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Restless Legs Syndrome: From Diagnosis to Treatment

Huzursuz Bacaklar Sendromu: Tanıdan Tedaviye

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ABSTRACT

Restless legs syndrome (RLS) can be described by an urge to move limbs that typically coincides with an uncomfortable sensation. When at rest or inactivity, signs may start or develop worse; they usually go away when one moves or gets up for a walk. RLS can be both idiopathic or secondary to many kinds of health conditions, such as deficiency of iron, diabetes, obesity, hypothyroidism, and chronic renal failure. At the admission, secondary causes and iron tests, such as transferrin saturation and ferritin, must be evaluated. Assessments should be repeated when symptoms worsen, or when augmentation develops. Augmentation is a significant adverse effect of therapy by levodopa and dopamine agonists. More severe signs, early appearance of symptoms, and spreading of symptoms from the legs to other body parts are indicative of augmentation. Non-pharmacological treatments help some RLS patients control their symptoms. Iron-replacement therapy is a first-line treatment option for patients with indications of low body iron stores. The use of α2δ ligands as initial treatments instead of dopamine agonists has been recommended recently.
Keywords: Restless legs syndrome; augmentation; iron deficiency; iron therapy.

ÖZ

Huzursuz bacaklar sendromu (HBS), uzuvları hareket ettirme dürtüsünün tipik olarak rahatsız edici bir hisle örtüşmesiyle tanımlanabilir. Dinlenme veya hareketsizlik sırasında belirtiler başlayabilir veya daha kötü gelişebilir; genellikle kişi hareket ettiğinde veya yürüyüşe çıktığında kaybolurlar. HBS hem idiyopatik hem de demir eksikliği, diyabet, obezite, hipotiroidizm ve kronik böbrek yetmezliği gibi birçok sağlık durumuna sekonder olabilir. İlk başvuruda ikincil nedenler ve transferrin saturasyonu, ferritin gibi demir testleri değerlendirilmelidir. Semptomlar kötüleştiğinde veya augmentasyon geliştiğinde değerlendirmeler tekrarlanmalıdır. Augmentasyon, levodopa ve dopamin agonistleriyle yapılan tedavinin önemli bir olumsuz etkisidir. Belirtilerin daha şiddetli olması, belirtilerin erken ortaya çıkması ve belirtilerin bacaklardan diğer vücut bölgelerine yayılması augmentasyonun göstergesidir. Farmakolojik olmayan tedaviler bazı HBS hastalarının semptomlarını kontrol altına almasına yardımcı olur. Demir replasman tedavisi, vücut demir depolarının düşük olduğu belirtileri olan hastalar için birinci basamak tedavi seçeneğidir. Son zamanlarda dopamin agonistleri yerine başlangıç tedavileri olarak α2δ ligandlarının kullanılması önerilmiştir.
Anahtar kelimeler: Huzursuz bacaklar sendromu; augmentasyon; demir eksikliği; demir tedavisi.

INTRODUCTION

Restless legs syndrome (RLS), which is also referred to as Willis-Ekbom illness, was first identified by Sir Thomas Willis in 1685 (1). Later, in the mid-twentieth century, Karl Ekbom determined and described the syndrome further (2). The characteristic symptoms of RLS, a sensorimotor disease, include an urge to move an individual's legs, usually accompanied by an uncomfortable feeling (3). Symptoms with a circadian pattern begin or worsen at moments of resting or inactivity, and tend to be most severe in the late afternoon or early part of sleeping (4). RLS symptoms are usually experienced concurrently and on both sides in the deep muscles and bones of the lower extremities (5).

The quality of life and sleep of RLS patients can be seriously affected (6). Patients with RLS frequently seek medical advice due to insomnia. Furthermore, the severity of RLS can exacerbate symptoms of depression and decrease the quality of life. For this reason, it's important to recognize, prevent, and manage RLS symptoms. Previous studies demonstrated that the diagnosis is frequently missed and prolonged by years (7).

The aim of this review was to increase awareness about RLS diagnosis and treatments. Therefore, if medical professionals are more knowledgeable about RLS diagnosis and treatment, early identification and treatment of patients may be possible.

EPIDEMIOLOGY

RLS is a widespread disease that more commonly affects females than males with a prevalence of 5-10.6% (8-10). However, the average frequency of RLS is found to go up to 18-23% in the elderly (11). RLS has been shown to affect females 1.5-2 times more frequently than males in the majority of epidemiological studies (12). The results of the research indicate that RLS involves 3.6% of the Turkish population, with a relatively high prevalence in younger people (13).

PATHOPHYSIOLOGY

RLS pathogenesis is yet uncertain. Several hypotheses have been described, such as the impact of abnormal metabolism of iron, various neurotransmitters, and the primary opiate function (14). Radiology investigations demonstrated a link between the metabolism of iron with RLS, particularly in the nervous system (15). Whether low levels of iron in the central or peripheral nerve systems cause RLS remains a topic of debate (2). In a previous study, patients with RLS were shown to have decreased ferritin and increased transferrin levels of cerebral fluid (16). It has been shown that RLS severity increases by decreased peripheral iron levels, and the frequency of RLS has been reported to be higher in individuals suffering from iron deficiency anemia (17). Despite this, many RLS patients have normal serum ferritin levels (14). The current consensus suggests a cerebral iron shortage is an essential biological component of RLS, probably caused by a combination of variables such as low peripheral iron and/or genetics (18).

The rate-limiting enzyme in the production of dopamine, tyrosine hydroxylase, also needs iron as a cofactor (16). The pathological function of the dopaminergic system is supported by the significant relief of RLS symptoms with low doses of dopaminergic treatments. Studies haven't found any differences in CSF levels of dopamine between RLS patients and healthy controls, however (19). Investigations have demonstrated dopaminergic system abnormalities in RLS patients, particularly in the striatum. Dopamine signaling and dopamine receptor modulation are shown to decrease to their lowest level at night while increasing up to the highest level in the morning so-called circadian rhythm of dopamine (16). In addition to dopaminergic mechanisms, the pathophysiology of RLS has also been linked to endogenous opioids, glutamate/glutamine, and gamma-aminobutyric acid systems (1,11).

CLINICAL PRESENTATION AND DIAGNOSIS

RLS is identified by an urge to move the legs, unpleasant and uncomfortable sensations described as “twitching and wiggling”, “burning and itching”, “tingling and prickling”, “cramp”, “pins and needles”, “tickling sensation deep inside the muscles”, “electric shock sensations”, “prickling and pain” (20). RLS symptoms typically occur in both legs, but may start in one of the legs and subsequently spread to the other, or they may appear in the other leg on a separate day (21). However, RLS may be purely unilateral, as well. The features of RLS include an uncontrollable urge to move the lower extremities, occurring at rest or inactivity (21). At night, symptoms can occur or get worse compared to the daytime (2). The characteristics and diagnostic criteria of this sensorimotor phenomenon, updated in 2014 by the International Restless Legs Syndrome Study Group (IRLSSG) are summarized in Table 1 (11,21).

In addition to basic clinical criteria, RLS has 4 supportive characteristics such as periodic leg movements (PLMs), dopaminergic therapy response, family history of RLS, and absence of predicted sleepiness during the day that can support a diagnosis (2,21-23). Previous studies demonstrated that severe PLMs occur in approximately 80% of patients with RLS examined in clinical practice (2,22). Most RLS patients have at least some initial clinical relief from dopaminergic medications. When asked about their family members' history, 50 to 60% of patients with RLS indicate

Table 1. Diagnostic criteria for restless leg syndrome (11,21).

1. An urge to move the legs usually but not always accompanied by uncomfortable and unpleasant sensations in the legs
2. Symptoms begin or worsen during periods of rest or inactivity such as lying down or sitting
3. Symptoms are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues
4. Symptoms occur or are worse in the evening or night than during the day
5. The occurrences of the above features are not solely accounted for as symptoms primary to another medical or behavioral condition (e.g. myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping).

a relative of the first degree who has RLS signs (11). Patients may experience fatigue, reduced concentration, and depression as an outcome of sleep deprivation, but they do not report a level of daytime sleepiness (21).

RLS primarily affects the limbs. However, other body parts, including the mouth, neck, arms, face, abdomen, and genitals, have also been reported to be affected by RLS (21,22,24-27). The bodily parts most commonly affected were the legs, arms, trunk, head, neck, and genital region, in that order (28). When limb symptoms are absent, the involvement of other body parts can sometimes make a diagnosis difficult (28). There have been reports of atypical cases of RLS in which the legs show little or no impairment while the arms are primarily involved (27). Up to 50% of RLS patients experience upper-limb impairment, usually in those who experience more severe RLS symptoms (29). Additionally, "phantom" RLS cases have been documented when people who have had limbs amputated experience symptoms of RLS in the area where the missing limb was supposed to be (30).

For research and treatment planning, more homogeneous subgroups were defined. The definition of "intermittent RLS" refers to restless legs which, although less frequent than twice a week on average, are severe enough to need medical treatment (31). Generally, non-pharmacologic or intermittent medical therapies are recommended. Chronic persistent RLS occurs at least twice a week and is sufficiently severe to require daily medication. RLS that is resistant to monotherapy with appropriate doses of initial medicines because of side effects, augmentation, or effectiveness is known as refractory RLS (11).

COMORBIDITIES

Restless legs syndrome can be both idiopathic or secondary to many kinds of health. Secondary RLS, as compared to idiopathic RLS, usually begins over the age of 45 and progresses rapidly (32). Before the age of 45, RLS, is known as early-onset RLS, which is not closely related to blood levels of iron and progresses slowly with age. It is also common in family members (32). One of the most significant secondary causes of RLS is anemia due to iron shortage, for which the prevalence of RLS is reported as high as 25% to 35% (33).

Since, being a blood donor, pregnancy and end-stage renal disease are the potential risks for iron deficiency, these conditions are considered as the important contributors of secondary RLS (34). Regarding this, among 500 pregnant women, the prevalence of RLS is reported to be 17% for the first trimester, 27% for the second, and 30% for the third (35). Pregnancy-related symptoms can occur or get worse for a variety of reasons, such as iron and folate shortages, changes in hormones, and causes for vascular congestion in the legs (36). Patients who require dialysis due to end-stage renal illness also frequently experience RLS. Other conditions that are shown to be linked with RLS can be summarized as obesity, migraine, stroke, polyneuropathy, Parkinson's disease, hyperphosphatemia, obstructive sleep apnea, insomnia, anxiety, depression, emotion-focused coping with stress, and excessive daytime sleepiness, as well as narcolepsy (Table 2, 33-35). In addition, many drugs have been reported to aggravate or cause RLS, such as neuroleptics, antidepressants, antihistamines, lithium, and dopamine antagonists (37).

TREATMENT

Secondary causes of RLS should be evaluated and restored in the first step of treatment strategies of RLS. RLS symptoms may be reduced by treating some treatable comorbid conditions such as diabetes, obesity, hypertension, hypothyroidism, iron deficiency anemia, magnesium deficiency, and others. Iron stores should be evaluated both initial diagnosis of RLS and subsequently while long-term therapy. In addition, iron stores should be assessed in cases where there is a decreasing benefit from previously successful therapy, signs of augmentation, or a general clinical increase of symptom prevalence or intensity. Early in the morning following an overnight fast, serum ferritin, iron, percentage transferrin saturation, and total iron-binding capacity should all be assessed. It is recommended that oral iron therapy be tried for all RLS patients with serum ferritin concentrations of 75 mg/L or less and transferrin saturation levels of less than 45% (38). In cases when a more rapid response is required, oral iron cannot be adequately absorbed and tolerated, or if a three-month trial of oral iron use is inadequate to improve the symptoms of restless legs, intravenous iron therapy may be considered (38).

Many non-pharmacological therapies are beneficial in the treatment of idiopathic (primary) RLS. Massage, stretching, walking, cognitive distraction, and warm or cold baths are examples of the non-pharmacological therapy (11). These non-pharmacological treatments are

Table 2. Restless legs syndrome comorbidities (33-35)

Medical Disorders	Neurologic Disease	Medications
Iron deficiency	Parkinson disease	Antidepressants
Anemia	Multiple sclerosis	Neuroleptics
Blood donors	Stroke	Dopamine antagonists
Pregnancy	Migraine	Antihistamines
Renal failure	Narcolepsy	Lithium
Cardiovascular diseases	Polyneuropathies	
Obesity	Lumbosacral polyradiculopathies	
Diabetes mellitus		
Hypothyroidism		
Magnesium deficiency		
Obstructive sleep apnea		
Chronic obstructive pulmonary disease		
Rheumatoid arthritis		

generally tolerable and safe for all RLS patients and may help to avoid higher doses of medications.

Standard pharmacological medications for RLS are carbidopa/levodopa, non-ergot dopamine agonists, α2δ ligands, opioids, and benzodiazepines. The Movement Disorders Society Task Force provided a new set of guidelines in 2016 that encouraged the use of α2δ ligands as first-line treatment rather than dopamine receptor agonists (39). α2δ ligands have the potential to alleviate anxiety and insomnia in addition to treating chronic pain. A dopamine agonist is a better option when depression and weight gain are present, as α2δ ligands have the potential to cause these side effects (38).

The appropriate drugs are determined by categorizing the disease into intermittent, chronic progressing, and refractory subtypes. Thus, for patients with intermittent or mild RLS symptoms, only non-pharmacological treatment may be required (31). In addition, for individuals with intermittent or mild RLS, an appropriate monotherapy strategy may be the first line of treatment. Intermittent use of these medications may be helpful for RLS that occurs intermittently in the evening, at bedtime, or while waking up during the night, and for RLS related to specific activities. A once-daily dose of medication is recommended for those with chronic persistent RLS symptoms. However, for some patients to effectively treat unpleasant daytime symptoms, a divided dose with earlier daytime usage is also needed. In the case of refractory RLS, other exacerbating factors should be sought. Adding a second agent and trying to reduce the dose of the initial drug should also be considered. Further details on management approaches and algorithms can be found in the RLS Foundation consensus statement on RLS management (32,40).

Augmentation is a significant adverse effect of therapy by levodopa and dopamine agonists. Decreasing the dose of dopaminergic medications, or cessation of the drug is beneficial in managing augmentation. Augmentation syndrome (AS) is often seen in RLS patients who have been chronically treated by dopamine agonists. It was first described by Allen and Earley (41) in RLS patients. It is a worsening of RLS symptoms with temporally earlier, increasingly intense, and typically geographically ascending characteristics, which should be considered as an adverse effect of treatment. The Max Planck Institute criteria were used to determine the diagnostic criteria of augmentation that was revised in 2006 (Table 3, 42). The frequency of AS is highest with L-DOPA, which has a short half-life, and up to 73%, compared to 6-47% with pramipexole (43), and is related to the dosages and duration of treatment. Iron deficiency, high doses of dopaminergic therapy, particularly with a short half-life, the initial severity of RLS, long exposure to the treatment, advanced age, and a higher risk of self-medication are the risk factors for augmentation that are most frequently reported (44). It is important to differentiate augmentation from tolerance, early morning rebound, RLS progression, or fluctuations in disease severity, as well as neuroleptic-induced akathisia (45).

CONCLUSION

The quality of life and sleep are both significantly affected by RLS, a sensorimotor condition. The general model of the disease's pathophysiology, which includes iron, various neurotransmitters, and opiate pathways, continues to be studied. Most cases are idiopathic RLS. Secondary RLS has been associated with a higher incidence in populations at risk for iron deficiency, including blood donors, pregnant women, and patients with end-stage renal illness. The initial management approach should include measuring iron stores. The initial course of treatment with α2δ ligands has recently been proposed. Additionally, useful, dopamine agonists may cause augmentation.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

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Table 3. Max Planck Institute criteria for the diagnosis of augmentation (42)

Augmentation requires criteria A + B, A + C or A + B + C to be met
A. Basic features (all of which need to be met) **1.** An increase in the severity of symptoms for at least 5 days a week **2.** No other factors explaining the aggravation **3.** There has been a prior positive response to treatment
B. A paradoxical response (although not immediate) to treatment: Symptoms worsen with increasing dose and better with decreasing dose
C. Earlier onset of symptoms. **1.** An earlier onset by at least 4 hours **2.** or symptoms start between 2-4 hours earlier in association with at least one of the following criteria compared to symptom status before treatment **a.** Shorter latency of symptoms at rest **b.** Spread of symptoms to other parts of the body **c.** Greater intensity of symptoms or increase in periodic limb movements via the polysomnography or immobilization test **d.** Shorter duration of relief from treatment

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Crocin Protects Mice Pancreatic Islets from Oxidative Stress Induced by Methylglyoxal and Increases Insulin Secretion

Krosin, Fare Pankreas Adacıklarını Metilglioksal Tarafından İndüklenen Oksidatif Stresten Korur ve İnsülin Sekresyonunu Arttırır

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ABSTRACT

Aim: Islets of Langerhans are more sensitive to oxidative damage because of their low antioxidant capacity. In diabetes, methylglyoxal (MG) accumulates in the pancreas. The present study examined the effect of crocin on oxidative stress induced by MG in isolated Langerhans islets from male mice.

Material and Methods: Twenty-four male mice weighing 20 to 25 g were prepared. The isolated Langerhans islets were transferred to the culture medium. Oxidative stress was induced through MG administration for 30 min, and then 10, 20, 30, and 40 µM of crocin was used for 2 h. Samples were divided into seven groups with 2.8, 5.6, and 16.7 mM glucose concentrations: control, MG 300 µM, MG+glibenclamide 10 µM, and MG+crocin in four doses of 10, 20, 30, and 40 µM. At the end, the islet's insulin, antioxidant levels, and lipid peroxidation were assessed by ELISA and calorimetry methods.

Results: Increased levels of malondialdehyde (MDA) in MG groups significantly decreased in 2.8 ($p=0.008$), 5.6 ($p=0.004$), and 16.7 ($p<0.001$) mM glucose concentrations, with administration of 30 and 40 µM crocin. Total antioxidant capacity (TAC) was reduced in MG groups ($p<0.001$) and significantly restored in all crocin-treated groups in 2.8, 5.6, and 16.7 mM glucose concentrations. Also, a significant decrease in insulin secretion and content was observed in MG groups of all three glucose concentrations ($p<0.001$). Crocin at high doses improved these alterations.

Conclusion: MG caused oxidative damage and reduced insulin secretion in isolated islets. Crocin improved the antioxidant defense system, diminished MDA, and increased insulin secretion.

Keywords: Beta-cell; crocin; diabetes; methylglyoxal; oxidative stress.

ÖZ

Amaç: Langerhans adacıkları, düşük antioksidan kapasiteleri nedeniyle oksidatif strese karşı daha duyarlıdır. Diyabette, pankreasta metilglioksal (MG) birikir. Bu çalışmada, erkek farelerden izole edilmiş olan Langerhans adacıklarında MG tarafından indüklenen oksidatif stres üzerinde krosinin etkisini araştırıldı.

Gereç ve Yöntemler: Ağırlıkları 20 ile 25 g arasında olan 24 adet erkek fare kullanıldı. İzole edilen Langerhans adacıkları kültür ortamına aktarıldı. 30 dakika boyunca MG uygulaması ile oksidatif stres indüklendi ve ardından 2 saat boyunca 10, 20, 30 ve 40 uM krosin kullanıldı. Örnekler, 2,8, 5,6 ve 16,7 mM glikoz konsantrasyonlarında yedi gruba ayrıldı: kontrol, MG 300 uM, MG+glibenclamide 10 uM ile 10, 20, 30 ve 40 µM'lik dört dozda MG+krosin. Son olarak adacığın insülin, antioksidan seviyeleri ve lipid peroksidasyonu ELISA ve kalorimetri yöntemleri ile değerlendirildi.

Bulgular: MG gruplarında artmış olan malondialdehit (MDA) düzeyleri, 30 ve 40 µM krosin uygulanmasıyla 2,8 ($p=0,008$), 5,6 ($p=0,004$) ve 16,7 ($p<0,001$) mM glikoz konsantrasyonlarında anlamlı olarak azaldı. Toplam antioksidan kapasite (TAC), MG gruplarında azalmıştı ($p<0,001$) ve krosinle tedavi edilen tüm gruplarda 2,8, 5,6 ve 16,7 mM glikoz konsantrasyonlarında önemli ölçüde düzeldi. Ayrıca MG gruplarında her üç glukoz konsantrasyonunda da insülin sekresyonu ve içeriğinde anlamlı azalma gözlendi ($p<0,001$). Yüksek dozlarda krosin bu değişiklikleri iyileştirdi.

Sonuç: MG, izole adacıklarda oksidatif hasara neden olmuş ve sonuç olarak insülin sekresyonunu azaltmıştır. Krosin antioksidan savunma sistemini iyileştirdi, MDA üretimini baskıladı ve insülin sekresyonunu artırdı.

Anahtar kelimeler: Beta hücresi; krosin; diyabet; metilglioksal; oksidatif stress.

INTRODUCTION

Diabetes is a common metabolic disease marked by hyperglycemia due to the inability of the pancreas to produce sufficient insulin or peripheral insulin resistance (1). Prolonged hyperglycemia has been found to cause excessive reactive oxygen species (ROS) production, which leads to pancreatic beta-cell damage and, finally beta-cell death (2,3). Also, the overproduction of ROS is an essential factor in the progression of diabetes and related complications (4,5). In biological systems, antioxidants defend cells against free radicals, especially ROS (6). Conversely, because pancreatic beta-cells have minor amounts of antioxidant enzymes under physiological conditions, they are highly vulnerable to oxidative stress (7). Lipid peroxidation is caused by the reaction of ROS with lipids in oxidative damage and plays a role in several diseases, including diabetes (8). Malondialdehyde (MDA) is produced in this process, and its content is a key indicator for the determination of oxidative stress (9).

Methylglyoxal (MG) is a reactive metabolite of glucose metabolism that accumulates during prolonged hyperglycemia (10). MG is known to produce free radicals in tissues. However, the glyoxalase system detoxifies MG under physiological conditions (11). Numerous evidences show that MG changes insulin structure and function, modulates the pathogenesis of insulin resistance (12,13), and is involved in various complications of diabetes (14).

The management of diabetes is complex due to its heterogeneous pathophysiology and various complications. Therefore, some drugs are used to treat and manage diabetes, including sulfonylureas. Glibenclamide (GLY) is an oral sulfonylurea that stimulates insulin secretion in beta-cells. However, efforts are underway to find effective drugs in controlling diabetes, especially natural antioxidants. Studies have shown that supplementing with natural products reduces the effects of hyperglycemia and maintains pancreatic beta-cell function (15,16). Flavonoids are major metabolites with multiple physiological and biological processes and mainly act as antioxidants to prevent ROS-induced damage under oxidative stress conditions (17-19). Various documents have indicated the valuable effects of flavonoids in treating diabetes and its complications (20-22).

Crocus sativus L. has been known for its various medical properties. This plant grows in various countries, including Iran, Egypt, Turkey, and Morocco, and is commonly known as saffron (23). Due to flavonoid content, saffron possesses antioxidant properties (24). Crocin is the major bioactive component of *Crocus sativa* L. and has particular antioxidant, radical scavenging, and anti-inflammatory properties (24-26). Chemically, crocin is a diester derived from the disaccharide gentiubiose and the dicarboxylic acid that intensely scavenges free radicals such as superoxide anions (27). In addition to its beneficial antidepressant and anti-anxiety effects (28,29), studies have reported that crocin exhibits neuroprotective (30), antihypertensive, and cardioprotective properties (31). According to a previous study, crocin effectively reduces oxidative stress markers, and blood glucose, and increases insulin levels in diabetic rats (32). Treatment with crocin has also been shown to reduce diabetic complications in rats with nicotinamide streptozotocin-induced diabetes (33). The present study was conducted to determine the protective effect of crocin as a potent antioxidant on insulin secretion against MG-induced oxidative damage in pancreatic islets under in vitro conditions.

MATERIAL AND METHODS

Animals

In this study, twenty-four male Naval Medical Research Institute (NMRI) mice weighing 25 to 35 g were obtained from the Ahvaz Jundishapur University of Medical Sciences. Animals were kept in an air-conditioned room at a temperature of 20-24 °C under 12 hours of light/dark cycle, and humidity of 70-80%, with free access to water and food. All experimental procedures were approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (27.04.2021, IR.AJUMS.ABHC.REC.1400.009).

Sample Size

Minitab software was used for determining the number of animals by considering the values of α=0.05 and β=0.2. Assuming a 35% drop, 24 mice were used in this study.

Chemicals

Crocin and MG were provided from Sigma (St. Louis, MO, USA), glibenclamide from Solar, bio (South Korea), xylazine 2%, and ketamine 10% (Alfasan Co, The Netherlands). Assay kits for MDA and TAC levels were obtained from Zell Bio GmbH (Germany). Collagenase type P was obtained from Roch Company (Germany). Glucose and insulin were purchased from Pars Azmoon (Tehran, Iran), and (Monobind Inc., USA), respectively. Bovine serum albumin, potassium chloride (KCl), sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), sodium bicarbonate ($NaHCO_3$), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and hydrochloric acid (HCL) all purchased from Merck, Germany.

Islet Isolation

After anesthetizing the animals with ketamine (70 mg/kg) and xylazine (10 mg/kg), the pancreas was excised and transferred to a Petri dish containing Krebs-bicarbonate buffer (KBB) (0.5% bovine serum albumin, KCl 5 mM, NaCl 115 mM, $CaCl_2$ 2.56 mM, $MgCl_2$ 1 mM, $NaHCO_3$ 10 mM, HEPES 15 mM, and stable with a mixture of 5% carbon dioxide, 95% oxygen, pH 7.4). The isolated pancreas was cut into minute pieces (1 mm) and then centrifuged for 5 min (at 100×g). To separate the islets, KBB plus P-type collagenase (12 mg per pancreas) was added to the deposition in a centrifuge conical tube. The conical tube was transferred to an 800 oscillating incubator for 5-10 min at 37 °C. To stop collagenase activity, cold KBB was added to the conical tube and centrifuged for 5 min (at 500×g). The islets were transferred to a petri and manually isolated under a stereo microscope (34). Eventually, the islets were cultured in the hanks' buffer and affected by different substances and solutions.

Experimental Design

This in vitro study was carried out in three different glucose concentrations, including 2.8, 5.6, and 16.7 mM, as hypoglycemic, normal, and hyperglycemic culture mediums, respectively. The grouping and islets treatment procedure was as follows:

MG: 10 isolated islets were incubated for 2 h and 30 min in 300 μM MG.

MG-GLY: 10 isolated islets were incubated for 30 min in 300 µM MG, and GLY was added to reach the final concentration of 10 µM and further incubated for 2 h.
MG-C10: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 10 µM and further incubated for 2 h.
MG-C20: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 20 µM and further incubated for 2 h.
MG-C30: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 30 µM and further incubated for 2 h.
MG-C40: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 40 µM and further incubated for 2 h.
At the same time, control groups for culture mediums were defined as 10 isolated islets incubated in 2.8, 5.6, and 16.7 mM glucose-containing culture medium for 2 hours and 30 min.

Insulin Secretion and Content Measurement

The isolated islets were placed in a microtube with a KBB (1 mL) in the mentioned groups. Briefly, after MG incubated for 30 min administration, in treated groups, samples were exposed to crocin or GLY for 2 h at 37 °C. The samples were centrifuged at 100×g for 5 min. Then, the supernatant was kept at -70 °C until insulin secretion was measured. The mentioned method was performed to assess the insulin content of the islets. Insulin extraction buffer was prepared [1 mL of HCL (0.18 M) in ethanol (96%)]. After the mentioned incubation steps, glass tubes re-pipet and vortex for 1 min and kept at 4 °C overnight. The samples were centrifuged at 2500 rpm for 5 min at 4 °C. Then, 900 µL of supernatant was kept at -70 °C until insulin content was measured using a colorimetric assay kit (35).

Antioxidant and Lipid Peroxidation Analysis

The level of lipid peroxidation (MDA content) and TAC levels were measured using a colorimetric method according to the manufacturer's instructions.

Statistical Analysis

Obtained data passed the normality assumption by using the Shapiro-Wilk test. Also, Levene's test was used for homogeneity of variances. GraphPad Prism Version 9 for Windows (GraphPad Software, San Diego, CA) was utilized to analyze the data. The results were presented as mean and standard deviation. The one-way analysis of variance followed by the post hoc Bonferroni test was used for the data analysis. A $p<0.05$ was considered significant.

RESULTS

Effects of Crocin on Islet's Lipid Peroxidation and Antioxidant Activity

2.8 mM Glucose-Containing Medium

The MDA as an indicator for peroxidation of lipids, increased in MG (p=0.008), MG-GLY (p=0.020), and MG-C10 (p=0.020) groups compared to the control group. Also, MG had a remarkable difference when compared with the MG-C40 group ($p<0.001$). The levels of lipid peroxidation in the MG-GLY (p=0.002), MG-C10 (p=0.002), and MG-C20 (p=0.009) groups were higher than in the MG-C40 group (Table 1, Figure 1A).

Measurement of TAC levels significantly decreased in the MG and MG-GLY groups when compared to the control group. Administration of GLY and crocin at all doses effectively recovered TAC levels compared to the MG group ($p<0.001$). Moreover, 40 µM of crocin positively improved the TAC levels of pancreatic islets compared to the GLY ($p<0.001$), 10 (p=0.010), 20 (p=0.002), and 30 (p=0.009) µM of crocin (Table 1, Figure 1B).

5.6 mM Glucose-Containing Medium

The MDA levels increased significantly in MG (p=0.004) and MG-C10 (p=0.005) groups compared to the control group. Treatment with GLY (p=0.006), 30 (p=0.005) and 40 (p=0.004) µM of crocin reduced the peroxidation process compared to the MG group. Also, there was a significant difference between MG-GLY and MG-C10 groups (p=0.007). Also, 40 µM of crocin had a better effect (p=0.004) than the other doses (Table 1, Figure 2A). At this glucose concentration, the TAC levels decreased in MG and MG-GLY ($p<0.001$) groups, but it was increased in MG-C30 (p=0.007) and MG-C40 ($p<0.001$) groups when compared to the control group. Administration of crocin at all doses improved the TAC levels compared to the MG and MG-GLY groups ($p<0.001$). The differences between MG-C30 (p=0.003) and MG-C40 ($p<0.001$) with the MG-C10 were significant. Moreover, the TAC content of pancreatic islets in the presence of 30 and 40 µM of crocin was higher than 20 µM (Table 1, Figure 2B).

16.7 mM Glucose-Containing Medium

Our findings indicated an elevation of lipid peroxidation in the MG, MG-C10, and MG-C20 groups compared to the control group ($p<0.001$), as recognized in the MDA levels. Administration of GLY, and 30 and 40 µM of crocin significantly diminished it ($p<0.001$). Among the

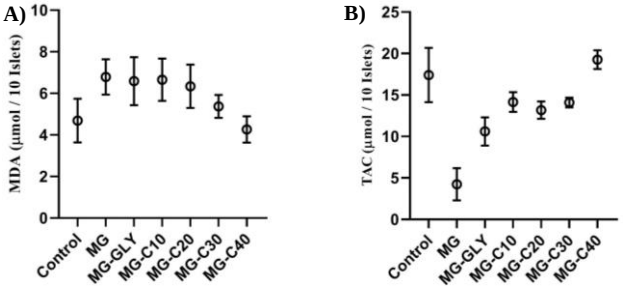

Figure 1. A) MDA and **B)** TAC levels of mice isolated islets incubated in 2.8 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 µM, MG-C20: methylglyoxal + crocin 20 µM, MG-C30: methylglyoxal + crocin 30 µM, MG-C40: methylglyoxal + crocin 40 µM

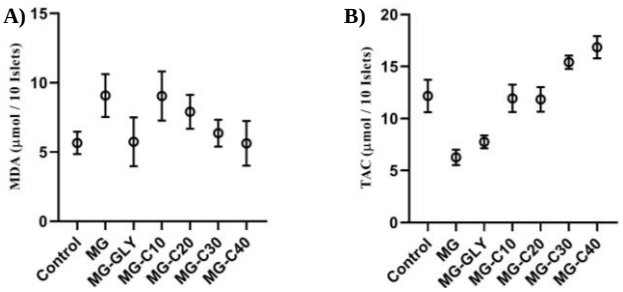

Figure 2. A) MDA and **B)** TAC levels of mice isolated islets incubated in 5.6 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 µM, MG-C20: methylglyoxal + crocin 20 µM, MG-C30: methylglyoxal + crocin 30 µM, MG-C40: methylglyoxal + crocin 40 µM

MG-treated islets, MG-C40, and MG-GLY had better effects than MG-10 and MG-C20 (p<0.001). Moreover, a significant difference was observed between the MG-C10 and MG-C30 (p=0.005) groups (Table 1, Figure 3A).

The decreased levels of TAC in the MG group were enhanced in the MG-GLY (p<0.001), MG-C10 (p=0.001), and the other crocin-treated groups (p<0.001). Furthermore, a high dose of crocin had a better effect than the MG-GLY (p=0.020), MG-C10 (p=0.020), and MG-C20 (p=0.030) groups (Table 1, Figure 3B).

Effects of Crocin on Insulin Secretion and Insulin Content in Isolated Pancreatic Islets

2.8 mM Glucose-Containing Medium

Treatment with MG reduced the insulin secretion in the MG (p<0.001), MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.003) groups compared to the control group. Treatment with GLY (p<0.001), and 30 (p=0.030), and 40 (p<0.001) μM of crocin effectively recovered the insulin levels when compared to the MG group. Also, the differences in insulin secretion between the MG-GLY group and the MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.020) groups were significant. In addition, our results demonstrated that the high dose of crocin treatment (p<0.001) was more beneficial than the other doses (Table 2, Figure 4A). Insulin content decreased in the MG, MG-C10, and MG-C20 groups compared to the control group (p<0.001). Among the treatments, only the high crocin dose improved the insulin content compared to the MG group (p<0.001). Moreover, insulin contents in the

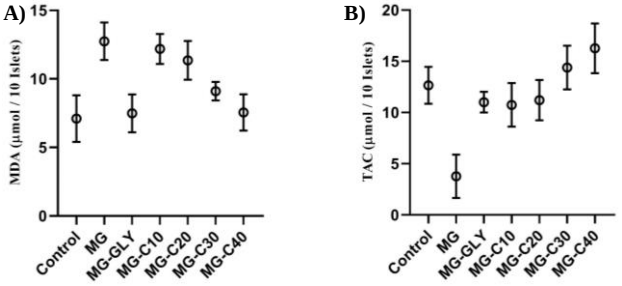

Figure 3. A) MDA and **B)** TAC levels of mice isolated islets incubated in 16.7 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

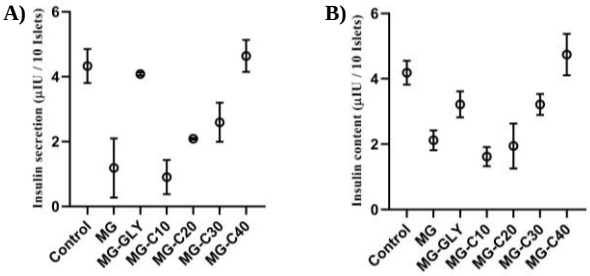

Figure 4. A) Insulin secretion and **B)** content of isolated islets incubated in 2.8 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

Table 1. Effect of crocin and glibenclamaide on islet's lipid peroxidation and antioxidant activity

	Control	MG	MG-GLY	MG-C10	MG-C20	MG-C30	MG-C40	p
MDA								
a	4.69±1.05	6.78±0.85*	6.58±1.15*	6.65±1.02*	6.34±1.04	5.37±0.55	4.26±0.63$^{\#\$\&@}$	**<0.001**
b	5.66±0.80	9.07±1.54*	5.74±1.76$^{\#}$	9.04±1.77$^{*\$}$	7.91±1.22	6.36±0.96$^{\#}$	5.63±1.61$^{\#\&}$	**<0.001**
c	7.10±1.70	12.75±1.37*	7.49±1.38$^{\#}$	12.19±1.09$^{*\$}$	11.36±1.41$^{*\$}$	9.10±0.67$^{\#}$	7.55±1.32$^{\#}$	**<0.001**
TAC								
a	17.4±3.27	4.24±1.93*	10.60±1.70$^{*\#}$	14.16±1.18$^{\#}$	13.18±1.04$^{\#}$	14.09±0.58$^{\#}$	19.27±1.13$^{\#\$\&@¥}$	**<0.001**
b	12.17±1.56	6.27±0.74*	7.75±0.62*	11.94±1.31$^{\#\$}$	11.85±1.18$^{\#\$}$	15.42±0.64$^{*\#\$\&}$	16.86±1.06$^{*\#\$\&@}$	**<0.001**
c	12.66±1.80	3.77±2.10*	11.01±1.01$^{\#}$	10.74±2.12$^{\#}$	11.21±1.96$^{\#}$	14.39±2.13$^{\#\&}$	16.27±2.42$^{\#\$\&@}$	**<0.001**

MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM, MDA: malondialdehyde, TAC: total antioxidant capacity, a: 2.8 mM, b: 5.6 mM, and c: 16.7 mM glucose-containing medium, superscript symbols denote significant differences of the group when compared with the *: control group, $^{\#}$: MG group, $^{\$}$: MG-GLY group, $^{\&}$: MG-C10 group, $^{@}$: MG-C20 group, $^{¥}$: MG-C30 group

Table 2. Effect of crocin and glibenclamaide on insulin secretion and content in MG-induced oxidative stress of islets

	Control	MG	MG-GLY	MG-C10	MG-C20	MG-C30	MG-C40	p
Insulin secretion								
a	4.33±0.53	1.18±0.91*	4.08±0.04	0.9±0.53$^{*\$}$	2.08±0.03$^{*\$}$	2.6±0.6$^{\&@}$	4.64±0.5$^{\#\$\&@¥}$	**<0.001**
b	9.99±0.58	1.15±0.66*	7.3±0.42$^{*\#}$	2.21±0.19$^{*\$}$	3.65±0.22$^{*\#\$}$	8.13±0.7$^{*\#\&@}$	9.45±1.15$^{\#\$\&@}$	**<0.001**
c	10.33±1.63	1.92±1.43*	8.73±1.02$^{\#}$	4.7±0.64$^{*\#\$}$	6.47±0.52$^{*\#}$	9.9±0.75$^{\#\&@}$	10.88±1.18$^{\#\&@}$	**<0.001**
Insulin content								
a	4.18±0.37	2.12±0.3*	3.22±0.4	1.62±0.3$^{*\$}$	1.94±0.7$^{*\$}$	3.21±0.32$^{\&@}$	4.74±0.63$^{\#\$\&@¥}$	**<0.001**
b	6.82±0.52	0.97±0.8*	4.13±0.53$^{*\#}$	2.94±0.73$^{*\#}$	2.9±0.66$^{*\#}$	6.08±0.83$^{\#\$\&@}$	8.02±0.75$^{\#\$\&@¥}$	**<0.001**
c	5.97±0.55	1.5±0.6*	4.17±0.22$^{*\#}$	1.98±0.4$^{*\$}$	2.47±0.29$^{*\$}$	4.76±0.54$^{*\#\$\&@}$	5.41±0.63$^{\#\&@}$	**<0.001**

MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM, MDA: malondialdehyde, TAC: total antioxidant capacity, a: 2.8 mM, b: 5.6 mM, and c: 16.7 mM glucose-containing medium, superscript symbols denote significant differences of the group when compared with the *: control group, $^{\#}$: MG group, $^{\$}$: MG-GLY group, $^{\&}$: MG-C10 group, $^{@}$: MG-C20 group, $^{¥}$: MG-C30 group

MG-C10 (p=0.001) and MG-C20 (p=0.020) groups were significantly lower than in the MG-GLY group. Treatment with a crocin dose of 40 μM had improving effects when compared to the MG-GLY (p=0.002) group, and other doses of MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.002) groups (Table 2, Figure 4B).

5.6 mM Glucose-Containing Medium

MG significantly reduced insulin secretion and insulin content (p<0.001). Administration of GLY (p<0.001), and 20 (p=0.002), 30 (p<0.001), and 40 (p<0.001) μM of crocin increased insulin secretion and content when compared to the MG. But, crocin 10 μM just improved the content of insulin in this condition (p=0.010). The difference in the levels of insulin secretion between the MG-GLY and MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C40 (p=0.002) groups were significant. The positive effects of 30 (p=0.020) and 40 (p<0.001) μM of crocin on insulin secretion were higher than the GLY. Moreover, significant differences between MG-C10 and MG-C20 groups with MG-C30 and MG-C40 groups were observed (p<0.001) in both insulin secretion and insulin content (Table 2, Figure 5A and 5B).

16.7 mM Glucose-Containing Medium

The insulin secretion decreased in the MG (p<0.001), MG-C10 (p<0.001), and MG-C20 (p=0.001) groups when compared to the control group. Administration of GLY, 20, 30, 40 (p<0.001) and 10 (p=0.040) μM of crocin improved it. Between the crocin-treated groups, the efficacy of the dose of 40 μM crocin was better than the other doses (p<0.001). There were significant differences between the MG-GLY and MG-C10, and also MG-C20 and MG-C30 (p<0.001) groups (Table 2, Figure 6A). On the other side, the content of insulin remarkably reduced in the MG (p<0.001), MG-GLY (p<0.001), MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.040) groups compared to the control group. The decreased levels of insulin content in the MG group recovered in the GLY, and 30 and 40 μM crocin-treated islets (p<0.001). Among the crocin-treated islets, the MG-C40 group had a better effect than the MG-GLY group (p=0.030). Furthermore, significant differences between the MG-C10 and MG-C20 groups and the MG-C30 and MG-C40 groups (p<0.001) were observed (Table 2, Figure 6B).

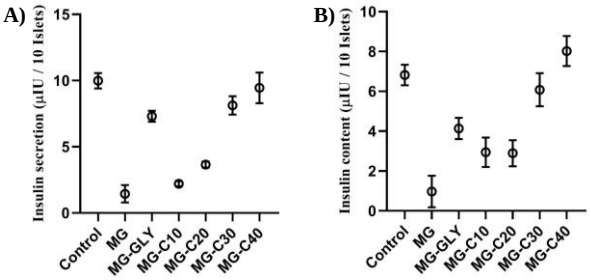

Figure 5. A) Insulin secretion and **B)** content of isolated islets incubated in 5.6 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

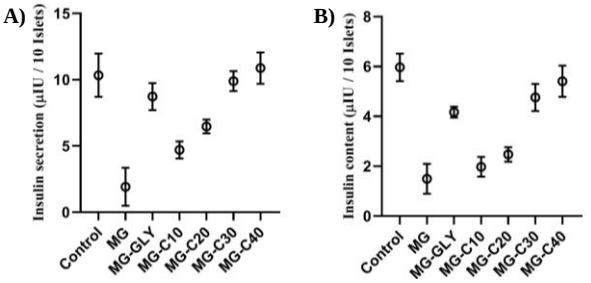

Figure 6. A) Insulin secretion and **B)** content of isolated islets incubated in 16.7 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

DISCUSSION

Several studies have been conducted on the anti-diabetic effect of flavonoids, including crocin (active constituent of *Crocus sativus*). Flavonoids are known as improving compounds for insulin production and secretion (36). In the pancreas, flavonoids mainly reduce oxidative stress, improve cell viability, and enhance insulin secretion (20). This in vitro study examined the effects of crocin on pancreatic beta-cells under MG-induced oxidative damage conditions. Our findings demonstrated that incubation with 300 μM MG for 30 min impaired insulin secretion and insulin content in islets in low, normal, or high glucose concentrations. Based on a previous result, MG can induce toxicity and decrease insulin secretion in pancreatic beta cells (37). In oxidative conditions, MG accumulates in cells and leads to oxidative damage. It is well known that oxidative stress plays an essential role in the development and onset of diabetes. One of the consequences of oxidative stress is beta-cell dysfunction, which leads to decreased insulin secretion and, eventually diabetes (38). Therefore, these results further supported the noxious effects of MG on insulin secretion.

We evaluated insulin secretion in beta-cells after MG exposure, followed by crocin treatment. According to our results, crocin improved insulin secretion in hypo, normal, and hyperglycemic conditions. Also, crocin effectively increased insulin content in three glucose concentrations. Consequently, crocin improved the secretion of pancreatic beta-cells by inhibiting the harmful effects of MG and possibly regenerating pancreatic beta-cells. A previous report showed that *Crocus sativus* extract increased plasma insulin levels in alloxan-induced diabetic rats (39). Based on the study by Samaha et al. (40), crocin enhances insulin secretion in beta islets in STZ-induced diabetic rats. These reports are similar to the results of our study.

The MDA levels as a lipid peroxidation indicator of the beta-cells were measured to prove the ameliorating effect of crocin on oxidative stress. We revealed that MG increased the formation of MDA in glucose-containing mediums at three different concentrations. Hyperglycemia can increase oxidative stress markers such as lipid peroxidation (41). Lipid peroxidation is defined as the oxidation of lipid compounds in the presence of excess amounts of free radicals producing toxic byproducts such as aldehydes. In diabetes, lipid peroxidation occurs due to the excessive production of free radicals and, so exacerbates oxidative stress (42). The production of MDA was attenuated when the islets were treated with crocin, 30 and 40 μM. This finding is in agreement with Yaribeygi et al. (43), which mentioned that treatment of STZ-induced diabetic rats with crocin significantly diminished the MDA levels.

According to a previous study, increased levels of ROS along with decreased levels of antioxidants have been observed in diabetics (38). Due to the low levels of antioxidants, pancreatic islets are more vulnerable to oxidative damage than other tissues. In conditions of defects in the antioxidant defense system, the balance tends to favor the increase of free radicals and oxidative stress (44). Our results showed that MG induced a significant oxidative load with decreased TAC levels. Besides, crocin improved the antioxidant defense system by increasing the TAC levels in MG-exposed pancreatic beta-cells. This is in agreement with a previous study reported that crocin increased the pancreatic TAC in STZ-induced diabetic rats (40). In addition, the protective effect of crocin has been revealed through an increase in TAC levels of liver tissue in diabetic rats (33).

CONCLUSION

The present study suggests that MG negatively affects beta-cell function. MG induces lipid peroxidation and oxidative stress in pancreatic beta islets and reduces insulin secretion. Crocin administration improves these alterations by increasing the TAC levels and decreasing the MDA levels.

Ethics Committee Approval: The study was approved by the Ethics Committee of Research Center & Experimental Animal House - Ahvaz Jundishapur University of Medical Sciences (27.04.2021, IR.AJUMS.ABHC.REC.1400.009).

Conflict of Interest: None declared by the authors.

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Muscarinic Receptors as Targets for Metronomic Therapy in Ovarian Cancer

Yumurtalık Kanserinde Metronomik Tedavi Hedefi Olarak Muskarinik Reseptörler

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ABSTRACT

Aim: In this study, the effects of muscarinic acetylcholine receptor (mAChR) agonist carbachol on the proliferation of cisplatin-resistant (A2780cis) and cisplatin-free (SKOV-3) ovarian cancer cell line were for the first time investigated to further evaluate the potential therapeutic effect of metronomic chemotherapy.

Material and Methods: The inhibitory effect of carbachol on cell proliferation was detected using the xCELLigence Real-Time Cell Analyzer (RTCA) dual plate (DP) system. A preliminary study was conducted to determine the dose of carbachol 100 µM, cisplatin 1 µM, and two combination studies were carried out with 100 µM carbachol + cisplatin 1 µM and 100 µM carbachol + 10 µM atropine, over cancer cells without drugs was used as the control group. The cell proliferation curve was monitored for 96 hours. The cell index value of inhibition in cell proliferation was automatically measured every hour for each well using RTCA 1.2.1 software.

Results: Co-administration of carbachol with cisplatin caused a decrease in cell number in both A2780cis and SKOV-3 cell lines in a time-dependent manner ($p<0.001$). Substantial cell death was observed in both cisplatin-resistant (A2780cis) and cisplatin-free (SKOV-3) cell lines within 24 hours after carbachol with cisplatin application and this continued at the 96th hour.

Conclusion: The findings of this study confirm the notion that mAChRs can be considered as therapeutic targets for metronomic therapy in ovarian cancer, as well as the usefulness of a muscarinic agonist as a repositioning drug in the treatment of such tumors.

Keywords: Ovarian cancer; mAChR; carbachol; SKOV-3; A2780cis; cisplatin.

ÖZ

Amaç: Bu çalışmada, muskarinik asetilkolin reseptörü (mAChR) agonisti karbakolun, sisplatine dirençli (A2780cis) ve sisplatine dirençsiz (SKOV-3) yumurtalık kanseri hücre hatlarının proliferasyonu üzerindeki etkileri, metronomik kemoterapinin potansiyel terapötik etkisini daha ileri düzeyde değerlendirmek üzere ilk kez araştırıldı.

Gereç ve Yöntemler: Karbakolün hücre proliferasyonu üzerindeki inhibitör etkisi, xCELLigence Real-Time Cell Analyzer (RTCA) dual plate (DP) sistemi kullanılarak tespit edildi. 100 µM karbakol ve 1 µM sisplatin dozunu belirlemek için bir ön çalışma yapıldı ve 100 µM karbakol + 1 µM sisplatin ve 100 µM karbakol + 10 µM atropin olmak üzere iki kombinasyon çalışması yapıldı, ilaçsız yumurtalık kanser hücreleri ise kontrol grubu olarak kullanıldı. Hücre proliferasyon eğrisi 96 saat izlendi. Hücre proliferasyonundaki inhibisyonun hücre indeksi değeri, RTCA 1.2.1 yazılımı ile her kuyu için her saat otomatik olarak ölçüldü.

Bulgular: Karbakolun sisplatin ile birlikte uygulanması hem A2780cis hem de SKOV-3 hücre hatlarında zamana bağlı olarak hücre sayısında azalmaya neden olmuştur ($p<0.001$). Sisplatin ile karbakol uygulamasından sonraki 24 saat içinde sisplatine dirençli (A2780cis) ve sisplatine dirençsiz (SKOV-3) her iki hücre hattında da önemli düzeyde hücre ölümü gözlenmiş ve bu durum 96. saatte de devam etmiştir ($p<0.001$).

Sonuç: Bu çalışmanın bulguları, mAChR'lerin yumurtalık kanserinde metronomik tedavi için terapötik hedefler olarak kabul edilebileceği fikrini ve ayrıca bu tür tümörlerin tedavisinde bir muskarinik agonistin bir yeniden konumlandırma ilacı olarak kullanışlılığını doğrulamaktadır.

Anahtar kelimeler: Yumurtalık kanseri; mAChR; karbakol; SKOV-3; A2780cis; sisplatin.

INTRODUCTION

G protein-coupled receptors (GPCRs) are involved in tumorigenesis, including abnormal cell growth, increased cell viability, angiogenesis, and metastasis (1). These receptors control basic physiological functions such as neurotransmission, enzyme and hormone release, immune reactions, muscle contraction, and blood pressure regulation (2). Functional differences in these receptors cause some diseases including cancer and therefore, GPCRs are the target of therapeutic agents used for many diseases.

Three muscarinic receptor subtypes (M1R, M3R, and M5R) that stimulate cellular signaling when expressed in proliferating cells are conditional oncogenes (3).

Carbachol is a cholinomimetic drug that binds and activates acetylcholine receptors, and therefore it is classified as a cholinergic agonist. Some studies have shown that the cholinergic agonist carbachol induces cancer cell proliferation (4,5). Activation of muscarinic acetylcholine receptors (mAChRs) by the agonist carbachol has been reported to cause two opposite types of responses in breast tumor cells: short-term stimulation promotes tumor progression, while long-term treatment induces cancer cell death without affecting normal cells lacking mAChRs (4). The latter effect was thought to be an unfulfilled goal of conventional chemotherapy, consisting of systemic delivery of the highest effective and tolerable dose to kill as many of the tumor cells as possible. It also produces undesirable effects on the normal tissues of cancer patients and requires chemotherapy to be administered at relatively free and long intervals between doses to allow normal cells to heal. Therefore, the application of metronomic therapy has come to the fore to improve the treatment of cancer patients. Metronomic therapy relies on the administration of low doses of a chemotherapeutic drug, alone or in combination with other drugs, at short inter-dose intervals to provide both effective treatment and reduce side effects. Long-term treatment of breast cancer cells with the muscarinic agonist carbachol promotes cell death, it was investigated whether low doses of this agonist combined with paclitaxel (PX) used in the treatment of breast cancer inhibit disease progression in human MCF-7 tumor cells and PX plus carbachol has been shown to reduce cell viability and tumor growth in vitro (6). The results of this study confirm that mAChRs can be considered as therapeutic targets for metronomic therapy in breast cancer and that a muscarinic agonist would also be useful in the treatment of such tumors.

Gynecological cancers are among the important health problems in terms of mortality and morbidity in all women (7). Ovarian cancer is the seventh most common cancer in women and the most lethal gynecological cancer (8). It is the seventh most common cancer among women and the eighth leading cause of cancer-related death. The typical age of diagnosis with ovarian cancer is 60, and the average lifetime risk for women is about 1 in 70. Studies have shown that GPCRs are involved in the progression and metastasis of ovarian neoplasms. The presence of mAChRs has been detected in different types of tumor cells, including ovarian cancer, and they are associated with tumorigenesis (9,10).

In a single study that was identified in the literature, the effects of carbachol and histamine on changes in cytosolic-free calcium and cell proliferation have been characterized in human ovarian cancer (OVCAR-3) and non-tumorigenic Chinese hamster ovarian (CHO) cells. Both carbachol and histamine-induced cell proliferation in OVCAR-3 cells but did not affect CHO cells (11). It was observed that the effects of carbachol and histamine on cell proliferation and Ca^{2+} increase on OVCAR-3 cells were completely blocked by atropine and the selective H-1 histaminergic receptor antagonist pyrilamine, respectively. In this study, it was planned to investigate whether the muscarinic receptor agonist carbachol combined with PX used for cancer treatment inhibits the progression of the disease in tumor cells of cisplatin-resistant (A2780cis) and cisplatin-free (SKOV-3) ovarian cancer cell lines.

MATERIAL AND METHODS

Cell Culture

Cell culture A2780 was obtained from the European Collection of Authenticated Cell Cultures, (93112519) and SKOV-3 over cancer cells was obtained from the American Type Culture Collection (ATCC® HTB-77™, Manassas, VA, USA). These cells were cultured in RPMI medium (Catalog number: 11875093, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum, penicillin/streptomycin, and amphotericin-B 1%. Cell culture was performed at 37 °C in a 5% CO_2 incubator. In a study aiming to evaluate the potential association of M3 muscarinic receptors with proliferation and cell death in the human chronic myelogenous leukemia K562 cell line, it was determined that 100 µM carbachol application caused a decrease in cell number (5). Exposure of K562 cells to carbachol for 24 hours reduced the number of early apoptotic cells but had no change in the number of necrotic cells. 100 µM carbachol treatment for 48 hours increased the number of necrotic cells and decreased the number of apoptotic cells. Taking the results of this study as a reference, the carbachol 100 µM (CCh group), cisplatin 1 µM (Cis group), 100 µM carbachol + cisplatin 1 µM (CCh+Cis group), and 100 µM carbachol + 10 µM atropine (CCh+Atr group) combinations were carried out, over cancer cells without drug was the control group. Cancer cells were incubated with carbachol, atropine, and cisplatin for 96 hours.

xCELLigence Real-Time Cell Analysis

The xCELLigence Real-Time Cell Analyzer (RTCA) dual plate (DP) system (Roche Diagnostics GmbH, Penzberg) was used for real-time monitoring of cell viability without labeling on the cells. $3x10^3$ cells were seeded in each well of the E-plate and the cell proliferation curve was monitored for 24 hours after that carbachol, atropine, cisplatin, and their combinations were added to the E-plate systems and they were monitored in real-time for 96 hours. The cell index (CI) value was automatically measured every hour for each well with RTCA 1.2.1 software.

Statistical Analysis

All statistical analyses were performed using the SPSS software v.21. The assumptions of normality were tested by the Shapiro-Wilk test. The homogeneity of variances with normal distribution was tested by the Levene test. Homogeneous data were analyzed with the ANOVA test for comparisons between groups, and non-homogeneous data were analyzed with the Welch test. The Bonferroni

post hoc test was used for comparisons between subgroups of homogeneous data, while the Dunnett T3 test was used in non-homogeneous data. The data were expressed as mean±standard deviation, and the p value of <0.05 was defined as statistically significant.

RESULTS

The effect of the non-selective muscarinic agonist carbachol (100 μM) added to cisplatin-resistant (A2780cis) and cisplatin-free (SKOV-3) ovarian cancer cells on cell proliferation were examined in this study. The time period in which it showed the best effect was determined by comparing the groups at 0, 24, 48, 72, and 96 hours.

Results for Cisplatin-Resistant (A2780cis) Cell Line

Comparison of the cell proliferation between the groups for the cisplatin-resistant (A2780cis) cell line at 0, 24, 48, 72, and 96 hours were shown in Table 1 and Figure 1. Any statistically significant difference was not seen when the cell index values taken at the 0th hour were compared between the groups (p=0.165). At the 24th hour, it was observed that the control and CCh groups decreased significantly compared to the Cis group (p<0.001). At 48th and 72nd hours, the groups administered only carbachol were statistically significantly reduced compared to the group administered only cisplatin (p<0.001). At the 24th, 48th, and 72nd hours, a statistically significant decrease was detected in the CCh+Cis group compared to the control, CCh, and Cis groups (p<0.001). Additionally, when the CCh+Atr group was compared with the Cis and CCh+Cis groups, there was a significant increase in the Cis group, while a statistically significant decrease was detected in the CCh+Cis group (p<0.001). At the 96th hour, there was a statistically significant decrease in the CCh+Cis group compared to the control, CCh, and Cis groups (p=0.009).

Results for Cisplatin-Free (SKOV-3) Cell Line

Comparison of the cell proliferation between the groups for the cisplatin-free (SKOV-3) cell line at 0, 24, 48, 72, and 96 hours were shown in Table 2 and Figure 2. Any statistically significant difference was not seen when the cell index values taken at the 0th hour were compared between the groups (p=0.329). At the 24th hour, when the control group was compared with the group administered only with carbachol, a significant decrease in cell viability was detected in the CCh group (p<0.001). In addition, when the control and CCh groups were compared with the Cis group, a statistically significant decrease was found in the control and CCh groups (p<0.001). When the CCh+Cis group was compared with the CCh group, a statistically significant decrease was observed in the CCh group, while there was a significant increase in the Cis group (p<0.001). When the CCh+Atr group was compared with the CCh group, a statistically significant decrease was observed in the CCh and CCh+Cis groups, and a significant increase was found in the Cis group (p<0.001). At the 48th hour, when the control group was compared with the group administered only with carbachol, a significant decrease in cell viability was detected in the CCh group (p<0.001). In addition, when the control and CCh groups were compared with the Cis group, there was a statistically significant decrease in the control and CCh groups (p<0.001). When the CCh+Cis group was compared with the group administered only cisplatin, a significant decrease in cell viability was detected in the CCh+Cis group (p<0.001). When the CCh+Atr group was compared with the control and only cisplatin-administered groups, it was observed that there was a statistically significant decrease in the CCh+Atr group (p<0.001). At the 72nd hour, when the groups administered only carbachol and only cisplatin were compared, a significant decrease was detected in the CCh group (p<0.001). When the CCh+Cis group was compared with the control, only carbachol and only cisplatin applied groups, a statistically significant decrease was observed in the CCh+Cis group (p<0.001). When the CCh+Atr group was compared with the Cis and CCh+Cis

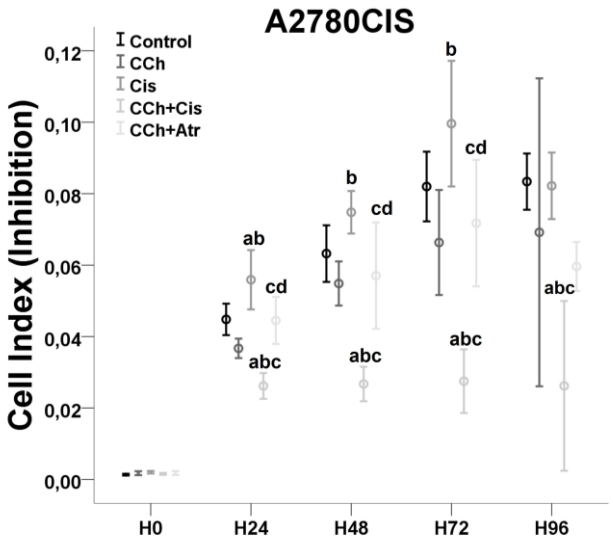

Figure 1. The effect of the cisplatin and carbachol on cisplatin-resistant (A2780cis) ovarian cell proliferation

CCh: carbachol applied group, Cis: cisplatin applied group, CCH+Cis: carbachol and cisplatin applied group, CCh+Atr: carbachol and atropine applied group, superscript letters denote significant differences of the group when compared with the [a]: control group, [b]: CCh group, [c]: Cis group, and [d]: CCh+Cis groups (p<0.001)

Table 1. Carbachol and cisplatin effects on cisplatin-resistant (A2780cis) ovarian cancer cell proliferation

	Control	CCh	Cis	CCh+Cis	CCh+Atr	p
H0	0.0013±0.0001	0.0017±0.0003	0.0020±0.0002	0.0015±0.0001	0.0018±0.0003	0.165
H24	0.0448±0.0022	0.0367±0.0014	0.0559±0.0041^{ab}	0.0261±0.0018^{abc}	0.0445±0.0032^{cd}	**<0.001**
H48	0.0632±0.0039	0.0548±0.0031	0.0747±0.0030^{b}	0.0267±0.0024^{abc}	0.0570±0.0074^{cd}	**<0.001**
H72	0.0820±0.0048	0.0663±0.0073	0.0996±0.0087^{b}	0.0275±0.0044^{abc}	0.0717±0.0088^{cd}	**<0.001**
H96	0.0833±0.0039	0.0691±0.0215	0.0821±0.0046	0.0261±0.0118^{abc}	0.0596±0.0034	**0.009**

CCh: carbachol applied group, Cis: cisplatin applied group, CCH+Cis: carbachol and cisplatin applied group, CCh+Atr: carbachol and atropine applied group, superscript letters denote significant differences of the group when compared with the [a]: control group (p<0.001), [b]: CCh group (p<0.001), [c]: Cis group (p<0.001), [d]: CCh+Cis group (p<0.001)

Table 2. Carbachol and cisplatin effects on cisplatin-free (SKOV-3) ovarian cancer cell proliferation

	Control	CCh	Cis	CCh+Cis	CCh+Atr	p
H0	0.0057±0.0002	0.0051±0.0003	0.0052±0.0004	0.0058±0.0005	0.0061±0.0004	0.329
H24	0.0390±0.0092	0.0116±0.0040[a]	0.0779±0.0039[ab]	0.0336±0.0026[bc]	0.0547±0.0073[bcd]	**<0.001**
H48	0.0551±0.0071	0.0394±0.0037[a]	0.0863±0.0045[ab]	0.0469±0.0038[c]	0.0324±0.0042[ac]	**<0.001**
H72	0.0740±0.0073	0.0606±0.0031	0.0849±0.0039[b]	0.0236±0.0034[abc]	0.0617±0.0030[cd]	**<0.001**
H96	0.0846±0.0077	0.0774±0.0071	0.0752±0.0025	0.0158±0.0033[abc]	0.0888±0.0038[d]	**<0.001**

CCh: carbachol applied group, Cis: cisplatin applied group, CCH+Cis: carbachol and cisplatin applied group, CCh+Atr: carbachol and atropine applied group, superscript letters denote significant differences of the group when compared with the [a]: control group (p<0.001), [b]: CCh group (p<0.001), [c]: Cis group (p<0.001), [d]: CCh+Cis group (p<0.001)

groups, there was a significant increase in the Cis group, while a statistically significant decrease was detected in the CCh+Cis group (p<0.001). At the 96th hour, when the CCh+Cis group was compared with the control, only carbachol applied and only cisplatin applied groups, a statistically significant decrease was determined in the CCh+Cis group (p<0.001). When the CCh+Atr group was compared with the CCh+Cis group, a significant decrease was observed in the CCh+Cis group (p<0.001).

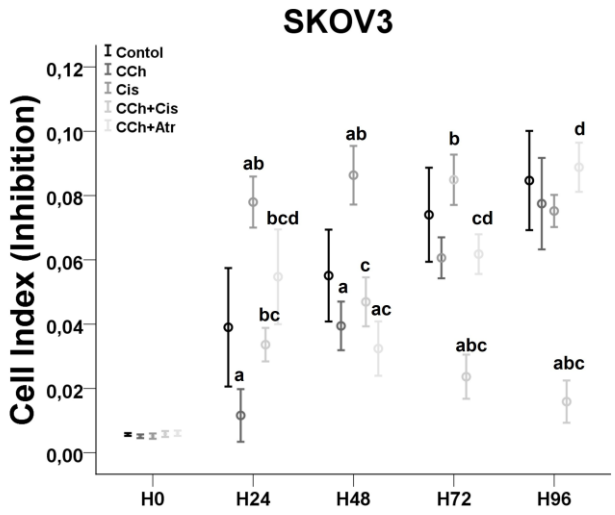

Figure 2. The effect of cisplatin and carbachol on cisplatin-free (SKOV-3) ovarian cell proliferation

CCh: carbachol applied group, Cis: cisplatin applied group, CCH+Cis: carbachol and cisplatin applied group, CCh+Atr: carbachol and atropine applied group, superscript letters denote significant differences of the group when compared with the [a]: control group, [b]: CCh group, [c]: Cis group, and [d]: CCh+Cis groups (p<0.001)

DISCUSSION

The term metronomic chemotherapy (MT) first used by Hanahan et al. (12), is the chronic administration of chemotherapy at low, minimal doses on a continuous schedule of administration (13). As ovarian cancer remains the most common cause of death from a gynecological malignancy, it is necessary to find better treatment strategies than traditional dosing of chemotherapy to cure this deadly disease. The traditional chemotherapy program requires episodic administration of a cytotoxic drug at the maximum tolerated doses (MTD) which targets tumor cells followed by periods of rest to allow non-cancer tissues to recover (14). However, these cytotoxic drugs also damage normal cells, especially in MTD (15). It has been reported that the success of chemotherapy may depend on the development of approaches for metronomic planning in order to minimize MTD toxicity and improve its anti-tumor effect (16). One of the main challenges in the treatment of ovarian cancer is the development of drug resistance. Metronomic chemotherapy also prevents the development of drug resistance as it reduces the extent of drug-free periods.

For the development of the MT strategy, some drugs were tested, especially those with an oral formulation. The common point of the studies conducted is that MT applications have promising results for ovarian cancer therapy. Nevertheless, the identification of the optimal dosage has yet to be established. Using an optimal metronomic dose of metronomic docetaxel has been shown to be effective in inhibiting tumor growth and prolonging survival using an ovarian cancer model (14). In addition, it has been reported that oral topotecan may be an ideal agent to be considered for clinical MT trial in ovarian cancer (17). In another study, pazopanib therapy in combination with metronomic topotecan therapy showed significant antitumor effects in preclinical ovarian cancer models (18). When the studies are reviewed, it is suggested that MT is a treatment option for ovarian cancer patients.

Muscarinic receptor agonists such as carbachol stimulate cell proliferation, survival, migration, and invasion, as shown by in vitro studies using human ovarian cancer cells. There is previous evidence suggesting that muscarinic receptors can regulate cell proliferation depending on the growth context of the cell. It has previously been reported that mAChRs are involved in ovarian cancer progression (9). mAChRs belong to the GPCR family which constitutes the largest family of cell surface receptors involved in signal transduction. The effects of carbachol and histamine on cytosolic-free calcium and changes in cell proliferation were characterized in OVCAR-3 cells and non-tumorigenic CHO cells (11).

No studies have yet been conducted on the effects of CCh in metronomic therapy applications for ovarian cancer. In a study evaluating cell proliferation has been reported that the cholinergic agonist carbachol inhibits the proliferation of human chronic myelogenous leukemia K562 cells, especially at the 48th hour after administration (5). A study investigating metronomic treatment approaches in breast cancer demonstrated that low doses of therapy combining PX with carbachol could be a useful strategy to treat triple negative (TN) breast tumors (19). To inhibit the action of

carbachol, cells also were treated with atropine at 10^{-9} M and this inhibitory effect was prevented in the presence of atropine.

In this study, it was investigated the action of a combination of low doses of the muscarinic agonist carbachol plus cisplatin, a chemotherapeutic agent frequently used in ovarian cancer treatment, in terms of effectiveness. Substantial cell death was observed in A2780cis and SKOV-3 cells within 24 h after carbachol application and this continued at 96th hour. It was observed that carbachol application caused a significant decrease in cell number at the 24th hour compared to cisplatin in cisplatin-free and cisplatin-resistant cell lines. As a result of atropine application, while carbachol decreased cell proliferation both alone and together with cisplatin, only cisplatin itself showed the opposite activity in the cisplatin-free cell line. The effect of carbachol on reducing cell proliferation was seen at the 72nd and 96th hours when it was administered together with cisplatin in the cisplatin-free cell line. In the cisplatin-resistant cell line, it was observed that the control and CCh groups significantly decreased compared to the Cis group at 24 hours. Carbachol with cisplatin significantly inhibited cell proliferation at the 24th, 48th, 72nd, and 96th hours. Co-administration of carbachol with cisplatin caused a more effective decrease in cell number than application of carbachol alone. Unlike other studies, the inhibitory effect of carbachol was not prevented by atropine application. It is demonstrated that low doses therapy combining cisplatin with a cholinergic agonist carbachol could be a useful strategy to treat ovarian tumors.

CONCLUSION

These results support the notion that the cholinergic agonist carbachol may have roles in cell death and affect cell proliferation by activating muscarinic receptors. The results of the study confirmed that mAChRs can be considered as therapeutic targets for metronomic therapy in ovarian cancer and the usefulness of a muscarinic agonist as a repositioning drug in the treatment of this type of tumor. However, in order to reach a definite conclusion, the results need to be supported by other methods. The next study is planned to evaluate the effects of carbachol on cell proliferation as well as its effects on cell cycle and apoptosis in breast, ovarian, and brain cancers.

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

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The Effect of Inflammatory Markers in the Hemogram Parameters of Pregnant Women with Thyroid Disease on Obstetric and Neonatal Outcomes

Tiroid Hastalığı olan Gebelerin Hemogram Parametrelerinde İnflamatuar Belirteçlerin Obstetrik ve Neonatal Sonuçlara Etkisi

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ABSTRACT

Aim: This study aimed to determine the differences in hemogram parameters, especially in inflammatory markers and perinatal and neonatal outcomes of pregnant women with thyroid disease, and to examine the effects of these differences on pregnancy outcomes.

Material and Methods: The data of 80 pregnant women diagnosed with thyroid disease according to the American Thyroid Association (ATA) criteria at the first admission between 2016 and 2019 and 100 pregnant women whose thyroid hormone levels were within the normal reference range were retrospectively analyzed. Obstetric outcomes such as type and time of delivery, and the presence of additional disease during pregnancy, and neonatal outcomes such as weight, gender, and Apgar score at birth were compared. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), white blood cell (WBC) count, and hemoglobin (Hb) and mean platelet volume (MPV) values, which are accepted as inflammation markers, were also compared between groups.

Results: There was no significant difference between hypothyroid and hyperthyroid pregnant women in terms of Hb (p=0.319) and PLR (p=0.341) values. Third-trimester WBC (p=0.015) values were higher and MPV (p=0.007) values were lower in the hypothyroid pregnant women compared to the control group. The highest NLR (p=0.005) value was observed in the hypothyroid group. Comorbidities were found to be 27.4% (n=20) in the hypothyroid group, 14.3% (n=1) in the hyperthyroid group, and 1.0% (n=1) in the control group (p<0.001).

Conclusion: Pregnant women with thyroid disease may have differences in hemogram parameters, especially in inflammatory markers, and these differences may affect pregnancy outcomes.

Keywords: Thyroid disease; NLR; PLR; perinatal and neonatal outcomes; pregnancy.

ÖZ

Amaç: Bu çalışmanın amacı, tiroid hastalığı olan gebelerin hemogram parametrelerinde, özellikle inflamatuar belirteçlerde ve perinatal ve neonatal sonuçlardaki farklılıkları belirlemek ve bu farklılıkların gebelik sonuçları üzerindeki etkisini incelemektir.

Gereç ve Yöntemler: 2016 ve 2019 yılları arasında ilk başvurusunda Amerikan Tiroid Derneği (American Thyroid Association, ATA) kriterlerine göre tiroid hastalığı tanısı alan 80 gebe kadın ile tiroid hormon düzeyleri normal referans aralığında olan 100 gebe kadının verileri geriye dönük olarak analiz edildi. Doğum tipi ve zamanı ve gebelikte ek hastalık varlığı gibi obstetrik sonuçlar ile doğumda kilo, cinsiyet ve Apgar skoru gibi neonatal sonuçlar karşılaştırıldı. Yine, nötrofil/lenfosit oranı (neutrophil-to-lymphocyte ratio, NLR), trombosit/lenfosit oranı (platelet-to-lymphocyte ratio, PLR), beyaz kan hücresi (white blood cell, WBC) sayısı ve inflamasyon belirteçleri olarak kabul edilen hemoglobin (Hb) ve ortalama trombosit hacmi (mean platelet volume, MPV) değerleri de gruplar arasında karşılaştırıldı.

Bulgular: Hipotiroidili ve hipertiroidili gebeler arasında Hb (p=0,319) ve PLR (p=0,341) değerleri açısından anlamlı bir farklılık yoktu. Hipotiroidili gebelerde kontrol grubuna göre 3. trimester WBC (p=0,015) değerleri daha yüksek ve MPV (p=0,007) değerleri daha düşüktü. En yüksek NLR (p=0,005) değeri hipotiroid grubunda gözlendi. Ek hastalık hipotiroid grubunda %27,4 (n=20), hipertiroid grubunda %14,3 (n=1) ve kontrol grubunda %1,0 (n=1) olarak saptandı (p<0,001).

Sonuç: Tiroid hastalığı olan gebelerin hemogram parametrelerinde özellikle inflamatuar belirteçlerde farklılıklar olabilir ve bu farklılıklar gebelik sonuçlarını etkileyebilir.

Anahtar kelimeler: Tiroid hastalığı; NLR; PLR; perinatal ve neonatal sonuçlar; gebelik.

INTRODUCTION

After diabetes, thyroid diseases are the second most common endocrine disorder in pregnancy. Thyroid hormone production during pregnancy increases by about 50% with a similar increase in total daily iodine requirement. In addition, following the physiological and hormonal changes caused by pregnancy and human chorionic gonadotropin (HCG), there is an increase of 50% in the daily iodine maternal requirement caused by an increase of up to 50% in thyroxine (T4) and triiodothyronine (T3) production, the thyroid stimulating hormone (TSH) level decreases, especially in the first trimester (1). Thyroid stimulation is started in the first trimester by HCG hormone, which has some structural similarities to TSH. While free T4 and free T3 are at normal or slightly higher elevated levels in the first trimester, they can be lower than normal in the third trimester. The increase in free T3 and free T4 levels in the early stages of pregnancy is associated with the thyrotropic effect of HCG, and related to this effect, TSH is suppressed. Thyroid-stimulating activity causes temporary hyperthyroidism in some women (2,3).

Thyroid hormones have important effects on the fetus. Maternal thyroid hormones play an important role in the development of the fetal brain at 1-20 weeks of gestation (4). Thyroid diseases in pregnancy affect pregnancy outcomes and neurophysiological development of the fetus at a serious rate (5,6). Insufficient thyroid hormone synthesis in the early weeks of pregnancy leads to significant impairments in motor skills and the intelligence of the fetus (7).

Thyroid diseases can be classified as hypothyroidism and hyperthyroidism. In regions where there is insufficient iodine intake, Hashimoto's thyroiditis is the most common cause of hypothyroidism in pregnancy (8). Endemic iodine deficiency is generally associated with hypothyroidism in pregnancy throughout the world. The prevalence of spontaneous hypothyroidism is between 2% and 3%, and of these cases, 3-5% present with evident hypothyroidism, and 2-2.5% with subclinical hypothyroidism (9). The results of untreated hypothyroidism during pregnancy include preterm delivery, preeclampsia, and gestational hypertension in the fetus. There may be postpartum hemorrhage, low birth weight, neurophysiological and cognitive dysfunctions in the fetus, and there can be an increase in perinatal morbidity and mortality (10).

The most common reason for hyperthyroidism seen in pregnancy is Graves' disease (11). Other less common causes are toxic multinodular goiter, toxic adenoma, and thyroiditis. Temporary gestational thyrotoxicosis associated with elevated HCG levels, which affects 1-3% of pregnancies in the first half of pregnancy, is another cause of gestational hyperthyroidism (3). Studies have shown that untreated hyperthyroidism can result in premature birth, spontaneous abortion, retarded intrauterine development, preeclampsia, low birth weight, and fetal malformations (12).

The aim of this study was to investigate the potential relationships between thyroid status measured by serum TSH levels during pregnancy and obstetric and perinatal outcomes, and to compare hemogram parameters and inflammatory markers in hemogram parameters of thyroid patients with those in the control group and to determine their effects on pregnancy outcomes.

MATERIAL AND METHODS

Approval for the study was granted by the Local Ethics Committee (Kocaeli Derince Training and Research Hospital, dated 11.11.2021, and numbered 2021-96). A retrospective review was made of the data of 73 patients diagnosed with hypothyroidism, and 7 diagnosed with hyperthyroidism during pregnancy, who gave birth in the Kocaeli Derince Training and Research Hospital between 2016 and 2019, and healthy pregnant women with no additional diseases.

Serum TSH concentration is the first and most reliable measurement in the evaluation of thyroid function in pregnancy. Physiological changes occur in TSH levels during pregnancy. According to the latest American Thyroid Association (ATA) guidelines, TSH levels were defined using population- and trimester-specific reference ranges (13). Inclusion criteria for the hypothyroid group were TSH above 2.5 mIU/ml in case the patient applied to the hospital in the first trimester, over 3 mIU/ml for the second and third trimesters (Table 1), and high free T4 value, for the hyperthyroid group, it was determined that the TSH level was below 0.45 mIU/ml and the free T4 value was above 1.8 ng/ml. TSH values of the control group were determined as being within normal ranges according to trimester reference intervals (6). The ATA recommends the use of test-specific, trimester-specific reference intervals obtained in women with no known thyroid disease, optimal iodine nutrition, and negative thyroid peroxidase (TPO) antibodies. Currently, such ranges are not widely available. In the absence of such pregnancy-specific reference ranges, the ATA recommends that the TSH lower limit be 0.1 mIU/L, for non-pregnant adults approximately 0.4 mIU/L lower than the lower limit, and the TSH upper limit 4 mIU/L (13).

Pregnant women in all three groups were compared in terms of hemogram parameters. In addition to comparing inflammatory markers during pregnancy, perinatal and neonatal outcomes, gestational age at birth, birth weight, infant sex, and Apgar scores were also compared.

Statistical Analysis

Data were analyzed using SPSS v.23.0 software. Whether the data showed normal distribution was analyzed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Since the assumption of normality was not met, the Kruskal-Wallis test was applied with the post hoc Bonferroni-Dunn test in the comparisons of continuous variables between groups. In the analysis of the relationship between categorical variables, the chi-square test was used. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

The study included a total of 180 pregnant women, 73 diagnosed with hypothyroidism, 7 diagnosed with hyperthyroidism, and a control group of 100 healthy pregnant patients. The median age of hypothyroid patients was determined to be statistically significantly older than that of the control group (30 years and 26 years, p=0.001). The rate of premature births (9.6%, n=7) in hypothyroid patients was found to be significantly higher than the rates in the other two groups (p=0.032). Cesarean section delivery rate (56.2%, n=41) was high enough to show a statistically significant difference in the hypothyroid patient group (p=0.018). The birth weights in the three

groups were statistically similar (p=0.108). The 1-minute Apgar score of the infants of hypothyroid patients was significantly higher than the infants in the hyperthyroid group (p=0.042), and the 5-minute Apgar score of the hypothyroid group was significantly higher than the control group (p<0.001). Maternal age, gestational week, infant gender, type of birth, birth weight, and Apgar scores were presented in Table 2.

The results of the analysis of the third-trimester hemogram parameters were reported in Table 3. There was no statistically significant difference between the three groups in terms of hemoglobin (Hb) and platelet-to-lymphocyte ratio (PLR) values (p=0.319, p=0.341, respectively). The white blood cell (WBC) counts of hypothyroid patients were significantly higher than the control group (p=0.015), while the mean platelet volume (MPV) was significantly lower (p=0.007) in the hypothyroid group. The highest neutrophil-to-lymphocyte ratio (NLR) was observed in hypothyroid patients (p=0.005).

Table 1. TSH reference ranges according to weeks of gestation by ATA guidelines (13)

Trimester	Reference Interval
First trimester	0.1-2.5 mIU/ml
Second trimester	0.2-3.0 mIU/ml
Last trimester	0.3-3.0 mIU/ml

TSH: thyroid stimulating hormone, ATA: American Thyroid Association

Comorbidities were determined in the hypothyroid group at the rate of 27.4% (n=20), in the hyperthyroid group at 14.3% (n=1), and in the control group at 1% (n=1). The comorbidity rate in the control group was statistically significantly lower than the other two groups (p<0.001). When the comorbidities were evaluated, there was observed to be a higher rate of gestational diabetes (GDM) in hypothyroid patients (p<0.001) and a higher rate of cholestasis in hyperthyroid patients (p=0.039). Maternal complications were observed in 2 (2.7%) patients in the hypothyroid group and there was no significant difference between the groups (p=0.240). Data related to the comorbidities and maternal complications in groups were presented in Table 4.

DISCUSSION

Thyroid hormones not only play a role in the neurological development of the fetus during pregnancy, but are also necessary for the normal development and differentiation of all cells, metabolic balance, and physiological function of tissues (14). Thyroid hormones regulate human hematopoiesis in bone marrow. Changes in hematological parameters such as Hb, hematocrit (Hct), mean corpuscular volume (MCV), and WBC are associated with thyroid dysfunction (15). There are studies in the literature showing that hyperthyroidism and hypothyroidism are associated with an increased risk of leukocytopenia, neutropenia, and thrombocytopenia (14,16).

In the study, the hemogram parameters of pregnant women with thyroid dysfunction and the differences between these

Table 2. Comparison of patient characteristics

	Normal (n=100)	Hypothyroid (n=73)	Hyperthyroid (n=7)	p
Maternal age (year)	26 (5) [20-40]^{a}	30 (4) [19-52]^{b}	29 (3) [20-38]^{a,b}	**0.001**
Gestational week	39 (1) [36-40]	39 (2) [35-41]	39 (1) [37-40]	0.589
Infant Gender, n (%)				
Male	52 (52.0)	42 (57.5)	4 (57.1)	0.775
Female	48 (48.0)	31 (42.5)	3 (42.9)	
Type of birth, n (%)				
Cesarean	35 (35.0)^{a}	41 (56.2)^{b}	3 (42.9)^{a,b}	**0.018**
Normal delivery	65 (65.0)^{a}	32 (43.8)^{b}	4 (57.1)^{a,b}	
Prematurity, n (%)				
Mature	99 (99.0)	66 (90.4)	7 (100)	**0.032**
Premature	1 (1.0)^{a}	7 (9.6)^{b}	0 (0)^{a}	
Birth weight (g)	3413 (457.5) [2800-4140]	3400 (345.5) [1560-4660]	3200 (423.5) [2900-3750]	0.108
Apgar 1-min	8 (1) [7-9]^{a,b}	9 (1) [5-9]^{a}	8 (1) [8-9]^{b}	**0.042**
Apgar 5-min	10 (1) [9-10]^{a}	10 (2) [7-10]^{b}	10 (1) [9-10]^{a,b}	**<0.001**

Descriptive statistics were presented as median (interquartile range) [minimum-maximum], ^{a,b}: different superscript letters denote significant differences between the groups

Table 3. Last trimester hemogram parameters of the patients

	Normal (n=100)	Hypothyroid (n=73)	Hyperthyroid (n=7)	p
Hb (g/dl)	10.9 (2.1) [8-15.7]	11.1 (1.9) [7.9-14.1]	11.5 (1.8) [10.6-13]	0.319
WBC ($\times 10^9$/L)	12.3 (3.8) [5-26.1]^{a}	13.6 (3.4) [6.7-30.7]^{b}	12.3 (3.9) [8.1-20]^{a,b}	**0.015**
MPV (fL)	8.9 (1.5) [6.1-11.6]^{a}	8.6 (1.3) [5.9-17.9]^{b}	8.7 (1.7) [7.8-13.9]^{a,b}	**0.007**
NLR	5.7 (3.7) [1.5-19.5]^{a}	7.4 (4.1) [2.2-21.4]^{b}	5.8 (2.3) [2.7-7.6]^{a}	**0.005**
PLR	114.8 (70.4) [1.6-481]	127 (46.6) [52.5-296]	108 (41.3) [54-167]	0.341

Hb: hemoglobin, WBC: white blood cell, MPV: mean platelet volume, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, descriptive statistics were presented as median (interquartile range) [minimum-maximum], ^{a,b}: different superscript letters denote significant differences between the groups

Table 4. Comorbidities and maternal complications of the patients

	Normal (n=100)	Hypothyroid (n=73)	Hyperthyroid (n=7)	p
Any comorbidity, n (%)	1 (1.0)[a]	20 (27.4)[b]	1 (14.3)[b]	**<0.001**
Gestational diabetes, n (%)	1 (1.0)[a]	18 (24.7)[b]	0 (0.0)[a]	**<0.001**
Celiac, n (%)	0 (0.0)	1 (1.4)	0 (0.0)	0.444
Asthma, n (%)	0 (0.0)	1 (1.4)	0 (0.0)	0.444
Cholestasis, n (%)	0 (0.0)[a]	0 (0.0)[a]	1 (14.3)[b]	**0.039**
Complication, n (%)	0 (0.0)	2 (2.7)	0 (0.0)	0.240

parameters, especially those accepted as inflammation markers, compared to the health group, and the effect of this difference on pregnancy outcomes was investigated. In the hemogram parameters of pregnant thyroid patients examined in the study, WBC, NLR, and PLR which are considered as especially inflammatory markers, and MPV and Hb values, which are an important focus of thyroid disease studies, were examined. In the third-trimester hemogram parameters of hypothyroid patients, WBC values were higher and MPV values were lower than the healthy control group. The highest NLR was found in the hypothyroid patient group. There was no significant difference between the groups in terms of Hb and PLR values.

It was determined that the rate of preterm birth was higher in the hypothyroid group with high levels of WBC and NLR, which are markers of inflammation, compared to the healthy control group and the hyperthyroid group. Similarly, in a study of 157 pregnant women, including patients with premature rupture of membranes (PPROM), which is one of the causes of preterm birth, the rate of NLR was found to be significantly higher in the PPROM patient group (17).

In another study involving 486 people, including 243 preterm and 243 term delivery patients, NLR, PLR, and MLR scores were found to be significantly higher in the patients who gave premature birth, and NLR had the highest value among the tested scores and had the highest sensitivity (71%) in the study (18).

The MPV value was higher in the patient group with hyperthyroidism in this study. Gestational cholestasis rates were found to be higher in the hyperthyroid patient group compared with the other groups. In a study including 84 pregnant women examining hematological inflammatory markers in mild and severe intrahepatic cholestasis of pregnancy, MPV was found to be significantly increased in severe intrahepatic cholestasis of pregnancy (19).

In another study involving 117 pregnant women with intrahepatic cholestasis, the MPV value was found to be higher in the hemogram parameters of the patient group with intrahepatic cholestasis compared with 100 healthy pregnant women (20).

In the study, the NLR value, which is accepted as an inflammation marker, was determined to be significantly high in the hypothyroid group compared with the values of the healthy control group, and the incidence of GDM was high enough to make a statistically significant difference in this group. In a study including 120 patients with 58 GDM and 62 healthy control groups, the NLR value, which is one of the inflammation markers, was found to be high, which supports our results (21).

Similarly, in the meta-analysis results of 11 studies including 1271 GDM and 1504 healthy control groups, NLR values were found statistically significantly high in the GDM group, compared to the healthy control group (22).

CONCLUSION

In this study, pregnant women with thyroid dysfunction were compared with healthy pregnant women and it was determined that there may be differences in perinatal and neonatal outcomes. The relationship of these differences with inflammatory markers in hemogram parameters was investigated. It was determined that these differences in hemogram parameters, especially in inflammatory markers, can affect pregnancy outcomes in this patient group.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Kocaeli Derince Training and Research Hospital (11.11.2021, 96).

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The Effect of Treatment on Weight Gain in Iron Deficiency Anemia and Its Association with Ghrelin and Hepcidin Levels

Demir Eksikliği Anemisinde Tedavinin Kilo Alımına Etkisi ve Ghrelin ve Hepsidin Düzeyleri ile İlişkisi

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ABSTRACT

Aim: Although loss of appetite in iron deficiency anemia (IDA) and weight gain during treatment are common complaints, there are very few studies in adults. This study aimed to determine the levels of ghrelin, one of the appetite-related hormones, and hepcidin, one of the main regulators of iron metabolism, in IDA, and to examine the effects of treatment on weight gain and the levels of these hormones.

Material and Methods: Eighty-seven adult patients with IDA and a control group of 50 healthy volunteers were included in the study. Anthropometric measurements and blood samples were obtained from the patient and control groups before treatment, and repeated after treatment in the IDA group.

Results: No significant difference was found in terms of weight, body mass index (BMI), and waist-to-hip ratio between groups but there was a significant increase in weight and BMI, in the patient group after treatment (both $p<0.001$). Pre-treatment hepcidin and ghrelin levels of the patient group were significantly lower than the control group ($p<0.001$, and $p=0.026$, respectively), and hepcidin levels increased significantly after treatment ($p<0.001$). The increase in ghrelin was not statistically significant but showed a positive weak correlation with both weight ($r=0.254$, $p=0.018$) and BMI ($r=0.231$, $p=0.031$) increase. Hepcidin levels were not correlated with weight and BMI changes.

Conclusion: These findings revealed low levels of ghrelin and hepcidin in adults with IDA and an increase in weight and BMI with treatment. Hepcidin increased with treatment but was not correlated with weight gain, ghrelin was weakly correlated.

Keywords: Iron deficiency anemia; appetite; weight gain; ghrelin; hepcidin.

ÖZ

Amaç: Demir eksikliği anemisi (DEA)'nde iştahsızlık ve tedavi sırasında kilo alımı sık yakınmalar olsa da bu konuda erişkinlerde yapılmış çok az çalışma mevcuttur. Bu çalışmada, DEA'da iştah ile ilgili hormanlardan biri olan ghrelin ve demir metabolizmasının temel düzenleyicilerinden biri olan hepsidin seviyelerinin belirlenmesi ile tedavinin kilo alımına ve bu hormonların düzeylerine etkisinin incelenmesi amaçlandı.

Gereç ve Yöntemler: Çalışmaya DEA tanılı 87 erişkin hasta ve 50 sağlıklı gönüllüden oluşan kontrol grubu dahil edildi. Hasta ve kontrol grubunda tedavi öncesi antropometrik ölçümler yapılarak kan örnekleri alındı ve tedavi sonrasında DEA grubunda tekrarlandı.

Bulgular: Gruplar arasında kilo, vücut kitle indeksi (VKİ), bel-kalça oranı açısından anlamlı fark yoktu ancak hasta grubunda tedavi sonrasında kilo ve VKİ'de anlamlı artış saptandı (her iki $p<0,001$). Hasta grubunun tedavi öncesi hepsidin ve ghrelin seviyesi kontrol grubuna göre anlamlı olarak daha düşüktü (sırasıyla $p<0,001$ ve $p=0,026$) ve tedavi sonrası hepsidin seviyeleri anlamlı olarak arttı ($p<0,001$). Tedavi sonrası ghrelin artışı istatistiksel olarak anlamlı değildi ancak hem kilo ($r=0,254$; $p=0,018$) hem de VKİ ($r=0,231$; $p=0,031$) artışı ile pozitif yönde zayıf korelasyon gösteriyordu. Hepsidin düzeylerinin kilo ve VKİ değişimleri ile korelasyonu saptanmadı.

Sonuç: Bu bulgular erişkinlerde DEA'da ghrelin ve hepsidinin düşük olduğunu ve tedavi ile hastalarda objektif bir kilo ve VKİ artışı olduğunu ortaya koydu. Hepsidin tedavi ile artış göstermekle beraber kilo alımı ile korele değildi, ghrelin zayıf korelasyon göstermekteydi.

Anahtar kelimeler: Demir eksikliği anemisi; iştah; kilo alımı; ghrelin; hepsidin.

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INTRODUCTION

Anemia is accepted as a worldwide public health problem by the World Health Organization (WHO) and iron deficiency anemia (IDA) is the most common type of anemia (1). IDA, which constitutes approximately 50% of all anemias (2), is an anemia that develops as a result of decreased erythropoiesis due to a significant decrease in the total amount of iron, which is essential for hemoglobin (Hb) production (3). In the patient management, investigation and, if possible, correction of the condition causing iron deficiency has an important place and iron replacement by oral or parenteral route is the treatment of choice. Although this treatment seems to be simple, problems are frequently encountered especially in compliance with oral iron treatment and it has been shown that the drugs are not used regularly or discontinued because of gastrointestinal complaints or weight gain during the treatment process (4). Although increased appetite and weight gain during iron therapy is a common complaint heard by clinicians, there are very few publications on the objective demonstration and causes of this.

Ghrelin and hepcidin are parameters related to appetite and iron metabolism associated with iron deficiency which have been investigated in recent years (5). Ghrelin is a hormone secreted from the gastric fundus that stimulates the feeling of hunger. It increases appetite and food intake centrally or peripherally (6). Hepcidin is a homeostatic regulator of iron absorption from the intestine. Increases in plasma iron levels and increases in tissue iron deposits activate hepcidin synthesis and then hepcidin decreases iron release from macrophages and enterocytes (7). There are studies reporting a significant increase in hepcidin and ghrelin levels with iron treatment in children (8). There are many studies reporting low ghrelin levels in the presence of IDA in children and associating this with anorexia (9,10). Increased ghrelin levels, weight gain, and acceleration in growth have also been reported with iron treatment in children (11-13). However, studies on whether there is an objective weight gain with treatment in adults and its determinants are limited and contradictory (14,15).

We planned this study to determine the levels of ghrelin, one of the hormones related to appetite, and hepcidin, one of the main regulators of iron metabolism, and the effect of treatment on weight gain and the levels of these hormones in IDA. We hope that this study will provide information that will contribute to explaining the decreased appetite seen in IDA and the compliance problems experienced during treatment.

MATERIAL AND METHODS

This study, which was approved by the decision numbered 2015/63 of the local Ethics Committee of Duzce University on 02.11.2015 and supported by the Scientific Research Projects Coordinatorship of Duzce University (Project no: 2015.04.03.396) was carried out in accordance with the principles of the Declaration of Helsinki. A total of 130 patients over the age of 18 who were admitted to the Internal Medicine or Hematology outpatient clinic of our hospital, who were diagnosed with IDA according to WHO and Turkish Hematology Association criteria (Hb <13 g/dl in males, Hb <12 g/dl in females, and ferritin <15 ng/mL) were evaluated for the study. Patients who are younger than 18 years of age or pregnant, who have received erythrocyte or whole blood transfusion in the last 3 months, who have used oral or parenteral iron preparations in the last month, who have any of the etiological causes that may cause anemia other than iron deficiency (vitamin B12 or folate deficiency), and patients with active inflammatory or infectious diseases, with another hematological disease (thalassemia, myelodysplastic syndrome, multiple myeloma, chronic myeloproliferative disease, etc.) or who did not give consent (43 subjects in total) were excluded from the study. After obtaining the consent of 87 IDA patients and 50 healthy volunteers, weight, height, body mass index (BMI), waist and hip circumference were measured and blood samples were taken. The patients were treated for iron deficiency with the dose and method (orally or parenterally) recommended by the responsible doctor, and the researchers had no influence on the treatment. After the Hb and mean corpuscular volume (MCV) levels were normalized and ferritin was >30 ng/mL according to the anemia parameters of the patient being followed up in the outpatient clinic, but not before the 3rd month of treatment, weight, height, waist and hip circumference were measured and blood samples were taken again.

Venous blood was collected from the patient and control groups (IDA patients before and after treatment, and from the control group only once) at 08:00-09:00 hours after 8 hours of fasting. For biochemical parameters, appropriate amounts of samples were collected using serum tubes containing a clot activator with a separator. Biochemical parameters, urea, creatinine, sodium, potassium, ferritin, iron, and total iron binding capacity (TIBC) were determined on ROCHE COBAS 6000 Hitachi c501 (Roche Diagnostics GmbH, Mannheim, Germany) auto analyzer using commercial kits (Roche Diagnostics, Germany) and hemogram was determined on the same day without waiting on BECMAN COULTER LH 780 (USA) using commercial kits. For hepcidin and ghrelin levels, samples were centrifuged by a single physician, serum was separated, portioned into Eppendorf tubes, and stored at -80 °C. Hepcidin was analyzed by the enzyme-linked immunosorbent assay (ELISA) method using Elabscience Biotechnology Co., Ltd (Wuhan, P.R.C.) brand Human Hepcidin-25 (bioactive) ELISA kit (REF: E-EL-H0077-96 Wells) according to the manufacturer's catalog. Ghrelin was analyzed in serum samples using Elabscience Biotechnology Co., Ltd (Wuhan, P.R.C.) brand Human Acylated Ghrelin ELISA kit (REF: E-EL-H1919-96 Wells) in Biotec L800 device according to the manufacturer's catalog.

Statistical Analysis

Descriptive statistics were presented as mean, standard deviation or median, first and third quartiles, and frequency and percentage, as appropriate for the type of data. The conformity of numerical data to the normal distribution was examined with the Kolmogorov-Smirnov test. The independent-sample t-test or Mann-Whitney U test was used to compare numerical variables between groups. Relationships between categorical variables were analyzed using Pearson's chi-square and Fisher's exact tests. Paired samples t-test or Wilcoxon test was used to

compare variables before and after treatment. The statistical significance level was taken as 0.05 and PASW v.18 was utilized for statistical analyses.

RESULTS

A total of 137 people, including 87 patients and 50 control, were included in the study. Parameters such as age, hemogram and anemia parameters, vitamin B12, folate, BMI, waist and hip circumference, waist-to-hip ratio, hepcidin, and ghrelin levels were evaluated in iron deficiency patients and control group, and details were given in Table 1. Females constituted 81.6% (n=71) of the patient group and 82.0% (n=41) of the control group and there was no statistically significant difference between the groups (p=0.955). The mean Hb level was 9.59±1.72 gr/dl, the MCV value was 69.38±7.29 fl, and the ferritin value was 6.57±3.32 ng/mL, and these values indicated significant iron deficiency. Other anemia parameters were significantly different from the control group (p<0.001) in accordance with IDA. BMI, waist-to-hip ratio, white blood cell (WBC), vitamin B12, and folate levels were not significantly different between the patient and control groups (Table 1).

When the pre- and post-treatment examinations of the patient group were compared, there was no significant difference in WBC values, but there was a significant increase in Hb and anemia-related parameters indicating the effectiveness of the treatment (Table 2). No significant difference was found between the pretreatment vitamin B12 and folate levels and the control group.

When the pre-treatment and post-treatment measurements were evaluated, it was found that the weight, BMI, waist and hip circumference measurements of the patients increased significantly, the patients gained an average of 1.15 kg after treatment (p<0.001), BMI increased from 25.86 kg/m^2 to 26.33 kg/m^2 (p<0.001), both waist and hip circumference increased significantly (0.81 cm increase for waist and 0.82 cm increase for hip, respectively, p<0.001), but the waist-to-hip ratio remained constant. While ferritin, iron, and transferrin saturation (TSAT) values increased significantly after treatment, TIBC decreased significantly (Table 2).

The pretreatment hepcidin level was 80 ng/ml in IDA patients and 179 ng/ml in the control group and was found to be statistically significantly (p<0.001) lower in IDA. Similarly, the ghrelin level was found to be 152 pq/ml in patients with IDA and 213 pq/ml in the control group and was found to be statistically significantly (p=0.026) lower in the IDA group.

When compared before and after treatment in patients, plasma hepcidin levels increased significantly (80 ng/ml and 92 ng/ml, respectively, p<0.001). The plasma ghrelin level was found 152 pq/ml before treatment and 164 pq/ml after treatment, but this increase did not reach statistical significance (p=0.589).

When the correlations of the change in weight and BMI of the patients with the change in anemia parameters or ghrelin and hepcidin levels were investigated, both weight and BMI changes were found to be positively correlated only with ghrelin change, albeit weakly (r=0.254, p=0.018 for weight change and r=0.231, p=0.031 for BMI change). No significant correlation was found with the change in anemia parameters or change in hepcidin level.

Table 1. Comparison of demographic and clinical characteristics in patients with IDA and control group

	IDA (n=87)	Control (n=50)	p
Gender, n (%)			
Female	71 (81.6)	41 (82.0)	0.955
Male	16 (18.4)	9 (18.0)	
Age (year)	42.74±15.09	41.22±13.14	0.554
WBC (x10^9/L)	6.99±1.75	6.87±1.37	0.672
Hb (g/dl)	9.59±1.72	13.48±1.27	**<0.001**
MCV (fl)	69.38±7.29	85.56±2.42	**<0.001**
Ferritin (ng/ml)	6.57±3.32	58.23±37.90	**<0.001**
Iron (mcg/dL)	25.86±11.14	83.92±26.85	**<0.001**
TIBC (mcg/dL)	413.47±65.52	246.78±40.71	**<0.001**
TSAT (%)	6.45±3.24	34.87±13.29	**<0.001**
B12 (pg/ml)	441.14±319.78	349.26±115.61	**0.017**
Folate (ng/ml)	10.39±4.56	10.05±4.27	0.667
BMI (kg/m^2)	25.86±6.21	25.13±4.60	0.472
WHR	0.7647±0.0789	0.8628±0.5128	0.082

IDA: iron deficiency anemia, WBC: white blood cell, Hb: hemoglobin, MCV: mean corpuscular volume, TIBC: total iron binding capacity, TSAT: transferrin saturation, B12: vitamin B12, BMI: body mass index, WHR: waist to hip ratio

Table 2. Comparison of anemia parameters and anthropometric measurements before and after treatment in patients with IDA

	Before	After	p
WBC (x10^9/L)	6.99±1.75	7.20±1.91	0.319
Hb (g/dl)	9.59±1.72	13.14±1.01	**<0.001**
MCV (fl)	69.38±7.29	82.64±7.58	**<0.001**
Ferritin (ng/ml)	6.57±3.32	106.81±135.57	**<0.001**
Iron (mcg/dL)	25.86±11.14	82.51±54.59	**<0.001**
TIBC (mcg/dL)	413.47±65.52	254.94±63.76	**<0.001**
TSAT (%)	6.45±3.24	42.19±68.01	**<0.001**
Weight (kg)	68.30±15.83	69.45±16.10	**<0.001**
BMI (kg/m^2)	25.86±6.21	26.33±6.34	**<0.001**
WC (cm)	80.33±14.38	81.14±14.61	**<0.001**
HC (cm)	104.52±10.94	105.34±11.40	**<0.001**
WHR	0.7647±0.0789	0.7664±0.0780	0.071

IDA: iron deficiency anemia, WBC: white blood cell, Hb: hemoglobin, MCV: mean corpuscular volume, TIBC: total iron binding capacity, TSAT: transferrin saturation, BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio

DISCUSSION

Anemia is a worldwide public health problem, approximately 25% of the world population is anemic, concentrated in preschool-aged children and women (16). Even though different etiologies come to the fore according to age groups in IDA, which is the most common cause of anemia, the most important cause in developing countries, as in Türkiye, is inadequate intake of iron-containing foods (9). On the other hand, one of the clinical findings of IDA observed by clinicians is decreased appetite and this may turn the condition into a vicious cycle.

The association of IDA with appetite, nutrition, and weight gain has been investigated primarily in children and adolescents. In a study by Naiman et al. (17) on 14 infants and children, the gastrointestinal systems of children with IDA were analyzed in detail including duodenal biopsies, and both functional and structural abnormalities were described. The fact that most of these abnormalities disappeared after iron treatment suggested that it caused diffuse and reversible enteropathy in children. In adolescent girls, a change in eating habits was observed with iron treatment given every other day, and a median weight gain of 2.66 kg was reported (12).

In our study, no significant difference was found in terms of weight when the patient and control groups were compared, but a statistically significant weight gain was found in the patient group with treatment in accordance with clinical experience and data in the literature on pediatric patients. This finding suggested that iron deficiency alone was not associated with low weight but iron treatment was associated with weight gain. In our study, BMI, waist and hip circumference, which are considered to be more objective indicators of subcutaneous and total body fat, increased significantly and it was shown that fat distribution was proportional according to the waist-to-hip ratio, which remained constant.

One of the parameters studied in the literature to reveal the causes of anorexia observed in IDA with objective measurements is ghrelin, which is also called the "appetite hormone". Ghrelin is a hormone released from the gastrointestinal system and is known to induce a feeling of hunger. Ghrelin hormone has orexigenic and adipogenic effects and is secreted in response to hunger and hypoglycemia (18). Endogenous ghrelin is a potentially important new regulator of complex systems controlling food intake and body weight (19). However, appetite has both biological and behavioral/psychological aspects and is difficult to assess quantitatively (20). Since appetite is a conscious desire for food, evaluation of the weight gained by measuring the amount of food eaten per day may be a way of measuring appetite. In a study by Shiiya et al. (21), 70 patients and 28 control groups were included and it was shown that plasma ghrelin concentration and BMI were negatively correlated. Many studies have been conducted to explain the association between iron and ghrelin levels. In a study conducted by Isguven et al. (9) in prebubertal children, 25 IDA and 25 control groups were included and ghrelin levels were found to be lower in children with IDA compared to the control group. Again, Akarsu et al. (10) investigated ghrelin levels in various periods of iron deficiency including hypoferritinemia, iron deficiency, and overt IDA in children, and reported that ghrelin levels decreased as iron deficiency became apparent. However, two more recent studies conducted in adults suggested findings in the opposite direction. Luo et al. (14) suggested that ghrelin increased with fasting in rats and healthy volunteers but iron levels decreased and there was a negative correlation between them. In their study in which 56 adult IDA patients and 51 healthy volunteers were examined, Ghrayeb et al. (15) reported that low Simplified Nutritional Appetite Questionnaire (SNAQ) scores indicated anorexia in the IDA group and acylated ghrelin (AG) levels and acylated ghrelin/unacylated ghrelin (AG/UAG) ratios were higher than in the control group. They reported that there was a significant increase in SNAQ scores and a significant decrease in AG levels and AG/AUG ratio after parenteral iron sucrose treatment was given to this patient group.

In our study, ghrelin level was found to be lower in patients with IDA compared to the control group, supporting the findings in studies conducted in children. In our study, the patient and control groups did not differ in terms of weight and BMI. This situation ensured that ghrelin changes which may be related to the effect of BMI were not a confounding factor and suggested that the decrease in ghrelin was an effect of iron deficiency. In other studies, decreased ghrelin levels were accompanied by lower weight and BMI values compared to the control group, but this difference with the other studies may have resulted from the age difference of the patient groups and the difference in sample sizes. However, our study reached different results from the study of Ghrayeb et al. (15), which is another longitudinal study conducted in adults. In this study, it is noteworthy that the BMI of the IDA group was higher than that of the control group and the gender distribution was different between the groups, but the authors also reported similar results with multivariate linear regression analysis. Since acylated ghrelin, which is thought to be the active form, was measured both in our study and in the study of Ghrayeb et al. (15), more extensive studies are required to clarify this issue.

In our study, although there was an increasing trend in ghrelin levels before and after treatment, this numerical increase did not reach statistical significance. This may be because ghrelin plasma concentration is negatively correlated with weight and BMI. In our study, weight and BMI increased significantly after treatment and it is known that weight gain suppresses ghrelin levels (22). Therefore, an increase in ghrelin may not have been seen as a net effect. When the association between the increase in ghrelin level of each patient and the weight gain of that patient was analyzed, a weak positive correlation was found.

Another parameter closely related to iron metabolism which has been studied recently is hepcidin. Hepcidin is mostly synthesized from the liver, but it has been shown that it is also synthesized from the kidneys, skeletal muscle, brain, and heart (23). Hepcidin is defined as the main homeostatic regulator of iron absorption in the intestines, the iron cycle in macrophages, and iron release from hepatic stores (24). The observation that hepcidin synthesis increased with dietary iron suggested that hepcidin was involved in iron metabolism, and it was shown that hepcidin level was a negative regulator of intestinal iron absorption, iron transport through the placenta, and iron release from macrophages (25).

Many studies have been performed to demonstrate the metabolism of hepcidin and iron. Dallalio et al. (26) showed a positive correlation between hepcidin and ferritin in patients with anemia. In a study where 94 patients with IDA and 91 control groups were included, it was found that hepcidin level was lower in the patient group with IDA compared to the control group (27). The largest studies performed with hepcidin to date are two large studies including 2998 patients in the Netherlands and 1577 patients in Italy. In these studies, hepcidin level was found to be low in correlation with low serum ferritin levels in premenopausal and postmenopausal women (28,29).

In our study, similar to these studies, when IDA and the control group were compared, there was a significant decrease in the level of hepcidin in the group with IDA compared to the control group. Since hepcidin expression in normal erythropoietic activity is directly related to hepatic iron stores, hepcidin expression is expected to increase when hepatocyte iron stores increase (30). Increased hepcidin levels have been reported with treatment in children who presented with IDA (31). In our study, pre-treatment hepcidin level was compared with post-treatment hepcidin level in patients with IDA, and a significant increase in post-treatment hepcidin level was found in support of these studies in the literature.

Although it has been suggested that hepcidin may have an effect on appetite and energy balance in iron deficiency, there are very few studies investigating this. It has been reported that some foods may have an effect on hepcidin levels (32), however, it is not clear whether the level of hepcidin affects food intake and through which pathways. In a study conducted in patients with newly diagnosed diabetes mellitus after pancreatitis without overt iron deficiency, a significant negative correlation of hepcidin with leptin, another determinant hormone of energy balance, and with leptin/ghrelin ratio in fasting was shown, but it was reported that it was not correlated with ghrelin (33). On the other hand, a study conducted in children with IDA reported that ghrelin and hepcidin levels were higher than controls before treatment, there was no difference in leptin levels and there was a significant increase only in hepcidin and ghrelin levels with treatment (31). Our study is the first study in the literature to investigate ghrelin and hepcidin changes together with weight changes in adults with IDA. In our study, weight change was not associated with parameters such as Hb, MCV, ferritin, and TSAT, which reflect the depth of anemia at baseline. Hepcidin, which was significantly lower than the pretreatment control and increased significantly with treatment, did not correlate significantly with either weight gain or BMI. These findings suggested that hepcidin is an important parameter that may contribute to the diagnosis of iron deficiency and may be used to show the adequacy of treatment, but it is not directly related to weight gain. Since hepcidin was significantly lower than in the control group, it seems possible that it has a role in the anorexia symptom seen in IDA patients, but large studies including other parameters related to appetite and energy balance may be necessary to clarify this role.

CONCLUSION

These findings suggested that decreased appetite, one of the symptoms frequently observed by clinicians in IDA, may be related to ghrelin levels, which were found to be lower in IDA patients compared to the control group. In our study, IDA patients, who were not different from the control group in terms of weight and BMI at baseline, showed a significant increase in weight and BMI with treatment, and the effect of treatment on weight gain attributed to treatment was demonstrated concretely. The increase in ghrelin with treatment did not reach statistical significance. This may be due to physiological suppression of ghrelin levels with weight gain. When weight gain was analyzed on a patient basis, a positive correlation was found with the increase in ghrelin. Hepcidin was significantly lower in the iron deficiency group compared to the control group and showed a significant increase with treatment, but was not associated with weight gain. Hepcidin can be considered as a parameter that can contribute to the diagnosis of iron deficiency and can be used to show the adequacy of treatment, but we did not find a direct relationship between hepcidin and appetite or weight gain. Although the results of our study overlap with the studies conducted in children, since contrasting findings were found with other recent adult studies, larger studies including other appetite-related hormones are needed to clarify the issue.

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Short-Term Outcomes of Carotid Artery Stenting versus Carotid Endarterectomy in the Treatment of Carotid Stenosis: An Up-Dated Meta-Analysis of Randomized Controlled Trials

Karotis Stenozu Tedavisinde Karotis Arter Stentleme ve Karotis Endarterektomi Yöntemlerinin Kısa Dönem Sonuçları: Randomize Kontrollü Çalışmaların Güncel Bir Meta-Analizi

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ABSTRACT

Aim: Carotid artery stenting is thought to result in better outcomes when compared to carotid endarterectomy. To evaluate this hypothesis, a far-reaching of published randomized controlled trials were performed to evaluate the short-term outcomes of carotid artery stenting versus carotid endarterectomy for patients undergoing carotid artery stenosis.
Material and Methods: A comprehensive search of trials published from 1994 until December 31, 2022, was performed using Science Direct, PubMed, Web of Science, Sage, Ebscohost, Scopus, and Cochrane Central electronic databases. Major endpoints (any stroke, myocardial infarction, and all-cause mortality) were extracted from the publications. Pooled risk ratio (RR) and 95% confidence interval (CI) were calculated using a fixed-effects model.
Results: 21 trials involving 15518 patients (8514 with stenting, 7004 with endarterectomy) were included in the meta-analysis. Stenting was associated with a significantly increased risk of short-term any stroke (RR=1.555, 95% CI: 1.307-1.851, p<0.001) yet a significantly decreased risk of short-term myocardial infarction (RR=0.458, 95% CI: 0.319-0.660, p<0.001) when compared with endarterectomy. No significant difference was found in all-cause mortality between the two interventions (RR=1.277, 95% CI: 0.835-1.952, p=0.259), but with a trend toward superiority favoring endarterectomy.
Conclusion: Endarterectomy was found to be superior in terms of any stroke and partially regarding all-cause mortality, whereas stenting was found to be superior in terms of myocardial infarction. Yet for robust results, further studies are needed to address the relative effectiveness of stenting versus endarterectomy in the future.
Keywords: Carotid endarterectomy; meta-analysis; randomized controlled trials; short-term outcomes; carotid artery stenting.

ÖZ

Amaç: Karotis arter stentlemenin karotis endarterektomiye kıyasla daha iyi sonuçlar ürettiği düşünülmektedir. Bu hipotezi sınamak için, karotis arter stenozu geçiren hastalarda karotis arter stentleme ve karotis endarterektominin kısa süreli sonuçlarını değerlendirmek üzere yayınlanmış randomize kontrollü çalışmaların geniş kapsamlı değerlendirmesi yapıldı.
Gereç ve Yöntemler: Science Direct, PubMed, Web of Science, Sage, Ebscohost, Scopus ve Cochrane Central elektronik veri tabanları kullanılarak 1994'ten 31 Aralık 2022'ye kadar yayınlanmış olan denemelerin kapsamlı bir araştırması yapıldı. Yayınlardan temel sonlanım noktaları (herhangi bir inme türü, miyokard enfarktüsü ve tüm nedenlere bağlı ölüm) çıkarıldı. Sabit etkiler modeli iler etki büyüklüğü risk oranı (RO) ve %95 güven aralığı (GA) hesaplandı.
Bulgular: Bu meta-analizine 15518 hastayı (8514 stentleme, 7004 endarterektomi) içeren 21 çalışma dahil edildi. Endarterektomi ile karşılaştırıldığında, stentleme, kısa süreli herhangi bir inme riskinde anlamlı derecede artma (RR=1,555; %95 GA: 1,307-1,851; p<0,001), ancak kısa süreli miyokard enfarktüsü riskinde ise azalma (RR=0,458; %95 GA: 0,319-0,660; p<0,001) ile ilişkiliydi. İki müdahale arasında tüm nedenlere bağlı ölüm açısından anlamlı bir fark yokken (RR=1,277; %95 GA: 0,835-1,952; p=0,259) endarterektomi lehine üstünlük eğilimi vardı.
Sonuç: Endarterektominin herhangi bir inme türü açısından ve kısmen de tüm nedenlere bağlı ölüm açısından üstün olduğu, stentlemenin ise miyokard enfarktüsü açısından üstün olduğu görüldü. Ancak daha sağlam sonuçlar için gelecekte stentlemenin endarterektomiye karşı göreceli etkinliğini ele alan daha fazla çalışmalara ihtiyaç vardır.
Anahtar kelimeler: Karotis endarterektomi; meta-analizi; randomize kontrollü çalışmalar; kısa-dönem sonuçlar; karotis arter stentleme.

INTRODUCTION

Cerebrovascular and cardiovascular diseases have become the leading cause of disability and mortality globally. Thus, the great and still growing burden of cerebrovascular and cardiovascular diseases on healthcare systems indicates an urgent need for preventive measures (1-3). Carotid artery stenosis (CS), an atherosclerosis disease, is a major cause of neurological and cardiological morbidity and mortality. The prevalence of CS disease was estimated to be 1.5% globally in 2020 (1).

Carotid artery stenting (CAS) was progressively preferred as a chance to carotid endarterectomy (CEA) for the operation of patients with CS in the 1994s onwards (2,3). Stroke is a leading cause of death worldwide (4), yet it was previously noted that about 20-25% of nearly all types of strokes are caused by CS (5). On the other hand, individuals with CS are at high risk of developing cardiovascular disease as well (1). In a review, it was indicated that approximately 63% of individuals with CS were found to be associated with cardiac events (6). In this regard, myocardial infarction is measured as another primary disease outcome in the treatment of CS. Likewise, mortality is generally measured after both CAS and CEA (1). In addition to these major outcomes mentioned here, of course, there are other complications such as restenosis, cranial nerve palsies, transient ischemic attack, cognitive decline, bleeding, etc. being tested after CAS and CEA treatment techniques (7).

CAS and CEA are feasible options for patients with symptomatic or asymptomatic CS. Even though higher side effects and death rates were associated with CAS than CEA in the early studies, developments such as new endovascular technologies, cerebral embolic protection device (EPD), and, trials with their larger sample sizes issued lately have improved efficacy with CAS. Yet, the exact role of CAS versus CEA in the treatment of CS remains controversial, according to some studies (8-12). Therefore, in this paper, we aimed to compare the risks and benefits of CAS and CEA with a particular focus on the short-term outcomes of any stroke, all-cause mortality, and myocardial infarction, which are frequently observed and measured immediately after operation.

MATERIAL AND METHODS

In this study, the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist and guidelines were used as a handbook (13).

Data Sources and Search Strategy

A wide-ranging literature search was carried out from 1994 (when Morris et al. first deployed metal stents in two patients with CS) to December 31, 2022, for all randomized controlled trials (RCTs) that compared CAS with CEA in the treatment of CS and reported peri- and post-procedural outcomes. We searched Science Direct, PubMed, Web of Science, Sage, Ebscohost, Scopus, and Cochrane Central electronic databases and keyword search terms for “carotid artery stenosis”, “carotid stenosis”, “endarterectomy”, “stenting”, “randomized controlled trial”, “controlled trial” “stroke”, “death”, “mortality”, and “myocardial infarction”. All search databases were screened using the advanced search options specific to those databases. For instance, while searching PubMed, we utilized medical subject headings (MeSH) terms. Moreover, the Peer Review of Electronic Search Strategies (PRESS) guideline, which focuses on the quality of the database search and is the core element in the health technology assessment, was used systematically for searching databases (9). All the studies were initially examined according to title, abstract, and finally the complete body text by researchers. In cases of disagreement, resolution was achieved through discussion. Finally, the articles were merely restricted to English.

Study Selection

The population, intervention, comparison, outcome, and study design (PICOS) criteria identified by researchers were used to construct a set of inclusion and exclusion guidelines. Eligible trials that met the subsequent predefined criteria were included in the analysis by consensus. Trials that meet the requirements are (i) RCTs of participants with symptomatic or asymptomatic CS comparing CAS with CEA, (ii) trials with or without EPD, (iii) participants with symptomatic CS of ≥50% and asymptomatic CS of ≥60%, (iv) participants aged ≥18 years, (v) participants who have not previously been treated for CS, and (vi) participants reporting the 30-day peri- and post-procedural any stroke, all-cause mortality, and myocardial infarction.

Non-randomized controlled trials, animal trials, and all other forms of studies were excluded. Participants who had coronary bypass concurrently with the CS and only those who underwent balloon angioplasty were also excluded. Additionally, the surgical risk conditions of participants and gender terms were disregarded as results of insufficient studies.

Data Extraction and Quality Assessment

Pre-specified data elements were extracted and evaluated independently by the all three researchers of the present study. For each included RCT, study characteristics such as year of publication, study type (single- or multi-center), total number of randomized patients, median length of follow-up, mean age, the proportion of symptomatic and asymptomatic participants, degree of stenosis, surgical risk, the use of EPD, and outcomes to be analyzed were classified.

The quality evaluation of the included studies and the risk of bias (Risk Of Bias VISualization, Robvis) before analysis were assessed by the Cochrane Collaboration assessment tools. More specifically, we evaluated each RCT’s sequence generation, allocation concealment, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other potential sources of bias. For each domain, every study was assigned a score of high, low, or unclear risk of bias (14,15).

Data Synthesis and Statistical Analysis

The main outcomes whose impacts were examined in our study are the incidence of any stroke, all-cause mortality, and myocardial infarction just after the operation. All outcomes in the analysis were limited to the information provided in each trial. For data analysis, we used Comprehensive Meta-Analysis (CMA) v.2.0 software. For the pooled effect size, the risk ratio (RR)-widely employed in health science- and 95% confidence interval (CI) were utilized in estimating results. $p<0.05$ were considered statistically significant. The Cochran Q-test was used to determine the presence of heterogeneity among studies. If

the Q statistic was greater than the degree of freedom (df), this would indicate the existence of heterogeneity. For heterogeneity between the RCTs, the Higgins I^2 statistic was used. I^2 represents significant heterogeneity, provided that $I^2 \geq 50\%$ is taken to represent significant heterogeneity. In the present study, as no evidence of significant heterogeneity was found in any analyses, the fixed-effect model (FEM) was applied. Finally, publication bias was evaluated using both the visually funnel plot along with the trim and fill statistic and the weighted regression test of Egger. Eventually, a sensitivity analysis was performed to evaluate the impact of higher-weight individual studies on the summary for any stroke, myocardial infarction, and all-cause mortality separately.

RESULTS

Search Results

Based on database searching, we initially identified 1,045 potentially relevant studies. After removing duplicates, abstracts, titles, reviews, protocols, costs, etc. Finally, 86 papers were predicted to meet the inclusion criteria. After a thorough screening and full-text readings by researchers, 21 strictly eligible trials comparing CAS with CEA were included in the meta-analysis (Figure 1).

Patient Characteristics and Quality Assessment

The design features and clinical characteristics of the individual studies are summarized in Table 1. In all included studies, basic criteria in individual studies and some institutions' guidelines (e.g., Peripheral Artery and Vein Diseases-National Treatment Guidelines, American Society of Cardiologists, American Heart Association Guidelines) were taken as references. Those 21 studies enrolled 15,519 (8,514 for CAS, 7,004 for CEA) Participants. Of these patients, 9,721 are asymptomatic, accounting for approximately 62.6% of the studies included, and 12 of them are multi-center RCTs. The mean age (68.2) of the patients was in the range of 63.0 to 72.6 years, and median follow-up durations ranged from 1 to 60 months. The majority of patients had high or moderate surgical risk. In addition, EPD was used in most of the patients, especially those published in recent years. The incidence of short-term outcomes after CAS and CEA was also given in Table 1. In the end, we found 21 studies comparing any stroke and all-cause mortality and 19 comparing myocardial infarction.

When analyzing the visual risk of bias (Robvis) of included trials, the quality of randomization was found to be high. As shown in Figure 2, however, some studies had no data about the risk of bias pointed out with yellow dots. Due to the nature of CAS and CEA treatment procedures, none of the trials involved blinding of participants or personnel. Yet, all individual studies were defined by the authors, who carried out the individual studies, as having a low risk of randomization bias.

Any Post-Procedural Stroke

A FEM was applied as the Q statistics for heterogeneity indicated an obvious trend for homogeneity (Q: 15.561, df(Q):20, I^2=0.001%, p=0.743) among the trials, indicating that most of the variance reflects sampling error. Upon performing FEM, CAS was associated with a significantly higher incidence of any stroke when compared to CEA (RR=1.555, 95% CI: 1.307-1.851, p<0.001). It could be said that the risk of any stroke after treatment in

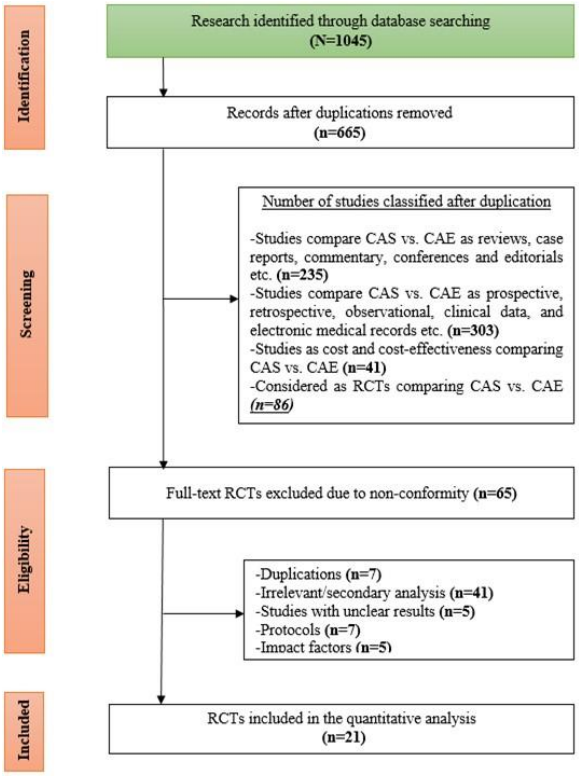

Figure 1. Flowchart of the study selection process
CAS: carotid artery stenting, CEA: carotid endarterectomy, RCT: randomized controlled trial

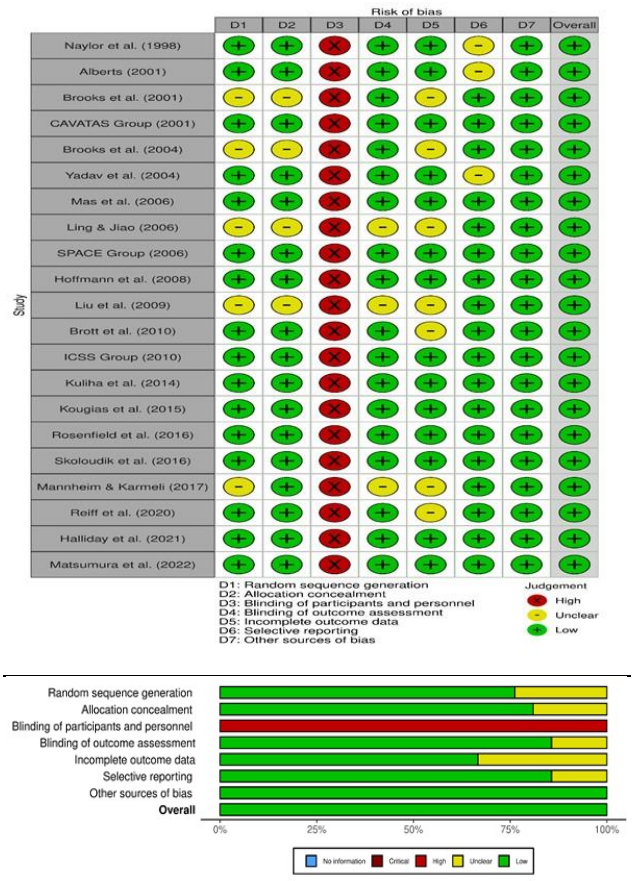

Figure 2. Risk of bias graph for randomized controlled trials (upper panel) and risk of bias summary of randomized controlled trials (lower panel)

Table 1. Characteristics and clinical outcomes of including randomized controlled trials comparing carotid artery stenting and carotid endarterectomy

Study	Year	Study Design	Population CAS/CEA (Total)	Mean Age (Year)	Any Stroke (CAS/CEA)	Myocardial Infarction (CAS/CEA)	All-cause Mortality (CAS/CEA)	Follow-up (Month)	Sym/Asy	Degree of Stenosis (%) (Sym/Asy)	Surgical Risk	Use of EPD
SPACE Group (10)	2006	MC	561/550 (1111)	67.9	42/34	0/0	4/5	24	1111/0	≥70/-	high	mixed
Alberts MJ. (16)	2001	MC	107/112 (219)	68.3	4/1	-	9/4	24	219/0	≥60/-	low	no
Naylor et al. (17)	1998	SC	7/10 (17)	67.2	5/0	-	0/0	1	17/0	≥70/-	moderate	yes
Brooks et al. (18)	2001	SC	53/51 (104)	68.0	0/0	0/1	0/1	24	104/0	≥70/-	low	no
Kougias et al. (19)	2015	SC	29/31 (60)	69.2	0/0	0/0	0/0	6	0/60	-/≥80	moderate	yes
CAVATAS Group (20)	2001	MC	240/246 (486)	67.0	18/21	0/3	7/4	60	437/49	≥50/≥50	moderate	no
Brooks et al. (21)	2004	SC	43/42 (85)	68.2	0/0	0/0	0/0	48	0/85	-/≥80	high	no
Yadav et al. (22)	2004	MC	159/151 (310)	72.6	5/5	3/10	1/3	36	91/219	≥50/≥80	high	yes
Mannheim et al. (23)	2017	SC	68/68 (136)	69.2	2/1	0/0	0/0	60	0/136	-/≥70	moderate	yes
Mas et al. (24)	2006	MC	247/257 (504)	69.7	22/9	1/2	2/3	6	504/0	≥60/-	moderate	yes
Liu et al. (25)	2009	SC	23/23 (46)	65.4	2/1	0/1	0/0	18	NA	≥50/≥70	NA	mixed
Ling et al. (26)	2006	MC	82/84 (166)	63.0	2/3	1/2	1/2	6	NA	≥50/≥70	moderate	yes
Hoffmann et al. (27)	2008	SC	10/10 (20)	70.0	0/1	0/0	0/0	24	20/0	≥70/-	high	yes
Reiff et al. (28)	2020	MC	197/203 (400)	70.0	5/5	0/0	0/0	60	0/400	-/≥70	moderate	mixed
ICSS Group (29)	2010	MC	828/821 (1649)	70.0	58/24	3/5	11/4	4	1649/0	≥50/-	high	mixed
Brott et al. (30)	2010	MC	1184/1118 (2302)	69.0	52/29	14/28	9/4	48	1217/1085	≥50/≥60	moderate	mixed
Kuliha et al. (31)	2014	SC	77/73 (150)	65.5	2/1	0/0	0/0	1	87/63	≥70/≥70	high	yes
Rosenfield et al. (32)	2016	MC	1032/343 (1375)	67.8	30/5	5/3	1/1	60	0/1375	-/≥70	high	yes
Školoudík et al. (33)	2016	SC	136/106 (242)	66.3	3/1	0/0	0/0	1	126/116	≥70/≥70	high	yes
Halliday et al. (34)	2021	MC	1811/1814 (3625)	69.5	61/41	5/8	2/2	60	0/3625	-/≥60	high	yes
Matsumura et al. (35)	2022	MC	1620/891 (2511)	68.0	43/13	9/15	2/2	48	0/2511	-/≥70	high	yes
Summation			**8514/7004 (15518)**	**68.2**	**356/198**	**41/88**	**50/35**	-	**5582/9721**			

CAS: carotid artery stenting, CEA: carotid endarterectomy, MC: multi-center randomized controlled trial, SC: single-center randomized controlled trial, Sym: symptomatic, Asy: asymptomatic, EPD: embolic protection device, NA: not available

the CEA group is approximately 55% less than treatment in CAS (Figure 3). In other words, being treated by CEA might be safer and more effective than CAS in terms of short-term any stroke.

As for publication bias for any stroke, a funnel plot distribution, and the result of an Egger's test (p=0.904) suggest that there was no publication bias, which indicates the results could be reliable. According to Duval and Tweedie's trim and fill statistic, complete symmetry will be achieved if only one imaginary study (red circles) is added to the right side of the funnel plot. Sensitivity analysis was performed by exclusion of trials that contributed the most number of patients (higher weight). After the exclusion of these trials, the results revealed no particular strong influence, while the effect size partly came down (RR=1.541, 95% CI: 1.283-1.705).

Post-Procedural Myocardial Infarction

Due to the lower heterogeneity (Q: 3.504, df(Q):18, I^2=0.001%, p<0.001) for myocardial infarction, a FEM statistic was applied. The pooled results (RR=0.458, 95% CI: 0.319-0.660, p<0.001) show that CEA was associated with a higher risk of myocardial infarction compared with CAS (Figure 4). These findings also show that the risk of myocardial infarction for post-operation could be reduced by almost 54% if treated with CAS.

There was no evidence of big-size study effects (publication bias) of funnel plots or Egger's regression test (p=0.116). Yet, for the exact symmetry, while considering Duval and Tweedie's trim and fill statistic, about four imaginary studies (red circles) are needed on the left side of the funnel plot. A sensitivity analysis also confirmed the consistency of our main findings. The odds of 30-day myocardial infarction remained in favor of CAS when data from the most number of trials was omitted (RR=0.514, 95% CI: 0.352-0.730).

Post-Procedural All-Cause Mortality

Since the homogeneity terms for 30-day all-cause mortality were met in the study (Q: 9.445, df(Q): 20, I^2=0.001%, p=0.977) we applied the FEM statistic. Compared with CAS, CEA was associated with a non-significant reduction in the risk of all-cause mortality (Figure 5). In other words, no significant difference in all-cause mortality was observed between the CAS group and the CEA group after operation (RR=1.277, 95% CI: 0.835-1.952, p=0.259).

However, the funnel plot distributions and the result of Egger's test (p=0.007) suggest that there was a publication bias and that the results are questionable in terms of reliability. According to the trim and fill statistics, when 10 virtual studies (red circles) are added to the right side of the funnel graph, the desired symmetry will be achieved. In addition to publication bias, a sensitivity analysis of all-cause mortality demonstrated that the exclusion of trials with the highest weight did not greatly affect the overall result of all-cause mortality in favor of CAS (RR=1.252, 95% CI: 0.702-1.452). Therefore, it is beneficial to be more careful and cautious when interpreting pooled effect size related to all-cause mortality. All the funnel plots of outcomes are demonstrated in Figure 6.

DISCUSSION

Aiming to summarize the effectiveness of CAS versus CEA with the evidence from RCTs, CAS was found to be associated with a significantly higher rate of any stroke within 30 days after the operation. The higher rate of any stroke in the stenting group was likely attributed to the minor strokes in accordance with some recent trials' findings (26-30). On the other hand, it was found that CAS is superior to CEA just in the incidence of myocardial infarction for 30 days after operation. Furthermore, both procedures appeared equivalent in their effects on all-cause mortality, despite a trend toward the superiority of CEA. These results suggest that CEA has more favorable effects on short-term any stroke and partially all-cause mortality and should remain the treatment of choice for patients with CS.

Even though there has been an observed increased rate of any stroke with CAS and an increased rate of myocardial infarction with CEA, according to some studies (16-20,36-38), much of this conclusion could be

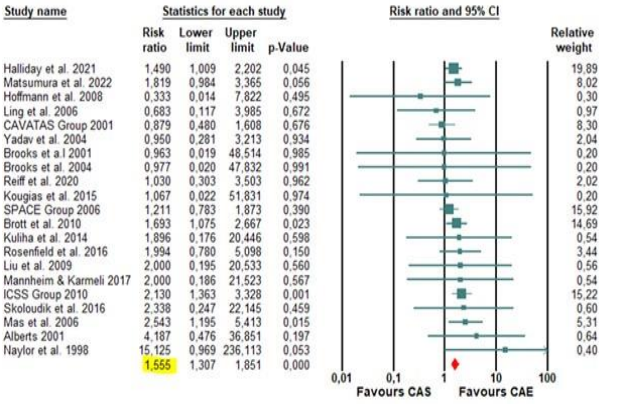

Figure 3. Forest plot of risk ratio of any stroke

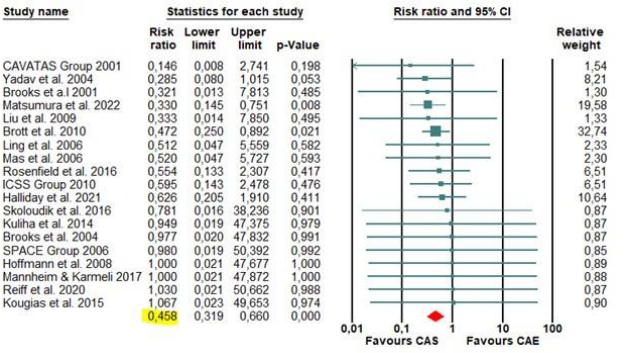

Figure 4. Forest plot of risk ratio of myocardial infarction

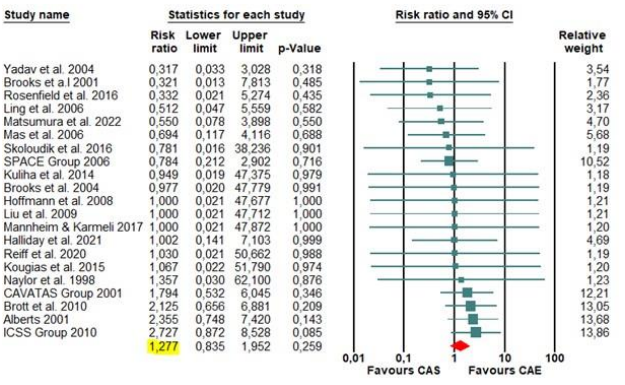

Figure 5. Forest plot of risk ratio of all-cause mortality

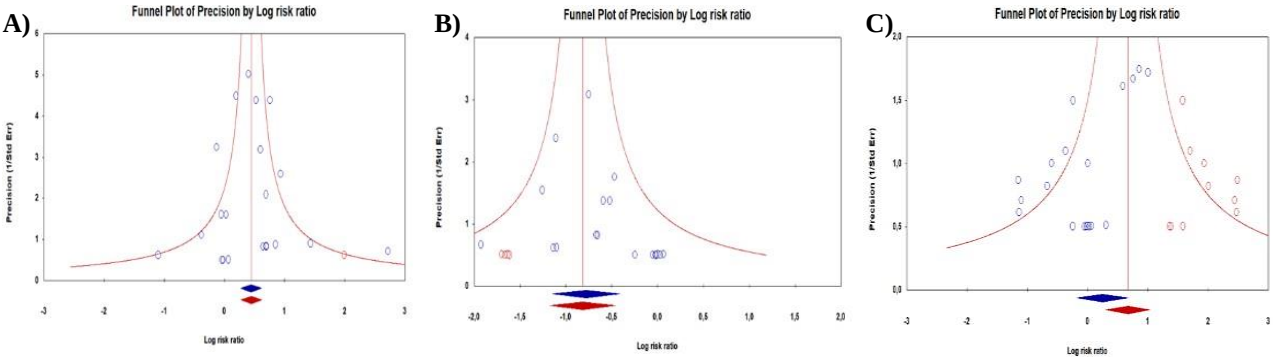

Figure 6. Funnel plots of the incidence of **A)** any stroke, **B)** myocardial infarction, and **C)** all-cause mortality

based on the majority of the symptomatic population. Yet, this gap may be attributable to the nature of the CAS and CEA techniques as well as the asymptomatic. Furthermore, as reported in some studies (10,20,36) surgical risk level might have also contributed to the expected outcomes in this study.

Technological advances in CAS and the use of distal EPD as well as mesh-covered stents might have reduced the incidence of post-procedural stroke in patients undergoing CAS but have not yet reached a comparative effectiveness over CEA (39). As for CEA, advances in preoperative cardiac evaluation, anesthesia, and quality improvement through standardized outcome analysis are areas of focus to reduce the risk of post-operative complications (40,41) Therefore, it could be said that our findings are in concordance with those views.

In this study, although we obtained results parallel to those of previous studies for patients with both symptomatic and asymptomatic CS at large, the current meta-analysis is the first far-reaching review with pooled outcomes from 21 RCTs. A summary comparison outcome of individual RCTs and some previous meta-analytical studies with similar design characteristics to the current study results are shown in Table 2.

Several limitations of this current study should be underlined. To begin with, as the number of trials included in the analysis was not enough, it was thus overlooked to perform subgroup analysis in terms of patient type (symptomatic or asymptomatic), use of an EPD, stent type, surgical risk, etc. Secondly, our conclusions were based on evidence predominantly from asymptomatic patients. Third, studies with both small and large samples included in this review may have affected the effect size. Therefore, all these limitations may have reduced the scientific precision of research. Thirdly, the differences in patient characteristics within the individual studies, and being both symptomatic and asymptomatic traits of studies might have affected outcomes. Another limitation is that the possible consequences of long-term results on the effectiveness of the methods are not included in the study. On the other hand, our study also has several strengths. Firstly, it is a comprehensive study conducted by different databases; data collection, summary methods, reporting biases, and explicit quality assessment represent the strengths of this work. Besides, the homogeneity across trials did reach a level of statistical significance, reinforcing the consistency of our findings. Taken together, the current analysis suggests that CAS and CEA seem to be complementary rather than competing modes of therapy with careful patient selection. Over and above, CEA is a reasonably safe treatment for CS in terms of any stroke and all-cause mortality in short-term results whereas CAS is a reasonable procedure for short-term myocardial infarction.

CONCLUSION

This study was designed to examine the safety and efficacy of compared with endarterectomy in patients with CS, with a particular focus on short-term outcomes. While stenting had a more favorable post-procedural outcome with respect to myocardial infarction, endarterectomy had a more favorable post-procedural any stroke outcome. For all-cause mortality, no significant differences were found between CAS and CEA, despite a trend toward superiority favoring CEA. The outcome-related all-cause mortality comparison of CAS and CEA must be interpreted cautiously, given the publication bias found. As a result, CAS may offer a viable alternative given its lower associated risk of myocardial infarction, whereas CEA offers a standard of care in the treatment of CS for the prevention of any stroke. To sum up, according to the findings, it could be said that CEA should be offered as the first alternative to CS, but more evidence is needed to reevaluate the absolute effectiveness of both techniques in terms of short-term results. For this assumption, further studies are needed to make a concrete comparison of CAS versus CEA in the future. Moreover, it is extremely important that, for payer institutions and policymakers it should be taken into consideration the economic effects of both procedures as well as intermediate and long-term outcomes.

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

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Author Contributions: Idea/Concept: İA, AEE; Design: İA, AEE; Data Collection/Processing: İA, İD; Analysis/Interpretation: İA, İD, AEE; Literature Review: İA; Drafting/Writing: İA; Critical Review: İD, AEE.

Table 2. Summary findings on the short-term clinical efficacy of carotid artery stenting versus carotid endarterectomy

Study	Year	Outcome	Pooled Effect	p
Sardar et al. (8)	2017	Any stroke	OR=2.07 (95% CI: 1.56-2.75)	**0.001**
		Myocardial infarction	OR=0.45 (95% CI: 0.27-0.75)	**0.002**
		All-cause mortality	OR=1.34 (95% CI: 0.60-3.02)	0.480
SPACE Group (10)	2006	Any stroke	OR=1.24 (95% CI: 0.79-1.95)	-
		All-cause mortality	OR=0.78 (95% CI: 0.15-3.64)	-
		Any stroke & all-cause mortality	OR=1.19 (95% CI: 0.71-1.92)	-
Kan et al. (12)	2018	Any stroke	RR=1.57 (95% CI: 1.25-1.97)	**0.001**
		All-cause mortality	RR=1.50 (95% CI: 0.83-2.74)	0.180
CAVATAS Group (20)	2001	Any stroke & all-cause mortality	HR=1.03 (95% CI: 0.64-1.64)	0.900
Mas et al. (24)	2006	Any stroke	RR=3.30 (95% CI: 1.40-7.50)	**0.004**
		Myocardial infarction	RR=0.50 (95% CI: 0.04-5.40)	0.620
		All-cause mortality	RR=0.70 (95% CI: 0.10-3.90)	0.680
ICSS Group (29)	2010	Any stroke	HR=2.13 (95% CI: 1.36-3.33)	**0.001**
		All-cause mortality	HR=2.73 (95% CI: 0.87-8.53)	0.072
		Any stroke & all-cause mortality	HR=1.83 (95% CI: 1.21-2.77)	**0.003**
Brott et al. (30)	2010	Any stroke	HR=1.79 (95% CI: 1.14-2.82)	**0.010**
		Myocardial infarction	HR=0.50 (95% CI: 0.26-0.94)	**0.030**
		All-cause mortality	HR=2.25 (95% CI: 0.69-7.30)	0.180
Yavin et al. (40)	2011	Any stroke	OR=1.72 (95% CI: 1.20-2.47)	**0.003**
		Myocardial infarction	OR=0.47 (95% CI: 0.29-0.78)	**0.003**
		All-cause mortality	OR=1.11 (95% CI: 0.56-2.18)	0.760
Murad et al. (41)	2008	Any stroke	RR=1.29 (95% CI: 0.73-2.26)	-
		Myocardial infarction	RR=0.43 (95% CI: 0.17-1.11)	-
		All-cause mortality	RR=0.61 (95% CI: 0.27-1.37)	-
Current study		Any stroke	RR=1.555 (95% CI: 1.307-1.851)	**0.001**
		Myocardial infarction	RR=0.458 (95% CI: 0.319-0.660)	**0.001**
		All-cause mortality	RR=1.277 (95% CI: 0.835-1.952)	0.259

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Application of Several Special Staining Methods for Paraffin Sections on Epon-Embedded Semithin Sections

Yarı-İnce Epon Kesitlere Parafin Kesitler İçin Önerilen Çeşitli Özel Boyama Yöntemlerinin Uygulanması

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ABSTRACT

Aim: This study aimed to compare several specific staining protocols recommended for paraffin sections and toluidine blue and light green double staining combination to be tried for the first time with routine toluidine blue staining on semithin epon sections.
Material and Methods: Samples of 1x1x1 mm were taken from the liver, skin, and aorta tissues of Wistar albino adult rats. Tissue samples were fixed with 5% glutaraldehyde at +4º C overnight, postfixed with 1% osmium tetroxide for one hour, and then, blocked with Epon 812 after processing. Semithin sections of 1 µm thickness were obtained from the epon blocks. Sections were stained with Altmann's method (for mitochondria), Verhoeff's method (for elastic fibers), Gordon&Sweets' silver impregnation method (for type III collagen), toluidine blue and light green double staining combination (for type I collagen) and routine toluidine blue method.
Results: In liver sections, mitochondria in hepatocytes were differentiated by the Altmann method, and stromal type III collagen fibers were distinguished with Gordon&Sweets' method. Elastic lamellar structures were easily observed in black in the aortic sections stained with the Verhoeff method. Successful results were obtained in the staining of dermal type I collagen with toluidine blue and light green double staining in skin sections.
Conclusion: Since the specific staining tried for the first time gave positive results in epon sections, it was concluded that these methods can be used to determine the localization of cellular and intercellular components that are aimed to be examined at the ultrastructural level.
Keywords: Epon section; special stainings; light green.

ÖZ

Amaç: Bu çalışmada, parafin kesitler için önerilen çeşitli spesifik boyama protokollerinin ve ilk kez denenecek toluidin mavisi ve açık yeşil ikili boyama kombinasyonunun yarı-ince epon kesitlerde rutin toluidin mavi boyamasıyla karşılaştırılması amaçlandı.
Gereç ve Yöntemler: Wistar albino türü erişkin sıçanlara ait karaciğer, deri ve aort dokularından 1x1x1 mm boyutlarında örnekler alındı. Alınan doku örnekleri %5 gluteraldehit ile bir gece süreyle +4º C'de fikse edildi, ardından bir saat %1 osmiyum tetroksit ile postfiksasyon uygulandı ve takip işlemi sonrası Epon 812 ile bloklandı. Epon bloklardan 1 µm kalınlıkta yarı-ince kesitler elde edildi. Kesitler Altmann yöntemi (mitokondri için), Verhoeff yöntemi (elastik lifler için), Gordon&Sweet gümüşleme yöntemi (tip III kollajen için), toluidin mavisi ve açık yeşil ikili boyama kombinasyonu (tip I kollajen için) ve rutin toluidin mavisi yöntemi ile boyandı.
Bulgular: Karaciğer kesitlerinde, Altmann yöntemi ile hepatositlerdeki mitokondriler, Gordon&Sweets yöntemi ile stromal tip III kollajen lifler belirgin bir şekilde ayırt edildi. Verhoeff yöntemi ile boyanan aort kesitlerinde elastik lamellar yapılar siyah renkte kolaylıkla izlendi. Deri kesitlerinde toluidin mavisi ve açık yeşil ikili boyaması ile dermal tip I kollajenin boyanmasında başarılı sonuçlar elde edildi.
Sonuç: İlk kez denenen spesifik boyanmalar epon kesitlerde olumlu sonuçlar verdiği için, bu metodlardan ultrastrüktürel düzeyde incelenmesi hedeflenen hücresel ve hücrelerarası bileşenlerin lokalizasyonunun belirlenmesinde yararlanılabileceği sonucuna varılmıştır.
Anahtar kelimeler: Epon kesit; özel boyamalar; light green.

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INTRODUCTION

Semithin sections occupy an important place in histological and clinical diagnosis studies. They act as links in between light microscopic images and transmission electron microscopy (TEM) images. Protecting the cellular compounds of tissues better than those embedded in paraffin and enabling further identification of them on the light microscopic level are the biggest advantages of semithin sections. TEM studies employ a double fixation procedure that applies a glutaraldehyde fixation followed by a postfixation, with which osmium tetroxide is generally used. Obtaining staining in different colors is very difficult in semithin sections of tissues fixed with osmium tetroxide. The presence of reducted osmium complicates the penetration of the dyes into the sections or blocks certain reactive groups in the tissues. For these reasons, glutaraldehyde fixation alone is often preferred when targeting lipids is not an objective (1-3).

The advantages of epoxy resins make them the most preferred embedding medium in TEM studies. Resin sections have plastic and hydrophobic structures, and therefore, do not show affinity for or are stained by the dyes used in many histological methods. Epon also limits the penetration of the staining agents used in light microscopic examination into tissues. In routine electron microscopy techniques, semithin sections are usually subjected to monochromatic staining methods. Basic dyes such as toluidine blue, methylene blue, basic fuchsin, azure II, crystal violet, safranin O, and thionin are used for this purpose. Increased alkalinity enhances the penetration of dyes into the tissues in plastic sections (2-6). Toluidine blue, which is the most widely used stain in this context, is a metachromatic, cationic thiazine dye (7,8). Alkaline solutions of toluidine blue are used to stain semithin epon sections. These solutions yield elaborate structural details, are easily prepared, and do not deteriorate for a long time. Polychromatic staining methods cannot be routinely used on epon sections because of disadvantages such as the need for numerous complicated reactive agents, long incubation period, difficulty in stabilizing the color tone, short duration of use of the stain solutions, dominance of the last stain, shedding of the sections due to multistep processes, and wrinkles or precipitates forming in the sections. This leads to the inability to identify specific tissue components in semithin sections. Fixative solutions, buffer solutions, pH, temperature, concentrations of the stain solutions, staining duration, section thickness, and properties of the embedding medium are the factors that affect differential staining in polychromatic staining methods (2-6).

This study aimed to i) demonstrate the general tissue morphology of the control sections of various tissues by routine monochromatic toluidine blue staining, ii) determine at the cellular/intercellular level the localization, quantification, and density of specific tissue components that cannot be completely identified with toluidine blue by utilizing the polychromatic staining methods proposed for paraffin sections, iii) identify type I collagen fibers with the combination of toluidine blue and light green, which this information was not found in the literature, and, iv) localize cellular/intercellular specific tissue components in semithin epon sections, thus allowing ultrastructural correlation.

MATERIAL AND METHODS

In this study, archived epon blocks of liver, aorta, and skin tissues of Wistar albino male and female adult rats that were prepared in the Bursa Uludağ University, Faculty of Medicine, Department of Histology and Embryology, Transmission Electron Microscopy Unit were used.

Tissue samples were fixed overnight in 5% glutaraldehyde buffered with 0.13 M Sörensen's phosphate buffer at 4° C and postfixed with 1% osmium tetroxide in the same buffer at 4° C for one hour. After postfixation, the tissues were washed with buffer solution and dehydrated in an alcohol series of increasing concentrations. The clearing was performed in a propylene oxide solution. Afterwards, they were kept in a mixture of propylene oxide:epon at a ratio of 1:1. After overnight impregnation in pure epon, tissues were embedded in Epon 812. The epon blocks were trimmed using an ultra-trim device (Reichert). Afterward, semithin sections of 1 µm thickness were obtained using glass knives and an ultramicrotome (Reichert Supernova). All sections were mounted on gelatin-coated slides to minimize shedding risks and applied etching by acetone before staining (9).

Staining Methods

Classical 1% toluidine blue with borax staining (3) was applied to all the tissue samples of the control sections. Liver sections were stained with Altmann's method (10) and Gordon&Sweets' silver impregnation method, aorta sections were stained with Verhoeff's method, and skin sections were stained with toluidine blue (3) and light green (10,11) double staining combination. The optimum durations and temperatures of the dyes were determined after preliminary studies. Photographs were taken using an Olympus BX50 photomicroscope.

RESULTS

Identification of the Mitochondria with Altmann's Method

The mitochondria could not be specifically observed in the semithin epon-embedded liver sections stained with the conventional toluidine blue stain (Figure 1A). After Altmann's protocol was applied to the sections, the mitochondrial profile could clearly be distinguished as granular structures in brilliant cyclamen color, contrasting on a pale pinkish-yellow cytoplasmic background (Figure 1B).

Identification of the Reticular (Type III Collagen) Fibers with Gordon&Sweets' Silver Impregnation Method

The reticular (type III collagen) fibers in the liver stroma could not be differentiated with toluidine blue (Figure 1A), while they could clearly be observed in black color with Gordon&Sweets' silver impregnation method (Figure 1C).

Identification of the Elastic Fibers with Verhoeff's Method

Elastic fibers were observed as bundles in their classic dark blue-violet in semithin epon-embedded aorta sections stained with the conventional toluidine blue (Figure 2A). However, they were differentiated as black bundles on a clear background in sections stained with Verhoeff's hematoxylin (Figure 2B).

Identification of the Type I Collagen Fibers with the Toluidine Blue and Light Green Combination

Type I collagen fibers present in the dense irregular connective tissue of the dermis (stratum reticulare) layer of skin were distinguished as light violet irregular bundles

in semithin epon-embedded sections stained with toluidine blue. Specific differentiation could not be achieved with this staining (Figure 3A). In the epon sections stained with the toluidine blue and light green combination, however, type I collagen fibers were observed to be stained a brilliant green color on a clear background and could be easily identified from other contrasting structures stained violet-dark blue with toluidine blue (Figure 3B).

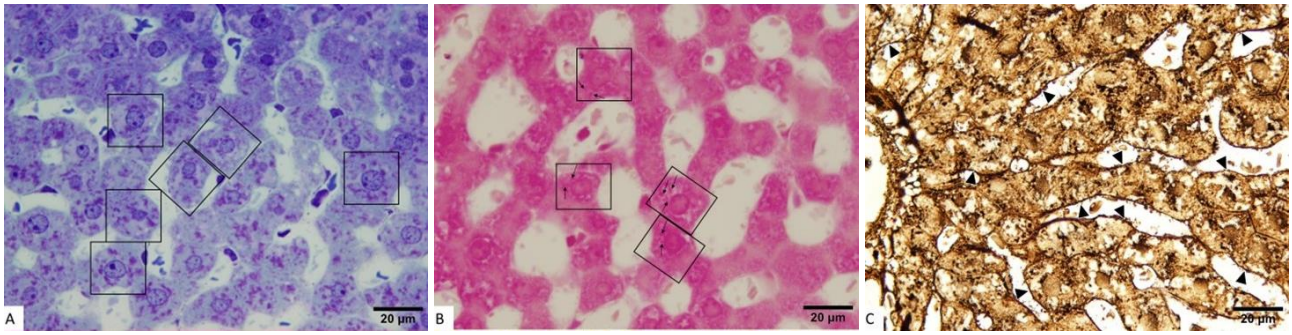

Figure 1. A) Toluidine blue staining, **B)** Altmann's method, and **C)** Gordon&Sweets' silver impregnation method in semithin epon sections of liver
black boxes: hepatocyte cells (A), arrows in black boxes: mitochondria in hepatocytes (B), black arrowheads: reticular fibers (C)

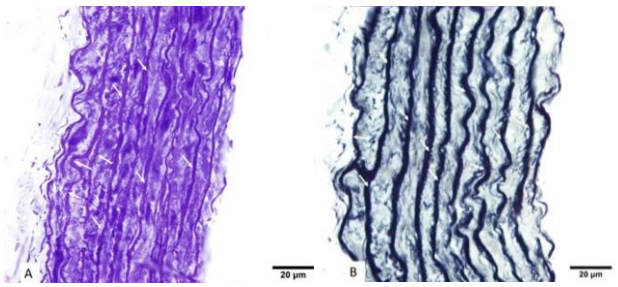

Figure 2. A) Toluidine blue staining and **B)** Verhoeff's hematoxylin staining in semithin epon sections of the aorta
white arrows: elastic bundles

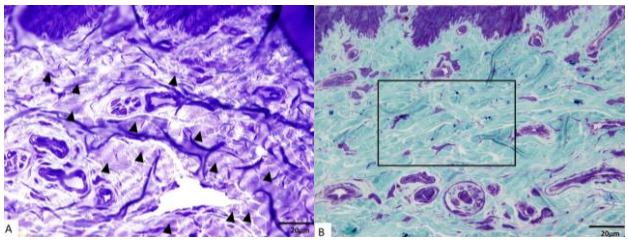

Figure 3. A) Toluidine blue staining and **B)** toluidine blue and light green combination in semithin epon sections of skin
black arrowheads: type I collagen bundles (A), black box: type I collagen bundles (B)

DISCUSSION

In the field of monochromatic stainings of plastic sections, toluidine blue was initially tested by a group of researchers for staining semithin epon-embedded sections obtained after osmium tetroxide postfixation (12). Their staining method utilizing toluidine blue's high alkaline solution and the positive results therefore initialized the current extensive usage of the protocol and stain in all the laboratories worldwide.

Toluidine blue is still used today for staining semithin sections. It is especially preferred in the morphological evaluation of the peripheral nerves (13-16). For this purpose, histopathological analysis of age-related changes, different damages (e.g., axonal degeneration, autolysis, swelling of myelinated fibers, and loss of the myelin sheath), and the effect of different treatment agents (e.g., axonal regeneration or nerve regeneration) in the sciatic nerve, sural nerve or phrenic nerve were investigated. In these studies, quantitative histomorphometric analyses were performed by evaluating the myelinated nerve fiber density (17-21). These sections are also used in the evaluation of demyelination and remyelination levels according to the results of G-ratio (the ratio of axon diameter and myelin sheath thickness) after damage, lesion, and treatments in the central nervous system (22).

Combined histological methods such as conventional light microscopy, non-conventional light microscopy (semithin sections stained with toluidine blue), TEM, and immunohistochemistry are very important for understanding and interpreting the examined tissue components. Correlations of these methods are used both in research and clinical practice (23). For example, the histological identification of M-cells in Peyer's plaques in the small intestine of albino rats (24), the identification of telocytes in the oviduct of mares (25), the correlative analysis of hard tissue and large pieces by light microscopy and electron microscopy (26), in improving visualization and interpretability of tooth cementum annulation (27), and the optimal diagnostic interpretation of epoxy-resin-embedded bone marrow biopsies (28) have been reported between routine light microscopy and TEM.

Many studies on factors like pH, temperature, and microwave radiation have been conducted in order to further increase the practical use of the classic basic dyes utilized in the field of staining plastic sections, which started in the 1960s. The studies in this field, which aim towards improving the current methods or the new

methods to be developed to be faster, easier, more practical, and more reliable and enabling the acquisition of the highest histological details, are still observed to be continuing. In one of the studies of semithin plastic sections, differential staining and distinction of spermium acrosome, mitochondrial sheaths in the midpiece, and tail were achieved with toluidine blue and basic fuchsin. Proceeding the protocol with phosphotungstic acid and light green further allowed the collagen to be stained in a separate color (29). In a dichromatic staining study, semithin sections were stained with borax methylene blue and basic fuchsin. On account of the observed staining of the nucleus, cytoplasm, collagen, elastin, myelin, and axoplasm, the method was especially recommended for peripheral nerve and blood vessel research (30). In another study, semithin epon-embedded sections of various tissues were stained with a one-step Mallory-Heidenhein stain. Intracytoplasmic components such as the nucleus, cytoplasm, collagen and elastic fibers, glycogen, and mucus were reported to be stained in different colors after nuclear staining with celestine blue (31). In a study that investigates a version of Mallory's phloxin B-methylene blue-azure II technique, the triple staining combination was carried out both unaltered and after phloxin B was removed from it, and the results of these two experiments were compared. Phloxin B was reported to have stained collagen and elastic fibers only (32). Fritsch (33), stained epon-embedded sections with a methylene blue-azure II solution and reported that following a counter-staining with basic fuchsin, cartilage, collagen fibers, elastic fibers, and muscle fibers could be stained in separate colors and easily distinguished. In another study, after a phosphotungstic acid-methyl green combination staining was applied to semithin epon sections, some mucin granules and the glycocalyx were observed to have stained in the same color, while other mucin granules, luminal mucin, and collagen fibers were stained in a different and contrasting color (34). Another study utilized microwave radiation on Bodian's silver staining, resorcin fuchsin, and later picrosirius red F3BA staining methods of nervous and connective tissue sections obtained after embedding into glycol methacrylate. The study reported that the staining durations were notably shortened and the nerve, elastic, and collagen fibers were stained in different colors, making their differentiation easier (35). In an experiment on semithin sections (36), Harris hematoxylin, silver methenamine, light green, eosin, and safranine stains were applied to the same section consecutively. The application was evaluated as a histological, histochemical, and immunocytochemical method that allows a thoroughly specific identification of the whole cellular tissue architecture. It was reported that following staining with carbol methylene blue-carbol gentian violet solution and a counter-staining with pararosaniline, vascular connective tissue, and elastic laminae were stained in separate colors and thus could easily be distinguished (37). In a dichromatic staining study aiming the demonstration of the collagen composition of peripheral nerves in semithin epon sections, the quantity and orientation of the collagenous connective tissue in horizontal and vertical nervous tissue sections could easily be distinguished with a toluidine blue staining followed by a basic fuchsin application (38). D'Amico (39) in his study, applied to semithin epon-embedded sections a methylene blue-azure B mixture and a succeeding basic fuchsin. He obtained separate colors differentiating cytoplasm, nucleus, collagen, elastin, mucus, and lipid components and evaluated the results positively. Another study reported that a mixture of azure B and basic fuchsin allowed the differentiation of many intra- and extracellular (collagen and elastic fibers) components in diverse colors and tones and that the method could also be useful for pathological tissues (40). Twort's staining method (neutral red and fast green FCF mixture) was adapted to epon-embedded sections in a study, which highlighted that in addition to many intracellular structures, extracellular collagen and elastic fibers could also be easily distinguished in many colors (41). A solution consisting of a toluidine blue and malachite green mixture and basic fuchsin for counter-staining was utilized in another study, in which discernible staining of nuclei, erythrocytes, mitochondria, collagen and elastic fibers, and cartilaginous structures was recorded (42). Light microscopic results were compared with electron microscopic results in a study that researches, following a methylene blue and sodium tetraborate application to semithin sections of heart muscle, and mitochondria morphometry in cardiomyocytes. The study was stated as quite successful, without further need for advancement towards the electron microscopic level (43).

In the literature, similar studies related to the specific staining of connective tissue fibers (elastic fibers, type I and type III collagen fibers) and mitochondria (Verhoeff hematoxylin, toluidine blue-light green combination, Gordon&Sweets' silver impregnation, Altmann's protocol; respectively) applied to epon sections were not found within the scope of the present study. However, within the scope of staining methods applied for general or different purposes, there are studies that mention the staining results of tissue components in the present study. Therefore, the results of the present study are in agreement with the studies on light green and fast green FCF dyes that only stain type I collagen, especially trichrome techniques, and the identification of collagen and elastic fibers (29,31,36,41).

We think that our study will be a guide in this field. However, as a limitation of our study, the stainings we applied were not studied on different tissues. We believe that applying the staining in our study on more tissue types would be more beneficial.

CONCLUSION

Some staining techniques recommended for paraffin sections (Altmann's method for mitochondria, Gordon&Sweets' silver impregnation method for type III collagen/reticular fibers, Verhoeff's method for elastic fibers) can also be applied to semithin epon sections. Toluidine blue-light green double staining, which we tried as a new combination, can be used to identify type I collagen fibers in semithin epon sections. Quantitative histomorphometric analyses of cell and tissue components can be easily performed at the light microscopic level in semithin epon sections. Epon-embedded tissues with osmium postfixation can be advanced to ultrastructural examination. Thus, light and electron microscopic correlation becomes possible.

Ethics Committee Approval: In this study, archived epon blocks of rats were used. Therefore, ethical approval was not required with the approval of the animal experiments local ethics committee of Uludağ University (01.08.2023).

Conflict of Interest: None declared by the authors.

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The Frequency of Fabry Disease in Acute Stroke Patients with Renal Insufficiency in Sakarya Province

Sakarya İli Akut İskemik İnme ve Kronik Renal Yetmezlikli Olgularda Fabry Hastalığı Sıklığı

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ABSTRACT

Aim: This study aimed to investigate the frequency, clinical and genetic characteristics, and therapeutic options associated with Fabry disease (FD) in individuals with acute stroke and concomitant renal insufficiency.
Material and Methods: An FD screening was performed on adult patients with renal dysfunction who were admitted to the neurology clinic due to acute stroke between 2015 and 2021. Screening was performed by a leukocyte α-galactosidase A (α-Gal A) enzyme activity assay using dried blood spot (DBS) samples from male patients. In cases where the enzyme activity was less than 2.5 nmol/ml/h, genetic analysis was performed. Female patients underwent direct genetic analysis.
Results: Renal dysfunction was detected in 39 ischemic stroke patients and 5 hemorrhagic stroke patients out of a total of 401 cases. The enzyme level was found low in only one of the male patients. The c.680G>A (p.R227Q) mutation was observed in this male patient and a female patient. In the later stages of the study, it was realized with the help of pedigree analysis that these two cases were first-degree relatives. The same mutation was also detected in 13 first-degree and 2 second-degree relatives. The frequency of FD in our study group, which included patients with cerebral and renal involvement regardless of consanguinity, was 4.54%.
Conclusion: Rapid detection of FD cases can be achieved by screening individuals presenting with multiple end-organ damages. To the best of our knowledge, this study highlights the underemphasized association between renal involvement and stroke in FD.
Keywords: Fabry disease; renal insufficiency; cerebrovascular disease.

ÖZ

Amaç: Bu çalışmada, böbrek yetmezliği olan akut inme hastalarında Fabry Hastalığı (FH) sıklığı ile klinik ve genetik özelliklerinin ve tedavi seçeneklerinin araştırılması amaçlandı.
Gereç ve Yöntemler: 2015 ve 2021 yılları arasında nöroloji kiniğine akut inme nedeniyle yatırılan ve böbrek fonksiyon bozukluğu tespit edilen erişkin hastalarda FH taraması yapıldı. Tarama, erkek hastalardan alınan bir kuru kan lekesi (dried blood spot, DBS) örnekleri ile lökosit α-galactosidase A (α-Gal A) enzimatik aktivite değerlendirmesine ile yapıldı. Enzim aktivitesi 2,5 nmol/ml/saat'in altında tespit edilen vakalarda genetik inceleme yapıldı. Kadın hastalarda ise doğrudan genetik analiz uygulandı.
Bulgular: Toplam 401 olgudan 39 iskemik inme olgusunda ve 5 hemorajik inme olgusunda renal disfonksiyon saptandı. Erkek hastalardan sadece birinde enzim düzeyi düşük bulundu. Bu erkek hastada ve bir de kadın hastada c.680G>A (p.R227Q) mutasyonu tespit edildi. Çalışmanın ilerleyen aşamalarında pedigri analizi ile bu iki olgunun birinci derece akraba olduğu fark edildi. Aynı mutasyon 13 birinci derece ve 2 ikinci derece akrabada da tespit edildi. Akraba evliliğinden bağımsız olarak hem beyin hem de böbrek tutulumu olan hastaları içeren çalışma grubumuzda FD sıklığı %4,54 idi.
Sonuç: Çoklu uç organ hasarı olan bireylerin taranması ile FH olgularının erken tespitinde başarı sağlanabilir. Bildiğimiz kadarıyla bu çalışma, FH'da böbrek tutulumu ve inme arasında kapsamlı bir şekilde vurgulanmamış olan bu ilişkiyi vurgulamaktadır.
Anahtar kelimeler: Fabry hastalığı; böbrek yetmezliği; serebrovasküler hastalık.

INTRODUCTION

Fabry disease (FD) is a rare X-linked progressive lysosomal storage disorder known for its multisystem manifestations, including neuronal and vascular complications. Its prevalence ranges from 1/3100 to 1/117,000 in European adults and has been reported to be as high as 1/1250 in newborns (1,2). If left untreated, the life expectancy for FD patients is approximately 60 years for males and 75 years for females (3).

FD is the result of mutations in the GLA gene located on the X chromosome (4). In patients with FD, dysfunction of the α-galactosidase A (α-Gal A) enzyme leads to the gradual accumulation of globotriaosylceramide (Gb3) and its by-products in various cellular compartments, particularly lysosomes (5).

Typical clinical manifestations of FD include early symptoms such as angiokeratomas, acroparesthesias, sweating irregularities, and gastrointestinal problems that typically begin in childhood or adolescence. Late-onset symptoms, which appear between the third and fifth decades of life, include vasculopathy, cerebrovascular disease, cardiomyopathy, and renal failure (6,7). In males, the diagnosis of FD is based primarily on the identification of reduced leukocyte α-Gal A enzyme activity, usually by dried blood spot (DBS) testing, followed by GLA gene analysis to identify the specific mutation. However, genetic analysis is recommended as the initial diagnostic approach for females (8).

Management of FD includes enzyme replacement therapy (ERT) and/or chaperone therapy (9). A multidisciplinary approach is required for the management of adult FD. In addition, individual disease phenotypes and organ involvement should be taken into account before starting ERT. ERT should not be delayed after diagnosis, as even asymptomatic cases require comprehensive follow-up and the administration of supportive treatments for FD-related symptoms (10). Nevertheless, renal failure, stroke, and sudden cardiac death are the most common causes of mortality in FD (11). Among adults with FD, stroke and renal dysfunction are the most common major organ impairments. To the best of our knowledge, no previous studies have investigated the prevalence of FD in patients with acute cerebrovascular stroke and concurrent renal dysfunction. Therefore, this study aimed to investigate the prevalence and clinical and genetic characteristics of FD patients with acute stroke and concomitant renal dysfunction.

MATERIAL AND METHODS

In this retrospective study, we enrolled patients who were admitted to the Neurology Department of Sakarya University Training and Research Hospital and diagnosed with acute ischemic or hemorrhagic stroke with concomitant renal dysfunction between January 2015 and November 2021.

Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of Sakarya University (January 03, 2022, and 558).

Inclusion criteria included individuals aged 18 to 80 years with acute stroke and renal dysfunction. Renal dysfunction was defined as the presence of microalbuminuria, previously diagnosed chronic renal failure, or participation in a regular dialysis program. We carefully collected demographic information and clinical characteristics, performed physical examinations, obtained laboratory test results, and documented radiologic and echocardiographic findings. Of particular importance during the physical examination were assessments for angiokeratoma and concentric ventricular hypertrophy, as these are commonly associated with FD.

In male patients, FD screening included the use of a DBS assay to measure leukocyte α-Gal-A enzymatic activity. Genetic analysis targeting the GLA gene was performed in males whose enzyme activity was <2.5 nmol/ml/hour. In contrast, female patients underwent direct genetic analysis. Screening for GLA gene mutations was performed by Sanger sequence analysis using polymerase chain reaction amplification followed by Sanger DNA sequencing (ARCHIMED Laboratory, Vienna, Austria). The GLA gene is located on Xq22 and spans 13 kb of genomic DNA (7 exons and a cDNA sequence of 1290 bases). Exons 1-7 of the GLA gene coding sequences, together with adjacent intronic sequences (minimum 20 base pairs), were amplified from pure genomic DNA and sequenced in both forward and reverse directions. Targeted mutation analysis of a single exon was possible. Patient sequences were compared with reference DNA sequences for comprehensive analysis.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS version 23.0. The descriptive statistics were calculated. The continuous variables were presented as mean±standard deviation, the categorical variables were as numbers or percentages. Student t-test was used to compare numerical data between groups, and the chi-square test was used to compare categorical data. All statistical tests reported were two-tailed, with the significance level at <0.05.

RESULTS

A total of 401 patients with acute stroke and renal dysfunction, who were hospitalized between January 2015 and November 2021, were initially considered for inclusion in this study. Forty-four patients who met the inclusion criteria were included in this study of this cohort of patients with acute stroke and concurrent renal dysfunction, 39 (88.6%) had ischemic stroke and the remaining five (11.4%) had hemorrhagic stroke. Both groups had a similar gender distribution and age profile (p=0.411, and p=0.361, respectively). Within this cohort, 42 (95.5%) patients had chronic renal insufficiency, eight of whom were on regular dialysis. The remaining two (4.5%) patients had proteinuria during their hospitalization.

The stroke type, demographic characteristics, and chronic diseases of the patients in the study were presented in Table 1. Two patients included in the study were diagnosed with FD. Notably, they were first-degree relatives. Their clinical, laboratory, radiologic, and genetic characteristics were presented in Table 2.

The enzymatic activity of α-Gal A enzyme was found to be <2.5 nmol/mL/hr (0.1 nmol/h/mL; normal >1.2) in the male patient with ischemic stroke, subsequent genetic analysis revealed the presence of the c.680G>A (p.R227Q) missense mutation. With these results, the patient was

Table 1. The characteristics of the patients with ischemic stroke and hemorrhagic stroke

	Ischemic Stroke (n=39)		Hemorrhagic Stroke (n=5)	
	Male (n=22)	**Female (n=17)**	**Male (n=2)**	**Female (n=3)**
Age (years)	60.5±14.6	62.3±16.5	59.0±2.8	75.3±23.5
Chronic Disease, n (%)				
Hypertension	14 (63.6)	11 (64.7)	2 (100)	2 (66.7)
Diabetes mellitus	9 (40.9)	7 (41.2)	0 (0.0)	2 (66.7)
Coroner artery disease	4 (18.2)	5 (29.4)	0 (0.0)	1 (33.3)
Cerebrovascular disease	2 (9.1)	6 (35.3)	0 (0.0)	1 (33.3)
Congestive heart failure	1 (4.5)	1 (5.9)	0 (0.0)	0 (0.0)
Cirrhosis	0 (0.0)	1 (5.9)	0 (0.0)	0 (0.0)
Atrial fibrillation	1 (4.5)	2 (11.8)	0 (0.0)	0 (0.0)

Table 2. The clinical, laboratory, radiologic, and genetic features of patients with Fabry disease

	Index Case	**Other Case**
Age	43 years	45 years
Symptom	Left 4+/5 hemiparesia, left hemihypoestesia	Hypoestesia on the right arm, right homonymous hemianopsia
ECG	Normal sinus rhythm	Normal sinus rhythm
Radiological Findings		
Brain CT	Isodense	Isodense
Brain MRI	a left side pontine ischemic lesion	left occipital lobe ischemic lesion
CDUS	bilateral non-stenotic atherosclerotic plaque	right vertebral artery flow 60 ml/min, left vertebral artery flow 70 ml/min, means vertebro-basillar insufficiency
Echo	60% ejection fraction, mild mitral regurgitation, atrial insufficiency, and left ventricular hypertrophic concentric cardiomyopathy (HCM)	60% ejection fraction and normally left systolic function
Laboratory Findings (nv)*		
Urea (17-43)	115 mg/dL	14 mg/dL
Cr (0.67-1.17)	10.24 mg/dL	0.58 mg/dL
UA (3.5-7.2)	5.6 mg/dL	3.9 mg/dL
LDL (<130)	123 mg/dL	143 mg/dL
HDL (>40)	31 mg/dL	51 mg/dL
VLDL	69.2 mg/dL	23.2 mg/dL
TG (0-200)	346 mg/dL	116 mg/dL
TC (0-200)	174 mg/dL	205 mg/dL
Proteinuria	1+	2+
Physical examination		
Angiokeratoma	none	none
Corneal symptoms	none	none
α-Gal A activity	0.1 nmols/h/mL	-
GLA gene analysis	c.680G>A (p.R227Q) missense mutation	c.680G>A (p.R227Q) heterozygote missense mutation

ECG: electrocardiography, CT: computed tomography, MRI: magnetic resonance imaging, CDUS: carotid vertebral Doppler ultrasonography, Echo: echocardiography, (nv)*: normal reference values of laboratory parameters were shown in brackets, Cr: creatinine, UA: uric acid, LDL: low-density lipoprotein, HDL: high-density lipoprotein, VLDL: very-low-density lipoprotein, TG: triglyceride, TC: total cholesterol, α-Gal A: α-galactosidase A

diagnosed with FD and defined as an index case (ZS). This patient had a history of recurrent fevers, neuropathic symptoms in the distal extremities, acroparesthesias, intolerance to temperature extremes, hypohidrosis, and gastrointestinal disturbances since childhood. There were no angiokeratomas or corneal symptoms. The patient received peritoneal dialysis. He was subsequently referred to our nephrology clinic for further evaluation. ERT was initiated with intravenous α-galactosidase beta (1.0 mg/kg) administered biweekly. However, his renal insufficiency progressed to end-stage renal failure, necessitating a switch from peritoneal dialysis to hemodialysis after one year. He then underwent hemodialysis three times a week for two years, after which he sought a kidney transplant from his spouse. Currently, he is 49 years old, has been on ERT for six years, and no longer requires dialysis. Importantly, he has had no recurrence of stroke.

The second patient, the sister of the index case (FT), was hospitalized with an ischemic stroke. She was found to have the same mutation as her sibling. At the time of diagnosis, she had only microproteinuria as a renal sign. ERT was initiated for her with intravenous α-galactosidase beta (1.0 mg/kg) administered biweekly. She was subsequently referred to our nephrology clinic for comprehensive follow-up.

FD is a genetically inherited disease so family screening was performed on the index case to detect asymptomatic cases. With the scope of the family screening, genetic analysis was performed on the siblings and offspring of our index case (ZS). But, we could not perform genetic analysis on the mother and father. The same genetic mutation was identified in a total of 9 females who shared first-degree consanguinity with the index case (including 6 sisters and 3 daughters), 4 males (his brothers), and 2 males who were his nephews, representing second-degree consanguinity (Figure 1). The whole family member's preference for cousin marriage caught our attention. We think that the reason why the X-linked recessive disease shows an autosomal dominant transmission pattern is due to consanguineous marriage preferences within the family. Notably, the sisters of the index case remained asymptomatic at the time of their FD diagnosis. However, one of them (FT) experienced an ischemic stroke in the second year after confirmation of FD and initiation of prophylactic ERT. The brothers of the index case were all asymptomatic at the time of diagnosis. Unfortunately, one of them succumbed to intraparenchymal hemorrhage during the second year of ERT at the age of 35. Among the 20 relatives, one nephew with a history of cryptogenic

stroke at a young age and proteinuria was identified as having FD. In addition, three children of the index case were diagnosed with FD during the asymptomatic phase and subsequently started prophylactic ERT.

In this study, two cases from the same family were included. We performed frequency analysis only on the male FD patients within the study group due to the consanguinity between the cases. The prevalence of FD in Sakarya province was calculated to be 4.54% within the cohort of patients characterized by dual end-organ damage involving both the kidneys and the brain. However, when individuals from the same lineage were included, the observed frequency increased to 8.8% (including patients with stroke and kidney injury as independent criteria). As a result, this approach allowed us to identify and evaluate a more concentrated group of patients.

DISCUSSION

Fabry disease is a rare metabolic disorder characterized by extensive organ involvement resulting from vascular damage. This involvement can lead to severe dysfunction of the nervous, renal, and cardiac systems, with potentially fatal consequences. Given its X-linked inheritance pattern, recent research has revealed differences in gene expression on the X chromosome between male and female ischemic stroke patients (12). The clinical manifestations of FD can vary significantly in heterozygous female patients due to X chromosome inactivation (13). The pedigree of our cases shows an autosomal dominant transmission pattern. We think that the preference of all family members for cousin marriage is effective in the OD transmission pattern in the x-linked recessive disease. Within the spectrum of FD, those with α-Gal A enzyme activity below 1% are classified as having the classical form, whereas those with enzyme activity between 1% and 30% are classified as having the atypical form (14). Classical FD typically presents with multiorgan involvement, while atypical FD often presents with single-organ involvement (7).

The hallmark symptoms of FD, such as angiokeratomas, corneal ectropion, and heat- and exercise-induced neuropathic pain (acroparesthesia), may become apparent in childhood (7). In contrast, adult FD complications, including proteinuria, chronic kidney disease, cryptogenic concentric left ventricular hypertrophy (LVH), young cryptogenic stroke, cerebral white matter lesions, and occlusive cerebrovascular events, tend to develop progressively over the following years (6).

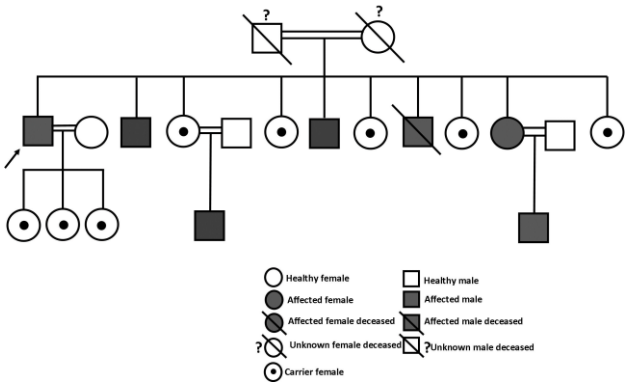

Figure 1. The pedigree of index case

Ischemic stroke is the most common type of cerebrovascular event in individuals with FD. The reported incidence of FD in the context of stroke varies in the literature. The estimates suggest that it accounts for approximately 1% of young stroke cases, 0.35% in ischemic stroke or transient ischemic attack (TIA), and 0.2% in unselected stroke populations. In our study, two patients were diagnosed with FD after ischemic stroke (15-17). No FD was found in patients with hemorrhagic stroke.

Notably, cerebrovascular events in FD patients are particularly common in individuals aged 25 to 44 years, in contrast to the general population (10,13). Our study is consistent with this pattern, as the patients diagnosed with FD after stroke were aged 43 and 45 years. Furthermore, our analysis showed that the incidence of stroke was significantly higher in individuals with FD than in the general population across all age groups (18).

Renal dysfunction is a significant complication of FD following cerebrovascular events. One of the most common symptoms is proteinuria, which typically manifests in the second or third decade of life. As individuals with FD age, the pathological accumulation of glycosphingolipid deposits progressively intensifies, leading to a gradual decline in renal function, a reduction in glomerular filtration rate (GFR), and eventually the need for dialysis or kidney transplantation (6). The prevalence of FD in hemodialysis patients is estimated to be 0.15% to 1.2%, and it is approximately 2% in individuals with chronic kidney disease who do not require dialysis (19,20). In our study, the male patient was undergoing peritoneal dialysis at the time of his FD diagnosis, whereas the female patient had only microproteinuria at the time of her FD diagnosis. Both patients were started on ERT. The American Heart Association/American Stroke Association guidelines for 2021 recommend α-galactosidase ERT for patients with ischemic stroke in FD, assigning it a Class 2 recommendation with a Level B-NR rating (21). ERT has demonstrated efficacy in alleviating neuropathic pain, improving GFR, and reducing QRS complex duration (22). In addition, ERT is recognized for its ability to attenuate vasculopathic lesions in the brain (23). However, the extent to which ERT can reduce the incidence of stroke remains controversial (22,24).

Nervous system disorders and renal dysfunction are life-threatening complications of FD. When both systems are affected simultaneously, they contribute to a more severe clinical phenotype. The pathophysiological mechanisms underlying renal and nervous system dysfunction in FD show similarities. Emerging evidence suggests that inadequate renal function may be associated with the presence of chronic white matter hyperintensities, highlighting a potential link between the two (23).

The coexistence of cerebrovascular and renal disease is an emerging area of investigation in the context of FD. There are studies in the literature showing the long-term effects of renal dysfunction in young stroke patients (25). Existing literature provides limited insight into the prevalence of FD associated with chronic renal failure and stroke in general. In a study by Rolfs et al. (26), FD was identified in 13 of 39 patients with cryptogenic stroke and concomitant proteinuria, none of whom were part of a hemodialysis program, and, these individuals represented 13 of 28 patients diagnosed with FD. In the present study,

the prevalence of FD was estimated to be 4.54% in patients with dual end-organ damage involving both the kidney and the brain. However, when individuals from the same lineage were included, this prevalence increased to 8.6% (only when individuals with both stroke and renal injury were considered). This highlights the practicality of screening for FD in the chronic renal failure cohort, regardless of whether the stroke is classified as cryptogenic or not.

Of the two patients diagnosed with FD in our study, one had early-stage renal disease, while the other had late-stage renal disease. This patient ultimately underwent kidney transplantation. We believe that diagnosing FD and initiating ERT in these individuals has potential benefits, including protecting the transplanted kidney and reducing the risk of further complications.

CONCLUSION

Studies examining renal dysfunction in adult stroke patients exist in the literature. However, the body of research investigating FD within this patient cohort is limited. Despite the study's limited sample size, it represents, to the best of our knowledge, the initial investigation of this issue and may serve as a foundational step toward future multicenter studies with larger and more diverse populations. Screening individuals presenting with multiple instances of end-organ damage has proven to be an effective means of promptly identifying FD cases. This approach holds particular significance for genetic counseling within affected families and for ensuring the timely provision of ERT to individuals at risk of organ failure.

Ethics Committee Approval: The study was approved by the Non-invasive Research Ethics Committee of Sakarya University (03.01.2022, 558).

Conflict of Interest: None declared by the authors.

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Investigating the Difference in Exercise Self-Efficacy According to the Grade of Muscle Injury

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ABSTRACT

Aim: Muscle injuries are common sports-related injuries that cause the most training loss. Self-efficacy refers to a person's personal belief that he/she can do the behavior necessary to reach his goal. This study aimed to examine the difference in exercise self-efficacy according to the grade of muscle injury in recreational and professional athletes.
Material and Methods: Ninety-three patients who applied to the sports medicine outpatient clinic and were diagnosed with muscle injury and doing sports were included in the study. Gender, age, weight, height, sports branch and exercise duration per week, injury grade, and Tegner activity level of the patients diagnosed with muscle injury were recorded. The patients were asked to fill in the sports fitness index and athlete self-efficacy scale.
Results: A significant difference was found in the sports fitness index score according to the grade of muscle injury (p=0.002), while there was no significant difference in the Tegner activity level (p=0.228) and athlete self-efficacy scale (p=0.791). A negative correlation was found between age and duration of exercise (r=-0.234, p=0.024), and age and Tegner activity level (r=-0.425, p<0.001). A positive correlation was found between exercise duration and Tegner activity level (r=0.308, p=0.003), and exercise duration and professional thought efficacy (r=0.251, p=0.015). Again, a positive correlation was found between the Tegner activity level and sports discipline efficacy (r=0.225, p=0.030) and professional thought efficacy (r=0.226, p=0.029) dimensions of the athlete self-efficacy scale.
Conclusion: No significant difference in exercise self-efficacy depending on the grade of muscle injury in recreational and professional athletes.
Keywords: Muscle injury; Tegner activity level; sports fitness index; exercise self-efficacy.

ÖZ

Amaç: Kas yaralanmaları en fazla antrenman kaybına yol açan ve sporla ilişkili olarak sık görülen yaralanmalardır. Öz yeterlilik, kişinin hedefine ulaşması için gerekli olan davranışı yapabileceğine olan kişisel inancını ifade eder. Bu çalışmanın amacı, rekreasyonel ve profesyonel sporcularda kas yaralanmasının evresine göre egzersiz öz yeterliliğindeki farkı araştırmaktır.
Gereç ve Yöntemler: Bu çalışmaya spor hekimliği polikliniğine başvurup kas yaralanması tanısı almış olan ve spor yapan 96 hasta dahil edilmiştir. Kas yaralanması tanısı almış olan hastaların cinsiyet, yaş, kilo, boy, spor branşı ve haftalık egzersiz süresi, yaralanma evresi ve Tegner aktivite düzeyi kaydedilmiştir. Hastalardan spora uygunluk göstergesi ve sporcu öz yeterlilik ölçeğini doldurmaları istenmiştir.
Bulgular: Kas yaralanmasının derecesine göre spora uygunluk göstergesi skorunda anlamlı bir farklılık bulunurken (p=0,002), Tegner aktivite düzeyi (p=0,228) ve sporcu öz yeterlilik ölçeğinde (p=0,791) anlamlı farklılık saptanmadı. Yaş ile egzersiz süresi (r=-0,234; p=0,024) ve yaş ile Tegner aktivite düzeyi (r=-0,425; p<0,001) arasında negatif korelasyon bulundu. Egzersiz süresi ile Tegner aktivite düzeyi (r=0,308; p=0,003) ve egzersiz süresi ile mesleki düşünce yeterliliği (r=0,251; p=0,015) arasında pozitif korelasyon bulundu. Tegner aktivite düzeyi ile sporcu öz yeterlik ölçeğinin spor disiplini yeterliliği (r=0,225; p=0,030) ve mesleki düşünce yeterliliği (r=0,226; p=0,029) boyutları arasında da pozitif korelasyon bulunmuştur.
Sonuç: Rekreasyonel ve profesyonel sporcularda kas yaralanmasının derecesine bağlı olarak egzersiz öz-yeterliliğinde anlamlı bir fark yoktur.
Anahtar kelimeler: Kas yaralanması; Tegner aktivite düzeyi; spora uygunluk göstergesi; egzersiz öz yeterliliği.

Preliminary results were presented orally at the 1st International & 4th National Health Services Online Congress (June 10-12, 2022).

INTRODUCTION

Muscle strain is the most common injury pattern in sports (1). Muscle injuries are more common in sports involving explosive movements and direction changes, such as football, basketball, and athletics (2). Muscle injuries account for approximately one-third of all injuries in professional-level football. The football player undergoes 0.6 per season muscle injuries. Therefore, a football team of 25 players can expect around 15 muscle injuries per season (3). Most muscle injuries in football are non-contact and occur at the muscle-tendon junctions (4,5). Due to the increased incidence of hamstring and calf muscle injuries in recent years (5,6), studies are still being conducted on the management of muscle injuries (5,7-9). The high injury load creates economic costs and affects player availability and team performance (5,10,11).

Self-efficacy refers to the individual's personal belief that he/she can perform the behavior required to achieve the desired result (12-15). Individuals with low self-efficacy report more pain during exercise even if they do not have a psychological problem. This affects exercise-based rehabilitation after injury. High self-efficacy increases the sustainability of physical activity and compliance with treatment in case of a sports injury. The self-efficacy level is a predictor of injury-induced disability and is prognostic for the success of exercise-based rehabilitation (16).

This study hypothesized that exercise self-efficacy and grades of previous muscle injuries would bidirectionally affect each other. There are few publications in the literature on exercise self-efficacy in muscle injuries. In light of all this information, the main purpose of this study was to examine the difference in exercise self-efficacy according to the grade of muscle injury in recreational and professional athletes.

MATERIAL AND METHODS

Ninety-three male and female volunteers aged between 18 to 50 years who applied to Health Sciences University Gülhane Training and Research Hospital and were diagnosed with muscle injuries were included in the study. Volunteers who did not have chronic or systemic problems were included in the study. Volunteers who had an acute illness affecting body balance and movement independent from the muscle injury and had current pregnancies were excluded from the study.

Health Sciences University Gülhane Training and Research Hospital Clinical Research Ethics Committee approved the study on 09.02.2022 (Approval number: 2022/4). Informed consent was given to all patients and signed.

Age, gender, height, weight, sports branch, exercise duration per week, grade of the muscle injury, and Tegner activity level were assessed at the first admission. The patients were asked to fill in the sports fitness index and the athlete self-efficacy scale. O'Donoghue classification was used for evaluating muscle injuries (17).

For an effect size of 0.80, a power of 95%, and a Type-I error rate of 5%, the required number of participants was calculated as a minimum of 70.

Tegner Activity Level

It is used to evaluate the sports activity level of the patients. This scoring system varies between 0 and 10 according to activities in daily life and sports. Activity level is graded from 0 points for those who quit the activity due to injury or dysfunction, to 10 points for those who play professional sports at a national team level (18).

Sports Fitness Index

Turkish version of the sports fitness index developed by Wilkerson in 2016 was used (19,20). In the Turkish version of the scale, the best cut-off point was 70 points, with a sensitivity of 96.6% and a specificity of 75%. Each item has a 6-level response option (from 0 to 5) with descriptors for problem frequency or problem severity. The total score is calculated by multiplying the sum of values for 8 items by 2. The higher the score obtained from the scale, the higher the fitness level for sports (20).

Athlete Self-Efficacy Scale

It is a 5-point Likert-type scale consisting of 16 questions in total, which includes the sub-dimensions of sports discipline efficacy, psychological efficacy, professional thought efficacy, and personality efficacy. Each of the dimensions is evaluated with 4 questions. The lowest score that can be obtained from the scale is 16, and the highest score is 80 (21).

Statistical Analysis

Analyses were made in the IBM SPSS v.22 package program (IBM Corp., Armonk, NY, USA). Mean and standard deviation, minimum, maximum, frequency (n), and percentage (%) were used for descriptive values in statistical analysis. Kolmogorov-Smirnov test was used to evaluate conformity to normal distribution. Independent samples t-test and chi-square test were used for group comparison and the Pearson test was used for correlations. The p-value was accepted as significant at the 0.05 level.

RESULTS

A total of 93 patients (23 female, and 70 male) were included in the study. Demographic data of the patients were shown in Table 1. Muscle injuries were divided into three grades. While 33 (35.5%) of the patients were classified as grade 1, 57 (61.3%) of them were grade 2. Only 3 (3.2%) patients with grade 3 muscle injury were present in the study. Since the small number of patients was not statistically significant, grade 3 patients were excluded when analyzing according to the injury grade. In addition, the results did not change when grade 2 and 3 muscle injuries were combined. The grade 3 muscle injury group was excluded from the statistical analysis due to the small number of participants. A significant difference was found in the sports fitness index score between grade 1 and grade 2 muscle injuries ($p=0.002$). No significant difference was found in the Tegner activity level and athlete self-efficacy scale according to the grade of muscle injury ($p=0.228$, and $p=0.791$, respectively).

There was a negative correlation between age and weekly exercise duration ($r=-0.234$, $p=0.024$), and between age and Tegner activity level ($r=-0.425$, $p<0.001$). There was a positive correlation between exercise duration and Tegner activity level ($r=0.308$, $p=0.003$), and exercise duration and professional thought efficacy dimension of the athlete self-efficacy scale ($r=0.251$, $p=0.015$). There was a positive correlation between the Tegner activity level and sports discipline efficacy ($r=0.225$, $p=0.030$) and professional thought efficacy ($r=0.226$, $p=0.029$) dimensions of the athlete self-efficacy scale.

There was no statistically significant difference between the male and female gender in terms of muscle injury grade, Tegner activity level, sports fitness index, and athlete self-efficacy scale (Table 2).

Patients were also divided into two groups according to Tegner activity level. The levels of 9 and 10 were accepted as high-intensity sports (n=44, 9.2±0.4) and 5 to 8 as moderate-intensity (n=46, 7.3±0.4). In terms of muscle injury, while 13 (29.5%) patients were classified as grade 1 and 31 (70.5%) were grade 2 in the high-intensity group, 20 (43.5%) patients were grade 1 and 26 (56.5%) were grade 2 in the moderate-intensity group. No significant difference was found between these two groups in terms of muscle injury grade (p=0.195), sports fitness index score (44.9±14.9 vs 42.2±13.1, p=0.546), and athlete self-efficacy scale score (67.7±9.9 vs 65.4±15.8, p=0.408).

DISCUSSION

In this study, there was no significant difference in exercise self-efficacy in recreational and professional athletes according to the grade of muscle injury. Similarly, there was no difference in the Tegner activity level and athlete self-efficacy scale according to the grade of muscle injury. There was a difference in the sports fitness index score according to the grade of muscle injury. There was no difference in the sports fitness index score, Tegner activity level, and athlete self-efficacy scale according to gender. Similarly, a negative correlation was found between age and weekly exercise duration, and age and Tegner activity level. However, no relationship was found between the grade of muscle injury and the athlete self-efficacy scale score.

Participation in high-intensity sports decreases with increasing age. This may be due to many different reasons; decreased muscle strength, joint stiffness, reduced flexibility, a decline in overall physical fitness, previous injuries, prolonged recovery period, additional health concerns, changing lifestyle/social factors, and fear of injury. As a result, instead of participating in intense sports, individuals modify their participation by reducing their exercise duration or intensity (22,23). Similar to the literature, this study revealed that with increased age, participation in high-intensity sports and duration of exercise decreases.

This study did not find a difference between male and female gender for muscle injury grade, Tegner activity

Table 1. Demographics of the patients and differences between the groups

	Grade 1 (n=33)	Grade 2 (n=57)	p	All patients
Age (years), mean±SD	23.3±6.8	24.6±7.8	0.430	24.3±7.5 (18-50)
Weight (kg), mean±SD	72.1±13.4	73.4±13.4	0.663	72.8±13.1 (44-103)
Height (cm), mean±SD	176.3±7.9	177.1±7.6	0.664	176.7±7.6 (159-195)
Gender, n (%)				
Female	11 (33.3)	12 (21.1)	0.198	23 (24.7)
Male	22 (66.7)	45 (78.9)		70 (75.3)
Exercise duration/week (min), mean±SD	442.7±241.7	495.2±343.0	0.441	473.8±306.4 (90-1800)
Sports Fitness Index, mean±SD	48.9±13.4	39.7±13.1	**0.002**	43.0±14.0 (5-37)
Athlete Self-Efficacy Scale	65.3±14.4	66.0±12.9	0.791	65.9±13.2 (16-80)
Sports Discipline Efficacy	16.1±4.3	16.2±4.0	0.929	16.3±4.0 (4-20)
Psychological Efficacy	15.6±3.8	16.4±3.6	0.326	16.2±3.7 (4-20)
Professional Thought Efficacy	16.3±4.1	16.2±3.4	0.890	16.2±3.7 (4-20)
Personality Efficacy	17.0±3.8	17.0±3.3	0.990	17.1±3.5 (4-20)
Tegner Activity Level, mean±SD	8.0±1.4	8.3±1.0	0.228	8.1±1.1 (5-10)

SD: standard deviation

Table 2. The differences between genders

	Female (n=23)	Male (n=67)	p
Grade, n (%)			
1	11 (47.8)	22 (32.8)	0.198
2	12 (52.2)	45 (67.2)	
Exercise duration/week (min), mean±SD	384.7±247.6	503.1±319.5	0.108
Sports Fitness Index, mean±SD	40.6±14.6	43.8±13.8	0.347
Athlete Self-Efficacy Scale, mean±SD	66.0±12.1	65.9±13.7	0.957
Sports Discipline Efficacy	16.4±3.6	16.2±4.2	0.812
Psychological Efficacy	15.8±3.5	16.4±3.7	0.546
Professional Thought Efficacy	16.5±3.6	16.1±3.8	0.700
Personality Efficacy	17.2±3.1	17.0±3.6	0.777
Tegner Activity Level, mean±SD	8.3±1.1	8.1±1.2	0.446

SD: standard deviation

level, sports fitness index, and athlete self-efficacy scale score. However, according to the literature on muscle injuries, the prevalence and severity change between males and females. Generally, males tend to have a higher incidence of muscle injuries, particularly in certain sports that require explosive power and strength. This can be attributed to differences in hormonal profiles, muscle mass, and biomechanics between the genders (24). Having only 23 females out of the 93 participants may be the reason for this result. However, in terms of the incidence of muscle injury, males constituted the majority in our study, similar to the literature.

The Tegner activity level is a scale used to assess an individual's activity level and participation in sports and recreational activities (18). While there might be variations within genders, studies have indicated that males generally tend to have higher Tegner activity level scores compared to females. This difference could be influenced by factors such as cultural expectations, societal norms, and variations in sports participation rates between males and females (25).

The sports fitness index is a measure of an individual's overall fitness level concerning their sport or athletic activities. It takes various fitness components into account such as strength, endurance, speed, and agility (19). Differences between males and females can exist due to factors like hormonal profiles, body composition, and physiological characteristics (26). Generally, males tend to exhibit higher levels of absolute strength and power, while females may excel in areas such as flexibility and endurance (27).

Self-efficacy refers to an individual's belief in their ability to successfully perform a specific task or activity (21). Studies have shown that males and females may differ in their self-efficacy levels, with males often exhibiting higher levels of self-efficacy in certain domains. These differences may be influenced by factors such as societal expectations, stereotypes, and past experiences. It's important to note that while there are general trends and differences between genders in these factors, individual variations exist within each gender. Moreover, these differences are not absolute and may be influenced by various factors such as training, experience, genetics, and personal motivation (28,29). Our study found similar results for Tegner activity level, sports fitness index, and athlete self-efficacy scale according to gender. This might be due to the male-to-female ratio of the study participants or the patient population presenting to the outpatient clinic of the study.

A higher sports fitness index score indicates better fitness and performance capabilities (20). On the other hand, the grade of muscle injury refers to the severity or extent of the injury, with higher grades indicating more severe injuries. In general, it is expected that individuals with more severe muscle injuries would have a lower Sports Fitness Index. This is because muscle injuries can significantly impact an individual's ability to engage in physical activity, reduce their overall fitness level, and limit their performance in sports. In other words, as the grade of muscle injury increases, the Sports Fitness Index tends to decrease. However, there is no study in the literature that supports this expectation. In the Turkish validity and reliability study of the sports fitness index, 70 points and above were found to be suitable for sports. In this study, the mean of the sports fitness index was found to be 43.0±14.0. Also, the sports fitness index of athletes with grade 1 injury was 48.9±13.4, and grade 2 was 39.7±13.1. In the study, the sports fitness index score decreased with the increase in the muscle injury grade. Despite being below the cut-off values of the sports fitness index score average, even those with lower scores can still be considered to have lower physical fitness compared to those with higher scores.

The cut-off value of the athlete self-efficacy scale is reported as 40 (21). This study found the mean of the participants' score as 65.9±13.2. Engaging in regular sports or exercise can already be a situation that individuals with exercise self-efficacy can achieve. Therefore it might not be possible to find a relationship with the grade of injury. In addition, the psychological rehabilitation of the injured athlete is of great importance in sports medicine. The most basic part of athlete rehabilitation is to explain each step and to determine the goals together with the athlete so that the psychology of the athlete is not affected (30). The result that there is no difference in exercise self-efficacy according to the stage of muscle injury showed that the grade of the muscle injury does not affect the exercise self-efficacy and might not so the sports rehabilitation's psychological phase or the performance.

Generally, individuals who engage in longer durations of exercise are more likely to have higher Tegner activity level scores. This is because longer exercise durations often indicate a greater commitment to physical activity and participation in sports, which can contribute to the higher activity levels and a wider range of sports involvement (31). Similarly, this study revealed that higher exercise duration is related to a higher Tegner activity level. Professional thought efficacy is a subgroup referring to self-efficacy related to professional thoughts or activities (21). Confidence in one's professional skills or decision-making abilities, and higher exercise durations can positively impact self-efficacy. Regular exercise has been associated with improved cognitive function, increased self-confidence, and enhanced overall well-being, which can translate into greater professional thought efficacy (32). In this study, it was revealed that the duration of exercise affects professional thought efficacy positively.

Sports discipline efficacy refers to an individual's belief in their ability to adhere to the rules, regulations, and behavioral expectations of a specific sport or athletic activity (21). In general, individuals with higher Tegner activity level, indicating a greater engagement in sports and physical activities, are more likely to have higher levels of sports discipline efficacy. This is because increased involvement in sports often requires a higher level of discipline, commitment, and adherence to the rules and standards of the sport (33). Professional thought efficacy refers to an individual's belief in their ability to successfully perform professional tasks or activities. While the Tegner activity level is primarily focused on sports and recreational activities, it can indirectly influence an individual's professional thought efficacy. Engaging in regular physical activity and sports participation can positively impact cognitive function, self-confidence, and overall well-being (32). These factors can contribute to

enhanced professional thought processes, decision-making abilities, and self-efficacy in professional domains. This study showed that if the Tegner activity level is high, the sports discipline efficacy and professional thought efficacy dimensions of the athlete self-efficacy scale will also be high.

Factors such as the intensity and type of exercise, personal motivation, and specific professional domains can influence the relationship. This can be named as a limitation of our study since these parameters were not evaluated.

CONCLUSION

Sports and exercise are factors that improve an individual's exercise self-efficacy. Increasing exercise duration increases Tegner activity level and professional thought efficacy. Increasing Tegner activity level, like dominos, increases the sports discipline efficacy and professional thought efficacy dimensions of the athlete self-efficacy scale. So that even when the athlete experiences muscle injury, exercise self-efficacy may not be affected.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Gülhane Training and Research Hospital (09.02.2022, 2022/4).

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Anatomical Investigation of the Flexor Retinaculum Covering the Tarsal Tunnel in Formaldehyde-Fixed Cadavers

Formaldehit Fikse Kadavralarda Tarsal Tüneli Kaplayan Retinaculum Flexorum'un Anatomik Olarak İncelenmesi

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ABSTRACT

Aim: The flexor retinaculum lies between the medial malleolus of the tibia and the medial process of the calcaneus. It converts grooves on the tibia and calcaneus into the tarsal tunnel. The aim of this study was to analyze the morphometry, the course, and the shape of the flexor retinaculum covering the tarsal tunnel in formaldehyde-fixed cadavers.
Material and Methods: Six lower extremities of four formaldehyde-fixed cadavers (four right sides, and 2 left sides) were examined to evaluate the morphometry of the flexor retinaculum. The cadavers were two females and two males with an age range of 60-89 years. A digital caliper (150 mm) and a light microscope were used for measuring parameters. The central length, proximal, midpoint, and distal width, thickness, course, and shape of the flexor retinaculum were examined in this study.
Results: The mean central length of the flexor retinaculum was found 42.26±5.18 mm. The mean proximal, midpoint, and distal width of the flexor retinaculum were 29.29±7.29 mm, 29.92±3.66 mm, and 29.76±8.13 mm, respectively. The mean of the thickness was measured at 234.94 µm. The flexor retinaculum coursed vertically in four extremities and coursed obliquely in two extremities. The shape of the retinaculum was triangular in five extremities and quadrangular in only one extremity.
Conclusion: The morphometric data obtained from this study can help surgeons during the operations of the tarsal tunnel syndrome. To our knowledge, the width and course of the flexor retinaculum were examined for the first time in this study.
Keywords: Flexor retinaculum; ankle joint; tarsal tunnel syndrome; morphometry.

ÖZ

Amaç: Retinaculum flexorum, tibia'nın malleolus medialis'i ile calcaneus'un processus medialis'i arasında uzanır. Retinaculum flexorum, tibia ve calcaneus üzerindeki olukları tarsal tünele dönüştürür. Bu çalışmanın amacı, formaldehit fikse kadavralarda tarsal tüneli kaplayan retinaculum flexorum'un morfometrisini, seyrini ve şeklini incelemektir.
Gereç ve Yöntemler: Dört formaldehit fikse kadavraya ait olan altı adet alt ekstremite (dört sağ taraf ve iki sol taraf), retinaculum flexorum morfometrisini değerlendirmek için incelendi. Yaş aralığı 60-89 yıl arasında olan kadavraların ikisi kadın ve ikisi ise erkek idi. Parametrelerin ölçümü için bir dijital kumpas (150 mm) ve bir ışık mikroskobu kullanıldı. Bu çalışmada retinaculum flexorum'un santral uzunluğu, proksimal, orta nokta ve distal genişliği ile kalınlığı, seyri ve şekli incelendi.
Bulgular: Retinaculum flexorum'un ortalama santral uzunluğu 42,26±5,18 mm olarak bulundu. Retinaculum flexorum'un proksimal, orta nokta ve distal ortalama genişliği ise sırasıyla 29,29±7,29 mm, 29,92±3,66 mm ve 29,76±8,13 mm olarak ölçüldü. Retinaculum flexorum'un kalınlığı ortalama olarak 234,94 µm olarak bulundu. Retinaculum flexorum'un seyri, dört ekstremitede dikey ve iki ekstremitede ise oblik olarak bulundu. Retinaculum'un şekli beş ekstremitede üçgen ve sadece bir ekstremitede ise dörtgen idi.
Sonuç: Bu çalışmadan elde edilen morfometrik veriler tarsal tünel sendromu ameliyatları sırasında cerrahlara yardımcı olacaktır. Bildiğimiz kadarıyla retinaculum flexorum'un genişliği ve seyri ilk kez bu çalışmada incelenmiştir.
Anahtar kelimeler: Retinaculum flexorum; articulatio talocruralis; tarsal tünel sendromu; morfometri.

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INTRODUCTION

The flexor retinaculum lies between the medial malleolus of the tibia and the medial process of the calcaneus bone. Proximally, there is no obvious boundary between its border and the deep fascia of the leg (1-4). Distally, its border is continuous with the plantar aponeurosis. Fibres of the abductor hallucis muscle are attached to the distal border of the flexor retinaculum. The flexor retinaculum converts grooves on the tibia and calcaneus into the tarsal tunnel (2). The structures pass through the tarsal tunnel from medial to lateral are the tibialis posterior muscle, flexor digitorum longus muscle, posterior tibial artery/vein, tibial nerve, and flexor hallucis longus muscle (2,5). The main function of the flexor retinaculum is to prevent dislocation of the flexor digitorum longus and the tibialis posterior tendons over the medial malleolus edge during ankle joint movements (6).

The tibial nerve may be compressed at the level of the tarsal tunnel as the nerve passes under the flexor retinaculum (2,7,8). This pathologic nerve entrapment is called "tarsal tunnel syndrome" (4,9). This syndrome was initially described by Kopell and Thompson in 1960 (10). The tibial nerve compression can occur by trauma, repetitive stress, external compression by osseous prominences, edema, perineural fibrosis, pseudoaneurysms, accessory muscles, ganglion cysts, and tumors (5,7,8,11-16). The most common symptoms of tarsal tunnel syndrome are burning, tingling, and shooting pain along the heel and medial aspect of the ankle joint (5,11,17).

The flexor retinaculum is a thin structure composed of three layers: the inner one is a smooth layer to enable gliding; a thick middle layer that contains collagen bundles, fibroblasts, and elastin fibers supports; and the outer layer that contains vascular channels covers the other two layers (1,18). This retinaculum is a broad, narrow collagen structure that reinforces the crural fascia inferiorly and posteriorly (18).

The aim of this study was to analyze the morphometry, the course, and the shape of the flexor retinaculum covering the tarsal tunnel in formaldehyde-fixed cadavers.

MATERIAL AND METHODS

Seven lower extremities of four formaldehyde-fixed cadavers were dissected carefully to evaluate the morphometry of the flexor retinaculum. In one extremity, the borders of the flexor retinaculum could not be distinguished from the crural fascia, and this extremity was excluded from the study. The cadavers included in this study were two females and two males with an age range of 60-89 years. Six lower extremities (four right, and two left sides) were examined for this study. All lower extremities were free from pathology, trauma, surgical incision, or deformity. Dissections were made in the gross anatomy dissection laboratory of Hacettepe University. Ethical approval for this study was obtained from the ethics committee of our institution (June 20, 2023; 11-36). The study was conducted following the Declaration of Helsinki.

A digital caliper (150 mm) was used for measuring the length and width of the flexor retinaculum. Before each measurement, correction of the caliper was done. The light microscope was used for measuring the thickness. The parameters evaluated in this study were as follows: i) the central length (Figure 1), ii) the proximal, iii) the midpoint, iv) the distal width (Figure 2), v) the thickness (Figure 3), vi) the course, and vii) the shape of the flexor retinaculum.

Statistical Analysis

Descriptive statistics analyses were performed using the IBM SPSS Statistics v.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) program. The descriptive statistics of parameters were summarized as the mean, standard deviation, minimum, and maximum values.

RESULTS

Six formaldehyde-fixed lower extremities (four right sides, and two left sides) were examined to evaluate the morphometry of the flexor retinaculum. It was difficult to identify the proximal and distal borders of the flexor retinaculum since the flexor retinaculum was a contiguity with the deep crural fascia. However, fine and careful dissection was performed starting from the middle part of the leg. Skin and crural fascia were dissected slowly. During fascia dissection, it was observed that the flexor retinaculum was thicker than the fascia on the medial side of the ankle joint. After the borders of the flexor retinaculum were determined by the decision of three anatomists, measurements were done. In one extremity, the borders of the flexor retinaculum could not be distinguished from the crural fascia, so this extremity was excluded from the study.

The mean central length of the flexor retinaculum was found 42.26±5.18 mm in general. The mean proximal, midpoint, and distal width of the flexor retinaculum were measured at 29.29±7.29 mm, 29.92±3.66 mm, and 29.76±8.13 mm, respectively. The thickness of the flexor retinaculum was measured from different regions using with light microscope and its mean value was found to be 234.94 (min-max: 225.60-241.95) μm. Measurements of the flexor retinaculum were summarized in Table 1.

After measuring the length, width, and thickness of the flexor retinaculum, we examined the course and shape of the retinaculum. The flexor retinaculum coursed vertically in four extremities and coursed obliquely in two extremities. The shape of the retinaculum was found to be triangular in five extremities and quadrangular in only one extremity.

Table 1. Morphometric measurements of the flexor retinaculum

Flexor Retinaculum Measurements	Right Side (mm)	Left Side (mm)	General (mm)
Central Length (AB)	43.34±2.94	40.09±9.69	42.26±5.18
Proximal Width (CD)	30.18±9.19	27.52±1.63	29.29±7.29
Midpoint Width (EF)	31.07±1.42	27.61±6.70	29.92±3.66
Distal Width (GH)	29.40±7.08	30.49±13.35	29.76±8.13

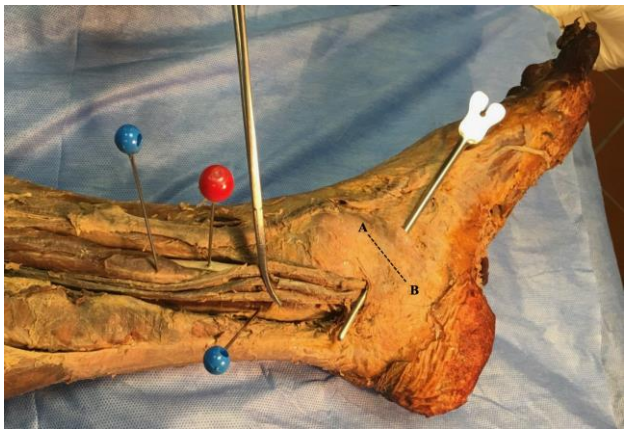

Figure 1. The central length of the flexor retinaculum (AB)

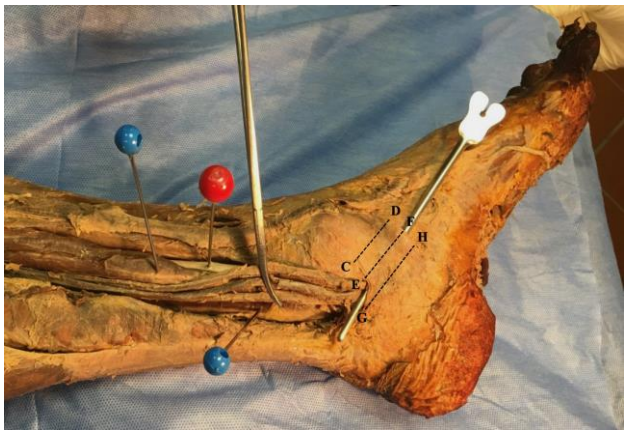

Figure 2. The proximal (CD), midpoint (EF), and distal width (GH) of the flexor retinaculum

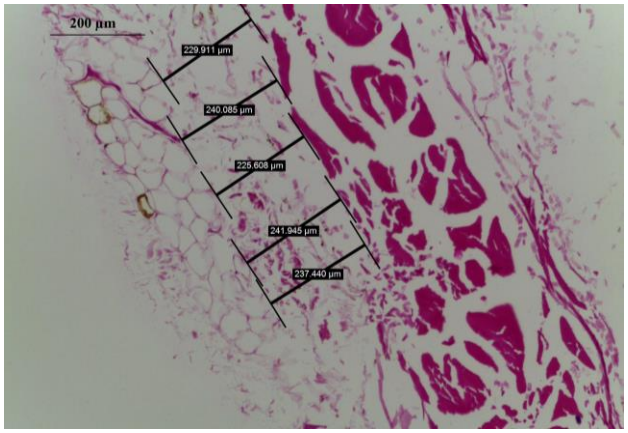

Figure 3. The thickness of the flexor retinaculum

DISCUSSION

The flexor retinaculum is placed on the medial side of the ankle joint and encloses the tarsal tunnel. It has a roughly triangular shape and extends from the medial malleolus of the tibial bone to the posterosuperior aspect of the calcaneus (1).

Mattos et al. (17) dissected 40 lower extremities, but only 9 specimens showed a denser consistency of flexor retinaculum. They measured the mean length of the flexor retinaculum 51.9 (min-max: 43-62) mm in 9 specimens. They found that the flexor retinaculum was an undistinguished extension of the crural fascia in 31 specimens. El Shazly et al. (7) dissected 12 intact adult lower limb specimens (seven left feet specimens, five right feet specimens) and they measured the length of the flexor retinaculum 29.58±1.88 (min-max: 26-32) mm. In our study, we found the central length of the flexor retinaculum 42.26±5.18 mm, higher than El Shazly et al.'s (7) study, but lower than Mattos et al.'s (17) study. The difference between our result and the literature was thought to be due to ethnic differences. These ethnic differences should be taken into consideration in surgical interventions to the medial side of the ankle joint.

Numkarunarunrote et al. (1) evaluated the flexor retinaculum on 10 fresh foot and ankle specimens using 1.5-T with magnetic resonance imaging (MRI). While they measured the thickness of the flexor retinaculum as 0.9 (min-max: 0.7-1.0) mm, they could not evaluate the shape of the flexor retinaculum in their study because of its very thin structure. Stecco et al. (6) analyzed MRI images of 7 voluntary subjects, 17 subjects with ankle sprain and 3 amputated legs. They measured the thickness of the flexor retinaculum as 1.15±0.16 mm around the Achilles tendon and as 1.4±0.22 mm on the tarsal tunnel. El Shazly et al. (7) performed an ultrasonographic study on 10 patients (11 feet) and 14 normal volunteers (28 feet) to examine the flexor retinaculum. They measured the thickness of the flexor retinaculum at 0.81±0.09 mm in the patients group and at 0.64±0.04 mm in the healthy subjects group. They found that there was a statistically significant difference in the thickness of the retinaculum between these two groups. In the present study, we measured the thickness of the flexor retinaculum as 234.94 µm (0.23 mm), lower than the other studies in the literature. This difference may be because of the use of different examination techniques. The other studies in the literature (1,6,7) used MRI and ultrasonographic methods to measure the thickness. However, we performed cadaveric dissections and measured the thickness using a light microscope.

The shape of the flexor retinaculum was described as roughly triangular in most studies in the literature (1,18). In our study, we found the shape of the flexor retinaculum triangular in most cadavers, which was consistent with the literature.

The structure of the lower extremity is generally affected by the body's characteristics such as height/weight and body type, but in our laboratory these properties of cadavers are unknown. Because of this, we couldn't evaluate these parameters. This is the limitation of our study. Maybe, further studies will be planned to evaluate these properties.

CONCLUSION

The flexor retinaculum is an important structure for the stable ankle joint. To our knowledge, the width and course of the flexor retinaculum were examined for the first time in our study. The morphometric data obtained from this study can help surgeons during the operations of the tarsal tunnel syndrome. The results of our study should serve as a foundation for future investigations dealing with pathologic conditions of the flexor retinaculum.

Ethics Committee Approval: The study was approved by the Non-invasive Clinical Research Ethics Committee of Hacettepe University (20.06.2023, 11-36).

Conflict of Interest: None declared by the authors.

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Classical Blind Percutaneous Dilatational Tracheostomy vs Fiberoptic Bronchoscopy Guided Percutaneous Dilatational Tracheostomy in the Intensive Care Unit: Complications, Mortality, and Outcomes

Yoğun Bakım Ünitesinde Fiberoptik Bronkoskopi Kılavuzluğunda Perkütan Dilatasyonel Trakeostomiye Karşı Klasik Kör Perkütan Dilatasyonel Trakeostomi: Komplikasyonlar, Mortalite ve Sonuçlar

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ABSTRACT

Aim: This study aimed to compare percutaneous dilatational tracheostomy (PDT) procedures performed with fiberoptic bronchoscopy (FOB) guidance and classical blind technique regarding complications, mortality, and patient outcomes.

Material and Methods: This study included 62 patients receiving mechanical ventilator support in the intensive care unit (ICU) between October 2022 and June 2023. Patients were randomized into two groups: those who underwent FOB-guided PDT (group FOB, n=31) and those who underwent PDT with the classical blind technique (group C, n=31). Demographic data, clinical characteristics, PDT procedure times, complications, and mortalities were analyzed.

Results: The median age was 64 (range, 19-94) years, and 67.7% (n=42) of the patients were male. Demographic data were found similar between groups. The most common primary diagnosis in patients who underwent PDT was intracranial hemorrhages (32.3%, n=20). While the median tracheostomy opening time in the entire study group was 13 (range, 3-31) days, there was no significant difference between the groups (p=0.637). The mean PDT procedure time (9.6±3.8 vs 12.6±5.4 min, p=0.015), median ICU stay (26 vs 37 days, p=0.004), and complication rate (6.4% vs 25.8%, p=0.038) were found to be significantly lower in group FOB. While the 28-day mortality in the entire study group was 17.7% (n=11), there was no significant difference between the groups (p=0.740).

Conclusion: In PDT procedures performed under FOB guidance, procedure time, length of stay in the ICU, and procedure-related complication rates were significantly lower, while no significant difference was observed in terms of mortality.

Keywords: Tracheostomy; bronchoscopy; intensive care unit; mechanical ventilation; ventilator weaning.

ÖZ

Amaç: Bu çalışmanın amacı, fiberoptik bronkoskopi (FOB) kılavuzluğu ile klasik kör teknikle gerçekleştirilen perkütan dilatasyonel trakeostomi (PDT) işlemlerinin komplikasyonlar, mortalite ve hasta sonuçları açısından karşılaştırılmasıdır.

Gereç ve Yöntemler: Bu çalışmaya Ekim 2022 ile Haziran 2023 tarihleri arasında yoğun bakım ünitesinde (YBÜ) mekanik ventilatör desteği alan 62 hasta dahil edildi. Hastalar FOB kılavuzluğunda PDT gerçekleştirilenler (grup FOB, n=31) ve klasik kör teknikle PDT gerçekleştirilenler (grup C, n=31) olarak iki gruba randomize edildi. Demografik veriler, klinik özellikler, PDT işlem süreleri, komplikasyonlar ve mortaliteler analiz edildi.

Bulgular: Ortanca yaş 64 (aralık, 19-94) yıl ve hastaların %67,7'si (n=42) erkek idi. Gruplar arasında demografik verilerin benzer olduğu saptandı. PDT işlemi gerçekleştirilen hastalarda en sık primer tanı intrakranyal hemorajiler (%32,3; n=20) idi. Tüm çalışma grubunda medyan trakeostomi açılma zamanı 13 (aralık, 3-31) gün iken gruplar arasında anlamlı bir farklılık yoktu (p=0,637). Ortalama PDT işlem süresi (9,6±3,8'e karşı 12,6±5,4 dakika, p=0,015), ortanca YBÜ kalış süresi (26'ya karşı 37 gün, p=0,004) ve komplikasyon oranı (%6,4'e karşı %25,8; p=0,038) grup FOB'da anlamlı olarak daha düşük saptandı. Tüm çalışma grubunda 28 günlük mortalite %17,7 (n=11) iken gruplar arasında anlamlı bir farklılık yoktu (p=0,740).

Sonuç: FOB kılavuzluğunda gerçekleştirilen PDT işlemlerinde, işlem süresi, YBÜ kalış süresi ve işleme bağlı görülen komplikasyon oranları anlamlı olarak düşük saptanırken, mortalite açısından anlamlı bir farklılık görülmedi.

Anahtar kelimeler: Trakeostomi; bronkoskopi; yoğun bakım ünitesi; mekanik ventilasyon; ventilatörden ayırma.

INTRODUCTION

Percutaneous dilatational tracheostomy (PDT) is frequently performed in patients who need prolonged mechanical ventilator follow-up in the intensive care unit (ICU). PDT has become a standard method, preferred over surgical tracheotomy due to its advantages, such as being able to be performed at the bedside in patients followed in ICU, avoiding complications during transport to the operating room, and limited tissue incision and damage (1). It has been reported that PDT is beneficial in avoiding complications related to prolonged intubation, ensuring airway safety, reducing work of breathing, clearing secretions in the airway more efficiently, reducing the need for sedation, increasing the comfort of the patient by enabling speech, and shortening the length of stay in ICU (2,3).

The development of PDT techniques has facilitated the spread and implementation of the procedure in ICUs. Thus, the PDT procedure has become one of the most frequently performed surgical procedures in patients receiving mechanical ventilator support (4). Various PDT methods are available. Although there are methods such as multiple dilatation (Ciaglia technique) and one-step dilatation (Ciaglia Blue Rhino), the Griggs method using forceps dilatation is one of the most frequently used methods (5). In the Griggs technique, tracheal dilatation is performed with specially designed forceps, and the cannula is placed in the trachea. Recently, fiberoptic bronchoscopy (FOB) in PDT procedures has become common. Thus, the trachea can be visualized, and the airway and the posterior tracheal wall can be seen during the placement of the tracheal cannula, thus ensuring the procedure's safety. Although it is reported in the literature that FOB reduces early complications, there needs to be more studies on its effect on late complications and mortality.

This study aimed to compare FOB-guided PDT and classical blinded PDT procedures regarding early and late complications, mortality, and patient outcomes in patients followed up in the ICU.

MATERIAL AND METHODS

This prospective randomized study was conducted following the principles of the Declaration of Helsinki after the approval of the local Clinical Research Ethics Committee (date: 12.10.2022, no: 208). Sixty-two patients who underwent elective PDT procedures between October 2022 and June 2023 at the University of Health Sciences Türkiye, İstanbul Kanuni Sultan Süleyman Training and Research Hospital ICU, were included in the study. Informed consent was obtained from the relatives of all patients included in the study. The patients were randomized into two groups of 31 using the sequentially closed envelope method. Randomization was performed by a healthcare professional other than those performing the tracheostomy (Figure 1). In the ICU of our hospital, PDT is performed both with the classical blind technique and under FOB guidance. The patients who underwent PDT with the blind technique were classified as group C (n=31), and those who underwent PDT with FOB accompaniment were classified as group FOB (n=31).

Inclusion criteria: Patients who were endobronchial intubated and received mechanical ventilator support, were 18 years or older, were not expected to be extubated soon, did not have complex neck anatomy, and had normal coagulation parameters regarding the procedure.

Exclusion criteria: They were patients under 18 years of age who needed urgent tracheostomy, had complicated neck anatomy (past neck surgery, abnormally large thyroid tissue, mass in the trachea or neck, or suspected infection), and abnormal coagulation parameters (INR>1.5 and platelet count <50,000 /µL). All PDT procedures were performed by two experienced Anesthesiology and Reanimation and Intensive care specialists (who had at least 5 PDT experience and performed tracheostomy with both methods) accompanied by two experienced (>5 years of ICU and tracheostomy experience).

Before the PDT procedure, the patients in both groups were placed on volume-controlled ventilation and ventilated with 100% oxygen for 5 minutes. Considering the vital signs of all patients, before the procedure, 1 µg/kg fentanyl (Talinat, Vem Pharma, Türkiye), 1-2 mg/kg propofol (Propofol-PF 1%, Polifarma, Türkiye), and 0.3 mg/kg rocuronium (Muscuron, Kocak Pharma, Türkiye) was given intravenously and 0.9% isotonic fluid resuscitation was performed. All patients were followed up with electrocardiogram, pulse oximetry, oxygen saturation, non-invasive blood pressure, or invasive arterial pressure monitoring during the PDT procedure. The patients were placed in the ideal position with the head slightly extended by placing support under their shoulders.

Classical Blind Percutaneous Dilatational Tracheostomy

The tracheostomy application site was sterilized with 10% povidone-iodine and covered with a perforated drape. To reduce bleeding with its vasoconstrictor effect and to facilitate the procedure, 2-3 mL of 2% lidocaine (Aritmal, Osel Pharma, Türkiye) with epinephrine diluted 1:100,000 was applied subcutaneously at the application site. All tracheostomy procedures were performed with the Grigss technique. The endotracheal tube cuff was deflated and retracted to remain between the vocal cords and allow ventilation. The cricoid process was palpated and advanced

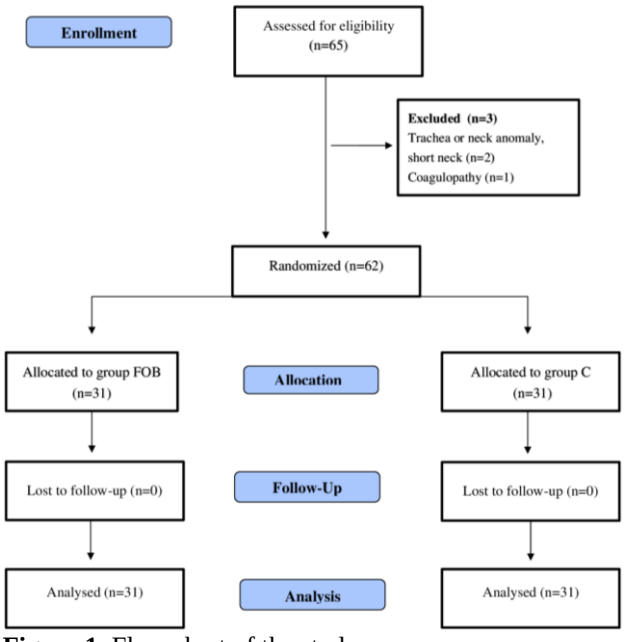

Figure 1. Flow chart of the study

approximately 1.5-2 cm below the second and third tracheal cartilages with a 14G cannula until air was aspirated and the tracheal lumen was entered. A horizontal incision of approximately 1-2 cm in diameter was created in the skin of the puncture site. After the guide wire was placed, the cannula was withdrawn, the 8F dilator was inserted over the guide wire, and the skin and tracheal rings were widened with forceps. Tracheostomy cannula 7F or 8F was inserted according to the patient's height and weight. After confirming the location of the cannula with chest movements and auscultation, the endotracheal tube was removed. Ventilation was started at volume-controlled Mv settings before the procedure. A bedside chest X-ray was taken 2-4 hours after the procedure, and possible complications were checked.

Fiberoptic Bronchoscopy Guided Percutaneous Dilatational Tracheostomy

As in the classical blind technique, the application area was sterilized with povidone-iodine, and a perforated cover was placed. Due to its vasoconstrictor and local anesthetic effect, 2-3 mL of 2% lidocaine with epinephrine was applied to the area where the tracheostomy was performed. A fiberoptic bronchoscope was inserted through a small hole by making a cross-shaped incision on the lid of the oral endotracheal tube. Thus, it was ensured that both followed the tracheostomy procedure, continued the procedure safely, and ventilated the patient mechanically. With FOB, the endotracheal tube was pulled up to the level of the vocal cords. The tip of the bronchoscope was left approximately 1 cm away from the end of the endotracheal tube to provide optimum view. As in the blind technique, the second and third tracheal cartilages were palpated 1.5-2 cm below the cricoid process. Simultaneously, the entry site was confirmed by transillumination of the FOB light by applying gentle pressure on the skin. As in the blind technique, air puncture and guide wire were placed under FOB guidance. A horizontal incision of approximately 1-2 cm diameter was created at the puncture site. After the guide wire was placed, the cannula was withdrawn, the 8F dilator was inserted over the guide wire, and the skin and tracheal rings were widened with forceps. Considering the patient's height, weight, and neck anatomy, a tracheostomy cannula 7F or 8F was inserted. After confirming the location of the cannula with chest movements and auscultation, the endotracheal tube was removed. Ventilation was started at volume-controlled Mv settings before the procedure. A bedside chest X-ray was taken 2-4 hours after the procedure, and possible complications were checked.

Demographic characteristics of the patients in both groups, time from ICU admission to tracheostomy, Glasgow coma scale (GCS) and acute physiology and chronic health evaluation II (APACHE-2) scores, duration of PDT, minor (small hemorrhages that stop with <10 mL pressure, hypoxemia; <88% SpO_2 in pulse oximetry) and major (>50 mL or major bleeding requiring suturing, paratracheal placement of the tracheostomy cannula; wrong lumen, pneumothorax, tracheoesophageal fistula, tracheal posterior wall injury) complications were analyzed by recording mortality and patient outcomes. In both groups, the procedure duration was determined as the time between starting the skin incision, inflating the cuff of the cannula, and seeing chest movements.

G*Power 3.1 program was used to calculate the sample size. The study's primary outcome is comparing 28-day mortality between groups. Secondary outcomes were determined as complication rates and procedure time. In this context, it was calculated that at least 31 patients in each group should be included in the 95% confidence interval when the effect size of 0.7 for the t-tests, and the power of the study was 85%. This sample size also includes other analyses within the scope of the study.

Statistical Analysis

IBM SPSS v.26.0 (IBM Corp., Armonk, NY) program was used to analyze the data. Descriptive statistics were expressed as mean±standard deviation, median, interquartile range (IQR=Q3-Q1), minimum-maximum, number of patients, and percentage. The conformity of the variables to the normal distribution was evaluated analytically (Shapiro-Wilk test) and visually (histogram). Independent sample t-test was used to analyze data with normal distribution, and the Mann-Whitney U test was used to analyze data that did not show normal distribution among the groups. The chi-square and Fisher's exact tests were used to evaluate qualitative data. The statistical significance limit was accepted as $p<0.05$.

RESULTS

A total of 62 patients, 31 in group FOB and 31 in group C were included in the study. The median age of the patients was 58 (range, 21-94) years in group FOB and 67 (range, 19-88) years in group C. Of the entire study group, 67.7% (n=42) were male. There was no significant difference between the groups regarding age (p=0.375), gender (p=0.587), and BMI (p=0.966). While 71% (n=44) of the entire study group had at least one comorbid disease, the most common comorbid diseases were hypertension (27.4%, n=17), diabetes mellitus (25.8%, n=16), and coronary artery disease/heart failure (17.7%, n=11). While the GCS score at the time of admission to the ICU was 7 (range, 3-12) in group FOB and 7 (range, 3-13) in group C, no significant difference was found between the groups (p=0.579). Similarly, APACHE-2 scores were similar between groups (21.6±6.5 vs 21.5±6.5, p=0.899). The median tracheostomy opening time after ICU admission was 13 (range, 5-31) days in group FOB and 13 (range, 3-28) days in group C (p=0.637). It was determined that the tracheostomy procedure was performed in a significantly shorter time in group FOB than in group C (9.6±3.8 vs 12.6±5.4 minutes, p=0.015). The median length of stay in the ICU was 26 (range, 6-74) days in group FOB and 37 (range, 14-100) days in group C. In group FOB, the length of ICU stay was significantly shorter (p=0.004). While 28-day mortality was 17.7% (n=11) and 90-day mortality was 40.3% (n=25) in the whole study group, there was no significant difference between the groups in terms of 28- and 90-day mortality (p=0.740 and p=0.796, respectively, Table 1).

Considering the primary admission diagnoses of patients who underwent tracheostomy in the ICU, 32.3% (n=20) of the entire group had intracranial hemorrhages (intraparenchymal, intraventricular, subdural, and subarachnoid hemorrhage) and 19.4% (n=12) of the acute ischemic strokes were hospitalized most frequently (Table 2).

Considering the complications between the groups during and after the tracheostomy procedure, complications were

Table 1. Demographic data and some clinical characteristics of the patients

	Group FOB (n=31)	Group C (n=31)	p	Overall (n=62)
Age (years)	58 (74-44) [21-94]	67 (76-55) [19-88]	0.375	64 (76-49) [19-94]
Gender, n (%)				
Female	11 (35.5)	9 (29)	0.587	20 (32.3)
Male	20 (64.5)	22 (71)		42 (67.7)
BMI (kg/m^2)	26.1 (27-23) [19.5-38.8]	25.7 (27-24) [19.1-39.1]	0.966	25.7 (27-23) [19.1-39.1]
Comorbidity, n (%)	21 (67.7)	23 (74.2)	0.576	44 (71)
GCS score	7 (11-5) [3-12]	7 (10-4) [3-13]	0.579	7 (11-5) [3-13]
APACHE-2 score	21.6±6.5	21.4±6.5	0.899	21.5±6.5
Tracheostomy time (days)	13 (17-9) [5-31]	13 (21-8) [3-28]	0.637	13 (21-8) [3-31]
Processing time (min)	9.6±3.8	12.6±5.4	**0.015**	11.1±4.9
Duration of ICU (days)	26 (35-22) [6-74]	37 (51-28) [14-100]	**0.004**	30 (46-25) [6-100]
Mortality (28-day)	6 (19.4)	5 (16.1)	0.740	11 (17.7)
Mortality (90-day)	13 (41.9)	12 (38.7)	0.796	25 (40.3)

Group FOB: fiberoptic bronchoscopy guided percutaneous dilatational tracheostomy, Group C: classical blind percutaneous dilatational tracheostomy, BMI: body mass index, GCS: Glasgow coma scale, APACHE-2: acute physiology and chronic health evaluation II, ICU: intensive care unit, descriptive statistics were reported as mean±standard deviation or median (interquartile range, IQR=75th-25th percentile) [minimum-maximum] for numerical variables, and number of patients and percentage for categorical variables

Table 2. Primary diagnosis of patients with tracheostomy

	Group FOB (n=31)	Group C (n=31)	p	Overall (n=62)
Primary diagnosis, n (%)				
Intracranial hemorrhages*	10 (32.3)	10 (32.3)		20 (32.3)
Acute ischemic strokes	5 (16.1)	7 (22.6)		12 (19.4)
Sepsis/septic shock	3 (9.7)	6 (19.4)		9 (14.5)
Pneumonia, respiratory failure	5 (16.1)	4 (12.9)	0.317	9 (14.5)
Multi-trauma	4 (12.9)	1 (3.2)		5 (8.1)
Post-CPR	4 (12.9)	1 (3.2)		5 (8.1)
Other**	0 (0.0)	2 (6.4)		2 (3.2)

Group FOB: fiberoptic bronchoscopy guided percutaneous dilatational tracheostomy, Group C: classical blind percutaneous dilatational tracheostomy, CPR: cardiopulmonary resuscitation, *: intraparenchymal, intraventricular, subdural, epidural, and subarachnoid hemorrhage, **: preeclampsia and ileus

observed in 6.4% (n=2) of the patients in group FOB. In comparison, minor and major complications were observed in 25.8% (n=8) of the patients in group C. The complication rate in group C was found to be significantly higher (p=0.038). While subcutaneous emphysema was seen in only 1 (3.2%) patient among major complications in group FOB, in group C, subcutaneous emphysema, which is one of the major complications, tracheostomy cannula placement in the wrong lumen, pneumothorax and tracheoesophageal fistula, which is one of the late complications, were detected in a total of 5 (16.1%) patients (Table 3).

Table 3. Complications seen in patients with tracheostomy

	Group FOB (n=31)	Group C (n=31)	p
Complication (total), n (%)	2 (6.4)	8 (25.8)	**0.038**
Minor complication, n (%)			
Minor bleeding (<10 mL)	1 (3.2)	2 (6.4)	
Hypoxemia	0 (0.0)	1 (3.2)	
Major complication, n (%)			
Subcutaneous emphysema	1 (3.2)	1 (3.2)	
Placement in wrong lumen	0 (0.0)	2 (6.4)	
Pneumothorax	0 (0.0)	1 (3.2)	
Tracheoesophageal fistula	0 (0.0)	1 (3.2)	

Group FOB: fiberoptic bronchoscopy guided percutaneous dilatational tracheostomy, Group C: classical blind percutaneous dilatational tracheostomy

DISCUSSION

In this prospective study in which FOB guidance and classical percutaneous tracheostomy procedures were analyzed in the ICU, it was determined that more tracheostomy was required in the male gender in the entire study group. In FOB-guided PDT, the duration of the procedure after skin sterilization and the rate of complications during and after the procedure were significantly reduced, and the duration of stay in the ICU was shorter in these patients. However, no significant difference was found in 28- and 90-day mortality rates in patients who underwent tracheostomy with both methods. Tracheostomy is one of the most frequently performed surgical procedures in the ICU, and its application rates may vary depending on the specialized structure of ICU and the characteristics of the patients followed. The rate of performing tracheostomy in an ICU where patients with neurological problems are followed has been reported as 28.6% (6). It has been reported that tracheostomy was performed in 6% of trauma patients in an ICU where a significant number of trauma patients were followed (7). There has yet to be a definite consensus on the indications, timing, method, and subject. In a multicenter survey study from Türkiye, it was reported that 70.4% of ICU physicians performed PDT with the Grigss method, and the most common indication for tracheostomy was prolonged mechanical ventilation (76.9%) and coma (14.8%) (8). FOB-guided PDT has become a frequently used method today because it reduces complications and provides

procedural safety (9). The application time may vary depending on the decision of the ICU specialist physicians to evaluate the patient's clinical condition daily. However, the process may be prolonged due to bleeding diathesis, the instability of the patient's clinical condition, and the indecision of the patient's relatives about the procedure. Although early tracheostomy applications may be associated with improvement in some clinical outcomes, it has been reported that an unnecessary tracheostomy procedure may lead to various complications and risks (10,11) and also reported that it is generally performed seven days after orotracheal intubation (11). Romero et al. (13) reported that approximately 60% of patients who underwent FOB-guided PDT were male, with a mean age of 64±18 years and a mean tracheostomy opening time of 11±3 days. In another study, it was reported that 70% of the patients who underwent tracheostomy were male, the mean age was 56.6±18 years, and all tracheostomies were opened due to the need for a prolonged mechanical ventilator (14). Consistent with the literature, in our study, while more tracheostomies were performed in males (67.7% of the entire study group), the median age was found to be 64 (range, 19-94) years in the entire group. The median duration of mechanical ventilation was 13 (range, 3-31) days, similar in both groups. While all PDT procedures were performed with the Griggs method, the need for prolonged mechanical ventilation and coma were the most common indications for tracheostomy. Considering that prolonged mechanical ventilation and coma are effective in opening tracheostomy in some of the patients followed in the ICU, it is difficult to state the indications clearly.

The duration of the tracheostomy procedure may be crucial in critically ill patients followed in the ICU. FOB-guided PDT procedure may increase the cost and prolong the procedure depending on the physician's experience using the bronchoscopy. Shen et al. (15) reported an average time of 9.8±1.2 minutes to perform FOB-guided tracheostomy. In another study, ultrasound-guided and FOB-guided tracheostomy was investigated, and the mean time to perform FOB-guided tracheostomy was 16.3±1.6 minutes (16). Batcik et al. (14) reported that the duration of tracheostomy procedures performed under FOB guidance was significantly higher than the classical technique (13.4±4.9 vs 8.1±6.1 minutes). In our study, the procedure times were similar to the literature. However, the procedure time was significantly lower in the FOB group compared to the classical blind technique (9.6±3.8 vs 12.6±5.4 minutes). The physician's experience using bronchoscopy, the type of bronchoscopy (rigid or flexible bronchoscopy), or how the duration is calculated may affect this situation. In our study, all bronchoscope procedures were performed by an experienced ICU or anesthesiology and reanimation specialist, and the starting time of the procedure was started by opening the plus sign at the end of the endotracheal tube after skin disinfection and placing the flexible bronchoscopy. All tracheostomy procedures were performed by senior assistants with experience in tracheostomy at least five times under the guidance of an expert. Confirming the place where the procedure is performed and guiding the people who perform the procedure, thanks to the translumination of the light on the fiberoptic bronchoscope tip, may be effective in shortening the procedure time.

Although PDT is a generally safe procedure, it has some complications. Major complications such as subcutaneous emphysema, pneumomediastinum, pneumothorax, paratracheal placement of the cannula (cannula in the wrong lumen), perforation of the tracheal posterior wall and tracheoesophageal fistula that may occur in the late period can be seen among minor complications such as minor bleeding that can stop with compression and hypoxemia. In studies comparing percutaneous and surgical tracheostomy, it was reported that complications such as hemorrhage, subcutaneous emphysema, pneumothorax, and tracheal stenosis were significantly less common in percutaneous techniques compared to the surgical technique (3,17). In a study comparing FOB guidance and standard blind PDT, it was reported that major complications, including tracheal posterior wall damage, were observed in the blind technique, and the use of FOB reduced both major and minor complications (18). Another study reported that FOB guidance in PDT did not make a difference in complications compared to the blind technique (19). A meta-analysis examining FOB-guided PDT procedures reported that the rates of serious complications could reach 35%, and the rates of minor complications could reach 65% (20). In our study, both minor and major complication rates were found to be low in the FOB-guided PDT group, consistent with the literature. One (3.2%) patient had minor bleeding, and one patient had subcutaneous emphysema, which could be classified as a major complication. In the classical blind technique group, major complications (subcutaneous emphysema, paratracheal placement of the cannula, pneumothorax, and tracheoesophageal fistula) were found in 5 (16.1%) patients. Following the literature, FOB guidance reduces complications. However, sufficient experience in using bronchoscopy is essential in not prolonging the procedure time and preventing complications related to bronchoscopy.

In the literature, auxiliary methods such as bronchoscopy and ultrasonography have been investigated regarding guiding surgical methods, bedside percutaneous techniques, or percutaneous techniques in patients who have undergone tracheostomy (14,18,21,22). In these studies, the early complications and the duration of the procedure were investigated, and the effects of the methods on mortality were not evaluated. Shen et al. (15) reported no significant difference in 28-day mortality between the groups in PDTs opened with FOB guidance and the classical blind technique, and the mortality was 20% in the whole population. The authors stated that the APACHE-2 scores of the patients in both groups in the study were similar, and 23±7 in their entire study group. In our study, consistent with the literature, APACHE-2 scores were similar between the groups and were found to be 21.5±6.5 in the entire study group. GCS scores also did not differ significantly; the median was 7 (range, 3-13) in whole patients. From this point of view, we can say that our patient groups consist of patients with similar characteristics. Our mortality rates did not differ significantly between the groups (28-day mortality rates were 19.4% in group FOB, and 16.1% in group C), consistent with the literature.

The main limitations of our study are the small sample size and its single-center design. In addition, although all

tracheostomy procedures were performed by the same intensive care or senior assistant physicians with tracheostomy experience, accompanied by an anesthesiology and reanimation specialist, not all tracheostomy procedures were performed by the same person.

CONCLUSION

FOB-guided percutaneous tracheostomy procedures, which are frequently performed in patients followed up in the ICU, may be beneficial in shortening the procedure time and reducing complication rates and length of stay in the ICU, although it does not affect mortality.

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul Kanuni Sultan Süleyman Training and Research Hospital (12.10.2022, 208).

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Performance and Safety Evaluation of Polypropylene Mesh Used in Inguinal Hernia Repairs

İnguinal Herni Onarımlarında Kullanılan Polipropilen Meshin Performansı ve Güvenlik Değerlendirmesi

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ABSTRACT

Aim: This study aimed to compare the data of patients who underwent open tension-free hernia repair using polypropylene mesh with the data obtained from the literature, and to evaluate the efficacy and safety of this mesh in the early postoperative period. The results of this study will provide additional data to the literature in terms of comparing different mesh materials.
Material and Methods: In this cross-sectional, and observational study, the early postoperative-period data of 96 patients who had undergone standard Lichtenstein tension-free hernia repair using polypropylene mesh in a tertiary-level hospital, using polypropylene mesh were evaluated and compared with the data obtained from the literature.
Results: The mean age of patients was 58.59±13.82 (range, 20-83) years. The median length of hospital stay was 1 (range, 1-4) day. The median of visual analogue scale (VAS) scores was 2 (range, 0-4) for the day of surgery, 0 (range, 0-4) for postoperative day-1, and 0 (range, 0-2) for postoperative day-2. A total of 6 complications developed in 5 of the patients, 3 (3.13%) were hematoma, 2 (2.08%) were wound site infections, and 1 (1.04%) was seroma. In one patient, both hematoma and wound infection were determined. No mortality was encountered.
Conclusion: Polypropylene mesh could be used effectively and safely in groin hernia operations. Although some complications with the use of synthetic mesh materials have been reported since the introduction of these materials into clinical use, none of these have yet been considered as conditions that will adversely affect the use of polypropylene mesh.
Keywords: Hernia; inguinal; mesh; polypropylene; herniorrhaphy; postoperative complications.

ÖZ

Amaç: Bu çalışmanın amacı polipropilen mesh kullanılarak açık gerilimsiz fıtık onarımı yapılan hastaların verilerini literatürden elde edilen verilerle karşılaştırmak ve bu meshin ameliyat sonrası erken dönemdeki etkinliğini ve güvenilirliğini değerlendirmektir. Bu çalışmanın sonuçları, farklı mesh malzemelerinin karşılaştırılması açısından literatüre ek veri sağlayacaktır.
Gereç ve Yöntemler: Bu kesitsel ve gözlemsel çalışmada, üçüncü basamak bir hastanede polipropilen mesh kullanılarak standart Lichtenstein yöntemiyle gerilimsiz fıtık onarımı uygulanmış olan 96 hastanın ameliyat sonrası erken dönem verileri değerlendirildi ve literatürden elde edilen verilerle karşılaştırıldı.
Bulgular: Hastaların ortalama yaşı 58,59±13,82 (aralık, 20-83) yıl idi. Hastanede kalış süresinin ortancası 1 (aralık, 1-4) gün idi. Görsel ağrı skalası (visual analogue scale, VAS) skorlarının ortancası ameliyat günü için 2 (aralık, 0-4) olarak belirlenirken, ameliyat sonrası 1. gün için 0 (aralık, 0-4) ve ameliyat sonrası 2. gün için ise 0 (aralık, 0-2) olarak belirlendi. Hastaların 5'inde, 3'ü (%3,13) hematom, 2'si (%2,08) yara yeri enfeksiyonu ve 1'i (%1,04) seroma olmak üzere toplam 6 komplikasyon gelişti. Bir hastada hem hematom hem de yara yeri enfeksiyonu bir arada belirlendi. Herhangi bir mortalite ile karşılaşılmadı.
Sonuç: Polipropilen mesh, kasık fıtığı ameliyatlarında etkin ve güvenli bir şekilde kullanılabilir. Sentetik mesh malzemelerinin klinik kullanıma girmesinden bu yana bazı komplikasyonlar rapor edilmiş olsa da bunların hiçbiri henüz polipropilen mesh kullanımını olumsuz etkileyecek durumlar olarak değerlendirilmemiştir.
Anahtar kelimeler: Fıtık; inguinal; mesh; polipropilen; fıtık onarımı; ameliyat sonrası komplikasyonlar.

INTRODUCTION

Hernia is defined as the protrusion or prolapsus of an organ through the wall of the cavity where it is ordinarily contained. They can be seen in a variety of shapes and sizes, with the abdominal wall being the area most prone to hernia development. Inguinal hernia is the most common hernia at the rate of 70-75%, followed by femoral (6-17%) and umbilical (3-8.5%) hernias. One of the most frequently performed surgical procedures worldwide is hernia repair. More than 20 million hernia repair surgeries are thought to be performed annually around the globe (1). Since the introduction of the Bassini procedure in 1887, more than 70 forms of pure tissue repair have been recorded in the surgical literature. The tissue approximation techniques of hernia repair have all but been abandoned due to high recurrence rates of up to 34%. Lichtenstein established the idea of tension-free repair for hernias (2). Currently, more than 80% of hernia surgeries in the United States use hernia mesh products. However, there are many different kinds of mesh, and there is much disagreement on the best use of surgical techniques and their success (1).

Currently, most surgeons concur that using a prosthetic mesh is the ideal method for soft tissue reinforcement and the treatment of hernias during open and laparoscopic surgeries (1). Despite tension-free mesh repair of ventral and groin hernias being widely accepted due to lower hernia recurrence rates than primary soft-tissue repair, the use of mesh for other surgical procedures is still a matter of debate because of the rare but serious complications that necessitate mesh removal and surgical repair (3). Infection, discomfort, pain, intestinal complications, seroma, local reaction, erosion/migration, adhesions, and mesh shrinkage are among the unfavorable outcomes associated with mesh use (4).

The aim of this study was to compare the data of patients who underwent open tension-free hernia repair using polypropylene mesh in a tertiary-level hospital with the data obtained from the literature and to evaluate the efficacy and safety of this mesh in the early postoperative period. With the development of technology, new materials and techniques are constantly being introduced and these innovations should be evaluated by comparing them with other applications. The results of this study will provide additional data to the literature in terms of making such comparisons.

MATERIAL AND METHODS

This retrospective, cross-sectional, and observational research included patients aged 18-99 years who met the study inclusion criteria, regardless of gender, who had undergone standard Lichtenstein tension-free inguinal hernia repair between August 2022 and December 2022, using polypropylene mesh, in the General Surgery Department of Health Sciences University Ankara Training and Research Hospital. The study was approved by the Ethics Committee of Ankara Training and Research Hospital (21.09.2022, 1086). The polypropylene mesh, used in this study was Polypropylene Mesh, which is a Class IIb medical device, certified since 2007, and manufactured by Altaylar Medikal, Ankara, Türkiye.

The evaluated parameters regarding the patient and the surgery during the preoperative, operative process and postoperative hospital stay were age, gender, body mass index (BMI), smoking status, additional diseases (diabetes mellitus, hypertension, chronic obstructive pulmonary disease, coronary artery disease, etc.), hernia type (direct, indirect, direct+indirect hernia, etc.), hernia side (right, left), anticoagulant use (such as aspirin, coumadin, Plavix, etc.), American Society of Anesthesiologists (ASA) score, anesthesia method (general, sedation, spinal), postoperative hospital stay, pain assessment during hospitalization according to the visual analogue scale (VAS) pain score, postoperative fever, morbidity, and mortality.

In accordance with standardized principles, all patients who were operated on in the General Surgery Clinic because of an inguinal hernia were invited to the outpatient clinic on the 10th day postoperatively for examination and removal of sutures. In addition to this routine practice, during discharge, the patients were informed that in the case of unexpected signs and symptoms such as severe pain, nausea-vomiting, redness-discharge at the wound site, gas-stool inability, or swelling at the incision site, they should immediately go to the General Surgery Department without waiting for the expiration of the 10-day period. According to the routine practice of the clinic, when patients come to the clinic on the 10th day or are admitted to the hospital due to a developing problem, they are questioned about current complaints, and physical examinations are performed. In patients with wound infection, the wound is drained and a sample is taken for culture-antibiogram, or when a complication such as a hematoma, hydrocele, or early recurrence is considered, ultrasonography is performed.

In the current study, the records were examined of the patients' routine admissions during the 10-day postoperative period and the patient admissions at the General Surgery Clinic in the one-month postoperative period, and complications including seroma, hematoma, wound infection, urinary tract infection, hydrocele, early recurrence, early mesh reaction, spermatic cord injury, testicular atrophy, orchitis, foreign body sensation, pain, or any other complications were determined. No problem was encountered in accessing information in this retrospective study, as all the parameters evaluated during the study were routine and mandatory data recorded in the hospital registry system by the physician evaluating the patient.

The patients with bilateral inguinal hernia, femoral hernia, incarcerated hernia, recurrent hernia, severe cardiopulmonary disease, chronic liver or kidney dysfunction, malignant tumor, serious diseases causing increased intra-abdominal pressure, and patients with incomplete data in the registry system were not included in the study.

Statistical Analysis

The IBM SPSS v.25 program was used for statistical analysis. For quantitative data, mean, standard deviation, median, minimum, and maximum values were used. Frequency tables were used for qualitative data.

RESULTS

The evaluation was made for a total of 96 patients with a mean age of 58.59±13.82 (range, 20-83) years, comprising 87 (90.6%) males with a mean age of 58.92±14.08 (range, 20-83) years, and 9 (9.4%) females with a mean age of 55.44±11.07 (range, 44-79) years.

The demographic and clinical characteristics of patients according to the parameters evaluated in the present study were given in Table 1. Most of the patients (n=53, 55.2%) had an ASA II score, and most (n=60, 62.5%) patients had a BMI of >25 kg/m^2. The median length of hospital stay was 1 (range, 1-4) day, and 79.2% (n=76) of the patients were discharged on postoperative day 1. The VAS scores were evaluated, on the night of the surgery day (VAS-0), postoperative day 1 (VAS-1), and day 2 (VAS-2) for patients who were not discharged. The median of the VAS scores were 2 (range, 0-4) for VAS-0, 0 (range, 0-4) for VAS-1, and 0 (range, 0-2) for VAS-2.

The most preferred anesthesia method was spinal anesthesia (n=81, 84.4%). Postoperative fever was observed in a total of 9 (9.4%) patients, but these patients did not have resistant fever and no additional treatment was required (Table 2).

A total of 6 complications developed in 5 patients, of which, 3 (3.13%) were hematoma, 2 (2.08%) were wound site infections, and 1 (1.04%) was seroma. In one patient, both hematoma and wound infection were determined. One of these patients with a 12-cm diameter hematoma was re-operated without mesh extraction. In the other patients, seroma, the other two hematomas, and the wound infections were drained by removing 2 or 3 sutures when these complications were diagnosed. The demographic and medical parameters of the patients with complications were given in Table 3. No mortality was encountered.

DISCUSSION

The goal of any hernia repair must be to fix the defect permanently with minimal risk. Avoiding recurrence, managing pain, and reducing infection rates are crucial concerns. The most notable improvement in inguinal hernia repair occurred in the 1980s, when Lichtenstein first used mesh to cover the inguinal canal floor, thereby enabling a realistic tension-free repair. Recurrence rates of <2% confirmed the effectiveness of the Lichtenstein Open Method as a mesh reinforcing technique. The Lichtenstein operation, which is technically simple and may be performed with a local anesthetic, has evolved into the standard repair method, and the mesh technique is by far the most used worldwide. With all mesh operations, the recovery time is shorter and less painful. The success of prosthetic repairs has led to much discussion regarding the ideal mesh properties and how the mesh can be fixed. An ideal mesh should be light, flexible, robust, resistant to contraction and infection, immunologically inert, and economical to produce (5,6).

The two types of materials that are employed to manufacture mesh are synthetic and biological. While all biological meshes are bio-degradable, synthetic meshes can either be degradable or permanent. Currently, the majority of meshes are made of carbon polymers such as expanded polytetrafluoroethylene, polyethylene terephthalate polyester, or polypropylene are used to make permanent mesh, which is strong and reasonably priced. There are already more than 100 different products, some

Table 1. Demographic and clinical characteristics of the patients

	Male (n=87)	Female (n=9)	Total (n=96)
Age (years), mean±SD (min-max)	58.92±14.08 (20-83)	55.44±11.07 (44-79)	58.59±13.82 (20-83)
BMI (kg/m^2), mean±SD (min-max)	26.35±3.60 (16.51-38.06)	27.72±7.15 (17.72-42.97)	26.47±4.03 (16.51-42.97)
BMI, n (%)			
<17 kg/m^2	1 (1.2)	0 (0.0)	1 (1.0)
17-25 kg/m^2	31 (35.6)	4 (44.4)	35 (36.5)
>25 kg/m^2	55 (63.2)	5 (55.6)	60 (62.5)
ASA Score, n (%)			
I	10 (11.5)	2 (22.2)	12 (12.5)
II	47 (54.0)	6 (66.7)	53 (55.2)
III	30 (34.5)	1 (11.1)	31 (33.3)
VAS-0, median (min-max)	2 (0-4)	0 (0-2)	2 (0-4)
VAS-1, median (min-max)	0 (0-4)	0 (0-2)	0 (0-4)
VAS-2, median (min-max)	0 (0-2)	0 (0-2)	0 (0-2)
Hospital stay (day), median (min-max)	1 (1-4)	1 (1-2)	1 (1-4)

SD: standard deviation, BMI: body mass index; ASA: American Society of Anesthesiologists, VAS: visual analogue scale

Table 2. Clinical characteristics of the patients

Smoking, n (%)	39 (40.6)
Comorbidities, n (%)	
Hypertension	30 (31.3)
Diabetes mellitus	16 (16.7)
COPD	9 (9.4)
CAD	24 (25.0)
Hernia Type, n (%)	
Direct	35 (36.5)
Indirect	57 (59.4)
Direct + Indirect	3 (3.1)
Other	1 (1.0)
Hernia Side, n (%)	
Right	57 (59.4)
Left	39 (40.6)
Anesthesia Method, n (%)	
General	5 (5.2)
Spinal	81 (84.4)
Sedation + spinal	7 (7.3)
General + spinal	3 (3.1)
Postoperative Fever, n (%)	9 (9.4)
Complications, n (%)	
Seroma	1 (1.0)
Hematoma	3 (3.1)
Wound site infection	2 (2.1)

COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease

Table 3. Demographic and clinical parameters of the patients with complications

Patient No	I	II	III	IV	V
Complication	Hematoma+ Wound Infection	Seroma	Wound Infection	Hematoma	Hematoma
Gender	Male	Female	Male	Male	Female
Age (years)	44	56	72	83	79
ASA Score	II	II	II	II	II
BMI (kg/m^2)	24.38	42.97	24.58	23.94	23.66
VAS Score	VAS-0: 4 VAS-1: 1 VAS-2: 1	VAS-0: 0 VAS-1: 0	VAS-0: 0 VAS-1: 0	VAS-0: 2 VAS-1: 0	VAS-0: 2 VAS-1: 0
Smoking Habit	Smoker	Non-smoker	Non-smoker	Non-smoker	Non-smoker
Comorbidity	None	HT+CAD	BPH	HT+BPH	None
Anticoagulant Usage	No	No	No	No	No
Anesthesia	Spinal	Spinal	Spinal	Spinal	Spinal
Day of Discharge	Postop 3	Postop 1	Postop 1	Postop 3	Postop 1

ASA: American Society of Anesthesiologists, BMI: body mass index, VAS: visual analogue scale, HT: hypertension, CAD: coronary artery disease, BPH: benign prostate hyperplasia

of which differ significantly and others just slightly. Mesh absorbability, weight, thickness, strength, and porosity are the main factors to be taken into account when selecting mesh material. The most popular synthetic prosthetic materials used in hernia repair are polypropylene and polyester, both of which have proven to be excellent for hernia surgery (5-7).

It is challenging to identify the exact influence of the material on the rates of complications because there are so many potential causes of issues following surgery. Even with the best low-risk mesh or without any mesh at all, any severe complication may be caused by poor surgery or a patient with compromised wound healing. Therefore, any other potential non-mesh-related risks must be excluded from any consideration of the specific material-associated risks. Any straightforward association between material and complications in clinical trials should generally be considered with caution because many of these confounding factors may not be known or may not have been appropriately recorded (8).

Bleeding, seroma, infection, urinary retention, damage to adjacent structures, and ileus are the most common complications of groin hernia repair. Hernia recurrence, chronic pubic and inguinal pain, and injury to the testis or spermatic cord are all specific complications of herniorrhaphy. The most often used indicator of postoperative success after inguinal hernia surgery is the incidence of recurrence. Complication rates, hospital stay, quality of life, and operative duration are additional important outcome indicators to consider when comparing the various procedures currently available. The Lichtenstein tension-free repair significantly reduces hernia recurrence. Mesh repair is associated with fewer recurrences, a faster return to normal activities, and shorter hospital stays when compared to open surgical tissue-based repairs. The Lichtenstein approach continues to be the most often performed procedure worldwide among alternative tension-free repairs (6).

There are many studies in the literature that have used different groups of meshes in hernia repair. In this part of the article, the data obtained from the current study will be compared with the data obtained from the literature in order to evaluate the efficacy and safety of polypropylene mesh in the early postoperative period.

Clancy et al. (9) reviewed current research studies on the auto-immune and systemic consequences of polypropylene mesh usage in hernia repair. They identified 23 studies and these studies supported the appropriate usage of mesh in hernia surgery and other procedures.

The outcomes of a retrospective study that evaluated the performance, biocompatibility, and short- and long-term results of meshes used in open inguinal hernia surgery were recently published. The objectives of that study and the current study were very similar, except that the current study only evaluated early post-operative outcomes and not long-term outcomes as the patients only had the operations between August and December 2022. The study conducted by Tanasescu et al. (10) included 255 patients over a 7-year period, who underwent the modified Lichtenstein procedure using a monofilament polypropylene mesh (Premiline Mesh™). At the day-2 visit, there were four cases (1.5%) of postoperative large hematoma that necessitated surgical re-intervention but did not require removal of the mesh. At the 7-day visit, seromas in 16 (6.3%) patients and hematomas in 9 (3.5%) patients were observed as a total of 25 patients (10). In the current study, there were 3 (3.13%) hematomas, and only 1 (1.04%) seroma. One of these patients with hematoma was re-operated without mesh extraction. In the other patients, the seroma and the other two hematomas were drained by removing 2 or 3 sutures. In conclusion, although the rates of hematoma were similar in both studies, the seroma ratio in the current study was significantly low. There was no postoperative wound infection in the previous study whereas two (2.08%) surgical site infections were encountered in the current study.

Some other early postoperative complications, which were not reviewed by Tanasescu et al. (10), were examined in the current study, including early recurrence, early mesh reaction, spermatic cord injury, testicular atrophy, orchitis, foreign body sensation, hydrocele, and urinary tract

infection, were also discussed, and none of these complications was encountered. Early mesh reaction, early recurrence, and foreign body sensation in particular are considered as complications directly associated with the performance and physical properties of meshes, which are directly related to the main aim of the current study. The fact that these complications were not encountered can be accepted as positive data in terms of the performance and safety of the mesh used in the current study.

There are many clinical studies about the usage of polypropylene mesh on different parts of the body. Cavalli et al. (11) retrospectively reviewed 22 patients who underwent open repair of a lateral abdominal wall complex hernia. No major complications developed and it was concluded that any lateral complex hernia, regardless of the size and location, might be repaired with polypropylene mesh in the extra-peritoneal plane.

A recent study showed that using synthetic mesh produced a safe and long-lasting repair and using polypropylene mesh in the infected setting produced results that were similar to clean repairs (12). Although polypropylene mesh was not employed in an infected environment in the current study, the study by Birolini et al. (12) provides highly important information on the safe usage of mesh even in contaminated settings.

Pande and Naidu (13) conducted a systematic observational prospective study to evaluate the incidence of mesh infections, determine the type of related organism, and analyze the results of patients with hernioplasty in order to assess the complications of polypropylene mesh usage. Of the 181 cases, 59 cases of mesh contamination and 9 (4.97%) cases of mesh infection were observed. Groin hernias were the most common type of case that became infected. Mesh extraction was not required in any of these cases (13). In the current study, two (2.1%) patients experienced wound infection, neither of which required mesh removal. This rate was seen to be low in comparison with the rate in the above-mentioned study by Pande and Maidu (13).

Polypropylene meshes have also been used effectively and safely for many different indications, including anterior chest wall reconstruction, abdominal-based free flap breast reconstruction, pelvic organ prolapsus, stress urinary incontinence, laparoscopic sacrocolpopexy, laparoscopic sacrohysteropexy, cystocele, and orbital floor fractures (14-23).

Emral et al. (24) compared the short- and long-term outcomes of the traditional polypropylene mesh and self-adhesive mesh in Lichtenstein repair in a prospective, randomized, controlled study. The findings revealed that except for operation time, the self-adhesive mesh did not provide any statistically significant advantages over the traditional polypropylene mesh in the Lichtenstein repair. As part of that study, 39 patients from the polypropylene group underwent 42 (3 bilateral operations) inguinal hernia procedures and all of these patients were discharged on the first postoperative day. The majority of the patients in the current study were discharged on postoperative day 1, but the length of hospital stay was longer (median, 1; range, 1-4 days) than in the previous study. In the polypropylene group, the mean first-day VAS score was 3.6±1.2 whereas the median VAS score was 2 (range, 0-4) in the current study. Wound infection developed in 3 (3.5%) patients; in two (4.8%) of the polypropylene group and one (2.3%) of the self-adhesive mesh group. In the polypropylene group, only one (2.4%) patient experienced seroma development. Hematoma developed in 4 (4.7%) patients overall; two (4.8%) in the polypropylene group and two (4.5%) in the self-adhesive mesh group. The ratios of hematoma (3.15%), seroma (1.05%), wound infection (2.08%), and median VAS score on postoperative day-1 were all lower in the current study compared with the results of the polypropylene group in the study by Emral et al. (24).

In the study conducted by Sun et al. (25), the incidence of foreign body sensation, incision inflammation, postoperative VAS pain score, orchitis, and hydrocele were assessed as secondary outcome measures. The following were the main clinical outcomes of polypropylene mesh in that study: there was no orchitis, incision inflammation in 2 (3.0%) cases, foreign body sensation in 4 (6.1% on day-1), hydrocele in 1 (1.6%), and a mean VAS pain score of 2.41±0.86 on day-1. When compared with that study, all complication rates and the median VAS score were lower, and there was also no development of orchitis in the current study.

CONCLUSION

Clinical studies are needed to evaluate risk-benefit ratios. According to the early (1-month postoperative period) results of the current study, the authors concluded that polypropylene mesh could be used effectively and safely in groin hernia operations. In addition to its use in hernia repair, which is the main indication for the use of polypropylene mesh, studies were reviewed that have examined different indications, side-effects, and undesirable effects of the product, and for comparisons with other mesh groups. Although some complications with the use of synthetic mesh materials have been reported since the introduction of these materials into clinical use, none of these have yet been considered as conditions that will adversely affect the use of polypropylene mesh. These meshes are still widely used for surgical repair of anatomic defects worldwide even in contaminated environments or in emergent operations.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Ankara Training and Research Hospital (21.09.2022, 1086).

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Morphometric Analysis of Greater Palatine Canal by Computed Tomography

Canalis Palatinus Major Morfometrisinin Bilgisayarlı Tomografi ile Değerlendirilmesi

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ABSTRACT

Aim: The greater palatine canal connects to the oral cavity through the greater palatine foramen. Preoperatively identifying the morphology of the greater palatine canal and greater palatine foramen is very important to avoid possible complications during surgery. This study aimed to evaluate the greater palatine canal and surrounding anatomical structures using computed tomography.

Material and Methods: Images from 100 patients (35 female and 65 male) who had previously undergone computed tomography for various reasons were evaluated. The study data were divided into three age groups, <20 years, 20-60 years, and >60 years. Morphological parameters measured in this study included; diameter measurement from the widest part of the canal, length of the canal, beginning diameter of the canal, the ends diameter of the canal, localization of the canal entrance with respect to the third molar tooth, distance of the canal entrance to palatine suture. The values obtained from the measurements were compared in terms of age group, gender, and side.

Results: The mean length of the canalis palatinus major was 15.19±4.38 mm. The diameter of the widest part of the canal and the end of the canal, and the distance between the canal entrance and the sutura palatina increased with age, but these increases were not statistically significant.

Conclusion: Proper administration of anesthesia through the greater palatine foramen in maxillofacial surgeries and related applications requires a detailed understanding of the anatomy of the greater palatine canal, and the results of the present study will contribute to the understanding of this anatomy.

Keywords: Greater palatine canal; anatomy; computed tomography.

ÖZ

Amaç: Canalis palatinus major, foramen palatinum majus aracılığı ile ağız boşluğuna bağlanır. Canalis palatinus major ve foramen palatinum majus morfolojisinin ameliyat öncesinde belirlenmesi, ameliyat sırasında ortaya çıkabilecek olan olası komplikasyonlardan kaçınmak için oldukça önemlidir. Bu çalışmanın amacı, canalis palatinus major ve çevresindeki anatomik yapıların bilgisayarlı tomografi ile değerlendirilmesidir.

Gereç ve Yöntemler: Daha önce çeşitli nedenler ile bilgisayarlı tomografisi çekilmiş olan 100 hastanın (35 kadın ve 65 erkek) görüntüleri incelendi. Çalışma verileri <20 yaş, 20-60 yaş ve >60 yaş olmak üzere üç yaş grubuna ayrıldı. Bu çalışmada ölçülen morfolojik parametreler; kanalın en geniş kısmından çap ölçümü, kanalın uzunluğu, kanalın başlangıç çapı, kanalın uç çapı, kanal girişinin üçüncü molar dişe göre lokalizasyonu ve kanal girişinin sutura palatinaya olan uzaklığıdır. Ölçümler sonucu elde edilen değerler, yaş grubu, cinsiyet ve taraf açısından birbiriyle karşılaştırıldı.

Bulgular: Canalis palatinus majorun ortalama uzunluğu 15,19±4,38 mm idi. Kanalın en geniş yerinin çapı ile bitiş yerinin çapı ve kanal girişi ile sutura palatina arasındaki mesafe, yaş ile birlikte artmış olmakla birlikte bu artışlar istatistiksel olarak anlamlı değildi.

Sonuç: Maksillofasiyal cerrahilerde ve bununla ilgili olan uygulamalarda anestezinin foramen palatinum majus yolu ile uygun bir şekilde uygulanması için canalis palatinus major anatomisinin ayrıntılı bir şekilde anlaşılmış olmasını gerekmektedir ve bu çalışmanın sonuçları bu anatominin anlaşılmasına katkı sunacaktır.

Anahtar kelimeler: Canalis palatinus major; anatomi; bilgisayarlı tomografi.

INTRODUCTION

The greater palatine canal (GPC) is mostly placed opposite the third molar. It connects with the oral cavity via the greater palatine foramen (GPF) (1). The GPC's location is important in terms of associated anatomical structures. It continues in a posterior-superior direction ending at the pterygopalatine fossa communicating with the middle cranial fossa, nasal cavity, and orbit via foramen rotundum, sphenopalatine foramen, and inferior orbital fissure respectively (2).

It goes on in a posterior-superior direction ending at the pterygopalatine fossa and connects with the middle cranial fossa, the nasal cavity, and the orbit via the foramen rotundum, the sphenopalatine foramen, and the inferior orbital fissure respectively (2).

Reaching the pterygopalatine fossa through GPC can be accomplished in maxillary division nerve block in maxillofacial procedures, hemostasis in endoscopic sinus surgery, and relief of sphenopalatine neuralgia (3,4).

The greater and lesser palatine nerves, their posterior inferior lateral nasal branches, and the descending palatine artery are located in this canal (5). Sensory innervation of all maxillary and mandibular teeth and surrounding tissues is provided by the trigeminal nerve.

The maxillary division of the trigeminal nerve (V2) exits the skull through the foramen rotundum and it innervates all maxillary teeth, maxillary palatal and gingival tissue, the nasal cavity, and sinuses (6). The nerve of the pterygoid canal enters the pterygopalatine fossa from the posterior to the foramen rotundum, and transmits the nerve of the pterygoid canal (7). The maxillary nerve receives the sensation of the maxillary teeth, palatal mucosa, and the anatomical structures associated with this region. In major surgical procedures related to the upper jaw, a maxillary nerve block is performed under local anesthesia (8).

The anatomy of these structures undoubtedly affects the anatomy of the GPC due to their proximal relationships. Knowing the anatomy of the GPC is essential for dentists, oral maxillofacial surgeons, and otolaryngologists performing procedures in this area (7,9). When performing surgical procedures, preservation of the descending palatine artery and palatine nerves is essential to avoid excessive bleeding and to maintain nerve supply to the maxilla (10).

The present study aimed to evaluate the morphometry of the GPC and the surrounding anatomical structures using computed tomography.

MATERIAL AND METHODS

This study has been approved by the Afyonkarahisar Health Sciences University, Clinical Research Ethics Committee with approval number 2020/446 and dated 02.10.2020, and was conducted following the Declaration of Helsinki Principles.

The current study was carried out in Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy. A total of 100 computed tomography (CT) images with no pathology of 35 female and 65 male subjects aged 12-85 years were selected randomly. The CT images of all individuals who were admitted to the Department of Radiology for any reason were evaluated retrospectively. CT images of individuals with nasal pathology that may affect the measurement and individuals with poor imaging quality were excluded from the study. The study data were divided into three age groups, <20 years, 20-60 years, and >60 years, as adolescent, adult, and elderly, respectively (11-13).

CT scans were performed with an 80-row Multidetector Computed Tomography (MDCT) scanner (Aquilion Prime, Toshiba Medical Systems, Nasu, Japan). The CT protocol was as follows: peak kilovoltage 120 kVp, tube current, 150-165 mAs; maximum collimation, 2.5 mm; slice thickness, 3 mm; and rotation time, 0.75 s. Images that included the GPC were analyzed retrospectively on a workstation (Aquarius, TeraRecon Inc., San Mateo, CA, USA). Reconstruction images of 0.5 mm slice thickness were created from the 3 mm slice thickness server images. Multiplanar reconstruction and 3D volume rendering (VR) images were obtained from 0.5 mm slice thickness sections. The anatomical landmarks were measured bilaterally on the sagittal and coronal plane (200 sides of 100 cases): a) the beginning diameter, b) the diameter from the widest part, c) the ends diameter, and d) the length of the GPC (Figure 1), e) the entry and f) exit angles of the GPC (Figure 2), g) localization of the GPC entrance with respect to the third molar tooth (distance between the GPC entrance and tooth border), and h) the distance between the GPC entrance and the palatine suture (Figure 3).

Statistical Analysis

All data were analyzed by using IBM SPSS Statistics for Windows, version 19.0 (Armonk, NY: IBM Corp). Descriptive statistics included mean, standard deviation, median, interquartile range, minimum, and maximum values. The Kolmogorov-Smirnov test was used to evaluate the suitability of the data for normal distribution. The differences among the age groups were analyzed with the Kruskal-Wallis test. Mann-Whitney U test was used in comparing the difference between the genders, and sides. The results were evaluated in the 95% confidence interval and $p<0.05$ were considered statistically significant.

RESULTS

In this study, eight anatomical landmarks related to GPC were measured bilaterally in CT images with no pathology of 100 individuals (35 female, 65 male) with an age range of 12-85 years. Individuals were divided into three groups according to their age; adolescent, adult, and elderly. While the first group consists of 15 people between the ages of 12-20 years, the second group consists of 56 people between the ages of 21-60 years, and the third group consists of 29 people over the age of 60 years.

Regardless of right or left, the mean lengths of GPC was 15.19±4.38 mm. No significant difference was found in any measurements in terms of both gender (Table 1) and sides (Table 2). It has been observed that the diameter measurement from the widest part of the GPC, the distance between the GPC entrance and the palatine suture, and the ends diameter of the GPC increases with age (Table 3).

DISCUSSION

A full understanding of the exact location of the GPC and GPF is required to properly administer anesthesia through this foramen in maxillofacial and related applications. Using GPF as a route for injection has many advantages in local anesthesia for surgeons. Anesthesia is applied in the

hard palate area by inserting a needle into the GPC through the GPF. Thus, the anesthetic solution reaches the pterygopalatine fossa where the maxillary nerve trunk is located. Neurovascular structures within the palatine canal may be at risk also during endoscopic surgery. Possible damage to this area may cause significant blood loss and anesthesia in the ipsilateral hard palate (14). Considering the substantial importance of the exact location of the GPC, this study aimed to determine the length, angle, and diameter of the GPC (2-4,15).

A total of 200 sides of 100 GPC morphologies were evaluated. The diameter of the GPC entrance was found statistically significantly larger in males than females in the young group. GPC length was found statistically significantly greater in females than males in the young group. It has been observed that the diameter measurement from the widest part of the GPC, the distance between the GPC entrance and the palatine suture, and the ends diameter of the GPC increase with age.

The length and angle of the GPC have been determined by using dry skulls, CT, and Conical Beam Computed Tomography (CBCT) studies for different populations.

The length of the GPC of 500 patients had been examined in sagittal sections. In this study, the pterygoid canal was selected as a superior limit of the GPC, and the mean length of the GPC was recorded to be 29±3 mm, ranging from 22 to 40 mm (7).

Sheikhi et al. (16) investigated the length of the GPC of 138 patients in sagittal sections and the mean length was found as 31.8 mm. There was no significant difference between age groups. Ozdede et al. (17) investigated the angle of GPC in different sections. The mean angles of the GPC were determined 156° and 169° in sagittal and coronal sections, respectively. Urbano et al. (18) examined the length of GPC with a dry skull and it was found that 36.40 mm in the female skull and 35.30 mm in the male skull. Tomaszewska et al. (1) conducted a study which is on 150 dry human skulls and 1200 archived adult head CT scans. The length of the right GPC was 29.60±2.50 mm and 32.60±2.80 mm; the left GPC was 29.90±2.70 mm and 32.40±2.80 mm in female and male, respectively. Hwang et al. (19) evaluated the CT scans of 50 patients retrospectively and the mean length of GPC was found 13.80±2.00 mm. Douglas and Wormald (3) investigated GPC length with CT of 22 cadaver heads, and the length of GPC was found 18.50 mm in female individuals. Bahşi et al. (20) examined the length of the GPF-CP with CBCT. The length of the right GPF-CP was 27.48±3.10 mm and 29.27±3.59 mm whereas the length of the left GPF-CP was 26.71±2.82 mm and 29.33±3.14 mm in females and males, respectively. In a CT study evaluating the relationship between facial types and GPC, Lacerda-Santos et al. (21) found that the distance between GPF and the palatine suture was 14.47±1.63 mm on the right and 15.16±1.67 mm on the left and this difference was significant, unlike this study. Ortug and Uzel (22) found the same distance of 14.64±2.20 mm on the right and 14.74±2.22 mm on the left in their measurements on 97 dry skulls and stated that this difference was not significant. Radošević et al. (23) measured the same distance on 174 bone plates and found no difference between right and left, but a difference between males and females.

Different results could have arisen from radiological methods, ethnic origin, the choice's superior limit of GPC, age groups, and genders. We think that the major differences between some studies were due to differences in measurement methodology.

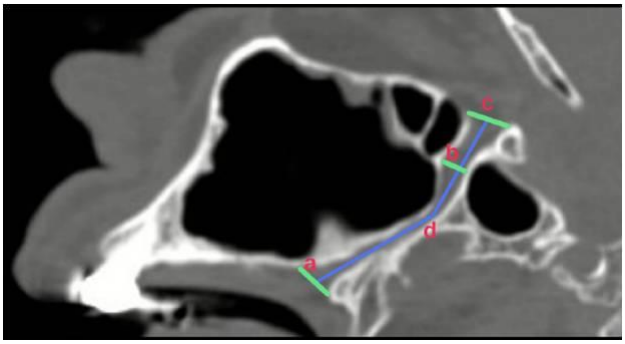

Figure 1. Measurements of the greater palatine canal (GPC) on the sagittal plane, the beginning diameter **(a)**, the diameter measurement from the widest part **(b)**, the ends diameter **(c)**, and the length **(d)** of the GPC

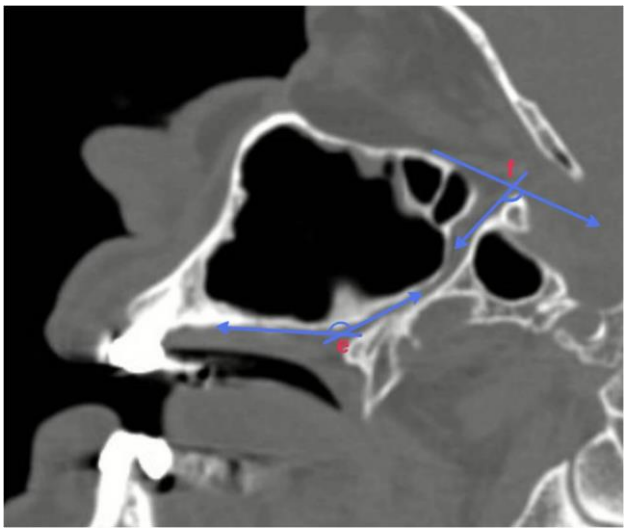

Figure 2. The entry **(e)** and exit **(f)** angles of the greater palatine canal (GPC) on the sagittal plane

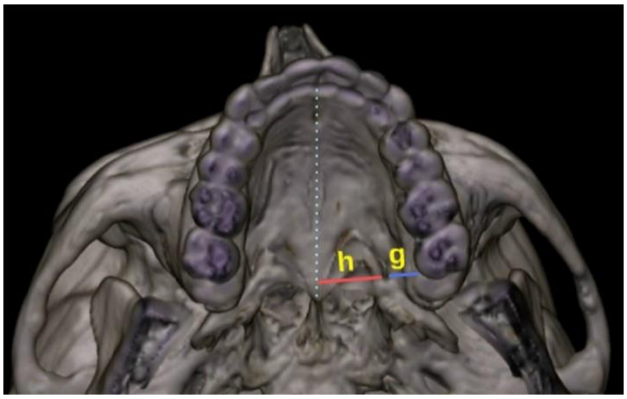

Figure 3. Localization of the greater palatine canal (GPC) entrance, the distance between the GPC entrance and third molar tooth border **(g)**, and palatine suture **(h)**

Table 1. Comparison between the male and female on the basis of all parameters (all measurements were given in mm)

	Female (n=35)		Male (n=65)		p
	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	
Diameter of the GPC	4.67±2.15	4.20 (3.07-6.31) [1.14-11.20]	4.99±2.02	4.54 (3.75-5.84) [1.12-11.80]	0.132
Beginning diameter of the GPC	2.55±1.18	2.47 (1.82-3.04) [1.02-6.68]	2.87±1.42	2.56 (2.02-3.36) [0.45-12.20]	0.906
Ends diameter of the GPC	2.48±1.02	2.62 (1.90-2.89) [0.82-5.13]	2.84±1.57	2.77 (1.89-3.42) [0.07-10.80]	0.134
Length of the GPC	15.53±4.04	16.67 (14.67-17.75) [2.72-22.60]	15.00±4.55	15.70 (12.52-17.75) [1.92-25.60]	0.259
Distance between the GPC entrance and tooth border	4.41±1.12	4.35 (3.54-4.95) [2.61-8.39]	4.64±1.56	4.40 (3.62-5.32) [1.73-9.86]	0.647
Distance between the GPC entrance and palatine suture	12.47±2.93	13.35 (11.58-14.22) [3.10-16.40]	13.24±2.20	13.50 (12.05-14.72) [6.50-17.30]	0.144

GPC: greater palatine canal, SD: standard deviation, IQR: interquartile range (25^{th}-75^{th} percentiles)

Table 2. Comparison between the left and right sides on the basis of all parameters (all measurements were given in mm)

	Left (n=100)		Right (n=100)		p
	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	
Diameter of the GPC	4.90±1.97	4.31 (3.56-5.75) [1.14-11.20]	4.85±2.17	4.48 (3.25-5.57) [1.12-11.80]	0.976
Beginning diameter of the GPC	2.64±1.12	2.48 (1.78-3.33) [0.65-5.73]	2.87±1.54	2.61 (1.84-3.52) [0.45-12.20]	0.302
Ends diameter of the GPC	2.81±1.60	2.60 (1.75-3.58) [0.54-10.80]	2.61±1.18	2.53 (1.69-3.23) [0.07-8.03]	0.519
Length of the GPC	15.16±4.29	15.70 (13.20-17.65) [1.92-25.60]	15.22±4.47	16.20 (13.10-17.95) [2.73-25.00]	0.823
Distance between the GPC entrance and tooth border	4.47±1.34	4.28 (3.48-5.28) [1.98-9.51]	4.64±1.49	4.37 (3.66-5.34) [1.73-9.86]	0.177
Distance between the GPC entrance and palatine suture	12.90±2.43	13.30 (11.85-14.70) [4.20-17.00]	13.03±2.59	13.50 (11.60-14.75) [3.10-17.30]	0.356

GPC: greater palatine canal, SD: standard deviation, IQR: interquartile range (25^{th}-75^{th} percentiles)

Table 3. Comparison of all parameters according to age groups (all measurements are given in mm)

	Group 1 (12-20 years, n=15)		Group 2 (21-60 years, n=56)		Group 3 (60+ years, n=29)		p
	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	
Diameter of the GPC	4.61±1.73	4.35 (2.91-6.02) [1.12-7.19]	4.53±1.93	4.16 (3.40-5.64) [1.75-9.05]	5.68±2.30	5.34 (3.97-6.89) [2.68-11.80]	0.713
Beginning diameter of the GPC	2.92±1.39	3.01 (1.53-4.02) [0.45-5.05]	2.61±1.41	2.39 (1.98-3.00) [1.11-7.79]	2.94±1.18	3.01 (2.18-3.85) [1.51-12.20]	0.416
Ends diameter of the GPC	2.28±0.93	1.99 (1.68-3.01) [0.07-3.10]	2.56±1.29	2.65 (1.89-3.08) [1.04-6.35]	3.22±1.69	3.01 (2.34-4.08) [1.35-10.80]	0.650
Length of the GPC	15.23±3.61	16.75 (13.10-17.60) [1.92-20.10]	15.27±4.59	15.45 (13.30-18.05) [5.13-25.10]	15.01±4.35	16.90 (12.55-17.75) [4.08-25.60]	0.783
Distance between the GPC entrance and tooth border	4.48±1.04	4.45 (3.46-4.94) [1.73-6.44]	4.69±1.46	4.65 (3.76-5.30) [2.73-9.15]	4.34±1.50	4.14 (3.38-5.21) [2.33-9.86]	0.508
Distance between the GPC entrance and palatine suture	11.28±3.13	11.95 (9.36-13.90) [3.10-13.10]	12.94±2.47	13.20 (11.60-14.65) [4.68-17.05]	13.89±1.65	13.95 (12.90-14.75) [9.73-17.30]	0.554

GPC: greater palatine canal, SD: standard deviation, IQR: interquartile range (25^{th}-75^{th} percentiles)

The limitation of our study can be considered as the small number of cases. Therefore, the number of subjects in the groups is not homogeneous.

CONCLUSION

Given all the distances measured, this study could help clinicians to more precisely localize the GPC in patients and predict to numb the maxillary nerve with low complication. It can be concluded that further CT-based studies are needed to estimate the length and other related measurements of the GPC in different ethnic groups.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Afyonkarahisar Health Sciences University (02.10.2020, 446).

Conflict of Interest: None declared by the authors.

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A Rare Manifestation of Leptospirosis: Long-Term Elevation in Liver Enzymes

Nadir Bir Leptospirozis Kliniği: Karaciğer Enzimlerinde Uzun Süreli Yükseklik

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ABSTRACT

Leptospirosis is a bacterial zoonosis that is endemic in many tropical and subtropical regions. The disease is transmitted to humans by contact with the urine or an environment contaminated with the urine of infected animals. The clinical manifestations of leptospirosis vary from subclinical infection to severe illness with multiorgan dysfunction. This case report aimed to present a leptospirosis case diagnosed with detailed anamnesis and progressed with long-term elevation in liver function tests, in a non-endemic region. A 28-year-old male patient was admitted with complaints of weakness, fever, and vomiting. As a result of the detailed anamnesis, it was learned that the patient with hyperbilirubinemia, elevated liver enzymes, and kidney failure had contact with polluted water in a rural area, and the patient was followed up with a preliminary diagnosis of leptospirosis. Leptospirosis may present with different clinical presentations and be confused with many diseases, risk factors should be carefully questioned.
Keywords: Acute kidney failure; leptospirosis; liver function tests.

ÖZ

Leptospiroz, birçok tropikal ve subtropikal bölgede endemik olan bakteriyel bir zoonozdur. Hastalık insanlara enfekte hayvanların idrarıyla veya idrarıyla kontamine olmuş bir ortamla temas yoluyla bulaşmaktadır. Leptospirozun klinik belirtileri subklinik enfeksiyondan çoklu organ fonksiyon bozukluğu ile seyreden ağır hastalık tablosuna kadar değişkenlik göstermektedir. Bu vaka raporunun amacı, endemik olmayan bir bölgede, detaylı anamnez ile tanı konulan ve karaciğer fonksiyon testlerinde uzun süreli yükselme ile seyreden bir leptospirosis olgusunu sunmaktır. 28 yaşında erkek hasta, halsizlik, ateş ve kusma şikayetleriyle başvurdu. Hiperbilirubinemi, karaciğer enzim yüksekliği ve böbrek yetmezliği olan hastanın ayrıntılı anamnez sonucu kırsal bölgede kirli su ile teması olduğu öğrenildi ve hasta leptospiroz ön tanısı ile takip edildi. Leptospirosis farklı klinik bulgularla ortaya çıkabildiği ve birçok hastalıkla da karışabildiği için risk faktörlerinin dikkatle sorgulanması gerekir.
Anahtar kelimeler: Akut böbrek yetmezliği; leptospirozis; karaciğer fonksiyon testleri.

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INTRODUCTION

Leptospirosis is a zoonotic infection caused by spirochetes of the genus of *Leptospira*. Although the disease is commonly seen in tropical and subtropical regions, it is a serious cause of mortality in low-income countries (1). The infection is transmitted not only from rodents but also from wild or domestic animals, especially from rodents. The disease is transmitted to humans by direct contact with

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the urine of infected animals or by contact with an environment contaminated with the urine of infected rodents. The clinical manifestation of leptospirosis varies from mild and self-limited illness to severe illness with multiorgan dysfunction. A mild, anicteric, and self-limiting febrile disease is seen in 90% of the cases. Weil's disease, the most severe form of illness, is characterized by jaundice, renal failure, and hemorrhage (2). The symptoms of leptospirosis can mimic other unrelated infections such as hepatitis, sepsis, hantavirus, and other viral hemorrhagic fevers. Thus, a significant amount of leptospirosis cases may go undetected or be misdiagnosed. The liver is not the main target of spirochetes infections. Hepatic dysfunction in leptospirosis usually presents with isolated direct hyperbilirubinemia with mild elevation transaminases and lever function usually improves without complications (3). Herein, we presented a case of leptospirosis with long-term elevation in liver function tests which was diagnosed by detailed anamnesis and serology.

CASE REPORT

A 28-year-old male patient with no medical history was admitted to the Infectious Diseases and Clinical Microbiology outpatient clinic with weakness, fever, and vomiting. Vital signs on admission were a fever of 39.2 °C, heart rate of 132 beats per minute, blood pressure of 110/60 mmHg, and respiratory rate of 22 breaths per minute. On physical examination, he was oriented and cooperative. He had scleral icteric and jaundiced skin. Other system examinations were normal. Upon further questioning, the patient was a teacher, who lived in the city center. He had no history of liver disease. He denied any recent travel, use of herbal supplements, or alcohol use. He had no contact with ticks and rodents. However, he reported that three days before the presentation, he was drinking natural spring water in rural settings near Mount Ararat.

The laboratory tests showed a white blood cell (WBC) count of 7060 /mm^3 (normal range, 4500-11000 /mm^3), aspartate aminotransferase (AST) level of 128 U/L (normal range, 10-40 U/L), alanine aminotransferase (ALT) level of 247 U/L (normal range, 10-40 U/L), alkaline phosphatase (ALP) level of 153 U/L (normal range, 44-147 U/L), gamma-glutamyl transferase (GGT) level of 1160 U/L (normal range, 0-65 U/L), total bilirubin level of 3.5 mg/dl (normal range, 0.2-1.2 mg/dl), direct bilirubin level of 1 mg/dl (normal range, 0.0-0.3 mg/dl), platelet count (PLT) of 240 x10^3/uL (normal range, 150-400 x10^3/uL), and creatinine level of 2.4 mg/dl (normal range, 0.5-1.2 mg/dl). Hepatobiliary ultrasonography (USG) showed normal liver size and no biliary dilatation. Anti-HAV IgG was positive, while anti-HAV IgM, Hbs-Ag, anti-HBc IgG, and anti-HCV were negative. Hence, antibiotic empiric treatment was initiated with ceftriaxone 2-gram IV once daily for preliminary diagnosis of leptospirosis. The highest values in the follow-up were as follows; AST: 1126 U/L, ALP: 245 U/L, GGT: 1716 U/L, total bilirubin: 10.6 mg/dl, and creatinine: 5.9 mg/dl. The examination results of the patient are shown in Table 1. On the fourth day, the fever regressed, and hyperbilirubinemia and renal function improved. Serum *Leptospira* PCR was positive, confirming the suspected diagnosis of leptospirosis. Hantavirus IgM and IgG were negative. In the follow-up, hemodialysis was not necessary due to acute renal failure, as a resolved renal function. The patient gradually improved and was discharged on the 10th day. The elevation in liver function tests continued for 6 weeks and completely regressed to normal values.

DISCUSSION

Leptospirosis is endemic in various parts of the world. Transmission to humans usually occurs as a result of contact with the urine or tissues of infected animals, which are long-term carriers, or contact with contaminated soil and water. Mice are the most commonly known reservoir of the disease. Shepherds, slaughterhouse workers, butchers, sewer and mine workers, hunters, veterinarians, and laboratory workers who are likely to come into contact with these reservoirs are risky occupational groups in terms of leptospirosis (3). Although he was not in the occupational risk group in our case, leptospira was transmitted as a result of contact with contaminated water in the rural area.

The clinical manifestation of leptospirosis in humans varies from an asymptomatic, self-limiting mild disease to severe illness with, hepatic dysfunction and acute renal failure. About 90% of leptospirosis is a subclinical and self-limiting febrile illness, while the severe form is presented by multiorgan dysfunction. The most severe form of leptospirosis is Weil's disease, characterized by jaundice, acute renal failure, and hemorrhage and can be fatal without treatment (4).

Hepatic involvement in leptospirosis can vary from an asymptomatic rise in transaminases to severe icteric hepatitis. However, detailed data on the frequency and type of hepatic dysfunction in leptospirosis are limited. Studies aiming to elucidate the pathogenesis of *Leptospira*

Table 1. Laboratory findings on admission and days later

Days	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	TBIL (mg/dL)	PLT (x10^3/uL)	Cr (mg/dL)
1st	128	247	153	1160	3.5	140	2.4
3rd	979	712	197	1340	7.8	82	3.2
5th	1126	918	245	1716	10.6	53	5.9
7th	878	754	179	1236	8.2	76	2.5
10th	500	357	161	886	5.2	97	2.0
21st	274	91	158	534	3.5	158	1.0
42nd	98	76	142	256	2.1	175	1.0

AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, TBIL: total bilirubin, PLT: platelet, Cr: creatinine

induced jaundice and hepatic disorders have shown that hyperbilirubinemia in leptospirosis matched with a cholestatic pattern, rather than hepatocellular injury or hemolysis (5,6). They have shown that conjugated bilirubinemia seen in leptospirosis could be due to hepatic infiltration of leptospires (6). Penetration of cells such as endothelial cells, kidney cells, and macrophages by leptospires was reported in in vitro studies (7). In a study based on this, it was investigated whether leptospires localized in hepatocytes, but couldn't find leptospires localized in hepatocytes. This finding suggests that pathogenic leptospires invade host hepatocytes intercellularly rather than intracellularly and hepatocytes are not the main target of leptospires (6). Also, some studies reported that *Leptospira* induced apoptosis of hepatocytes.

Studies investigating clinical manifestation and laboratory test abnormalities in patients who had severe leptospirosis and case reports reported in the literature confirm that elevated liver enzymes in leptospirosis usually resolve without long-lasting effects (8). In our case, high liver enzyme elevation for a long time is remarkable. Transaminases, which were elevated 10 times in the first week, continued to be elevated 2–3 times in the 6th week. By the eighth week, it was completely normal.

While spontaneous recovery is observed in most mild leptospirosis cases, it has been shown that early antibiotic treatment in severe leptospirosis cases reduces mortality (9). Doxycycline or amoxicillin is generally recommended in moderate cases, and penicillin or ceftriaxone is recommended in severe cases (10). In our case, the patient who applied with fever, weakness, and nausea was accepted as having leptospirosis with clinical findings and anamnesis, and antibiotic treatment was started with a preliminary diagnosis of Weil's disease. A fever response was obtained on the fourth day of treatment, and clinical symptoms and urea and creatinine values became completely normal. Hemodialysis was not necessary for acute renal failure although there was a serious increase in urea and creatinine, This suggested that it may be due to early antibiotic treatment and supportive treatment.

Our case is important in terms of early diagnosis as a result of detailed anamnesis in the non-endemic region. Leptospirosis may present with different clinical presentations and may be confused with many diseases with similar clinical presentations. Therefore, risk factors should be carefully questioned in the anamnesis. Our case is interesting in that the elevation in liver function tests is a case of leptospirosis that lasts for a very long time.

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Telenutrition Support in Rare Diseases May Have Positive Effects: Spinal Muscular Atrophy Type 2 Case Report

Nadir Hastalıklarda Telenütrisyon Desteği Olumlu Etkiler Oluşturabilir: Spinal Musküler Atrofi Tip 2 Olgu Sunumu

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ABSTRACT

In this case report, the 6-month telenutrition counseling process of a 13-year-old male patient diagnosed with spinal muscular atrophy type 2 was discussed. Severe malnutrition was observed in the patient, who used a wheelchair and was fed orally. As a result of examining all the findings, a 6-month telenutrition counseling process was started with a diet containing 50 kcal/kg of energy and 1.85 g/kg of protein per day. At the end of the process, the patient's body weight increased from 30.5 kg to 35 kg, the body mass index increased from 13.6 kg/m^2 to 15.6 kg/m^2, and the body mass index Z-score value for age increased from -3.04 to -1.75. The family expressed satisfaction with the counseling process. Patients diagnosed with spinal muscular atrophy require medical nutritional therapy counseling. Telenutrition counseling is considered beneficial in reaching patients and ensuring the treatment process.
Keywords: Rare diseases; spinal muscular atrophy; nutrition; telenutrition.

ÖZ

Bu olgu sunumunda, sipinal muskuler atrofi tip 2 tanısı olan 13 yaşında erkek hastanın 6 ay süren telenütrisyon danışmanlık süreci tartışılmıştır. Tekerlekli sandalye kullanan ve ağızdan beslenen hastada ağır malnütrisyon görülmüştür. Tüm bulguların incelenmesi sonucunda günlük 50 kkal/kg enerji ve 1.85 g/kg protein içeren diyet ile 6 aylık telenütrisyon danışmanlık süreci başlatılmıştır. Süreç sonunda hastanın vücut ağırlığı 30,5 kg'den 35 kg'ye, beden kütle indeksi 13,6 kg/m^2'den 15,6 kg/m^2'ye ve yaşa göre beden kütle indeksi Z-skoru değeri ise -3,04'den -1,75'e yükselmiştir. Aile danışmanlık sürecinden memnun kaldığını belirtmiştir. Sipinal muskuler atrofi tanılı hastalar tıbbi beslenme tedavisi danışmanlığına ihtiyaç duymaktadır. Hastalara ulaşmak ve tedavi sürecini sağlamak için telenütrisyon danışmanlığının faydalı olabileceği düşünülmektedir.
Anahtar kelimeler: Nadir hastalıklar; spinal muskuler atrofi; beslenme; telenütrisyon.

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INTRODUCTION

Spinal muscular atrophy (SMA) is a rare autosomal recessive neuromuscular disease characterized by progressive muscle atrophy, weakness, paralysis, and degeneration of the alpha motor neurons in the spinal cord. Among its various types, nerve, skeletal, and muscular system problems are prevalent, particularly in patients with type 1 and type 2 SMA (1). Most patients experience respiratory and feeding difficulties due to muscle atrophy, which affects chewing and swallowing functions. Consequently, expert dietitian counseling is crucial during the medical nutrition therapy process for patients with SMA (2). Telenutrition leverages electronic information and telecommunication technologies provided by licensed dietitians from

authorized institutions to manage all stages of medical nutrition therapy. This includes anamnesis, patient nutritional status determination, evaluation of anthropometric, laboratory, and physical findings, and the planning and monitoring of medical nutrition therapy (3,4). This case report aimed to elucidate the medical nutrition therapy process facilitated through a telenutrition system in a patient diagnosed with SMA type 2 in Türkiye.

CASE REPORT

A thirteen-year-old male patient sought telenutrition support via the website smailebeslen.com due to concerns about weight loss. The patient was diagnosed with SMA type 2 at the age of 11 months, has three copies of the survival motor neuron-2 gene, and regularly received Nusinersen treatment. A history of scoliosis surgery at the age of nine was noted, after which weight gain ceased. Currently, the patient uses a wheelchair and engages in physical therapy exercises twice a week. The patient, who has regular measurements taken at the family health center, was reported to have a body weight of 30.5 kg and a height of 150 cm. According to the World Health Organization (WHO) criteria, his body mass index (BMI) for age stands at 13.6 kg/m^2, with a BMI Z-score for age of -3.04. In terms of the biochemical parameters, his plasma creatinine value was noted as 0.09 mg/dL. The vitamin, mineral, and enzyme levels within his nutritional profile were within normal ranges.

The patient's family had previously implemented the Gut and Psychology Syndrome (GAPS) diet. The GAPS diet is an elimination diet based on the exclusion of all grains, pasteurized dairy products, starchy vegetables, and refined carbohydrates from the diet for an extended period. In its place, a dietary plan is followed, which includes homemade bone broths, vegetable juices, animal products, and fats. The family discontinued the implementation of the GAPS diet due to its adverse effects on the patient. At the first interview, it was reported that the patient ate three meals a day and had no chewing or swallowing problems. In general, it has been reported that there is no food selectivity when consuming all meals cooked at home. The patient experienced occasional constipation. When the three-day food record was examined, it was observed that he received an average of 1100 kcal (36 kcal/kg) of daily energy from nutrition. It has been calculated that 52.5% of the energy intake comes from carbohydrates, 14.5% from proteins, and 33% from lipids. It was determined that the vegetable intake is low and the individual consumes 8 grams of fiber daily. An initial diet containing 50 kcal/kg of energy and 1.85 g/kg of protein was planned to ensure weight gain. In the prepared 1500-kcal diet, 50% of the energy comes from carbohydrates, 15% from proteins, and 35% from lipids. The fibre content of the diet was 21 g. Daily fluid consumption is recommended to be at least 1500 ml. The patient was weighed before each interview.

The telenutrition counseling process consisted of monthly follow-ups conducted via the Zoom Pro (https://zoom.us/) program. The initial interview lasted for 60 minutes, during which a detailed anamnesis was conducted. Based on the patient's evaluation, a weight increase of 15% (5 kg) was targeted over a six-month period with diet monitoring. The patient and family were introduced to the 'Food Exchange List', explaining how their daily diet could be modified using this list. Written materials were provided to the patient, concerning the management of the diet process, daily meal plans, menu samples, a diet exchange list, and recommendations. Subsequent interviews were conducted for 30 minutes. Adherence to the diet during this process was assessed based on the information provided by the patient and the family. Recommendations were given to address difficulties encountered in adhering to the diet program. The patient's dietary follow-up process is summarized in Table 1.

The high educational level and motivation of the family had a significant impact on the adherence process to the diet. After six months of telenutrition counseling, the patient's severe malnutrition improved to a moderate level. He reported enhanced arm mobility during physical activities. The patient also expressed good compliance with the dietary regimen and expressed satisfaction with the monitoring process. The family requested continuation of dietary monitoring through telenutrition counseling.

Table 1. Telenutrition consultancy process

Interviews (Every month)	Body Mass (kg)	BMI (kg/m^2)	BMI for age Z-score	Interview details
1. Interview	30.5	13.6	-3.04	Detailed anamnesis was performed, and the anthropometric and biochemical parameters were evaluated. A targeted diet was developed, and the corresponding goals were established. Subsequently, nutritional education was provided to the patient.
2. Interview	31.5	14.0	-2.71	A question-and-answer session was conducted, and no problems were identified.
3. Interview	32.1	14.3	-2.54	A question-and-answer session was conducted, and constipation was observed.
4. Interview	32.3	14.4	-2.50	A question-and-answer session was conducted. An increase in body temperature was noted for several days following the administration of drugs for scoliosis treatment. This side effect disrupted the patient's adherence to the dietary regimen.
5. Interview	33.0	14.7	-2.30	A question-and-answer session was conducted, and no problems were identified.
6. Interview	33.9	15.1	-2.06	A question-and-answer session was conducted, and no problems were identified.
7. Interview	35.0	15.6	-1.75	A question-and-answer session was conducted, and no problems were identified. It was also noted that the Nusinersen treatment would be administered later in the month.

BMI: Body mass index

DISCUSSION

Nutrition is a very important issue for patients with SMA, and no dietary model has been developed based on scientific evidence. The medical nutrition process is managed by expert dietitians who prepare a personalized plan according to the patient's symptoms. Throughout this process, detailed anamnesis is taken from the patient, anthropometric measurements are conducted, biochemical and physiological parameters are assessed, and nutrition programs tailored to socio-cultural values are prepared (2). To plan a diet, it is necessary to first determine the daily energy required by the patient. Patients with SMA have a lower basal metabolic rate than their healthy peers because of muscle wasting or weakness. Simultaneously, movement restrictions reduce physical activity. Therefore, the daily energy requirements of children with SMA are lower than those of their healthy peers (5,6). Some clinics recommend 9-11 kcal/cm of energy intake for patients, according to their height (2). Energy calculation for height was not performed in this patient because of bone problems. When the patient's three-day food record was examined, he received an average of 37 kcal/kg of energy. International organizations recommend an average energy intake of 60 kcal/kg per day for healthy 13-year-old boys (7). To increase the weight of the patient, a diet program containing an average of 50 kcal/day (1500 kcal) of energy was determined in the first stage, taking into account the daily food consumption amount. Evidence of the percentage of energy from macronutrients in the diet of patients with SMA is limited. In general, the macronutrient distributions in SMA Type 2 patients were similar to those of their healthy peers. In addition, considering muscle loss, it is important to provide 1-2 g/kg of protein intake (2).

While planning the nutrition program for patients, the number and content of meals should be planned according to the patient's living conditions. In general, the energy requirement per body weight of children is higher than that of adults. In addition, their stomach capacity is lower than that of the adults. For this reason, the number of meals is increased to meet the high daily energy requirement. They are fed little and often. At the same time, it is necessary to provide a variety of food groups such as meat, milk, vegetables, fruits, and cereals at every meal, especially to avoid micronutrient deficiencies (8). The number of meals for the patient was planned according to living standards and was determined to be three main meals and two snacks, with high nutritional diversity provided at each meal. In addition, fibre and fluid consumption are increased to prevent constipation. In general, the recommendation for 14 g of dietary fibre per 1000 kcal has been adhered to (8).

Factors such as weight, height, BMI, blood parameters, and body tissue distribution are used in the follow-up of patients with severe malnutrition. Follow-up was performed according to the WHO growth curves. In these curves, it is recommended to follow up BMI values according to age in children over 10 years of age. (9). Height measurements and BMI values can be misinterpreted because of wheelchair use and scoliosis problems. Therefore, blood parameters and weight follow-up are prioritized during patient follow-up. The fact that telenutrition counseling is carried out remotely does not allow for physical examinations and measurements. The patient's anthropometric measurement data were regularly provided by the family in the same place and with the same measurement tool.

Telenutrition offers great opportunities for healthcare professionals, especially for patients with limited mobility (3). Although our patient lived in a different city, he regularly participated in his appointments. The appointment times were flexible and could be arranged. The patient had participated in their appointments using only the Internet and computers without experiencing transportation problems, so he saved time and money. It survived the stress factors of the hospital environment and transportation. During the COVID-19 process, similar positive results were seen in a study examining the medical nutrition treatment of phenylketonuria patients in Türkiye (10). Similar benefits have been reported in patients with obesity, cancer, spinal cord injury, kidney disease, and surgery (3).

The educational level of the family holds significant importance in the adherence process to medical nutritional therapy. This process is also valid for telenutrition counseling. It is necessary for the family to understand their education, actively utilize telecommunication systems, and possess the required educational level to maintain adherence to the diet (11). The high level of education of both parents in this case has increased the effectiveness of telenutrition counseling, facilitating adherence to the diet.

For patients who have difficulty reaching experts, such as those with rare diseases, telenutrition systems should be developed and dietitians should be able to actively use these systems (12,13). In addition, telenutrition systems have legal problems such as patient rights, ethical issues, health payments, or taxation. International organizations have supported the development of telenutrition systems by publishing various guidelines (3,14). Developed in Türkiye, smailebeslen.com is the first and only free telenutrition system and serves SMA patients.

Studies related to the nutrition of patients with spinal muscular atrophy have predominantly focused on tube feeding or identifying the nutrition-related challenges faced by these individuals. There is a lack of extensive research involving the detailed calculations required for planning the medical nutrition process for patients and monitoring adherence to the diet. Particularly, there is a scarcity of detailed studies concerning oral nutrition and the management of the nutritional process (15,16). It is believed that this case study, providing detailed insights into the management of oral nutrition in a spinal muscular atrophy patient, would contribute significantly to the literature in this area.

CONCLUSION

Nutritional problems are common in patients with SMA. It can be difficult for patients to access medical nutrition therapy services due to their mobility limitations. Telenutrition support has positive effects on patients' nutrition. Hence, the development of customized telenutrition systems for SMA patients is recommended. Training of dietitians to work within these systems and the involvement of expert dietitians in the counseling process are necessary.

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Propafenone Induced Takotsubo Cardiomyopathy: A Mere Coincidence or A New Causal Relationship?

Propafenona Bağlı Gelişen Takotsubo Kardiyomiyopatisi: Sadece Bir Tesadüf mü, Yeni Bir Nedensel İlişki mi?

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ABSTRACT

Propafenone is a class 1C antiarrhythmic drug that blocks sodium channels and is used in the treatment of arrhythmia. Because of its rapid effect on terminating paroxysmal episodes of atrial fibrillation, it can be used as a pill-in-the-pocket. In patients with structural heart disease, it is less preferred due to cardiotoxic effects in long-term use. Although propafenone use is known to cause several cardiovascular side effects, the development of Takotsubo cardiomyopathy is unknown. Propafenone toxicity at standard doses is a rare condition. Propafenone plasma concentrations may increase through inhibition of cytochrome P450 2D6 and complete inhibition of 2D6 metabolism can increase propafenone levels by up to 3 to 10 times. In this case report, we aimed to present a 37-year-old female patient who developed Takotsubo cardiomyopathy and cardiogenic shock after the first dose of propafenone use and recovered with medical treatment.
Keywords: Takotsubo; propafenone; cardiomyopathy.

ÖZ

Propafenon, sodyum kanallarını bloke eden ve aritmi tedavisinde kullanılan, sınıf 1C antiaritmik bir ilaçtır. Atriyal fibrilasyonun paroksismal ataklarını sonlandırmadaki hızlı etkisi nedeniyle "cep hapı" olarak kullanılmaktadır. Uzun süreli kullanımlarda meydana getirdiği kardiyotoksik etkilerinden dolayı yapısal kalp hastalığı olan bireylerde daha az tercih edilmektedir. Propafenon kullanımının çeşitli kardiyovasküler yan etkilere neden olduğu bilinmesine rağmen, Takotsubo kardiyomiyopatisi gelişimi bilinmemektedir. Standart dozlarda propafenon toksisitesi nadir olarak gözlenen bir durumdur. Propafenon plazma konsantrasyonları, sitokrom P450 2D6'nın inhibisyonu yoluyla artabilir ve 2D6 metabolizmasının tamamen inhibisyonu, propafenon düzeylerini 3 ila 10 kata kadar arttırabilir. Bu vaka sunumunda ilk doz propafenon kullanımı sonrasında Takotsubo kardiyomiyopatisi ve kardiyojenik şok gelişen ve medikal tedavi ile düzelen 37 yaşında kadın hastanın sunulması amaçlanmıştır.
Anahtar kelimeler: Takotsubo; propafenon; kardiyomiyopati.

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INTRODUCTION

Takotsubo cardiomyopathy (TC) is a reversible cardiomyopathy in which the left ventricular apical region is akinetic and basal parts are hyperkinetic. Although it may rarely have a mortal course, it usually resolves within a few weeks with supportive treatment (1). Although the pathophysiology is not known exactly, it has been reported that it may be secondary to high catecholamine release, rheumatological

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diseases such as systemic lupus erythematosus, and drug use (1-3). Although the development of TC secondary to anti-arrhythmic drugs such as flecainide has been reported, the development of TC due to propafenone use with the same mechanism of action is unknown (4,5). Development or exacerbating of TC secondary to propafenone use was first seen in our case.

CASE REPORT

A 37-year-old female patient was admitted to the emergency room with a presyncope. She had reported feeling unwell the evening of the presentation, with palpitation and chest pain. In the emergency service, she was found to be hypotensive with a systolic blood pressure of 50/30 mmHg and a heart rate of 73 beats/min. Other vital signs were normal (respiratory rate: 10/minute, SaO_2: 93% -without oxygen support-, and body temperature: 36.8 °C). Physical examination showed: pale color, cold extremities, low-amplitude pulses, and normal cardiac examination with no additional sounds and murmurs. There was also nothing remarkable in her lung examination.

In history, she had been seen by a cardiologist due to palpitation one year ago, and her cardiac examination was normal, but she had paroxysmal atrial fibrillation in a 24-hour Holter evaluation. She had been prescribed Propafenone 300 mg (Rytmonorm, Abbott) but she did not take the drug. Further questioning revealed that she had lost her child three years ago and had not had any other stress factors in recent days. Upon the onset of palpitation, she remembered the medicine prescribed by the doctor and took 4 tablets - 600 mg propafenone for the first time ever. Later, she was brought to the emergency service as her condition worsened and she was about to faint.

In the laboratory tests, her blood glucose level: 97 mg/dL, creatinine: 0.9 mg/dL, urea: 33 mg/dL, AST: 83 IU/L, ALT: 106 IU/L, sodium: 141 mEq/L, potassium: 3.9 mEq/L, magnesium: 1.9 mg/dL, calcium: 8.3 mg/dL, albumin: 3.4 g/dL, pH: 7.21, pO_2: 76 mmHg, pCO_2: 35 mmHg, HCO_3: 14 mEq/L, SaO_2: 92%, lactate: 4.2 mmol/L in arterial blood gas. Other biochemical tests were normal. D-dimer was 287 ng/mL (upper limit: 500 ng/mL) and troponin was 0.04 ng/mL (upper limit: 0.06 ng/mL). Her electrocardiography (ECG) showed slow atrial fibrillation and a wide QRS segment (180 ms) along with a long corrected QT interval (574 ms) (Figure 1A). Bedside transthoracic echocardiographic examination revealed akinetic left ventricular apex and hyperkinetic basal segments (Figure 2, Video 1). Cerebrovascular disease was excluded by cranial tomography and magnetic resonance imaging. The patient was hospitalized with a pre-diagnosis of cardiogenic shock and TC. Intravenous isotonic fluid with an infusion rate of 70 ml/h, dopamine with an infusion rate of minimal tolerated dosage -4 mcg/kg/min- to keep blood systolic pressure >100 mmHg and supportive sodium bicarbonate were administrated. The vital signs of the patient were followed up with continuous monitoring. Her ECG showed ST-segment elevation in D2, D3, and aVF with reciprocal ST-segment depression in D1 and AVL (Figure 1B) seen in control ECG upon she had stated chest pain. The patient was taken to the catheter laboratory with the diagnosis of acute inferior MI. Normal coronary arteries were observed in coronary angiogram while ventriculography revealed apical ballooning and hyperkinetic basal segments supporting the TC (Figure 3, Video 2). Later in her ECG follow-ups, V1 and V2 leads showed coved ST segment elevation that mimics channelopathy syndrome. An upper lead ECG was taken to confirm changes and the Brugada pattern became evident (Figure 1C). Following initial sodium bicarbonate administration with two ampules, continuous sodium bicarbonate infusion was started with one ampule/hour dosing. Within supportive treatment, her hypotension had resolved, and she was weaned off the dopamine and sodium bicarbonate infusions after 4 hours. The patient's hemodynamic values were maintained normal without any support. Blood gas parameters, QRS complex (78 ms), and corrected QT interval (473 ms) returned to normal on the day after hospitalization (Figure 1D). Liver function tests and

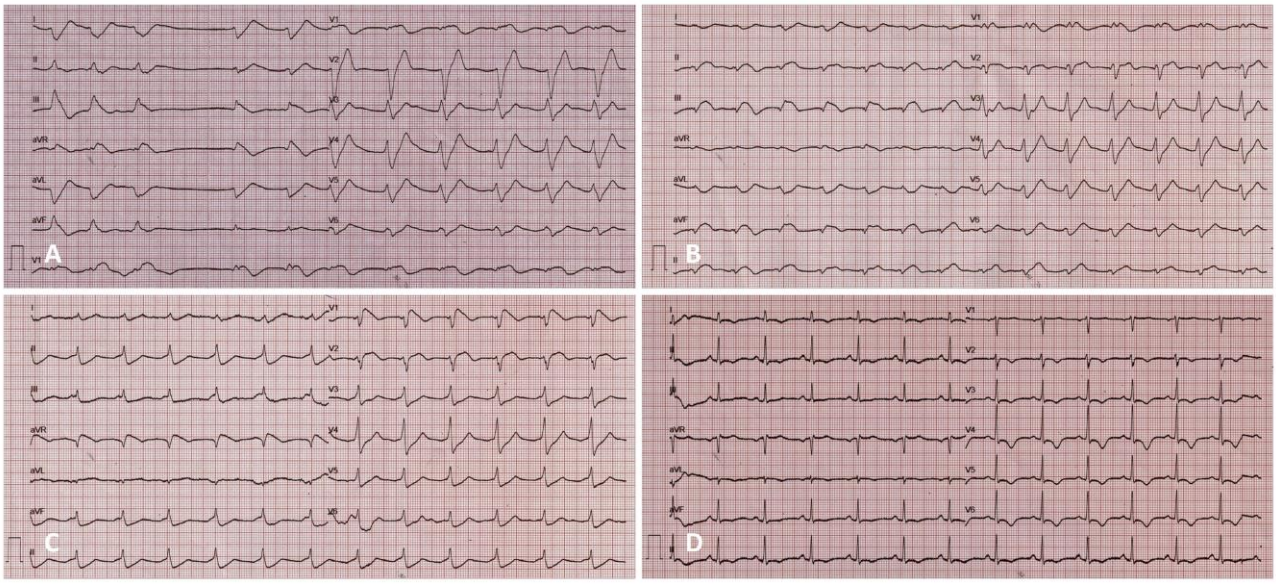


Figure 1. Electrocardiography; **A)** on arrival, **B)** ST-segment elevation in inferior leads, **C)** Brugada pattern, **D)** after treatment

echocardiographic parameters returned to normal on the 3rd day of hospitalization (Video 3). At discharge (day 7), ST segment depression in D1, aVL, V5, and V6 and T negativity were maintained with normal QRS and QTc durations. An electrophysiological study was recommended for the patient.

DISCUSSION

Propafenone can provoke the development of congestive heart failure with a negative inotropic effect (1). In cases of acute toxicity, cardiovascular deterioration is observed mainly in the form of hypotension, convulsion, bradycardia, ventricular arrhythmias, QRS widening, and heart blocks (6). In this case, it was the first time being described propafenone toxicity and the development of TC accompanied by cardiogenic shock after ingestion of 600 mg of propafenone orally. Typical propafenone-related ECG changes have supported TC was dependent on propafenone use. Since it was learned that the patient had taken normal doses of propafenone and more than two hours had passed since the use of the drug, gastric lavage, activated charcoal, or aspiration was not performed. Temporary pacemaker implantation was not required as her condition improved with medical treatment. Sepsis, meningitis, pulmonary embolism, and acute myocardial infarction which may cause confusion and hypotension were not considered, since troponin, D-dimer, C-reactive protein, brain tomography, and magnetic resonance imaging were close to normal limits. There was no increase in troponin in our case. It has been reported that 10% of TC patients do not have an increase in troponin (4). Interestingly, it is observed that there is no increase in troponin in almost all TC patients developing secondary to drug use.

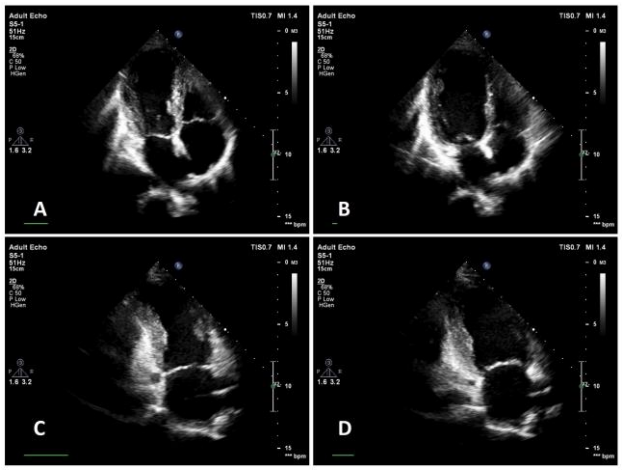


Figure 2. Transthoracic echocardiography, apical view **A)** 4 chamber, left ventricular apex aneurysmatic basal segment hyperkinetic, **B)** 4 chamber, normalized, **C)** 2 chamber, left ventricular apex aneurysmatic basal segment hyperkinetic, **D)** 2 chamber, normalized

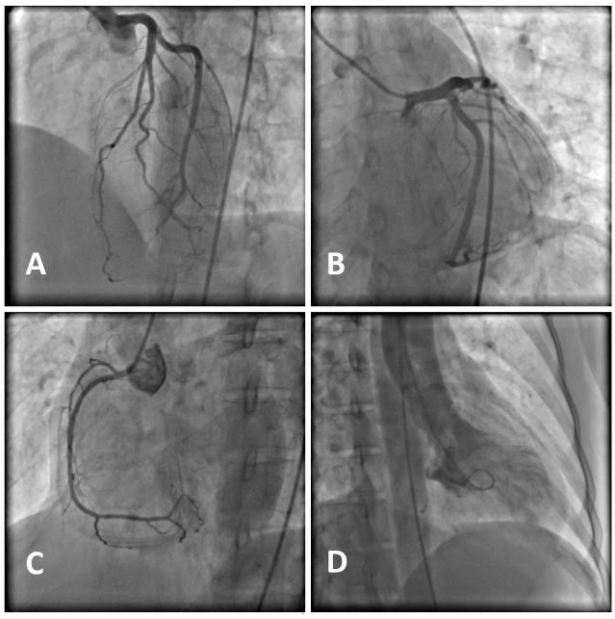


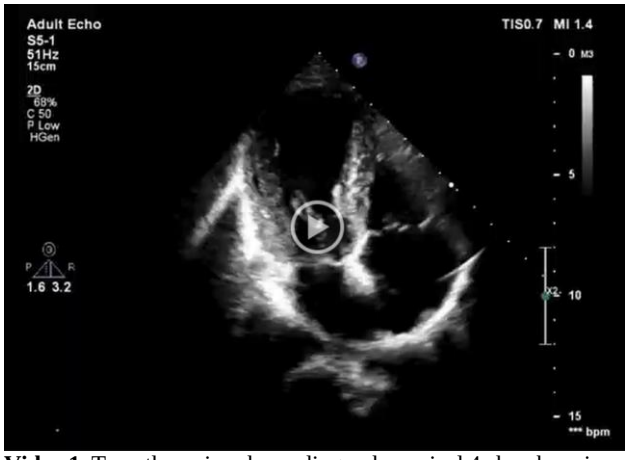
Figure 3. A, B, C) Coronary angiography of normal coronary arteries, **D)** ventriculography left ventricular apex aneurysmatic basal segment hyperkinetic

It is known that propafenone plasma concentrations may increase through inhibition of cytochrome P450 2D6. Due to the low bioavailability, steady-state and/or peak concentrations of propafenone may increase significantly in the presence of a cytochrome P450 inhibitor. Assuming a 10% to 30% bioavailability, complete inhibition of 2D6 metabolism can increase propafenone levels by up to 3 to 10 times (7). Acute toxicity has been reported between the range of 675 mg daily therapeutic dosage to 8.1 g one-time ingestion (7,8).

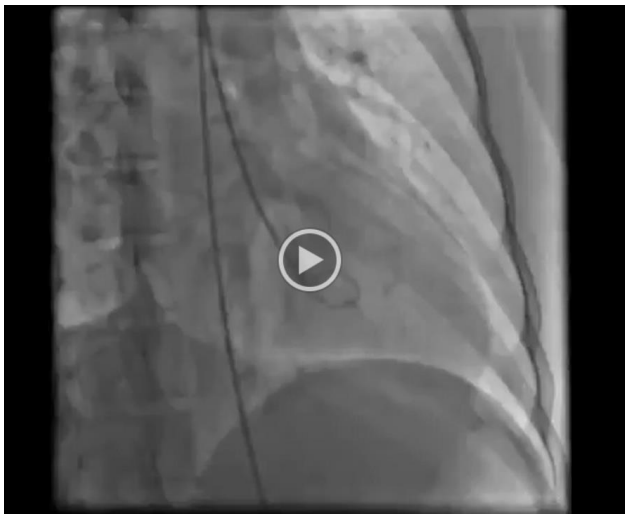
As the facility did not have the necessary equipment to measure the serum propafenone level, the diagnosis of propafenone toxicity could not be made quantitatively. However, the clinical condition of the patient at the time of admission, the absence of electrolyte imbalance, the observed ECG changes, and the fact that she did not use any medication other than propafenone focused us on this diagnosis. Opinions are controversial about the necessity of measuring serum propafenone levels in cases of acute toxicity. The patient's recovery with sodium bicarbonate administration and positive inotropic supportive treatment, which was applied with a pre-diagnosis of acute toxicity, strengthened the diagnosis. The exact incidence of such complications at therapeutic doses, especially at first use, is unknown. The possibility of such complications even at this dose is an important point to be kept in mind during propafenone use.

Propafenone can trigger heart failure by blocking sodium channels in cardiomyocytes (9). For this reason, it is not recommended in patients with heart failure. Viland et al. (10) reported a case of TC due to flecainide overdose in support of our case. Like propafenone, flecainide is also a negative inotropic anti-arrhythmic drug blocking Na^+ channels in cardiomyocytes. These cases show that Na^+ channel blockade in cardiomyocytes may play a role in TC pathogenesis. The ECGs of the case of Viland et al. (10) are similar to our case. Both cases were admitted to the emergency service with cardiogenic shock and clinical manifestation of TC. Although propafenone was taken at a normal dose in our case, flecainide was taken in toxic doses in the case of Viland et al. (10). Therefore, their case was more aggressive. The case was intubated and developed pulmonary edema, and the length of hospitalization was longer (7 days vs. 17 days).

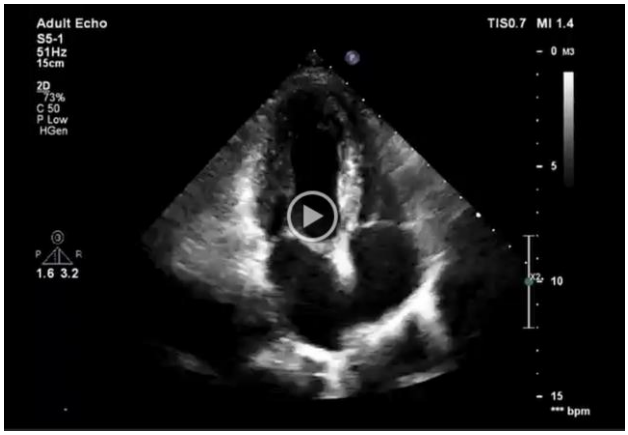
Since propafenone's affinity for sodium channels decreases at high pH levels and exhibits competitive binding on sodium channel, sodium bicarbonate application is widely recommended in the treatment (11). Another important point was not to use high-dose prolonged positive inotropic agents. In this case, we used dopamine as a positive inotropic agent with close monitoring to find the proper dosage to maintain blood pressure and hemodynamics normal and ceased as soon as possible. As TC can occur due to high catecholamines and positive inotropic agents, using these agents as they were merely needed is crucial.



Video 1. Transthoracic echocardiography, apical 4 chamber view, left ventricular apex aneurysmatic basal segment hyperkinetic



Video 2. Ventriculography left ventricular apex aneurysmatic basal segment hyperkinetic



Video 3. Transthoracic echocardiography, apical 4 chamber view, normal left ventricular systolic function

Brugada pattern and acute ST-segment elevation myocardial infarction-like ECG development secondary to propafenone intake has been reported (12-18). The detection of the Brugada pattern and acute inferior ST-segment elevation myocardial infarction-like ECG in the ECG follow-ups of our case supports the current publications. Although Brugada-like ECG changes have generally been reported with propafenone intake above therapeutic doses, there are publications reporting that this pattern can also be observed in therapeutic doses (15,17). A Brugada type 1 ECG pattern was observed but the patient did not meet any clinical criteria for Brugada Syndrome. Therefore, these changes were attributed to propafenone-induced Brugada phenocopy.

Due to the first-pass hepatic elimination effect, bioavailability is not predictable and the elimination half-life of propafenone varies depending on whether the patient's metabolizing pathways are weak or vigorous (18-20). These individual differences and important clinical changes necessitate close ECG follow-up after propafenone initiation. Especially, having the history of achieving a therapeutic effect with very high or very low doses for a disease is another important point that the toxic effects of propafenone in these individuals may occur at lower doses.

CONCLUSION

Propafenone, a medication preferred for arrhythmia control in cases where cardiac functions are known to be normal, can lead to fatal outcomes in sensitive individuals. Even though the goal is treatment, individuals taking it should be cautioned about potential side effects and worsening of their clinical condition at the beginning of treatment. On the other hand, TC is a trending clinical condition and should be always considered when inconsistent cardiac disturbances and arrhythmias cannot be explained by routine clinical reasons.

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

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Simultaneous Intratumoral Hemorrhage in Multiple Brain Metastases of Endometrioid Carcinoma: A Rare Presentation of A Rare Metastasis

Endometrioid Karsinomanın Çoklu Beyin Metastazında Eşzamanlı Tümöriçi Kanama: Nadir Bir Metastazın Nadir Bir Bulgusu

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ABSTRACT

Metastasis of endometrioid carcinomas to the brain is rare, usually solitary, and has a poor prognosis. Intratumoral bleeding is also a poor prognostic factor for metastatic brain tumors but rarely occurs in gynecological cancers. A female patient who was under chemotherapy for endometrioid carcinoma was admitted to the emergency department for recent deterioration and seizures. She had confusion and left hemiparesis, along with an elevated systolic blood pressure and platelet count of 45,000 /mL. An emergent computerized tomography and subsequent magnetic resonance imaging revealed multiple hemorrhagic metastatic lesions in both hemispheres and cerebellum. Brain metastasis of endometrioid carcinoma is rare and can present with intratumoral hemorrhage, which is associated with a poor prognosis and is more likely to occur in the setting of low platelet counts. This case highlights the importance of monitoring brain metastasis in patients with endometrioid carcinoma and considering the possibility of intratumoral hemorrhage.
Keywords: Endometrioid carcinoma; brain metastasis; hemorrhage; thrombocytopenia.

ÖZ

Endometrioid karsinomanın beyin metastazı nadir, genellikle tekil ve kötü prognozludur. Tümör içi kanama da metastatik beyin tümörleri için kötü prognoz faktörü olmakla birlikte jinekolojik kanserlerde nadiren gerçekleşir. Endometrioid karsinoma nedeniyle kemoterapi tedavisi devam etmekte olan bir kadın hasta, bilinçte kötüleşme ve nöbet nedeniyle acil servise getirildi. Konfüzyon ve sol hemiparezi mevcut idi. Sistolik kan basıncı yüksek, trombosit sayısı ise 45.000 /mL idi. Acil bilgisayarlı tomografi ve takiben çekilen manyetik rezonans görüntülemede her iki hemisferde ve serebellumda multipl hemorajik metastatik lezyon saptandı. Endometrioid karsinomanın beyin metastazı nadirdir ve tümör içine kanama ile prezente olabilir -ki kötü prognozla ilişkilidir ve daha çok düşük trombosit sayısı durumunda ortaya çıkabilir. Bu vaka endometrioid karsinomalı hastaların beyin metastazı açısından takip edilmesinin ve böyle durumlarda tümör içi kanama olasılığının göz önüne alınmasının önemini vurgulamaktadır.
Anahtar kelimeler: Endometrioid karsinoma; beyin metastazları; hemoraji; trombositopeni.

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INTRODUCTION

Endometrioid carcinomas are the most common type of endometrial cancer, but they may also arise from ovaries. Regardless of origin, they have similar, though not identical, properties and behavior (1). They usually spread locally and distant metastases are rare and mostly involve the lung, liver, and bone (2,3). The metastasis

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of endometrioid cancer to the brain is extremely rare and has an incidence of 0.3-1.16% in endometrial type and 0.49-11.54% in ovarian type (4,5). These metastases are associated with a poor prognosis and tend to occur as solitary lesions (6-9).

Since these tumors are rare, our knowledge of these metastases is limited (10,11). This case report aimed to present an unusual case that admitted with simultaneous hemorrhage in multiple metastatic lesions.

CASE REPORT

A 64-year-old female patient with right abdomen pain was diagnosed with a right adnexial mass. A tru-cut biopsy revealed a low-grade endometroid carcinoma, but its origin organ could not be determined histopathologically. Since concurrent metastatic thorax lesions were detected neither a surgical treatment nor an endometrial sampling was performed to determine its origin. She was put into a chemotherapy regimen of 9 cycles. Apart from occasional delays due to hematological disturbances, the treatment continued as planned.

One and a half months after the 7th chemotherapy cycle, which was six months after the initial diagnosis, she had progressive deterioration for 3 days. After rapid worsening accompanied by 2 episodes of generalized tonic-clonic convulsions, she was admitted to the hospital by her relatives. Her Glasgow Coma Scale score was 10/15 (E3M5V2) and she had left hemiparesis. An emergent computed tomography (CT) scan revealed multiple foci of hyperdense lesions suggestive of metastatic hemorrhage (Figure 1). Magnetic resonance imaging (MRI) revealed multiple contrast-enhancing mass lesions in the left temporooccipital, right temporoparietal, right frontal, and right frontoparietal area, of which the right temporoparietal one was the largest with 46 mm diameter. The lesions compressed the right lateral and 3rd ventricles and caused a midline shift of 12 mm towards the left (Figure 2). Her platelet (PLT) count was 45000 /mL.

The first-degree relatives of the patient were informed about the poor prognosis regardless of surgical treatment and offered decompressive craniectomy for palliative purposes but they rejected it. In-depth history from relatives revealed that she was having an argument prior to deterioration and systolic blood pressure measured at the emergency department was higher than her baseline values. Conservative medical management was initiated in the intensive care unit, but the patient eventually deceased. Written informed consent for this study was obtained from a first-degree relative of the patient since the patient was not able to cooperate.

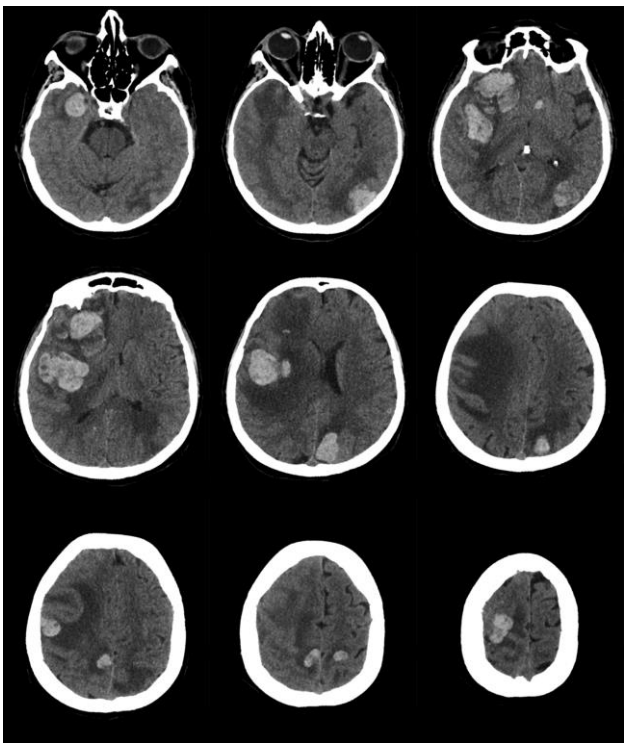

Figure 1. Axial CT scan shows significant hemorrhage and swelling in the frontal, temporal, and parietal lobes of the right hemisphere, with the largest lesion measuring 4x4.5 cm located in the parietal lobe. Additionally, smaller lesions with similar characteristics are observed in the left hemisphere, specifically in the parietal and occipital lobes as well as the left caudate nucleus. The edema causes partial effacement of the right lateral ventricle and a midline shift to the right by 12 mm.

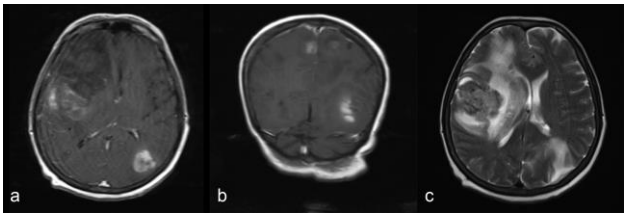

Figure 2. MRI images of the metastatic lesions are seen in **(a)** contrast-enhanced T1-weighted axial, and **(b)** coronal sequences. The extent of edema and midline shift is best visualized in **(c)** T2-weighted axial sequence.

DISCUSSION

The most frequently seen gynecological malignancies are endometrial and ovarian cancers (12). 70-80% of endometrial carcinomas and 10% of ovarian carcinomas are endometrioid carcinoma. They evolve from similar precursor cells and though they are not identical (as a result of different microenvironments), they have similar clinicopathological features (1).

Though brain metastasis is rare in endometrial and even rarer in ovarian endometrioid cancers, it is associated with poor prognosis (8). This may be due to the fact that most patients with brain metastasis are already in the advanced stages of the disease (10). Since this type of metastasis is rare, there is currently no standard treatment algorithm for brain metastasis, but treatment options include whole-brain radiotherapy, surgical resection, stereotactic radiotherapy, chemotherapy, and molecular targeting (9).

Since brain metastasis is rare, brain imaging is not typically included in routine follow-up. This type of metastasis is rarely diagnosed incidentally and patients often present with neurological symptoms depending on the location of the lesions (4,11). In our case, the patient had no neurological symptoms until intratumoral hemorrhage occurred.

Overall and gross intratumoral hemorrhage incidence in central nervous system tumors is 14.6% and 5.4% respectively. And these hemorrhages generally present in acute-on-chronic settings, rather than acute collapse (13). In a recent study, the incidence of intratumoral hemorrhage in metastatic brain tumors was 12.3% (14). Such hemorrhage is associated with poor prognosis in metastatic brain tumors and is more common in certain types of cancer such as melanoma, choriocarcinoma, thyroid carcinoma, hepatocellular carcinoma, and renal cell carcinoma (15,16). Hemorrhagic brain metastasis of endometrioid carcinoma, on the other hand, is rarely reported in the literature (17,18).

The exact mechanism behind intratumoral hemorrhage is not well understood, however, it is thought to be caused by a combination of factors such as endothelial proliferation with obliteration, vessel compression or distraction due to rapid tumor growth, and the abnormal characteristics of tumor vessels such as thin walls, poor development, and dilation, which contribute to the fragile structure of these vessels (13,19). Tumor invasion and necrosis can also lead to bleeding (20). Anticoagulation therapy is a known risk factor for intratumoral hemorrhage, while chronic hypertension is not (13,21). Additionally, Kondziolka et al. (13) reported thrombocytopenia (PLT <50000 /mL) in 4 out of 49 cases of macroscopic intratumoral hemorrhage. Although spontaneous hemorrhage is not typically expected unless the PLT count falls below 10000 /mL, the brittle nature of tumor vessels may predispose them to bleeding at a PLT count above 10000 /mL. In the case discussed, the bleeding was not restricted to a single lesion but occurred simultaneously in multiple lesions suggesting a global cause. It is possible that a temporary hypertensive period led to an increase in intracranial pressure, causing the rupture of fragile tumor vessels, and the bleeding was aggravated by improper hemostasis due to low PLT count. To the best of our knowledge, this case is among rare examples of hemorrhagic brain metastasis of endometrioid adenocarcinoma. Moreover, while previous cases had solitary lesions, this case had multiple metastatic lesions that bleed simultaneously.

CONCLUSION

Brain metastasis of endometrioid carcinoma is rare but should not be overlooked. Routine brain imaging should be considered to diagnose the disease at an early stage before multiple metastases occur. It is also important to note that the abnormal vasculature of metastatic brain tumors can make them prone to bleeding in thrombocytopenia, even at levels that would not typically result in spontaneous bleeding in healthy individuals.

Informed Consent: Written informed consent was obtained from a first-degree relative of the patient (since the patient was not able to cooperate) for publication and images.

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AUTHOR GUIDELINES

Please visit the webpage to Submit Manuscript and also for detailed information about the Author Guidelines, Ethical Principles and Publication Policy, and Review Process (https://dergipark.org.tr/en/pub/dtfd).

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YAZARLARA BİLGİLENDİRME

Makale Göndermek için ve ayrıca Yazar Yönergeleri, Etik İlkeler ve Yayın Politikası ve Değerlendirme Süreci hakkında ayrıntılı bilgi için lütfen web sayfasını ziyaret edin (https://dergipark.org.tr/en/pub/dtfd).

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Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörleri Komitesi (ICMJE, http://www.icmje.org/recommendations/), Dünya Tıbbi Editörler Birliği (WAME, https://www.wame.org/policies) ve Yayın Etiği Komitesi (COPE, https://publicationethics.org/guidance/Guidelines) kriterlerine uygun olarak hazırlanmalıdır.

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

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uyulması zorunludur. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
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- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- İngilizce anahtar kelimeler Medical Subject Headings (MESH, http://www.nlm.nih.gov/mesh/MBrowser.html) ve Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT, http://www.bilimterimleri.com) ile uyumlu olarak verilmelidir.

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

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

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

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

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

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