. The questionnaire should be easily understandable and short and contain essential questions about the disease.

We conducted the validity and reliability study of the Turkish version (RUIS-TR), considering that the Revised Urinary Incontinence Scale (RUIS) is adequately equipped for UI.

## MATERIALS AND METHODS

After obtaining permission via e-mail from Sansoni et al.[10], who developed the RUIS, and approval of the Süleyman Demirel University Local Ethics Committee (decision dated: 05.05.2021 and number: 11/203), the research began. An informed consent form was obtained from each volunteer participating in the study.

RUIS was translated from its original language, English, into Turkish by three academicians (two field experts and one non-field expert). During the translation, attention was paid to the use of the daily Turkish language structure that our society could easily understand without moving away from the meaning expressed in the original language.

The three translated texts of the scale, which were translated independently of each other, were created as the first Turkish version of the scale after the consensus meeting held by the three academicians who did the translations. This version was translated back into its original language by a native English translator, and language compatibility was examined and evaluated in terms of semantic shift. It was agreed that the content and validity of the last version of the scale were suitable for evaluation.

### CONTENT VALIDITY

The clarity of the first Turkish version of the scale was evaluated using the Davis method on ten patients (eight women, two men) who applied to our hospital with the complaint of UI. The clarity of the first Turkish version of the scale was examined with a four-point Likert-type form. The scores given to the form were used to calculate the item content validity indices and the scale content validity index. While the critical value of the item content validity index was 0.78, the critical value of the scale content validity index was accepted as 0.80[11,12].

Item content validity indices were calculated as 0.89 for item 1, 1.00 for items 2 and 4, and 0.78 for items 3 and 5. The content validity index of the scale was determined as 0.89. These values indicated that the scale provided content validity by exceeding the recommended critical values. With the qualitative feedback given to the form, the Turkish version of the scale to be used in the pilot scheme was made ready.

### PILOT SCHEME

To evaluate the Turkish validity and reliability of the scale, it was aimed to reach individuals with UI complaints at least ten times higher than the number of items[13].

Patients over the age of 18 years, who applied to the urology outpatient clinic with the complaint of UI, were included in the study on a voluntary basis. Exclusion criteria of the study were; pregnancy, urinary tract infection, symptomatic urinary stone disease, previous history of urethra/prostate/bladder/uterus/vagina or lumbar hernia operation, diabetes mellitus, diuretic drugs, and receiving medical treatment for UI. In addition, patients with physical or mental disabilities who could not fill in the questionnaire on their own were not included in the study.

A total of 56 volunteers, whose 83.9% (n=47) were female and 16.1% (n=9) were male, the mean age was 59.2±14 years, and the body mass index was 26.8±4.9 kg/m$^2$, who met the inclusion criteria of the study were applied International Consultation on Incontinence Questionnaire Short Form (ICIQ-SF) Turkish version, Urogenital Distress Inventory (UDI-6) Turkish version and RUIS-TR, which is tried to be adapted into Turkish with this study, by using face-to-face interview techniques (Table 1).

### Statistical Analysis

The data obtained at the end of the pilot scheme were analyzed with Statistical Package for the Social Sciences v.23 and AMOS v.24 package program. Descriptive statistical analyses, explanatory and confirmatory factor analysis, Cronbach's alpha analysis, quarterly difference analysis, and correlation analysis of fit were performed on the data. Results are presented as frequency, percentage, and mean ± standard deviation.

## RESULTS

In our study, the mean score from RUIS-TR was 8.71±4.11, and the median and mode values were 8 points. The 25% percentile score of the sample was 6, the 50% percentile score was 8, and the 75% percentile score was 12.

In the Turkish version of the scale, the Cronbach alpha coefficient was calculated as 0.810, and when it was removed from the scale, the item that caused an increase in the Cronbach alpha coefficient was not detected. Thus, the scale was found to have high reliability (Cronbach's alpha coefficient=0.80-1.00) (Table 2).

It was observed that the item-total correlation coefficients in the scale were greater than 0.25, the index of distinctiviness values were positive, and the p value obtained in the difference test between the lower and upper 27% groups was determined as 0.0001. It was determined that the rate of those who got 0 points, which is the lowest score that can be obtained from RUIS-TR, was 5.4% (n=3) and there was no floor effect. The rate of those who got 16 points, which is the highest score that can be obtained, was 1.8% (n=1) and there was no ceiling effect.

The Kaiser-Meyer-Olkin value of the scale was 0.762, the Bartlett Sphericity test chi-square value was 94.583, and the p value was 0.0001, the 'anti-image' correlation values were in the range of 0.709-0.884. In the light of these results, the analyses were continued using the principal components method. The explained variance rate of RUIS-TR, which preserves its single sub-dimensional structure, was calculated as 57.943% and its eigenvalue as 2.897.

Within the scope of convergent validity of RUIS-TR, its correlation with Turkish versions of ICIQ-SF and UDI-6

**Table 1. 'Revised Urinary Incontinence Scale' adapted into Turkish**

**Revised Urinary Incontinence Scale-Türkçe versiyon**

İdrar kaçırma şikayetinizle ilgili olarak son 4 haftayı düşündüğünüzde aşağıdaki kutucuklardan kendiniz için en uygun olanı işaretleyiniz.

Aşağıdaki durum başınıza geldi mi, öyle ise bu durum sizi ne kadar rahatsız etti?:

1. Ani sıkışma hissi ile ilişkili olan idrar kaçırma
   - Asla (0)
   - Nadiren (1)
   - Ara sıra (2)
   - Çoğu zaman (3)
2. Fiziksel aktivite, öksürme veya hapşırma ile ilişkili olan idrar kaçırma
   - Asla (0)
   - Nadiren (1)
   - Ara sıra (2)
   - Çoğu zaman (3)
3. Az miktarda idrar kaçırma (damlama)
   - Asla (0)
   - Nadiren (1)
   - Ara sıra (2)
   - Çoğu zaman (3)
4. İdrar kaçırma hangi sıklıkta başınıza gelmektedir?
   - Hiçbir zaman (0)
   - Ayda bir kereden az (1)
   - Ayda birkaç kez (2)
   - Haftada birkaç kez (3)
   - Her gün ve/veya her gece (4)
5. Her seferinde ne kadar miktarda idrar kaçırmaktasınız?
   - Hiç (0)
   - Birkaç damla (1)
   - Hafif sızıntı (2)
   - Çok fazla (3)

was examined. Accordingly, a high level of positive linear relationship was determined between the total score of the RUIS-TR and the other two measurement tools, in which the convergent validity was evaluated, and it was found that it had an excellent concordance (r=0.80-1.00) (Table 3).

It was observed that the scale, whose reliability, structure and fit validity was ensured, also met the model goodness of fit values in confirmatory factor analyses (Figure 1, Table 4).

## DISCUSSION

According to the data obtained from the study, it was seen that RUIS-TR was adapted to Turkish, and its validity and reliability was ensured. During the clinical application of the Turkish scale; It is recommended that patients with 0-6 points be classified as 'mild', patients with 7-11 points as 'moderate', and patients with 12-16 points as 'severe' UI.

In the development study of RUIS by Sansoni et al.[10], the total score obtained from the scale was calculated as 10.92±3.33, based on the answers given by patients (86% female and 14% male). Although the gender distribution of the patients participating in the original study was slightly higher (10.92±3.33 points vs. 8.71±4.11), the mean values obtained from the scale were compatible with our study, which is the Turkish version of the scale. During the clinical application of the original scale, the recommended cut-off values were 0-3 points (extremely mild), 4-8 points (mild), 9-12 points (moderate), and 13-16 points (severe)[10]. In the Turkish version of the scale, 0-6 points were considered as mild, 7-11 points as moderate, and 12-16 points as severe. We thought this variation occurred due to the difference in the mean, median, and mode values of the scores given to the scale by the samples in the studies.

The most important indicator of the reliability of the scales is the value of the Cronbach alpha coefficient. In this context, the Cronbach alpha coefficient obtained from the pre-treatment sample group of the original study was 0.73 and the Cronbach alpha coefficient obtained from the post-treatment sample

**Table 2. Validity and reliability results of Revised Urinary Incontinence Scale-Turkish version**

| | RUIS-TR Item 1 | RUIS-TR Item 2 | RUIS-TR Item 3 | RUIS-TR Item 4 | RUIS-TR Item 5 | RUIS-TR |
|---|---|---|---|---|---|---|
| Mean | 1.18 | 1.73 | 1.55 | 2.66 | 1.59 | 8.71 |
| Standard deviation | 1.15 | 1.19 | 0.97 | 1.24 | 0.85 | 4.11 |
| Item-total correlation | 0.538 | 0.584 | 0.509 | 0.729 | 0.675 | - |
| Item discrimination strength index | 7.977 | 10.057 | 5.675 | 8.721 | 6.693 | 14.165 |
| Factor load | 0.705 | 0.742 | 0.663 | 0.864 | 0.814 | - |
| Cronbach alpha | 0.792 | 0.779 | 0.798 | 0.729 | 0.761 | 0.810 |

RUIS-TR: Revised Urinary Incontinence Scale-Turkish version

**Table 3. Convergent validity results of Revised Urinary Incontinence Scale-Turkish version**

| | | ICIQ-SF total score | UDI-6 total score |
|---|---|---|---|
| | | 8.9±5.9 | 6.8±4.3 |
| RUIS-TR Item 1 | r | 0.534** | 0.793** |
| | p | 0.0001 | 0.0001 |
| RUIS-TR Item 2 | r | 0.656** | 0.799** |
| | p | 0.0001 | 0.0001 |
| RUIS-TR Item 3 | r | 0.511** | 0.777** |
| | p | 0.0001 | 0.0001 |
| RUIS-TR Item 4 | r | 0.788** | 0.680** |
| | p | 0.0001 | 0.0001 |
| RUIS-TR Item 5 | r | 0.753** | 0.573** |
| | p | 0.0001 | 0.001 |
| RUIS-TR total score | r | 0.854** | 0.866** |
| | p | 0.0001 | 0.0001 |

**: p value is significant at the level of 0.01.

RUIS-TR: Revised Urinary Incontinence Scale-Turkish version, ICIQ-SF: International Consultation on Incontinence Questionnaire Short Form-Turkish version, UDI-6: Urogenital Distress Inventory-Turkish version

group was 0.90, and the scale was found reliable[10]. In the Turkish version of the scale, this value was calculated as 0.810, and it was observed that RUIS-TR provided high reliability.

The condition for the applicability of factor analysis during the analyses performed in scale development and adaptation studies was Kaiser-Meyer-Olkin value above 0.60 and the Bartlett Sphericity test chi-square test p value less than 0.05[13]. The applicability condition for factor analysis was met in the original study and in this study, which is an adaptation study into Turkish. In this context, while the factor load explained in the original study was 49%, the eigenvalue was 2.43, and the factor loads of the items were in the range of 0.64-0.80, these values were found to be 57.943, 2.897 and the range of 0.663-0.864, respectively, in the Turkish version of the scale, which showed the validity of RUIS-TR.

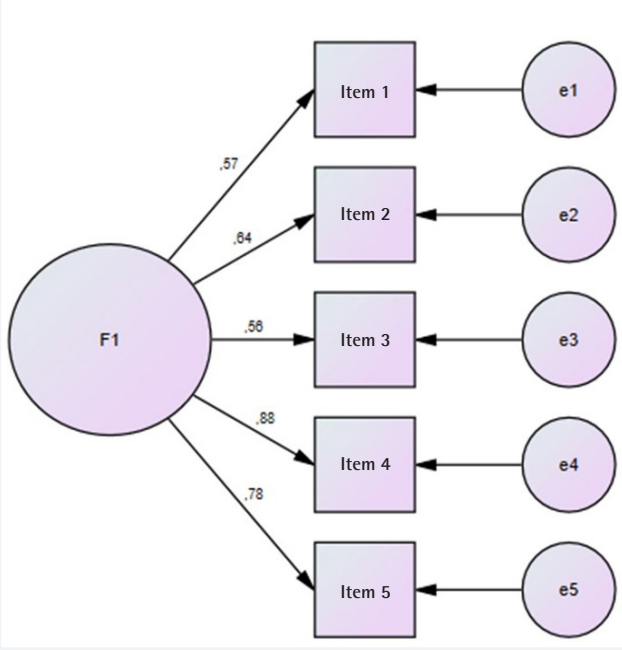


**Figure 1.** Confirmatory factor analysis diagram of the Revised Urinary Incontinence Scale-Turkish version

**Table 4. Confirmatory factor analysis results of the Revised Urinary Incontinence Scale-Turkish version**

| Model fit indices | Value in RUIS-TR |
|---|---|
| $\chi^2$/df | 1.364[i] |
| RMSEA | 0.081[k] |
| SRMR | 0.043[i] |
| CFI | 0.980[i] |
| GFI | 0.958[i] |
| AGFI | 0.875[k] |
| IFI | 0.981[i] |
| TLI | 0.959[i] |

[i]: good fit, [k]: Acceptable fit.

RUIS-TR: Revised Urinary Incontinence Scale-Turkish version, RMSEA: Root Mean Square Error of Approximation, SRMR: Standardized Root Mean Squared Residual, CFI: Comparative Fit Index, GFI: Goodness-of-Fit Index, AGFI: Adjusted Goodness-of-Fit Index, IFI: Incremental Fit Index, TLI: Tucker-Lewis Index

In the original study, its correlation coefficient with the UDI-6, in which the concordance validity was evaluated, was 0.76 ($p<0.01$), and its correlation coefficient with the ICIQ-SF was 0.74 ($p<0.01$)[10]. In our study, both correlation coefficients remained above 0.80 with the Turkish version of UDI-6 and ICIQ-SF, and RUIS-TR showed excellent concordance with other scales.

In scale development and adaptation studies, it is desired that the floor and ceiling effects in the sample be below 15%[14]. In this context, while the rate of those who got a base score was 0.5% in the original study, the rate of those who got a ceiling score was 5.6%[10]. In our study, these rates were 5.4% and 1.8%, respectively, below the 15% limit value desired to be applied in the literature.

When adapting the original scales to a different culture and language, confirmatory factor analysis is recommended as well as explanatory factor analysis and reliability analysis[15]. In this study, confirmatory factor analysis was applied after the explanatory factor analysis, and it was observed that the scale met the model goodness of fit values.

### Study Limitations

The main limitation of our study is its single-center conducted nature. However, it was observed that RUIS-TR was adapted to Turkish, and its validity and reliability were ensured.

## CONCLUSION

The use of the internationally standardized RUIS-TR scale form in the evaluation of patients who apply with UI complaints will strengthen the clinicians' armamentarium both in practice and in future studies. At the same time, we believe that analyzing the obtained data in a common ground will prevent conceptual confusion in diagnosis and treatment.

### Ethics

**Ethics Committee Approval:** The study were approved by the Süleyman Demirel University of Local Ethics Committee (decision dated: 05.05.2021 and numbered: 11/203).

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

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

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

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

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# Detection of Biofilm Production in *Candida* Species from the Vagina by Two Different Methods

## Vajenden Soyutlanan *Candida* Türlerinde Biyofilm Üretiminin İki Farklı Yöntemle Araştırılması

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**ABSTRACT**

**Aim:** The incidence of fungal infections has increased today, and antifungal resistance has increased in such infections. It is known that most infections produced by *Candida* species are associated with biofilm formation.

**Materials and Methods:** In this study, 192 patients diagnosed with yeast cytologically in cervico-vaginal smears between September 2015 and August 2020 were investigated.

**Results:** When all *Candida* species studied with Congo Red Agar were evaluated, biofilm positivity was found to be 61.4% in non-albicans *Candida* species and 38.6% in *C. albicans*. Biofilm positive non-albicans *Candida* species were identified as 11 (15.7%) *C. glabrata*, 11 (15.7%) *C. tropicalis*, 6 (8.6%), *C. guillermondii* and 6 (8.6%) *C. krusei*.

**Conclusion:** In fungal infections, the biofilm produced by the agent is directly proportional to antifungal resistance and invasion of the infection. Therefore, determining the biofilm production of the causative fungus is important in planning the treatment.

**Keywords:** Biofilm production, *Candida* species, *Candida* vaginitis

**ÖZ**

**Amaç:** Günümüzde mantar enfeksiyonlarının görülme sıklığı ve antifungal dirençleri artmıştır. *Candida* türleri tarafından üretilen enfeksiyonların çoğunun biyofilm oluşumu ile ilişkili olduğu bilinmektedir.

**Gereç ve Yöntem:** Bu çalışmada Eylül 2015 ile Ağustos 2020 tarihleri arasında Sitonet Sito-Patoloji Merkezi'nde serviko-vajinal smearlerinde sitolojik olarak maya tanısı saptanan 192 hasta araştırıldı.

**Bulgular:** Kongo Kırmızısı Agar ile çalışılan tüm *Candida* türleri değerlendirildiğinde biyofilm pozitifliği non-albicans *Candida* türlerinde %61,4 iken *C. albicans*'ta %38,6 olarak bulundu. Biyofilm pozitif olan non-albicans *Candida* türleri ise 11 (%15,7) *C. glabrata*, 11 (%15,7) *C. tropicalis*, 6 (%8,6), *C. guillermondii* ve 6 (%8,6) *C. krusei* olarak tanımlandı.

**Sonuç:** Mantar enfeksiyonlarında etkenin ürettiği biyofilm, antifungal direnç ve enfeksiyonun invazyonu ile doğru orantılıdır. Bu nedenle etken mantarın biyofilm üretiminin saptanması tedavinin planlanması açısından önem taşımaktadır.

**Anahtar Kelimeler:** Biofilm production, *Candida* species, *Candida* vaginitis

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## INTRODUCTION

The incidence of fungal infections and also antifungal resistance in these types of infections have currently increased. *Candida* species are important fungal pathogens of humans that cause mucosal infections, especially in the vagina. *Candida* species are the most common pathogen seen in cervicovaginal smear. Ascomycola, Aspergillus, Sporidiobolaceae, Basidiomycota, Coprinellus, and Paracoccidioides are rarely found. Many pieces of evidence suggest that the majority of infections produced by *Candida* species are associated with biofilm growth. Biofilm formation is a major virulence factor in the pathogenicity of *Candida* species. Any biofilm or slime factor facilitates the colonization or invasive infections of *Candida* species in host cells, catheters, and prosthetic devices. Biofilm production is also associated with a high level of antifungal resistance in *Candida* species. When *Candida* species grow in vaginal samples, the detection of biofilm production as a criterion for antifungal resistance and virulence will guide the clinician for treatment. The method to be preferred for the detection of biofilm production should yield results in a short time, be easy to evaluate and be inexpensive. We, therefore, compared isolates from clinical vaginal discharge, representing different *Candida* species to each other for the capacity to form biofilms in glucose-containing medium such as 8% Sabouraud Dextrose Broth and Congo Red Agar (CRA).

## MATERIALS AND METHODS

In our study, there were 192 routine patients who were examined in Sitonet Cyto-Pathology Center between September 2015 and August 2020. Two sets of examples were obtained using conventional smear cytology and wet smear (Figure 1). Conventional cervico-vaginal smears were scanned in Sitonet Cyto-Pathology and wet smears were studied in İstanbul Okan University Medical Faculty Hospital Microbiology Laboratory.

One hundred ninety-two *Candida* strain biofilm productions extracted from vaginal discharge samples were evaluated in the microbiology laboratory via two different methods. Vaginal samples were inoculated into Sabouraud Dextrose Agar (SDA) (Condalab, Spain) and then incubated for 24 hours at 37 °C. The suspected yeast colonies were subjected to Gram staining and then germ tube test. Subsequently, they were inoculated into Corn Meal Agar plate (Dalmau plate-Condalab, Spain) containing Tween-80 (Merck. Millipore, Germany). Isolates forming chlamydospore on germ tube and Corn Meal Agar plate were defined as *C. albicans*. Yeast samples from other non-albicans *Candida* (NAC) were identified with the VITEK 2 (BioMérieux, France) YST automated identification system.

Biofilm production in *Candida* strains was investigated using the tube adherence method (Sabouraud broth with 8% glucose/Sabouraud Dextrose Broth-SDB-) and CRA with glucose[1,2]. SDB (Condalab, Spain) was prepared in accordance with the manufacturer's recommended method by adding 60 g of glucose per liter (glucose density 80 g/L or 8%) and each sample was incubated at 35 °C for 24 hours at SDA. Then the grown yeast colonies were suspended in sterile saline, the concentration of $3x10^7$ CFU/mL was added to 9 mL of methylene blue and SDB suspension in 10 mL glass tubes and diluted to $3x10^6$ CFU/mL, incubated at 35 °C without shaking. Specimens were observed macroscopically at 24$^{th}$ and 48$^{th}$ hours. The specimens stained with methylene blue at the tube rim and the bottom were considered "positive" for biofilm production. In CRA, after 48 hours of incubation at 35 °C, the presence of black colonies and visually biofilm production was detected[1-5].

## RESULTS

In the study, 70 of 192 *Candida* strains were identified as *C. albicans* (36.5%) and 122 as NAC (63.5%). Of the NAC species, 51 (41.8%) *C. glabrata,* 35 (28.7%) *C. tropicalis*, 18 (14.7%) *C. pseudotropicalis*, 9 (7%) 4) *C. guillermondii* and 9 (7.4%) were identified as *C. krusei*.

Fifty-eight (30.2%) biofilm positivity was detected at the 24$^{th}$ hour in all *Candida* species in SDB and 109 (56.8%) at the 48$^{th}$ hour (Figure 2). In CRA, 97 (50.05%) biofilm-positive *Candida* species were detected (Figure 3). Biofilm-positive *Candida* strain was found to be 88 (45.8%) and biofilm-negative *Candida* strain was found to be 74 (38.6%) with both methods (Table 1, 2, 3).

Of the 4 *Candida* strains that were positive at the 48$^{th}$ hour in SDB and negative in CRA, 22 were found to be negative at the 24th hour and weakly positive (+1 positive) at the 48$^{th}$ hour. No *Candida* strain that was positive in CRA but negative in SDB was found.

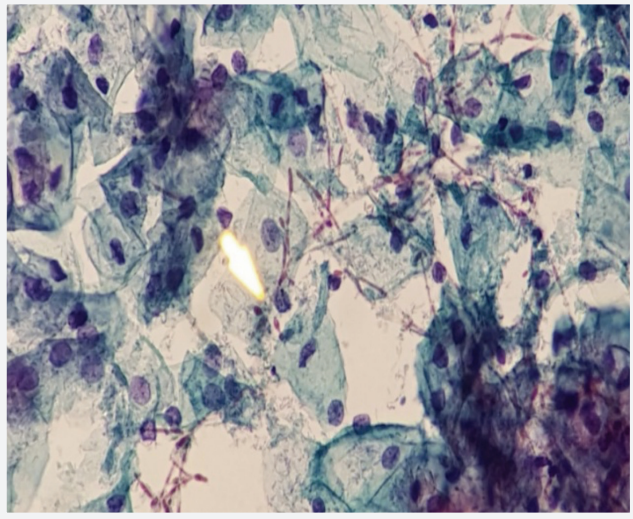

**Figure 1.** Yeasts, hyphae and vaginal epithelium in Papanicolau smear. X20, Pap stain

When all *Candida* species studied with CRA were evaluated, biofilm positivity was found to be 57.4% in NAC species and 38.6% in *C. albicans*. Biofilm positive NAC species were identified as 11 (15.7%) *C. glabrata*, 11 (15.7%) *C. tropicalis*, 6 (8.6%), *C. guillermondii* and 6 (8.6%) *C. krusei* (Figure 4).

**Figure 2.** Biofilm production by Christensen et al.[1] standard tube method with SDB positive (left) and negative (right) glass tubes -close up photo-

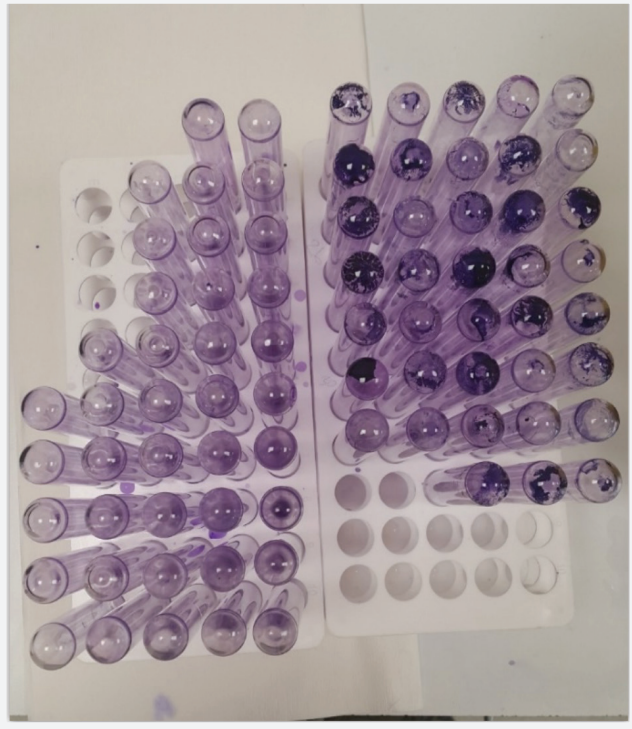

**Figure 3.** Biofilm production by Christensen standard tube method with Sabouraud Dextrose Broth positive (right) and negative (left) glass tubes

**Table 1. Biofilm productions by standard tube method with SDB at the 24th and 48th hours**

| Medium | Positive | Negative | Total |
|---|---|---|---|
| SDB (24th hour) | 58 (30.2%) | 134 (69.8%) | 192 (100%) |
| SDB (48th hour) | 109 (56.8%) | 83 (43.2%) | 192 (100%) |
| CRA | 97 (50.5%) | 95 (49.5%) | 192 (100%) |
| CRA and SDB together | 88 (45.8%) | 74 (38.6%) | |

SDB: Sabouraud Dextrose Broth, CRA: Congo Red Agar

**Table 2. Distribution of biofilm positive and negative samples by standard tube method with SDB at the 48th hour**

| Species | Biofilm positive | Biofilm negative | Total |
|---|---|---|---|
| *C. albicans* | 29 (41.4%) | 41 (58.6%) | 70 (100%) |
| *Non-albicans Candida* | 80 (65.6%) | 42 (34.4%) | 122 (100%) |
| Total | 109 (56.8%) | 83 (43.2%) | 192 (100%) |

SDB: Sabouraud Dextrose Broth

**Table 3. Distribution of biofilm positive and negative samples by CRA method**

| Species | Biofilm positive | Biofilm negative | Total |
|---|---|---|---|
| *C. albicans* | 27 (38.6%) | 43 (61.4%) | 70 (100%) |
| *Non-albicans Candida* | 70 (57.4%) | 52 (42.6%) | 122 (100%) |
| Total | 97 (50.5%) | 95 (49.5%) | 192 (100%) |

CRA: Congo Red Agar

**Figure 4.** Biofilm positive black colonies on CRA
CRA: Congo Red Agar

## DISCUSSION

The fact that some microorganisms, especially coagulase-negative staphylococci, produce biofilms under the name of slime factor was first reported by Christensen et al.[1]. The determination of slime factor production in coagulase-negative staphylococci by the CRA method has been first described by Freeman et al.[2] in 1989. Today, to detect biofilm production in coagulase-negative staphylococci, microdilution plate and standard tube methods described by Christensen or CRA method are used. The effects of biofilm production on adhesion and antibiotic resistance in bacteria and therefore its clinical importance have been shown in various studies[3,4].

Similar studies were conducted on yeasts after coagulase-negative staphylococci. Dolapçi and Tekeli[5] detected 15.14% slime factor positivity with the modified tube adherence method in a total of 350 *Candida* species (246 throat, 19 vaginal and 85 blood samples), and they also showed that the slime factor positivity was statistically significant. In the same study, they found that the slime factor positivity was significantly higher in *C. famata, C. guillermondii*, and *C. krusei*.

Li et al.[6] demonstrated the quantitative variability of adhesion and biofilm formation that allowed adhesion to polystyrene plastic surfaces in 115 *C. albicans* strain, including 47 from the oral cavity of healthy volunteers, 31 from the environment and 37 from vaginal samples of patients with candidiasis, and its relationship with genotypes. Harriott et al.[7] demonstrated *in vivo* biofilm formation and biofilm structure in the vaginal epithelium for the first-time. Calışkan et al.[8] showed that *Candida* species in the vagina could cause vaginal infections by forming biofilms and that biofilm structures that might form in intrauterine devices might cause resistance to antifungal drugs.

In the study of Yücesoy and Karaman[9], they investigated the biofilm production between *C. albicans* and NAC species with modified tube adherence and microplate methods. They showed a positivity rate of 65% with both methods. In our study, the biofilm positivity rate was found to be 45.8% with the standard tube adherence method and CRA. In the same study conducted by Yücesoy and Karaman[9], the biofilm positivity rate in different *Candida* species was between 17% and 55% in the tube adherence test. In the microplate method, it varied between 0% and 48%. In our study, SDB was found between 41.4% and 65.6% in the tube adherence test and 38.6% and 57.4% in the CRA test among *Candida* species. In the study of Yücesoy and Karaman[9] it was reported that there was no significant difference between *C. albicans* and NAC species in the tube adherence test. However, in our study, biofilm positivity was 41.4% for *Candida albicans* and 65.6% for NAC species biofilm positivity. Biofilm positivity was found to be 38.6% for *C. albicans* and 57.4% for NAC species in the CRA test. In the studies of Yücesoy and Karaman[9], it has been shown that biofilm production is a potential virulence factor for *Candida* species and is important in terms of antifungal sensitivity. In the study of Houdaii et al.[10], a positive relationship was found between biofilm formation and amphotericin B resistance.

Kumari et al.[11] detected 30.6% *Candida* species as causative agents in vulvovaginitis samples obtained from a total of 232 patients. 32.4% of them were identified as *C. albicans*, 45.07% as *C. parapsilosis*, and 22.53% as *C. glabrata*. The NAC rate of 67.6% found by Kumari et al.[11] is consistent with the rate of 63.5% in our study. While biofilm formation among *Candida* species was 70.4% in the study in question, it was found as 56.8% in the SDB method and 50.5% in the CRA method in our study.

Araurio Paulo de Medeiros et al.[12] examined their adhesion capacity to epithelial cells in 62 *C. albicans* samples isolated from the vagina and anus, biofilm formation in polystyrene microtiter plates, and proteinase activities, and they showed that virulence factors played an important role in the transition from colonization to infection in vulvovaginal candidiasis.

Kalaiarasan et al.[13], examined different virulence factors such as hemolysis, protease activity, and biofilm production, and they emphasized the importance of virulence factors, especially in NAC species. In our study, biofilm formation as a virulence factor in NAC species was found to be 63.5%.

In the study of Kıvanç and Er[14] , although there were differences between CRA and SDB microtiter plate methods for detecting biofilm production, it was stated that biofilm production was higher in *Candida* species isolated from the vagina. In the

same study, it was shown that lactic acid bacteria inhibited the production of biofilms formed by *Candida* species through competition in the adhesion area[14].

## CONCLUSION

In fungal infections, the biofilm produced by the agent is directly proportional to antifungal resistance and the invasion of the infection. Therefore, determining the biofilm production of the causative fungus is important in planning the treatment. According to the values obtained from our study, the probability of biofilm positive for those with positive CRA results is 100%, while it is 87% for those with negative CRA results. CRA method with glucose is a method that can be preferred in determining biofilm production as a virulence factor in NAC species, which is increasingly common among *Candida* species, because of its simpler and shorter application compared to other methods and its objective evaluation.

### Ethics

**Ethics Committee Approval:** The study were approved by the Okan University of Local Ethics Committee (protocol number: 13, date: 23.06.2021).

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

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: A.A., G.V., Concept: A.A., Design: G.V., Data Collection or Processing: A.A., G.V., Analysis or Interpretation: A.A., G.V., Literature Search: A.A., Writing: A.A., G.V.

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

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ORIGINAL ARTICLE

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# Measurable Residual Disease in Chronic Lymphocytic Leukemia: Experience in Real-Life Setting with Dry Tube Flow Cytometric Method

## Kronik Lenfositik Lösemide Ölçülebilir Kalıntı Hastalık: Kuru Tüp Akım Sitometri Metoduyla Gerçek Yaşam Deneyimi

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**ABSTRACT**

**Aim:** Undetectable measurable residual disease (uMRD) after chemoimmunotherapy (CI) is associated longer progression-free survival (PFS) and overall survival. However, it remains to be demonstrated whether uMRD translates into survival benefit in patients treated outside of clinical trials. Pipetting-free antibody staining procedures such as dry antibody tube method can reduce process-related errors and provide a better standardization. However, there are no clinical data about this method. The aim of the study was to evaluate the impact of dry antibody tube-based MRD analysis in the management of chronic lymphocytic leukemia (CLL).

**Materials and Methods:** We retrospectively reviewed the data of CLL patients, who were treated with CI regimens and had MRD analysis within 6 months after therapy. Forty-six patients were included in the study. MRD was assessed by multi-color flow cytometry panels with a sensitivity level of $10^{-4}$, mostly with dry tube.

**Results:** uMRD was achieved in 30 (65.2%) of the patients. The median PFS of patients who achieved uMRD was significantly longer compared to patients who did not. Twenty-nine patients were analyzed only by dry tube throughout study period. In the patients studied with the dry tube method, the median PFS of the ones who achieved uMRD was also significantly longer than those who did not.

**Conclusion:** Our study has indicated that flow cytometry based MRD surveillance of CLL patients in real-life setting provides prognostic information regarding PFS in accordance with clinical studies. In addition, clinical data of dry antibody panel (DuraClone RE CLB Tube) were presented for the first time.

**Keywords:** Measurable residual disease, chemoimmunotherapy, chronic lymphocytic leukemia, flow cytometry

**ÖZ**

**Amaç:** Kemoimmünoterapi (Kİ) sonrası ölçülebilir kalıntı hastalığın (ÖKH) negatifleşmesi uzun dönem progresyonsuz sağkalım (PS) ve genel sağkalım ile ilişkilidir. Ancak klinik çalışmalar dışında tedavi edilen hastalarda ÖKH negatifliğinin sağkalım üzerine etkisi belirsizdir. Kuru antikor tüp metodu gibi pipet kullanılmayan antikor boyama yöntemleri işlem ilişkili hataları azaltabilir ve daha iyi standardizasyon sağlayabilir. Fakat bu yöntemin kullanıldığı klinik veri bulunmamaktadır. Çalışmamızda kronik lenfositik lösemi (KLL) olgularının tedavisinde kuru antikor tüp metodunun etkililiğinin belirlenmesi amaçlandı.

**Gereç ve Yöntem:** Kİ uygulanan ve tedavinin bitiminden sonraki 6 ayda ÖKH analizi yapılan KLL hastalarının verileri geriye dönük olarak analiz edildi. Çalışmaya 46 hasta dahil edildi. ÖKH, çoğunlukla kuru tüp metodu kullanılarak, hassasiyeti $10^{-4}$ olan akım sitometri ile değerlendirildi.

**Bulgular:** Otuz (%65,2) hastada ÖKH negatifliği sağlandı. ÖKH negatifleşenlerde medyan PS süresi negatifleşmeyenlere kıyasla daha uzundu. Çalışma sürecinde 29 hasta yalnızca kuru tüp metodu ile değerlendirildi. Kuru tüp metodu ile çalışılan hastalar ayrıca değerlendirildiğinde ÖKH negatifliğinin uzamış PS ile ilişkili olduğu görüldü.

**Sonuç:** Çalışmamız KLL hastalarında akım sitometri temelli ÖKH izleminin klinik çalışmalardakine benzer şekilde PS açısından prognostik önemini ortaya koydu. Ayrıca kuru antikor paneli (DuraClone RE CLB Tube) yöntemi klinik pratikte ilk kez kullanıldı.

**Anahtar Kelimeler:** Ölçülebilir kalıntı hastalık, kemoimmünoterapi, kronik lenfositik lösemi, akım sitometri

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## INTRODUCTION

In recent years, the treatment of chronic lymphocytic leukemia (CLL) has radically changed with the introduction of new biological agents[1]. However, chemoimmunotherapy (CI) agents such as fludarabine, cyclophosphamide and rituximab (FCR) and rituximab-bendamustin (RB) are still being used in first line treatment of CLL, especially in patients presenting with mutated-immunoglobuin heavy-chain variable (IGHV) or in those who do not have high-risk cytogenetic abnormalities such as del(11)(q22-23) and del(17)(p13.1)[2].

Measurable residual disease (MRD) in CLL is defined as the persistence of leukemia below the detection limit of standard assays following treatment. Undetectable MRD (uMRD) in CLL is currently defined as the presence of less than 1 CLL cell in 10,000 leukocytes ($<10^{-4}$)[3]. In CLL patients treated with CI, achieving uMRD has been found to be an independent predictor for longer progression-free survival (PFS) and overall survival (OS)[4-6]. Consequently, European Regulatory Agency (EMA) accepted the achievement of MRD negativity as an intermediate endpoint of phase III clinical trials for approval of new agents[7].

After the emergence of the new biological agents such as ibrutinib, which provide long term disease control in CLL patients but, surprisingly with persistent detectable disease[8], it seems that MRD evaluation might not have the same predictive value in patients receiving CI. However, after the inclusion of venetoclax to treatment armamentarium of CLL and novel chemo-free combination strategies started being evaluated in clinical trials, MRD evaluation attracted the attention of scientific community again[9,10].

MRD status can be evaluated via multicolor flow cytometry (FCM), real-time quantitative polymerase chain reaction (RQ-PCR) and high-throughput sequencing (HTS). Even though RQ-PCR and HTS are more sensitive methods, FCM is undoubtedly the most widely applied procedure because of the recent standardization, and the general applicability[11]. In the beginning, residual disease was assessed with 2- or 3- color FCM with low sensitivity, then the definition and applicability of MRD has dramatically changed. European Research Initiative on CLL (ERIC) in collaboration with US and Australian centers has proposed several standard assays regarding MRD evaluation with FCM, which are also recognized by regulatory agencies[11-14].

It has been claimed that the use of pipetting-free antibody staining procedures, such as dry antibody cocktails which reduce the influence of pipetting-originating errors, may result in better standardization of detection[15]. Recently, an eight-color tube with dried reagent, which is specific for detection of MRD in CLL samples, was developed by Beckman Coulter. This new technology provides tubes that contain a dry antibody panel coating adhered to the bottom of the tube[16]. This panel was compared to the method purposed by ERIC group, which uses liquid reagents, and it had been shown that the analysis of MRD in CLL samples was sensitive and feasible with this new method[16]. However, no clinical data about this method have been published until now.

Although the prognostic significance of uMRD has been demonstrated in prospective clinical trials, it is still unknown whether uMRD translates into a real benefit in patients treated off-study. Accordingly, the aim of our study was to investigate whether the end-of-treatment MRD would contribute to the management of CLL patients in real life setting. In addition, we aimed to demonstrate the validity of the dry tube method in clinical patient samples.

## MATERIALS AND METHODS

### Study Design

We retrospectively reviewed data of CLL patients who were treated with CI regimens [RFC, RB and R-Clorambucil (R-CLB)] at our department between June 2013 and January 2021. We included all consecutive CLL patients (aged ≥18 years) who were diagnosed as CLL according to the International Workshop on CLL (iwCLL)[17] and had received at least four cycles of CI as first line therapy. Patients were only included if data for clinical follow-up were available, and if they had complete response (CR), CR incomplete recovery (CRi) or partial response without absolute lymphocytosis according iwCLL criteria at the end of therapy and had MRD analysis performed within 6 months after therapy.

Baseline characteristics of the patients were recorded at the time of starting CI. This included age, gender, Rai stage, the indication of treatment, the interval between diagnosis and the start of CI, complete blood count values, beta-2 microglobulin, albumin, creatinine, lactate dehydrogenase, and genetic features like del(13q14), trisomy 12, del(17p), del(11q). Moreover, the cycle's number of CI, response to treatment, remission duration and other time parameters were recorded.

### MRD Assessment

Fresh peripheral blood samples were used for all MRD analyses with FCM. Until March 2015, four-color two tubes FCM method with antibody panel including anti-CD19, anti-CD20, anti-CD5, anti-CD43, anti-CD81, anti-CD79b, anti-CD3 was used on a FACS Calibur (BD Biosciences, USA). Between March 2015 and February 2017, eight colors FCM, with one tube, antibody panel including anti-CD19, anti-CD20, anti-CD5, anti-CD43, anti-CD81, anti-CD79b, anti-CD3, anti-CD45 was used on a BC Navious FCM (Beckman Coulter, FL, USA). Both methods were in line with CLL ERIC recommendations[13].

After February 2017, we used eight-color DuraClone RE CLB Tube includinganti-CD81, anti-ROR1, anti-CD79b, anti-CD19, anti-CD5, anti-CD43, anti-CD20, and anti-CD45, which was in line with the latest ERIC recommendations[11]. The study procedure was performed according to the instructions of the manufacturer. Briefly, 300 micL of whole blood was added directly to the dried reagent tube and incubated in dark at room temperature for 15 minutes. Then, samples were lysed with VersaLysing Solution (Beckman Coulter) for 20 minutes, centrifuged and washed once with phosphate buffered saline (PBS). They were re-suspended in 500 micL PBS. After that, the samples were acquired in a Navios Flow Cytometer (Beckman Coulter, FL, USA). The setting and compensation matrix for samples were performed using the Compensation Kit provided in the kit according to the manufacturer's instructions. Data analysis was performed on Kaluza Flow Cytometry Analysis Software (Beckman Coulter, USA). Irrespective of the methodology used regarding flow cytometric MRD evaluation through study period, we analyzed at least 500000 cells in every case to reach a sensitivity level below $10^{-4}$ as recommended by ERIC. All participants gave written informed consent for the use of clinical/laboratory data for research purposes. The study was approved by Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (date: 29/09/2020 protocol number: 2020.219.09.06) and conducted in accordance with Helsinki's declaration.

### Statistical Analysis

All statistical analyses were performed using the IBM Statistical Package for the Social Sciences Statistics (version 25.0; IBM Corp., USA). A descriptive analysis of continuous and qualitative variables was performed. PFS was defined as the time from the start of treatment until disease progression. Survival curves were calculated using the Kaplan-Meier method and cross-group comparisons were made using the log rank test. Univariable and multivariable analyses for association between pretreatment characteristics and PFS were performed using the Cox regression analysis. Multivariable analysis for MRD status (binary outcome) was performed using logistic regression analysis. Clinical and biological characteristics between groups were analyzed with the $\chi^2$ test or Kruskal-Wallis test. All p-values were two-sided, and $p<0.05$ was considered statistically significant.

## RESULTS

Forty-six patients were included in the study. The numbers of the patients in the FCR, RB and R-CLB groups were 24, 17 and 5, respectively. The patients' characteristics and outcomes are presented in Table 1. As expected, there was a difference in terms of age between study arms. FCR group included younger patients with normal renal function compared to RB and R-CLB arms. The same trend was observed in serum β-2-microglobulin which might be explained with differences of serum creatinine levels among study cohort. In total, results of cytogenetic assessment were available for only 22 patients (47.8%). None of the patients had del (17p). Since IGHV mutation status and ZAP 70 expression were not studied in most patients, they were not included in the analysis.

Twenty-nine patients were analyzed only by dry tube throughout the study period. Seventeen patients were analyzed previously by means of other methods but after February 2017, the analyses were repeated with dry tube method in all these cases. All the non-dry tube method evaluated positive MRD patients were found to be positive again with dry tube method. At the end of therapy, uMRD was achieved in 30 (65.2%) of the patients. Eighteen patients in the FCR group and 12 patients in the bendamustine/rituximab (BR) group achieved uMRD; however, none of the patients in the R-CLB group achieved uMRD (Table 1). Pretreatment characteristics including age, stage, CD38 expression and β-2 microglobulin were not associated with the end of treatment uMRD in multivariable analysis (data not shown).

When all cases were evaluated together, the estimated median PFS was 56 months (Figure 1A). The median follow-up time was 34.5 months (9-100 months). Only one of 46 patients died within the follow-up period. The median PFS of patients who achieved uMRD was significantly longer (not reached) compared to patients who did not (29 months) (Figure 1B). In the treatment groups, age, β-2 microglobulin, albumin and creatinine were associated with PFS in univariate analysis (Table 2) but only albumin was associated with PFS in multivariate analysis (p: 0.008, hazard ratio: 15.54, confidence interval: 2,030-119.1). Relapse occurred in only 4 of the patients who achieved uMRD, 2 of them were in the RB group, and two in the RFC group. Moreover, 13 patients who were unable to achieve uMRD relapsed.

The patients studied with the dry tube method were also evaluated separately. The median follow-up period in these patients was 25 months. Other characteristics of these patients were given in Table 3. The median PFS of the patients who achieved uMRD was significantly longer (not reached) than those who did not (22 months) (Figure 2).

## DISCUSSION

Our study, which included CLL patients who were treated in real-life setting, has indicated that achievement of uMRD at the end of first line treatment of CLL with CI is significantly associated with longer PFS.

Firstly, the FCR300 and the CLL8 studies indicated the efficacy of FCR as front-line therapy for CLL[18,19]. Aforementioned studies

clearly showed that a subset of patients who had mutated IgHV and did not have high-risk cytogenetic abnormalities, such as del(17p13.1) and del(11q22.3), achieved long-term durable remissions with FCR. Whilst FCR is known to result in longer PFS compared to RB in younger patients, the benefit was not seen in patients over 65 years of age and a lower rate of serious infections were observed in the BR cohort[20]. Therefore, for older CLL patients with standard risk over the

**Table 1. Characteristics and outcome of study cohort**

| Variable* | All patients, n=46 | RFC group, n=24 | RB group, n=17 | R-CLB group, n=5 | p value |
|---|---|---|---|---|---|
| Age (years) | 65 (45-83) | 58.5 (45-68) | 68 (60-83) | 76 (75-77) | <0.0001 |
| Sex, n (%) | | | | | |
| Male | 27 (58.7) | 14 (58.3) | 10 (58.8) | 3 (60) | 0.998 |
| Female | 19 (41.3) | 10 (41.7) | 7 (41.2) | 2 (40) | |
| RAI stage; n, (%) | | | | | |
| 0-2 | 24 (52.2) | 13 (54.2) | 8 (47.1) | 3 (60) | 0.844 |
| 3,4 | 22 (47.8) | 11 (45.8) | 9 (52.9) | 2 (40) | |
| Indication of treatment** n, % | | | | | |
| 1 | 20 (43.5) | 10 (41.7) | 7 (41.2) | 3 (60) | 0.602 |
| 2 | 8 (17.4) | 5 (20.8) | 3 (17.6) | 0 (0) | |
| 3 | 8 (17.4) | 5 (20.8) | 3 (17.6) | 0 (0) | |
| 4 | 2 (4.3) | 0 (0) | 1 (5.9) | 1 (20) | |
| 5 | 8 (17.4) | 4 (16.7) | 3 (17.6) | 1 (20) | |
| Time from diagnosis to treatment, months, median, (range) | 12 (1-156) | 7 (1-48) | 23 (1-81) | 18 (8-156) | 0.108 |
| Cycles number, n (%) | | | | | |
| 4 | 3 (6.5) | 2 (8.3) | 1 (5.9) | 0 (0) | 0.79 |
| 5 | 6 (13) | 4 (16.7) | 2 (11.8) | 0 (0) | |
| 6 | 37 (80.4) | 18 (75) | 14 (82.4) | 5 (100) | |
| Hemoglobin, (gr/dL) | 11.8 (8.2-16.26) | 11.72 (8.3-14.4) | 11.84 (8.2-14.4) | 11.66 (10-16.26) | 0.974 |
| Lymphocyte count, ($10^9$/L) | 50.35 (5.2-138.8) | 47.3 (9.6-138.8) | 59.3 (5.83-126.2) | 43.97 (12.9-61.5) | 0.262 |
| Platelet count, ($10^9$/L) | 149.5 (23.0-505.0) | 180.5 (84.0-505.0) | 139.0 (23.0-261.0) | 120.5 (91.0-257.0) | 0.291 |
| B2M, (mg/L) | 4.4 (2.1-8.0) | 3.15 (2.1-6.4) | 4.7 (2.6-8.0) | 6.6 (3.5-7.1) | 0.002 |
| Creatinine, (mg/dL) | 0.91 (0.5-1.6) | 0.77 (0.5-1.31) | 1.08 (0.63-1.6) | 1.08 (0.55-1.49) | 0.016 |
| Albumin, (gr/dL) | 4.47 (2.30-5.23) | 4.35 (3.0-4.9) | 4.6 (4.0-5.23) | 4.7 (4.4-5.1) | 0.232 |
| LDH (U/L) | 249 (120-568) | 235 (127-475) | 248 (178-568) | 251 (232-407) | 0.423 |
| CD38 positive (>%30), n (%) | 5 (11.4) | 4 (16.6) | 0 (0) | 1 (20) | 0.197 |
| FISH (available: 22), n (%) | | | | | - |
| Del (17p) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Del (11q) | 1 (4.54) | 0 (0) | 1 (8.33) | 0 (0) | |
| Del (13q) | 7 (31.8) | 2 (25) | 5 (41.6) | 0 (0) | |
| Trisomy 12 | 2 (9.0) | 0 (0) | 2 (16.6) | 0 (0) | |
| No aberrations | 11 (50) | 6 (75) | 4 (33.3) | 1 (100) | |
| Responses, n (%) | | | | | |
| CR | 31 (67.4) | 16 (66.7) | 12 (70.6) | 3 (60) | 0.22 |
| CRi | 10 (21.7) | 6 (25) | 4 (23.5) | 0 (0) | |
| PR | 5 (10.9) | 2 (8.3) | 1 (5.9) | 2 (40) | |
| uMRD patients, n (%) | 30 (65.2) | 18 (75) | 12 (70.6) | 0 (0) | 0.005 |
| Relapsed patients, n (%) | 17 (37.0) | 7 (29.2) | 7 (41.2) | 3 (60) | 0.388 |

*Parametric variables are expressed as median (minimum-maximum).

**1: Evidence of progressive bone marrow failure 2: Massive/symptomatic or progressive splenomegaly/lymphadenomegaly 3: progressive lymphocytosis with an increase of >50% over a 2-month period or lymphocyte doubling time of <6 months. 4: Autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids 5: Constitutional symptoms.

B2M: β-2 microglobulin, LDH: Lactate dehydrogenase, FISH: Fluorescence *in situ* hybridization, CR: Complete response, PR: Partial response, CRi: CR with incomplete bone marrow recovery, uMRD: Undetectable measurable residual disease, RFC: Rituximab, fludarabine, cyclophosphamide, RB: Rituximab, bendamustine, R-CLB: Rituximab, clorambucil

age of 65 years, BR is a logical first line treatment option with a good risk-benefit ratio, unless there is a contraindication to bendamustine. In this case, chlorambucil plus rituximab could be used and preferred over BR[20]. The CLL11 trial evaluated the impact of obinituzumab in older patients with co-morbidities[21]. Treatment with obinutuzumab-CLB compared with R-CLB and CLB monotherapy increased response rates and prolonged PFS. Finally, although the role of CI in CLL treatment has narrowed with the introduction of venetoclax and BTK inhibitors into CLL treatment, CI is still used especially in CLL patients with good prognostic features[2]. As we have mainly treated CLL patients with CI until recently, CLL patients who were followed and treated with CI between the years of 2013 and 2020 were included in our study.

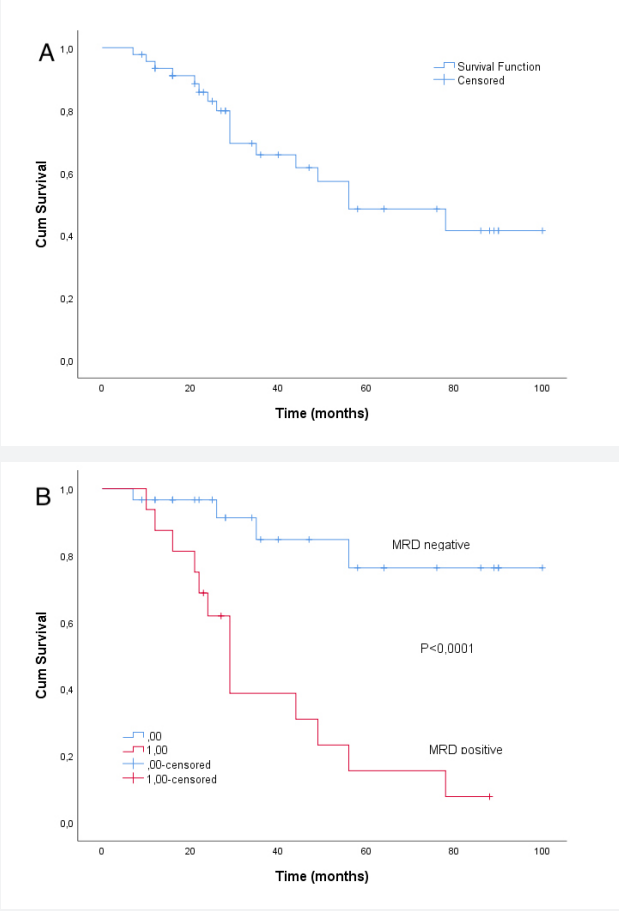


**Figure 1.** Progression free survival of all patients (A) and by MRD status (B)

*MRD: Measurable residual disease*

Most frequently used methods to measure MRD are FCM and PCR. Although HTS and more specific assays are being investigated, FCM remains the gold standard to assess MRD[11]. MRD analysis in CLL with FCM started on the basis of 2-color staining in which the classic markers CD19 and CD5 co-positivity or the unbalanced in kappa/lambda light chain ratio were evaluated, but this method did not go beyond what could be obtained by immunohistochemistry[22-24]. With the initiatives of the ERIC group and other international participants, 4, 6 and finally 8 colored FCM protocols have been developed, that remains at the moment the gold standard in prospective clinical trials[11,13]. In our study, four-color two tubes FCM

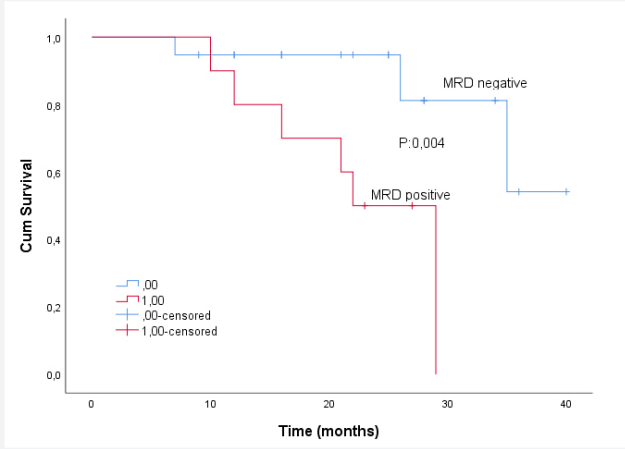


**Figure 2.** Progression free survival of the patients who were evaluated with dry tube method by MRD status

*MRD: Measurable residual disease*

**Table 2. Univariate analysis on variable in relation to progression-free survival**

| Variable | HR (95% CI) | p value |
|---|---|---|
| Age | 1.055 (1.003-1.109) | 0.039 |
| Creatinine | 5.823 (1.068-31.73) | 0.042 |
| Albumin | 5.339 (1.332-21.40) | 0.018 |
| β-2 microglobulin | 1.590 (1.119-2.259) | 0.01 |
| Treatment group | | |
| RFC | 9.52 | 0.009 |
| RB | 4.297 (1.331-13.87) | 0.015 |
| R-CLB | 9.855 (2.072-46.86) | 0.004 |

HR: Hazard ratio, CI: Confidence interval, RFC: Rituximab, fludarabine, cyclophosphamide, RB: rituximab, bendamustine, R-CLB: Rituximab, clorambucil.
Analyses performed using univariate Cox models

method had been used until 2015, eight colors one tube FCM method was used after 2015, both of which were in line with ERIC recommendations.

**Table 3. Characteristics and outcome of the patients who were analyzed by try tube method**

| Variable* | The patients, n=29 |
|---|---|
| Age (years) | 66 (45-77) |
| Sex, n (%) | |
| Male | 17 (58.6) |
| Female | 12 (41.4) |
| RAI stage; n, (%) | |
| 0-2 | 15 (51.7) |
| 3,4 | 14 (48.3) |
| Indication of treatment** n, % | |
| 1 | 12 (41.4) |
| 2 | 4 (13.8) |
| 3 | 6 (20.7) |
| 4 | 2 (6.9) |
| 5 | 5 (17.2) |
| Time from diagnosis to treatment (months) | 13 (1-156) |
| Treatment, n (%) | |
| RFC | 9 (31) |
| RB | 15 (51.7) |
| RCLB | 5 (17.2) |
| Cycles number, n (%) | |
| 5 | 2 (6.9) |
| 6 | 27 (93.1) |
| Hemoglobin, (gr/dL) | 12.15 (8.2-16.26) |
| Lymphocyte count, ($10^9$/L) | 53.20 (5.4-126.2) |
| Platelet count, ($10^9$/L) | 150.0 (23.0-505.0) |
| B2M, (mg/L) | 5.7 (2.33-8.0) |
| Creatinine, (mg/dL) | 1.01 (0.55-1.6) |
| Albumin, (gr/dL) | 4.60 (3.0-5.23) |
| LDH (U/L) | 254 (167-568) |
| CD38 positive (>%30), n (%) | 1 (3.4) |
| Responses, n (%) | |
| CR | 20 (69) |
| CRi | 5 (17.2) |
| PR | 4 (13.8) |
| uMRD patients, n (%) | 19 (65.5) |
| Relapsed patients, n (%) | 11 (37.9) |

*Parametric variables are expressed as median (minimum-maximum)

**1: Evidence of progressive bone marrow failure 2: Massive/symptomatic or progressive splenomegaly/lymphadenomegaly 3: Progressive lymphocytosis with an increase of >50% over a 2-month period or lymphocyte doubling time of <6 months. 4: Autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids 5: Constitutional symptoms.

B2M: β-2 microglobulin, LDH: Lactate dehydrogenase, CR: Complete response, PR: Partial response, CRi: CR with incomplete bone marrow recovery, uMRD: Undetectable measurable residual disease, RFC: Rituximab, fludarabine, cyclophosphamide, RB: Rituximab, bendamustine; R-CLB: rituximab, clorambucil

In recent years, dry tube methods, which eliminate the stages such as pipetting and washing that cause cell loss and make standardization difficult, have been developed for MRD analysis[14]. Recently, Beckman Coulter has developed an eight-color tube with dried reagents which is specific for the detection of MRD in CLL samples by FCM. These tubes contain a dry antibody panel coating adhered to the bottom of tube. This tube contains ROR-α in addition to the core markers suggested by the ERIC group. In our study, this dry tube method was used in MRD analysis after March 2017. MRD was studied again with this method in the follow-up of all cases. The validity of the dry tube method in CLL samples was shown in the comparison with the method suggested by the ECIL group, but not as a clinical datum[15]. In our study, when we analyzed the patients studied with the only dry tube method separately, we observed the results similar to the total study population. Achievement of uMRD at the end of first line treatment was also significantly associated with longer PFS in patients evaluated by try tube method. With our study, clinical data of this dry tube method were presented for the first time.

Although the recently developed 8-color single-tube method reached $10^{-5}$ sensitivity, $10^{-4}$ sensitivity, which is the level proposed also by European Medicines Agency (EMA), has been used in the majority of prospective clinical studies[4-6,25-30]. To reach $10^{-5}$ sensitivity, at least 1 million cells must be studied. In our study, we had withdrawn an average of 500,000 cells. Therefore, in spite of that we used 8-color single-tube method, our sensitivity level was $10^{-4}$.

Several large randomized controlled trials (RCT) in CLL patients have shown that MRD status after induction treatment is an independent predictor of progression-free survival and overall survival[4-6]. In 2016, after the publication of these trials, the EMA allowed the use of uMRD as an intermediate endpoint in RCTs that were used for drug approval[7] but it is still an ongoing debate whether routine MRD testing should also be a part of clinical practice. In the German CLL8 trial, MRD was analyzed in patients receiving FC or FCR treatment, with 35% of FC-treated patients achieving uMRD ($<10^{-4}$) in PB vs 63% after FCR CI. uMRD after the end of treatment was associated with significantly longer PFS than intermediate ($\geq 10^{-4}$ to $<10^{-2}$) or high MRD ($\geq 10^{-2}$)[4]. In the same study, patients who attained low-level MRD by FC chemotherapy had PFS similar to that of patients who achieved the low CLL cell levels with FCR. Therefore, authors concluded that achievement of uMRD, not the type of treatment, was the key factor for durable remissions. Similarly, in our study, low relapse rates were observed in patients who achieved uMRD irrespective of treatment regimen (RB or RFC).

In our study, uMRD was achieved in 18 (75%) of 24 patients who received FCR. The absence of patients with 17pdel and only 1

patient with 11q deletion among our patients may explain the higher rate of uMRD obtained in our study. In GCLLSG CLL10 trial, the superiority of FCR in achieving uMRD was shown compared to RB CI[19]. In our study, 12 of 17 patients (70.6%) treated with BR had uMRD. In our retrospective study, patients who received RB were unsuitable for FCR because of their age and comorbidities. Therefore, it was not appropriate to compare the two groups, although the high uMRD percentage with RB was notable in our study. None of the patients who received R-CLB had uMRD and their PFS was inferior as expected, which supports the notion that this regime is not strong enough.

In the study, the relationship between genetic changes and obtaining uMRD could not be evaluated due to the small number of patients included. IGHV mutation and ZAP 70 expression were not studied in most of the patients. CD38, which is other important prognostic factor in the studies, was not found to be related to uMRD in our study. In addition, there was no correlation with other baseline disease characteristic of the patients and obtaining uMRD.

### Study Limitations

The major limitations of the study are its retrospective single-center design and limited number of patients. We were also unable to evaluate important prognostic parameters in most of our study cohort like IGHV mutation status and ZAP 70 expression.

## CONCLUSION

In conclusion, MRD analysis was used in the follow-up of CLL patients outside of a clinical trial in our study and it was revealed that it could provide information about PFS in accordance with clinical studies. In addition, clinical data of dry antibody method (DuraClone RE CLB Tube) were presented for the first time.

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### Ethics

**Ethics Committee Approval:** Approval was obtained from Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (date: 29/09/2020 protocol number: 2020.219.09.06).

**Informed Consent:** All participants gave written informed consent for the use of clinical/laboratory data for research purposes.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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

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

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# Evaluation of Culture Results in Pediatric Clinics of the Training and Research Hospital

## Bir Eğitim ve Araştırma Hastanesi Çocuk Kliniklerinde Alınan Kültür Sonuçlarının Değerlendirilmesi

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**ABSTRACT**

**Aim:** Infections due to drug resistant microorganisms are increasing and it is important to evaluate culture specimens for early recognition, fast and effective therapy, epidemiological and prognosis of the infections. The aim of the study is to evaluate the results of blood and urine cultures taken from patients hospitalized in pediatric services outside the neonatal intensive care unit.

**Materials and Methods:** In this study, 2277 blood cultures and 857 urine cultures taken between the years of 2007 and 2008 were evaluated retrospectively. 6.8% (n=156) positive blood cultures and 6% (n=52) positive urine cultures were included in the study. Blood cultures were put onto Bact-Alert 3D automotized blood culture systems and urine cultures were put onto MacConkey and 5% sheep blood agar plates. Microorganisms were identified with routine bacteriological procedures and antibiotic sensitivity tests were performed.

**Results:** In pediatrics clinics, 79.4% gram positive microorganisms (69% *coagulase-negative staphylococcus CNS*) among-positive blood cultures 86.5% gram negative microorganisms (61.5% *Escherichia coli)* among positive urine cultures were produced. Microorganisms grew in blood cultures were as follows: 48% (n=75) *Methicillin-resistant CNS*, 21% (n=33) *Methicillin-sensitive CNS*, 7.5% (n=12) *Klebsiella* species (4.5% ESBL positive), 2.6% (n=4) *Streptococcus pneumoniae*, 2.6% (n=4) *Methicillin-sensitive staphylococci* 2% (n=3) *Acinetobacter*, 2.6% (n=4) *Candida tropicalis*, 2% (n=3) *Escherichia coli*, 2% (n=3) *Enterobacter*, 1.3% (n=2) *Haemophilus influenzae*, 0.6% (n=1) *Brucella* spp. Microorganisms detected in urine cultures are as follows: 61.5% (n=32) *Escherichia coli* (40% ESBL positive), 11.5% (n=6) *Enterococcus* spp., 5.8% (n=3) *Proteus* spp., 3.8% (n=2) *Pseudomonas* spp., 3.8% (n=2) *Stenotrophomonas maltophilia*, 2% (n=1) *Methicillin-sensitive staphylococci*, 2% (n=1) *Acinetobacter baumannii*, 2% (n=1) *Klebsiella* spp.

**Conclusion:** Identifying the infectious agents and their antibiotic susceptibility and resistance rates is important for adequate and effective initial empiric antimicrobial therapy and treatment of infections.

**Keywords:** Children, bacteremia, urinary tract infection

**ÖZ**

**Amaç:** Çoklu ilaç direnci olan mikroorganizmalar ile enfeksiyon sıklığı giderek artmakta olup; enfeksiyonların erken tanınması, etkili tedavisi ve prognoz açısından kültür örneklerinin değerlendirilmesi önemlidir. Bu çalışmanın amacı, yenidoğan yoğun bakım ünitesi dışındaki çocuk servislerinde yatan hastalardan alınan kan ve idrar kültürü sonuçlarının değerlendirilmesidir.

**Gereç ve Yöntem:** Bu çalışmada 2007-2008 yılları arasında bakılan 2277 kan kültürü ve 857 idrar kültürü retrospektif olarak değerlendirilmiştir. Kan kültüründe %6,8 (n=156), idrar kültüründe ise %6 (n=52) üreme anlamlı görülerek çalışmaya dahil edilmiştir. Alınan kan kültürleri Bact-Alert 3D otomatize kan kültür sistemlerinde, idrar örnekleri MacConkey ve %5 koyun kanlı agar besiyerlerine ekimi yapılarak üreyen mikroorganizmalar tanımlanmış, antibiyotik duyarlılık testleri yapılmıştır.

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**Bulgular:** Çocuk kliniklerinde bakteriyemi saptanan hastalarda %79,4 gram pozitif (%69'u *Koagülaz-negatif stafilokok, KNS*), idrar kültüründe ise %86,5 gram negatif (%61,5 *Escherichia coli,* %11,5 *Enterokoklar)* mikroorganizma üremiştir. Kan kültürlerinde üreyen mikroorganizmalar: %48 (n=75) *Metisilin-dirençli KNS,* %21(n=33) *Metisilin-hassas KNS*, %7,5 (n=12) *Klebsiella pneumoniae* (%4,5 *genişlemiş spektrumlu beta-laktamaz,* GSBL pozitif), %4,5 (n=7) *Pseudomonas* suşları, %3,2 (n=5) *Enterococcus* spp., %2,6 (n=4) *Streptococcus pneumoniae*, %2,6 (n=4) *Metisilin-hassas Staphylococcus aureus*, %2 (n=3) *Acinetobacter,* %2,6 (n=4) *Candida tropicalis,* %2 (n=3) *Escherichia coli,* %2 (n=3) *Enterobacter,* %1,3 (n=2) *Hemofilus influenza,* %0,6 (n=1) *Brucella* spp. idi. İdrar kültürlerinde saptanan mikroorganizmalar; %61,5 (n=32) *E. coli*, (%40 *genişlemiş spektrumlu beta-laktamaz,* GSBL pozitif), %11,5 (n=6) *Enterococcus* spp., %5,8 (n=3) *Proteus* spp., %3,8 (n=2) *Pseudomonas* spp., %3,8 (n=2) *Stenotrophomonas maltophilia*, %2 (n=1) *Metisilin-hassas Staphylococcus aureus,* %2 (n=1) *Acinetobacter baumannii,* %2 (n=1) *Klebsiella* spp. idi.

**Sonuç:** Enfeksiyon etkeni mikroorganizmaların ve antibiyotik duyarlılıkları ile direnç oranlarının belirlenmesi uygun ve etkili ampirik antimikrobiyal tedavi başlanmasında ve enfeksiyonların tedavisinde önemlidir.

**Anahtar Kelimeler:** Çocuk, bakteriyemi, idrar yolu enfeksiyonu

## INTRODUCTION

The epidemiology of infectious agents detected in the bloodstream changes in parallel with the development of antibiotic resistance[1]. Today, the frequency of infection is increasing due to multi-drug resistant microorganisms. Bacteremia continues to be a common cause of febrile diseases in the world and adversely affects morbidity and increases mortality, especially in hospitalized patients[1,2]. Mortality is related to the severity of the infection, the presence of concomitant disease, age, and inappropriate use of antibiotics[3,4]. Although gram-positive microorganisms are frequently reported as the causative agent of bacteremia in childhood, recent studies have shown that the frequency of gram-negative microorganisms has increased[5-7]. Infections should be recognized, treated quickly and effectively, and culture samples should be taken in terms of prognosis[8]. It is necessary to reduce the risk of contamination with skin bacteria by paying attention to sterility while taking culture samples, to interpret the results correctly and to pay attention to the correlation with the clinic. On the other hand, false-positive blood culture results cause errors in the interpretation of circulatory system infections, and may lead to inappropriate antibiotic use, additional laboratory tests, prolonged hospitalization, and increased costs[9].

Antibiotic-resistant microorganisms, on the other hand, limit the availability of effective treatment options, cause difficulties in the treatment of some frequently encountered bacterial infections, including urinary tract infection (UTI), and may lead to increase in mortality, morbidity, and health costs[10-12]. In the treatment of urinary tract infection, it is important to apply appropriate and adequate empirical treatment in order to prevent complications such as scar formation in the urinary system.

## MATERIALS AND METHODS

In this study, blood and urine cultures taken from patients in pediatric services outside the neonatal intensive care clinic between 2007 and 2008 were retrospectively analyzed. The growth in the blood culture samples taken from the patients was evaluated as bacteremia in the presence of clinical findings. If the microorganism grown in a single blood culture was not clinically compatible or there were no predisposing factors such as immunodeficiency, or if there was growth in only one of the many blood culture samples taken, this was considered as contamination. If coagulase-negative staphylococci or viridans streptococci grew, it was accepted as significant bacteremia in the presence of at least two positive results in culture[13].

In the study, 2277 blood cultures and 857 urine cultures were evaluated. Growth was detected in 10.1% (n=231) of the blood culture samples; 1% (n=24) blood cultures were not included in the study because patient information could not be reached or the same agent was produced twice. Of the 9% (n=207) blood cultures with growth, 6.8% (n=156) were considered significant and included in the study; 2.2% (n=51) were considered as contamination. Of the growths that were accepted as contamination, 84% (n=43) did not receive treatment. In 857 urine cultures taken, 6% (n=52) of the growths were considered significant and included in the study; 1.7% (n=15) of them were considered as contamination. In the study, 156 blood cultures and 52 urine cultures were analyzed.

## BLOOD CULTURE METHOD

Blood cultures were evaluated in Bact-Alert 3D automated blood culture systems; hemoculture samples were incubated for five days; aerobe cultures of samples showing positive signals were made on blood agar, EMB agar and sabouraud dextrose agar at 37 ºC.

Urine culture was taken with the help of a urinary catheter or urinary bladder in infants, with midstream urine after standard cleaning in older age groups and with the help of a catheter from patients who were not compatible; urine samples sent to the laboratory were inoculated on MacConkey and 5% sheep blood agar media and incubated at 35-37 ºC for 18-24 hours. Microorganisms that grew at the end of the incubation

period were identified by routine bacteriological methods, and antibiotic susceptibility tests were performed to investigate the resistance against commonly used antibiotics.

### Statistical Analysis

Statistical analyses were made with the NCSS 2007 package program in the study, descriptive statistical methods were used in the evaluation of the data as well as the Fisher reality test in the comparison of qualitative data. The value of $p<0.05$ was considered significant.

## RESULTS

Of the patients, 56% (n=117) were male and 44% (n=91) were female. The mean age was 3.4±3 years in patients from whom blood cultures were taken and 3.92±3.87 years in patients from whom urine cultures were taken.

Diagnostic distribution of patients with bacteremia in blood culture is summarized in Table 1; mean leukocyte count in laboratory parameters was 11971±10243/uL; leukocyte with polymorphic nuclei was 6093±5522/uL; lymphocyte count was 3080±3008/uL, and C-reactive protein (CRP) was 51±87 mg/L.

Mean leukocyte count in laboratory tests of patients with significant growth in urine culture was 12,908±7993/uL; leukocyte with polymorphic nuclei was 6827±5991/uL; lymphocyte count was 4698±4100/uL, and CRP was 65±78 mg/L.

In blood cultures in which bacteremia was detected in pediatric clinics, 79.4% gram-positive and 20.6% gram negative microorganisms were found; in urine cultures, 86.5% gram negative and 13.5% gram positive microorganisms were grown.

Significant bacteremia causative microorganisms in blood cultures and significant infectious causative microorganisms grown in urine cultures are shown in Table 2.

There was no difference in the distribution of microorganisms grown in blood culture according to pediatric services ($p>0.05$). Considering the antibiotic resistance of microorganisms causing bacteremia in blood culture; vancomycin resistance was not detected in MSCNS, MSSA, and *Streptococcus pneumoniae* (*St. pneumoniae*) strains. In *Acinetobacter baumannii* strains, 100% resistance to cefuroxime and ceftriaxone was found. In MRCoNS strains, 84% erythromycin, 60% clindamycin, 40% ciprofloxacin, 49.3% fusidic acid resistance; 97.3% linezolid and 100% vancomycin sensitivity were detected. In MSCNS strains, 42% penicillin, 48.5% erythromycin, 21% clindamycin, and 33.3% trimethoprim-sulfamethoxazole (TMP-SMX) resistance were found; 63.6% amoxicillin clavulanic acid, 90% ciprofloxacin, and 97% gentamicin were sensitive. It was found that *St. pneumoniae* strains had 25% penicillin and erythromycin resistance and 75% trimethoprim-sulfamethoxazole resistance; vancomycin, teicoplanin and ciprofloxacillin were found to be 100%

**Table 1. Diagnostic distribution of patients with bacteremia in blood culture**

| Diagnosis distribution | |
|---|---|
| Sepsis | 47.4% (n=74) |
| Pneumonia | 30% (n=47) |
| Febrile neutropenia | 19.2% (n=30) |
| Deep neck infection | 1.9% (n=3) |
| Brucella infection | 0.6% (n=1) |
| Encephalitis | 0.6% (n=1) |

**Table 2. The causative microorganisms in blood culture and their distribution by frequency**

| Microorganism | Blood culture | Causative microorganism | Urine culture |
|---|---|---|---|
| *Methicillin-resistant CNS* | n=75 (48%) | *Escherichia coli* | n=32 (61.5%) (40% ESBL positive) |
| *Methicillin sensitive CNS* | n=33 (21%) | *Enterococcus* spp. | n=6 (11.5%) |
| *Klebsiella pneumoniae* | n=12 (7.5%) | *Klebsiella* spp. | n=5 (9.6%) (80% ESBL positive) |
| *Pseudomonas* spp. | n=7 (4.5%) | *Proteus* spp. | n=3 (5.8%) |
| *Enterococcus* spp. | n=5 (3.2%) | *Pseudomonas* spp. | n=2 (3.8%) |
| *Streptococcus pneumoniae* | n=4 (2.6%) | *St. maltophilia* | n=2 (3.8%) |
| *Methicillin-sensitive coagulase-negative staphylococcus (MSSA)* | n=4 (2.6%) | *Methicillin-sensitive coagulase-negative staphylococcus (MSSA)* | n=1 (2%) |
| *Acinetobacter* spp. | n=4 (2.6%) | *Acinetobacter baumannii* | n=1 (2%) |
| *Candida tropicalis* | n=3 (2%) | | |
| *Escherichia coli* | n=3 (2%) | | |
| *Enterobacter* | n=3 (2%) | | |
| *Hemophilus influenza* | n=2 (1.3%) | | |
| *Brucella* spp. | n=1 (0.6%) | | |

CNS: Coagulase negative staphylococcus, MSSA: *Methicillin-sensitive Staphylococcus aureus, St. maltophilia: Stenotrophomonas maltophilia*, ESBL: Extended spectrum beta-lactamase

sensitive. In *methicillin-susceptible Staphylococcus aureus* (MSSA) strains, 50% erythromycin, penicillin and amoxicillin clovulanic acid resistance and 25% penicillin resistance were detected (Figure 1).

In *Haemophilus influenzae* (*H. influenzae*) strains, 50% erythromycin, ceftriaxone and trimethoprim-sulfamethoxazole resistance were detected; ampicillin and amoxicillin-clovulanic acid were 100% sensitive. Klebsiella strains were 100% susceptible to ciprofloxacin, meropenem and imipenem, and 40% resistant to ceftriaxone, 20% to tobramycin, and 20% to piperacillin-tazobactam.

Broad-spectrum beta-lactamase positive Klebsiella strains (4.5%) were 88% susceptible to meropenem and imipenem. In these strains, 62.5% ceftazidime, 12.5% ciprofloxacin, and 50% piperacillin-tazobactam resistance were detected. *E. coli* strains were 100% susceptible to amikacin, meropenem and imipenem, and were found to be resistant to cefuroxime and ciprofloxacin at a rate of 33% and to cefotaxime at a rate of 16%.

*Pseudomonas* spp strains were 87.5% susceptible to piperacillin-tazobactam, amikacin and cefepime, and 62.5% to ceftazidime; it was found 25% resistant to ceftazidime, and 25% to gentamicin and tobramycin, 12.5% to piperacillin-tazobactam, meropenem and imipenem (Figure 2).

In *Enterococcus* strains, 60% erythromycin and teicoplanin resistance, 40% gentamicin, ciprofloxacin and vancomycin resistance were detected; linezolid was found to be 100% susceptible, and gentamicin and vancomycin was 60% susceptible.

Of the patients with growth in blood culture, 69.2% had a history of other concomitant disease; 40% (n=45) of these diseases were hemato-oncological diseases. It was observed that 88.5% (n=138) of the patients with growth in blood culture received appropriate treatment according to the culture results and treatment response.

In the study, microorganisms produced in patients with mortality (5.7%, n=9) who received bacteremia treatment were 20% (n=2) *MRCNS*, 20% (n=2) *E. coli*, 20% (n=2) *Klebsiella pneumoniae*, 10% (n=1) *Candida tropicalis*, 10% *MRCNS* and *Candida tropicalis*, 10% (n=1) *Acinetobacter baumannii*, and these patients had hematologic-oncological disease.

The distribution of microorganisms accepted as 2.2% (n=51) contamination in blood culture is as 28% *MSCNS*, 17% *Alpha hemolytic streptococci*, 15% *diphtheroid bacillus*, 13% *Micrococcus* spp., 7.6% *MRCNS*, 5.7% *Bacillus* spp., 3.8% *non-hemolytic streptococci*, 2% *Cytrobacter*, 2% *Enterobacter*, 2% *E. coli* and 2% *MSSA*. Of these microorganisms, 24% (n=12) were grown under antibiotic treatment, and 76% (n=40) were grown in cultures taken before antibiotic treatment.

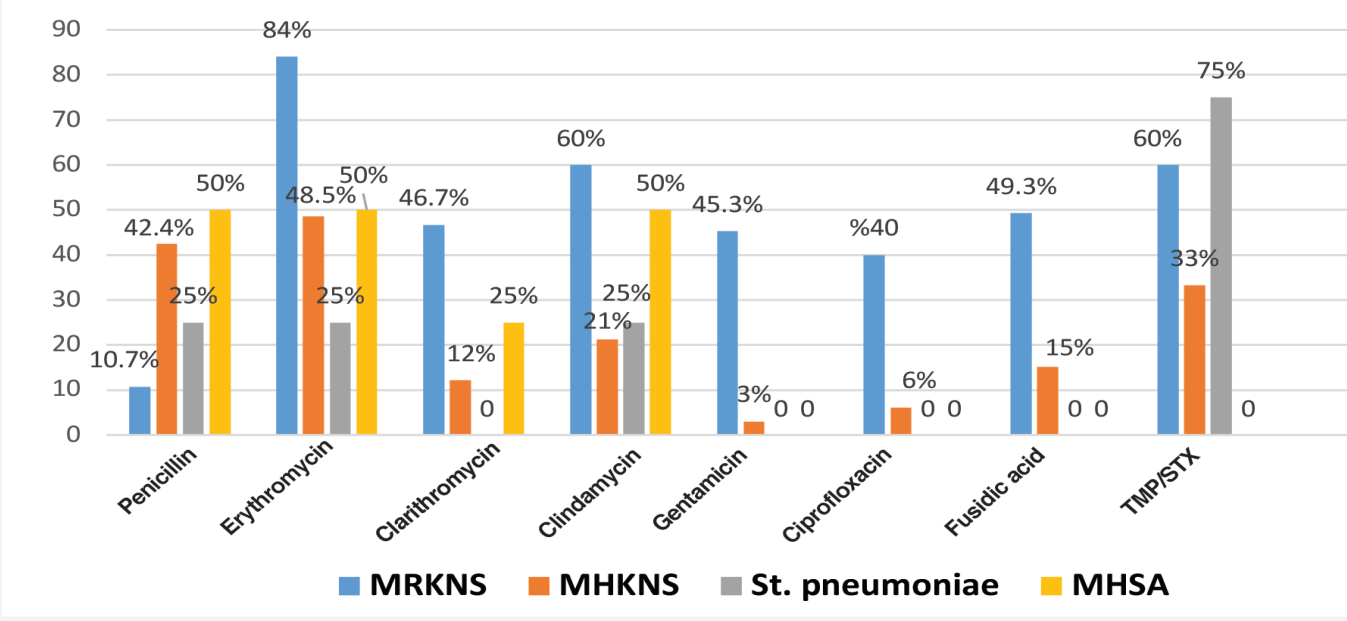


**Figure 1.** Antibiotic resistance of gram-positive microorganisms (coagulase negative staphylococcus, *Staphylococcus aureus* and *Streptococcus pneuomoniae*) grown in blood culture

*MHKNS: Methicillin-sensitive coagulase-negative staphylococci, MRKNS: Methicillin-resistant coagulase-negative staphylococci, St. pneumoniae: Streptococcus pneumoniae, MHSA: Methicillin sensitive Staphylococcus aureus*

Most specifically, trimethoprim-sulfamethoxazole (37%) resistance was found in *E. coli* strains in microorganisms grown in urine culture. In *Klebsiella* strains, resistance to cephalosporin derivatives and ciprofloxacin was seen with a high probability. ESBL positivity was detected in 80% of *Klebsiella* strains and 40% of *E. coli* strains. Ceftazidime, amikacin, and ciprofloxacin resistance were not observed in *Pseudomonas* strains (Figure 3).

No resistance to piperacillin-tazobactam, meropenem, and imipenem was detected in the microorganisms grown in urine culture. Of *proteus* strains, 33.3% were resistant to gentamicin, netilmicin and amikacin; were 100% susceptible to

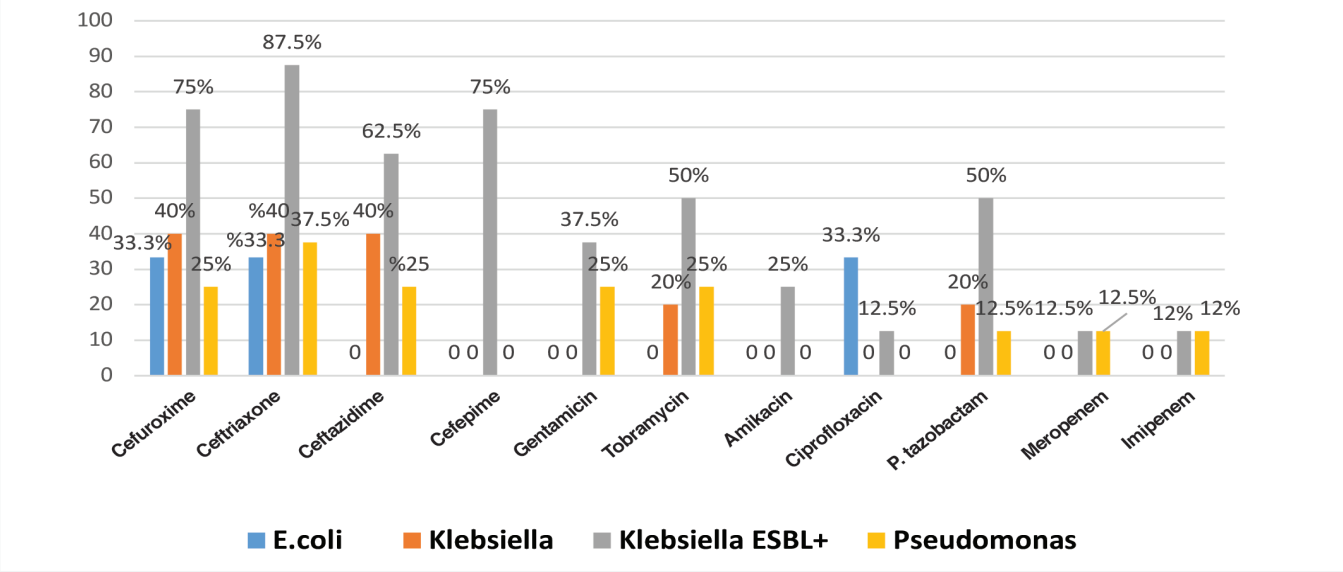


**Figure 2.** Antibiotic resistance of gram-negative microorganisms (*E. coli*, *Klebsiella* spp., *Pseudomonas* spp.) grown in blood culture

*E. coli: Escherchia coli, K. pneumoniae: Klebsiella pneumoniae, P. tazobactam: Piperacillin-tazobactam, ESBL: Extended spectrum betalactamases*

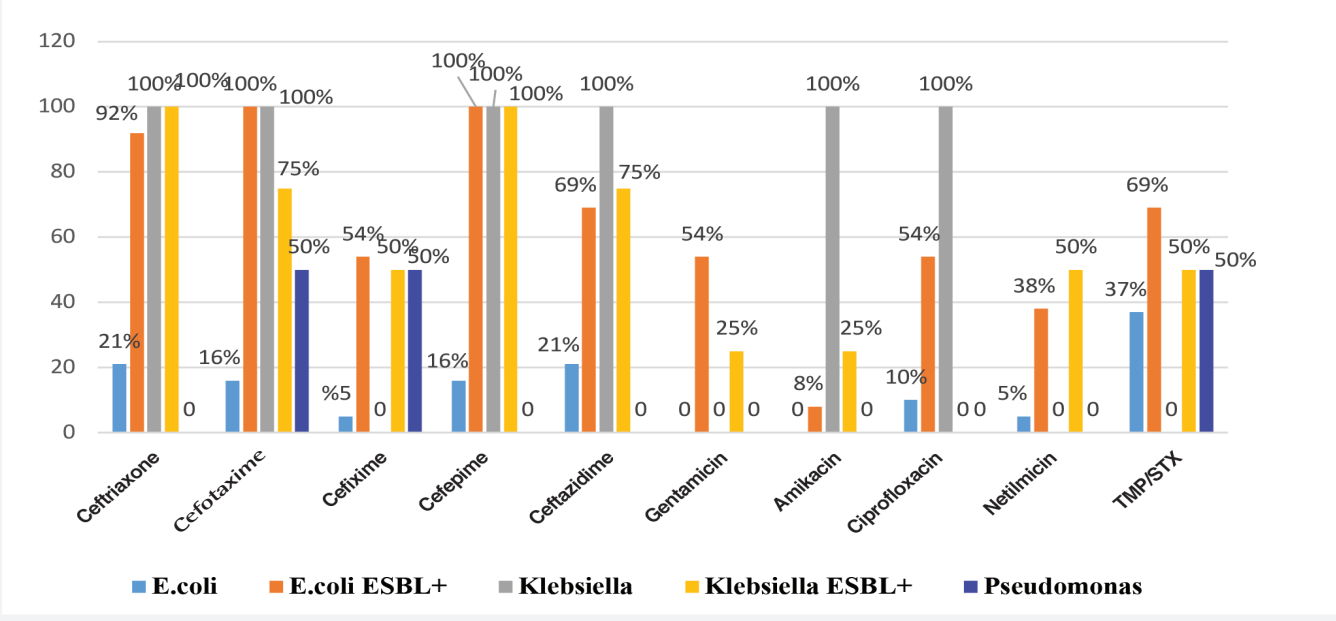


**Figure 3.** Antibiotic resistance of microorganisms grown in urine culture

*E. coli: Escherichia coli, ESBL: Extended spectrum betalactamases, TMP-SMX: Trimetoprim-sulfametaksazol*

ciprofloxacin, cefotaxime, ceftazidime, meropenem, imipenem, and were 66.7% susceptible to gentamicin, tobramycin, trimethoprim sulfamethoxazole. For *Acinetobacter baumanni*, 100% resistance was seen with piperacillin tazobactam, ceftazidime and meropenem; it was found to be 100% sensitive to gentamicin, tobramycin and netilmicin. In *Stenotrophomonas maltophilia* strain, 50% resistance to cefotaxime, cefepime and meropenem was observed. It was determined that *Enterococcus* strains had 83.3% ampicillin and penicillin resistance, and 16.7% vancomycin and teicoplanin resistance. The *MSSA* strain grown in the urine culture was penicillin resistant; it was found to be sensitive to Gentamicin, ciprofloxacin, vancomycin, trimethoprim-sulfamethoxazole, cefazolin, amikacin and netilmicin.

Reproduction was considered as contamination in 1.7% (n=15) of the patients whose urine cultures were taken. *E. coli* (40%, n=6) was found to be the most common among the microorganisms evaluated as contamination.

In the study, urinary system ultrasonography of the patients with growth in urine culture was found to be normal in 67.3%; 23.1% of the cases had hydronephrosis, 3.8% had mild ectasia, 1.9% had grade 1 parenchymal disease, 1.9% had renal calculus, and 1.9% had grade 3 ectasia.

It was observed that 90.4% (n=47) of the patients with significant growth in the urine culture received appropriate treatment according to the culture results and treatment response.

## DISCUSSION

In recent studies, culture positivity has been reported as 8.9-11% (4.7-27.3%) in blood culture samples taken, and in this study, blood culture positivity was found to be 10.1% (n=231), which is compatible with the literature[14]. In blood cultures, 79.4% of gram-positive microorganisms were found to be bacteremia causative, which is compatible with the literature[5,6].

In the study, the most common microorganism in blood culture was *CNS* at a rate of 69.2%, and 69.4% of them were methicillin resistant, 30.6% were methicillin susceptible. Methicillin resistance of *CNS*s was found as 80.4% by Edmond et al.[15] as 78.5% by Johnson et al.[16] and as 76.3% by Nahaei et al.[17] in a study conducted by various clinical samples from different centers.

In the study, 84% erythromycin, 60% clindamycin, 40% ciprofloxacin resistance was detected in *MRCNS* strains produced in blood culture; linezolid was found 97.3% susceptible and vancomycin 100% susceptible. In the studies of Hope et al.[18] including pediatric and adult age groups, methicillin resistance of *CNS* strains was found to be 67%; 80.2% erythromycin, 67% ciprofloxacin, and 25.5% clindamycin resistance was reported in *MRCNS* strains. As in the study of Buckingham et al.[19], no vancomycin resistance was found in *CNS*s.

*Streptococcus pneumoniae* is one of the major agents of pneumonia, otitis media, and bacteremia infections in children and it is reported that multi-antibiotic resistance is increasing[20,21]. In this study, *St. pneumoniae* strains were found to have 75% trimethoprim sulfamethoxazole resistance, 25% penicillin and erythromycin resistance; no resistance to clarithromycin, vancomycin and ciprofloxacillin was observed. In the study of Gür et al.[22], moderate penicillin resistance was found to be 30%. In a study conducted in Spain, it was shown that penicillin resistance of invasive pneumococcal strains was 33%, erythromycin resistance was 25.7%, and cefotaxime resistance was 8.4%.[23] In a study conducted in Switzerland, penicillin resistance was found to be 7%.[24] Unlike this study, in the study of Opintan and Newman[14] trimethoprim-sulfamethoxazole resistance was not observed in pneumococci, and high resistance was found with ciprofloxacin (50%) and erythromycin (66.7%).

In recent years, it has been reported that the frequency of bacteremia with gram-negative microorganisms increases, and ESBL-producing *Enterobacteriaceae* and resistant *Pseudomonas* and *Acinetobacter* isolates pose a significant problem in treatment[1,10]. In blood cultures of Nivesvivat et al.[25], ESBL production that can hydrolyze penicillins, most cephalosporins and monobactam antibiotics was reported as 28.9% in *E. coli* and as 25.8% in *K. pneumoniae*[25]. In this study, ESBL positive *Klebsiella* strains were seen at a low rate (4.4%) in blood culture; however, ESBL positivity was detected in 58% of *Klebsiella* strains. ESBL positivity was not detected in *E. coli* strains. Opintan and Newman[14] reported in their study that *E. coli* strains had 87.5% cefuroxime resistance, 88.9% cefotaxime resistance, 60% ciprofloxacin resistance and 12.5% meropenem resistance; in this study, however, meropenem resistance was not detected, cefotaxime (16%) and cefuroxime (33%) resistance was lower; similarly, amikacin resistance was not observed.

In the study, 7.6% (n=12) *Klebsiella* spp. were detected and *Klebsiella* strains not producing ESBL were resistant to ceftriaxone at a rate of 40% and to piperacillin-tazobactam at a rate of 20%; ESBL producing strains were found to be 87.5% resistant to ceftriaxone, 75% to cefepime, 62.5% to ceftazidime, 12.5% to ciprofloxacin, and 50% to piperacillin-tazobactam.

The increase in antibiotic resistance of *Enterococcus* species and the occurrence of infections with multiple resistant strains cause difficulties in treatment. In particular, the increase in vancomycin resistance draws attention[26]. In a multicenter study, glycopeptide resistance of *Enterococcus* species was found as 9.7%, ciprofloxacin resistance as 27.4%, and

gentamicin resistance as 28.2%[27]. In this study, teicoplanin resistance (60%), gentamicin, ciprofloxacin and vancomycin resistance (40%) of *Enterococcus* species were found to be high in blood culture.

*Pseudomonas* spp. is an important bacterium that needs attention due to its multiple antibiotic resistance and can cause severe clinical pictures. *Pseudomonas aeruginosa*, which is the cause of hospital-acquired bacteremias caused by gram-negative microorganisms with a frequency of 20%, is reported to be resistant to most penicillins and cephalosporins.[6,28] Although antipseudomonal penicillins, cefoperazone, ceftazidime, cefepime, quinolones and carbapenems are effective against pseudomanas; in a multicenter study, 50% ciprofloxacin and piperacillin resistance, 30% ceftazidime resistance, and 26% amikacin resistance were found[29]. In this study, 25% ceftazidime resistance, 12.5% piperacillin-tazobactam and meropenem resistance were determined in pseudomonas species grown in blood cultures.

The causative agents of urinary tract infections are gram-negative bacteria and *E. coli* is the most frequently isolated among them (61.5%), as also found in this study. According to the results of studies obtained from various regions in our country, the frequency of *E. coli* isolation in children varies between 43-66.6%[30,31]. Bean et al.[32] found 55% ampicillin resistance and 40% trimethoprim-sulfamethoxazole resistance in *E. coli* strains. Resistance to third generation cephalosporins has been reported in the treatment of urinary tract infections. Yüksel et al.[33] found 7.5% ceftriaxone resistance in *E. coli* strains; Pape et al.[34] found 53% ampicillin resistance, 42% trimethoprim-sulfamethoxazole resistance, 12% amikacin resistance, and 6% cefuroxime resistance. Grude et al.[35] found 28% ampicillin, 12% trimethoprim-sulfamethoxazole, and 12% cefuroxime resistance. In this study, 37% trimethoprim-sulfamethoxazole, 21% ceftriaxone, and 10% ciprofloxacin resistance were found in *E. coli* strains in urine culture; no resistance was found to gentamicin and amikacin. In ESBL positive *E. coli* strains, 69% trimethoprim-sulfamethoxazole resistance, 54% gentamicin and ciprofloxacin resistance, and 38% netilmicin resistance were found.

Today, it has been reported that gram-negative strains expressing ESBL are becoming common in UTI in many countries[36,37]. In a meta-analysis conducted for urinary tract infections, the rate of ESBL positive *Enterobacteriaceae* in urine was found to be 15%; in this study, ESBL-producing *Enterobacteriaceae* in urine was found as high as 32.6%[38].

In the study, ESBL was found positive in 40% of *E. coli* strains. Of ESBL positive *E. coli* strains, 69.2% were resistant to trimethoprim-sulfamethoxazole, 53.8% to gentamicin and 7.7% to amikacin. On the other hand, 36.8% of ESBL negative strains were found to be resistant to trimethoprim-sulfamethoxazole.

Contamination rates in blood culture are reported as 0.6-3%; in this study, the frequency was 2.2% (n=51), which was consistent with the literature, and the most frequent contamination was MSCNS (28%)[39,40].

### Study Limitations

The limitations of this study are that it was conducted retrospectively and it was a single-center study.

## CONCLUSION

Since antibiotic-resistant microorganisms are an important problem in the diagnosis and treatment of infectious diseases, knowing the antibiotic resistance and susceptibility rates against microorganisms is necessary for the regulation of appropriate empirical treatment. Evaluating the results of the culture samples taken and reviewing the appropriateness of the empirical treatment according to the culture antibiogram is important for the success of the treatment.

In this study, the importance of initiating appropriate and adequate empirical treatment and preventing the development of antibiotic resistance was emphasized by investigating the microorganisms grown in blood and urine cultures and their antibiotic susceptibility.

### Ethics

**Ethics Committee Approval:** Retrospective study.

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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# Anesthesia Evaluation of Non-COVID-19 Oncological - Non-Oncological Operations in Our Operating Room in the First Year of the COVID-19 Pandemic: A Retrospective Study

## COVID-19 Pandemisinin İlk Yılında Ameliyathanemizin Non-COVID-19 Onkolojik - Onkolojik Tanı Alabilecek Ameliyatlarının Anestezi Değerlendirilmesi: Retrospektif Çalışma

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**ABSTRACT**

**Aim:** The Coronavirus disease-2019 (COVID-19) pandemic has caused serious changes in health services in our country as well as all over the world. The most affected group includes those who will undergo surgery with an oncological diagnosis or who can be diagnosed with an oncological diagnosis. It is aimed to observe the changes in the operations with oncological diagnoses, which will require pathological examination in the operating room of our tertiary university hospital, in terms of demographics, surgical branch, duration of the operation and type of anesthesia in the first year of the pandemic.

**Materials and Methods:** The retrospective scanning method was used to scan the surgical patient files of the patients who met the criteria (Retrospective cross-sectional study).

**Results:** While the first 3 branches of our operating room with the highest number of cases did not change, there was a difference in the American Society of Anesthesiology (ASA) II-III scoring ($p<0.05$), neuraxial and trunk blocks ($p<0.05$) in which general anesthesia was applied alone as an anesthesia type or it was added. In addition, a change was observed in 13.3% of cases with oncological diagnosis and 32% in elective surgeries requiring pathological examination.

**Conclusion:** In the COVID-19 pandemic, more expected cancellation rates did not occur by adapting to the "new normal", but there were differences in ASA scores and anesthesia type during this period.

**Keywords:** COVID-19, oncological surgery, ASA score, type of anesthesia

**ÖZ**

**Amaç:** Koronavirüs hastalığı-2019 (COVID-19) pandemisi tüm dünyada olduğu gibi ülkemizde de sağlık hizmetlerinde ciddi değişimlere neden olmuştur. En çok etkilenen grup, onkolojik bir tanı ile ameliyat olacak veya onkolojik bir tanı ile teşhis edilebilecek olanlardır. Çalışmanın amacı pandeminin ilk yılında üçüncü basamak üniversite hastanemizin ameliyathanesinde patolojik inceleme gerektirecek veya onkolojik tanılı ameliyatlarda demografik, cerrahi branş, ameliyat süresi ve anestezi tipi açısından değişiklikleri gözlemlemektir.

**Gereç ve Yöntem:** Kriterleri sağlayan hastaların ameliyat hasta dosyaları retrospektif tarama yöntemiyle incelendi (retrospektif kesitsel çalışma).

**Bulgular:** Ameliyathanemizin olgu sayısının en fazla olduğu ilk 3 cerrahi branş değişmezken, Amerikan Anesteziyoloji Derneği (ASA) II-III skorlamasında ($p<0,05$) anestezi türünde tek başına uygulanan genel anestezi veya genel anestezi eklenen nöroaksiyel ve gövde bloklarında ($p<0,05$) farklılık mevcuttur. Ayrıca onkolojik tanılı olgularda %13,3 ve patolojik inceleme gerektiren elektif ameliyatlarda %32 oranında değişim gözlenmiştir.

**Sonuç:** COVID-19 pandemisinde "yeni normal"e uyum sağlanarak daha fazla beklenen iptal oranları oluşmamış ancak bu dönemde ASA skorlarında ve anestezi tipinde farklılıklar olmuştur.

**Anahtar Kelimeler:** COVID-19, onkolojik cerrahi, ASA skoru, anestezi türü

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## INTRODUCTION

Coronavirus disease-2019 (COVID-19), which started in Wuhan in December 2019, was declared as a pandemic by the World Health Organization (WHO) on March 11, 2020, as a result of its rapid spread to the world after China[1]. The COVID-19 pandemic, as it is known all over the world, has led to interruptions in general health services. During the intense periods of the COVID-19 pandemic, priority was given to emergency and cancer surgeries, and the cancellation of elective surgeries was prioritized[2]. Elective surgeries were suspended when necessary in hospitals selected as pandemic hospitals (COVID-19 treatment centers) in our country, whereas, in hospitals that did not operate as pandemic hospitals (COVID-19-free centers)[3] (our hospital is in this group), priority was given to COVID-19 patients, emergency and oncological surgeries during the peak periods of the pandemic. In the rest of the year, surgical procedures that needed to be evaluated in terms of pathology were allowed by the pandemic commission of the hospital.

Towards the end of the first year of the COVID-19 pandemic, vaccination for the pandemic started in the world, and studies were carried out on the application strategies and changes in oncological surgeries for certain periods of this period (especially the peak periods)[2,4-11]. We aimed to evaluate the change in demographics, surgical branch, duration of operation and anesthesia type of patients who were operated in our operating room with a non-COVID-19 oncological diagnosis in the first year of COVID-19 in our hospital, which is a tertiary hospital, and without oncological diagnosis, which needed to be evaluated (elective) by pathology.

## MATERIALS AND METHODS

This is a retrospective observational single-center study conducted between January 1, 2019 and December 31, 2020 in the operating room of our hospital. In our study, the case data (anesthesia file data) of 11 surgical branches [Departments of General Surgery, Obstetrics and Gynecology (OG), Orthopedics and Traumatology (Orthopedics), Urology, Thoracic Surgery, Ophthalmology, Brain and Nerve Surgery (BNS), Otorhinolaryngology, Plastic Reconstructive and Aesthetic Surgery, Cardiovascular Surgery and Pediatric Surgery] using our operating room were scanned and used. Cardiovascular Surgery, Pediatric Surgery and Plastic Reconstructive and Aesthetic Surgery were excluded from the study due to the high number of changes in faculty members during this period. Anesthesia files in the archive were used while collecting our data. Emergency surgeries, surgeries with COVID-19 disease, and surgical procedures that did not require pathological necessity were excluded from the study. With the WHO's declaration of the pandemic, the number of rooms used for elective surgery in the operating room was reduced due to the density in the COVID-19 units in some periods. Nasopharyngeal swab samples were taken from each patient to be operated within 72 hours before the operation, and hospitalization was ensured if there were no preoperative symptoms. Between the cases, 20 minutes were awaited for operating rooms after sterilization. The rooms of COVID-19 patients and the operating rooms of non-COVID-19 patients were kept separate. COVID-19 and non-COVID-19 intensive care units were separated.

In this period, the data of our patients such as date of operation, age, gender, American Society of Anesthesiology (ASA) score, oncological diagnosis, surgical branch, duration of operation (time considered as the sum of anesthesia and surgical duration), type of anesthesia and the condition or surgical procedure that caused the operation were recorded.

### Statistical Analysis

Descriptive statistics were presented as mean, standard deviation, median (minimum-maximum), frequency and percentage. The distribution of the variables was measured using the Kolmogorov-Smirnov test. Quantitative independent data were analyzed using the Mann-Whitney U test. The chi-square test was used in the analysis of qualitative independent data whereas the Fisher's exact test was used when the chi-square test requirements were not met. The Statistical Package for the Social Sciences 27.0 program was used for analyses. The level of significance was taken as $p<0.05$.

## RESULTS

When the cases not included in the study in 2019-2020 were excluded, the files of 2,559 patients were scanned. Of these, 1,055 patients (41.2%) had an operation with a definite oncological diagnosis, while the remaining 1,504 patients (58.8%) were examined pathologically (can be diagnosed). The first 3 clinics that received the most patients in the surgical branch were General Surgery (33.4%), OG (23%), and Urology (21.8%) clinics. The most preferred type of anesthesia was general anesthesia (with 77%). Demographic, surgical branch, anesthesia type and operation time data of all patients are shown in Table 1.

The age of the patients did not differ significantly ($p>0.05$) in the year before the COVID-19 pandemic (2019) and in the first year of the pandemic (2020). The ASA score of the patients in 2020 was found to be significantly higher ($p<0.05$) than that in 2019. In the first year of the pandemic (2020), there was a statistical difference ($p=0.003$) with a 13.3% decrease in the number of patients with oncological diagnosis and a 32% decrease in the group requiring pathological examination (can be diagnosed). While the rate of general surgery cases in 2020 was significantly higher ($p<0.05$) than that in 2019, the rates of otolaryngology, chest diseases and orthopedics were

significantly lower than in 2019 (p<0.05). While the rate of patients in the general surgery department was 30.7% in 2019, this rate increased to 36.9% in 2020. While the rate of patients in the orthopedics department was 3.4% in 2019, this rate was 0.5% in 2020. While the rate of patients in the thoracic surgery department was 4.7% in 2019, this rate became 2.6% in 2020. While the rate of patients in the ENT department was 13.4% in 2019, this rate was 8.5% in 2020. The ratio of OG, urology, neurosurgery, eye branches did not differ significantly (p>0.05) in the years of 2019 and 2020 (Table 2).

In 2020, the rates of general anesthesia + neuraxial block and general anesthesia + trunk block were significantly higher (p<0.05) than in 2019. The rate of general anesthesia type of patients in 2020 was significantly (p<0.05) lower than in 2019. The rates of neuraxial block, sedoanalgesia, and peripheral nerve block did not differ significantly between 2019 and 2020 (p>0.05). The duration of surgery did not differ significantly (p>0.05) between 2019 and 2020 (Table 2).

When we compared the number of operations in the operating room monthly during the year, there was a statistical difference in the rates of oncological operations in April (p<0.001) and December (p=0.004) compared to the previous year (although there was a decrease in the number of operations in April and an increase in the interval) (Table 3).

## DISCUSSION

As it is known, the COVID-19 pandemic has caused interruptions in health services all over the world. During the intense periods of the COVID-19 pandemic, priority was given to emergency and cancer surgeries, and cancellation of elective surgeries was prioritized[2]. We aimed to examine cancer surgeries that were not diagnosed with COVID-19 in the first year of the pandemic, and elective surgeries that required pathological examination (which can be diagnosed) in the first year of the pandemic, in which we served as a tertiary university and hospital that did not function as an active pandemic hospital (except during the peak periods of the pandemic). Three surgical branches out of

**Table 1. Demographic, surgical branch, anesthesia type and operation time data of all patients**

| | | Min.-Max. | | | Median | Mean±SD/n-% | | |
|---|---|---|---|---|---|---|---|---|
| **Age** | | 1.0 | - | 94.0 | 55.0 | 53.7 | ± | 14.5 |
| **Gender** | Male | | | | | 999 | | 39.0% |
| | Female | | | | | 1,560 | | 61.0% |
| **ASA Score** | I | | | | | 182 | | 7.1% |
| | II | | | | | 1,777 | | 69.4% |
| | III | | | | | 589 | | 23.0% |
| | IV | | | | | 11 | | 0.4% |
| **Diagnosis** | With oncological diagnosis | | | | | 1,055 | | 41.2% |
| | Can be diagnosed | | | | | 1,504 | | 58.8% |
| **Surgical Branch** | | | | | | | | |
| General Surgery | | | | | | 854 | | 33.4% |
| Obstetrics and Gynecology | | | | | | 588 | | 23.0% |
| Urology | | | | | | 559 | | 21.8% |
| Otorhinolaryngology | | | | | | 288 | | 11.3% |
| Brain and Nerve Surgery | | | | | | 106 | | 4.1% |
| Thoracic Surgery | | | | | | 98 | | 3.8% |
| Orthopedics | | | | | | 55 | | 2.1% |
| Eye Diseases | | | | | | 11 | | 0.4% |
| **Type of Anesthesia** | | | | | | | | |
| General Anesthesia | | | | | | 1,971 | | 77.0% |
| Neuraxial Block | | | | | | 259 | | 10.1% |
| General Anesthesia + Neuraxial Block | | | | | | 169 | | 6.6% |
| General Anesthesia + Trunk Block | | | | | | 95 | | 3.7% |
| Sedoanalgesia | | | | | | 54 | | 2.1% |
| Peripheral Nerve Block | | | | | | 11 | | 0.4% |
| Duration of operation (min) | | 5.0 | - | 760.0 | 75.0 | 97.7 | ± | 84.5 |

SD: Standard deviation, ASA: American Society of Anesthesiology, min: Minute, Min.: Minimum, Max.: Maximum

11 surgical branches in our operating room did not meet the study criteria, so they were excluded from the study (Pediatric Surgery, Cardiovascular Surgery and Plastic Reconstructive and Aesthetic Surgery).

In the first year of the pandemic, as before the pandemic, the first 3 departments that performed the most surgical procedures did not change (General Surgery, Gynecology, and Urology). Comparing the two years, it is seen that there is a significant increase in ASA II-III in 2020. It is thought that the decrease in ASA I is due to canceled elective surgeries, the decrease in ASA IV is due to the additional comorbid diseases of this group and the fact that this group also includes the patients in the highest risk group for COVID-19 disease[12].

Compared to the same period, the decrease rate in oncological cases was 13.3%, and this rate was 32% in the other group that could be considered as elective. While the period we evaluated was one year, in studies comparing shorter periods, reductions between 20-60%[2,4,13] in oncological surgery and up to 81.7%[2] in elective surgeries were detected. We think that, among the most important reasons why our rate is lower, it covers the wider time interval and periods outside the peak periods of COVID-19, as well as the adaptation process to the "new normal".

The ratios of surgical branches in the number of cases were affected by the pandemic, and since the cancellation rate in the General surgery[2] branch was less, the case rate of some branches (OG, Urology, BNS and Ophthalmology) did not change, while other branches (Orthopedics, Thoracic surgery, Otorhinolaryngology) differed (Table 2).

In this period, regional anesthesia techniques were recommended as a type of anesthesia, especially in order to prevent viral transmission and aerosolization[14-16]. In our study, there was no difference in regional techniques, but there was a difference in the group of blocks applied in addition to general

**Table 2. General comparison of operating room for the years 2019-2020**

| | Year 2019 | Year 2020 | | | | p value | | | | p value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean±SD /n-% | | | Median | | Mean±SD /n-% | | Median | | | |
| **Age** | | 53.5 | ± | 14.8 | 55.0 | 53.9 | ± | 14.2 | 55.0 | 0.549 | m |
| **Gender** | Male | 588 | | 40.3% | | 411 | | 37.4% | | 0.140 | $X^2$ |
| | Female | 872 | | 59.7% | | 688 | | 62.6% | | | |
| **ASA score** | I | 134 | | 9.2% | | 48 | | 4.4% | | **0.000** | $X^2$ |
| | II | 997 | | 68.3% | | 780 | | 71.0% | | | |
| | III | 321 | | 22.0% | | 268 | | 24.4% | | | |
| | IV | 8 | | 0.5% | | 3 | | 0.3% | | | |
| **Diagnosis** | Oncological diagnosis | 565 | | 38.7% | | 490 | | 44.6% | | **0.003** | $X^2$ |
| | Can be diagnosed | 895 | | 61.3% | | 609 | | 55.4% | | | |
| **Surgical Branch** | | | | | | | | | | | |
| General Surgery | | 448 | | 30.7% | | 406 | | 36.9% | | **0.001** | $X^2$ |
| Obstectrics and Gynecology | | 328 | | 22.5% | | 260 | | 23.7% | | 0.478 | $X^2$ |
| Urology | | 308 | | 21.1% | | 251 | | 22.8% | | 0.291 | $X^2$ |
| Otorhinolaryngology | | 195 | | 13.4% | | 93 | | 8.5% | | **0.000** | $X^2$ |
| Brain and Nerve Surgery | | 57 | | 3.9% | | 49 | | 4.5% | | 0.486 | $X^2$ |
| Thoracic Surgery | | 69 | | 4.7% | | 29 | | 2.6% | | **0.006** | $X^2$ |
| Orthopedics | | 49 | | 3.4% | | 6 | | 0.5% | | **0.000** | $X^2$ |
| Eye diseases | | 6 | | 0.4% | | 5 | | 0.5% | | 0.866 | $X^2$ |
| **Type of Anesthesia** | | | | | | | | | | | |
| General Anesthesia | | 1177 | | 80.6% | | 794 | | 72.2% | | **0.000** | $X^2$ |
| Neuraxial Block | | 136 | | 9.3% | | 123 | | 11.2% | | 0.119 | $X^2$ |
| General Anesthesia + Neuraxial Block | | 73 | | 5.0% | | 96 | | 8.7% | | **0.000** | $X^2$ |
| General Anesthesia + Trunk Block | | 34 | | 2.3% | | 61 | | 5.6% | | **0.000** | $X^2$ |
| Sedoanalgesia | | 32 | | 2.2% | | 22 | | 2.0% | | 0.741 | $X^2$ |
| Peripheral Nerve Block | | 8 | | 0.5% | | 3 | | 0.3% | | 0.293 | $X^2$ |
| Duration of operation (min) | | 93.6 | ± | 78.4 | 75.0 | 103.2 | ± | 91.7 | 75.0 | 0.075 | m |

m: Mann-Whitney U test, $x^2$: Chi-square test, SD: Standard deviation, ASA: American Society of Anesthesiology, min: Minute

anesthesia for general anesthesia or postoperative analgesia (General anesthesia + neuraxial block and General anesthesia + body blocks).

When we evaluate the effect in the pandemic period as months, it was found that there was a significant decrease in the number of cases (54.8% in the oncological group and 83% in the other group) in the month after the WHO's declaration of the pandemic (March 11, 2021), and there was a difference in the rate of oncological and elective diagnoses. With oncological cases becoming a priority, the number of oncological cases increasing throughout the year also made a difference in December compared to the previous year, proportionally (Table 3).

### Study Limitations

The most important limitation of our study is that it is a single-center and retrospective study. A multicenter study could make a difference in terms of interaction from covid according to the population of the regions. In addition, a prospective study could improve the quality of the study, but all healthcare professionals in the world were working under difficult and uncertain conditions in the follow-up and control of this challenging process.

## CONCLUSION

We presented the report of surgeries with oncological diagnosis and pathological examination (elective) in the operating room of the tertiary university hospital in the COVID-19 pandemic. The effect of the pandemic in our operating room is seen especially in the period after the cases in our country and the WHO's declaration of the pandemic (March 2021). Compared to the same period, an increase in the ASA II-III group rates and an increase in the case rates of surgical branches and anesthesia techniques (in the blocks added for anesthesia and analgesia in addition to general anesthesia and general anesthesia) were observed. Towards the end of the first year of the pandemic, it is seen that the number of cases that underwent surgery (especially with oncological diagnosis) and our hospital and operating room adapted to this "new normal",

**Table 3. Comparison of the change in the number of surgeries by month**

| | | 2019 (n=1.460) (%) | 2020 (n=1.099) (%) | Total (n=2.559) | $p^{X^2}$ |
|---|---|---|---|---|---|
| January | With oncological diagnosis | 53 (39) | 49 (34.5) | 102 (36.7) | 0.440 |
| | Can be diagnosed | 83 (61) | 93 (65.5) | 176 (63.3) | |
| February | With oncological diagnosis | 53 (34.4) | 56 (39.7) | 109 (36.9) | 0.346 |
| | Can be diagnosed | 101 (65.6) | 85 (60.3) | 186 (63.1) | |
| March | With oncological diagnosis | 51 (40.5) | 20 (28.6) | 71 (36.2) | 0.097 |
| | Can be diagnosed | 75 (59.5) | 50 (71.4) | 125 (63.8) | |
| April | With oncological diagnosis | 42 (36.8) | 19 (86.4) | 61 (44.9) | **<0.001** |
| | Can be diagnosed | 72 (63.2) | 3 (13.6) | 75 (55.1) | |
| May | With oncological diagnosis | 53 (39.6) | 9 (47.4) | 62 (40.5) | 0.516 |
| | Can be diagnosed | 81 (60.4) | 10 (52.6) | 91 (59.5) | |
| June | With oncological diagnosis | 37 (42.5) | 36 (43.4) | 73 (42.9) | 0.911 |
| | Can be diagnosed | 50 (57.5) | 47 (56.6) | 97 (57.1) | |
| July | With oncological diagnosis | 63 (41.4) | 42 (47.7) | 105 (43.8) | 0.345 |
| | Can be diagnosed | 89 (58.6) | 46 (52.3) | 135 (56.3) | |
| August | With oncological diagnosis | 25 (36.8) | 43 (43) | 68 (40.5) | 0.419 |
| | Can be diagnosed | 43 (63.2) | 57 (57) | 100 (59.5) | |
| September | With oncological diagnosis | 44 (38.9) | 45 (464) | 89 (42.4) | 0.276 |
| | Can be diagnosed | 69 (61.1) | 52 (53.6) | 121 (57.6) | |
| October | With oncological diagnosis | 44 (35.5) | 55 (47.8) | 99 (41.4) | 0.053 |
| | Can be diagnosed | 80 (64.5) | 60 (52.2) | 140 (58.6) | |
| November | With oncological diagnosis | 51 (39.5) | 48 (44.9) | 99 (41.9) | 0.409 |
| | Can be diagnosed | 78 (60.5) | 59 (55.1) | 137 (58.1) | |
| December | With oncological diagnosis | 49 (39.8) | 67 (58.8) | 116 (48.9) | **0.004** |
| | Can be diagnosed | 74 (60.2) | 47 (41.2) | 121 (51.1) | |

$x^2$: Chi-square test

reducing the oncological and elective case cancellation rates that are expected to cause more of the pandemic.

## Ethics

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Tekirdağ Namık Kemal University Faculty of Medicine (approval no: 2021.206.07.14, date: 27.07.2021).

**Informed Consent:** Retrospective cross-sectional study.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: A.G., A.Ş., İ.Y., O.B., C.A., Concept: A.G., A.Ş., Design: A.G., A.Ş., Data Collection or Processing: A.G., A.Ş., İ.Y., Analysis or Interpretation: A.G., A.Ş., O.B., Literature Search: A.G., A.Ş., Writing: A.G., A.Ş., C.A.

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# Comparative Evaluation of Systemic Immune Indexes in Infants Born to COVID-19 PCR Positive and Negative Mothers - Can Neonatal Effects Be Predicted?

## COVID-19 PCR Pozitif ve Negatif Anneden Doğan Bebeklerin Sistemik İmmün İndekslerinin Karşılaştırmalı Değerlendirilmesi - Neonatal Etkiler Öngörülebilir mi?

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**ABSTRACT**

**Aim:** The Coronavirus disease-2019 (COVID-19), declared as a pandemic by the World Health Organization on March 11, 2020, is a condition caused by Severe acute respiratory syndrome-Coronavirus-2, and the number of cases is increasing day by day. The aim of this study is to investigate the differences in systemic inflammatory indices of newborn babies born to COVID-19 polymerase chain reaction (PCR)-positive mothers, who constitute a sensitive population during the COVID-19 pandemic period, compared to the normal population.

**Materials and Methods:** Between March 2019 and November 2021, in Ankara City Hospital, newborns who were born at ≥37 weeks of gestation to COVID-19 PCR positive mothers in the two weeks before birth and whose COVID-19 PCR tests were negative and were given usual care with

mothers, basal hematological parameters were compared by taking healthy newborns born to COVID-19 PCR negative mothers at ≥37 weeks of gestation in the control group.

**Results:** The rate of cesarean delivery was higher in the group (n=86) of babies of COVID-19 PCR positive mothers ($p<0.05$). Considering the hemogram parameters, total white blood cell, neutrophil, and hemoglobin/hematocrit counts were lower in the control group (n=94), and platelet/plateletcrit values were higher ($p<0.05$). Neutrophil lymphocyte ratio and systemic immune-inflammation index were statistically significantly higher in the infants of COVID-19 PCR-positive mothers, neutrophilia and neutrophil lymphocyte ratio was determined as independent predictive variables in logistic regression analysis ($p=0.048$ and $p=0.011$).

**Results:** Vertical viral transmission was not observed in babies born to COVID-19 PCR positive mothers. Compared to the control group, it was thought that the high neutrophil lymphocyte ratio and neutrophilia in babies born to COVID-19 PCR positive mothers might be due to maternal cytokine release. Our study will shed light on further research for the elaboration of this situation and longer follow-up.

**Keywords:** COVID-19, pandemic, newborn, systemic inflammatory indexes

**ÖZ**

**Amaç:** Dünya Sağlık Örgütü'nün 11 Mart 2020 tarihinde pandemi olarak ilan ettiği Koronavirüs hastalığı-2019 (COVID-19), Şiddetli akut respiratuvar sendrom-Koronavirüs-2'nin neden olduğu bir durumdur ve olgu sayısı her gün giderek artmaktadır. Çalışmanın amacı COVID-19 pandemisi döneminde hassas popülasyon olan COVID-19 polimeraz zincir reaksiyonu (PCR) pozitif anneden doğan yenidoğan bebeklerinin SİSTEMİK immün-enflamasyon indeksleri (Sİİ) normal popülasyona göre farklılıklarının araştırılmasıdır.

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**Gereç ve Yöntem:** Mart 2019 ve Kasım 2021 tarihleri arasında Ankara Şehir Hastanesi'nde doğumdan önceki iki haftada COVID-19 PCR testi pozitif saptanan annelerden, ≥37 gestasyon haftasında doğan, nazal sürüntü ile alınan COVID-19 PCR testi negatif saptanan ve anne yanında olağan bakım verilen yenidoğanlar ile kontrol grubunda ≥37 gestasyon haftasında COVID-19 PCR testi negatif anneden doğan sağlıklı yenidoğanların bazal hematolojik parametreleri karşılaştırıldı.

**Bulgular:** COVID-19 PCR pozitif anne bebekleri grubunda (n=86) sezaryen yöntemiyle doğum oranı daha fazlaydı (p<0,05). Hemogram parametrelerine bakıldığında toplam beyaz küre, nötrofil, hemoglobin/hematokrit sayıları kontrol grubunda (n=94) daha düşük, trombosit/plateletkrit değerleri daha yüksekti (p<0,05). Nötrofil lenfosit oranı ve Sİİ COVID-19 PCR pozitif anne bebekleri grubunda istatistiksel anlamlı olarak yüksek, multivariate lojistik regresyon analizinde nötrofili ve nötrofil lenfosit oranı ise bağımsız prediktif değişkenler olarak saptandı (p=0,048 ve p=0,011).

**Sonuç:** COVID-19 PCR pozitif anneden doğan bebeklerde vertikal viral geçişi gözlenmedi. Kontrol grubuna göre COVID-19 PCR pozitif anneden doğan bebeklerde nötrofil lenfosit oranı yüksekliği ve nötrofili maternal sitokin salınıma bağlı olabileceği düşünüldü. Çalışmamız bu durumun detaylandırılması ve daha uzun izlem için ileri araştırmalara ışık tutacaktır.

**Anahtar Kelimeler:** COVID-19, pandemi, yenidoğan, sistemik immün-enflamasyon indeksi

## INTRODUCTION

The Coronavirus disease-2019 (COVID-19), declared as a pandemic by the World Health Organization on March 11, 2020, is a condition caused by Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2), and the number of cases is increasing day by day. As a result of this disease, which causes an epidemic as a social health problem, pregnant women and their babies are greatly affected.

Pregnant women constitute a vulnerable population for this disease due to their suppressed immune systems. Although it can be asymptomatic in pregnant women, severe cases with lung involvement can result in death. This virus, which causes disease through angiotensin-converting enzyme 2 (ACE2) receptors, may allow placental damage and subsequent vertical transmission of the virus due to the high number of placental ACE2 receptors[1,2]. Although vertical transmission of SARS-CoV-2 has been reported in some case-based series, postpartum neonatal PCR positivity is not observed in most of the pregnancies with positive COVID-19 PCR[3-7]. In studies conducted during the COVID-19 pandemic period (INTERCOVID), it was found that there was an increase in the rates of maternal death, abortion, preterm, cesarean section and infant birth with low birth weight[8,9].

It is known that COVID-19 progresses with milder symptoms in the pediatric age group compared to the adult population, but the frequency of Kawasaki-like syndrome/macrophage activation syndrome or multisystem inflammatory syndrome (MIS), which develops secondary to 'cytokine storm', continues to increase in children. Leukocytosis, lymphopenia and thrombocytopenia have been associated with the severity of the disease especially in pediatric patients and lymphocytosis in infants[10]. Although it is said that the severity of the disease may be higher in newborns compared to older children as a result of inadequate immune response, mortality and morbidity rates were found to be lower[11]. In the meta-analyses, the immunoglobulin G (IgG) levels in babies born to COVID-19 PCR positive mothers were found to be higher than the normal population, and it was hypothesized that COVID-19 antibodies transmitted from the mother via the placental route protect the baby from active disease[12].

Low fibrinogen, high ferritin, high troponin, high pro-BNP, high lactate dehydrogenase, high D-Dimer, and high C-reactive protein (CRP) are pathological laboratory parameters that can be seen in patient groups diagnosed with MIS. Inflammatory indices such as the neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and systemic immune-inflammation index (SII) have been widely used recently to determine the severity of the disease[13-18]. The aim of this study was to compare the basal hematological parameters and inflammatory indices of babies born to COVID-19 PCR positive mothers with the normal population.

## MATERIALS AND METHODS

This retrospective observational study was conducted in Ankara City Hospital between March 2019 and November 2021. It was planned to include newborns of mothers who were found to have positive COVID-19 PCR test in the two weeks before birth, those who were born at ≥37 weeks of gestation, those whose nasal swab was found to be negative, and those who were given usual care. It was planned to include healthy newborns born to mothers with a negative COVID-19 PCR test at ≥37 weeks of gestation in the control group. Cases with maternal (preeclampsia, hypertension, poly- or oligohydramniosis, pregestational and gestational diabetes, early rupture of membranes, other chronic diseases), placental (previa, accreta, intrauterine growth restriction) and neonatal (intrauterine hypoxia/asphyxia, early neonatal sepsis, congenital and chromosomal anomalies) disease were not included in the study. Demographic, clinical and laboratory data of the patients were recorded from the hospital data system and patient files. The study was approved by the Ethics Committee of the same center (Ankara City Hospital Clinical

Research Ethics Committee no. 2, date: 16.06.2021, ethics committee no: E2-21-606).

### Systemic Immune-Inflammation Indexes

Hemogram parameters evaluated in the patient and control groups were taken in the postnatal first 6 hours. It was calculated with the ratio of NLR- absolute neutrophil count (N; $10^9$/L) to the absolute lymphocyte count (L; $10^9$/L) (with the formula NLR=N/L), the ratio of PLR- absolute platelet count (P; $10^9$/L) to the absolute lymphocyte count (L; $10^9$/L) (with the formula PLR=P/L) and the multiplication of SII-NLR value by absolute platelet count (with SII=NLR×P/1000 formula)[19].

### Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 22.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY). The Kolmogorov-Smirnov test was used to determine the normal distribution of the data and the Student's t-test was used to compare parametric variables. The Mann-Whitney U and chi-square tests were employed for non-parametric variables. In addition, the Pearson's and Spearman's tests were used for correlation analysis. Receiver operating characteristic (ROC) analysis was performed to determine the cutoff values, sensitivity and specificity of systemic inflammatory tests, and logistic regression analysis was conducted to identify independent risk factors/predictors. A p value of <0.05 was considered significant for all tests.

## RESULTS

Eighty-six newborns born to mothers with positive COVID-19 PCR tests were included in the study. 94 healthy newborns were taken as the control group. Weeks of gestation, birth weights and genders of the patients were similar in both groups. The rate of C/S delivery was higher in the group of infants born from COVID-19 PCR positive mothers (p<0.05) (Table 1). Considering the hemogram parameters, total white blood cell, neutrophil, and hemogram/hematocrit counts were lower in the control group, and platelet/plateletcrit values were higher in the control group (p<0.05). NLR and SII were found to be statistically significantly higher in the infants of COVID-19 PCR positive mothers (Table 1). There was no difference between the groups in terms of other hematological parameters (Table 1).

**Table 1. Demographic and laboratory values of the groups**

| | Infants born to COVID-19 PCR positive mothers (n=86) | Infants born to COVID-19 PCR negative mothers (n=94) | p value |
|---|---|---|---|
| **Demographic features** | | | |
| Week of gestation, (mean) | 38±1 | 38±1 | 0.388 |
| Birth weight, (mean), g | 3213±486 | 3294±450 | 0.250 |
| Male, n (%) | 46 (53.5) | 50 (53.2) | 0.968 |
| C/S delivery, n (%) | 57 (66.3) | 46 (48.9) | **0.019** |
| **Laboratory** | | | |
| WBC, (mean), (x$10^9$/L) | 16.434±5.752 | 11.321±4.409 | **0.000** |
| NEU, (mean), (x$10^9$/L) | 10.361±5085 | 5.160±3.937 | **0.000** |
| LYM, (mean), (x$10^9$/L) | 3.946±1.742 | 4.297±1.352 | 0.132 |
| HGB, (mean), gr/dL | 17.8±2.6 | 16.9±2.3 | **0.014** |
| HCT, (mean), % | 55.4±8.6 | 52.3±7.6 | **0.012** |
| MPV, (mean), fL | 8.4±0.9 | 8.5±1.2 | 0.818 |
| PLT, (mean), (x$10^9$/L) | 280.348±88.801 | 316.244±113.262 | **0.020** |
| PCT, (mean), % | 0.22±0.07 | 0.27±0.09 | **0.000** |
| RDW, (mean), % | 17.0±1.2 | 16.9±1.3 | 0.702 |
| NRBC, (mean), (x$10^9$/L) | 1.1±2.5 | 0.63±2.5 | 0.216 |
| PLR, (mean) | 77±32 | 80±59 | 0.635 |
| NLR, (mean) | 3.0±1.9 | 1.3±1.2 | **0.000** |
| SII, (mean) | 831.610±626.117 | 414.555±469.307 | **0.000** |

WBC: White blood cell, NEU: Neutrophil, LYM: Lymphocyte, HGB: Hemoglobin, HCT: Hematocrit, MPV: Mean platelet volume, PLT: Platelet, PCT: Plateletcrit, RDW: Distribution width of erythrocytes, NRBC: Nucleated erythrocytes, PLR: Platelet/lymphocyte ratio, NLR: Neutrophil/lymphocyte ratio, SII: Systemic immune-inflammation index, COVID-19: Coronavirus disease-2019, PCR: Polymerase chain reaction

ROC analyses are shown in Figure 1, and area under curve values and 95% confidence intervals are shown in Table 2. Optimal sensitivity and specificity cutoff values for neutrophil, NLR, and SII were determined as 5320, 1,12, and 427,289, appropriately (Table 2).

Neutrophilia and NLR were determined as independent predictive variables in the multivariate logistic regression analysis (p=0.048 and p=0.011) (Table 3).

## DISCUSSION

Although SARS-CoV-2 is a disease transmitted through droplet, there is a risk of transmission to the baby born from a COVID-19 PCR positive mother by the transplacental route during the intrauterine period, or during aspiration of secretions at the time of delivery and during postnatal breastfeeding[2]. In the studies conducted by Yang et al.[14] and Chen et al.[4], no coronavirus was found in the amniotic fluid, placenta, and breast milk of COVID-19 PCR-positive mothers. In the studies of Akyıldız and Çamur[8] and Cappoza et al.[5], no coronavirus positivity was found in the nasopharyngeal swab samples of the babies of COVID-19 PCR positive mothers. In our study, in consistency with the literature, no positivity was detected in the COVID-19 PCR tests taken by nasal swab in the first 48 hours in babies born to COVID-19 PCR positive mothers.

Spontaneous vaginal delivery is recommended by the World Health Organization for COVID-19 PCR positive pregnant women, except for obstetric indications[20]. However, in our study, consistent with some studies in the literature, the rate of cesarean delivery was higher in babies born to COVID-19 PCR-positive mothers compared to the control group, and the majority of cesarean section indications in our patient group consisted of repeated cesarean section[4,14,21].

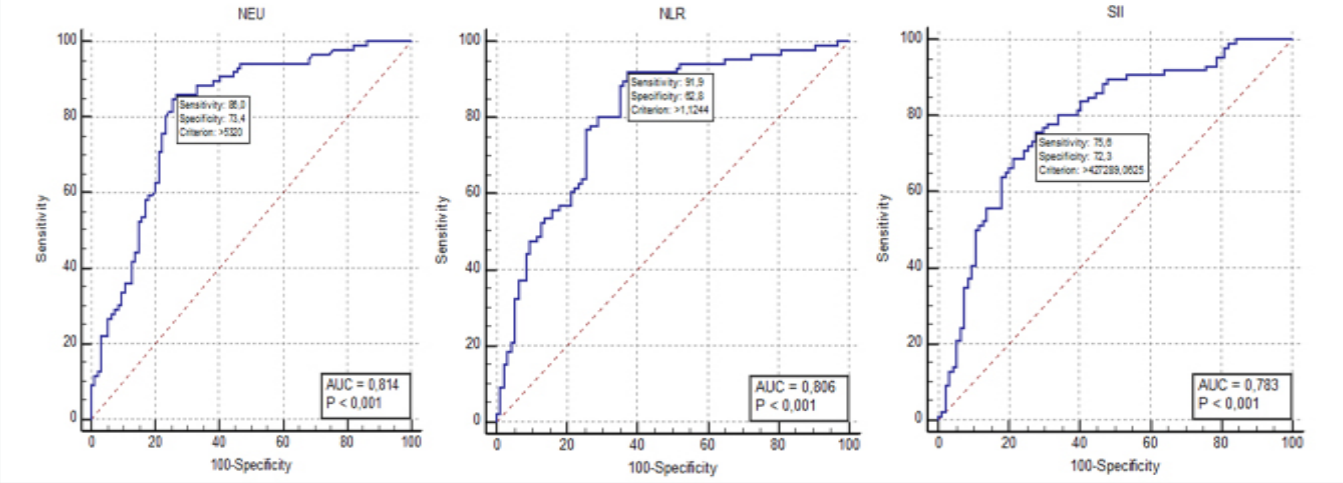


**Figure 1.** ROC curves for neutrophil, NLR, and SII

*NEU: Neutrophil, NLR: Neutrophil/lymphocyte ratio, SII: Systemic immune-inflammation index, ROC: Receiver operating characteristic*

**Table 2. Cutoff, sensitivity, specificity, AUC and 95% confidence interval values of hemogram parameters of babies born to COVID-19 PCR positive mothers**

| | Cutoff | Sensitivity | Specificity | AUC | 95% CI | | p |
|---|---|---|---|---|---|---|---|
| | | | | | Lower | Upper | |
| WBC, (x10$^9$/L) | ≥11.340 | 82.6% | 68.1% | 0.770 | 0.701 | 0.829 | **<0.001** |
| NEU, (x10$^9$/L) | ≥5.320 | 86% | 73.4% | 0.814 | 0.749 | 0.868 | **<0.001** |
| HGB, gr/dL | >18.5 | 40.7% | 77.7% | 0.592 | 0.516 | 0.664 | **=0.031** |
| HCT, % | >58.5 | 33.7% | 83.0% | 0.590 | 0.514 | 0.662 | **=0.035** |
| PLT, (x10$^9$/L) | ≤268.000 | 50.0% | 69.1% | 0.594 | 0.518 | 0.666 | **=0.027** |
| PCT, % | ≤0.31 | 90.7% | 20.2% | 0.661 | 0.587 | 0.729 | **<0.001** |
| NLR | >1.12 | 91.9% | 62.8% | 0.805 | 0.740 | 0.861 | **<0.001** |
| SII | >427.289 | 75.6% | 72.3% | 0.783 | 0.716 | 0.841 | **<0.001** |

WBC: White blood cell, NEU: Neutrophil, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelet, PCT: Plateletcrit, NLR: Neutrophil/lymphocyte ratio, SII: Systemic immune-inflammation index, AUC: Area under curve, COVID-19: Coronavirus disease-2019, PCR: Polymerase chain reaction, CI: Confidence interval

**Table 3. Logistic regression analysis for independent predictors**

| | OR | 95% CI | | p |
|---|---|---|---|---|
| | | Lower | Upper | |
| WBC, (x10$^9$/L) | 0.028 | 0.163 | 4.614 | 0.867 |
| NEU, (x10$^9$/L) | 4.100 | 0.054 | 0.685 | **0.048** |
| C/S | 1.038 | 0.310 | 1.448 | 0.308 |
| PCT, % | 0.099 | 0.372 | 3.920 | 0.753 |
| NLR | 6.454 | 0.008 | 0.872 | **0.011** |
| SII | 0.097 | 0.403 | 3.502 | 0.756 |

WBC: White blood cell, NEU: Neutrophil, CI: Confidence interval, C/S: Cesarean delivery, PCT: Plateletcrit, NLR: Neutrophil/lymphocyte ratio, SII: Systemic immune-inflammation index, CI: Confidence interval, OR: Odds ratio

Considering the relationship between COVID 19 infection and lymphopenia, lymphopenia is observed very frequently and is associated with disease severity in most of the studies on adult coronavirus patients, whereas lymphopenia occurs less frequently in the pediatric age group[22]. While lymphopenia was observed in more than 80% of critically ill adults, leukopenia was found at the rate of 47% and neutropenia at the rate of 52% in an analysis including 80 children with COVID-19 PCR positivity[21,23]. Lymphopenia develops due to consumption of lymphocytes, apoptosis and increased cytokine damage during the disease. The reason why lymphopenia is rare in pediatric and infant age groups can be explained by the inadequate immune response. Although lymphopenia is not frequently seen in the pediatric age group during the disease, it is known that its presence plays an important role in determining the severity of the disease[10]. The absence of leukopenia and neutropenia in infants born to COVID-19 PCR-positive mothers in our study may be explained by the very low rate of direct vertical transmission of coronavirus infection.

In the meta-analysis of babies born to COVID-19 PCR-positive mothers, neutrophil levels were found to be higher than the control group, as in our study[12]. It has been shown that high neutrophil count, increased CRP values, PLR and NLR ratios in adult patient groups are very informative in determining the severity of the disease and in the follow-up of treatment[13-16,18]. In our study, the high NLR ratio in babies born to COVID-19 PCR positive mothers is related to the increase in neutrophil count rather than a decrease in lymphocyte count, which may be due to maternal cytokine activation, not infection in infants.

While the SII has been used for a long time in the evaluation of disease severity, course and treatment response in adult patient group in oncology practice, it has recently been shown to be associated with the identification of risk groups for COVID-19 and mortality[17]. It is known that it has recently been used to predict disease severity in diseases such as perinatal hypoxic ischemic encephalopathy and retinopathy in newborn babies[19,23]. In our study, SII was found to be significantly higher in babies born to COVID-19 PCR positive mothers compared to the control group, but it could not be shown to be an independent marker. Studies with larger numbers of patients are required to demonstrate this.

In a study conducted by Lamba et al.[24], it was found that the risk of postnatal viral transmission from the mother was low in newborns whose mothers were positive for COVID-19 PCR. It has been shown that the implementation of strict rules such as keeping babies of COVID-19 PCR-positive mothers separate from the mother, taking early baths, and encouraging formula feeding instead of breastfeeding has negative effects on mother-infant bonding and decrease in breast milk rather than reducing the risk of postnatal viral transmission[25,26]. In our clinic, babies are followed up with their mothers by following the mask and hygiene rules.

### Study Limitations

The limitations of the study include that it was a retrospective cohort study, the number of cases was low, neonatal acute phase reactants and antibody levels were not evaluated, the severity of maternal COVID-19 and the treatments given were not recorded, babies born to COVID-19 PCR positive mothers were not followed up in terms of neonatal COVID-19 and MIS-N. The reason for not checking neonatal antibody levels is that the normal interval was not determined in the neonatal period.

Although vertical transmission of maternal infection is very rare, it is important to know the effects of possible maternal inflammation and cytokine activation on the baby. In this study, it was shown that some inflammatory tests of even asymptomatic infants born to mothers with infections and who did not require intensive care follow-up differed from those of healthy infants born to COVID-19 PCR negative mothers. Although there is no detailed study in the literature about the period of postnatal life until when this difference may last, in a study by More et al.[27] in which 20 MIS-N cases were included, the SARS-CoV-2 IgG levels were found to be high, but the antigen levels were negative and it was shown that MIS-N condition might develop in case babies born to mothers with COVID-19 disease encountered the SARS-CoV-2 virus. In line with this information, it can be thought that inflammatory indices may be useful in the prediction of MIS-N and in the follow-up of treatment. Our study will shed light on further research for the elaboration of this issue and longer follow-up.

## CONCLUSION

Although morbidity and mortality due to COVID-19 are low in newborn babies, they should be followed closely due to their

being sensitive population, and NLR and SII should be included in future studies as markers that can predict the disease and be used in treatment and follow-up.

## Ethics

**Ethics Committee Approval:** The study were approved by the Ankara City Hospital Clinical Research Ethics Committee no. 2, date: 16.06.2021, ethics committee no: E2-21-606.

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: E.T., G.K.Ş., H.G.K.K., Concept: S.E., F.E.C., N.Y., Design: S.E., F.E.C., N.Y., Data Collection or Processing: S.E., N.Y., Analysis or Interpretation: E.T., G.K.Ş., H.G.K.K., Literature Search: S.E., E.T., N.Y., Writing: S.E.

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

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# Long-term Prognostic Significance of Pentraxin-3 in Patients with Non-ST Elevation Myocardial Infarction and Coronary Stenting

## Koroner Stent Uygulanan ST Elevasyonsuz Miyokard İnfarktüsünde Pentraksin-3'ün Uzun Vadeli Prognostik Önemi

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### ABSTRACT

**Aim:** We aimed to investigate the relationship of serially measured pentraxin-3 (PTX3) levels with Gensini risk score and cardiovascular mortality in long-term follow-up in patients who underwent percutaneous coronary intervention (PCI) with the diagnosis of non-ST elevation myocardial infarction (NSTEMI) and stable angina pectoris (SAP).

**Materials and Methods:** Our study was planned retrospectively, and the long-term cardiovascular mortality results of patients with NSTEMI and SAP, who underwent PCI, were evaluated. Our study consisted of two groups, including the study and the control groups. Eighteen patients with NSTEMI who underwent PCI were included in the study group, and 37 patients with a diagnosis of SAP were included in the control group. Blood samples were taken from all patients for PTX3 measurements at the time of admission, at the 8$^{th}$ and 24$^{th}$ hours. Gensini scores were calculated before PCI.

**Results:** PTX3 levels measured at the eighth hour were found to be numerically and statistically significant in NSTEMI patients compared to SAP patients [13.37 (5.47-27.75) and 5 (3.83-12.42), p=0.006]. PTX3 values measured at the time of admission were found to be associated with Gensini score (r=0.299, p=0.026). PTX3 values measured at the eighth hour were found to be independent predictors of long-term cardiovascular mortality (Hazard ratio: 1.294, 95% confidence interval: 1024-1.653, p=0.039).

**Conclusion:** PTX3 may be helpful in identifying individuals at high risk for cardiovascular mortality in the long term in NSTEMI patients.

**Keywords:** Acute coronary syndrome, Gensini risk score, pentraxin-3, long-term prognosis

### ÖZ

**Amaç:** ST elevasyonu olmayan miyokard infarktüsü (NSTEMI) ve stabil anjina pektoris (SAP) tanısıyla perkütan koroner girişim (PKG) yapılmış hastalarda ardışık ölçülen pentraksin-3 (PTX3) düzeylerinin Gensini risk skoru ve uzun dönem takiplerde kardiyovasküler mortalite ile ilişkisini araştırmayı amaçladık.

**Gereç ve Yöntem:** Çalışmamız retrospektif olarak planlanmış olup PKG yapılan NSTEMI ve SAP tanılı hastaların uzun dönem kardiyovasküler mortalite sonuçları değerlendirildi. Çalışmamız, çalışma ve kontrol grubu olmak üzere iki gruptan oluşmaktadır. PKG yapılan NSTEMI tanılı 18 hasta çalışma grubuna, SAP tanılı 37 hasta ise kontrol gurubuna dahil edildi. Tüm hastalardan başvuru anı, 8. ve 24. saatte PTX3 ölçümleri için kan örnekleri alındı. Gensini skorları PKG öncesi hesaplandı.

**Bulgular:** Sekizinci saatte bakılan PTX3 düzeyleri NSTEMI hastalarında SAP tanılı hastalara göre sayısal ve istatistiksel olarak anlamlı saptandı [13,37 (5,47-27,75) ve 5 (3,83-12,42), p=0,006]. Başvuru anında bakılan PTX3 değerleri Gensini skoru ile ilişkili saptandı (r=0,299, p=0,026). Sekizinci saatte bakılan PTX3 değerleri uzun dönem kardiyovasküler mortalite için bağımsız öngördürücü olarak saptandı (Hazard oranı: 1,294, %95 güven aralığı: 1,024-1,653, p=0,039).

**Sonuç:** PTX3, NSTEMI hastalarında uzun dönemde kardiyovasküler mortalite için yüksek riskli bireylerin belirlenmesinde yardımcı olabilir.

**Anahtar Kelimeler:** Akut koroner sendrom, Gensini risk skoru, pentraksin-3, uzun vadeli prognoz

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## INTRODUCTION

Acute myocardial infarction (AMI) is an important cause of death worldwide. The onset of critical processes for coronary artery stenosis is partial or complete coronary artery occlusion due to rupture of atherosclerotic plaque[1]. Biomarkers such as creatine kinase and troponin have been used for the early diagnosis of myocardial damage, and it has been shown that these markers can also be used in the prognosis of patients[2].

Vascular inflammation plays an important role in the pathophysiology of atherosclerosis and coronary artery disease (CAD) (stable or acute coronary syndrome)[3]. Pentraxin 3 (PTX3) is a multimeric acute phase protein and is an inflammatory glycoprotein like C-reactive protein (CRP)[4]. CRP is in the short pentraxin group; PTX3 is in the long pentraxin group. Unlike CRP synthesized by hepatocytes, PTX3 can be directly synthesized by a variety of cells, such as cells found in atherosclerotic lesions, vascular endothelial cells, smooth muscle cells, and fibroblasts[5]. It has been shown in previous studies that there is an increase in PTX3 levels in patients with AMI and unstable angina pectoris[6,7].

The Gensini score system is a scoring system developed for the assessment of the severity of CAD[8]. The morphology and anatomy of the coronary arteries and the severity of the stenosis are evaluated in the scoring system[9]. Strong correlations were observed between the risk of cardiovascular disease in the long and short term and the Gensini score[10].

In this study, we aimed to investigate the relationship between consecutively measured the Gensini score of serum PTX3 levels and cardiovascular mortality in long-term follow-up in patients with non-ST elevation myocardial infarction (NSTEMI).

## MATERIALS AND METHODS

### Study Design

In our retrospectively planned study, 119 patients were included. As a result of regular periodic follow-ups (clinic and polyclinic), a total of 55 cases, 18 of whom were NSTEMI and 37 of whom had stable angina pectoris (SAP) (with evidence of coronary artery ischemia, myocardial perfusion scintigraphy or treadmill exercise test) were included in the study. Percutaneous coronary intervention (PCI) was applied to all patients. Our study consists of patients included in the study between February and August 2010 in a tertiary health center.

NSTEMI was defined as ST segment depression or transient ST segment elevation, T wave inversion and troponin positivity in at least two adjacent leads on electrocardiography in addition to typical chest pain lasting longer than 10 minutes. All NSTEMI patients consisted of patients who were hospitalized within 24 hours of the onset of chest pain.

The SAP group was defined as those with at least one coronary artery lesion requiring PCI in coronary angiography (CAG).

Coronary blood flow after PCI was evaluated with the thrombolysis in myocardial infarction (TIMI) frame number[11]. Patients with TIMI 3 after the procedure were included in the study.

PTX3 levels were measured 3 times for each patient. They were measured at baseline (before CAG), at 8$^{th}$ hour (after PCI), and at 24$^{th}$ hour (time after the first measurement).

Patients with a history of persistent ST segment elevation, newly developing left bundle branch block, malignancy, renal failure (serum creatinine >2.0 mg/dL), acute or chronic inflammatory disease were not included in the study.

Approval was obtained from the Çanakkale Onsekiz Mart University Local Ethics Committee for the study (decision no: 2011-KAEK-27/2021-2100169941, date: 24.11.2021), and our study was carried out in accordance with the Declaration of Helsinki.

### Coronary Angiographic Analysis and Interventional Procedure

CAG was performed via the femoral or radial artery using the Judkins technique. Coronary arteries were evaluated from images obtained from at least two different angles. PCI was performed using standard technique. Non-ionic low osmolality contrast material was used during the procedure. Angiographic images were evaluated by two interventional cardiologists who were unaware of the study. Quantitative analyses of angiographic images were completed using an automated system (GE Medical Systems). Stenoses greater than 50% for the left main coronary artery and more than 70% for the other coronary arteries were considered clinically significant. The CAG procedure and treatments of the patients were performed within the framework of the current American College of Cardiology/American Heart Society recommendations in the years of the study[12].

### Blood Sample Collection and Laboratory Analyses

Blood samples were taken into ethylenediaminetetraacetic tubes and centrifuged and stored at -70 °C until the day the blood samples were to be studied. PTX3 was measured by enzyme-linked immunosorbent assay using the assay kit of Perseus Proteomics Inc., Tokyo, Japan.

### Gensini Score Calculation

Gensini score was calculated by considering the degree of stenosis of the lesion in the coronary artery and segment in which it is located. Scoring was done according to the percentage of the degree of stenosis. One point was given for

a 0-25% stenosis, 4 points for a 25-50% stenosis, 8 points for a 75-90% stenosis, 16 points for a 90-99% stenosis, and 32 points for a 100% fully occluded stenosis. The Gensini score was obtained by multiplying the scores given with the coefficients for each segment defined in the literature[8].

### Study Endpoints and Follow-up

Follow-up continued for 10 years. The endpoint of the study was cardiovascular mortality. Cardiovascular mortality was defined as decompensated heart failure, fatal arrhythmias disrupting hemodynamics, and deaths due to AMI or unexplained sudden death.

### Statistical Analysis

Statistical data were obtained using the Statistical Package for the Social Sciences (SPSS) 20.0 (SPSS Inc, Chicago, IL, USA) application. The Kolmogorov-Smirnov test was used to evaluate the distribution of continuous variables. Continuous variables obtained as a result of the analysis were expressed as mean±standard deviation; categorical variables were expressed as percentages and numbers. Data that failed the Kolmogorov-Smirnov test of normality were expressed as median and width between quartiles of 25-75%. The t-test and Mann-Whitney tests were used to compare the parameters conforming to normal distribution, respectively. Chi-square analysis was used to compare categorical variables and Spearman correlation analysis was used for correlation analysis. Multivariate Cox regression analysis was performed to identify independent predictors of cardiovascular mortality. Kaplan-Meier analysis was performed for PTX3 values measured at the eighth hour for cardiovascular mortality. 95% confidence intervals (CI) were calculated with standardized beta coefficients. P values below 0.05 were considered statistically significant.

## RESULTS

Our study consisted of 55 patients in two groups, NSTMI (11 men, 7 women) and SAP (24 men, 13 women). While the median age of the NSTEMI group was 62 (50-64), the median age of the SAP group was 59 (54-64). Demographic and laboratory data of the patients are shown in Table 1. There were no differences between the groups in terms of medical treatments before admission to the cardiology clinic. When PTX3 levels were examined; while no difference was observed between baseline and 24$^{th}$-hour values, PTX3 values measured at the eighth hour were higher in the NSTEMI group compared to the SAP group (p=0.006). When the responsible occlusive lesions were compared between the groups, no statistical difference was observed (p=0.947) (Table 1). After 10 years of follow-up, cardiovascular mortality was observed in 6 patients in the NSTEMI group and 2 patients in the SAP group (p=0.011). The median length of life was 63 (49-76) months in the NSTEMI group and 84 (80-88) months in the SAP group (p=0.009) (Table 1).

In the correlation analysis, a significant correlation was observed between baseline PTX3 levels and Gensini score (r=0.299, p=0.026) (Table 2).

In Cox regression analysis, PTX3 levels measured at the eighth hour were found to be an independent predictor of cardiovascular mortality in the NSTEMI group (Hazard ratio: 1.294, 95% CI: 1.024-1.653, p=0.039) (Table 3).

As a result of receiver operating characteristic analysis, the cut-off for PTX3 values measured at the eighth hour was determined as 10.37 (75% sensitivity and 97% specificity p<0.001). Kaplan-Meier analysis for cardiovascular mortality was performed and is shown in Figure 1.

## DISCUSSION

In this study, we investigated the relationship of serum PTX3 levels with Gensini score and cardiovascular mortality in long-term follow-up in NSTEMI patients. As a result of the study; while PTX3 levels measured at the time of admission were found to be associated with the severity of CAD, PTX3 measured at the eighth hour was observed to predict cardiovascular mortality in 10-year follow-ups.

PTX3 is an acute phase protein that is similar in structure and function to CRP and belongs to the same family. PTX3 is associated with cardiovascular diseases and is released at high rates from atherosclerotic lesions[13,14]. Previous studies have shown that PTX3 is increased in ACS patients and that PTX3 can be used in the risk classification, especially in NSTEMI patients[15,16].

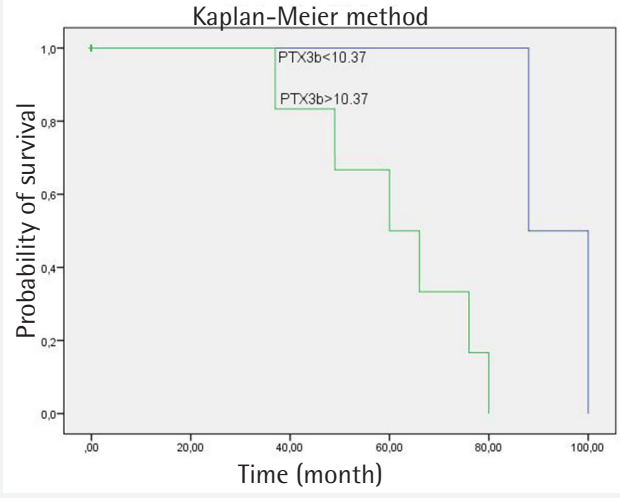


**Figure 1.** Kaplan-Meier analysis for cardiovascular mortality

**Table 1. Baseline characteristics and pentraxin-3 levels of study patients**

| Clinical characteristics | NSTEMI (n=18) | SAP (n=37) | p value |
|---|---|---|---|
| Age, year | 62 (50-64) | 59 (54-64) | 0.495 |
| Gender (female/male) | 7/11 | 13/24 | 0.786 |
| Diabetes (n, %) | 6 (33.3) | 12 (32.4) | 0.947 |
| Hypertension (n, %) | 12 (66.7) | 29 (78.4) | 0.510 |
| Body mass index (kg/m$^2$) | 27.7 (25-31.4) | 28 (26.1-30.4) | 0.788 |
| Smoking status (n, %) | 10 (55.6) | 16 (43.2) | 0.391 |
| SBP, mmHg | 132.33±24.13 | 128.68±16.72 | 0.568 |
| DBP, mmHg | 75.89±12.39 | 73.70±7.22 | 0.495 |
| Heart rate. minute | 76±16.19 | 75.16±13.32 | 0.850 |
| **Biochemical variables** | | | |
| Glucose (mg/dL) | 106 (97-138.5) | 111 (97-132.5) | 0.969 |
| Creatinine (mg/d) | 0.94±0.17 | 1.08±0.28 | 0.066 |
| Hemoglobin (g/dL) | 13.32±1.57 | 13.53±1.52 | 0.520 |
| White blood cell (x10$^3$/mL) | 7.9 (6.8-9.8) | 8.1 (6.7-9.3) | 0.603 |
| LDL (mg/dL) | 98 (76-151.5) | 121 (98-143) | 0.384 |
| HDL (mg/dL) | 39.29±10.77 | 42.28±8.44 | 0.311 |
| NYHA | 1 (1-2.5) | 1 (1-2) | 0.717 |
| LVEF | 60 (53.75-62.5) | 60 (53.5-61.5) | 0.760 |
| Gensini score | 17 (11.12-32) | 10 (2.25-48.75) | 0.364 |
| $PTX3_a$ | 5.01 (3.40-7.44) | 3.62 (2.04-8.61) | 0.206 |
| $PTX3_b$ | 13.37 (5.47-27.75) | 5 (3.83-12.42) | 0.006 |
| $PTX3_c$ | 4.44 (3.41-7.59) | 4.48 (3.12-8.52) | 0.747 |
| **Medical treatment before admission (n)** | | | |
| Aspirin | 17 | 29 | 0.244 |
| Statin | 12 | 18 | 0.208 |
| Beta blocker | 14 | 20 | 0.089 |
| **Responsible artery with lesion, n** | | | 0.947 |
| LAD | 7 | 16 | |
| Cx | 6 | 11 | |
| RCA | 5 | 10 | |
| **Cardiovascular mortality (n)** | 6 | 2 | 0.011 |
| Length of life (month) | 63 (49-76) | 84 (80-88) | 0.009 |

NSTEMI: Non-ST elevation myocardial infarction, SAP: Stable angina pectoris, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, LVEF: Left ventricular ejection fraction, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, NYHA: Functional classification of the New York Heart Association, $PTX3_a$: Serum blood level at the time of admission, $PTX3_b$: Serum blood level measured at the eighth hour, $PTX3_c$: Serum blood level measured at the 24$^{th}$ hour, LAD: Left anterior descending artery, Cx: Left circumflex, RCA: Right coronary artery

**Table 2. PTX3 levels and correlation analysis between variables**

| | $PTX3_a$ | | $PTX3_b$ | | $PTX3_c$ | |
|---|---|---|---|---|---|---|
| | r | p | r | p | r | p |
| Age | 0.276 | 0.459 | 0.059 | 0.668 | 0.107 | 0.435 |
| Gensini score | 0.299 | 0.026 | 0.264 | 0.051 | 0.203 | 0.138 |
| NYHA | 0.025 | 0.855 | 0.253 | 0.063 | 0.134 | 0.328 |

$PTX3_a$: Serum blood level at the time of admission, $PTX3_b$: Serum blood level measured at the eighth hour, $PTX3_c$: Serum blood level measured at the 24$^{th}$ hour, NYHA: Functional classification of the New York Heart Association

**Table 3. Cox regression analysis showing independent predictors of cardiovascular mortality**

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | HO (CI % 95) | p | HR (CI %95) | p |
| Age | 1.052 (0.941-1.176) | 0.372 | | |
| LVEF | 1.126 (0.875-1.145) | 0.356 | | |
| Gensini score | 1.000 (0.969-1.033) | 0.978 | | |
| $PTX3_a$ | 0.867 (0.585-1.287) | 0.479 | | |
| $PTX3_b$ | 1.123 (1.008-1.251) | 0.035 | 1.294 (1.014-1.653) | 0.039 |
| $PTX3_c$ | 1.009 (0.894-1.138) | 0.889 | | |

$PTX3_a$: Serum blood level at the time of admission, $PTX3_b$: Serum blood level measured at the eighth hour, $PTX3_c$: Serum blood level measured at the 24th hour, HR: Hazard ratio, CI: Confidence interval

Identifying high-risk groups in ACS patients and initiating optimal treatment in the early period are important to prevent cardiovascular events. Cardiac troponins and CRP are among the most studied biomarkers in ACS patients[17,18]. The most important disadvantage of CRP is its elevation in various conditions such as inflammation, malignancy and vasculitis[19]. PTX3 is released more specifically from the cells in atherosclerotic region[20]. Following AMI; while PTX3 plasma levels peak in approximately seven and a half hours, CRP levels peak within 50 hours[21]. In the light of all this information, PTX3 is a special biomarker in ACS patients, unlike CRP. As a matter of fact; PTX3 which was examined in patients with NSTEMI and unstable angina pectoris in the first six hours after the onset of chest pain was found to be more specific in a study in which it was compared with neutrophil activating peptide 2 and cardiac troponin I[22].

As can be understood from the case studies in the literature, it is possible that biomarkers have certain limitations in themselves. In our study, we thought that it would be more beneficial to study blood samples in consecutive time periods, not just once, in order to minimize this. When our study results were evaluated, especially when basal PTX3 levels were evaluated, no difference was observed between the NSTEMI and SAP groups. Although the Gensini score, which we used to evaluate the severity of CAD, was numerically higher in the NSTEMI group than in the SAP group in our study, it was not statistically significant. When the previous literature data is interpreted; although the lesions in the coronary arteries are evaluated anatomically and morphologically with the Gensini score, it is not possible to evaluate the content of the plaque causing the stenosis and the inflammatory activity. Therefore, although a correlation was observed with Gensini score and baseline PTX3 values in our study, it would be more accurate for PTX3 values measured consecutively after PCI to provide information about the structure of plaque in the coronary arteries and in terms of the correlation with long-term results. In our study, the eighth hour PTX3 level in the patients after PCI was found to be statistically and numerically significant in the NSTEMI group. As a matter of fact, it has been shown that the deterioration of the vessel layers after coronary stenting causes an increase in PTX3 levels. We think that a similar mechanism is effective in monitoring PTX3 levels higher than basal PTX3 values in our patients. It is known that neutrophils, monocytes and macrophages cause an increase in PTX3 levels in arterial thrombus in patients with AMI[23]. In addition to coronary artery stenting, the higher values that were measured at the eighth hour in the NSTEMI patient group compared to the SAP group may be responsible for the increase in PTX3 levels of increased neutrophil, monocytes and macrophage counts in the coronary arteries in NSTEMI patients. In addition, autopsies performed in patients with ACS have shown that inflammatory activity and extracellular matrix compositions differ phenotypically. Larger necrotic nuclei and higher macrophage activity have been demonstrated, particularly in ruptured plaques[24]. When all this information is evaluated, it is possible that there will be an increase in PTX3 levels after PCI, although vascular patency is achieved with PCI in unstable plaques in NSTEMI patients.

Information on the long-term prognostic value of PTX3 is limited in the literature. It has been shown that PTX3 values measured at admission in ST-elevation myocardial infarction (STEMI) patients are associated with 2-year all-cause mortality, and another study found that PTX3 values measured at the time of admission in patients with STEMI and NSTEMI were associated with cardiovascular mortality in five-year long-term follow-up[25,26]. In our study, it is an important advantage to have 10-year follow-up data, and another important difference is that consecutive PTX3 values were examined. Although PTX3 values measured at the time of admission were associated with Gensini score, PTX3 values measured at the eighth hour in long-term follow-up were found to be an independent predictor of cardiovascular mortality.

In-hospital mortality rates are lower in patients with NSTEMI than in patients with STEMI. However, long-term mortality rates are higher in NSTEMI patients compared to STEMI patients[27]. Therefore, early risk assessment in NSTEMI patients is important in long-term follow-up. PTX3, measured consecutively, can provide clinicians with important information about both the assessment of CAD severity and long-term cardiovascular mortality in NSTEMI patients.

### Study Limitations

The most important limitation of our study is that it was conducted with a limited number of patients. However, it was seen in which time period PTX3 levels measured in NSTEMI patients could be used to predict cardiovascular mortality. In our study, superior imaging devices such as 3-dimensional optical coherence tomography could not be used to evaluate the plaque causing stenosis in the coronary arteries and the severity of CAD. However, the Gensini score was used to evaluate the severity of CAD, as suggested in the literature.

## CONCLUSION

PTX3 levels are associated with long-term adverse cardiovascular outcomes in hospitalized and treated patients with a diagnosis of NSTEMI. PTX3 can be used for risk classification in NSTEMI patients.

### Ethics

**Ethics Committee Approval:** The study were approved by the Çanakkale Onsekiz Mart University Local Ethics Committee for the study (decision no: 2011-KAEK-27/2021-2100169941, date: 24.11.2021).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices - Concept – Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: U.K., B.K., E.E.

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# Evaluation of Femoral Anteversion and Femoral Neck-Shaft Angles in Cerebral Palsy and a Review of the Literature

## Serebral Palsili Pediatrik Hastalarda Femoral Anteversiyon ve Femur Boyun-Şaft Açılarının Değerlendirilmesi ve Literatürün Tekrar Değerlendirilmesi

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### ABSTRACT

**Aim:** Cerebral palsy (CP) is a chronic, sensorimotor disease as a result of damage in the brain that has not completed its development. In this study, we evaluated femoral anteversion (FA) and femoral neck-shaft (FNS) angles in pediatric patients with CP and studied whether there was a difference between the healthy population and patient group according to involvement subtypes or Gross Motor Functional Classification System (GMFCS).

**Materials and Methods:** Thirty patients (20 females, 10 males; mean age 10 years 8 months; range 4 to 14 years) diagnosed with spastic CP, who had undergone surgery, were evaluated retrospectively. Of thirty patients, eleven were quadriplegic, ten were diplegic and nine were hemiplegic. Fifty-one hips of 30 patients were investigated in the study. According to the GMFCS, there were nine patients at level II, eight at level III, six at level IV, and seven at level V. X-rays and computed tomograhy images of the patient and control group were evaluated by radiological files of the hospital. FA and FNS angles of all patients were measured and the obtained values were compared.

**Results:** The FNS angle ($p<0.05$) and the FA angle ($p<0.05$) were significantly larger in the patient group than in the control group. The FNS angle ($p<0.05$) and the FA angle ($p<0.05$) were significantly larger in the quadriplegic group than in the diplegic and hemiplegic groups. The FNS angle ($p<0.05$) and FA angle ($p<0.05$) were significantly larger in GMFCS level IV/V patients than in GMFCS level II/III patients.

**Conclusion:** FA and FNS angles are increased in CP compared to normal population due to the spasticity in some muscles acting on the hip and the resulting imbalance in muscle strength. In quadriplegics, angles are significantly higher compared to diplegics and hemiplegics, GMFCS level IV/V had higher angle values compared to GMFCS II/III.

**Keywords:** Cerebral palsy, femoral anteversion angle, femoral neck-shaft angle

### ÖZ

**Amaç:** Serebral palsi (SP), gelişimini tamamlamamış beyin hasarı sonucu oluşan kronik, sensorimotor bir hastalıktır. Bu çalışmada pediatrik SP'li hastalarda femoral anteversiyon (FA) ve femur boyun-şaft (FNS) açılarını değerlendirdik ve sağlıklı popülasyon ve hasta grubunun kendi içinde tutulum alt tiplerine ve Kaba Motor Fonksiyonel Sınıflandırma Sistemi'ne (GMFCS) göre bir fark olup olmadığını araştırdık.

**Gereç ve Yöntem:** Spastik SP tanısı alan ve cerrahi uygulanan 30 hasta (20 kadın, 10 erkek; ortalama yaş 10 yıl 8 ay; dağılım 4-14 yıl) geriye dönük olarak değerlendirildi. Çalışmada 30 hastanın 51 kalçası incelendi. Otuz hastanın 11'i kuadriplejik, 10'u diplejik ve dokuzu hemiplejikti. GMFCS'ye göre II. düzeyde dokuz, III. düzeyde sekiz, IV. düzeyde altı ve düzey V'te yedi hasta bulunmaktaydı. Hasta ve kontrol grubunun bilgisayarlı tomografi görüntüleri hastanenin radyolojik dosyalarından değerlendirildi. Tüm hastaların FA ve FNS açıları ölçüldü ve elde edilen değerler karşılaştırıldı.

**Bulgular:** FNS açısı ($p<0,05$) ve FA açısı ($p<0,05$) hasta grubunda kontrol grubuna göre anlamlı olarak daha büyüktü. Kuadriplejik hastalarda FNS açısı ($p<0,05$) ve FA açısı ($p<0,05$) diplejik ve hemiplejik gruba göre anlamlı olarak daha büyüktü. FNS açısı ($p<0,05$) ve FA açısı ($p<0,05$), GMFCS seviye IV/V hastalarında, GMFCS seviye II/III hastalarına göre anlamlı olarak daha büyüktü.

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**Sonuç:** Kalçaya etki eden bazı kaslarda spastisite ve buna bağlı olarak kas gücünde dengesizlik nedeniyle SP'de normal popülasyona göre FA ve FNS açıları artar. Kuadriplejiklerde açılar diplejiklere ve hemiplejiklere göre belirgin olarak daha yüksektir, GMFCS seviye IV/V, GMFCS II/III'e göre daha yüksek açı değerlerine sahiptir.

**Anahtar Kelimeler:** Serebral palsi, femoral anteversiyon açısı, femur boyun-şaft açısı

## INTRODUCTION

Cerebral palsy (CP) is proposed as a group of permanent disorders of the development of movement and posture, causing activity limitation, whereas the disturbances in fetal or infant brain are non-progressive. In CP, the spinal cord and muscles are structurally and biochemically normal so called all of the pathology is attributed to brain. The abnormality of the brain results in motor impairment[1].

The prevalence of CP is 2 in 1000 live births and the most common type of involvement is spastic diplegia constituting 80% of the patients[2]. The lesion in the brain creates problems in muscle tone and coordination. Over time, secondary disorders in the form of joint contractures develop due to unbalanced distribution of muscle strength in the musculoskeletal system[1-3].

Spasticity, which is an increase in the physiological tension of the muscle to passive movements, is caused by lesions in the cerebral cortex. Although the lesion in the brain does not show progression, adaptive changes secondary to spasticity occur over time in the musculoskeletal system[4].

Although muscle spasticity and contractures need physical therapy or surgical treatments like tendon lenghtenings and contracture releases for treatment, femoral anteversion (FA) and femur neck-shaft angle (FNS) increment of which are commonly seen in CP patients, leading to joint luxations or gait imbalance need purely surgical corrections for treatment[1,5]. Being aware of these angular changes, which can be detected by various measurement methods and corrected surgically, is extremely important in terms of the course of disease and determining the degree of planned surgical corrections to prevent hip instability and as well as hip joint luxation[4].

The Gross Motor Functional Classification System (GMFCS) is the most reliable classification system of CP patients according to capacity of mobility and independent movement and have been described for various bands[6].

The aim of this retrospective study is to evaluate FA and FNS angles of CP patients by comparing a healthy control group and also comparing the same angles within the patient group according to GMFCS levels.

## MATERIALS AND METHODS

Files of sixty patients (32 females, 28 males; mean age 11 years 2 months; range 4 to 22 years) with a diagnosis of spastic CP who had already been operated for surgical correction of FA and FNS angles between February 2000 and July 2004 were retrospectively evaluated after the approval of the Tekirdağ Namık Kemal University of Ethics Committee (protocol number: 2021.115.04.10, date: 27.04.2021).

In order to make the evaluation as objective as possible, patients who had no visual and cooperative problems, no significant mental retardation, and no extrapyramidal system involvement were chosen. Furthermore, patients using medications affecting motor functions (e.g. baclofen, botulinum toxin, and benzodiazepine) were not included in the study. At the end, thirty patients (20 females, 10 males; mean age 10 years 8 months; range 4 to 14 years), who met the criteria and had both X-rays and computed tomography (CT) images that had already been taken as a matter of fact before their surgeries to plan the correction amount, were included in the study. A control group was formed with 30 age-matched patients (18 females, 12 males; mean age 10 years 3 months; range 4 to 16 years) having no history of CP and being admitted to the emergency department of the hospital because of polytrauma and having undergone AP pelvic X-ray and whole body CT during evaluation. Their pelvic X-rays and CT views were investigated through radiological files of the hospital.

According to involvement subtypes, eleven were quadriplegic, ten were diplegic and nine were hemiplegic. While both hips of diplegic and quadriplegic patients were included in the study, only the involved hips of the hemiplegic patients were added. As a sum, 51 hips of 30 patients were investigated in the study.

According to the GMFCS, there were nine patients at level II, eight at level III, six atlevel IV, and seven at level V.

Twenty two of the patients in the study group were born before 36$^{th}$ gestational week, 4 had prenatal complications, and the other 4 patients had complications in 3 months and further after birth. So called prematurity was the most seen etiologic factor in the patient group.

On the AP pelvic X-rays, a straight line was drawn from the midline of the femoral neck by accepting the midline according to two reference points. The femoral anatomical axis was also drawn and the angle at the medial side of the two lines was measured with a goniometer, providing the FNS angle (Figure 1)[7].

In order to measure the FA angle, the longitudinal axis of the femoral neck was drawn in the scan where the femoral neck appeared longest in the hip (Figure 2) in CT scans. Then, the bicondylar axis was drawn in the femoral condylar region (Figure 3). The angle of the bicondylar axis with the longitudinal axis of the femoral neck was measured[8]. This angle provided the FA value.

Data related to the study and control groups are given in Table 1 and Table 2, respectively.

For all comparisons, only the involved sides of hemiplegic patients were included in the study group (11 quadriplegic patients-22 hips, 10 diplegic patients-20 hips, 9 hemiplegic patients-9 hips; a total of 30 patients-51 hips).

Patients in the study and control groups were compared using the parameters listed below.

1. Homogeneity of variables in the study and control groups,

2. Age distribution between the study and control groups,

3. FNS and FA angle values between the study and control groups,

4. Comparison of FNS and FA angle values in the study group based on CP involvement types,

5. Comparison of FNS and FA angle values in the study group based on GMFCS types.

## Statistical Analysis

To evaluate the number of the patients in the study group, G*power programme was used. The sample size was evaluated by large effect size (d=0.8) and the mean error level of $\alpha$=0.05 and at the end of analysis, it was evident that at least 52 people had to be involved in the study, 26 for the study group and another 26 for the control group. For this reason, the sample size of the research was determined to be at least 52. Within the scope of data analysis, independent samples t-test, which is one of the parametric tests, was used while comparing the experimental and control groups according to variables. Before analyzing, Q-Q plot and P-P plots were examined for the assumption of normality, and it was seen that the normality assumption was met. For the assumption of homogeneity of variances, the Levene's test value was examined and it was found that the variances were not homogeneous.

For this reason, the results of the Wald test were used to calculate the t value.

The analyses were carried out in the PASW statistics 18 (2009; SPSS Inc. Chicago, IL, ABD) program and the level of significance was determined as $\alpha$=0.05.

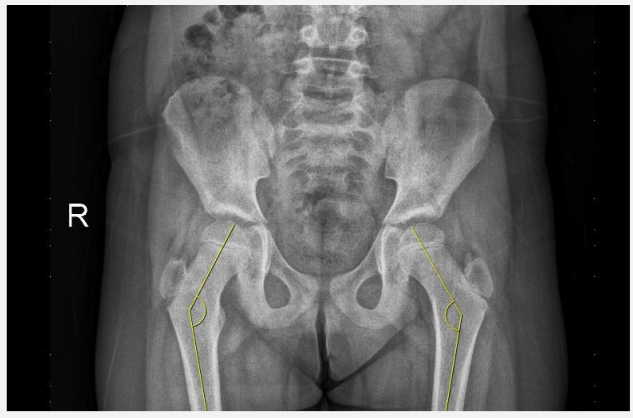


**Figure 1.** Femoral neck-shaft angle measurement in pelvis AP X-ray in a qudriplegic patient. Right side is measured as 143 degree, left side is measured as 141 degree

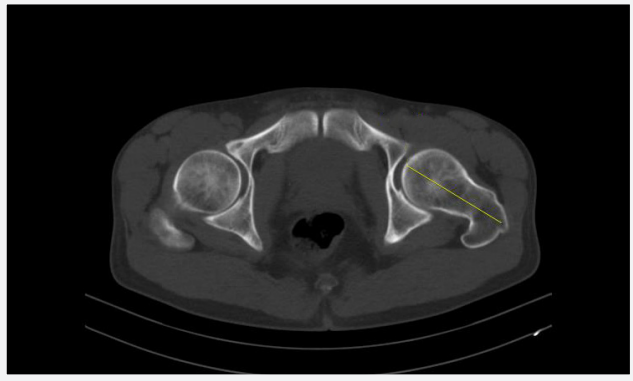

**Figure 2.** Reference points for left hip in computed tomography scan for measuring femoral anteversion

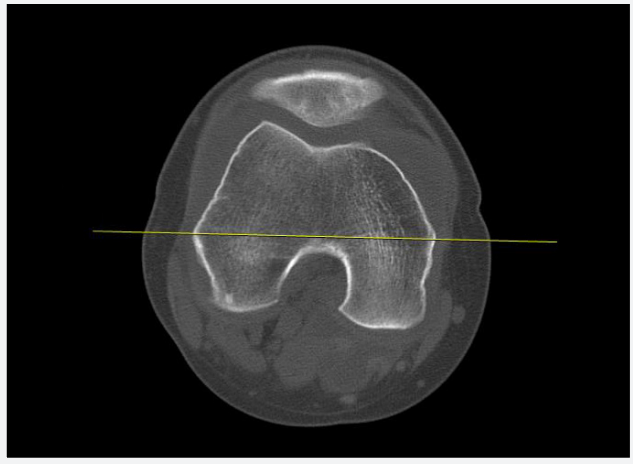

**Figure 3.** Reference points for knee in computed tomography scan for measuring femoral anteversion

## RESULTS

All variables in the study and control groups were found to be homogeneous ($p<0.05$). There was no significant difference between the study and control groups in terms of age distribution ($p>0.05$). The FNS angle ($p<0.05$) and the FA angle ($p<0.05$) were significantly larger in the patient group than in the control group. The FNS angle ($p<0.05$) and the FA angle ($p<0.05$) were significantly larger in the quadriplegic group than in the diplegic and hemiplegic groups. The FNS angle ($p<0.05$) and FA angle ($p<0.05$) were significantly larger in the GMFCS level IV/V patients than in the GMFCS level II/III patients.

All of the variables and standard deviations are seen in Table 3.

## DISCUSSION

Adduction, flexion and internal rotation deformities are the most commonly seen deformities in the hips of CP patients[1]. The type of deformity in CP patients varies depending on the type of involvement as well as the severity and distribution of spasticity. Since spasticity is more severe in quadriplegic and diplegic patients compared to hemiplegic patients, deformities may also be more severe in these patients[9]. In our study, we also revealed that quadriplegic ones had more bony deformities than the others in the patient group.

At birth, every infant, even CP patients, have a FA angle of about 40 degrees[10]. During the development process, in a healthy child, FA and its angle gradually decrease in accordance

**Table 1. Patient group data**

| | Age | Type | Femoral anteversion angle (degrees) | | Femoral neck-shaft angle (degrees) | | GMFCS level |
|---|---|---|---|---|---|---|---|
| | | | Right | Left | Right | Left | |
| 1 | 4 | Quadriplegic | 24 | 28 | 150 | 154 | V |
| 2 | 5 | Right hemiplegic | 29 | 15 | 146 | 138 | II |
| 3 | 5 | Quadriplegic | 37.6 | 38.8 | 142 | 142 | IV |
| 4 | 6 | Quadriplegic | 56 | 44 | 152 | 156 | IV |
| 5 | 6 | Diplegic | 50 | 50 | 142 | 142 | III |
| 6 | 7 | Quadriplegic | 30 | 34 | 158 | 162 | V |
| 7 | 8 | Left hemiplegic | 22 | 31 | 136 | 142 | V |
| 8 | 9 | Quadriplegic | 30 | 26 | 152 | 150 | III |
| 9 | 9 | Diplegic | 26 | 34 | 142 | 142 | III |
| 10 | 9 | Right hemiplegic | 21 | 10 | 142 | 136 | II |
| 11 | 9 | Diplegic | 32 | 30 | 140 | 142 | II |
| 12 | 9 | Right hemiplegic | 33 | 21 | 142 | 136 | IV |
| 13 | 10 | Diplegic | 45 | 41 | 142 | 142 | IV |
| 14 | 10 | Quadriplegic | 21 5 | 29 5 | 160 | 158 | III |
| 15 | 10 | Diplegic | 64 | 53 | 140 | 140 | V |
| 16 | 11 | Diplegic | 32 | 25 | 140 | 140 | III |
| 17 | 11 | Right hemiplegic | 40 | 26 | 142 | 128 | II |
| 18 | 11 | Quadriplegic | 40 | 50 | 140 | 140 | V |
| 19 | 12 | Diplegic | 49 | 46 | 141 | 142 | II |
| 20 | 12 | Left hemiplegic | 19 | 37 | 135 | 142 | III |
| 21 | 12 | Quadriplegic | 21 | 38 | 142 | 141 | IV |
| 22 | 12 | Left hemiplegic | 18 | 42 | 136 | 142 | II |
| 23 | 13 | Diplegic | 31 | 28 | 143 | 141 | II |
| 24 | 13 | Diplegic | 39.5 | 42.5 | 138 | 138 | III |
| 25 | 13 | Quadriplegic | 32 | 30 | 140 | 140 | IV |
| 26 | 13 | Left hemiplegic | 14 | 20 | 132 | 142 | II |
| 27 | 14 | Quadriplegic | 40 | 46 | 156 | 161 | V |
| 28 | 14 | Left hemiplegic | 14 | 26 | 132 | 142 | II |
| 29 | 14 | Quadriplegic | 30 | 32 | 139 | 138 | V |
| 30 | 14 | Diplegic | 53.5 | 52 | 138 | 140 | III |

GMFCS: Gross Motor Functional Classification System

with the Wolf's law due to the extensive tension of the muscle forces to the proximal femur and the plasticity of the skeletal system in the child until the age of 16 years, and decrease to the physiological level of 15 degrees in adults[10]. Whereas in CP patients, FA angle remains the same and high as a value. The main reason for the increase in FA angle in patients with CP is increased spasticity in the iliopsoas muscle[7]. Another responsible factor is the partially or completely diminished strength of the gluteal muscles adhering to the apophysis of the trochanter major[11-14]. In our study, we also concluded that FA angle was higher in the patient group compared to the healthy control group.

Although FNS angles of healthy children and children with CP are similar in the first years of life, the angles of children with CP barely change or even increase while the angles of healthy children decrease as they progress towards adult age[10]. Miller et al.[9] declared that increase of FNS angle was associated with the shortening of the femoral neck due to an increase in FA. We also declared that FNS angles were higher in the study group compared to the control group.

FA and FA angle can be measured with a numerous methods including physical examination[5,15,16], biplanar radiology X-ray[17,18], fluoroscopy[10], ultrasonography[9], 2 or 3-dimensional CT[15,19-21], and magnetic resonance imaging[22].

There are some methodological interactions for the evaluation or validation of FA angle. Chung et al.[23] revealed that physical examination combined with AP pelvic X-ray was enough for FA angle evaluation and this diminished the need for CT and excess radiation.

**Table 2. Control group data**

| | Age | Femoral anteversion angle (degrees) | | Femoral neck-shaft angle (degrees) | |
|---|---|---|---|---|---|
| | | Right | Left | Right | Left |
| 1 | 4 | 30 | 30 | 132 | 132 |
| 2 | 4 | 30 | 30 | 130 | 130 |
| 3 | 6 | 27 | 27 | 132 | 132 |
| 4 | 6 | 27 | 27 | 134 | 134 |
| 5 | 7 | 24 | 24 | 132 | 132 |
| 6 | 7 | 23 | 23 | 130 | 130 |
| 7 | 7 | 24 | 24 | 132 | 132 |
| 8 | 7 | 23 | 23 | 128 | 130 |
| 9 | 8 | 24 | 24 | 130 | 130 |
| 10 | 8 | 25 | 25 | 130 | 130 |
| 11 | 8 | 24 | 24 | 130 | 130 |
| 12 | 9 | 21 | 20 | 130 | 130 |
| 13 | 9 | 22 | 22 | 128 | 130 |
| 14 | 9 | 22 | 22 | 132 | 130 |
| 15 | 9 | 21 | 21 | 130 | 130 |
| 16 | 9 | 22 | 22 | 128 | 128 |
| 17 | 10 | 21 | 21 | 128 | 128 |
| 18 | 10 | 21 | 20 | 130 | 128 |
| 19 | 11 | 20 | 21 | 129 | 129 |
| 20 | 11 | 20 | 21 | 130 | 130 |
| 21 | 12 | 20 | 20 | 129 | 129 |
| 22 | 12 | 20 | 20 | 130 | 130 |
| 23 | 12 | 20 | 20 | 129 | 129 |
| 24 | 12 | 21 | 21 | 130 | 130 |
| 25 | 13 | 20 | 21 | 130 | 130 |
| 26 | 13 | 20 | 20 | 129 | 129 |
| 27 | 14 | 15 | 16 | 130 | 130 |
| 28 | 14 | 15 | 16 | 129 | 129 |
| 29 | 16 | 14 | 14 | 129 | 129 |
| 30 | 16 | 15 | 15 | 130 | 130 |

| Table 3. Femoral anteversion angles and femoral neck-shaft angles of patient group according to cerebral palsy type | | | | |
|---|---|---|---|---|
| | N (patients) | N (hips) | Femoral anteversion angle (degrees) | Femoral neck-shaft angle (degrees) |
| Total | 30 | 51 | 58.50±33.67 (15.50-58.50) | 142.98±6.91 (135-160) |
| CP type | | | | |
| Quadriplegic | 11 | 22 | 34.47±8.29 (25.50-50) | 148.77±8.52 (138.50-160) |
| Hemiplegic | 9 | 9 | 24.33±6.01 (15.50-33) | 138.39±1.93 (135-142) |
| Diplegic | 10 | 20 | 41.18±10.96 (28.50-58.50) | 140.75±1.44 (138-142) |
| GMFCS | | | | |
| II/III | 17 | 27 | 31.13±10.92 (15.50-52.75) | 141.35±5.71 (135-159) |
| IV/V | 13 | 24 | 36.98±10.22 (26-58.50) | 145.12±7.94 (138.50-160) |
| GMFCS: Gross Motor Functional Classification System, CP: Cerebral palsy | | | | |

Miller et al.[24] revealed that ultrasonography of the hip performed in internal rotation position gave better results than CT. In an experimental investigation on femora models, Riccio et al.[25] found that 3D reconstruction of CT was necessary and gave more accurate results about FA angles independent of FNS angles.

Davids et al.[21] revealed that the most important was the position of the patient during CT scanning. If the position is inaccurate then even 3D reconstruction will give false results vice versa 2D CT is enough for correct results.

In case of FNS angles, Kay et al.[26] revealed in a cadaveric study that positioning of the extremity in 15 degrees of internal rotation would give the most accurate information about FNS angle.

Davids et al.[15] reported that trochanteric prominence angle test and 3D CT scans gave similar increasing of FA and FNS angle results in CP patients so there was no need for CT to evaluate these patients.

In contrast to all these, Scorcelletti et al.[8] have revealed that there is not a reliable evaluation method for FA and FNS angles and CT gives high doses of radiation to patients so must not be used.

There is still a debate in the literature about the accurate measurement technique of FA and FNS in CP patients and a consensus could not been achieved yet. In our study, we used 2 dimensional CT for the measurement of FA angles and plain pelvic X-rays for FNS angle measurements.

Yamaguchi et al.[14] revealed that in non-ambulatory patients, FNS and FA were found in higher values than in ambulatory patients.

Bobroff et al.[10] stated that FA angle was higher in ambulatory CP patients compared to non-ambulatory ones but in contrast, FNS was smaller as an angle value in ambulatory ones compared to non-ambulatories, and they concluded this with fluoroscopy method.

In a biplanar radiographic study, Laplaza and Root[17] concluded that there was not any difference between ambulatory and non-ambulatory CP patients in means of FA angles but they also reported that FNS angle was higher in non-ambulatory ones.

Gose et al.[27] found with 3D CT that FA angle change was controversial but FNS angle was higher in non-ambulatory ones and they also revealed that in quadriplegic and GMFCS IV-V levels, FNS and FA angles were higher compared to diplegics and GMFCS II-III respectively.

Massaad et al.[28] revealed that FA and FNS angle increased in CP patients compared to a healthy control group and they concluded this with low dose biplanar X-ray technique.

Robin et al.[5] concluded that FA angles were higher in GMFCS I-II patients than in healthy control group but were lower than GMFCS III to V group and they also concluded that FNS angles increased as GMFCS level increased.

Laplaza et al.[7] reported that GMCSF level was the main factor affecting FA and FNS angles so called when level increased the angles also increased.

In our study, we found that FA and FNS angles were both higher in non-ambulatory group of CP patients compared to ambulatory ones by means as GMFCS level increased both of the angles also increased and we also concluded that FA and FNS angles were higher in quadriplegics compared to diplegics and hemiplegics.

## Study Limitations

This article has some limitations. The age variability in a large range is a limitation. Another limitation is that measurement of FNS angle on AP X-ray alone is not sufficient, these measurements cause errors especially in the hips with increased FA. Actually, this measurement on the AP X-ray gives the projected angle, while the true angle is obtained from the intersection points of the projected FNS angle in AP pelvic and lateral hip X-rays taken at 90 degrees of hip flexion and 20 degrees of hip abduction on corrected tables[29].

## CONCLUSION

CP patients are coming in front of orthopedic surgeons with increasing in number by the development and wellness of newborn intensive care units so called they care premature newborns much better compared to past. As the number of CP patients increases, their evaluation and surgeries also increase both in number and in importance.

FA and FNS angles are important for well-being of the hips of CP patients. FA and FNS angles increase in this patient group and this will bring their hips in danger for instability and luxation. If there is any suspicion of a change in FA and FNS angles in the clinical examinations performed during the periodic visits of patients with CP, these angles should be examined. If FA increase causing apparent toeing-in gait pattern in patients or FA and FNS increase showing tendency of subluxation is detected, soft tissue and osseous surgical interventions should not be avoided in order to prevent further hip pathologies. The progression of coxa valga is more critical and grave in quadriplegics and GMFCS IV/V group.

### Ethics

**Ethics Committee Approval:** The study were approved by the Tekirdağ Namık Kemal University of Local Ethics Committee (protocol number: 2021.115.04.10, date: 27.04.2021).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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

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# Can Hamstring Tendons be Used as Autografts in Peroneal Tendon Reconstruction? A Cadaveric Study

## Peroneal Tendon Rekonstrüksiyonunda Otogreft Olarak Hamstring Tendonları Kullanılabilir mi? Kadavra Çalışması

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### ABSTRACT

**Aim:** Tendon transfers and autografts can be used in the reconstruction of chronic peroneal tendon tears. This cadaveric study aimed to evaluate the use of autograft hamstring tendons to reconstruct peroneal tendons in terms of diameter suitability.

**Materials and Methods:** In this study, 13 hamstring tendons (gracilis, semitendinosus) without macroscopic injury and degeneration from the lower extremity of 13 fresh frozen cadavers were harvested and measured by standard methods. Then, peroneal tendons (peroneus longus, peroneus brevis) of the same cadavers were harvested and measured by standard methods. Tendon diameters were measured from the middle region of the tendon using a digital micro-caliper. After the measurements were completed, the thickness of the hamstring tendons and both peroneal tendons were statistically evaluated.

**Results:** The mean age of the cadavers included in the study was 74.07±12.25 (minimum: 51, maximum: 94) years, and the mean body mass index was calculated as 25.38±6.07. There was no statistically significant difference by gender in the evaluated tendon diameters ($p>0.05$ for each). A positive correlation was found between hamstring tendons (gracilis and semitendinosus) and peroneus longus and brevis tendons in terms of size ($p<0.01$ for each). In addition, in the measurement of the mean tendon diameter from the middle region, the mean diameter of the semitendinosus tendon was found to be closer to the mean diameter of the peroneal tendons.

**Conclusion:** In the reconstruction of chronic peroneal tendon rupture, the semitendinosus tendon's being used as an autograft for both peroneal tendons might be more appropriate according to the evaluation of the tendon diameter from the middle region.

**Keywords:** Peroneal tendon, peroneal tendon reconstruction, hamstring autograft

### ÖZ

**Amaç:** Kronik peroneal tendon yırtıklarının rekonstrüksiyonunda tendon transferleri ve otogreftler kullanılabilir. Bu kadavra çalışmasında peroneal tendonların rekonstrüksiyonu için otogreft hamstring tendonlarının kullanımının çap uygunluğu açısından değerlendirilmesi amaçlandı.

**Gereç ve Yöntem:** Çalışmada 13 (4 kadın, 9 erkek) taze donmuş kadavra alt ekstremitesinden otogreft olarak 13 adet makroskopik yaralanması ve dejenerasyonu olmayan, hamstring tendonları (gracilis, semitendinosus) standart yöntemler ile elde edildi ve ölçüme alındı. Ardından aynı kadavraların peroneal tendonları (peroneus longus, peroneus brevis) standart yöntemler ile elde edilerek ölçüme alındı. Tendon çap ölçümleri tendonların en kalın olduğu orta bölgesinden dijital mikro kumpas yardımıyla yapıldı. Ölçümler sonucunda hamstring tendonları ile her iki peroneal tendon kalınlıkları istatistiksel olarak değerlendirildi.

**Bulgular:** Çalışmaya dahil edilen kadavraların yaş ortalaması 74,07±12,25 (minimum: 51, maksimum: 94) yıl iken vücut kitle indeksi ortalaması 25,38±6,07 olarak bulundu. Çapları değerlendirilen tendonlar ile cinsiyet arasında istatistiksel olarak anlamlı bir fark bulunmadı (her biri için $p>0,05$).

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Hamstring tendonları (grasilis ve semitendinosus) boyutları ile, peroneus longus ve brevis tendonları arasında pozitif bir korelasyon mevcuttu (her biri için p<0,01). Ayrıca tendon orta çap değerlendirmesi sonucuna göre semitendinozus tendon çap ortalamasının peroneal tendonların çap ortalamasına daha yakın olduğu tespit edilmiştir.

**Sonuç:** Kronik peroneal tendon yırtıklarının rekonstrüksiyonunda otogreft olarak, her iki peroneal tendonun rekonstrüksiyonu için semitendinozus tendonunun kullanılması tendon orta çapları değerlendirmesine göre daha uygun olabileceği kanaatine varıldı.

**Anahtar Kelimeler:** Peroneal tendon, peroneal tendon rekonstrüksiyonu, hamstring otogreft

## INTRODUCTION

Problems associated with the peroneal tendon constitute an essential part of posterolateral ankle complaints and are often associated with anatomical abnormalities that predispose to chronic lateral ankle instability[1]. Peroneal tendon disorders can be encountered clinically as tendinitis, chronic tenosynovitis, subluxation, wear, longitudinal fissures, partial tears, and complete tears[2,3]. One study reported that only 60% of peroneal tendon disorders could be diagnosed correctly at the first clinical examination[4]. Although the exact prevalence of peroneal tendon tears in the general population is unknown, it has been reported that 11-38% of the samples were ruptured in cadaver studies. Left untreated, these disorders can cause persistent lateral ankle pain and significant functional disability[5,6].

Although there is no standard protocol in treating peroneal tendon disorders, conservative treatment or surgical treatment are among the options. Surgical treatment is preferred, especially when conservative treatment is not sufficient, such as tears and tendon subluxations[7].

Although tubularization and primary repair can be applied in acute partial tears, chronic tendon injuries require different treatment methods such as tendon transfers, tendon lengthening, allograft reconstructions, or synthetic graft reconstruction[8-10].

It has been reported that satisfactory results were obtained with allograft in peroneal tendon repair[10]. However, allograft tendon transfer is accompanied by several concerns such as tissue compatibility, sterilization, disease transmission, and cost[11]. A study has reported that hamstring autografts are an excellent option in peroneal tendon repair; while providing a biomechanical advantage for the patient, it results in better outcomes biologically than allograft reconstruction[12].

This cadaveric study aims to evaluate the compatibility of semitendinous and gracilis tendon autografts in terms of tendon size in peroneal tendon reconstruction.

## MATERIALS AND METHODS

Ethics committee approval was given to the Medical Research Evaluation Board (ATADEK) study with the date 09.07.2020 and the decision number 2020-15/12. This anatomical study included 13 unpaired fresh frozen cadaver legs (four females, nine males) stored at +4 C. No evidence of skin incision, scar tissue, external deformity, or trauma was observed around the knee and ankle in any of the legs. The mean preservation time from death to dissection was one month. Preoperative ankle range of motion (ROM) was measured with a goniometer, and ankle movements were regular. Exclusion criteria included significant osteoarthritis (>Stage 3), ligament damage at the medial or lateral ankle, and damage to the hamstring tendons. Hamstring tendons were harvested in the supine position while peroneal tendon dissections were performed in the prone position. The width of the peroneal tendons was measured using calipers at three regions standardized in each tendon, and mean values were used. The same person performed all dissections to eliminate inter-observer variability.

### Hamstring Tendon Preparation

An anteromedial approach harvested hamstring tendons. A standard release followed by a closed scraper was used to harvest the gracilis and semitendinosus tendons after they were identified at the tibial attachment sites. Following graft preparation and cleaning from adherent muscle and adipose tissue, a load of 89 newtons was applied to each doubling tendon for 15 minutes, and each tendon diameter was measured with a digital micro-caliper (Neiko 01407A Electronic Digital Caliper, Neiko Tools, China) with a resolution of 0.1 and a precision of 0.02 mm. The thickness was measured from three different points of each sample, and the mean value was recorded[13,14].

### Peroneal Tendon Preparation

Peroneal tendons were harvested by palpation, followed by a retro-malleolar posterolateral approach. A standard release followed by a closed scraper was used to harvest the peroneus longus and peroneus brevis tendons. Following graft preparation and cleaning from adherent muscle and adipose tissue, a load of 89 newtons was applied to each doubling tendon for 15 minutes, and each tendon diameter was measured with a digital micro-caliper (Neiko 01407A Electronic Digital Caliper, Neiko Tools, China) with a resolution of 0.1 and a precision of 0.02 mm. The thickness was measured from three different

points of each sample, and the mean value was recorded[13,14].

### Statistical Analysis

SPSS v20 program was used for data evaluation. The Shapiro Wilk-W test was used to determine the conformity of the data to the normal distribution. Variables are given as mean±standard deviation or frequency (percent). The normality t-test or Mann-Whitney U test was used to compare continuous variables. Correlation between tendon diameters from the middle region and peroneal tendons was evaluated with the Pearson correlation test. The significance level was accepted as $p<0.05$.

## RESULTS

Of the 13 cadavers included in the study, 9 (69.23%) were male, and 4 (30.76%) were female, with a mean age of 69.07±10.35 (minimum: 51, maximum: 94) years. The mean body mass index of the cadavers was calculated as 23.38±6.47 (Table 1). There was no statistically significant difference by gender in the evaluated tendon diameters ($p>0.05$ for each) (Table 2).

The mean tendon diameters were 5.65±0.66 mm, 4.22±0.37 mm, 6.56±0.49 mm, and 5.22±0.33 mm for semitendinosus, gracilis, peroneus longus, and peroneus brevis, respectively. A positive correlation was found between hamstring tendon (gracilis and semitendinosus) diameters and peroneus longus and brevis tendon diameters ($p<0.01$) (Table 3).

## DISCUSSION

Krause and Brodsky[15] are the first authors to present a classification system that guides the treatment of rare irreparable peroneal tendon tears. According to their definition, in cases where more than 50% of the tendon is affected, tendonesis can be performed on the remaining healthy tendon after segmental resection. Although tendonesis is a simple procedure, there is insufficient evidence for its clinical results. In some studies, it has been stated that tendonesis applied after an irreparable tear will not provide the normal tension of the peroneal tendons effectively and can be repaired with allograft and autograft reconstruction[12,16]. In 2010, Ousema and Nunley[17] published the first successful results of allograft reconstruction of the peroneus brevis in a series of 4 cases. Again, Mook et al.[10] reported successful clinical results in a retrospective series of 14 patients who underwent peroneal tendon reconstruction

**Table 1. Anthropometric features of cadavers**

| | | |
|---|---|---|
| **Age (years)** | **Min-Max (median)** | **51-94 (76)** |
| | **Av.±SD** | **69.07±10.35** |
| **Gender, n (%)** | Male | 9 (69.23) |
| | Female | 4 (30.76) |
| **Side, n (%)** | Left | 4 (30.76) |
| | Right | 9 (69.23) |
| **BMI** | Min-Max (median) | 19-39 (26) |
| | Av.±SD | 23.38±6.47 |
| **Weight (pound)** | Min-Max (median) | 70-240 (155) |
| | Av.±SD | 153.33±50.40 |
| **Size (inch)** | Min-Max (median) | 60-77 (65) |
| | Av.±SD | 64.33±8.40 |

Min-Max: Minimum-maximum, Av.: Average, SD: Standard deviation, BMI: Body mass index

**Table 2. Distribution of mean tendon diameters by gender**

| | Semitendinosus | Gracilis | P. longus | P. brevis |
|---|---|---|---|---|
| Male | 5.74±0.71 | 4.29±0.39 | 6.62±0.56 | 5.24±0.38 |
| Female | 5.45±0.45 | 4.06±0.29 | 6.42±0.30 | 5.16±0.18 |
| p | 0.49 | 0.31 | 0.51 | 0.68 |

**Table 3. Correlation between the diameter from the middle region of hamstring tendons and the diameter from the middle region of peroneus tendons**

| | Peroneus longus | | Peroneus brevis | |
|---|---|---|---|---|
| | r | p | r | p |
| Semitendinosus | 0.97 | <0.001 | 0.84 | <0.001 |
| Gracilis | 0.84 | <0.001 | 0.83 | <0.001 |

with a peroneal or semitendinosus allograft. However, in the study of Mook et al.[10], only the semitendinosus tendon was used, and no comparison was made with gracilis. Literature for peroneal tendon reconstruction with autograft is rare. In 2018, Ellis and Rosenbaum[12] were the first authors to describe the surgical technique for reconstructing the peroneus brevis with semitendinosus autograft without any clinical consequences. In this study, the compatibility of the diameter of the autogenous hamstring tendons in peroneal tendon reconstruction was evaluated with a cadaveric study.

A positive correlation was found in the study between the diameter from the middle region of the semitendinosus and gracilis tendons and the peroneus tendons. In addition, it was determined that the mean diameter of the semitendinosus tendon was closer to the mean diameter of the peroneus longus and peroneus brevis tendon. In another study, hamstring autograft was used to reconstruct the superior peroneal retinaculum injured in chronic dislocations, although not directly for peroneal tendon repair[18]. Many studies report that hamstring tendons are used primarily for anterior cruciate ligament repair, and good results are obtained[19-21]. In another study comparing hamstring tendons, it was shown that semitendinosus is superior to gracilis both in cross-sectional area width and biomechanics[22]. Zhao and Huangfu[23], in a biomechanical study in which they compared the peroneus longus anterior half with the hamstring tendon for use as autograft, showed that the endurance of the peroneus longus anterior half (7.8 N/mm) was similar to that of the semitendinous endurance (8.6 N/mm) but higher than the gracilis endurance (4.1 N/mm). In our study, semitendinosus was measured wider than gracilis in terms of tendon width. A closer value was found in the comparison of semitendinosus and peroneal tendon diameter measurements. In our study, the width of the semitendinosus tendon was more significant than the width of the gracilis. The diameters of semitendinosus and peroneal tendons were almost similar. In 2019, Nishikawa et al.[24] reported successful results with a short follow-up in their series of 3 cases where they performed peroneus brevis reconstruction with semitendinosus autograft. However, research on the use of hamstring autografts for peroneal tendon repair is minimal.

### Study Limitations

Like most laboratory studies, this research has methodological limitations. First of all, the compatibility of the tendons was evaluated only by measuring the diameter. The lack of biomechanical comparison with autograft after reconstruction is one of the limitations of our study. Secondly, the cadavers used in the study are of advanced age, and the number of cadavers is low. The fact that the cadavers were fresh frozen was an advantage.

## CONCLUSION

Gracilis tendon diameter was found to be smaller than those of the peroneus longus and peroneus brevis tendon, and the semitendinosus autograft is considered to be a more suitable option for peroneal tendon reconstruction in terms of diameter compatibility. There are still not enough studies on this subject. There is a need for biomechanical studies and clinical studies with long-term follow-up on the use of hamstring tendons as autografts in peroneal tendon reconstruction.

### Ethics

**Ethics Committee Approval:** The study were approved by the Mehmet Ali Aydınlar University of Ethics Committee (protocol number: ATADEK-2020/5, date: 09.07.2020).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: M.K., B.Y., Concept: D.P.K., B.Y., Design: M.K., E.D.Ç., E.N.K., Data Collection or Processing: D.P.K., E.N.K., Analysis or Interpretation: N.K., Literature Search: M.K., E.N.K., Writing: M.K., E.N.K.

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# Are Patients with Different Rheumatologic Diseases Under Immunosuppressive Therapies Adequately Screened and Protected Against Viral Hepatitis?

## İmmünosupresif Tedaviler Altında Farklı Romatolojik Hastalıklarla İzlenen Hastalar Viral Hepatitlere Karşı Yeterince Taranıyor ve Korunuyor mu?

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**ABSTRACT**

**Aim:** The aim of this study is to determine the screening rates for hepatitis B (HBV) and C virus (HCV) in patients with rheumatologic diseases who receive immunosuppressive therapies, to evaluate the prevalence of HBV reactivation during the regimens and also to reveal the frequency of vaccination.

**Materials and Methods:** This retrospective study included the patients who were followed-up with different rheumatologic diseases in two rheumatology outpatient clinics. The immunosuppressive regimens were categorized into two groups as biologic (bDMARDs) and conventional synthetic disease modifying anti rheumatic drugs (csDMARDs). The markers of HBsAg, anti-HBs, anti-HBc-IgM and anti-HBc-IgG, HBV DNA, anti-HCV levels were all taken from the patients' charts checked prior and during the immunosuppressive treatments.

**Results:** There were 451 patients [61.9% female, mean age 41.1 years, (standard deviation: 13.78)] who were taking bDMARDs (n=348) and csDMARDs (n=103). The data for HBV for 20 (4.4%) patients and HCV for 23 (5%) patients were missing, all in the csDMARDs group. Also, HBV serology tests were found to be incomplete in 51 patients (14.7%) in the bDMARDs group, as not checking the anti-HBc-IgM and anti-HBc-IgG. During the follow-up, HBV reactivation was not observed in the whole cohort. In the bDMARDs group, there were 39 patients who did not receive prophylaxis despite having HBsAg negative phase of chronic HBV infection; no HBV reactivation was observed also in this group. One hundred twenty nine (28.6%) of the patients were evaluated as never infected and unvaccinated prior to immunosuppressive therapies. Recurrent HBV serology controls were performed in nearly half (n=75) of them during their follow-up and it was observed that all were still non-immune.

**Conclusion:** The screening rates of HBV and HCV serology were detected as successful in rheumatology patients under immunosuppressive therapies. No HBV reactivation was observed in the entire group. Also, the study showed that there was a significant deficiency in immunizing patients against HBV in follow-up.

**Keywords:** Hepatitis B virus, hepatitis C virus, immunosuppressive therapies, anti-rheumatic drugs, hepatitis B immunization

**ÖZ**

**Amaç:** Çalışmanın amacı immünosupresif tedavi altındaki romatoloji hastalarının viral hepatit B (HBV) ve C (HCV) için taranma sıklığının değerlendirilmesi, HBV reaktivasyon sıklığının tespit edilmesi, HBV aşılanma oranlarının değerlendirilmesidir.

**Gereç ve Yöntem:** Bu retrospektif çalışmaya romatoloji polikliniğinde izlenmekte olan immünosupresif tedavi alan hastalar dahil edilmiştir. İmmünosupresif tedaviler biyolojik (bDMARD) ve konvansiyonel sentetik (ksDMARD) hastalığı modifiye eden antiromatizmal ilaçlar olarak sınıflandırılmıştır. Hastaların İmmünosupresif tedavi başlanmadan hemen önce veya tedavi esnasında bakılan serum HBsAg, anti-HBs, anti HBc-IgM, anti HBc-IgG, HBV DNA ve anti-HCV belirteçleri dosya taramalarından elde edilmiştir.

**Bulgular:** Çalışmada 451 hasta yer almıştır [%61,9 kadın, ortalama yaş 41,1 yıl, (standart sapma: 13,78)]. Bu hastalar bDMARD (n=348) ve ksDMARD (n=103) kullananlar olarak iki gruba ayrılmıştır. Tüm hasta grubunda 20 (%4,4) hastanın HBV verisi, 23 (%5) hastanın ise HCV verisi olmadığı

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izlendi, bu hastaların tamamının ksDMARD grubunda olduğu görüldü. Ayrıca bDMARD grubundaki 51 hastanın (%14,7) HBV seroloji tetkiklerinin yeterli ayrıntıda olmadığı izlendi (anti-HBc-IgM ve anti HBc-IgG tetkikleri izlenmedi). Tüm kohortta izlem esnasında akut HBV enfeksiyonu veya HBV reaktivasyonu hiç izlenmedi. bDMARD hasta grubunda HBsAg negatif fazda kronik HBV'si olmasına rağmen profilaksi almayan 39 hastada da HBV reaktivasyonu izlenmediği görüldü. Hastalardan 129'unun (%28,6) immünosupresif tedavi öncesinde non-immün/aşısız olduğu görüldü. Bu hastalardan izlemleri esnasında tekrarlayan HBV serolojisi kontrol edilenlerin (n=75) halen non-immün/aşısız olduğu gözlendi.

**Sonuç:** İmmünosupresif tedavi altındaki romatoloji hastalarının yapılan HBV ve HCV serolojilerinin tarama sonuçları başarılı olarak değerlendirildi. Tüm grupta hiç HBV reaktivasyonu izlenmedi. Bu çalışmanın sonuçları ayrıca HBV non-immün olan romatoloji hastalarının takipte aşılanmalarının yetersiz düzeyde olduğunu göstermiştir.

**Anahtar Kelimeler:** Hepatit B virüsü, hepatit C virusü, immünosupresif tedavi, anti romatizmal ilaçlar, hepatit B aşılama

## INTRODUCTION

Reactivation of hepatitis B (HBV) during immunosuppressive treatments could present as serious clinical conditions such as fulminant hepatitis and liver failure[1]. It has been shown that many immunosuppressive treatments used in rheumatology practice may be associated with HBV reactivation. Anti-CD20 regimens [e.g. rituximab (RTX)][2], corticosteroids (CS)[3], methotrexate (MTX)[4] and tumor necrosis factor alpha inhibitors (TNFi)[1] could enhance the chance of HBV reactivation to varying degrees in hepatitis B surface antigen (HBsAg) positive patients. Despite the lack of consensus, current guidelines recommend screening patients for HBV with serum HBsAg and anti-HBc prior to immunosuppressive therapies[5].

The high risk of reactivation for HBsAg positive HBV cases and the necessity of having prophylaxis under immunosuppressive therapy have taken their place as very strong recommendations in the guidelines[6-8]. However, the evidence for HBsAg negative and anti HBc positive occult HBV cases that will receive TNFi is not so clear. Studies report a lower risk of reactivation (1.7-5%) in this group when compared to HBsAg positive patients[1,9]. It is therefore recommended that the decision between prophylactic antiviral therapies versus follow-up should include risk stratification according to immunosuppressive regimen that the patient is taking.

Hence, the aim of this retrospective cohort study was to determine HBV screening rates, the frequency of HBV reactivation rates and also the vaccination and prophylaxis rates in rheumatology patients under different immunosuppressive therapies.

## MATERIALS AND METHODS

This study included 451 patients who were followed-up with rheumatologic diseases in rheumatology outpatient clinics in Mardin State Hospital and Bursa City Hospital. The data of patients were taken from the charts of the patients who were admitted to Rheumatology outpatient clinics in a time period of 3 months between October 2019 and January 2020 for Mardin State Hospital and 1 month in March 2020 for Bursa City Hospital retrospectively.

In the patient group, the demographic data, diagnosis, the medications [all the immunosuppressive agents as csDMARDs, CS, nonsteroid anti-inflammatory drugs (NSAIDs), bDMARDs] were all recorded.

Exclusion criteria for patient group were determined as,

1. Being younger than 18 years old,

2. Having previous diagnosis of malignancy, immunodeficiency syndromes, chronic infections as tuberculosis,

3. Having previous treatments for malignancy as cytotoxic therapies chemotherapy or radiotherapy,

4. Having signs and symptoms of rheumatologic diseases, but with diagnosis not yet clarified,

5. Having a diagnosis of a rheumatologic disease but no immunosuppressive therapy use.

The diagnosis of rheumatologic diseases and medications were recorded and categorized into two groups as bDMARDs and csDMARDs. All the TNFi, RTX, small molecules [tofacitinib (TOFA)], and IL-1 antagonists were categorized as bDMARDs. And, MTX, sulfasalazine (SLZ), azathioprine (AZA), hydroxychloroquine sulfate (OHQ), leflunomide (LEF), mycophenolate mophetil (MMF), and colchicine were categorized as the csDMARDs group. Also, the status of taking CSs and NSAIDs was recorded in both group of patients. If the patient was using a csDMARDs in addition to biologics, this patient was evaluated in the bDMARDs group.

At the inclusion visit, HBsAg, anti HBs, anti HBc-IgM and anti HBc-IgG, Anti HCV, HBV DNA, HCV RNA levels were all taken from the patients' charts, which were recorded at the initiation of immunosuppressive therapies and during the use of therapies. In addition, patients with disorders of liver function tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase, gamma-glutamyl transferase, bilirubin levels] during the period of immunosuppressive therapies were noted with etiologies (drug induced, viral, toxic, etc.). Patients who received viral hepatitis prophylaxis during their follow-up and the agents they received were noted.

Viral hepatitis reactivation was defined as reverse seroconversion of HBsAg in HBsAg negative patients or a rise of serum HBV DNA level by one log or greater compared to the pre-exacerbation baseline period, or a new detection of HBV DNA in patients with previously undetectable HBV DNA in patients.

The study was performed according to the Declaration of Helsinki and Bursa City Hospital Ethics Board approved the study with approval number 2021-13/8, date: 14.07.2021.

### Statistical Analyses

Data were statistically analysed with Statistical Package for the Social Science 13.0 (SPSS) program. Results were expressed as mean with standard deviation (SD) and median with minimum (min), maximum (max) values and interquartile range according to the distribution of the data. Baseline characteristics in terms of categorical variables were compared using the Mann-Whitney U test. Categorical variables were analyzed using the chi-square test. A 5% type-1-error level was used to infer statistical significance.

## RESULTS

There were 451 patients from two centers from two different cities in Turkey [61.9% female, mean age 41.1 years, (SD: 13.78)]. The diagnosis of the patients was mainly rheumatoid arthritis and ankylosing spondylitis (Table 1).

There were two main groups of patients, as the patients in the cohort who were taking bDMARDs (n=348) and those who were taking csDMARDs (n=103).

The biological therapies were adalimumab (n=101), etanercept (n=54), certolizumab (n=47), infliximab (n=42), golimumab (n=41), TOFA, n=20, RTX, n=17, tocilizumab (n=11), secucinumab (n=7), Anakinra (n=3), canacinumab (n=3), abatacept (n=2). The majority of patients (n=277) who had bDMARDs were taking the first-line biologics, also 38 of them were taking second-line, 24 of them were taking third-line, 8 of them were taking fourth-line and 1 of them was taking the fifth-line biological therapy. Moreover, in the bDMARDs group, 58 (16.7%) patients were taking LEF, 46 (13.2%) patients were taking MTX, 43 (12.4%) patients were taking SLZ, 42 (12.1%) patients were taking OHQ, 21 (6%) patients were taking colchicine, 11 (3.2%) patients were taking AZA, and 5 (1.4%) patients were taking MMF; concomitant to bDMARDs. Totally 226 (64.9%) patients were using concomitant csDMARDs during biologic use.

In the csDMARDs group, 47 patients (45.6%) were taking MTX, 41 (39.8%) patients were taking OHQ, 23 (22.3%) were taking LEF, 17 (16.5%) patients were taking SLZ, 14 (13.6%) patients were taking colchicine, 11 (10.6%) were taking AZA, and 7 (6.8%) patients were taking MMF in different combinations.

Furthermore, 190 (42.1%) patients were taking CS (the mean dosage was 7.16 mg prednisone equivalent SD 4.80, min: 5 max: 25 mg) and 239 (53%) patients were taking NSAIDs in all bDMARDs and csDMARDs groups. One hundred and fourteen (32.8%) patients were exposed to CSs during bDMARDs use and 76 (73.8%) patients were exposed to CSs during only-csDMARDs use.

The data for viral hepatitis B for 20 patients were missing, all in the csDMARDs group. Also, viral hepatitis B serology tests were found to be incomplete in 51 (14.7%) patients in the bDMARDs group. In all of these patients, the missing test was determined as not checking the anti HBc-IgM and anti HBc-IgG, which were recommended by guidelines. In the csDMARDs group, viral hepatitis B serology was only evaluated with HBsAg and anti-HBs in 26 (%25.2) patients and only with HBsAg in 30 (%29.1) patients as simple screening (Table 2). Also, the data for viral hepatitis C (HCV) were missing in 23 (5.1%) of the patients, all in the csDMARDs group, with not checking anti-

**Table 1. The rheumatologic diagnoses of the patients**

| Diagnosis | Patients (n) | Patients total (n, %) |
|---|---|---|
| AS | 169 | 185 (41%) |
| AS/Behçet | 4 | |
| AS/Crohn | 8 | |
| AS/UC | 1 | |
| AS/Crohn/FMF | 1 | |
| AS/FMF | 2 | |
| RA | 176 | 186 (41.2%) |
| RA/SLE | 4 | |
| RA/Scl | 1 | |
| RA/SjS | 3 | |
| RA/adult Still disease | 2 | |
| SLE | 15 | 19 (3.3%) |
| Behçet | 18 | 22 (4.9%) |
| Peripheral Spa | 10 | 10 (2.2%) |
| FMF | 6 | 9 (2%) |
| EGPA | 1 | 1 (0.22%) |
| GPA | 1 | 1 (0.22%) |
| PM/Scl | 1 | 2 (0.44%) |
| PM/SjS | 1 | |
| PsA | 13 | 13 (2.88%) |
| Scl | 5 | 7 (1.55%) |
| SjS | 2 | 6 (1.33%) |
| Adults Still disease | 1 | 3 (0.66%) |
| TAK | 6 | 6 (1.33%) |
| Total | 451 | |

AS: Ankylosing spondylitis, RA: Rheumatoid arthritis, UC: Ulcerative colitis, FMF: Familial Mediterranean Fever, SLE: Systemic lupus erythematosus, Scl: Scleroderma, SjS: Sjögren's syndrome, Spa: Spondyloathritis, EGPA: Eosinophilic granulomatosis polyangiitis, GPA: Granulomatosis polyangiitis, PM: Polymyositis, PsA: Psoriatic arthritis, TAK: Takayasu arteritis

HCV. The test was evaluated as negative in all 428 patients who were tested or anti-HCV. Therefore, it was observed that HCV-RNA testing was not considered necessary in the entire patient group.

It was determined that HBV DNA follow-up was performed in 28 patients in the whole group. HBV DNA positivity was observed in only 4 of these patients, as not meeting the reactivation criteria.

The immunization rates for HBV in patients under bDMARDs were higher than patients under csDMARDs (p<0001). The rate of not being screened for HBV or being screened with simple tests for HBV (as checking only the HBsAg with/without anti-HBs) was detected higher in patients under csDMARDs than bDMARDs (p<0001) (Table 2). Patients under the bDMARDs group were detected as screened with detailed tests for HBV compared to the csDMARDs group (Table 2).

Antiviral prophylaxis for hepatitis B was given to 52 (11.4%) patients in total group with tenofovir (n=42), entecavir (n=7), and lamivudine (n=3). These patients were predominantly in the bDMARDs group (n=50). While 7 patients of the prophylaxis group consisted of HBsAg positive patients, all the remaining prophylaxis patients were detected in the HBsAg negative phase of chronic HBV infection.

The mean follow-up time was 27 months (min: 5 months, max: 58 months). During the follow-up of 451 patients, acute HBV infection was not observed in any of the patients. No hepatitis B reactivation was observed in the whole cohort. In the bDMARDs/TNFi group (non-RTX), there were 39 patients who did not receive prophylaxis despite having HBsAg negative phase of chronic HBV infection; no hepatitis B reactivation was also observed in this group.

One hundred twenty nine (28.6%) of the patients were evaluated as unvaccinated prior to immunosuppressive therapies. Recurrent HBV serology controls were performed in nearly half (n=75, 58.1%) of them during their follow-up and it was observed that all were still unvaccinated/non-immune.

Elevated liver enzymes (AST-ALT) were detected in 11 of the patients during the follow-up. It has been determined that toxic hepatitis due to drugs (4 isoniazid, 3 colchicine, 1 MMF), disease involvement in liver (1 myositis, 1 SLE), and primary biliary cirrhosis (1) were involved in the etiology.

## DISCUSSION

This retrospective study showed that rheumatologists had satisfactory screening rates for HBV and HCV in patients prior to immunosuppressive therapies. No HBV reactivation was observed in the cohort, as a possible indicator of adequate screening and the appropriate use of prophylaxis. But also, the study showed that there was a significant deficiency in immunizing patients against HBV in follow-up.

Despite the lack of consensus, current guidelines recommend screening for HBV and HCV infection prior to all immunosuppressive therapy[5]. Guideline recommendations aside, real-life data in studies have actually shown that viral hepatitis screening is not optimal in immunosuppressive patients. There were two interview-based studies investigating rheumatologists' awareness of HBV and screening practices for HBV prior to immunosuppressive therapies. One of them detected that only 69% reported performing appropriate screening before bDMARDs[10]. In the other study, 93.8% of the physicians thought that screening should be performed before immunosuppressive therapies[11]. A study from Turkey showed

**Table 2. Viral hepatitis B serology in patients in the bDMARDs and csDMARDs groups**

| Serology | | | | | bDMARDs (n, %) | csDMARDs (n, %) | p value |
|---|---|---|---|---|---|---|---|
| HBsAg | Anti-HBs | Anti-HBc-IgG | Anti-HBc-IgM | | | | |
| (+) | (-) | (+) | (-) | Chronic HBV infection | 6 (1.7%) | 3 (2.9%) | 0.43 |
| (+) | (-) | (+) | (+) | Acute HBV infection | 0 (0%) | 0 (0%) | -- |
| (-) | (-) | (+) | (-) | Resolved HBV infection | 27 (7.7%) | 4 (3.9%) | 0.26 |
| (-) | (+) | (+) | (-) | Natural immunity after exposure HBV | 56 (16%) | 2 (1.9%) | <0.0001 |
| (-) | (-) | (-) | (-) | Not infected, no immunization | 119 (34.2%) | 10 (9.7%) | <0.0001 |
| (-) | (+) | (-) | (-) | Immunization | 89 (25.6%) | 8 (7.8%) | <0.0001 |
| (-) | (-)/(+) | Missing | Missing | Simple screening | 23 (6.6%) | 26 (25.2%) | <0.0001 |
| (-) | Missing | Missing | Missing | Simple screening | 28 (8%) | 30 (29.1%) | <0.0001 |
| Missing | Missing | Missing | Missing | No screening | 0 (0%) | 20 (19.4%) | <0.0001 |
| | | | | | Total (n) 348 | Total (n) 103 | |

bDMARDS: Biologic synthetic disease modifying anti rheumatic drugs, csDMARDs: Conventional synthetic disease modifying anti rheumatic drugs, HBV: Viral hepatitis B, HBsAg: Hepatitis B surface antigen, anti-HBs: Hepatitis B surface antibody, anti HBc-IgM/G: Hepatitis B core antibody Immunoglobulin M and G, n: Number

the overall HBV screening rate in patients before receiving TNFi as 82.3%, and this rate had an increasing trend during the years (64% in 2010, 87.4% in 2019)[12]. In a large cohort study from Germany, the HBV screening rate was found to be 94% in patients using bDMARDs, and it was considered successful[13]. In our cohort, the data for viral hepatitis B for 20 patients were missing, all in the csDMARDs group (4.4% of total patients). Viral hepatitis B serology scans were performed on all the patients in bDMARDs (100%). However, 107 (23.7%) of total patients were evaluated with incomplete examination as simple screening (Table 2). This screening rates of viral hepatitis serology was detected as successful in patients both in the bDMARDs and csDMARDs groups in this study.

The risk of reactivation of HBsAg-positive patients who are under immunosuppressive therapy has been known for long years. The guidelines of the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of Liver Diseases (EASL), and the Asian Pacific Association for the Study of Liver Diseases (APASL) all recommend prophylaxis in HBsAg positive patients under immunosuppressive therapy[5,8,14].

However, over time, it has been determined that besides HBsAg positive patients, those who are HBsAg negative but have positive antibodies against the core antigen (anti-HBc-IgM and IgG) carry the risk of reactivation under certain immunosuppressive therapies. These patients actually have chronic hepatitis B (CHB) with HBsAg seroclearance but they still carry HBV DNA material in the liver. They may or may not have antibodies to anti-HBs. The only positive serologic marker indicating previous HBV exposure could be having anti-HBc. The risk of reactivation differs with the type of immunosuppressive therapy in this group. Treatments such as RTX, CSs, TNFi, and MTX used in rheumatology practice are some immunosuppressive regimens that have been shown to increase the risk of HBV reactivation at different rates (>10%, 1-10%, 1%, <1%, respectively) in HBsAg negative phase CHB patients. The guidelines of AASLD, APASL, and EASL include different recommendations for this patient group, such as close monitoring, HBV DNA control or giving prophylaxis directly according to immunosuppressive drug regimen with weak evidence. For a patient with natural immunity from prior exposure to HBV (Table 2), the American College of Rheumatology guidelines strongly recommend that treatments should be the same as that of unexposed patients, as long as the patient's viral load is monitored regularly every 6-12 months[15,16].

In the follow-up of the patients in this study, the national viral hepatitis screening and treatment recommendations of the Turkish Society for Rheumatology were applied[17]. The need for antiviral prophylaxis was arranged according to the factors of the patients and the immunosuppressive therapy they received. There were 39 patients who did not receive prophylaxis despite having HBsAg negative phase of chronic HBV infection; no HBV reactivation was observed in this group of patients using TNFi. The study of Fidan et al.[12] supported this finding and showed that the risk of reactivation in occult HBV cases was very low (0.4%) in patients using TNFi. Similarly, Lee et al.[9] showed HBV occult carriers had HBV reactivation risk with a rate of 1.7% when treated with TNFi. This rate rises to 11.3-18.9% (reactivation according to virologic endpoints) and 41.5% (reactivation according to HBV DNA) in patients taking RTX in previous studies[18,19]. In our cohort, all the patients with occult HBV under RTX were detected as taking prophylaxis.

For this reason, appropriate risk stratification is needed for individual types of immunosuppressive therapies[20]. Since the seroprevalence of anti-HBc could be very high among HBV endemic regions (>40%)[21], it would not be cost effective to use HBV prophylaxis directly in all patients taking immunosuppressive therapies[20]. Some researchers state that the necessity of routine anti-HBc screening also needs more evidence in the patients who will use immunosuppressive therapies associated with a low risk of HBV reactivation[12]. Similarly, this study showed that in daily practice, rheumatologists only screen HBsAg and anti-HBs for HBV in patients under immunosuppressive treatments with a low risk of HBV reactivation (Table 2). The conditions of national health insurances can also be effective in adopting this approach by limiting the blood tests per visit. In this study, the higher vaccination rates and the higher screening rates with detailed tests in the bDMARDs group compared to the csDMARDs group showed that the physicians acted more cautiously, considering the bDMARDs group to be more risky for HBV (Table 2). Similarly, the screening rates for HCV were found to be higher in the bDMARDs group.

Another remarkable finding in this study was the low HBV vaccination rates of the patients. One hundred twenty nine (28.6%) of the patients were evaluated as never infected and unvaccinated prior to immunosuppressive therapies; also, nearly half of them were still non-immune for HBV during their follow-up. These data showed that rheumatologists had suboptimal HBV immunization rates in patients with rheumatologic diseases receiving immunosuppressive therapies. The frequency of recommendation of HBV vaccine to non-immune patients by physicians might be low, the efficacy of vaccines administered under immunosuppressive therapies might be weak, or vaccines might not be double dosed in this patient group as the guidelines suggested. These findings indicated that whatever the cause, insufficient attention was paid to immunizing patients against HBV in follow-up. In the literature, these are the first study notifying data on vaccination rates in the follow-up of the immunosuppressive patient group.

### Study Limitations

As strength, this study was a cohort study based on real-life data. In the literature, it was observed that most of the studies investigating the approaches of physicians to HBV screening and treatment were interview-based studies. However, more detailed results can be obtained by keeping the number of participating rheumatologists and clinical centers larger. As limitations, in the study, the follow-up time was short and the design of the study was in a retrospective nature.

## CONCLUSION

In conclusion, this study suggested that the screening rates of viral hepatitis serology were detected as satisfactory in patients under immunosuppressive therapies. Although the cohort included cases of occult hepatitis B using TNFi and not receiving prophylaxis, no HBV reactivation was observed in the entire group. Moreover, this study showed that there was a significant deficiency in vaccination against HBV in non-immune patients in follow-up.

### Ethics

**Ethics Committee Approval:** The study was performed according to the Declaration of Helsinki and Bursa City Hospital Ethics Board approved the study with approval number 2021-13/8, date: 14.07.2021.

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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

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# Evaluation of the Relationship Between Polypharmacy and Malnutrition in Diabetic Elderly

## Diyabetik Yaşlılarda Polifarmasi ve Malnütrisyon İlişkisinin Değerlendirilmesi

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### ABSTRACT

**Aim:** Type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases in older adults. With advancing age, polypharmacy and protein-energy malnutrition associated with chronic diseases can be seen frequently in T2DM patients due to metabolic causes and may adversely affect the prognosis. In this study, it was aimed to evaluate the relationship between polypharmacy and malnutrition in T2DM patients.

**Materials and Methods:** Three hundred and twenty-one patients aged 65 years and over, diagnosed with T2DM and receiving oral anti-diabetic drug therapy, who applied to the Internal Medicine and Geriatrics outpatient clinic between February and November 2021, were included in the study. The data of the patients were obtained retrospectively from their medical files. The use of 5 or more drugs was considered as polypharmacy. Mini Nutritional Assessment-short form (MNA-SF) was used for nutritional status assessment.

**Results:** The median number of concomitant medications used in patients followed up with the diagnosis of T2DM was 5. Polypharmacy was found in 209 (65.1%) patients, and severe polypharmacy was found in 21 (6.5%) patients. Malnutrition was found in 43 (20.6%) patients with polypharmacy, while 17 (80.1%) of 21 patients with severe polypharmacy had malnutrition. A positive correlation was found between the number of drugs and HbA1c, and a negative correlation with the MNA-SF score ($r=0.792$, $p<0.001$, $r=-0.317$, $p<0.001$, respectively). According to the logistic regression analysis, the presence of HbA1c and polypharmacy were found to be effective factors in the development of malnutrition ($p=0.009$, $p=0.002$).

**Conclusion:** Current findings show that polypharmacy is quite common in elderly T2DM patients and often accompanies malnutrition. It is important to review the drugs used in each visit and to evaluate malnutrition that may be related to newly started or currently used drugs so that intertwined polypharmacy and malnutrition are not overlooked in elderly patients.

**Keywords:** Diabetes mellitus, polypharmacy, malnutrition, elderly patients

### ÖZ

**Amaç:** Tip 2 diabetes mellitus (T2DM), yaşlı yetişkinlerde sık rastlanan kronik hastalıklardandır. İlerleyen yaşla birlikte kronik hastalıklarla ilişkili polifarmasi ve protein-enerji malnütrisyonu metabolik nedenlere bağlı olarak T2DM hastalarında sık görülebilir ve prognozu olumsuz etkileyebilir. Bu çalışmada T2DM hastalarında polifarmasi ve malnütrisyon ilişkisinin değerlendirilmesi amaçlanmıştır.

**Gereç ve Yöntem:** Şubat-Kasım 2021 tarihleri arasında İç Hastalıkları ve Geriatri Polikliniği'ne başvuran, 65 yaş ve üzeri T2DM tanısı olan ve oral anti-diyabetik ilaç tedavisi alan 321 hasta çalışmaya dahil edildi. Hastaların verileri tıbbi dosyalarından retrospektif olarak elde edildi. Beş ve üzeri ilaç kullanımı polifarmasi olarak kabul edildi. Beslenme durum değerlendirmesinde Mini Nutrisyonel değerlendirme- kısa form (MNA-SF) kullanıldı.

**Bulgular:** T2DM tanısı ile takip edilen hastalarda eşzamanlı kullanılan ilaçların medyan sayısı 5 idi. Hastaların 209'unda (%65,1) polifarmasi, 21'inde (%6,5) ise şiddetli polifarmasi saptandı. Polifarmasisi olan hastaların 43'ünde (%20,6) malnütrisyon saptanırken şiddetli polifarmasisi olan 21 hastanın 17'sinde (%80,1) malnütrisyon vardı. İlaç sayısı ile HbA1c arasında pozitif, MNA-SF puanı ile negatif korelasyon saptandı (sırasıyla $r=0,792$, $p<0,001$, $r=-0,317$, $p<0,001$). Yapılan lojistik regresyon analizine göre HbA1c ve polifarmasi varlığı malnutrisyon gelişiminde etkili faktörler olarak bulundu ($p=0,009$, $p=0,002$).

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**Sonuç:** Mevcut bulgular yaşlı T2DM hastalarında polifarmasinin oldukça yaygın olduğunu ve sıklıkla malnütrisyona eşlik ettiğini göstermektedir. Yaşlı hastalarda birbiri ile iç içe geçmiş polifarmasi ve malnütrisyonun gözden kaçırılmaması için her vizitte kullanılan ilaçların gözden geçirilmesi, yeni başlanan veya kullanılmakta olan ilaçlarla ilişkili olabilecek malnütrisyonun değerlendirilmesi önemlidir.

**Anahtar Kelimeler:** Diabetes mellitus, polifarmasi, malnütrisyon, yaşlı hastalar

## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases among older adults due to aging and the gradual increase in life expectancy[1]. Elderly patients have multiple comorbidities such as hypertension, dyslipidemia, coronary heart disease and chronic kidney disease[2]. As the most important consequence of this, more than one drug is often needed to adequately and appropriately treat T2DM and associated comorbidities. Commonly, the "use of five or more drugs per day" is defined as polypharmacy[3]. Polypharmacy increases the risk of increased hypoglycemia, decreased medication adherence, drug-drug interactions, and higher hospitalization, mortality, and healthcare costs[4]. In these patients, individualized treatments should be applied to control/balance other comorbid conditions and/or complications besides T2DM and to minimize and/or prevent drug-related risks; therefore, it is a complex process[5]. Although non-pharmacological interventions for the management of T2DM and related comorbidities are an integral part of the treatment plan, pharmacotherapy actually remains the cornerstone of management.

Malnutrition is defined as "a nutritional state in which a lack of energy, protein and other nutrients causes measurable adverse effects on tissue and body form (body shape, size and composition) and function and clinical outcomes"[6]. With advancing age, especially protein-energy malnutrition is common, and it also causes personal, social and economic burden[7]. The number and scope of drugs used in the elderly, especially symptoms such as loss of appetite or constipation related to the drugs used, are the most important factors that directly or indirectly affect the risk of malnutrition through the development of tolerance/unwillingness for food in patients[8]. A positive correlation has been observed between polypharmacy and malnutrition in cross-sectional studies[9].

Many factors have been associated with malnutrition in older adults and the most notable of them in recent years is undoubtedly polypharmacy. Since the emergence of diseases that require pharmacological treatment is more common with aging, the prevalence of polypharmacy is likely to increase in advanced ages[10]. The relationship between malnutrition and polypharmacy is quite striking, especially in elderly T2DM patients using different drugs that affect appetite[11]. Therefore, in our study, we aimed to evaluate the frequency and relationship of polypharmacy and malnutrition in elderly T2DM patients who applied to the Internal Medicine and Geriatrics Outpatient Clinic.

## MATERIALS AND METHODS

### Participants of the Study

In this study, 321 patients aged 65 years and over, who applied to İnönü University Medical Center Internal Medicine and Geriatrics Polyclinic between February 2021 and November 2021, were diagnosed with T2DM and were using oral anti-diabetic (OAD) drug therapy, were included. The study was designed as a retrospective, cross-sectional study. The demographic (age, gender, comorbidities), anthropometric, clinical and laboratory data of the patients and the comprehensive geriatric examination test results performed at each patient visit were retrospectively scanned from the medical files of the patients and recorded in the forms. The files of 450 patients who applied to the outpatient clinic were evaluated. According to this evaluation, 129 patients who were not diagnosed with T2DM, were diagnosed with T2DM but received insulin therapy and could not complete comprehensive geriatric tests (due to neurological or psychiatric diseases) were excluded from the study. Body weight was measured in kilograms. Body mass index (BMI) was calculated with the formula of body weight/height$^2$ (kilogram/meter$^2$).

### Comprehensive Geriatric Evaluation

Among the comprehensive geriatric assessment tests, the Katz Index of Independence in Activities of Daily Living, Lawton Instrumental Activities of Daily Living Scale (IADL), Mini-Mental State Examination (MMSE), Geriatric Depression Scale (short form consisting of 15 questions) (GDS) and the Mini Nutritional Assessment Short Form (MNA-SF) were used. The activities of daily living were evaluated with the Katz ADL. This index evaluates the functions of dressing, bathing, going to the toilet, getting out of bed, eating and urinary incontinence over 6 points[12]. Instrumental activities of daily living were evaluated using the Lawton IADL. In this scale, activities such as phone use, shopping, meal preparation, housework, laundry, urban transportation, and correct drug use are evaluated over eight points[13,14]. Cognitive functions were investigated by MMSE. Low scores obtained from this test, which is evaluated over

30 points, indicate deterioration in cognitive functions[15,16]. In the non-cognitive evaluation, the 15-item short form GDS of Yesevage was used[17]. Nutritional status was investigated with MNA-SF. Considering the Turkish validity and reliability of this test, 0-7 points indicate malnutrition, 8-11 points indicate malnutrition risk, and 12-14 points indicate normal nutrition[18].

Data on the number of drugs prescribed were recorded during the patient visit. Medications taken daily or at regular intervals were defined as regular use. Occasionally taken drugs were defined as drugs taken when needed and were not included in the number of drugs in the study. Polypharmacy status was defined as two subgroups. Severe polypharmacy was defined as the use of ten or more drugs, and polypharmacy was defined as the use of five to nine drugs[8].

### Biochemical Measurements

Blood samples taken from the patients after at least 8 hours of fasting were taken into a 4 cc gel biochemistry tube and a hemogram tube containing 2 cc citrate. Fasting blood glucose, urea, creatinine, total protein, albumin, complete blood count, 25 hydroxy vitamin D (25-OH Vit D), C reactive protein and HbA1c levels were studied in these tubes.

### Statistical Analysis

Statistical analyses were performed with SPSS for Windows version 22.0 (IBM SPSS Statistics, Armonk, NY). The distribution of normality was checked using the Shapiro-Wilk test. The Mann-Whitney U test was employed to compare two groups of independent variables that did not have normal distribution, the chi-square test was used to evaluate the relationship between categorical variables, and the Spearman's rank correlation coefficients were used to evaluate the relationship between numerical variables. In order to determine the independent predictors of malnutrition, first of all, linear regression analysis was performed to calculate the variance inflation factor, and the factors causing the multicollinearity problem were removed from the model, then multivariate logistic regression analysis was performed.

### Ethical Principles

The study was approved by the Ethics Committee of Non-Interventional Clinical Researches of İnönü University with the date of 14.12.2021 and the decision number of 2021/2837. This study was carried out in accordance with the ethical standards of the Declaration of Helsinki. Volunteer participants were included in the study and their personal identity information was kept confidential. A voluntary consent form was obtained from each of the participants.

## RESULTS

The demographic information of the patients is summarized in Table 1. A total of 321 patients, 105 of whom were male, were included in the study. The mean age of the patients was 71.66±6.17 years. The median number of concomitant medications used in patients followed up with the diagnosis of T2DM was 5 (IQR 2-12), and 209 (65.1%) of the participants had polypharmacy and 21 (6.5%) had severe polypharmacy (Table 1). The laboratory and comprehensive geriatric evaluation results of the groups with and without polypharmacy are shown in Table 2. Accordingly, 67.9% (n=147) of the patients with polypharmacy were women. Fasting blood glucose and HbA1c were found to be significantly higher in the polypharmacy group (Table 2).

Grouping according to the number of drugs used by the patients is shown in Table 2. Of 188 patients with polypharmacy (number of drugs 5-9), malnutrition was found in 22 (11.7%) and malnutrition risk was found in 65 (34.6%) patients. On the other hand, of 21 patients with severe polypharmacy, 17 (80.1%) had malnutrition and 4 (19.9%) had malnutrition risk.

**Table 1. Socio-demographic characteristics of the patients**

| **Age (year)** | **71.66±6.17** |
|---|---|
| | **n (%)** |
| **Gender** | |
| Male | 105 (32.7%) |
| Female | 216 (67.3%) |
| **Educational status** | |
| Illiterate | 130 (40.8%) |
| Primary school | 116 (36.4%) |
| Middle school | 33 (10.3%) |
| High school | 19 (6.0%) |
| University | 21 (6.6%) |
| **Marital status** | |
| Married | 218 (68.3%) |
| Single | 24 (7.4%) |
| Widow | 72 (22.6%) |
| Divorced | 5 (1.6%) |
| **With whom they live** | |
| Alone | 36 (11.2%) |
| With spouse | 181 (56.4%) |
| With relatives | 80 (24.9%) |
| With caregiver | 21 (6.5%) |
| Aged care facility | 3 (1.0%) |
| **Number of drugs used** | |
| 1-4 | 112 (34.8%) |
| 5-7 | 157 (48.8%) |
| 8-9 | 31 (9.6%) |
| ≥10 | 21 (6.5%) |

**Table 2. Laboratory findings and comprehensive geriatric evaluation results of the patients according to their polypharmacy status**

| | Polypharmacy | | |
|---|---|---|---|
| | Yes (n=209) | No (n=112) | p |
| Female (%) | 142 (44.2%) | 74 (23.1%) | 0.034* |
| Male (%) | 67 (20.9%) | 38 (11.8%) | 0.020* |
| Waist circumference (cm) | 106.52±7.92 | 101.14±6.68 | 0.448 |
| BMI (kg/m$^2$) | 29.56±1.03 | 28.44±1.81 | 0.599 |
| Number of drugs | 6.62±0.14 | 3.61±0.05 | <0.001* |
| **Laboratory values** | | | |
| Fasting blood glucose (mg/dL) | 211.56±14.42 | 135.85±8.74 | 0.001* |
| Creatinine (mg/dL) | 0.87±0.57 | 0.85±0.84 | 0.285 |
| Uric acid (mg/dL) | 5.92±0.33 | 5.98±0.81 | 0.941 |
| Albumin (g/dL) | 3.91±0.13 | 3.71±0.11 | 0.360 |
| Hemoglobin (g/dL) | 13.44±0.31 | 13.72±1.79 | 0.649 |
| 25 hydroxy vitamin D (ng/mL) | 16.08±7.96 | 17.64±6.27 | 0.247 |
| HbA1c (%) | 8.11±0.44 | 5.98±0.23 | <0.001* |
| C-reactive protein (mg/L) | 3.74±0.70 | 3.75±1.24 | 0.653 |
| ADL | 4.98±1.93 | 5.03±1.80 | 0.258 |
| IADL | 6.27±2.04 | 6.63±1.91 | 0.056 |
| MMSE | 24.50±4.59 | 24.69±4.79 | 0.976 |
| GDS | 5.54±4.20 | 4.83±4.60 | 0.2670 |
| MNA-SF | 11.19±2.29 | 11.24±2.90 | 0.689 |
| **Comorbid diseases** | | | |
| Hypertension | 134 (64.1%) | 60 (53.6%) | 0.066 |
| Coronary artery disease | 89 (42.6%) | 20 (17.9%) | <0.001* |
| Hyperlipidemia | 74 (35.4%) | 25 (22.3%) | <0.016* |
| Asthma/COPD | 33 (15.8%) | 11 (9.8%) | 0.138 |
| Dementia | 17 (8.1%) | 1 (0.9%) | 0.007* |

*p<0.05 statistically significant.

BMI: Body mass index, ADL: Katz index of independence in activities of daily living, IADL: Lawton instrumental activities of daily living scale, MMSE: Mini-mental state examination, GDS: Geriatric depression scale (short form consisting of 15 questions), MNA-SF: Mini Nutritional Assessment Short Form

**Table 3. Evaluation of the groups according to the MNA-SF score**

| | Malnutrition (n=48) | Malnutrition risk (n=103) | Normal (n=170) | p |
|---|---|---|---|---|
| Age (year) | 73.22±8.95 | 72.02±6.31 | 70.98±5.43 | 0.062 |
| BMI (kg/m$^2$) | 24.09±6.12 | 26.22±5.22 | 27.54±5.83 | 0.035* |
| Waist circumference (cm) | 92.96±15.15 | 96.17±10.57 | 100.70±12.46 | 0.013* |
| Albumin (g/dL) | 3.55±0.67 | 3.58±0.34 | 3.65±0.47 | 0.397 |
| Hemoglobin (g/dL) | 14.58±0.65 | 14.93±0.62 | 14.67±0.67 | 0.415 |
| C-reactive protein (mg/L) | 1.65±1.79 | 1.49±1.69 | 1.60±1.58 | 0.614 |
| HbA1c (%) | 7.41±1.89 | 7.33±1.83 | 7.21±2.01 | 0.780 |
| Polypharmacy n (%) | | | | |
| Yes | 39 (81.3%) | 69 (67.0%) | 101 (59.4%) | 0.017* |
| No | 9 (18.7%) | 34 (33.0%) | 69 (40.6%) | |

*p<0.05 statistically significant.

MNA-SF: Mini nutritional assessment short form, BMI: Body mass index

According to MNA-SF scores, patients were divided into three groups as normal, malnutrition risk and malnutrition. Accordingly, 48 (14.9%) patients were diagnosed with malnutrition, while 103 (32.1%) patients were considered to be at risk for malnutrition. Thirty-five (72.9%) of 48 patients with malnutrition and 73 (70.9%) of 103 patients with malnutrition risk were female. A statistically significant difference was found among the three groups in terms of the number of drugs used, BMI, and waist circumference (p<0.001, p<0.007, and p<0.013, respectively) (Table 3). The number of drugs in the malnutrition group was found to be significantly higher than the malnutrition risk group and the normal group (p<0.001, p<0.001, respectively).

The correlation of number of drugs and MNA-SF with age, BMI, HbA1c and albumin was analyzed. Accordingly, there was a positive correlation of HbA1c with the number of drugs and a negative correlation with the MNA-SF score (r=0.792, p<0.001, r=-0.317, p<0.001, respectively).

According to multivariate logistic regression analysis, HbA1c and polypharmacy were found to be independent variables in the development of malnutrition [p=0.009, Odds ratio (OR)=1.41, p=0.002, OR: 1.93, respectively].

## DISCUSSION

In our study, we evaluated the relationship between polypharmacy and malnutrition in elderly patients followed up with the diagnosis of T2DM. In addition to the detection of polypharmacy in 209 patients, we found malnutrition in 48 patients and malnutrition risk in 103 patients. In 188 patients with polypharmacy (number of drugs 5-9), malnutrition was found in 22 (11.7%) and malnutrition risk was found in 65 (34.6%) patients. On the other hand, of 21 patients with severe polypharmacy, 17 (80.1%) had malnutrition and 4 (19.9%) had malnutrition risk. The BMI and waist circumference of the patients with malnutrition were significantly lower than the other patients, and the number of drugs used was significantly higher. There was a positive correlation between the number of drugs used by the patients and HbA1c, and a negative correlation with the MNA-SF score. In addition, HbA1c and polypharmacy were found to be independent factors in the development of malnutrition in elderly T2DM patients. Our study is the first in our country to evaluate polypharmacy and malnutrition in elderly patients with T2DM.

**Table 4. Analysis of independent risk factors affecting malnutrition**

| Risk factors | OR (95% CI) | p |
|---|---|---|
| Age | 1.03 (0.95-1.12) | 0.453 |
| Gender (female) | 1.67 (0.58-4.83) | 0.342 |
| ADL | 1.01 (0.98-1.03) | 0.593 |
| HbA1c | 1.41 (1.09-1.92) | 0.009* |
| Polypharmacy | 1.93 (1.21-2.47) | 0.002* |
| Hypertension | 1.41 (0.42-4.74) | 0.573 |
| Hyperlipidemia | 0.62 (0.22-1.75) | 0.364 |
| Coronary artery disease | 0.62 (0.22-1.71) | 0.355 |
| Dementia | 1.61 (0.42-6.22) | 0.491 |
| GDS | 1.12 (0.98-1.28) | 0.087 |

*p<0.05 statistically significant.

ADL: Katz Index of Independence in Activities of Daily Living, GDS: Geriatric Depression Scale (short form consisting of 15 questions), CI: Confidence interval, OR: Odds ratio

Both malnutrition and polypharmacy are phenomena that are frequently encountered and that significantly affect the quality of life and mortality rate, especially in older age groups. In addition to age-related physiological, pathological, and environmental changes, polypharmacy put older adults at risk for malnutrition. Apart from these changes, other factors include the increased prevalence of chronic medical conditions, decreased thirst and sense of taste (which may increase the risk of fluid and electrolyte imbalance), dry mouth, and lack of access to a nutritionally adequate diet due to disability or increased difficulty in consuming it[19]. Evaluations have shown an inverse relationship between increased drug use and nutritional status. Accordingly, at least half of the elderly who use 10 or more drugs have malnutrition or malnutrition risk[20]. In our study, we found the malnutrition rate to be 80.1% in patients with severe polypharmacy, which supports this. As increasing age is generally associated with a greater burden of disease, it is associated with increased and often overused medication. A cohort study emphasized that the most important social and health determinants affecting disease-related malnutrition are living alone, polypharmacy and dysphagia, and that these strategies should be focused on to improve patient care[21]. In a large cohort of more than 1300 participants, it was stated that 51% of older adults with T2DM, who participated in the study, were using five or more drugs, and malnutrition and malnutrition risk were twice higher in these patients. In the study, polypharmacy was found to be higher in patients with malnutrition or malnutrition risk[22]. The judgment that it is unclear which is a risk factor for the other, or whether interventions targeting either one will have a significant positive effect, is valid for this study. Another population-based cohort study involving more than 700 community-dwelling older adults in Spain found a statistically significant association between malnutrition or malnutrition risk and increased use of prescription drugs. Furthermore, of the five main predictors of malnutrition (lower BMI, depressive symptoms, frailty, poor self-assessment of health, and polypharmacy), polypharmacy was shown to be a strong predictor in malnutrition assessment for both men and women[23]. A systematic review revealed that severe polypharmacy was a statistically significant risk factor for malnutrition in older women, but not in older men[24]. In

our study, the number of drugs used was found to be higher in the group diagnosed with malnutrition. In addition, the entire group with severe polypharmacy was malnourished or at risk of malnutrition. Our study suggests that polypharmacy significantly increases the risk of malnutrition.

As the incidence of diseases requiring pharmacological treatment increases with aging, the prevalence of polypharmacy also increases[25]. It is known that it has the potential to adversely affect the nutritional status due to changes in taste, absorption of vitamins and minerals from the intestines, and changes in the metabolism associated with the drugs used[26]. The content of drug or drugs directly or indirectly affects the risk of malnutrition. In a study, using one or two drugs seems to be protective for the development of malnutrition when compared to not using drugs. The most plausible explanation for this situation is that those who use drugs alone are likely to use preventive drugs for their diagnosis, which can have a beneficial effect on health[27]. However, a positive relationship was observed between the use of multiple drugs and malnutrition. However, many of the findings on polypharmacy are difficult to compare because they are obtained from cross-sectional studies, and many studies do not differentiate between the levels of polypharmacy[24]. We reached similar findings in our study. The rate of malnutrition rate was associated with the number of drug used. Moreover, additional comorbid diseases were found to be higher in the polypharmacy group. Based on this, it can be suggested that the development of additional comorbid conditions affects the development of polypharmacy, and polypharmacy affects the development of malnutrition.

Treatment of T2DM requires prescribing multiple drugs for glycemic control, cardiovascular risk management, and management of common comorbidities. In conclusion, patients followed with the diagnosis of T2DM are at risk for polypharmacy and severe polypharmacy. According to studies, the estimated prevalence of polypharmacy among T2DM patients ranges from 57% to 99%[28-31]. In addition, polypharmacy in T2DM is associated with suboptimal glycemic control, which increases the risk of long-term complications of diabetes[32,33]. Similarly, in our study, while fasting blood glucose and HbA1c levels were found to be significantly higher in the polypharmacy group, there was a positive correlation between the number of drugs and HbA1c. Many studies investigating the prevalence or characteristics of polypharmacy have been performed in selected T2DM populations of older adults[34]. Noale et al.[22] evaluated more than 1300 elderly patients with the diagnosis of T2DM from 57 diabetes centers and found the frequency of polypharmacy to be 57.1%. Moreover, female patients with a BMI ≥30 kg/m$^2$ were associated with polypharmacy in the study. In another study in which more than two thousand T2DM and T1DM patients were evaluated, the frequency of polypharmacy was found to be 56%. In addition, according to the results of the logistic regression analysis, it has been reported that being over 40 years old, having poor or very poor self-perceived health status, the presence of five or more comorbidities, and a mean diabetes mellitus diagnosis of more than ten years are associated with polypharmacy[31]. Polypharmacy in elderly patients should be regularly evaluated at each visit, since it is one of the most common geriatric syndromes, especially in T2DM patients. Examination of drugs at regular intervals should be an absolute part of treatment in elderly patients with T2DM. After a comprehensive geriatric evaluation in the elderly, individual goals should be determined according to the guidelines and potential interactions and adverse reactions should be identified, although it is often not possible to reduce the number of drugs.

### Study Limitations

Because the study was conducted in a tertiary health center, the patients who applied to the center may be composed of patients who require more uncontrolled forms of diabetes or more complex medical treatments, or these patients may experience more diabetes-related complications. All these reasons may cause an increase in the prevalence of polypharmacy in patients. The inclusion of only patients using OAD drugs in the study may mean more drug use for T2DM, which is tried to be controlled in these patients.

## CONCLUSION

This study, as expected, is important in terms of showing that polypharmacy is common in elderly patients with T2DM and that it often accompanies malnutrition in the elderly. In addition, it underlines that intertwined polypharmacy and malnutrition should not be overlooked in elderly patients, and that malnutrition that may be associated with newly started or currently used drugs should be evaluated, as well as reviewing the drugs used in each visit.

### Ethics

**Ethics Committee Approval:** Ethics committee approval was obtained for the study from İnönü University Non-Interventional Clinical Research Ethics Committee (date: 14.12.2021, decision number: 2021/2837).

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

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: F.D.Y., Concept: F.D.Y., Design: A.E., Data Collection or Processing: F.D.Y., Analysis or Interpretation: A.E., Literature Search: F.D.Y., A.E., Writing: F.D.Y., A.E.

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# Is Loss of Residual Renal Function Related to Longitudinal Uric Acid and CRP Levels in Peritoneal Dialysis Patients?

## Periton Diyalizi Hastalarında, Rezidüel Renal Fonksiyonların Kaybı Boylamsal Ürik Asit ve CRP Düzeyleri ile İlişkili midir?

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**ABSTRACT**

**Aim:** Residual renal functions have positive effects on morbidity and mortality among patients undergoing peritoneal dialysis. Our aim is to investigate the effects of baseline laboratory data and longitudinal uric acid and C-reactive protein (CRP) levels on loss of residual renal functions within the first three years of peritoneal dialysis.

**Materials and Methods:** This is a retrospective cohort study. Thirty-four patients who started peritoneal dialysis due to end-stage renal disease were included. The primary endpoint was loss of residual renal function and was defined as residual urine volume of less than 200 mL/24 hours. Patients were followed for three years after the onset of peritoneal dialysis or until loss of residual renal functions. Demographic and clinical data were recorded retrospectively.

**Results:** The follow-up period was 32.7 (12.9-36) months. Ten patients lost residual renal function 22.1±9.8 months after the initiation of peritoneal dialysis. Longitudinal uric acid level was 6.1±1.2 mg/dL and longitudinal CRP level was 0.5 (0.3-0.7) mg/dL. In patients with residual renal function loss, baseline sodium and triglyceride were lower, while parathormone were higher. There was no difference between the groups in terms of longitudinal uric acid and CRP levels. Baseline parathyroid hormone [hazard ratio (HR), 1,003; 95% confidence interval (CI) 1,001-1,006; p=0.013], body mass index (HR 0.817; 95% CI 0.684-0.975; p=0.025), and baseline sodium level (HR 0.801; 95% CI; 0.665- 0.965; p=0.019) were risk factors for residual renal function loss.

**Conclusion:** In peritoneal dialysis patients, residual renal function loss were associated with baseline sodium, triglyceride, and body mass index. There was no correlation between residual renal function loss and longitudinal CRP and uric acid levels. Prospective studies are needed to determine the optimal uric acid and CRP levels.

**Keywords:** C-reactive protein, peritoneal dialysis, residual renal functions, sodium, uric acid

**ÖZ**

**Amaç:** Periton diyalizi yapan hastalarda rezidüel renal fonksiyonların morbidite ve mortalite üzerinde olumlu etkileri vardır. Amacımız, periton diyalizi başlanmadan önceki bazal verilerin ve başlandıktan sonra ilk üç yıldaki boylamsal ürik asit ve C-reaktif protein (CRP) düzeyinin rezidüel renal fonksiyon kaybı üzerindeki etkilerinin araştırılmasıdır.

**Gereç ve Yöntem:** Retrospektif kohort bir çalışmadır. Son dönem böbrek yetmezliği nedeni ile periton diyalizi başlanan 34 hasta çalışmaya dahil edildi. Primer sonlanım noktası rezidüel renal fonksiyon kaybıydı ve rezidüel idrar miktarının 200 mL/24 saatten az olması olarak tanımlandı. Hastalar, periton diyalizi başlandıktan sonra üç yıl boyunca veya RRF kaybı olana kadar takip edildi. Hastaların klinik ve laboratuvar verileri hasta dosyalarından kaydedildi.

**Bulgular:** Takip süresi 32,7 (12,9-36) aydı. Periton diyalizi başlandıktan 22,1±9,8 ay sonra 10 hastada rezidüel renal fonksiyon kaybı oldu. Boylamsal ürik asit düzeyi 6,1±1,2 mg/dL ve boylamsal CRP düzeyi 0,5 (0,3-0,7) mg/dL idi. Rezidüel renal fonksiyon kaybı olan hastalarda, bazal sodyum,

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trigliserid düzeyleri daha düşük iken parathormon düzeyi daha yüksekti. Her iki grup arasında boylamsal ürik asit ve CRP düzeyi açısından fark yoktu. Periton diyalizi başlanmadan önceki parathormon [tehlike oranı (HR), 1.003; %95 güven aralığı (GA) 1.001-1.006; p=0,013], sodyum (HR 0,801; %95 GA 0,665-0,965; p=0,019) ve vücut kitle indeksi (HR 0,817; %95 GA 0,684-0,975; p=0,025) rezidüel renal fonksiyon kaybı için risk faktörüydü.

**Sonuç:** Periton diyalizi hastalarında, bazal sodyum, trigliserid ve vücut kitle indeksi rezidüel renal fonksiyon kaybı için risk faktörü olarak bulunmuştur. Rezidüel renal fonksiyon kaybı ile boylamsal CRP ve ürik asit düzeyi arasında ilişki gösterilememiştir. İdeal ürik asit ve CRP düzeyinin belirlenmesi için prospektif çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** C-reaktif protein, periton diyalizi, rezidüel renal fonksiyon, sodyum, ürik asit

## INTRODUCTION

Peritoneal dialysis (PD) is one of the renal replacement therapies in end-stage renal disease (ESRD). In patients undergoing PD, residual renal function (RRF) has positive effects on anemia, blood pressure and volume control, bone metabolism, and patient survival[1-3]. Therefore, it is important to maintain RRF in PD patients. Loss of RRF occurs earlier in patients with a history of diabetes mellitus (DM), heart failure, and peritonitis[4]. In addition, inflammation has also been found to be associated with loss of RRF[5].

Uric acid is the end product of purine metabolism. It has been shown that uric acid causes endothelial dysfunction, vascular smooth muscle cell damage and inflammation[6-8]. Hyperuricemia is a risk factor for chronic kidney disease, diabetes and hypertension[9-11]. It has been reported that mortality is higher in hemodialysis and PD patients with high uric acid levels[12,13]. In epidemiological studies, it has been shown that the risk of chronic kidney disease increases in people with high uric acid levels[11,14]. In patients with chronic kidney disease, inconsistent results have been reported regarding the effect of uric acid on disease progression[14-17]. In two different studies examining the relationship between the basal uric acid level at the onset of PD and RRF, it was reported that high serum uric acid levels were associated with loss of RRF[18,19]. The number of studies examining the relationship between RRF loss and the longitudinal uric acid level measured until the loss of RRF and the loss of RRF is quite limited. In this study, it was aimed to investigate the effects of clinical and laboratory data before the initiation of PD and longitudinal uric acid and C-reactive protein (CRP) levels in the first three years after the initiation of PD on RRF.

## MATERIALS AND METHODS

In this retrospective cohort study, patients older than 18 years of age, who started PD due to ESRD between January 01, 2010 and October 31, 2018, were evaluated retrospectively. Exclusion criteria were: 1. Follow-up of less than three months after the initiation of PD (n=6), 2. Having a kidney transplant (n=12), and 3. Residual urine less than 200 mL/24 hours at the time of the initiation of PD and within three months (n=6). After excluding a total of 24 patients, 34 patients were included in our study. Ege University of Local Ethics Committee (protocol number: 21-11T/21, date: 04.11.2021) was obtained for our study. Patients underwent continuous ambulatory PD or automated PD.

In general, continuous ambulatory PD consisting of 4-5 exchanges/24 hours was applied to the patients. Patients who were treated with automated PD underwent six or more exchanges/24 hours. Dialysis type, dialysate and number of exchanges were determined according to the peritoneal equalization test, Kt/Vurea and ultrafiltration need of the patients. Dialysate glucose concentration and icodextrin requirement were adjusted according to the patient's ultrafiltration need.

In our study, the primary endpoint was loss of RRF. RRF loss was defined as a residual urine volume of less than 200 mL/24 hours. Patients were followed for three years after the initiation of PD or until residual urine volume was <200 mL/24 hours. Patients who switched to another renal replacement therapy, died or were lost to follow-up were followed up to their last registered visit.

Demographic and clinical data (age, gender, height, weight, cause of ESRD, office blood pressure, DM, cardiovascular disease) and medications before the initiation of PD were recorded from patient files. Laboratory data (urea, creatinine, sodium, potassium, calcium, phosphorus, uric acid, albumin, CRP, triglyceride, low-density lipoprotein, parathormone (PTH), ferritin, hemoglobin) belonging to one month before starting PD were obtained from patient files and electronic hospital information management system. The type of PD at the initiation of PD and the weekly total Kt/Vurea measured between the third and sixth months after the initiation of PD were recorded. Body mass index (BMI) was obtained by dividing the patient's body weight (kg) by the square of the height (m). Peritonitis attacks were also recorded. During the follow-up period, serum uric acid and CRP levels which were obtained during approximately quarterly visits were recorded from the patient files. If the patients had active infection during the visit, the CRP and uric acid levels at that visit were not included in the study. If the patient did not attend the visit, the CRP and uric acid levels evaluated within a month before or after that visit were recorded.

### Statistical Analysis

Data analysis was performed with the statistical package program of IBM Statistical Package for the Social Sciences Statistics 25.0 (IBM Corp., Armonk, New York, USA). The distribution of normality for continuous variables was evaluated with the Shapiro-Wilk test. Descriptive statistics were given as frequency (n), percentage (%), mean, standard deviation, median *(M)*, 25$^{th}$ percentile *($C_1$)*, 75$^{th}$ percentile *($C_3$)*. Longitudinal uric acid and CRP levels were calculated as the mean of uric acid and CRP levels measured during the follow-up period. Independent sample t-test, Mann-Whitney U test and Fisher's exact chi-square test were used for comparisons between the groups. Risk factors for RRF loss were evaluated by cox regression analysis. A p value of <0.05 was considered statistically significant.

## RESULTS

The study included 34 patients. Baseline demographic, clinical and laboratory data of the patients are shown in Table 1. The mean age of the patients was 49.1±13.1 years and 58.8% were female. The cause of ESRD was unknown in 38.2% of the patients. The most common causes of ESRD were diabetic nephropathy, hypertensive nephrosclerosis and chronic glomerulonephritis. Three patients had autosomal dominant polycystic kidney disease. At the time of initiation of PD, six patients had DM and two had cardiovascular disease. In tour study, 88.2% of the patients were undergoing continuous ambulatory PD. At the time of the initiation of PD, five patients were receiving allopurinol and 25 patients were receiving diuretics. Before the initiation of PD, baseline creatinine level was 7.5 (6.6-8.7) mg/dL, sodium level was 139.4±3.3 mEq/L, uric acid level was 7.3±1.9 mg/dL, and CRP level was 0.3 (0.2-0.7) mg/dL.

The mean follow-up period was 32.7 (12.9-36) months (Table 2). Longitudinal uric acid level was 6.1±1.2 mg/dL and longitudinal CRP level was 0.5 (0.3-0.7) mg/dL. The rate of patients who had peritonitis attack was 52.9%. RRF loss was observed in 10 patients 22.1±9.8 months after the initiation of PD.

The baseline within data of patients with and without loss of RRF during the follow-up are shown in Table 3. There was no significant difference between the two groups in terms of age, gender, BMI and history of cardiovascular disease. Urea, creatinine, and hemoglobin levels were similar before the initiation of PD. Compared to patients without RRF loss, baseline sodium level was lower in those with RRF loss. Triglyceride was lower in those with RRF loss, while PTH was higher. BMI was lower in those with RRF loss, although it was not statistically significant. There was no difference between the two groups in terms of longitudinal uric acid and CRP levels.

Baseline uric acid, baseline CRP, longitudinal uric acid and longitudinal CRP levels before the initiation of PD were not risk factors for loss of RRF (Table 4). The presence of DM, history of peritonitis and use of diuretics were also not

**Table 1. Demographic and clinical data of the patients before starting peritoneal dialysis**

| | |
|---|---|
| **Age (years)** | 49.1±13.1 |
| **Gender** | |
| Female (n, %) | 20 (58.8%) |
| **Cause of end-stage renal disease** | |
| Diabetic nephropathy (n, %) | 5 (14.7%) |
| Hypertensive nephropathy (n, %) | 5 (14.7%) |
| Chronic glomerulonephritis (n, %) | 5 (14.7%) |
| Autosomal dominant polycystic kidney disease | 3 (8.8%) |
| Unknown (n, %) | 13 (38.2%) |
| Other (n, %) | 3 (8.8%) |
| Diabetes mellitus | 6 (17.6%) |
| Cardiovascular disease | 2 (5.9%) |
| **PD type** | |
| Continuous ambulatory PD (n, %) | 30 (88.2%) |
| Automated PD (n, %) | 4 (11.8%) |
| **Drugs** | |
| Allopurinol (n, %) | 5 (14.7%) |
| Antihypertensive drug (n, %) | 27 (79.4%) |
| Diuretic (n, %) | 25 (73.5%) |
| Phosphorus binding agent (n, %) | 29 (85.3%) |
| Vitamin D analogue (n, %) | 17 (50%) |
| **Kt/Vurea** | 2.5±0.4 |
| Systolic blood pressure (mmHg) | 141.2±17.9 |
| Diastolic blood pressure (mmHg) | 85 (80-90) |
| Body mass index (kg/m$^2$) | 24.9±4.9 |
| Urea (mg/dL) | 170.6±64.3 |
| Creatinine (mg/dL) | 7.5 (6.6-8.7) |
| Sodium (mEq/L) | 139.4±3.3 |
| Potassium (mEq/L) | 4.7±0.8 |
| Calcium (mg/dL) | 9.1 (8.6-9.6) |
| Phosphorus (mg/dL) | 6 (4.9-6.8) |
| Uric acid (mg/dL) | 7.3±1.9 |
| Albumin (g/dL) | 4.3 (3.9-4.5) |
| CRP (mg/dL) | 0.3 (0.2-0.7) |
| Triglyceride (mg/dL) | 150±54 |
| LDL (mg/dL) | 120 (99-144) |
| PTH (pg/mL) | 337.5 (192.1-477) |
| Ferritin (ng/mL) | 244 (114-430) |
| Hemoglobin (g/dL) | 10.4±1.6 |

CRP: C-reactive protein, LDL: Low density lipoprotein, PD: Peritoneal dialysis, PTH: Parathormone

associated with RRF loss. Before the initiation of PD, baseline sodium (HR 0.801; 95% CI 0.665-0.965, p=0.019), baseline PTH (HR, 1.003, 95% CI 1.001-1.006, p=0.013), and BMI (HR 0.817; 95%CI 0.684-0.975, p=0.025) were the risk factors for RRF loss. In the multivariate cox regression analysis including age at the initiation of PD, gender, DM, history of peritonitis, baseline PTH, baseline sodium, and baseline BMI, none of these parameters were risk factors for RRF loss.

**Table 2. Follow-up data in the first three years of peritoneal dialysis**

| | |
|---|---|
| Follow-up time (months) | 32.7 (12.9-36) |
| Those with RRF loss (n, %) | 10 (29.4%) |
| Time of RRF loss (month) | 22.1±9.8 |
| Longitudinal uric acid (mg/dL) | 6.1±1.2 |
| Longitudinal CRP (mg/dL) | 0.5 (0.3-0.7) |
| History of peritonitis (n, %) | 18 (52.9%) |

CRP: C-reactive protein, RRF: Residual renal function

## DISCUSSION

In this study, 29.4% of the patients had loss of RRF 22.1±9.8 months after the initiation of PD. Compared to patients without RRF loss, patients with RRF loss had lower baseline sodium, lower triglyceride levels and higher PTH levels. Longitudinal uric acid and CRP levels were similar between the two groups. Baseline uric acid and CRP and longitudinal uric acid and CRP levels were not risk factors for RRF loss. Low sodium level, high PTH level, and low BMI at the initiation of PD were risk factors for RRF loss.

**Table 3. Baseline clinical and laboratory data of patients with and without residual renal function loss at follow-up**

| | Without RRF loss (n=24) | With RRF loss (n=10) | p |
|---|---|---|---|
| Age (years) | 46.7 (42.1-60) | 54.5 (29-63.3) | 0.926 |
| Male (n, %) | 8 (33.3%) | 6 (60%) | 0.252 |
| Diabetes mellitus (n, %) | 5 (20.8%) | 1 (10%) | 0.644 |
| Cardiovascular disease (n, %) | 2 (8.3%) | 0 (0%) | 1 |
| History of peritonitis (n, %) | 11 (45.9%) | 7 (70%) | 0.270 |
| **Peritoneal dialysis type** | | | |
| Continuous ambulatory PD (n, %) | 21 (87.5%) | 9 (90%) | 1.000 |
| Automated PD (n, %) | 3 (12.5%) | 1 (10%) | |
| Antihypertensive drug use (n, %) | 5 (20.8%) | 2 (20%) | 1.000 |
| Allopurinol use (n, %) | 3 (12.5%) | 2 (20%) | 0.618 |
| Diuretic use (n, %) | 7 (29.2%) | 2 (20%) | 0.692 |
| Duration of follow-up (months) | 36 (9.2-36) | 22 (15.1-31.9) | 0.079 |
| Kt/Vurea | 2.5±0.5 | 2.4±0.3 | 0.370 |
| Systolic blood pressure (mmHg) | 139.4±17.9 | 146.1±18.3 | 0.349 |
| Diastolic blood pressure (mmHg) | 82.5 (80-90) | 90 (80-100) | 0.437 |
| Body mass index (kg/m$^2$) | 25.9±5.1 | 22.4±3.8 | 0.052 |
| Urea (mg/dL) | 168.3±45 | 176.1±99.8 | 0.754 |
| Creatinine (mg/dL) | 7.8 (7-8.7) | 6.7 (5.4-7.3) | 0.101 |
| Sodium (mEq/L) | 140.3±3.2 | 137.7±3.2 | **0.042** |
| Potassium (mEq/L) | 4.8±0.9 | 4.7±0.8 | 0.682 |
| Calcium (mg/dL) | 9±0.9 | 9±1.4 | 0.871 |
| Phosphorus (mg/dL) | 6 (5.4-6.7) | 5 (4.7-7.1) | 0.592 |
| Uric acid (mg/dL) | 7.3±1.8 | 7.3±2.2 | 0.997 |
| Albumin (g/dL) | 4.4 (4-4.6) | 4 (3.8-4.3) | 0.142 |
| CRP (mg/dL) | 0.3 (0.2-0.4) | 0.7 (0.3-0.9) | 0.068 |
| Triglyceride (mg/dL) | 161.6±50.5 | 115.3±52.6 | 0.048 |
| LDL (mg/dL) | 121 (108-144) | 99.5 (82-130) | 0.175 |
| PTH (pg/mL) | 288.7±153.8 | 605±253.7 | 0.016 |
| Hemoglobin (g/dL) | 10.6±1.4 | 10.1±2.2 | 0.461 |
| Ferritin (ng/mL) | 224 (119-413) | 222 (95-714) | 0.964 |
| Longitudinal uric acid (mg/dL) | 6.4±1.2 | 5.6±1.2 | 0.099 |
| Longitudinal CRP (mg/dL) | 0.6 (0.3-0.7) | 0.5 (0.3-0.8) | 0.669 |

CRP: C-reactive protein, LDL: Low density lipoprotein, PD: Peritoneal dialysis, PTH: Parathormone

**Table 4. Risk factors associated with loss of residual renal function**

| | HR (95% CI) | p | Model p |
|---|---|---|---|
| Age (years) | 0.997 (0.949-1.047) | 0.905 | 0.905 |
| Female gender | 1.556 (0.438-5.528) | 0.494 | 0.491 |
| Diabetes mellitus | 0.439 (0.055-3.470) | 0.435 | 0.422 |
| Body mass index (kg/m$^2$) | 0.817 (0.684-0.975) | 0.025 | 0.023 |
| Automated PD | 0.792 (0.100-6.262) | 0.825 | 0.825 |
| Allopurinol use | 1.426 (0.302-6.745) | 0.654 | 0.653 |
| Diuretic use | 1.086 (0.230-5.122) | 0.917 | 0.917 |
| Kt/Vurea | 0.638 (0.133-3.065) | 0.574 | 0.573 |
| Sodium (mEq/L) | 0.801 (0.665-0.965) | 0.019 | 0.018 |
| Uric acid (mg/dL) | 1.054 (0.675-1.646) | 0.816 | 0.816 |
| CRP (mg/dL) | 1.673 (0.415-6.754) | 0.469 | 0.464 |
| Triglyceride (mg/dL) | 0.981 (0.962-1.001) | 0.068 | 0.075 |
| PTH (pg/mL) | 1.003 (1.001-1.006) | 0.013 | 0.006 |
| Longitudinal uric acid (mg/dL) | 0.685 (0.412-1.137) | 0.143 | 0.141 |
| Longitudinal CRP (mg/dL) | 1.064 (0.401-2.822) | 0.901 | 0.901 |

CRP: C-reactive protein, LDL: Low density lipoprotein, PD: Peritoneal dialysis, PTH: Parathormone

Loss of RRF develops in PD patients over time due to risk factors such as peritonitis, DM, and heart failure. In PD patients, RRF was found to be associated with better volume and blood pressure control, improvement of anemia, phosphorus control, and better survival[1-3]. Therefore, long-term preservation of RRF is sought in PD patients.

In our study, patients with RRF loss had lower sodium and triglyceride levels. The BMI and albumin levels of these patients were also lower, although not statistically significant. These laboratory findings are parameters related to nutrition. In addition, low triglyceride and sodium levels were found to be risk factors for RRF loss. Our findings support that RRF loss may be associated with malnutrition. In one study, malnutrition-inflammation complex syndrome was found to be associated with loss of RRF[20]. Palomo-Piñón et al.[21] showed that baseline CRP level is high and albumin level is low in patients with RRF loss, and baseline CRP and albumin levels are risk factors for RRF loss. However, in our study, no correlation was found between baseline and longitudinal CRP levels and RRF.

Hyperuricemia causes inflammation, endothelial dysfunction and oxidative stress[22]. As hyperuricemia is associated with inflammatory markers such as interleukin-6, CRP, and tumor necrosis factor-alpha, it has been suggested that uric acid has a role in inflammatory processes[8]. In the general population, it has been shown that hyperuricemia is associated with hypertension, cardiovascular diseases and mortality[23,24]. In the CKD process, the results of studies on its relationship with disease progression and patient survival are contradictory[14-17].

The number of studies examining the relationship between uric acid level and clinical outcomes in PD patients is very few. Park et al.[18] Showed that there was a correlation between baseline uric acid level and loss of RRF at the end of 24-month follow-up. In a more recent study, a U-shaped relationship was found between baseline uric acid level and loss of RRF[19]. Most of the studies in the literature have evaluated the relationship between uric acid level at the initiation of PD and loss of RRF. In the only study examining the relationship between longitudinal uric acid levels and RRF after the initiation of PD, it was reported that longitudinal uric acid levels of <6.77 mg/dL and ≥7.64 mg/dL were associated with loss of RRF[25]. In our study, however, no relationship could be demonstrated between longitudinal uric acid level and loss of RRF. In our study, a relationship between RRF loss and longitudinal uric acid level may not have been determined due to the lower mean uric acid level and the small number of patients.

### Study Limitations

The limitations of our study are its retrospective nature and the small number of patients. On the other hand, the longitudinal evaluation of uric acid and CRP levels can be considered as the strengths of our study.

## CONCLUSION

In conclusion, while loss of RRF was associated with low baseline sodium, triglyceride and BMI in PD patients, no relationship could be shown between longitudinal CRP and uric acid levels and RRF loss. Larger, prospective studies are

needed to determine the effects of hyperuricemia on RRF loss and, in this respect, to determine the target uric acid level.

## Ethics

**Ethics Committee Approval:** The study were approved by the Ege University of Local Ethics Committee (protocol number: 21-11T/21, date: 04.11.2021).

**Informed Consent:** It is a retrospective cohort study.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

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

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# The Effect of Mindfulness Level on Drug Adherence in Hypertension Patients

## Hipertansiyon Hastalarında Bilinçli Farkındalık Düzeyinin İlaç Uyumuna Etkisi

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**ABSTRACT**

**Aim:** The effect of mindfulness levels of hypertensive patients on drug adherence was studied.

**Materials and Methods:** Hypertensive patients between the ages of 18 and 65 years, who gave their consent to participate in the study, were literate and had been using antihypertensive drugs for at least one year, were included in the study. Those who had mental retardation and/or mental disease that prevented filling the scale were excluded from the study. It is a cross-sectional and descriptive study. Socio-demographic data form and the Mindful Attention Awareness scale (MAAS) and Modified Morisky Adherence scale were applied to the participants.

**Results:** Drug adherence of the patients with a low mean age was found to be low. There was no significant difference between the groups in terms of drug compliance when it was examined in terms of gender, education level, employment status and marital status. In those with a family history of hypertension, the rate of low drug adherence was significantly higher compared to the rate of medium-high drug adherence, there was no statistically significant difference in drug adherence scores in terms of hypertension duration of disease. The mean MAAS scores were significantly higher in those with medium- high drug adherence.

**Conclusion:** Our study results provide evidence that drug adherence was low in the hypertensive patient group and there was a significant relationship between mindfulness and adherence. As in other chronic diseases, it is vital to increase drug adherence in hypertensive patients and to increase the level of mindfulness affecting drug adherence for this purpose. Our study data will raise awareness for clinical interventions in this area.

**Keywords:** Hypertension, mindfulness, drug adherence

**ÖZ**

**Amaç:** Çalışmada hipertansiyon hastalarının bilinçli farkındalık düzeylerinin antihipertansif ilaç uyumuna olan etkisi araştırılmıştır.

**Gereç ve Yöntem:** Çalışmaya katılmaya onam veren kişilerden okur yazar olan, en az bir yıldır antihipertansif kullanıyor olan 18-65 yaş aralığındaki hipertansif hastalar çalışmaya dahil edilmiştir. Ölçek doldurmayı engelleyecek düzeyde mental yetersizliği ve/veya mental hastalığı bulunanlar çalışma dışında bırakılmıştır. Kesitsel ve tanımlayıcı bir araştırmadır. Katılımcılara sosyo-demografik veri formu, Bilinçli Farkındalık ölçeği (BFÖ) ve Modifiye Morisky Tedavi Uyum ölçeği uygulanmıştır.

**Bulgular:** Yaş ortalaması düşük olan hastaların ilaç uyumu düşük olarak saptandı. Cinsiyet, eğitim düzeyi, çalışma durumu ve medeni durum açısından bakıldığında gruplar arasında ilaç uyumu açısından anlamlı fark yoktu. Ailesinde hipertansiyon öyküsü olanlarda ilaç uyumunun düşük olma oranı orta-yüksek olma oranına kıyasla anlamlı şekilde yüksekti. İlaç uyumu skorlarında hipertansiyon hastalığının süresi açısından istatistiksel açıdan anlamlı bir fark yoktu. BFÖ skor ortalamaları ilaç uyumu orta-yüksek olanlarda anlamlı şekilde daha yüksekti.

**Sonuç:** Çalışma sonuçlarımız hipertansif hasta grubunda ilaç uyumunun düşük olduğu, farkındalık ve uyum arasında anlamlı bir ilişki olduğu yönünde kanıt sunmaktadır. Diğer kronik hastalıklarda olduğu gibi hipertansiyon hastalarında da ilaç uyumunun artırılması, bu amaçla ilaç uyumunu etkileyen farkındalık düzeyinin artırılması hayati önem arz etmektedir. Çalışma verilerimiz bu alanda yapılacak klinik müdahaleler için farkındalık oluşturacaktır.

**Anahtar Kelimeler:** Hipertansiyon, bilinçli farkındalık, ilaç uyumu

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## INTRODUCTION

Hypertension is the most important preventable cause of morbidity and mortality globally, yet there are relatively few data collected using standardized methods. The aim of hypertension therapy is to reduce the mortality rate and prevent hypertension-related diseases such as cerebral hemorrhage, stroke and ischemic heart disease by managing blood pressure (BP). However, despite advances in the prevention and treatment of hypertension, the percentage of people with high or uncontrolled BP is high[1].

Assuming clinically valid BP values, the two main factors contributing to the control of hypertension in treated patients are the prescription of adequate numbers and doses of BP medications and treatment adherence. Suboptimal adherence to treatment is also associated with a variety of target organ changes associated with a greater risk of cardiovascular events, including vascular stiffness, left ventricular hypertrophy, and microalbuminuria. Besides, suboptimal adherence is associated with many adverse cardiovascular events such as acute coronary syndromes, stroke, transient ischemic attack, and chronic heart failure, and mortality[2].

Long-term chronic diseases such as hypertension are often associated with progressive reductions in therapy over months and years[3]. Adherence with pharmacotherapy for hypertension is typically reported, 50% after one year from baseline[4].

Contemporary pharmacological treatment strategies are insufficient in some individuals, and there may be patients who cannot achieve results in the treatment of hypertension, as in other chronic diseases, due to many factors that may be directly or indirectly related. Up to the present, with the cooperation of cardiology and psychiatry, psychiatric drugs have also played an important role in the treatment of hypertension. With this pharmacological treatment approach, it is aimed to eliminate the direct biological effects of pathological mood in BP regulation. However, the point where psychiatry has reached shows that in many patients, only the pharmacological approach is insufficient, it is necessary to treat the patient with psychotherapeutic interventions, poor lifestyle that causes the continuation of high BP, breakage of treatment resistance and drug incompatibility. Lifestyle changes alone or in combination with pharmacological therapy are usually sufficient to achieve BP control[5].

Although complementary behavioral therapies for BP control are not a substitute for conventional therapy, they can be evaluated for their potential to reduce BP. Stress reduction through meditation is a potentially important non-pharmacological therapy that can both reduce polypharmacy and improve BP control[6]. As it is known, the main effect of stress and depression is acute or chronic sympathetic nervous system activation that leads to the development of hypertension in susceptible individuals or worsening of BP levels if they are already hypertensive. An easily accessible, low-cost and reproducible technique such as mindfulness can reduce this pathology and the resulting organ damage[7]. Adding mindfulness-based strategies to the pharmacological treatment management of hypertensive individuals may contribute to BP control both directly and indirectly through lifestyle changes and increased drug adherence.

The aim of this study is to examine the effect of conscious awareness levels on drug compliance in patients with hypertension. To the best of our knowledge, there is no other study in the literature examining the effect of individuals' mindfulness levels on drug adherence. While we re-discuss the importance of multidimensional approach together with pharmacotherapy in hypertensive patient management with mindfulness, we hope that the results will accelerate mindfulness-based approaches in the clinical management of hypertensive patients.

## MATERIALS AND METHODS

The study is a cross-sectional and descriptive study and it was conducted with 68 hypertensive patients who were consecutively included in the study from individuals who were admitted to a university hospital cardiology outpatient clinic, met the research criteria, and gave written consent to participate in the study. Ethics committee approval was obtained from Marmara University Faculty of Medicine Clinical Research Ethic Committee (number: 09.2019.414, date: 05.04.2019) to conduct the study. The Helsinki Declaration Principles were complied with at every stage. Patients with hypertension, who were between the ages of 18 and 65 years, who were literate and gave consent to participate, who had received anti-hypertensive treatment for at least one year were included in the study. Those who had a mental retardation and/or mental disease that would hinder interview and filling the scale were excluded from the study. The evaluation of the clinician who performed the examination and the patient's statement regarding a previous diagnosis were taken into consideration in determining the mental status of the patients. Socio-demographic data form and the Mindful Attention Awareness scale (MAAS) and Modified Morisky Adherence scale (MMAS) were applied to the participants.

**Socio-demographic Data Form:** It is a form containing sociodemographic data prepared by the researchers in a content suitable for the purpose of the study.

**The MAAS:** The scale was developed to measure attention and awareness skills in daily life[8]. It is used to evaluate individual differences in being able to focus attention on the time lived and the body, not the past or the future. It consists of 15 items

in total. It is a 6 point Likert type scale. Turkish adaptation study has been done[9].

**The MMAS:** The original Morisky scale provides sufficient information on drug use habits. However, it was seen insufficient about the continuity of drug use during the long-term treatment of chronic diseases. Besides, the questionnaire was not designed to classify the knowledge and motivation levels of the patients. It contains questions answered as yes and no. As a result, the MMAS was developed with the addition of two new questions[10]. The Turkish validity and reliability study of the scale was conducted[11].

### Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences 20.0 statistical package for Windows. Continuous data were expressed as mean±standard deviation or median (minimum-maximum), while categorical data were presented as number of patients and percentage. The chi-square test was used for the comparison of categorical variables, while the Student's t-test or Mann-Whitney U test was used to compare parametric and nonparametric continuous variables, respectively. Normal distribution was assessed by the Kolmogorov-Smirnov test. Multivariate logistic regression analysis was performed to determinate the predictors of MMAS. A value of $p<0.05$ was considered statistically significant.

## RESULTS

Sixty-eight patients diagnosed with hypertension were included in the study. Socio-demographic characteristics are shown in Table 1. The mean age of the participants was 58.6±11.7 years; 70.6% (n=48) of them were male. Only 32.3% of them had high school and above education level. The mean MAAS score was 48.2±16.3. According to MMAS, drug adherence was low in 70.6% (n=48) of the group.

Comparison of demographic and clinical data of the study population by MMAS scores is given in Table 2. The mean age of the patients with low drug adherence was statistically significantly lower than that of those with medium-high drug adherence (p=0.01). No statistically significant difference was found between the two genders in terms of drug adherence (p=0.945). Regarding drug adherence in terms of educational status, employment status, and marital status, there was no significant difference between the groups with low and medium-high drug adherence (p=0.241, p=0.123, p=0.221). Considering drug adherence in patients with comorbid cancer, those with high adherence were significantly higher (p=0.006). The rate of low drug adherence was significantly higher in those with a family history of HT compared to the medium-high rate (p=0.011). There was no statistically significant difference in drug adherence scores in terms of HT duration (p=0.665). The mean MAAS scores were significantly higher in those with medium-high drug adherence (p=0.004, Figure 1).

The determinants of drug adherence in HT patients are shown in Table 3. Mindfulness was found to be an independent predictor according to age, family history of HT and MAAS results (p=0.002, p=0.019, p=0.005, respectively).

## DISCUSSION

Studies investigating the effect of mindfulness-based approaches on treatment adherence in hypertensive patients have been included in the literature in recent years[6,12]. Treatment adherence is an important problem in chronic diseases. There are many studies in the literature investigating the drug adherence of hypertensive patients[13]. It was reported that adherence to hypertension pharmacotherapy was below 50% one year after initiating the medication[4]. Studies have reported higher levels of drug non-adherence in hypertensive patients with uncontrolled BP[13,14].

**Table 1. Baseline characteristics and clinical data of study population (n=68)**

| | |
|---|---|
| Age (years) | 58.6±11.7 |
| Sex (male %) | 48 (70.6) |
| Education status (n %) | |
| Literate | 14 (20.6) |
| Primary school | 20 (29.4) |
| Middle school | 12 (17.6) |
| High school | 16 (23.5) |
| University | 6 (8.8) |
| Working status (n %) | |
| Working | 24 (35.3) |
| Not working | 16 (23.5) |
| Retired | 24 (35.3) |
| Disabled retired | 4 (5.9) |
| Married (n %) | 60 (88.2) |
| Living with nuclear family (n %) | 50 (73.5) |
| Diabetes mellitus (n %) | 26 (38.2) |
| Coronary artery disease (n %) | 28 (41.2) |
| Pulmonary disease (n %) | 8 (11.8) |
| Psychiatric disease (n %) | 4 (5.9) |
| Malignancy (n %) | 4 (5.9) |
| Family history of hypertension (n %) | 54 (79.4) |
| Family history of other medical condition (n %) | 30 (44.1) |
| Duration of hypertension (years) | 12.4±9.5 |
| Mindful Attention Awareness scale | 48.2±16.3 |
| Morisky Medication Adherence scale | |
| Low | 48 (70.6) |
| Moderate-high | 20 (29.4) |

In a study investigating the predictors of low drug adherence in the treatment of hypertension, it was found that 36.6% of the patients had good drug adherence[15]. In line with the literature, it was found that 70.6% of our patient group had low drug adherence in our study. It can be thought that the fact that hypertension is a chronic disease and that it is difficult to use drugs for a lifetime reduces adherence with drug therapy. Considering that the mean duration of hypertension in our patient group was 12.4±9.5, the concept of disease and treatment was not new for our patient group, and it was assumed that they were informed about the course of the disease and possible complications during this period; however, the treatment adherence was still poor.

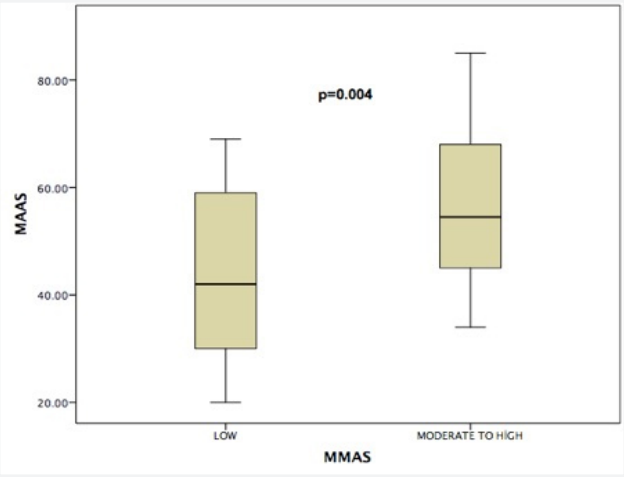


**Figure 1.** Comparison of MAAS and MMAS

*MMAS: Morisky Medication Adherence scale, MAAS: Mindful Attention Awareness scale*

Factors related to drug adherence have been the subject of many studies in the literature[16,17]. In a study, it was stated that the level of adherence was not dependent on gender, that the level of adherence increased with the age of the patient, and that there was no statistically significant relationship between drug adherence and education level[17]. Another study found that low drug adherence in hypertensive patients was associated with advanced age[18]. This may be related to factors such as decreased cognitive flexibility and the need to use medication for a longer period of time. There are also studies which found that young patients have lower medication adherence compared to older patients[15,19]. Similar to these studies, in our

**Table 2. Comparison of baseline characteristics and clinical data of the study population according to Morisky Medication Adherence scale**

| | Low (n=48) | Moderate to high (n=20) | p value |
|---|---|---|---|
| Age (years) | 55.7±11.5 | 65.5±9.1 | 0.001 |
| Sex (male %) | 34 (70.8) | 14 (70.0) | 0.945 |
| Education Status (n %) | | | |
| Literate | 8 (16.7) | 6 (30.0) | |
| Primary school | 12 (25.0) | 8 (40.0) | |
| Middle school | 10 (20.8) | 2 (10.0) | 0.241 |
| High school | 14 (29.2) | 2 (10.0) | |
| University | 4 (8.3) | 2 (10.0) | |
| Working status (n %) | | | |
| Working | 20 (41.7) | 4 (20.0) | |
| Not working | 10 (20.8) | 6 (30.0) | |
| Retired | 14 (29.2) | 10 (50.0) | 0.123 |
| Disabled retired | 4 (8.3) | 0 (0) | |
| Married (n %) | 44 (91.7) | 16 (80.0) | 0.221 |
| Living with nuclear family (n %) | 34 (70.8) | 16 (80.0) | 0.553 |
| Diabetes mellitus (n %) | 16 (33.3) | 10 (50.0) | 0.198 |
| Coronary artery disease (n %) | 18 (37.5) | 10 (50.0) | 0.340 |
| Pulmonary disease (n %) | 4 (8.3) | 4 (20.0) | 0.221 |
| Psychiatric disease (n %) | 4 (8.3) | 0 (0) | 0.312 |
| Malignancy (n %) | 0 (0) | 4 (20.0) | 0.006 |
| Family history of hypertension (n %) | 42 (87.5) | 12 (60.0) | 0.011 |
| Family history of other medical condition (n %) | 24 (50.0) | 6 (30.0) | 0.130 |
| Duration of hypertension | 12.7±10.5 | 11.6±6.8 | 0.665 |
| Mindful Attention Awareness scale | 44.6±15.3 | 56.8±15.5 | 0.004 |

**Table 3. Logistic regression analysis to determinate the predictors of MMAS**

| | Odds ratio | 95% Confidence interval | p value |
|---|---|---|---|
| Age | 1.105 | 1.036-1.178 | 0.002 |
| Family history of hypertension | 6.443 | 1.352-30.702 | 0.019 |
| MAAS | 1.076 | 1.023-1.133 | 0.005 |

MMAS: Morisky Medication Adherence scale, MAAS: Mindful Attention Awareness scale

study, it is observed that the drug adherence of those with a low average age is lower than those with a high average age. This may be due to the fact that younger patients take the disease or possible complications less seriously than the elderly. The fact that younger patients are likely to be more intense in activities of daily living and have more responsibilities may also be another factor that impairs drug adherence.

There are studies showing that women with resistant hypertension have more drug non-adherence than men[20]. In a study conducted with ischemic heart patients; women's belief in treatment and adherence was found to be better than men[21]. Another study found that young active men and the elderly with cognitive deficits were at high risk for drug non-adherence[22]. In another study investigating adherence to antihypertensives, it was shown that adherence to treatment was better in women than in men[23]. One study found that poor drug adherence in hypertensive patients was associated with male gender[18]. Considering the results of this study, it is seen that there are conflicting findings in the literature regarding the relationship between drug adherence and gender. In our study, no significant relationship was found between gender and drug adherence. This result may be due to the small sample size in our study. However, the obtained results suggest that gender is not an effective demographic factor on the perception of the severity of the disease and drug compliance.

It is mentioned in the literature that low education level, low income level and unemployment may decrease antihypertensive treatment adherence[19,20]. Contrary to these results, no statistically significant difference was found between drug adherence and education level and employment status in our study. It is particularly striking that no correlation in the same direction was found between education level and drug adherence. This result suggests that accessing information, having more information, and being able to access treatment economically are not sufficient for drug adherence. While it can be predicted that the socioeconomic level has a positive effect in terms of reaching and continuity of drugs, it can be predicted that this will increase drug adherence, our study results indicate that these factors are not effective and/or sufficient. As per our results, individuals with a good education level appear to be at risk for complications as well as those with low levels. When the literature findings and the results of our study are evaluated together, the importance of the mindfulness of individuals from all ages, genders and sociocultural levels for drug adherence can be understood. One study noted that a higher level of adherence was observed in patients taking longer-term hypertension medications[17]. As the duration of the disease increases, more awareness, being able to internalize the disease, and being exposed to complications may explain this result. However, contrary to this finding, in our study, no statistically significant difference was found in drug adherence scores in terms of disease duration. The fact that adaptation behavior has not developed despite the duration of the disease suggests the presence of other factors that make it difficult to adapt. One of these may be insufficient and/or disabled skills such as mindfulness, attention, organizing, managing time and work consciously, despite having information about the disease and possible risks. Many factors affecting these abilities in chronic diseases should be reviewed and studied. One of these factors may be the presence of comorbid mental and physical diseases. In a study examining the relationship between comorbidity and drug adherence in hypertensive patients, it was found that patients with no or only one comorbid state had more medication adherence than patients with two or more comorbid conditions[24]. According to this result, it can be thought that the number of comorbidities negatively affects drug adherence and the patient has difficulty in managing the current situations together. In a meta-analysis study in which 25 studies were included, it was found that 31.2% of hypertensive patients with comorbidity did not have drug adherence[13]. In one study, the presence of chronic heart failure was associated with low drug adherence[20]. In our study, when we looked at comorbid diseases in hypertensive patients, it was seen that they did not make a difference in terms of drug adherence except cancer. When drug adherence was examined in patients with cancer diagnosis, it was found that those with high adherence were significantly higher than those with low adherence. This result suggests that the higher the motivation to survive, the greater the awareness of drug adherence. The coexistence of another serious illness may be a factor that will increase conscious awareness. One study reported that hospitalization positively affected the treatment adherence process[20]. This result supports our aforementioned view. No data were found in the literature regarding the effect of hypertension in the family on adherence to antihypertensive medications. The result of our study is surprising contrary to what was expected. In our study, the rate of low drug adherence

was significantly higher in those with a family history of hypertension compared to the moderate-high rate. This result also supports our view that having knowledge, knowing the disease and its course may not be a sufficient factor for the development of regular drug use behavior. In one systematic review, age was an independent predictor of decreased systolic BP and diastolic BP values, regardless of technique and method of measuring BP. BP is present as older people may be more motivated to adhere to behavioral interventions in their desire to reduce pharmacological dependence[25]. Age, family history of HT and awareness levels were found to be independent determinants in our study. Potential reasons for low drug adherence, such as forgetting the drug, complicated drug regimen and side effects, are cited[26]. In a study, it was stated that the most important reason for low adherence was forgetting the drug with a rate of 51.4%[15]. Higher levels of depressive symptoms have been associated with low drug adherence[20]. Drug resistance and low drug adherence have been strongly associated with psychological profiles. While basic clinical characteristics were not reliable predictors of drug adherence and treatment resistance, psychological factors were found to be predictors[27]. In some studies conducted with individuals with chronic diseases, it was found that the patient's decision and responsibility for own treatment was directly proportional to better clinical outcomes[28,29]. It was suggested that self-monitoring and self- regeneration of drugs by patients in patients with high cardiovascular risk might help improve BP control[30]. This situation seems to be related to the ability to participate in the treatment effectively and consciously and to maintain it. High level of mindfulness is important in terms of high drug adherence and positive clinical outcomes in chronic diseases. For example, diabetic patients with higher levels of mindfulness have been shown to have higher normal glucose levels. In the study, it was mentioned that the level of mindfulness might be associated with better glucose regulation because it provided a better control[31]. There are publications reporting that mindfulness practice reduces symptoms in medical conditions such as cancer disease[32] and rheumatoid arthritis[33]. Clinically positive results have been reported in studies based on mindfulness in cardiovascular diseases[34].

In a study conducted in prehypertensive patients who did not receive treatment in recent years, it was found that there was a statistically and clinically significant decrease in BP and BMI after an eight-week mindfulness-based course and this decrease continued for another three months after the interview[7]. It was reported that mindfulness-based interventions could be viewed as preventive and complementary interventions in diabetes mellitus, especially for the relief of symptoms related to depression and anxiety in diabetic patients, and also in the management of other factors, including mindful eating, physical exercise, and adherence to therapy[35]. To the best of our knowledge, no study has been found in the literature that examines the relationship between the level of mindfulness and drug adherence in hypertensive patients. High MAAS scores in patients with medium-high drug adherence in our study supports our study hypothesis. It can be thought that high awareness enables the forgetfulness factor to be eliminated, to be organized, to be better aware of body sensations, to manage time correctly, and to follow the treatment process consciously. In our study, finding the awareness as per the MAAS scores as an independent predictor for drug adherence is significant. For drug adherence, it is necessary to remember the dosing hours, not to have trouble remembering, not to stop the treatment spontaneously on the assumption that the disease is recovered or the drug is not good without managing the process in cooperation with the physician. It can be said that mindfulness ensures that all components of this process are managed correctly by the patient. In chronic diseases, anxiety or depressive symptoms can be added to the process and may impair drug compliance. Although it was not evaluated in our study, it may be thought that drug adherence increased due to the positive effects of mindfulness on mood.

### Study Limitations

The small sample size in our study is one of our limitations. The fact that the scales used are self-report scales may also have affected the results of the study. In our study, the type and number of antihypertensive patients used were not studied, and the mood of the patients was not evaluated. Drug side effects that may lead to drug incompatibility have not been questioned. Studies in which these factors are also evaluated will contribute to other studies based on awareness.

## CONCLUSION

Our study results revealed that drug adherence was low in the hypertensive patient group, and there is a significant relationship between conscious awareness and drug adherence. Increasing drug adherence in patients with hypertension and increasing the level of mindfulness affecting drug adherence for this purpose is vital. Interventions to increase awareness in hypertensive patients should be noticed, and the patient should be supported in a multidisciplinary system.

### Ethics

**Ethics Committee Approval:** Ethics committee approval was obtained from Marmara University Faculty of Medicine Clinical Research Ethic Committee (number: 09.2019.414, date: 05.04.2019) to conduct the study.

**Informed Consent:** Informed consent was obtained.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Concept: P.Ş.G., E.A.S., T.G., M.S., Design: P.Ş.G., E.A.S., T.G., M.S., Data Collection or Processing: T.G., Analysis or Interpretation: P.Ş.G., M.S., Literature Search: P.Ş.G., E.A.S., Writing: P.Ş.G., E.A.S., T.G., M.S.

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

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# Effects of Quercetin on Cisplatin-Induced Renal Damage in Wistar Albino Rats

## Wistar Albino Sıçanlarda Sisplatin ile Oluşan Böbrek Hasarında Kuersetinin Etkileri

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**ABSTRACT**

**Aim:** Cisplatin is one of the effective antineoplastic drugs widely used in the treatment of many types of cancer. Cisplatin has harmful effects such as nephrotoxicity, ototoxicity and cardiomyopathy. Quercetin is an antioxidant of the flavonoid group. In this study, it was aimed to investigate the therapeutic effects of quercetin against cisplatin-induced kidney damage in rats.

**Materials and Methods:** Twenty-eight male Wistar albino rats were randomly selected and divided into 4 groups: Group 1: Control (no application), Group 2: Quercetin (25 mg/kg/7 days/intraperitoneal), Group 3: Cisplatin (7 mg/kg/single dose/ intraperitoneal), Group 4: Cisplatin+quercetin (7 mg) /kg/single dose/ intraperitoneal cisplatin followed by 25 mg/kg/7 days/ intraperitoneal quercetin). After routine histological follow-up, hematoxylin eosin and periodic acid-schiff staining were performed. Histopathological damage score was calculated. Caspase-3 immunostaining was performed and scored.

**Results:** Control and quercetin groups had normal histological appearance. In the cisplatin group, dilatation of the tubules, epithelial shedding, vacuolization of the tubular epithelial cells, and loss of microvilli in the proximal tubules were detected. In addition, infiltration areas were also found in places. In addition, an increase in caspase-3 immunostaining intensity was detected in this group (p=0.000). Histopathological findings were significantly reduced in the cisplatin+quercetin group compared to the cisplatin group (p=0.001).

**Conclusion:** In this study, we think that quercetin is histopathologically beneficial in the treatment of cisplatin-induced kidney damage.

**Keywords:** Cisplatin, quercetin, caspase-3, kidney toxicity, apoptosis

**ÖZ**

**Amaç:** Sisplatin birçok kanser türünün tedavisinde yaygın olarak kullanılan etkili antineoplastik ilaçlardan biridir. Sisplatinin nefrotoksisite, ototoksisite ve kardiyomiyopati gibi zararlı etkileri vardır. Kuersetin flavonoid grubu bir antioksidandır. Bu çalışmada, sıçanlarda sisplatin ile oluşturulan böbrek hasarına karşı kuersetinin tedavi edici etkilerinin incelenmesi amaçlanmıştır.

**Gereç ve Yöntem:** Wistar albino cinsi 28 adet erkek sıçan rastgele seçilerek 4 gruba ayrıldı. Grup 1: Kontrol (uygulama yapılmadı), Grup 2: Kuersetin (25 mg/kg/7 gün/intraperitoneal), Grup 3: Sisplatin (7 mg/kg/tek doz/ intraperitoneal), Grup 4: Sisplatin+kuersetin (7 mg/kg/tek doz/ intraperitoneal sisplatin ardından 25 mg/kg/7 gün/ intraperitoneal kuersetin). Rutin histolojik takipten sonra hematoksilen-eozin ve periodic acid-schiff boyamaları yapıldı. Histopatolojik hasar skoru hesaplandı. Caspase-3 immün boyaması yapılarak skorlandı.

**Bulgular:** Kontrol ve kuersetin grupları normal histolojik görünümdeydi. Sisplatin grubunda tubuler dilatasyon, tubul epitelinde dökülme, tubul epitel hücrelerinde vakuolizasyon ve proksimal tubullerde mikrovillus kaybı tespit edildi. Ayrıca yer yer infiltrasyon alanlarına da rastlandı. Sisplatin grubunun caspase-3 immün boyanma yoğunluğunda kontrol grubuna göre anlamlı artış tespit edildi (p=0,000). Sisplatin+kuersetin grubunda histopatolojik bulgular sisplatin grubuna kıyasla anlamlı derecede azalmıştı (p=0,001).

**Sonuç:** Bu çalışmada, sisplatinin sebep olduğu böbrek hasarının tedavisinde kuersetinin histopatolojik açıdan yararlı olduğu düşüncesindeyiz.

**Anahtar Kelimeler:** Sisplatin, kuersetin, caspase-3, böbrek toksisitesi, apoptoz

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## INTRODUCTION

Cisplatin is one of the potential and widely used drugs in the treatment of various solid cancers such as testicular, ovarian, head and neck, bladder, lung, lymphoma, cervical cancer, and melanoma[1]. The anticarcinogenic effect of cisplatin occurs through the interaction with purine bases on DNA, causing deoxyribo nucleic acid (DNA) damage and activation of signal transduction pathways that lead to apoptosis (programmed cell death)[1]. It has been shown that cisplatin plays a role in the formation of reactive oxygen species, thus inducing apoptosis via intrinsic caspases and causing mitochondrial dysfunction[2]. Cisplatin fights tumors through the induction of apoptosis mediated by activation of various signal transduction pathways, including calcium signaling, death receptor signaling, and activation of mitochondrial pathways[2]. The most important limiting factor in the use of anticarcinogenic drugs is their side effects. Cisplatin is characterized by toxic effects such as nephrotoxicity, cardiotoxicity, hepatotoxicity, neurotoxicity and myelosuppression[3].

Quercetin (3,3',4',5,7-pentahydroxyflavone), a member of the flavonoid family, is one of the polyphenolic compounds found in various foods[4]. Quercetin is found in onions, apples, strawberries, cauliflower, cabbage and many other foods[5]. Studies have shown that quercetin has anticarcinogenic effects, but it reduces oxidative damage by inhibiting the activity of xanthine oxidase, a form of xanthine oxidoreductase, which is a type of enzyme that produces reactive oxygen species, and has anti-inflammatory activity by inhibiting the production of tumor necrosis factor alpha depending on the dose[6-8].

Caspase-3, a cysteine-aspartic acid protease, is not activated until it is cleaved by initiator caspases during the apoptotic flux[9]. After activation, it cuts non-caspase target proteins in cells from their specific regions[10,11]. Thus, it plays an important role in programmed cell death[12].

In this study, it was aimed to histopathologically examine the therapeutic effects of quercetin against cisplatin-induced kidney damage in rats.

## MATERIALS AND METHODS

### Groups

Approval for the study was obtained from the Animal Experiments Local Ethics Committee of İnönü University Medical Faculty (ethics committee no: 2012/A-103, date: 09.06.2012). Animal rights were protected in line with the principles of the 'Guide for the Care and Use of Laboratory Animals'. In line with these principles, 28 healthy male Wistar albino rats, 28-30 days old and weighing 300-350 grams, obtained from Inonu University Faculty of Medicine Experimental Research Laboratory, were used. The rats were left in a room with 12 hours of light and 12 hours of darkness, in a daylight rhythm, in a ventilated environment with a temperature of 21 °C and a humidity of 55-60% for 21 days. They were fed ad libitum with standard pellet feed and tap water in special cages. Randomly selected rats were divided into 4 equal groups, each with7 animals. Groups were organized as Group 1: Control (no application), Group 2: Quercetin (25 mg/kg/intraperitoneal for 7 days), Group 3: Cisplatin (single dose 7 mg/kg/intraperitoneal), Group 4: Cisplatin+quercetin (single dose 7 mg/kg/intraperitoneal cisplatin, then 25 mg/kg/intraperitoneal quercetin for 7 days). Quercetin (CAS Number: 117-39-5) and cisplatin (CAS number: 15663-27-1) were obtained from Sigma Chemical Co. (St. Louis, MO). Cisplatin and quercetin doses used in this study were determined according to previous studies in the literature[13,14].

### Histopathological Analysis

At the end of the experiment, the rats were sacrificed under ketamine (90 mg/kg/intraperitoneal) anesthesia. Their kidneys were removed, rinsed with saline and fixed in 10% neutral buffered formalin solution for histological evaluation. After the tissues were fixed in formalin for 72 hours, they were dehydrated by passing through increasing alcohol series (70%, 80%, 96% and 100%). Finally, they were kept in xylene and embedded in paraffin. Tissue sections with 5 µm thickness were obtained using a fully automated microtome. Hematoxylin-eosin staining method was used to examine the general histological structure and periodic acid-schiff (PAS) staining method was used to observe glycogen accumulation.

### Histopathological Evaluation

Evaluation was done in a double-blind fashion by a histologist in the study. Renal damage was determined semi-quantitatively according to the degree and extent of histopathological changes. Tissues were examined for dilation of tubules, shedding of tubular epithelium, vacuolization of tubular epithelial cells, peritubular infiltration and loss of microvillus in proximal tubules. All sections were examined at 20X magnification in 10 different fields and scored as 0 (no change), 1 (mild), 2 (moderate), and 3 (severe) for each parameter[15]. The maximum mean histopathological damage score was 15. Tissue sections were examined with a Leica DFC 280 light microscope and Leica Q Win image analysis system (Leica Microscope Imaging Solution Ltd, Cambridge, UK) and evaluated, and their photographs were taken.

### Immunohistochemical Analysis

Immunohistochemical (IHC) staining was performed using caspase-3 antibody (ab13847; Abcam, Kimera, Turkey). Tubular and glomerular caspase-3 immunoreactions were examined semi-quantitatively under a Leica DFC 280 light microscope.

To determine the staining intensity, 10 fields from each section were examined at X20 magnification and scored as (0) no staining, (1) weak staining, (2) moderate staining, and (3) severe staining. Tissue sections were examined with Leica DFC 280 light microscope and Leica Q Win image analysis system, scored and photographed.

### Statistical Analysis

Statistical analyses were performed with SPSS (SPSS for Windows version 13.0) software. All results were expressed as arithmetic mean±standard error. In comparison of the groups, the Kruskal Wallis analysis of variance, which is one of the non-parametric tests, was used to compare all groups for all variables, while the Mann-Whitney U test was used for pairwise comparison of variables. A p value of <0.05 was considered statistically significant.

## RESULTS

### Histopathological Findings

The control group had normal histological appearance. The quercetin group had normal histological appearance like the control group. There was no statistically significant difference between the control group and the quercetin group in terms of histopathological findings (p>0.05). The mean histopathological damage score in the cisplatin group (Figure 1F) was statistically significantly increased compared to the control (Figure 1A) and quercetin (Figure 1B) groups (p=0.001). In the cisplatin group, dilatation of tubules, epithelial shedding into tubules (tubular caste), swelling and vacuolization of tubular epithelial cells and loss of microvillus in proximal tubules were detected (Figure 1C, 1E, 1H). In addition, increased PAS (+) staining intensity in the glomeruli, extensive hemorrhage, and occasionally peritubular infiltration areas were also observed in the cisplatin group (Figure 1D, 1F, 1G, 1H). All histopathological findings evaluated in the Cisplatin+Quercetin group were significantly reduced compared to the cisplatin group (p=0.001) (Table 1) (Figure 1C-K). The mean histopathological damage scores of all groups are given in Table 1.

### Immunohistochemical Findings

Mild staining was detected in the control and quercetin groups with anti-Caspase-3 antibody (Figures 2A, 2B). In the cisplatin group, the intensity of the staining was noticeably increased,

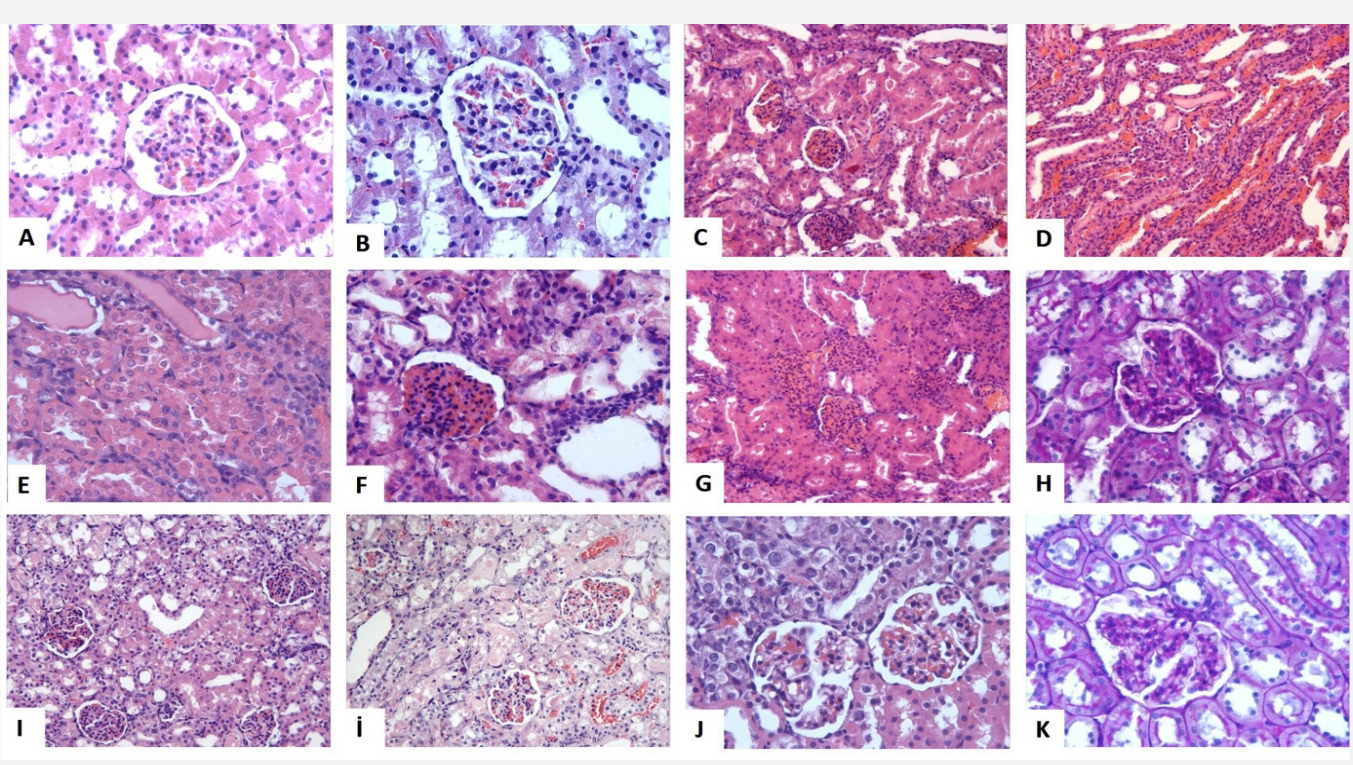


**Figure 1.** Kidney histopathology. Control group (A) and quercetin group (B) had normal histological appearance [hematoxylin-eosin (H-E) X20]. Many histopathological findings were found in the cisplatin group (C-H). In this group; dilatation in tubules (C), diffuse hemorrhage (D), epithelial shedding into tubules (E), swelling and vacuolization of tubular epithelial cells (E), peritubular infiltration (F, G), loss of microvillus in proximal tubules and increased PAS (+) staining in glomeruli (H) were detected. C. D. Cisplatin (H-E X10). E-F. Cisplatin (H-E X20). G. Cisplatin (H-E X10). H. Cisplatin (PAS X20). Histopathological findings were decreased in the cisplatin+quercetin group. I-J. Cisplatin+quercetin (H-E X20). K. Cisplatin+quercetin (PAS X20)

especially in the proximal tubules. When the Cisplatin and Cisplatin+Quercetin groups were compared, a statistically significant decrease in Caspase-3 IHC staining intensity was observed (p<0.005) (Figures 2C, 2D). The IHC scores of all groups are given in Table 2.

## DISCUSSION

One of the most common side effects of cisplatin is dose-dependent renal toxicity[16]. Cisplatin nephrotoxicity may present with very different manifestations such as acute kidney injury, distal renal acidosis, hyperuricemia, hypomagnesemia, hypocalcemia or chronic kidney failure[17-22]. Cisplatin nephrotoxicity is the result of transport of cisplatin to renal epithelial cells, injury of nuclear and mitochondrial DNA, activation of cell death pathways, and initiation of a strong inflammatory response[23].

In a study, tubular degeneration and necrosis, hyaline eruptions in the tubules, intertubular hemorrhage, glomerular obstruction, and vacuolization were reported in the kidneys of male Sprague-Dawley rats weighing 200 grams that were administered a single intraperitoneal dose of 7 mg/kg

**Table 1. Table of histopathological damage scores for all groups**

| Groups | Histopathological damage score |
|---|---|
| Group 1: Control | 0.50±0.26 |
| Group 2: Quercetin | 0.87±0.35 |
| Group 3: Cisplatin | 11.37±0.56[a] |
| Group 4: Cisplatin+quercetin | 7.62±0.37[b] |

Data were expressed as arithmetic mean±standard error (n=7).
[a]Group 3 vs group 1 and group 2 p=0.001
[b]Group 4 vs group 3 p=0.001

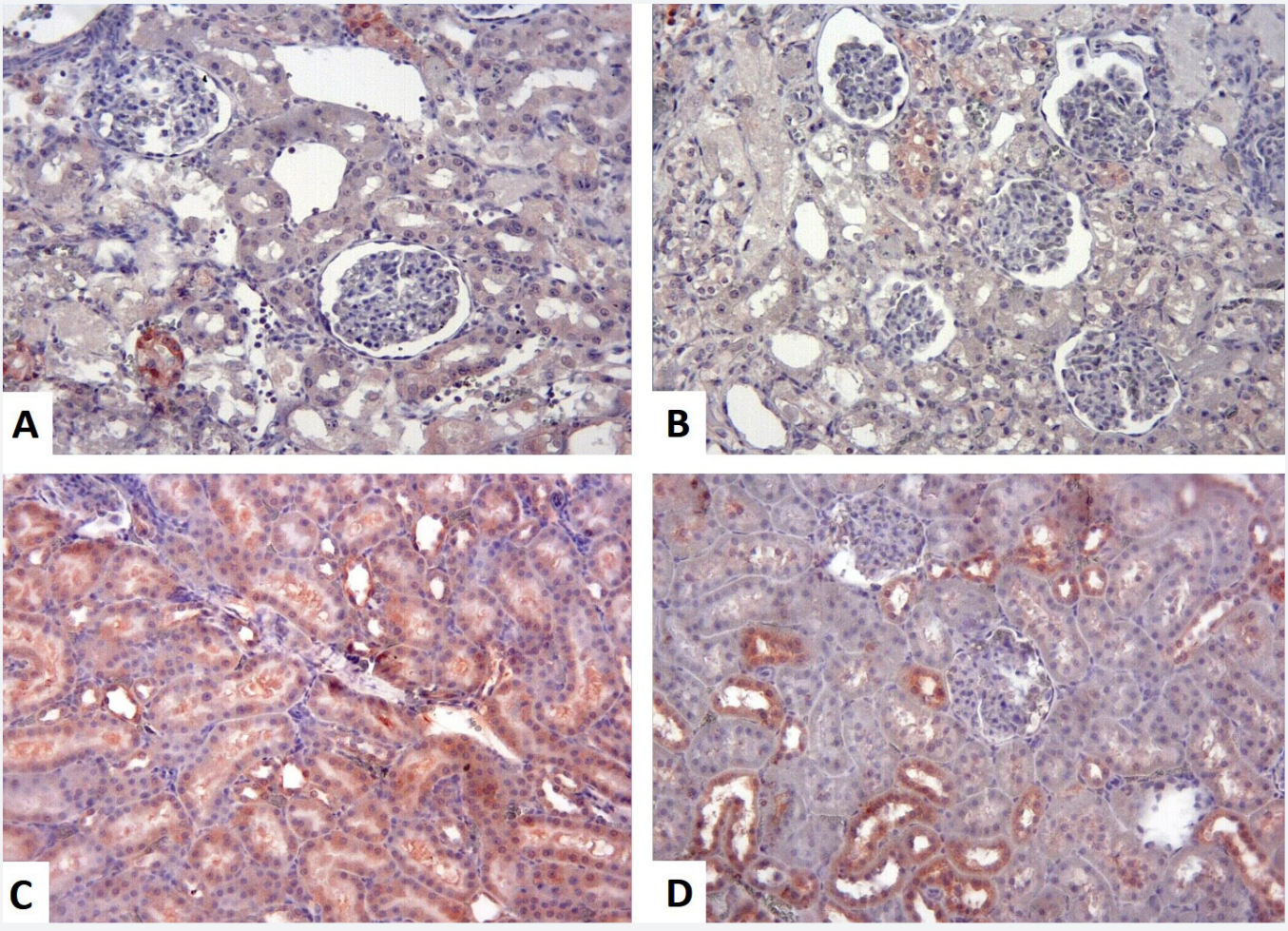


**Figure 2.** Immunohistochemical (IHC) staining with anti-caspase-3 antibody. Mild staining was detected in the control group (A) and quercetin group (B) (anti-caspase-3 X20). C. In the cisplatin group, the intensity of staining with anti-caspase-3 was increased especially in the proximal tubules (anti-caspase-3 X20). D. A decrease in IHC staining intensity was observed in the cisplatin+quercetin group (anti-caspase-3 X20)

**Table 2. Caspase-3 immune score table for all groups**

| Groups | Caspase-3 staining intensity |
|---|---|
| Group 1: Control | 0.37±0.18 |
| Group 2: Quercetin | 0.62±0.18 |
| Group 3: Cisplatin | 2.37±0.18[a] |
| Group 4: Cisplatin+quercetin | 1.37±0.18[b] |

Data were expressed as arithmetic mean±standard error (n=7).

[a]Group 3 vs group 1 and group 2 p=0.000

[b]Group 3 vs group 4 p<0.005

cisplatin[24]. We also applied the same dose of cisplatin in our study and observed the findings of dilatation and epithelial shedding (tubular caste) in the renal tubules. In another recent study, perivascular inflammatory cell infiltration, as well as tubular vacuolar degeneration and hyaline desquamation in the lumen of the tubules, was detected in the kidneys of rats administered the same dose of cisplatin as ours[25]. These findings also support our study.

In a study conducted to observe the antioxidant effects against cisplatin renal toxicity, microvillus deformation in the tubules and focal loss were observed in electron microscopic imaging of kidney tissue[26]. In our study, we observed loss of microvillus in the proximal tubules in sections with PAS staining.

Due to the serious side effects of cisplatin, various agents with antioxidant activity are being tested with this chemotherapeutic drug[27]. Quercetin is one of the most common dietary polyphenolic compounds, which is abundant in many foods. It is an effective antioxidant against radical oxygen species that prevents oxidation of low-density lipoproteins by scavenging free radicals and chelating transition metal ions[6,28]. It has anti-inflammatory, anticarcinogenic and antiviral properties[29-31].

In a study conducted to reverse the effects of nephrotoxicity, it was observed that histopathological findings such as renal tubular degeneration caused by lead element, necrosis, vacuolization and mononuclear cell infiltration regressed with 10 mg/kg quercetin and returned to normal histological appearance[32]. In our study, we found that dilatation in the renal tubules and vacuolization in tubular epithelial cells improved in the Cisplatin+quercetin group compared to the cisplatin group.

In renal damage induced by cyclosporine, quercetin has been found to reduce the findings of interstitial fibrosis, arteriopathy, glomerular basement membrane thickening, vacuolization of tubular epithelial cells and desquamation into the tubular lumen[33]. In our study, we observed that epithelial shedding (tubular caste) and vacuolization findings decreased with the application of quercetin.

In a study investigating the effects of quercetin in a diabetic nephropathy model, it was reported that histopathological findings such as epithelial desquamation, intracytoplasmic vacuolization, loss of brush border in the proximal tubules, and peritubular infiltration were reduced by the administration of quercetin[15]. In our study, we found that tubular dilatation, epithelial shedding in the tubule lumen (tubular caste), vacuolization in tubular epithelial cells, and loss of microvilli in the proximal tubules improved with the application of quercetin.

Caspase-3 is a key zymogen in cell apoptosis[9]. In a study, rats with experimental mammary adenocarcinoma were administered 4 mg/kg cisplatin and then 50 mg/kg quercetin. It has been observed that cisplatin causes shedding of renal tubular epithelial cells, loss of brush border, hyaline deposition in the tubule lumen, and enlargement of tubules. It has been reported that as a result of caspase-3 immunostaining, the staining intensity increased with cisplatin, but the staining intensity decreased in the cisplatin+quercetin group[34]. In our study, 7 mg/kg cisplatin and 25 mg/kg quercetin were administered and it was determined that the staining intensity increased with caspase-3 antibody.

### Study Limitations

Although the current study has given the expected results, it has some limitations. The most important limitation is that there had to be biochemical data because quercetin is an antioxidant substance. In addition, validation of data obtained from examinations with electron microscopy can make the results even more reliable.

## CONCLUSION

As a result, it is seen that quercetin is histopathologically beneficial in the treatment of kidney toxicity caused by cisplatin and has a positive effect on apoptotic pathways. We think that further studies on this subject with different doses and durations will contribute to the literature.

### Ethics

**Ethics Committee Approval:** The study was approved by the İnönü University Medical Faculty Animal Experiments Local Ethics Committee (ethics committee no: 2012/A-103, date: 09.06.2012).

**Informed Consent:** It is an animal experiment.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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

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

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

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LETTER TO THE EDITOR

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# Iatrogenic Coronary Artery Dissection: Early or Late Intervention?

## İyatrojenik Koroner Arter Diseksiyonu: Erken Müdahale mi Geç Müdahale mi?

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**Dear Editor,**

In clinical practice, coronary artery dissections mostly arise spontaneously or as iatrogenic complications [due to percutaneous coronary interventions (PCIs), etc.] and appear to be associated with significant morbidity and mortality[1-5]. Importantly, the guidance of intravascular imaging tools [intravascular ultrasound (IVUS), etc.] has been strongly recommended for the invasive management of coronary artery dissections[4]. Unfortunately, these imaging modalities may not always be readily available in all cardiovascular clinics potentially leading to a significant therapeutic challenge particularly in cases requiring urgent management. On the other hand, conservative management of these dissections (followed by a deferred PCI, where necessary) might also be considered as an efficient alternative in the absence of high-risk clinical characteristics (including malignant arrhythmias, hemodynamic compromise and intractable angina pectoris)[1]. Herein, we report conservative management of an iatrogenic coronary artery dissection (due to a PCI) in a 47-year-old male patient. Written informed consent for publication was obtained from the patient.

A 47-year-old male was referred to our clinics for a failed PCI of the right coronary artery (RCA) after his admission with a non-ST segment elevation myocardial infarction presentation in another center. Coronary angiographic (CAG) records demonstrated an iatrogenic dissection of the RCA (Figure 1A). The patient admitted to the coronary intensive care unit had a blood pressure value of 130/80 mmHg along with a pulse rate of 96/min. Electrocardiogram exhibited a normal sinus rhythm with inferior q waves and T wave inversion. A repeat CAG revealed a long spiral dissection starting from the ostium and extending to the mid-distal segments of the RCA, and to a minor extent, to proximal aorta (Figure 1B). Owing to the stable clinical status of the patient and apparently challenging features for further interventional modalities, the patient was managed with a conservative strategy alone (receiving necessary medications including acetyl salicylic acid, clopidogrel, metoprolol, ramipril, benidipine, trimetazidine, isosorbide mono nitrate). During the hospital stay, there was no further progression of the proximal aortic dissection as demonstrated on computed tomography (Figure 2A, 2B). CAG at 3 months demonstrated complete recovery of the RCA and aortic dissections along with a critical stenosis in the distal RCA (Figure 3A) that was subsequently managed with a drug-eluting stent (2.5x16 mm) (Figure 3B).

The CAG characteristics of iatrogenic coronary artery dissections might help predict clinical outcomes and hence, potentially allow the implementation of the most efficient management strategy[5]. In this regard, harnessing the National Heart Lung and Blood Institute classification may be quite practical[5]. Notably, double-wire technique may also be tried in certain cases with high-risk features particularly in the absence of intravascular imaging guidance with IVUS, etc.[6].

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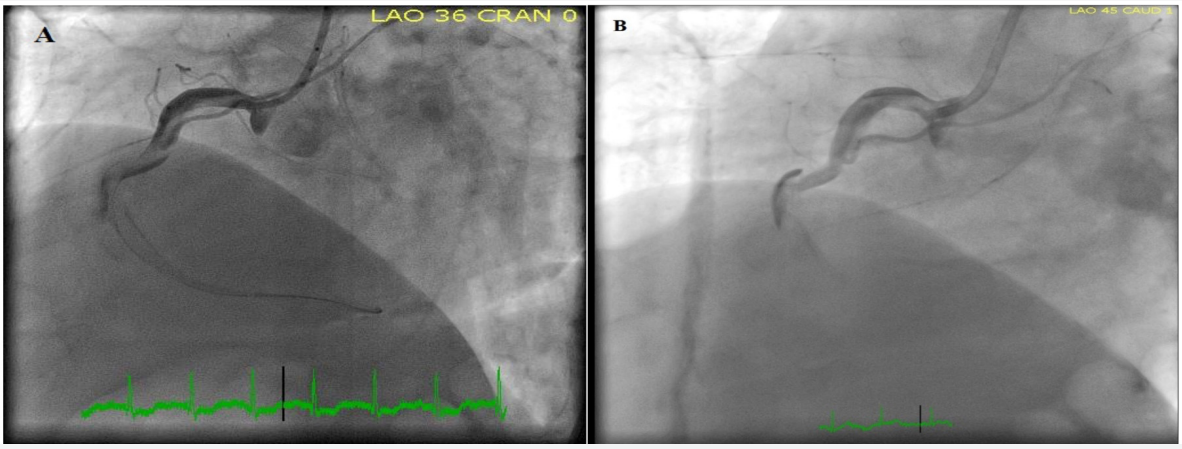


**Figure 1.** Iatrogenic dissection of the right coronary artery in external center, A. Spiral dissection due to the catheter in the procedure, B. A repeat control angiography image in internal center

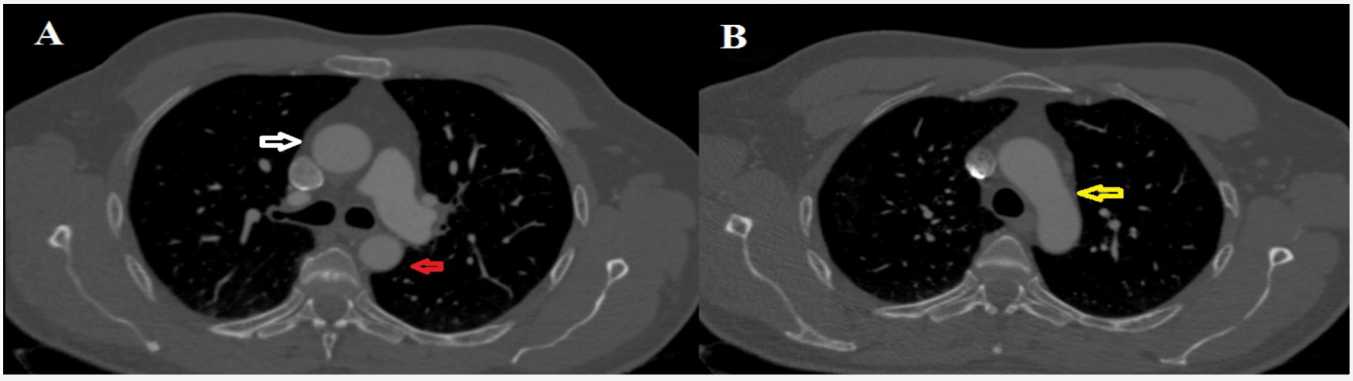


**Figure 2.** Aortic images in computed tomography angiography, A. There was no progress in dissection of the ascending aorta (white arrow) and descending aorta (red arrow). B. Arcus aorta (yellow aorta)

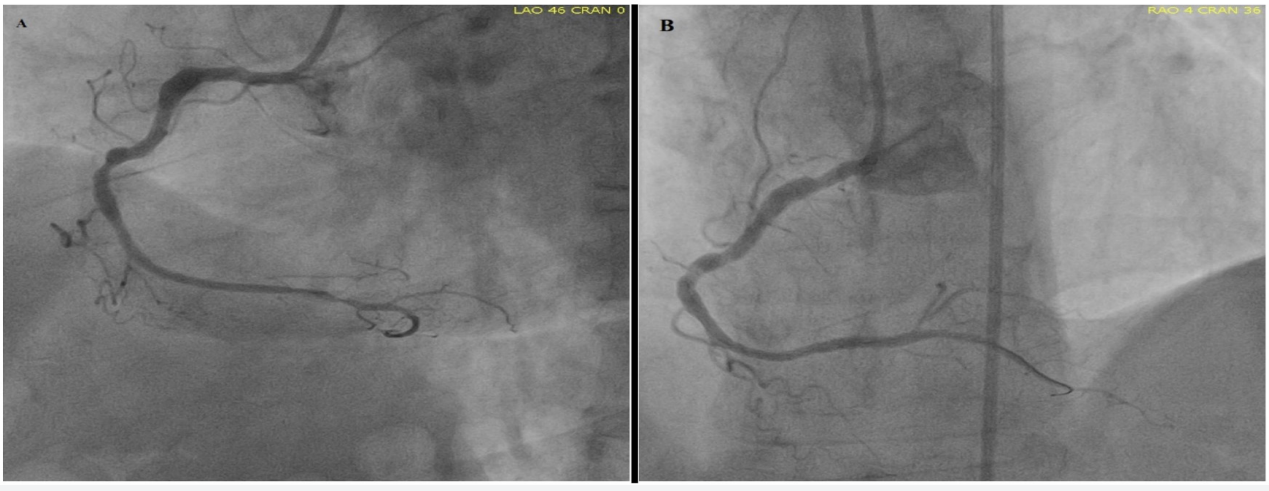


**Figure 3.** A. Control angiography image at the 3rd month after the procedure, B. Angiographic image after directly right coronary artery distal drug-eluting stent (2.5x16 mm) implant

In conclusion, the present case clearly demonstrates the feasibility of conservative management of iatrogenic coronary artery dissections (followed by a deferred PCI, where necessary) in the absence of high-risk clinical features. In this context, conservative strategy might possibly prevent further complications and unnecessary stent implantations potentially associated with adverse outcomes in the short and long-terms.

### Ethics

**Informed Consent:** Consent form was filled out by a participant.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

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