ISSN: 1305-9319 | e-ISSN: 1305-9327 | Number: 2 | Volume: 17 | Year: 2021

MEDICAL JOURNAL OF BAKIRKÖY

www.bakirkoytip.org

ISSN: 1305-9319 | e-ISSN: 1305-9327 | Number: 2 | Volume: 17 | Year: 2021

MEDICAL JOURNAL OF BAKIRKÖY

Medical Journal of Bakirköy of is an official scientific Journal of University of Health Sciense Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital (BMJ)

It is published quarterly as 4 issues every year (March, June, September, December).

Medical Journal of Bakirköy is an open Access, free and peer-reviewed journal and indexed in ESCI, EMBASE, Scopus, EBSCO and ULAKBİM TR Dizin.

Instructions for Authors and publication policy is available on our website.

www.bakirkoytip.org

Galenos Publishing House
Owner and Publisher
Derya Mor
Erkan Mor

Publication Coordinator
Burak Sever

Web Coordinators
Fuat Hocalar
Turgay Akpınar

Graphics Department
Ayda Alaca
Çiğdem Birinci
Gülşah Özgül

Finance Coordinator
Sevinç Çakmak

Project Coordinators
Aysel Balta
Duygu Yıldırım
Gamze Aksoy
Gülay Akın
Hatice Sever
Melike Eren
Meltem Acar
Özlem Çelik Çekil
Pınar Akpınar
Rabia Palazoğlu

Research&Development
Nihan Karamanlı
Melisa Yiğitoğlu

Digital Marketing Specialist
Seher Altundemir

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Turkey
Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr **Publisher Certificate Number:** 14521

Printing Date: Haziran 2021
ISSN: 1305-9319 **E-ISSN:** 1305-9327
International scientific journal published quarterly.

Advisory Board

Advisory Board

Advisory Board

Advisory Board

Advisory Board

Advisory Board

Contents

Review

Research

Advisory Board

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:108-114
DOI: 10.4274/BMJ.galenos.2021.57804

Review

Inherited Metabolic Myopathies: Current Diagnosis and Treatment Approaches

Kalıtsal Metabolik Miyopatiler: Güncel Tanı ve Tedavi Yaklaşımları

Melike Ersoy

University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Pediatrics, İstanbul, Turkey

ABSTRACT

Inherited metabolic myopathies (IMMs) are a heterogeneous group of diseases characterized by inherited defects of enzymatic pathways involved in muscle cell energy metabolism. The worldwide incidence of genetic myopathies is about 1/3500, but the incidence of IMMs is unknown. Although it is considered rare compared with other hereditary myopathies, the expansion of neonatal screening programs and the increase in next-generation sequencing genetic methods for diagnosis have shown that the frequency is above the predicted rate. IMM is summarized as the name given to the group that includes defects in glycogen catabolism (glycogenolysis and glycolysis), fatty acid oxidation, Krebs cycle, or mitochondrial respiratory chain and oxidative phosphorylations. They have a broad clinical spectrum that can present symptoms of different severity at any stage of their lifetime. It differs from other myopathies in that they have unique clinical findings. Hence, it requires specific laboratory diagnostic methods and has specific treatments.

This review aims to make the differential diagnosis of metabolic myopathies from other structural myopathies and present current diagnosis and treatment approaches.

Keywords: Inherited metabolic myopathy, diagnosis, treatment

ÖZ

Kalıtsal metabolik miyopatiler (KMM), kas hücresi enerji metabolizmasında rol oynayan enzimatik yolların kalıtsal kusurları ile karakterize heterojen bir hastalık grubudur. Dünya çapında genetik miyopatilerin insidansı yaklaşık 1/3500, ancak gerçek KMM insidansı bilinmemektedir. Yenidoğan tarama programlarının yaygınlaşması ve yeni nesil dizileme genetik yöntemlerinin artması ile diğer genetik miyopatilerle kıyaslandığında nadir olduğu düşünülse de, sıklığın tahmin edilen oranın üzerinde olduğu tahmin edilmektedir. KMM, glikojen katabolizması (glikojenoliz ve glikoliz), yağ asidi oksidasyonu, Krebs döngüsü veya mitokondriyal solunum zinciri ve oksidatif fosforilasyondaki kusurları içeren gruba verilen isim olarak özetlenmektedir. Yaşamın herhangi bir anında farklı şiddette semptomlarla ortaya çıkabilen geniş bir klinik yelpazeye sahiptirler. Kendine özgün klinik bulgulara sahip olmaları, spesifik laboratuvar tanı yöntemlerine ihtiyaç duymaları ve özgün tedavi şekilleri olmasıyla diğer miyopatilerden farklılık gösterirler.

Bu derlemenin amacı, metabolik miyopatilerin diğer yapısal miyopatilerden ayırıcı tanısını yapmak ve güncel tanı ve tedavi yaklaşımlarını sunmaktır.

Anahtar Kelimeler: Kalıtsal metabolik miyopati, tanı, tedavi

Address for Correspondence: Melike Ersoy, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Pediatrics, İstanbul, Turkey
Phone: +90 212 414 7171 E-mail: zeynepcey@hotmail.com ORCID ID: orcid.org/0000-0002-2316-0790

Cite as: Ersoy M. Inherited Metabolic Myopathies: Current Diagnosis and Treatment Approaches. Med J Bakırköy 2021;17:108-114

Received: 18.05.2021
Accepted: 17.06.2021

INTRODUCTION

Myopathies include the clinical disorders of the abnormalities of muscle cell structure or metabolism that lead to dysfunction. Disruption of muscle cells' structural integrity and metabolic status can result from inherited congenital abnormalities, external/internal toxins, inflammation, infection, and electrolyte imbalances. In addition, they can develop due to acquired or hereditary causes. Although they have a broad etiological spectrum, they are classified as the underlying causes (Table1).

Metabolic myopathies are a heterogeneous group of diseases with inherited defects of enzymatic pathways and signaling disorders involved in muscle cell metabolism (1). Furthermore, metabolic myopathies have only isolated muscle involvement and metabolic myopathies that may be associated with other system involvements (2). Metabolic myopathies have severe early manifestations in early childhood as well as late-onset adult types with mild symptoms. Both skeleton and cardiac muscle are high energy-consuming tissues. Exercise intolerance, muscle pain, muscle weakness and stiffness, rhabdomyolysis, myoglobinuria, cardiomyopathy (dilated, hypertrophic, restrictive) constitute general findings (3). Other accompanying involvements can also be related to liver, brain, eye, and glucose hemostasis with high energy requirements.

Energy Metabolism Physiology of Muscle

To understand the clinical findings of metabolic muscle diseases, explaining which substrates and enzymatic pathways the muscle uses during rest and exercise will facilitate understanding its pathology and preparing treatment protocols: Glucose, free fatty acids, and some amino acids and muscle creatine form the energy resources of the muscle (4). During the resting phase, the primary energy source is the mitochondrial beta-oxidation of free fatty acids (5). In the first phase of the exercise, glycogen stored in the muscle is used only for the muscle itself, unlike the liver. In the first few minutes of exercise, sufficient blood flow cannot be achieved yet. Energy is obtained by anaerobic destruction of muscle glycogen (6). Therefore, muscle glycogen, local blood glucose, high energy phosphate compounds are used in the intense short-term exercise. At the same time, free fatty acids are the main energy source in prolonged exercise and fasting.

Defects in any of these pathways glycogen catabolism (glycogenolysis and glycolysis), fatty acid oxidation, Krebs cycle, or mitochondrial respiratory chain and oxidative phosphorylation may cause myopathy (7,8).

Table 1. Classification of myopathies

Metabolic myopathies	**Muscular dystrophies**
Glycogen storage diseases	Duchenne and Becker MD
Fatty acid oxidation defects	
Respiratory chain disorders	
Congenital myopathies	**Inflammatory myopathies**
Central-core disease	Dermatomyositis
Nemaline myopathy	Polymyositis
Centronuclear (myotubular) myopaty	Inclusion body myositis
Congenital dystrophyies	Necrotizing myopathy
Muscle fiber (tybe 1-2) Distribution disorder	Eosinophilic myositis
	Granulomatous myositis
Endocrinological and toxic myopathies	**Myopathies associated with periodic paralysis**

Glycogen Storage Diseases

Glycogen storage diseases (GSDs) are a large group of inherited metabolic diseases with abnormal storage or utilization of glycogen. Of the fifteen, GSD II (acid alpha-glucosidase, Pompe), III (amylo-1,6 glucosidase, Cori, Forbes), V (myophosphorylase deficiency, Mc Ardele), VII [phosphofructokinase (PFK) deficiency, Tarui disease], VIII (phosphorylase b kinase deficiency), and X (phosphoglycerate mutase deficiency) are named "muscle glycogenosis" (9,10).

GSD II: (Pompe disease) is a kind of lysosomal storage disorder caused by an accumulation of glycogen in the lysosome due to a deficiency of the acid alpha-glucosidase enzyme. The incidence of the disease is about 1/40,000 according to the identified cases but is estimated to be more common than is detected by the widespread use of newborn screenings (11). It is classified in classic (infantile form), childhood, juvenile, and adult (late-onset forms) (12). Enzyme replacement therapy (ERT), which is a recombinant human GAA (rhGAA), has given a reasonable positive response (13,14). The histopathological diagnostic findings are the vacuolization and autophagy in the muscle. The diagnosis of Pompe disease can be implemented by dried blood spot screening in suspected patients with high creatine kinase with or without cardiac involvement (15). In addition to ERT, positive, supportive effects of diet (high protein and branched-chain amino acids diets have been used as alternative energetic substrates) and benefits of the antioxidant treatments have also been reported (16).

GSDV: GSDV (Mc Ardel) is characterized by exercise intolerance, muscle cramps, fatigue, weakness, with onset in late childhood. In half of the patients, muscle exercise results in massive creatine kinase elevation and rhabdomyolysis, leading to acute kidney failure. A block of muscle glycogen causes the disease to glucose-6- phosphate due to muscle glycogen phosphorylase deficiency (17).

After a few minutes of rest, patients with GSDV experience muscle pain and fatigue called the "second wind phenomenon." As indicated in the energy metabolism of muscle, fatty acids, the incapable of activating muscle glycogen stores, and patients' continuation of exercise are the leading causes of the "second wind phenomenon." The second wind does not occur in patients with other disorders associated with exercise intolerance (18). A recommended activity management targets to increase both capacity and muscle strength with the moderate-intensity exercise of 150 minutes per week distributed over 5 days per week to increase heart rate by 60%-70% (19,20). The diagnosis is based on the clinical features, subsarcolemmal vacuoles, and glycogen storage with absent myophosphorylase in the muscle biopsy.

Glucose or sucrose intake before exercise ameliorates symptoms in McArdle disease because the metabolic block is upstream of glucose catabolism, whereas it exacerbates muscle symptoms (21). Benefits from diets with high protein or a ketogenic diet, creatine monohydrate supplementation have been reported (22). Since myophosphorylase binds to vitamin B6, pyridoxine with fortified branched-chain amino acids diet may benefit GSDV (23).

GSD VII: GSDVII is characterized clinically by exercise intolerance, muscle cramps, weakness and stiffness in muscles, rhabdomyolysis as seen in GSDV, and an additional finding is a hemolysis (24). The deficiency of the muscle isoform of PFK results in loss of muscle cells and red cell PFK activity, respectively. Although it is generally diagnosed at advanced ages and with mild findings, it has been reported that it has a rare, rapidly progressive, fatal infantile form (25). Unlike GSDV, it is essential for the patient to listen to their body and avoid intense exercise and a diet containing high protein instead of glucose and fructose (26).

Respiratory Chain Disorders (Mitochondrial Myopathies)

Mitochondrial myopathies are the specific myopathies manifested by disruption in ATP synthesis due to genetic reasons that cause dysfunction of oxidative phosphorylation (OXPHOS) (27). The respiratory chain comprises four subunit enzymatic complexes (I, II, III, and IV), which generate a proton gradient across the inner mitochondrial membrane that drives ATP synthesis by complex V (28). CoQ10 and riboflavin are critical components of the mitochondrial respiratory chain, serving as "electron shuttles" between the complexes (29,30). Mitochondrial function is under the control of two genomes; mitochondrial genome (mtDNA) and nuclear genome (nDNA); there may be mitochondrial myopathy caused by pathogenic genetic variants found in any of these genomes. This dual genetic control causes different inheritance patterns [maternal (mtDNA), X-linked, autosomal recessive, autosomal dominant]. In addition, some common mitochondrial myopathies occur de novo (31).

Isolated muscle disease is rare in mitochondrial myopathies. It may affect one or more organs and systems with high energy consumption, such as the central nervous system, heart, liver, and eyes. Muscular findings of the patients can create different clinical phenotypes:

Chronic progressive external ophthalmoplegia (CPEO) can usually be associated with progressive ptosis and ophthalmoplegia with or without double vision and "myopathic face" appearance. CPEO can be seen as an isolated symptom or proximal myopathy, endocrinopathy, as in a Kearns-Sayre syndrome (32).

Proximal myopathy is the most common form of mitochondrial myopathy. Muscle weakness is variable and associated with fatigue. While some patients have a static course, in some patients, this weakness can progress gradually and affect the diaphragm and respiratory muscles, which can be life-threatening.

Exercise-induced muscle pain is a common finding and limits exercise tolerance. Unfortunately, this clinical variability is often confused with other types of muscle diseases by causing rhabdomyolysis.

Fatigue is one of the most common symptoms reported by patients. These cases constitute the most difficult group to diagnose. Disruption in performing simple everyday activities such as cutting with a knife, dressing, hair combing. This can cause psychiatric findings to accompany the clinic in individuals who had fatigue without myopathy.

It may also appear as a part of mitochondrial syndromes with combined system involvement such as Kearns-Sayre syndrome, Mitochondrial encephalomyopathy lactic acidosis, and stroke-like episodes syndrome, myoclonus epilepsy with ragged red fibers (33-35).

Diagnosis of mitochondrial myopathies requires a multidisciplinary approach. After history and physical examination, it includes routine biochemical tests, semi-specific metabolic tests, histopathological and

immunohistochemical tests, enzyme levels of complexes, and genetic tests involved in specific oxidative phosphorylation. One of the hallmarks of mitochondrial myopathies is physiological tests, including oxygen production, consumption, and redox measurements (32). In addition, 31P nuclear magnetic resonance spectroscopy can measure decreased basal levels of high energy phosphate compounds. Today, for patients who cannot be diagnosed with targeted next-generation sequence analysis, gene tests for definitive diagnosis, WES or WGS tests are preferred.

Antioxidant effective coenzimq10, idebenone, riboflavin, nicotinamide, vitamins C and E, lipoic acid, dichloroacetate, and ketogenic diet are applied the treatment with appropriate exercise programs. However, their effectiveness is limited, except for coenzimQ10 deficiency.

Fatty Acid Oxidation Disorders

Fatty acid oxidation (FAO) disorders are inborn lipid metabolism disorders caused by a deficiency of the enzymes needed to utilize fatty acids in mitochondria (36). The five types of FAO disorders present myopathic symptoms are primary carnitine deficiency, defects of beta-oxidation and carnitine transport, multiple acyl-coenzyme A dehydrogenase deficiency, neutral lipid storage disease with ichthyosis (NLSD-I), and neutral lipid storage disease with myopathy (NLSD-M) carnitine palmitoyltransferase deficiency (CPT-II deficiency) (37,38). Carnitine and acylcarnitine profiles include diagnostic findings in tandem MS-MS spectrophotometry for FAO disorders. Its association with dicarboxylic aciduria (suberic, sebacic, adipic acid) in the organic acid profile supports suspicion.

Systemic primary carnitine deficiency: Primary systemic carnitine deficiency is due to a defect in the carnitine transporter (OCTN2) expressed in muscle, heart, kidney, and fibroblasts. This results in impaired FAO in skeletal and heart muscle in the foreground. In addition, renal and bowel wasting of carnitine results in low serum levels and diminished hepatic uptake of carnitine by passive diffusion, which impairs ketogenesis (39). Hypotonia, myopathy, cardiomyopathy (dilated, hypertrophic, or both), and hypoketotic hypoglycemia are the main symptoms. It is diagnosed with a markedly low level of free carnitine in tandem MS. However, it should not be confused with the nutritional carnitine deficiency in preterm and vegan mothers' babies. Carnitine replacement provides complete improvement of clinical findings. According to other FAO disorders, carnitine dosage may have to be increased up to 400-500 mg/kg/day. It is necessary to follow up with routine echocardiography to prevent heart failure due to cardiomyopathy.

Defects of beta-oxidation: Inborn errors of FAO result in energy failure, especially in the heart and skeletal muscle, by causing urinary excretion of acyl-carnitine and acyl-glycine conjugates resulting in secondary carnitine deficiency. Four acyl-CoA dehydrogenases are involved in mitochondrial FAO: short-chain, medium-chain, long-chain, and very-long-chain acyl-CoA-dehydrogenases (SCAD, MCAD, LCAD, VLCAD) (40). Patients usually present an acute clinical attack with severe hypoketotic hypoglycemia, Reye-like acute liver and cardiac failure, and myoglobinuria due to rhabdomyolysis. In some cases, symptoms of muscle cramps, fatigue, and weakness can be observed without an acute metabolic attack. During acute metabolic decompensation, affected individuals should receive an intravenous glucose infusion at the rate of physiological glucose release by the liver according to the patient's age. It has been shown that carnitine intravenous replacement treatment can prevent secondary deficiency. Avoid prolonged fasting and excessive muscle egzersize in long-term treatment and provide energy with a dietary therapy based on fractionated meals rich in carbohydrates and medium-chain triglyceride (MCT) enriched low in fat. It is important for neurological development to supplement the patient with fish oil and walnut oil to avoid essential fatty acid deficiency (41).

Multiple acyl-coenzyme a dehydrogenase deficiency (MADD; glutaric aciduria type 2, GA2): MADD is an autosomal recessively inherited disorder of fatty acid, amino acid, and choline metabolism caused by mutations in 3 different genes: ETFA, ETFB, and ETFDH, which are involved in electron transfer in the mitochondrial respiratory chain. Certain type of FAO defect is also considered a mitochondrial disease. MADD is divided into 3 different groups depending on the heterogeneity of clinical findings: the neonatal-onset form with congenital anomalies; the neonatal-onset form without congenital anomalies; the late-onset form (42). Some of the neonatal-onset conditions can be lethal. Symptoms and age at presentation of late-onset MADD are highly variable and characterized by recurrent episodes of lethargy, vomiting, hypoglycemia, metabolic acidosis often preceded by metabolic attacks. Muscle involvement in the form of pain, weakness, and lipid storage myopathy occurs (43).

Riboflavin (vitamin B2) and CoenzimQ10 supplement often marked improvement of clinical weakness (100 mg 2-3 times daily, 15 mg/kg/day 2 times daily, respectively). A diet high in carbohydrates and low in fat and protein, with carnitine and MCT supplementation, avoiding long fasting periods are the long-term treatment targets.

Carnitine palmitoyltransferase deficiency (CPT-II deficiency): CPT-II deficiency is the most commonly diagnosed disorder of fatty acid metabolism, in which a wide age range and spectral findings are detected. Although it is expected, many cases cannot be analyzed due to difficulties in diagnosis. The symptomatology usually consists of recurrent myalgias and muscle stiffness attacks with weakness and often associated with rhabdomyolysis. The patients are generally asymptomatic between attacks. Clinical symptoms usually are triggered by prolonged exercise or fasting, high fat intake, exposure to cold, viral infections, and even emotional stress, general anesthesia, or medications such as diazepam and ibuprofen. Besides carnitine and diet therapy, Triheptanoin, most likely, can correct the shortage of anapleurotic intermediates to the Krebs cycle (44).

Neutral lipid storage diseases (NLSDs): Refer to two different inherited disorders characterized by the enzymatic deficiencies affecting the lipase adipose triglyceride lipase and its coactivator CGI58 (45). NLSD-I (NLSD with ichthyosis) and NLSD-M (NLSD with myopathy) result in massive lipid accumulation in the leukocytes, skin, muscle, liver, bone marrow, and intestine. NLSD-I presents early-onset ichthyosis associated with mild myopathy, while NLSD-M presents with muscle weakness and muscular atrophy in advanced cases (46). The effects of this defect are the alteration of energy production and the involvement of skeletal muscle that causes progressive myopathy and rarely cardiomyopathy. Muscle weakness is triggered by exercise, fasting, or infections. Unfortunately, no effective treatment exists to date for both NLSD-M and NLSD-M.

Diagnosis in inherited metabolic myopathies: Metabolic myopathies have a broad clinical spectrum, from infantile severe multisystemic disorders to adult-onset myopathies. To suspect these disorders, clinical features such as exercise intolerance and recurrent myoglobinuria need investigation while another group presents fixed weakness and cardiomyopathy as a clinical pattern. Therefore, when presented with such patients, it is most important to "think metabolic."

Before laboratory experiments, an exhaustive individual and family history, a neurological exam, exercise test, and neurophysiological exams are required. The inborn errors of metabolic disorders often present with muscle, heart, and central nervous system involvement. Therefore, their diagnostic workup includes sophisticated techniques ranging from simple biochemical tests to semi-specific and specific metabolic tests (Table 2).

However, now invasive procedures have been replaced by new-generation genetic (next-generation sequencing) diagnostic methods. In cases where structural and metabolic myopathies cannot be distinguished, next-generation sequencing-based "myopathy panels" that include many structural and metabolic genes can be studied.

Using algorithms in diagnosis will speed up the diagnosis and provide the rational use of expensive and time-consuming genetic tests (Figure 1).

Table 2. Diagnostic workup in inherited metabolic myopathies

First-line tests	Second-line tests (functional tests)
- Glucose, urea, creatinine, LDH, CK, ALP, ALT, AST, phosphate, calcium, magnesium	- Ischemic exercise test
- Thyroid function tests	- Exercise or bike ergometry test
- Myoglobin (urine)	- Fasting tests
- Free and acylcarnitines (dry blood)	Third-line tests
- Lactate, pyruvate, ammonia	- EMG
- Amino acids (serum)	- Thorax radiography
- Organic acids (urine)	- ECG, ECHO
	- Muscle biopsy
	Genetic tests (NGS, WES, WGS)

ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CK: Creatine kinase, ECG: Electrocardiography, ECHO: Echocardiography, EMG: Electromyogram, LDH: Lactate dehydrogenase, NGS: Next-Generation Sequencing, WES: Whole exome sequencing, WGS: Whole-genome sequencing

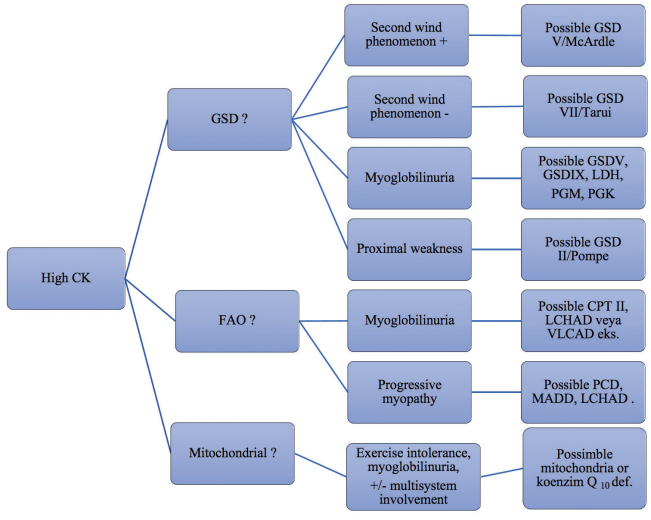

Figure 1. Diagnostic algorithm in inherited metabolic myopathies
CK: Creatine kinase, CPT: Carnitine palmitoyltransferase, FAO: Fatty acid oxidation, GSD: Glycogen storage disease, LCHAD: Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency, LDH: Lactate dehydrogenase, MADD: Multiple acyl-CoA dehydrogenase deficiency, PCD: Primary carnitine deficiency, PGK: Phosphoglycerate kinase deficiency, PGM: Phosphoglucomutase, VLCAD: Very long-chain acyl-CoA dehydrogenase

CONCLUSION

Metabolic myopathies should be considered in the differential diagnosis of exercise intolerance. A detailed clinical approach will help to determine which of the three main disorders (glycogenosis, lipid-related disorders, or mitochondrial diseases) is the underlying cause. Metabolic screening evaluates the second wind phenomenon and other related events, such as fasting, infections, and other catabolic situations. Further pre-exercise carbohydrate intake may provide additional clues to restrict the differential diagnosis. After "think metabolic" in diagnosis, metabolic screening tests should be included in the evaluation.

Treatment of metabolic myopathies primarily relies on avoiding precipitating factors and dietary supplements to bypass the metabolic block.

ETHICS

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

REFERENCES

1. Koo P, Sethi JM. Metabolic Myopathies and the Respiratory System. Clin Chest Med 2018;39:401-10.
2. Tarnopolsky MA. Metabolic Myopathies. Continuum (Minneap Minn) 2016;22:1829-51.
3. Finsterer J. An update on diagnosis and therapy of metabolic myopathies. Expert Rev Neurother 2018;18:933-43.
4. van Loon LJ, Greenhaff PL, Constantin-Teodosiu D, Saris WH, Wagenmakers AJ. The effects of increasing exercise intensity on muscle fuel utilisation in humans. J Physiol 2001;536:295-304.
5. Perry CG, Kane DA, Herbst EA, Mukai K, Lark DS, Wright DC, et al. Mitochondrial creatine kinase activity and phosphate shuttling are acutely regulated by exercise in human skeletal muscle. J Physiol 2012;590:5475-86.
6. Ørtenblad N, Westerblad H, Nielsen J. Muscle glycogen stores and fatigue. J Physiol 2013;591:4405-13.
7. van Adel BA, Tarnopolsky MA. Metabolic myopathies: update 2009. J Clin Neuromuscul Dis 2009;10:97-121.
8. DiMauro S, Lamperti C. Muscle glycogenoses. Muscle Nerve 2001;24:984-99.
9. Hedberg-Oldfors C, Mensch A, Visuttijai K, Stoltenburg G, Stoevesandt D, Kraya T, et al. Polyglucosan myopathy and functional characterization of a novel GYG1 mutation. Acta Neurol Scand 2018;137:308-15.
10. Preisler N, Haller RG, Vissing J. Exercise in muscle glycogen storage diseases. J Inherit Metab Dis 2015;38:551-63.
11. Tang H, Feuchtbaum L, Sciortino S, Matteson J, Mathur D, Bishop T, et al. The First Year Experience of Newborn Screening for Pompe Disease in California. Int J Neonatal Screen 2020;6:9.
12. van der Ploeg AT, Kruijshaar ME, Toscano A, Laforet P, Angelini C, Lachmann RH, et al. European consensus for starting and stopping enzyme replacement therapy in adult patients with Pompe disease: a 10-year experience. Eur J Neurol 2017;24:768-31.
13. Angelini C, Semplicini C, Ravaglia S, Bembi B, Servidei S, Pegoraro E, et al. Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years. J Neurol 2012;259:952-8.
14. Angelini C, Semplicini C, Ravaglia S, Moggio M, Comi GP, Musumeci O, et al. New motor outcome function measures in evaluation of late-onset Pompe disease before and after enzyme replacement therapy. Muscle Nerve 2012;45:831-4.
15. Nascimbeni AC, Fanin M, Tasca E, Angelini C. Molecular pathology and enzyme processing in various phenotypes of acid maltase deficiency. Neurology 2008;70:617-26.
16. Finsterer J. An update on diagnosis and therapy of metabolic myopathies. Expert Rev Neurother 2018;18:933-943.
17. Lucia A, Ruiz JR, Santalla A, Nogales-Gadea G, Rubio JC, García-Consuegra I, et al. Genotypic and phenotypic features of McArdle disease: insights from the Spanish national registry. J Neurol Neurosurg Psychiatry 2012;83:322-8.
18. Angelini C, Semplicini C. Metabolic myopathies: the challenge of new treatments. Curr Opin Pharmacol 2010;10:338-45.
19. Godfrey R, Quinlivan R. Skeletal muscle disorders of glycogenolysis and glycolysis. Nat Rev Neurol 2016;12:393-402.
20. Haller RG, Wyrick P, Taivassalo T, Vissing J. Aerobic conditioning: an effective therapy in McArdle's disease. Ann Neurol 2006;59:922-8.
21. Quinlivan R, Martinuzzi A, Schoser B. Pharmacological and nutritional treatment for McArdle disease (Glycogen Storage Disease type V). Cochrane Database Syst Rev 2014;2014:CD003458.
22. Vorgerd M, Zange J. Treatment of glycogenosys type V (McArdle disease) with creatine and ketogenic diet with clinical scores and with 31P-MRS on working leg muscle. Acta Myol 2007;26:61-3.
23. Sato S, Ohi T, Nishino I, Sugie H. Confirmation of the efficacy of vitamin B6 supplementation for McArdle disease by follow-up muscle biopsy. Muscle Nerve 2012;45:436-40.
24. Musumeci O, Bruno C, Mongini T, Rodolico C, Aguennouz M, Barca E, et al. Clinical features and new molecular findings in muscle phosphofructokinase deficiency (GSD type VII). Neuromuscul Disord 2012;22:325-30.
25. Raben N, Sherman JB. Mutations in muscle phosphofructokinase gene. Hum Mutat 1995;6:1-6.
26. Haller RG, Vissing J. No spontaneous second wind in muscle phosphofructokinase deficiency. Neurology 2004;62:82-6.
27. Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, et al. Mitochondrial diseases. Nat Rev Dis Primers 2016;2:16080.
28. Alston CL, Rocha MC, Lax NZ, Turnbull DM, Taylor RW. The genetics and pathology of mitochondrial disease. J Pathol 2017;241:236-50.
29. Elston T, Wang H, Oster G. Energy transduction in ATP synthase. Nature 1998;391:510-3.
30. Noji H, Yoshida M. The rotary machine in the cell, ATP synthase. J Biol Chem 2001;276:1665-8.
31. Zhu X, Peng X, Guan MX, Yan Q. Pathogenic mutations of nuclear genes associated with mitochondrial disorders. Acta Biochim Biophys Sin (Shanghai) 2009;41:179-87.
32. Taivassalo T, Jensen TD, Kennaway N, DiMauro S, Vissing J, Haller RG. The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients. Brain 2003;126:413-23.
33. Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, Rowland LP. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. Neurology. 1988 Sep;38(9):1339-46. doi: 10.1212/wnl.38.9.1339. PMID: 3412580.
34. Sproule DM, Kaufmann P. Mitochondrial encephalopathy, lactic acidosis, and strokelike episodes: basic concepts, clinical phenotype, and therapeutic management of MELAS syndrome. Ann N Y Acad Sci 2008;1142:133-58.

35. Wiedemann FR, Bartels C, Kirches E, Mawrin C, Wallesch CW. Unusual presentations of patients with the mitochondrial MERRF mutation A8344G. Clin Neurol Neurosurg 2008;110:859-63.
36. Goetzman ES. Advances in the Understanding and Treatment of Mitochondrial Fatty Acid Oxidation Disorders. Curr Genet Med Rep 2017;5:132-42.
37. Vasiljevski ER, Summers MA, Little DG, Schindeler A. Lipid storage myopathies: Current treatments and future directions. Prog Lipid Res 2018;72:1-17.
38. Angelini C. Molecular update and therapeutic trials in muscle disorders of glycogen and lipid metabolism. Paediatria Croatica 2003;47:61-7.
39. Lamhonwah AM, Olpin SE, Pollitt RJ, Vianey-Saban C, Divry P, Guffon N, et al. Novel OCTN2 mutations: no genotype-phenotype correlations: early carnitine therapy prevents cardiomyopathy. Am J Med Genet 2002;111:271-84.
40. Goetzman ES. Advances in the Understanding and Treatment of Mitochondrial Fatty Acid Oxidation Disorders. Curr Genet Med Rep 2017;5:132-42.
41. Madsen KL, Preisler N, Orngreen MC, Andersen SP, Olesen JH, Lund AM, et al. Patients with medium-chain acyl-coenzyme a dehydrogenase deficiency have impaired oxidation of fat during exercise but no effect of L-carnitine supplementation. J Clin Endocrinol Metab 2013;98:1667-75.
42. Olsen RK, Andresen BS, Christensen E, Bross P, Skovby F, Gregersen N. Clear relationship between ETF/ETFDH genotype and phenotype in patients with multiple acyl-CoA dehydrogenation deficiency. Hum Mutat 2003;22:12-23.
43. Olsen RKJ, Koňaříková E, Giancaspero TA, Mosegaard S, Boczonadi V, Mataković L, et al. Riboflavin-Responsive and -Non-responsive Mutations in FAD Synthase Cause Multiple Acyl-CoA Dehydrogenase and Combined Respiratory-Chain Deficiency. Am J Hum Genet 2016;98:1130-45.
44. Corti S, Bordoni A, Ronchi D, Musumeci O, Aguennouz M, Toscano A, et al. Clinical features and new molecular findings in Carnitine Palmitoyltransferase II (CPT II) deficiency. J Neurol Sci 2008;266:97-103.
45. Pennisi EM, Garibaldi M, Antonini G. Lipid Myopathies. J Clin Med 2018;7:472.
46. Tavian D, Colombo R. Improved cytochemical method for detecting Jordans' bodies in neutral lipid storage diseases. J Clin Pathol 2007;60:956-8.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:115-120
DOI: 10.4274/BMJ.galenos.2021.29494

Research

Assessment of Follow-up and Treatment Outcomes of Eyes With Wet Age-Related Macular Degeneration (Wet AMD) for 2 Years in a Real-Life Clinical Practice Setting

Yaş Tip Yaşa Bağlı Maküla Dejeneransı'nda (YBMD) Ranibizumab Pro Re Nata (PRN) Rejimi Benimsenen Olgularda İki Yıllık Gerçek Yaşam Sonuçlarımız

İsmail Umut Onur[1], Mehmet Fatih Aşula[2], Ulviye Yiğit[1], Utku Furuncuoğlu[3], Ozan Sonbahar[1]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Ophthalmology
[2]Tosya State Hospital, Clinic of Ophthalmology, Kastamonu, Turkey
[3]Kastamonu Training and Research Hospital, Clinic of Ophthalmology, Kastamonu, Turkey

ABSTRACT

Objective: The objective of this study is to assess the follow-up and treatment outcomes of eyes with wet age-related macular degeneration (wet AMD) for two years in a real-life clinical practice setting.

Methods: In total, 37 eyes of 37 patients with wet AMD treated with 0.5 mg of intravitreal ranibizumab as needed and with at least 2 years of follow-up were retrospectively evaluated. Analyses included best-corrected visual acuity (BCVA) and central foveal thickness measurements (CFT) by optical coherence tomography at baseline, sixth month, first year, and second year along with the number of injections and follow-up visits.

Results: A total of 37 eyes of 37 patients (23 women and 14 men) with a mean age of 74.6±7.9 years were evaluated in this study. The mean BCVAs were 58.1±26.7 letters at baseline, 59.9±29.3 letters at the sixth month, 58.8±28.9 letters at the first year, and 59.2±28.5 letters at the second year. No statistically significant difference in BCVA was detected among the scores at baseline, sixth month, first year, and second year (p=0.214, 0.791, and 0.945, respectively). The CFTs averaged 340.6±89.7 µm at baseline, 316.9±87.8 µm at the sixth month, 330.2±95.8 µm at the first year, and 323.4±97.2 µm at the second year. Only the CFT at sixth month showed a statistically significant improvement over the baseline value (p=0.031).

Conclusion: Considering the number of injections and follow-up visits, along with the course of outcomes in BCVA and CFT, our real-life outcomes remained far below the outcomes reported in pivotal randomized clinical trials. However, recent papers related to real-life performance in wet AMD show similar results to those of our study.

Keywords: Wet AMD, ranibizumab, PRN, real-life outcomes

ÖZ

Amaç: Kliniğimiz retina birimince takip edilen yaş tip yaşa bağlı maküla dejeransı (YBMD) hastalarının 2 yıllık gerçek yaşam takip ve tedavi parametrelerinin değerlendirilmesi.

Gereç ve Yöntem: Çalışma kriterlerine uygun şekilde takipte kalan ve intravitreal 0,5 mg ranibizumab tedavisi uygulanan yaş tip YBMD tanısı almış 37 hastanın 37 gözü retrospektif olarak değerlendirildi. Demografik özelliklerin yanında başlangıç, 6. ay, 1. yıl, 2. yıl en iyi düzeltilmiş görme keskinlikleri (EİDGK), optik kohorens tomografi (OKT) santral foveal kalınlık (SFK) değerlerindeki değişim ile 1. yılda ve 2. yılda gerçekleştirilebilmiş muayene ve enjeksiyon sayıları irdelendi.

Bulgular: Yirmi dördü kadın 14'ü erkek 37 hastanın yaş ortalaması 74,6±7,9 iken, görme keskinliği skoruna göre EİDGK sırası ile başlangıçta ortalama 58,1±26,7 harf, 6. ayda 59,9±29,3 harf, 1. yılda 58,8±28,9 harf, 2. yılda 59,2±28,5 harf olarak gerçekleşti. EİDGK başlangıç değerleri ile 1. yıl, 2. yıl değerleri arasında istatiksel olarak anlamlı farklılık izlenmedi (p=0,791, p=0.945). OKT SFK değerleri ise sırası ile başlangıçta ortalama

Address for Correspondence: Mehmet Fatih Aşula, Tosya State Hospital, Clinic of Ophthalmology, Kastamonu, Turkey
Phone: +90 532 404 41 46 E-mail: fatihasula@gmail.com ORCID ID: orcid.org/0000-0003-3856-9297

Cite as: Onur İU, Aşula MF, Yiğit U, Furuncuoğlu U, Sonbahar O. Assessment of Follow-up and Treatment Outcomes of Eyes With Wet Age-Related Macular Degeneration (Wet AMD) for 2 Years in a Real-Life Clinical Practice Setting. Med J Bakırköy 2021;17:115-120

Received: 17.08.2020
Accepted: 25.06.2021

340,6±89,7 µm, 6. ayda 316,9±87,8 µm, 1. yılda 330,2±9,8 µm, 2. yılda 323,4±97,2 µm olarak seyretti. OKT SFK değerlerinde 6. aydaki SFK değeri başlangıç değerine göre anlamlı ölçüde azalmış (p=0,031) olmakla beraber; 1. ve 2. yıllardaki SFK değerleri başlangıç değerlerine göre anlamlı bulunmamıştır (p=0,594, p=0,233).

Sonuç: Kliniğimiz retina birimince takip/tedavi edilen gözlere uygulanan enjenksiyon sayısı ve muayene sayısı konu ile ilgili rehber niteliği taşıyan çok merkezli randomize çalışmalarda bildirilen rakamlardan düşük olarak izlenmektedir. Buna karşılık gerçek yaşam pratiğinde tedavi ve takip performansı ile 2 yıllık süreçte başlangıç görme düzeyleri ve OKT parametrelerinin en azından korunmuş olduğu görülmektedir. Bu sonuç, benzer gerçek yaşam çalışmaları ile uyumludur.

Anahtar Kelimeler: Yaş tip YBMD, ranibizumab, PRN, gerçek yaşam sonuçları

INTRODUCTION

Age-related macular degeneration (AMD) is reported as the most common cause of legal blindness that affects 10%-13% of adults aged above 65 years in North America, Europe, Australia and, recently, Asia (1,2). AMD imposes a crucial medical and socioeconomic burden on resources worldwide, and its incidence is expected to double by 2020 because of the greater longevity, diversification of environmental risk factors, and, in particular, the negative effects of arteriosclerosis, obesity, and smoking (3-7).

AMD is classified in two subtypes: dry and wet (or neovascular). Dry AMD is more common, whereas 80% of the patients who experience severe visual loss suffer from wet AMD (8,9). Wet AMD is characterized by an abnormal growth of newly formed blood vessels by processes that are not fully understood; however, the stimulation of pathological choroidal neovascularization (CNV) appears to involve the vascular endothelial growth factor (VEGF) (10). VEGF blockade is an effective treatment in patients affected by neovascular AMD (11,12).

CNV subtypes observed in wet-type AMD are characterized as type 1 (occult), type 2 (classical), type 3 (retinal angiomatous proliferation), and polypoid choroidal vasculopathy (PCV) lesions (13). The inhibition of VEGF-A has been reported to mitigate the pathophysiological process of AMD, reverse the retinal damage partially, and sustain the neurosensory function in most eyes with neovascular AMD (11,14). One potent inhibitor is ranibizumab (Lucentis, Novartis, Switzerland), which is a recombinant, humanized, and monoclonal VEGF antibody specifically developed for use in the eye.

Ranibizumab enhances angiogenesis and vascular permeability by binding and inactivating all the isoforms of VEGF-A for suppressing the formation of CNV lesions (15,16). This drug has undergone multi-centered, randomized, and prospective trials for licensing, and these studies have reported similar responses or no statistically significant differences for monthly application, three consecutive loadings, and treat-as-needed or treat-extend protocols (12,17-19). However, a significant difference could be anticipated between the "achievable" results of real-life practice and the "maximized" results of these previous trials conducted under ideal conditions.

In this context, the objective of this study is to present the anatomical and functional real-life outcomes of eyes with AMD upon treatment with a "treat-as-needed" regimen following three consecutive monthly loading doses [pro re nata (PRN)] during a two-year course of injections and follow-ups.

METHODS

The study was conducted in accordance with the tenets of the Declaration of Helsinki and after receiving the approval of the The study were approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital of Local Ethics Committee (Protocol number: 2017/68). Informed consent was obtained from all individual participants included in the study.

The records of eyes with AMD treated with intravitreal ranibizumab between October 2013 and October 2016 and followed up for 24 months in our tertiary eye clinic were retrospectively reviewed in this study. Patients were enrolled if they had developed CNV because of AMD and had not been treated previously anywhere else. Only the eye with the lower visual acuity was selected in the cases of bilateral involvement. The eyes of patients aged under 50 years; eyes having any comorbidity such as diabetic retinopathy, retinal vein occlusion, inflammatory eye disease, or previous intraocular surgery other than cataract surgery; or eyes with optical media obscuring the visual axis were excluded from the study. In addition, eyes with suspicious possible distinctive diagnosis, such as high myopia, choroid rupture, and angioid streak that could lead to secondary CNV, were also excluded from this study.

The best-corrected visual acuities (BCVAs) of the eyes were converted to the Early Treatment Diabetic Retinopathy Study (ETDRS) letter scores. Intraocular pressure measurements, biomicroscopic anterior segment examination findings, and dilated fundus findings of all eyes were reviewed and entered onto data forms.

Central foveal thickness (CFT) measurements obtained by the optical coherence tomography (OCT) (RTVue Optovue Inc., Fremont, California, USA) MM5 protocol were assessed. Importantly, the CNV subtypes were identified according to fundus fluorescein angiography (FFA) and OCT (Raster scans) images.

In our practice, 0.5 mg/0.05 mL of intravitreal ranibizumab (Lucentis, Novartis, Switzerland) was injected following oral/written consent before each session. Patients were treated with a PRN regimen following three loading doses administered one month apart. If stabilization was not achieved, then the ranibizumab injection was repeated monthly in the cases with a visual acuity reduction of five letters or more, the persistence of intraretinal or subretinal fluid on OCT, or the detection of an increase in macular thickness or hemorrhage. The patients were strictly instructed to come to treatment sessions and follow-ups at intervals not exceeding 4-6 weeks.

The data for the eyes followed up for two years after three consecutive loading doses and the PRN regime were assessed. In addition to the anatomic-functional parameters, such as at baseline, sixth month (±1 month), first year (±2 months), and second year (±2 months) BCVA and CFT, other performance parameters obtained in real-life conditions, such as the time from the first visit to the FFA imaging, the time from the diagnosis/treatment decision to the first intravitreal injection, the completion time of the first three loading doses, the total number of injections, and the total number of follow-up examinations achieved were also considered. The mean, standard deviation, median, lowest and highest values, frequencies, and ratios were used in the descriptive statistics of the data. The distribution of variables was evaluated with the Kolmogorov-Smirnov test, followed by the Wilcoxon test for analyzing the dependent quantitative data. A value of $p<0.05$ was considered statistically significant.

Statistical Analyses

The SPSS 22.0 (SPSS Inc. Chicago, USA) software was used in the analysis.

RESULTS

Data were evaluated from the 37 eyes of 37 patients with wet AMD who received ranibizumab injections and remained on regular follow-ups for 2 years between October 2013 and October 2016. Of the 37 patients, 14 (38%) were males and 23 (62%) were females. The mean age was 74.6±7.9 years. The CNV subtypes comprised type 1 lesions in 21 eyes (57%), type 2 lesions in 11 eyes (30%), type 3 lesions in 3 eyes (8%), and PCV (5%) in 2 eyes. The mean duration between the patients' first examinations to FFA imaging was 40.5±64.1 days (0-283). The consecutive and monthly administration of the first three loading doses per protocol were completed in a mean of 18.8±12.5 weeks. During the course of follow-ups, a mean of 3.3±1.5 injections were administered at the end of the first year and 5.0±2.5 injections were administered at the end of the second year. The first three loading dose targets per protocol could not be reached in eight patients at the end of the first year, and that number dropped to two patients at the end of the second year. The mean number of visits at the end of the first and second years were 5.9±2.2 and 12.5±7.4, respectively.

The ETDRS letter scoring indicated a mean BCVA of 58.1±26.7 letters before the injection, 59.9±29.3 letters at the sixth month after treatment, 58.8±28.9 letters at the first year, and 59.2±28.5 letters at the end of the second year. The mean BCVA did not differ significantly from baseline to the scores at the sixth month, first year, and second year ($p=0.214$, 0.791, and 0.945). Table 1 summarizes the changes in BCVA.

In total, 9 eyes (24%) lost 15 letters or more in the first year and 13 (35%) lost 15 letters or more in the second year. Moreover, 8 eyes (21%) gained 15 letters or more in the first year and 10 (27%) gained 15 letters or more in the second year.

The OCT measurements indicated a mean CFT of 340.6±89.7 µm at baseline before treatment, 316.9±87.8 µm at the sixth month after treatment, 330.2±95.8 µm at the first year, and 323.4±97.2 µm at the second year. The only significant decrease in CFT as compared to the baseline was observed at 6 months ($p=0.031$). In the first and second years, the reductions in SFT were not statistically significant as compared to the baseline level ($p=0.594$, 0.233). Table 2 summarizes the changes in the CFT over time.

Table 1. Changes in BCVA (ETDRS Letters)

	Q1-Q3	Med	Mean ± SD	p
Baseline	43-80	65	58.1±26.7	
Sixth month	35-87	59	59.9±29.3	0.214[w]
First year	35-89	59	58.8±28.9	0.791[w]
Second year	35-85	59	59.2±28.5	0.945[w]

[w]Wilcoxon test, Med: Median, SD: standard deviation, Q1, Q3: quartile 1, quartile 3, BCVA: Best-corrected visual acuity

DISCUSSION

The widespread use of ranibizumab in routine clinical practice has raised concerns regarding the efficacy that can

Table 2. Changes in CFT (µm)

	Q1-Q3	Med	Mean±SD	p
Baseline	296-377	340	340.6±89.7	
Sixth Month	264-348	301	316.9±87.8	**0.031**[w]
First Year	266-389	299	330.2±95.8	0.594[w]
Second Year	258-357	309	323.4±97.2	0.233[w]

[w] Wilcoxon test, CFT: Central foveal thickness, Med: Median, SD: Standard deviation, Q1, Q3: quartile 1, quartile 3

be achieved in real-life practice. In recent years, a number of studies have shown that the results obtained with ranibizumab in clinical practice do not correspond well with the results reported in company-sponsored multi-centered, randomized phase 3 clinical trials (20,21) For example, the Kaiser et al. (17) and Brown et al. (12) studies, which used fixed monthly treatment regimens, reported the gains of +6.6 and +10.7 letters, respectively, as compared to the baseline at the end of 2 years. The Ho et al. (22) study, which applied three consecutive monthly loading doses plus a PRN regimen, reported the gains of +7.9 letters after 2 years and this result was similar to the results obtained with a fixed monthly treatment regimen. The comparison of a fixed monthly treatment regimen with one comprising three consecutive monthly loading doses plus PRN regimen itself in the CATT (23) and HARBOR studies revealed a difference of +1.2 letters in favor of the monthly regimen after 2 years, but the difference was not statistically significant.

By contrast, significant differences were reported for the results of the multi-centered Lalwani et al. (18) and Ho et al. (22) randomized trials that adopted the PRN treatment regimen, in agreement with the results of our real-life clinical practice values obtained at the end of the second year after providing the same treatment regimen to 37 patients. The comparison of the pre-treatment baseline results and the results at the end of the first year in the Lalwani et al. (18) and Ho et al. (22) studies revealed a mean letter gain in the BCVA of +11.1 letters and +8.2 letters, respectively. By contrast, in our study, this difference was only + 0.7 letters. At the end of the second year, the letter gains in the Lalwani et al. (18) and Ho et al. (22) studies were +9.3 and +7.9 letters, respectively, whereas this number was +1.1 letters in our study.

The Lalwani et al. (18) and Ho et al. (22) trials reported the rates of 35% and 30.2% for cases that gained 15 letters and above in BCVA in the first year, whereas that rate was 21.6% in our study. Similarly, in the second year, the number of cases that gained 15 letters or more were 43% and 33.1% in the Lalwani et al. (18) and Ho et al. (22) studies, respectively, whereas it was 27% in our study. In the first year, the prevention of the loss of more than 15 letters in BCVA was 95% and 94.5% in the Lalwani et al. (18) and Ho et al. (22) trials, respectively, but only 75.6% in our study. In the second year, these values were 97.5% and 90.9% for the Lalwani et al. (18) and Ho et al. (22) studies, respectively, but it was only 64.8% in our study.

The comparison of the pre-treatment baseline values in OCT/CFT measurements revealed a significant decrease only in the sixth month in our study, with no significant decreases below baseline in the OCT/CFT measurements performed in the first and second years. However, the Lalwani et al. (18) and Ho et al. (22) studies reported decreases in SFT of 215 µm and 172 µm, respectively, in the second year.

The examination of the number of injections that could be performed in our study identified eight patients for whom the first three consecutive loading doses could not be completed in the first year; this number dropped to two patients in the second year. In our study, a mean of 3.3 injections were performed in the first year and a mean of 5.0 at the end the second year. By contrast, the reported average number of injections for the first year in the multi-centered, randomized Lalwani et al. (18) and Ho et al. (22) trials, which used the same treatment regimen as in our study, were 5.6 and 7.7, respectively, in the first year and 9.9 and 13.3, respectively, in the second year. The Lalwani et al. (18) and Ho et al. (22) studies requested strict monthly follow-ups, even if no injections were administered. We achieved a mean of 5.9 visits in the first year and 12.5 visits in the second year in our study.

The accumulation of clinical practice studies reporting the outcomes of ranibizumab treatments for wet AMD is now revealing gaps between the results reported in the multi-centered, randomized licensing trials and those obtained in the real-life studies. For example, the observational phase 4 Kaiser et al. (17) study, which evaluated the application of ranibizumab in clinical practice, reported an average letter loss of -1.3, whereas the mean number of injections was 6.2 (4.4 in the first year) at the end of the second year. In the series by Özkan et al. (24) in which they adopted a PRN protocol with two years of follow-up, the final level of vision was reported to be same as the initial baseline level, whereas the average number of injections was 5.8 (first year). The series by Chavan et al. (25) conducted according to the UK-based PRN dosing regimen, reported a mean letter loss of -2.3 letters at the end of the second year with a mean number of 9.9 injections. Another study by Frennesson and Nilsson (26) conducted according to the Swedish-based PRN dosing regimen, reported an average letter gain of +1 letter at the end of the second year, whereas the average

number of injections was 7.9. A meta-analysis of 20 studies examined by Chong (20) reported a first year letter gain ranging from 2.0 to +5.5 and a weighted mean gain of +1.95 letters for the real-life results of ranibizumab application in wet AMD. At the end of the first year, the rate of cases that gained 15 or more letters was 19±7.5%, whereas the rate of cases that lost 15 or fewer letters was 89±6.5% (74.4%-97.4%) (20). The number of injections varied between 4.2 and 7.5 at the end of the first year, with a mean value of 5.5±0.8 (20).

The comparison of the number of injections and vision outcomes of this retrospective two-year real-life study with the results reported by the company-sponsored, multi-centered, and randomized trials reveal a significant difference. However, this difference is no longer significant when the results are compared with the results of similar real-life studies. In real-life studies, other parameters that influence the performance and outcomes can have pivotal roles; these can include the quality and performance of the health care providers and third parties, the quality of patient-physician communication, and the sociocultural features of the patients. In our study, the time from the first visit to FFA was clearly so long that it might have retarded the time to start the injections. However, FFA and OCT evaluations are prerequisites for the reimbursement process by our social security institution (SGK). Increasing patient loads, insufficient hospital conditions, and insufficient staff may also have negative effects on access to regular follow-up visits, thereby reducing the number of injections. The preferences of the physicians or patients and the course of the disease dictate the flexibility in the protocols. However, similar problems are foreseen in all countries and institutions.

The main limitations of this study are its retrospective design, the inclusion of lower baseline BCVA scored eyes as finger counting, and a small number of patients. These all may explain why the results of our study were persistently lower than the values reported in company-sponsored, multi-centered trials. In addition, a number of patients dropped out of the study during treatment and follow-ups from the FFA stage.

Regardless of the current follow-up and treatment protocols, the treatment follow-up cycle places a serious financial and moral burden on the patients. When the expression "real-life results" is defined in a broader sense to cover all patients unable to continue treatment and follow-ups, the outcomes are likely to show a greater underperformance than anticipated.

CONCLUSION

In summary, an increase in the number of studies reporting "real-life" results by relevant clinics and physicians for the treatment of wet AMD would enable the exposure of the achievable treatment and follow-up performance related to this disease. The consideration of real-life results could also force the pharmaceutical industry to expedite its work in developing new drug formulations that require fewer injections. Alternatively, the development of customized treatment and follow-up protocols may be possible that consider AMD risk factors, such as the characteristics and course of CNV lesions on the diseased eye, the involvement of fellow eye, and so on. This would allow fewer follow-up visits and injections in low-risk eyes and more frequent follow-ups and injections in high-risk eyes, along with an increased patient motivation.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the tenets of the Declaration of Helsinki and after receiving the approval of the institutional ethical review board (approval number: 2017/68).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Authorship Contributions

Surgical and Medical Practices: İ.U.O., U.Y., U.F., O.S., M.F.A., Concept: İ.U.O., U.Y., U.F., M.F.A., Design: İ.U.O., U.Y., M.F.A., O.S., Data Collection or Processing: İ.U.O., U.Y., U.F., O.S., Analysis or Interpretation: : İ.U.O., U.Y., M.F.A., U.F., O.S., Literature Search: İ.U.O., U.Y., M.F.A., U.F., O.S., Writing: İ.U.O., U.Y., M.F.A.

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

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

REFERENCES

1. Smith W, Assink J, Klein R, Mitchell P, Klaver CC, Klein BE, et al. Risk factors for age-related macular degeneration: Pooled findings from three continents. Ophthalmology 2001;108:697-704.
2. Kawasaki R, Yasuda M, Song SJ, Chen SJ, Jonas JB, Wang JJ, et al. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. Ophthalmology 2010;117:921-7.
3. Friedman DS, O'Colmain BJ, Muñoz B, Tomany SC, McCarty C, de Jong PT, et al. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol 2004;122:564-72.
4. Seddon JM, Willett WC, Speizer FE, Hankinson SE. A prospective study of cigarette smoking and age-related macular degeneration in women. JAMA 1996;276:1141-6.

5. Seddon JM, Cote J, Davis N, Rosner B. Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist-hip ratio. Arch Ophthalmol 2003;121:785-92.
6. Klein R, Li X, Kuo JZ, Klein BE, Cotch MF, Wong TY, et al. Associations of candidate genes to age-related macular degeneration among racial/ethnic groups in the multi-ethnic study of atherosclerosis. Am J Ophthalmol 2013;156:1010-20.e1.
7. Gemmy Cheung CM, Li X, Cheng CY, Zheng Y, Mitchell P, Wang JJ, et al. Prevalence and risk factors for age-related macular degeneration in Indians: a comparative study in Singapore and India. Am J Ophthalmol 2013;155:764-73.
8. Augood CA, Vingerling JR, de Jong PT, Chakravarthy U, Seland J, Soubrane G, et al. Prevalence of age-related maculopathy in older Europeans: the European Eye Study (EUREYE). Arch Ophthalmol 2006;124:529-35.
9. Ferris FL 3rd, Fine SL, Hyman L. Age-related macular degeneration and blindness due to neovascular maculopathy. Arch Ophthalmol 1984;102:1640-2.
10. Kliffen M, Sharma HS, Mooy CM, Kerkvliet S, de Jong PT. Increased expression of angiogenic growth factors in age-related maculopathy. Br J Ophthalmol 1997;81:154-62.
11. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, et al. . Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 2006;355:1419-31.
12. Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T, et al. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. Ophthalmology 2009;116:57-65.e5.
13. Farecki ML, Gutfleisch M, Faatz H, Rothaus K, Heimes B, Spital G, et al. Characteristics of type 1 and 2 CNV in exudative AMD in OCT-Angiography. Graefes Arch Clin Exp Ophthalmol 2017;255:913-21.
14. Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY, et al. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med 2006;355:1432-44.
15. Blick SK, Keating GM, Wagstaff AJ. Ranibizumab. Drugs 2007;67:1199-206.
16. Kenneth TE, Kertes PJ. Ranibizumab in neovascular age-related macular degeneration. Clin Interv Aging 2006;1:451-66.
17. Kaiser PK, Blodi BA, Shapiro H, Acharya NR; MARINA Study Group. Angiographic and optical coherence tomographic results of the MARINA study of ranibizumab in neovascular age-related macular degeneration. Ophthalmology 2007;114:1868-75.
18. Lalwani GA, Rosenfeld PJ, Fung AE, Dubovy SR, Michels S, Feuer W, et al. A variable-dosing regimen with intravitreal ranibizumab for neovascular age-related macular degeneration: year 2 of the PrONTO Study. Am J Ophthalmol 2009;148:43-58.e1.
19. Gupta OP, Shienbaum G, Patel AH, Fecarotta C, Kaiser RS, Regillo CD. A treat and extend regimen using ranibizumab for neovascular age-related macular degeneration clinical and economic impact. Ophthalmology 2010;117:2134-40.
20. Chong V. Ranibizumab for the treatment of wet AMD: a summary of real-world studies. Eye (Lond) 2016;30:270-86.
21. Pagliarini S, Beatty S, Lipkova B, Perez-Salvador Garcia E, Reynders S, Gekkieva M, et al. A 2-Year, Phase IV, Multicentre, Observational Study of Ranibizumab 0.5mg in Patients with Neovascular Age-Related Macular Degeneration in Routine Clinical Practice: The EPICOHORT Study. J Ophthalmol 2014;2014:857148.
22. Ho AC, Busbee BG, Regillo CD, Wieland MR, Van Everen SA, Li Z, et al. Twenty-four-month efficacy and safety of 0.5 mg or 2.0 mg ranibizumab in patients with subfoveal neovascular age-related macular degeneration. Ophthalmology 2014;121:2181-92.
23. CATT Research Group, Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Engl J Med 2011;364:1897-908.
24. Özkan GÜ, Ünlü N, Acar MA, Hazırolan D, Yalnız ZA, Örnek F. Yaşa Bağlı Maküla Dejeneresansında Ranibizumab Tedavisinin Uzun Dönem Sonuçları. Ret-Vit 2013;21:178-82.
25. Chavan R, Panneerselvam S, Adhana P, Narendran N, Yang Y. Bilateral visual outcomes and service utilization of patients treated for 3 years with ranibizumab for neovascular age-related macular degeneration. Clin Ophthalmol 2014;8:717-23.
26. Frennesson CI, Nilsson SE. A three-year follow-up of ranibizumab treatment of exudative AMD: impact on the outcome of carrying forward the last acuity observation in drop-outs. Acta Ophthalmol 2014;92:216-20.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:121-129
DOI: 10.4274/BMJ.galenos.2021.04127

Research

Effect of Pulmonary Rehabilitation on Patients With Severe and Very Severe COPD and Emphysema

Amfizem Baskın Ağır ve Çok Ağır KOAH Hastalarında Pulmoner Rehabilitasyonun Etkisi

Cantürk Taşçı, Deniz Doğan Mülazimoğlu, Deniz Doğan, Nesrin Öcal, Yakup Arslan

University of Health Sciences Turkey, Gülhane Training and Research Hospital, Clinic of Chest Diseases, Ankara, Turkey

ABSTRACT

Objective: Chronic obstructive pulmonary disease (COPD) is one of the significant causes of death worldwide. Exercise-induced dyspnea is a common symptom among patients with emphysema dominant-COPD. Decreased exercise capacity and dyspnea are the basis of morbidity of the disease. Pulmonary rehabilitation (PR) is an effective therapy for patients with COPD. Evidence shows, PR improves exercise capacity and the course of the disease.

Methods: Fifty-eight patients with severe and very severe COPD in an 8 week-PR program were evaluated retrospectively. Change in spirometric measurements, 6-minute walking test (6-MWT) results, and modified Medical Research Council (mMRC) dyspnea scores were compared pre and post PR.

Results: Thirty-four of fifty-eight patients have met the inclusion criteria. Pre- and post-PR measurements of percent predicted forced vital capacity (FVC) were 76.7±4.6 vs. 77.4±4.6 (p=0.207); FEV1 were 33.2±7.1 vs. 37.5±7.6 (p<0.001) and FEV1/FVC were 43.1±9.7 vs. 48.2±10.7 (p<0.001). Distance on 6-MWT were 254.9±77.6 m vs. 328.1±93.3 m (p<0.001); mMRC dyspnea scores were 3.14±0.74 vs. 2.26±0.66 (p<0.001) pre- and post-PR.

Conclusion: PR is an underrated yet very effective therapy for patients with COPD. Instead, of drug-only treatment models, PR is an essential option for the management of COPD. The PR effect on respiratory function and exercise capacity can be more apparent with a more extensive study population.

Keywords: COPD, emphysema, rehabilitation, pulmonary rehabilitation, spirometry

ÖZ

Amaç: Kronik obstrüktif akciğer hastalığı (KOAH), tüm dünyada en önemli ölüm nedenleri arasında yer almaktadır. Özellikle amfizem baskın KOAH hastalarında en önemli semptom egzersiz dispnesidir. Hastalığın temelinde yatan patoloji ile birlikte düşünüldüğünde bu semptom en önemli mortalite nedenleri arasinda yer almaktadır. Pulmoner rehabilitasyon (PR) KOAH hastalarında başta egzersiz dispnesi üzerine olumlu etkileri ile birlikte hastalığın seyri üzerine olumlu bir tedavi yöntemidir.

Gereç ve Yöntem: Bu çalışmada, çalışma kriterlerine uygun toplam 58 ağır ve çok ağır KOAH hastasının dosya verileri geriye dönük olarak değerlendirildi. Ortalama sekiz hafta süren PR programı öncesi ve sonrasında hastaların spirometrik verileri, altı dakikalik yürüme mesafeleri, modifiye Medikal Araştırma Kurulu (mMRC) puanları kaydedildi ve istatistiksel olarak karşılaştırıldı.

Bulgular: Hastaların PR öncesi ve sonrası FEV1 değerleri, sırası ile beklenenin %33,2±7,1'e karşılık %37,5±7,6 (p<0,001). FEV1/FVC değerleri 43,1±9,7'e karşılık 48,2±10,7 (p<0,001), mMRC puanları 3.14±0.74'e karşılık 2.26±0.66 (p<0.001), 6-dakika yürüme mesafeleri 254,9±77,6 metreye karşılık 328,1±93,3 metre (p<0,001) idi. Diğer yandan PR öncesi ve sonrası FVC beklenenin %76,7±4,6'a karşılık 7,4±4,6 (p=0,207) olarak bulundu.

Sonuç: KOAH hastalarının takip ve tedavisinde PR programları çoğu zaman gözden kaçmakta ve hastalar bu tedavi yöntemlerinden uzak kalmaktadır. Farkındalığın artırılması ile sadece ilaç tedavisinin KOAH'li hasta yönetiminde yeterli olmadığının gösterildiği çalışmamızda, olgu sayılarının da arttırıldığı çalışmalar ile PR etkinliği daha da belirgin bir şekilde gösterilecektir.

Anahtar Kelimeler: KOAH, amfizem, rehabilitasyon, pulmoner rehabilitasyon, spirometri

Address for Correspondence: Deniz Doğan Mülazimoğlu, University of Health Sciences Turkey, Gülhane Training and Research Hospital, Clinic of Chest Diseases, Ankara, Turkey
Phone: +90 505 749 90 80 E-mail: denizdoganmulazim@gmail.com ORCID ID: orcid.org/0000-0001-6254-0369

Cite as: Taşçı C, Doğan Mülazimoğlu D, Doğan D, Öcal N, Arslan Y. Effect of Pulmonary Rehabilitation on Patients With Severe and Very Severe COPD and Emphysema. Med J Bakırköy 2021;17:121-129

Received: 22.08.2020
Accepted: 16.06.2021

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the third major cause of death worldwide, characterized by airflow limitation, persistent respiratory symptoms, and high morbidity (1). Acute exacerbations of COPD and hospitalizations are observed frequently as the disease severity increases. Exercise-induced dyspnea is a common symptom among patients with COPD and emphysema. Loss of elasticity in the lungs is the primary cause of dyspnea on emphysema. Due to the early closing in small airways on expiration, air trapping occurs, and consequently, inspiratory capacity decreases. Decreased inspiratory capacity is the spirometric manifestation of reduced exercise capacity. Intrathoracic pressure rises with the increased air trapping, so cardiac functions are affected negatively, and mortality increases. Despite the medication, exercise intolerance is the least improvable symptom in patients with emphysema. Pulmonary rehabilitation (PR) is a multidisciplinary approach for improving exercise capacity and quality of life. With the help of a PR program, exercise tolerance, daily physical activity, self confidence can improve, while anxiety and depression diminish. Due to such alterations, healthcare costs can be reduced (2,3). In this study, the effectiveness of PR on patients with COPD and emphysema is investigated.

METHODS

This retrospective, cross-sectional, analytical study was performed between January 01, 2017 and December 31, 2019. This study was conducted following the amended Declaration of Helsinki. The parameters were recorded after obtaining Gülhane Research and Training Hospital's non-interventional ethics board approval.

Fifty-eight patients with severe and very severe emphysema dominant-COPD were referred to the PR program by the outpatient clinic. All of them had been using long-term oxygen therapy. It is planned 3 times a week for 8-week duration. The exclusion criteria were inability to complete the 8 week-PR program, suspicion of infection by the referral time, acute coronary syndrome, congestive heart failure (ejection fraction <40%), cardiac or thoracic surgery within the 3 months by referral time. After these exclusion criteria, 34 patients were included in the analysis for this study (Table 1). Pre and post-PR spirometric measurements, modified Medical Research Council (mMRC) dyspnea scores, and 6-minute walking test (6-MWT) results were recorded.

Statistical Analysis

R software was used for the statistical analysis. Variables were analyzed with the Kolmogorov-Smirnov test to evaluate the distribution. Results for descriptive statistics are expressed as mean ± standard deviation. Continuous variables of pre and post-PR change were analyzed with paired t-test or Wilcoxon Signed-rank test. Statistical significance was accepted as $p<0.05$.

RESULTS

Fifty-eight patients with severe and very severe emphysema dominant COPD attended the PR program in Pulmonary Rehabilitation Unite of Pulmonary Diseases Clinic from January 01, 2017 to December 31, 2019. For this study, the patient files were examined. 34 patients have met the inclusion criteria (Figure 1). Mean age was 63.4±3.5 years of all study population, it was 64.6±3.2 years for men and 63.3±3.6 years for women. Only 3 of 34 patients were women. All spirometric parameters except forced vital capacity (FVC) were improved significantly after PR. In addition, a significant increase in mMRC dyspnea scores and distance of 6-MWT were noted. The results of the study are summarized in Table 1.

DISCUSSION

This study shows patients with emphysema dominant-COPD benefit from PR. Respiratory function test parameters, exercise capacity, and dyspnea improve significantly with PR.

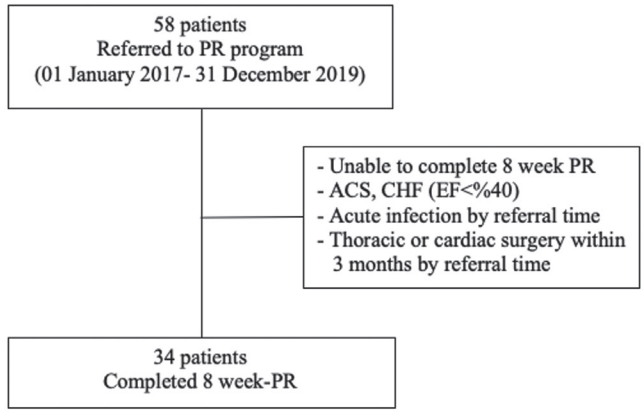

Figure 1. Study flow diagram
Definiton of abbreviations: PR: Pulmonary rehabilitation, ACS: Acute coronary syndrome, CHF: Congestive heart failure, EF: Ejection fraction

The major goal of COPD treatment is diminishing symptoms and increasing quality of life. Many patients with COPD have a limitation of activity due to dyspnea. PR is an essential treatment option for this group of patients. Physiotherapy reduces work of breathing and oxygen consumption, thus

Table 1. Characteristics of the patients (n=34)

Variable		Value ± SD	p
Age, years		63.4±3.5	-
FEV1, % predicted	Pre-PR	33.2±7.1	<0.001
	Post-PR	37.5±7.6	
	Difference	4.3±4.2	
FVC, % predicted	Pre-PR	76.7±4.6	0.207
	Post-PR	77.4±4.6	
	Difference	0.61±2.8	
FEV1/FVC	Pre-PR	43.1±9.7	<0.001
	Post-PR	48.2±10.7	
	Difference	5.2±5.7	
FEF25-75, % predicted	Pre-PR	40.7±5.9	0.002
	Post-PR	43.6±7.1	
	Difference	2.9±5.1	
PEF, % predicted	Pre-PR	54.3±8.5	<0.001
	Post-PR	61.0±7.4	
	Difference	6.7±4	
mMRC score*	Pre-PR	3.14±0.74	<0.001
	Post-PR	2.26±0.66	
	Difference	-0.88±0.68	
6-minute-walk distance, m	Pre-PR	254.9±77.6	<0.001
	Post-PR	328.1±93.3	
	Difference	73.2±63.4	

SD: Standard deviation, FEV1: Forced expiratory volume in one second, FVC: Forced vital capacity, PR: Pulmonary rehabilitation, mMRC: Modified medical research council

*mMRC dyspnea score scale ranges from 0 to 4, with higher scores indicating more severe dyspnea

diminishes dyspnea. Many studies use PR for lung cancer, idiopathic pulmonary fibrosis, and chronic respiratory diseases (4). However, unfortunately, many patients do not have access to PR.

Thirty-four patients constitute this study population, completed the 3 times a week, 8 week-PR programs. Pre-PR predicted % FEV1 mean was 33.2±7.1. Lower FEV1 means lower exercise capacity and quality of life for patients with COPD. Post-PR predicted % FEV1 mean rose to 37.5±7.6 in this study. Although it may be seen as a slight increase, this change of FEV1 increases exercises capacity. It can be understood from the increased distance of 6-MWT pre and post PR. This statistically significant change in predicted FEV1% was + 4.3. Notably, three major PR studies show no significant increase in FEV1 % pre and post PR (5-7).

The 6MWT is a safe, inexpensive, widely used tool to assess the functional status of patients with COPD. The difference in 6MWT is 54 mt for patients with COPD to notice an improvement (8). In this study Δ6MWT was 73.2±63.4 m, substantially higher than the threshold.

Our study population distinguishes this study from others. Only patients with severe and very severe emphysema dominant-COPD were included in this study. In patients with chronic bronchitis dominant-COPD, it is not expected that significant improvement on overserved spirometric parameters. Pre-PR mean mMRC dyspnea score was 3.14±0.74, post-PR, it declined to 2.26±0.66. This shows that PR improves not only spirometric measurements also the sense of dyspnea. Although decreasing dyspnea and increasing exercise capacity with PR can be associated with life expectancy, the literature shows no clear connection (9). A study by Bowen et al. showed that after PR, 3-year life expectancy is 69%-85% for patients with COPD (10). Nevertheless, it is known that PR diminishes dyspnea in patients with COPD (11,12).

PR program was planned for 8 weeks for patients with COPD in our daily practice. Only patients who completed 8 week-program have been included in the study. Based on the current literature, it is recommended to apply the PR program for at least 8 weeks, and for the optimum effect of the treatment, more than 8 weeks is required (13).

The study has some limitations. First, its retrospective methodology was a significant limitation. The study population was minimal, and there is no information about their comorbidities and pharmacological treatments. We believe that a prospective study with a large study population will overcome these limitations.

CONCLUSION

Our comprehensive, outpatient, 8 week-PR programs are effective for patients with severe and very severe COPD and emphysema component. Besides spirometric parameters, dyspnea scores and exercise capacity were all improved.

ETHICS

Ethics Committee Approval: Approval of the Local Research Ethics Committee of our tertiary hospital was obtained before initiating the study (University of Medical Sciences Turkey, Gülhane Training and Research Hospital, project no: 2020-13, date: 07.01.2020).

Informed Consent: Is a retrospective study.

Authorship Contributions: Surgical and Medical Practices: D.D., C.T., Y.A., Concept: D.D., C.T., N.Ö., Design: D.D.,

N.Ö., Data Collection or Processing: D.D.M., Y.A., Analysis or Interpretation: D.D., D.D.M., Y.A., Literature Search: D.D.M., C.T., N.Ö., Writing: D.D., D.D.M, C.T.

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

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

REFERENCES

1. Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019. Eur Respir J 2019;53:1900164.
2. Neder JA, Arbex FF, Alencar MC, O'Donnell CD, Cory J, Webb KA, et al. Exercise ventilatory inefficiency in mild to end-stage COPD. Eur Respir J 2015;45:377-87.
3. American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med 2002;166:518-624.
4. Prunera-Pardell MJ, Padín-López S, Domenech-Del Rio A, Godoy-Ramírez A. Effectiveness of a respiratory rehabilitation programme in patients with chronic obstructive pulmonary disease. Enferm Clin (Engl Ed) 2018;28:5-12.
5. Young P, Dewse M, Fergusson W, Kolbe J. Respiratory rehabilitation in chronic obstructive pulmonary disease: predictors of nonadherence. Eur Respir J 1999;13:855-9.
6. Selzler AM, Simmonds L, Rodgers WM, Wong EY, Stickland MK. Pulmonary rehabilitation in chronic obstructive pulmonary disease: predictors of program completion and success. COPD 2012;9:538-45.
7. Fischer MJ, Scharloo M, Abbink JJ, van 't Hul AJ, van Ranst D, Rudolphus A, et al. Drop-out and attendance in pulmonary rehabilitation: the role of clinical and psychosocial variables. Respir Med 2009;103:1564-71.
8. Redelmeier DA, Bayoumi AM, Goldstein RS, Guyatt GH. Interpreting small differences in functional status: the Six Minute Walk test in chronic lung disease patients. Am J Respir Crit Care Med 1997;155:1278-82.
9. Li Y, Qian H, Yu K, Huang Y. Nonadherence in Home-Based Pulmonary Rehabilitation Program for COPD Patients. Can Respir J 2020;2020:5146765.
10. Bowen JB, Votto JJ, Thrall RS, Haggerty MC, Stockdale-Woolley R, Bandyopadhyay T, et al. Functional status and survival following pulmonary rehabilitation. Chest 2000;118:697-703.
11. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. Am J Respir Crit Care Med 2017;195:557-82.
12. Wilson AM, Browne P, Olive S, Clark A, Galey P, Dix E, et al. The effects of maintenance schedules following pulmonary rehabilitation in patients with chronic obstructive pulmonary disease: a randomised controlled trial. BMJ Open 2015;5:e005921.
13. Houben-Wilke S, Janssen DJA, Franssen FME, Vanfleteren LEGW, Wouters EFM, Spruit MA. Contribution of individual COPD assessment test (CAT) items to CAT total score and effects of pulmonary rehabilitation on CAT scores. Health Qual Life Outcomes 2018;16:205.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:125-129
DOI: 10.4274/BMJ.galenos.2021.62634

Research

Radiological Evaluation of Age- and Gender-Related Changes in the Blumensaat Line

Blumensaat Çizgisinde Yaş ve Cinsiyetle İlişkili Değişikliklerin Radyolojik Olarak Değerlendirilmesi

Veysel Kaplanoğlu[1], Hatice Kaplanoğlu[2]

[1]University of Health Sciences Turkey, Keçiören Training and Research Hospital, Clinic of Radiology, Ankara, Turkey
[2]University of Health Sciences Turkey, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Clinic of Radiology, Ankara, Turkey

ABSTRACT

Objective: The position of the patella relative to the femur is critical in the evaluation of patellofemoral joint diseases. Blumensaat defined a line to evaluate patellofemoral congruence, which is still used clinically. This study aimed to evaluate age- and gender-related changes in the blumensaat line (BL).

Methods: Images of 229 patients, who underwent standard lateral knee radiography at 30° flexion, were retrospectively evaluated. The relationship between BL and interior pole of the patella was examined, and the variability of the measurements according to gender and age groups was investigated using statistical methods.

Results: Two hundred and twenty-nine patients (128 men; 101 women) were included in the study. The mean age was 41.96±13.41 years (39.63±13 years for men and 44.90±12.2 years for women). BL passed through the lower pole of the patella in only two (0.9%) of the 229 patients. No statistically significant difference was found in the BL measurement of men and women (p>0.05). There was also no statistically significant relationship between age and these distance values (r=-0.112; p>0.05).

Conclusion: It was concluded that there was no difference between genders and different age groups in terms of BL measurements.

Keywords: Blumensaat line, patellar height, gender, age, radiography

ÖZ

Amaç: Patella-femoral eklem hastalıklarının değerlendirilmesinde, patellanın femura göre pozisyonu çok önemlidir. Blumensaat (BS) patella femoral uyumu değerlendirmek için BS hattını tanımladı. Bu metod klinik kullanımda hala değerli olan bir yöntemdir. Bu çalışmanın amacı BS çizgisinde, yaş ve cinsiyetle ilgili değişiklikleri değerlendirmektir.

Gereç ve Yöntem: 30° fleksiyonda standart lateral diz radyografisi çekilen 229 hastanın görüntüleri retrospektif olarak değerlendirildi. BS çizgisi ve patella alt kutbu arasındaki ilişki incelendi, ölçümlerin cinsiyet ve yaş gruplarına göre değişkenliği istatistiksel yöntemlerle araştırıldı.

Bulgular: İki yüz yirmi dokuz hasta (128 erkek ve 101 kadın) çalışmaya alındı. Hastaların yaş ortalaması 41,96±13,41 idi (kadınlarda ve erkeklerde sırasıyla 44,90±12,2, 39,63±13). BS hattı 229 hastanın sadece 2'sinde (% 0.9) patellanın alt kutbundan geçmekteydi. BS ölçümü ile kadın ve erkekler arasında istatistiksel olarak anlamlı fark bulunmadı (p>0,05). Hastaların yaş grupları ile bu mesafe değerleri arasında istatistiksel olarak anlamlı bir ilişki yoktu (r=-0,112, p>0,05).

Sonuç: Çalışmamızda, BS ölçümünde farklı cinsiyet ve yaş grupları arasında fark olmadığı sonucuna varıldı.

Anahtar Kelimeler: Blumensaat çizgisi, patella yüksekliği, cinsiyet, yaş, radyografi

Address for Correspondence: Hatice Kaplanoğlu, University of Health Sciences Turkey, Keçiören Training and Research Hospital, Clinic of Radiology, Ankara, Turkey
Phone: +90 505 892 30 61 E-mail: hatice.altnkaynak@yahoo.com.tr ORCID ID: orcid.org/0000-0003-1874-8167

Cite as: Kaplanoğlu V, Kaplanoğlu H. Radiological Evaluation of Age- and Gender-Related Changes in the Blumensaat Line. Med J Bakırköy 2021;17:125-129

Received: 28.08.2020
Accepted: 22.06.2021

INTRODUCTION

The position of the patella relative to the femur is important for the evaluation of patellofemoral joint diseases. Most of patellofemoral joint diseases are based on a mismatch between the patella and femoral components (1). In 1938, on a lateral knee X-ray, Blumensaat described a line, in which the inferior pole of the patella was aligned with a line drawn from the roof of the intercondylar notch to the anterior of the knee joint (2). The vertical height above this line should be measured with the inferior pole of the patella, and the normal distance is defined as zero. Values greater than 10 mm are classified as patella alta (2) (Figure 1).

Patellar height measurements based on the blumensaat line (BL) are not affected by the patellar bone length or the angle between the BL and femoral shaft (BL-FS). Therefore, the BL method is more practical and reliable than indirect methods (3,4). However, some researchers suggest that BL varies at different BL-FS and flexion angles, resulting in inaccurate measurements (5-8).

Although the BL method is reported to be inconsistent with other patellar height measurement methods, the correlation between other measurement methods is also weak (9). In the clinical use of direct methods, BL remains to be an important parameter despite the controversial findings. Therefore, recently, it has been used more as a suitable reference point in newly described methods (3,4).

A limited number of studies, showing the accuracy of the BL method and affected variables, exist in the literature. This study aimed to investigate changes in BL measurements according to gender and age.

METHODS

Patients

The study included 229 patients, aged 18 to 60 years, who presented to our hospital with an anterior knee pain between January 2019 and July 2020 and underwent standard lateral knee radiography at the radiology clinic. Patients who had a history of previous knee surgery and those with developmental knee joint pathology or posttraumatic knee joint deformity, effusion, soft tissue pathology, or severe degeneration were excluded from the study. The demographic data of the patients were recorded.

To standardize the radiological measurements, lateral knee X-rays taken at 30° flexion were examined. The flexion angle was obtained by measuring the angle between the FS and tibia (Figure 2). This ensures that the slack is in the patellar tendon and determines the relationship between

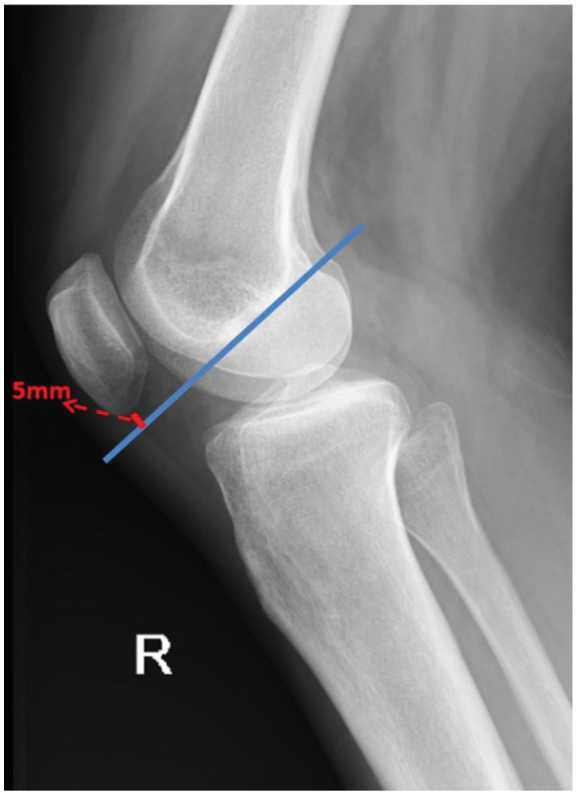

Figure 1. Blumensaat line

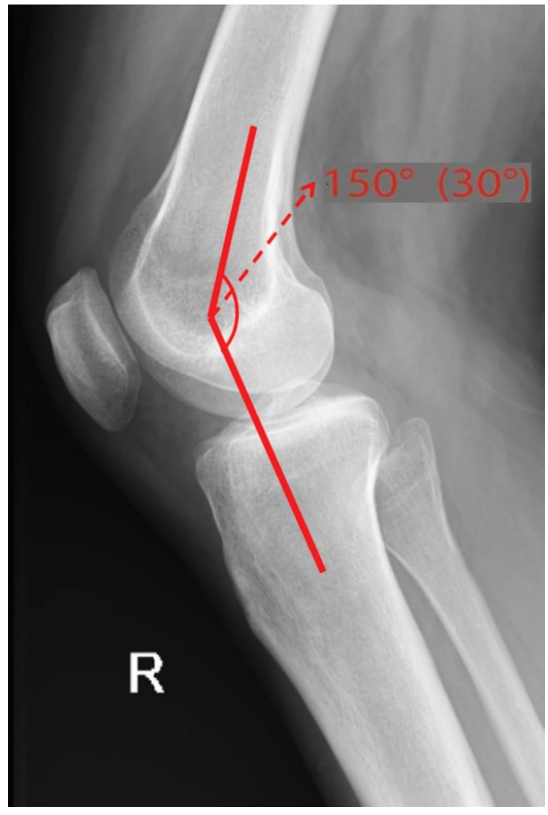

Figure 2. Knee joint flexion angle

BL and inferior pole of the patella (10). Measurements were undertaken directly on the X-rays by the same radiologist for each patient, using the digital picture archiving system (Figure 3). Before the study, approval was received from the clinical research ethics committee of our hospital, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all individuals participating in the study.

Statistical Analysis

For the descriptive statistics related to the continuous data, mean, standard deviation, median, and minimum and maximum values were used, while discrete data were expressed as percentages. The Shapiro-Wilk test was used to analyze the conformance of the data to a normal distribution. The Mann-Whitney U test was conducted to compare age and knee BL-patella values according to gender. The chi-square test was used in the group comparisons (cross-tables) of nominal variables. The relationship between age and knee BL-patella values was examined using Spearman's correlation coefficient. IBM SPSS Statistics v. 20 was used for statistical evaluations, and $p<0.05$ was considered the statistical significance limit.

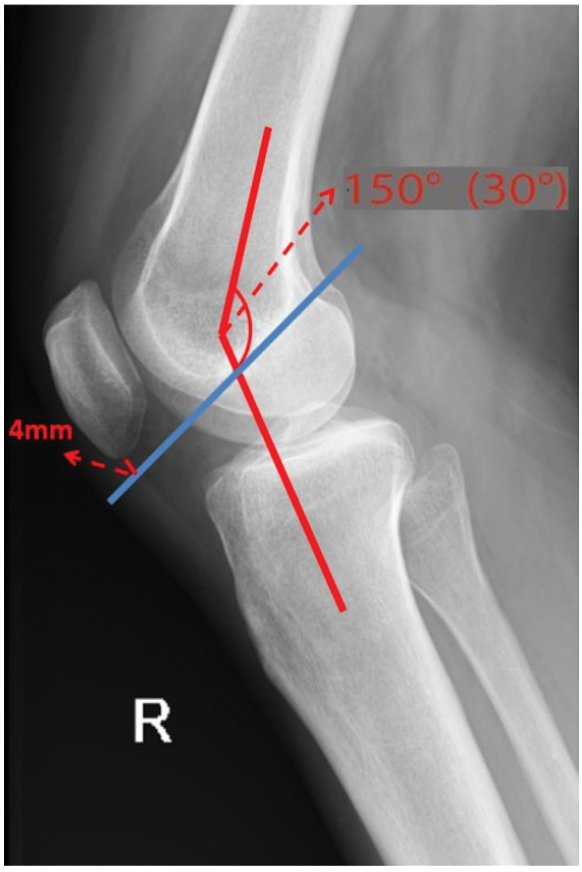

Figure 3. Digital measurement on the direct X-rays using the digital image archiving system

RESULTS

The ages of the 229 patients included in the study ranged from 18 to 60 years, and the mean age was 41.96±13.41 years. Of the patients, 44.1% were women, with a mean age of 44.90±12.2 years, and 55.9% were men, with a mean age of 39.63±13.89 years. Meniscal tears existed in 40.6% of the patients, degenerative changes, 47.2%, meniscal degeneration, 51.5%, and degeneration in the anterior cruciate ligament, 7.4% (Table 1).

When determining the height of the patella using the BL method, the position of the inferior pole of the patella (high or low) and its distance in millimeters are measured according to BL. In this study, the mean distance between the BL and inferior pole of the patella was 7.62±4.92 mm (7.06±4.59 mm in women; 8.06±5.14 mm in men). There was no significant difference in terms of the BL measurement values between men and women ($p>0.05$) (Table 2).

In only two (0.9%) of the 229 patients, BL passed straight through the inferior pole of the patella. Patella alta was identified in 49 patients (21.4%) (Figure 4). This condition was seen in 21 (20.8%) female patients and 28 (21.9%) male patients (Table 3). Patella baja was not detected in any patient. There was no significant relationship between the age groups of the patients and these distance values ($r=-0.112$; $p>0.05$) (Table 4).

Table 1. Distribution of gender, meniscal tear, degenerative changes, and anterior cruciate ligament among the patients

	n	%
Gender		
Female	101	44.1
Male	128	55.9
Meniscal tear		
Absent (0)	136	59.4
Present (1)	93	40.6
Degenerative changes		
Absent (0)	121	52.8
Present (1)	108	47.2
Meniscal degeneration		
Absent (0)	111	48.5
Present (1)	118	51.5
Degeneration in ACL		
Absent (0)	212	92.6
Present (1)	17	7.4

ACL: Anterior cruciate ligament

Table 2. Comparison of the age and blumensaat line measurements between women and men

	Women		Men		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	p
Age, years	44.90±12.21	48 (18-60)	39.63±13.89	42 (18-60)	0.005
Blumensaat line measurement	7.06±4.59	6.5 (0-22)	8.06±5.14	7.75 (0-26)	0.152

SD: Standard deviation, Min-max: Minimum-maximum

Table 3. Distribution of the patients according to the patellar height

	n	%
Knee BL-patella		
=0	2	0.9
≤10 mm	180	78.6
>10 mm	49	21.4

BL: Blumensaat line

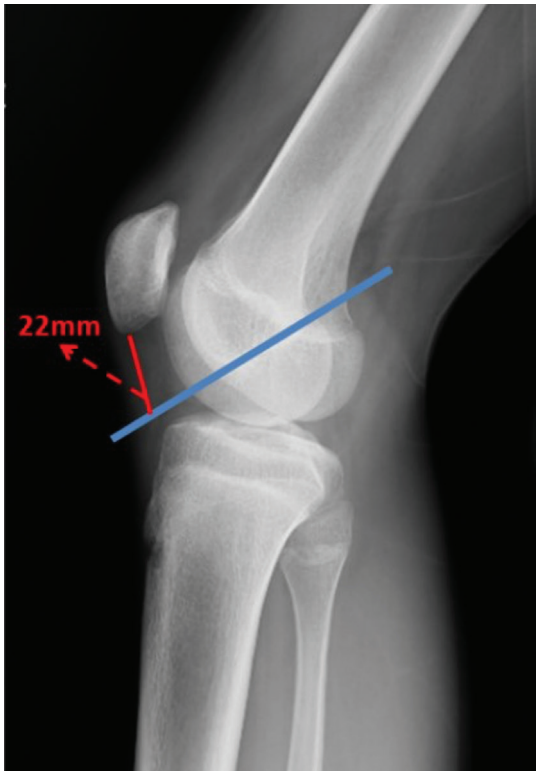

Figure 4. Measurement of the Blumensaat line in patients with patella alta

Table 4. Correlation between the patients' age and BL measurements

	Age	
	r	p
Knee BL-patella	-0.112	0.090

BL: Blumensaat line

DISCUSSION

Patellofemoral congruence is important for the etiology of anterior knee pain. The use of BL is accepted as a pioneering method in measuring the patellar height (2). According to Jacobsen et al., (10) since BL was first defined, this measurement has not been standardized, and thus, it provides varying results, depending especially on the knee flexion angle. However, Seyahi et al., (4) who compared the accuracy of BL methods in different BL-FS angles, determined that the accuracy of the BL method and size of the patellar bone were not affected by different BL-FS angles, but they also noted that the intercondylar notch depth might impact BL measurements. In this study, we examined whether the depth of the intercondylar notch may vary according to age and gender (11).

For patellar height measurements, many direct methods using patellar and femoral reference points were described, which mostly used BL as the reference point (3,12,13). Therefore, BL remains important in clinical use (11). In Andersen et al., (14) study, where they measured the patellar height based on BL in 256 knees, 207 patients were diagnosed with patella alta. Based on the study, it was revealed that the intercondylar notch roof-femoral diaphyseal angle affected the position of BL relative to the patella, which could provide different values varying from one person to another.

In another study, patella alta or baja was detected on lateral radiograph images, at 30° flexion, in 7.5% of patients. When the correlation of these values with other indirect methods was evaluated, the results were found to be consistent (1). According to the literature, the BL method shows the most significant correlation with the Insall-Salvati method (4,15,16). In a similar study conducted on Turkish patients, patella alta was detected on lateral radiograph images, at 30° flexion, in 9.47% of the patients (11). In the current study, patella alta was detected in 21.4% of the patients, while patella baja was not observed. Compared with the other study in Turkey, due to the higher number of patients in the sample, patella alta was detected in more patients.

Studies existing in the literature show that patellar height measurements may be affected by personal characteristics, such as ethnicity, age, and gender. Karadimas et al. (17) and Leung et al. (18) stated that ethnicity played an important role in patellar height. They reported that patellar height was higher, especially in Arabian, African, and Chinese populations, compared with the European population.

Norman et al. (5) and Egund et al. (19) emphasized that patellar height was affected by gender differences in direct measurements. Farrow et al. (20) stated that the intercondylar notch structure was narrower and shallower in women. The reference point of BL is the roof of the intercondylar notch; therefore, it was considered that the difference in the notch depth between genders might have affected the results (11). Değirmenci et al. (11) found a significant difference in terms of patellar height between genders. They reported that women had a higher patella than men, but the height of the patella did not significantly differ between the age groups (11). Moreover, studies in the literature show that patellar height is not affected by gender differences when measured using the BL method (16,21,22). Thus, there is still no consensus on the effect of gender differences in BL measurements. In this study, no difference was found between genders or different age groups in terms of the patellar height measurements using the BL method.

CONCLUSION

There was no difference between genders and different age groups in terms of BL measurements.

ETHICS

Ethics Committee Approval: The study were approved by the Health Sciences University Dışkapı Yıldırım Beyazit Training and Research Hospital of Local Ethics Committee (Protocol number: 10.08.2020/93/10).

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

Authorship Contributions

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

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

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

REFERENCES

1. Seil R, Müller B, Georg T, Kohn D, Rupp S. Reliability and interobserver variability in radiological patellar height ratios. Knee Surg Sports Traumatol Arthrosc 2000;8:231-6.
2. Blumensaat C. Die Lageabweichungen und Verrenkungen der Kniescheibe. Ergebnisse der Chirurgie und Orthopädie. Springer: Heidelberg; 1938, 149-223.
3. Hanada M, Takahashi M, Koyama H, Matsuyama Y. Assessing the validity of the modified Blumensaat method for radiographic evaluation of patellar height. Eur J Orthop Surg Traumatol 2015;25:757-62.
4. Seyahi A, Atalar AC, Koyuncu LO, Cinar BM, Demirhan M. Blumensaat çizgisi ve patella yüksekliği [Blumensaat line and patellar height]. Acta Orthop Traumatol Turc 2006;40:240-7.
5. Norman O, Egund N, Ekelund L, Rünow A. The vertical position of the patella. Acta Orthop Scand 1983;54:908-13.
6. Brattström H. Patella alta in non-dislocating knee joints. Acta Orthop Scand 1970;41:578-88.
7. Carson WG Jr, James SL, Larson RL, Singer KM, Winternitz WW. Patellofemoral disorders: physical and radiographic evaluation. Part II: Radiographic examination. Clin Orthop Relat Res 1984;185:178-86.
8. Igbigbi PS, Msamati BC, Ng'Ambi TM. Intercondylar shelf angle in adult black Malawian subjects. Clin Anat 2001;14:254-7.
9. Ng JP, Cawley DT, Beecher SM, Lee MJ, Bergin D, Shannon FJ. Focal intratendinous radiolucency: A new radiographic method for diagnosing patellar tendon ruptures. Knee 2016;23:482-6.
10. Jacobsen K, Bertheussen K, Gjerloff CC. Characteristics of the line of Blumensaat. An experimental analysis. Acta Orthop Scand 1974;45:764-71.
11. Değirmenci E, Yücel İ, Özturan KE, Karaduman ZO, Karaca E. Evaluation of the age and gender related changes in the Blumensaat line. Surg Radiol Anat 2020;42:641-5.
12. Phillips CL, Silver DA, Schranz PJ, Mandalia V. The measurement of patellar height: a review of the methods of imaging. J Bone Joint Surg Br 2010;92:1045-53.
13. Biedert RM, Albrecht S. The patellotrochlear index: a new index for assessing patellar height. Knee Surg Sports Traumatol Arthrosc 2006;14:707-12.
14. Andersen PT. Congenital deformities of the knee joint in dislocation of the patella and achondroplasia. Acta Orthop Scand 1958;28:27-50.
15. Lu W, Yang J, Chen S, Zhu Y, Zhu C. Abnormal Patella Height Based on Insall-Salvati Ratio and its Correlation with Patellar Cartilage Lesions: An Extremity-Dedicated Low-Field Magnetic Resonance Imaging Analysis of 1703 Chinese Cases. Scand J Surg 2016;105:197-203.
16. Verhulst FV, van Sambeeck JDP, Olthuis GS, van der Ree J, Koëter S. Patellar height measurements: Insall-Salvati ratio is most reliable method. Knee Surg Sports Traumatol Arthrosc 2020;28:869-75.
17. Karadimas JE, Piscopakis N, Syrmalis L. Patella alta and chondromalacia. Int Orthop 1981;5:247-9.
18. Leung YF, Wai YL, Leung YC. Patella alta in southern China. A new method of measurement. Int Orthop 1996;20:305-10.
19. Egund N, Lundin A, Wallengren NO. The vertical position of the patella. A new radiographic method for routine use. Acta Radiol 1988;29:555-8.
20. Farrow LD, Chen MR, Cooperman DR, Victoroff BN, Goodfellow DB. Morphology of the femoral intercondylar notch. J Bone Joint Surg Am 2007;89:2150-5.
21. Berg EE, Mason SL, Lucas MJ. Patellar height ratios. A comparison of four measurement methods. Am J Sports Med 1996;24:218-21.
22. Udoaka AI, Bienonwu EO. Assessment of the patellar height ratios in normal adult Nigerians. Asian J Biomed Pharm Sci 2013;3:1-3.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:130-134
DOI: 10.4274/BMJ.galenos.2021.54254

Research

Investigation of Antifungal Susceptibility of Trichosporon Asahii Isolated From Urine Samples

İdrar Örneklerinden İzole Edilen Trichosporon Asahii İzolatlarının Antifungal Duyarlılığının Araştırılması

Deniz Turan[1], Ayşe Barış[2], Fatma Özakkaş[3], Şölen Daldaban Dinçer[3], Sebahat Aksaray[4]

[1]University of Health Sciences Turkey, Haydarpaşa Numune Training and Research Hospital, Clinic of Medical Microbiology, İstanbul, Turkey
[2]University of Health Sciences Turkey, Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Medical Microbiology, İstanbul, Turkey and İstanbul University Faculty of Medicine, Department of Medical Microbiology, İstanbul, Turkey
[3]University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Clinic of Medical Microbiology, İstanbul, Turkey
[4]University of Health Sciences Faculty of Medicine, Department of Medical Microbiology, İstanbul, Turkey

ABSTRACT

Objective: As part of the normal human flora of the skin and gastrointestinal tract, Trichosporon species may lead to opportunistic infections through underlying facilitating factors. Urinary tract infections (UTIs) are the most common infections occurring in intensive care units (ICUs), where catheterization procedures are performed extensively. The aim of the study investigates the antifungal susceptibility of T. asahii strains isolated from urine samples.

Methods: Isolates were identified to the species level using the MALDI-TOF MS system (VITEK MS; bio-Mérieux). Antifungal susceptibility tests were conducted using the broth microdilution method, in accordance with the recommendations of the "Clinical and Laboratory Standards Institute (CLSI)".

Results: At a 48-hour assessment of the 100 T.asahii isolates included in the study, the minimal inhibitory concentration ($MIC)_{50}$ and MIC_{90} values were (2µg/mL, 8µg/mL) for fluconazole, (0.06µg/mL, 0.12µg/mL) for voriconazole, (0.25 µg/mL, 1 µg/mL) for posaconazole, (0.12 µg/mL, 0.25 µg/mL) for itraconazole and isavuconazole, (2 µg/mL) for amphotericin B and (>8) for micafungin.

Conclusion: The lowest and highest MIC values among the triazole antifungal agents were determined for voriconazole and fluconazole, respectively. Considering the high MIC values, care should be taken to prevent breakthrough infections of Trichosporon in at-risk patients undergoing empirical or prophylactic echinocandin or fluconazole therapies.

Keywords: Trichosporon asahii, urinary tract infection, antifungal susceptibility

ÖZ

Amaç: İnsanda deri ve gastrointestinal sistemin normal florasında bulunan Trichosporon türleri, altta yatan kolaylaştırıcı faktörlerin etkisi ile fırsatçı enfeksiyonlara neden olabilmektedir. Üriner sistem enfeksiyonları (ÜSE), yoğun kateterizasyon işlemlerinin uygulandığı yoğun bakım ünitesinde (YBÜ) en sık karşılaşılan enfeksiyonlardır.Trichosporon, ÜSE'de Candida' dan sonra en sık izole edilen maya cinsidir. Bu çalışmada idrar örneklerinden izole edilen T. asahii izolatlarının antifungal duyarlılıklarının araştırılması amaçlanmıştır.

Gereç ve Yöntem: İzolatların tür tanımı MALDI-TOF MS (VITEK MS; bio-Mérieux) sistemi ile yapıldı. Antifungal duyarlılık testleri "Clinical and Laboratory Standards Institute (CLSI)" önerileri doğrultusunda sıvı mikrodilüsyon yöntemi ile yapılmıştır.

Bulgular: Çalışmaya dahil edilen 100 T.asahii izolatının 48. saatte yapılan değerlendirmesinde izolatların minimum inhibitör konsant ($MİK)_{50}$, $MİK_{90}$ değerleri; flukonazol için (2µg/mL, 8µg/mL), vorikonazol için (0.06µg/mL, 0.12µg/mL), posakonazol için (0.25 µg/mL, 1 µg/mL), itrakonazol ve isavukonazol için (0.12 µg/mL, 0.25 µg/mL), amfoterisin B için (2 µg/mL) ve mikafungin için (>8) olarak belirlenmiştir.

Sonuç: Triazol grubu antifungal ilaçlar içinde en düşük MİK değerleri vorikonazol'de, en yüksek MİK değerleri flukonazolde saptanmıştır. Yüksek MİK değerleri göz önünde bulundurulduğunda; ekinokandin ve flukonazolün ampirik veya profilaktik olarak kullanıldığı, risk faktörleri bulunan hastalarda tedavi altında gelişebilecek Trichosporon enfeksiyonlarına karşı dikkatli olunmalıdır.

Anahtar Kelimeler: Tichosporon asahii, üriner sistem enfeksiyonu, antifungal duyarlılık

Address for Correspondence: Deniz Turan, University of Health Sciences Turkey, Haydarpaşa Numune Training and Research Hospital, Clinic of Medical Microbiology, İstanbul, Turkey
Phone: +90 216 542 32 32 E-mail: dennizturan@hotmail.com ORCID ID: orcid.org/0000-0001-7943-7536

Cite as: Turan D, Barış A, Özakka F, Daldaban Dinçer Ş, Aksaray S. Investigation of Antifungal Susceptibility of Trichosporon Asahii İsolated From Urine Samples. Med J Bakırköy 2021;17:130-134

Received: 08.09.2020
Accepted: 21.06.2021

INTRODUCTION

Trichosporon species are found widely in nature, comprising of yeast-like fungi belonging to the phylum Basidiomycota (1). A recent taxonomic revision identified 20 species within the genus, using IGS1 rDNA sequence analysis (2). Among these species, *T. asahii, T. asteroides, T. inkin, T. ovoides*, and *T. faecale* were reported as infectious in humans. The most common cause of invasive trichosporonosis and urinary tract infections (UTIs) is *T. asahii* (3,4).

Trichosporon species, which are members of saprophytic flora of the skin or found in the respiratory, gastrointestinal, and genitourinary tracts of humans, are causing superficial as well as invasive infections with increasing frequency (1,4). Nosocomial UTIs are the most common infections, particularly in intensive care units (ICUs). Most of these infections are reported to be related to the presence of a urinary catheter and tend to develop following urinary catheterization (5). *Candida* species are the most common types of yeasts isolated in UTIs, followed by *T. asahii* (6).

Virulence factors play an important role in the development of infection. *Trichosporon* species produce extracellular enzymes such as lipase, protease, and esterase and form biofilms (1,7). Most UTI-causing *T. asahii* isolates were shown to form biofilm on polystyrene plates (8,9). Moreover, studies have reported that a significant relationship exists between biofilm formation and antifungal resistance (8,10).

Amphotericin B and triazole antifungal agents are usually used for the treatment of trichosporonosis (1). Previous studies have reported that amphotericin B has inadequate fungicidal activity and limited *in vivo* activity with evidence of *in vitro* resistance (11). Triazole drugs, particularly voriconazole, are effective for treatment (12,13). Moreover, echinocandins, another drug group, are naturally ineffective against *Trichosporon* species (14-16). Thus, the present study aimed to determine the susceptibility of 100 *T. asahii* strains, isolated from urine samples, to various antifungal agents.

METHODS

Non-Invasive Research Ethics Committee approval was obtained from Haydarpasa Numune Education and Research Hospital (02.09.2019, HNHEAH-KAEK 2019/103-955). Among the 1,442 urine samples sent to the Central Laboratory of the Department of Public Hospital Services-2 in İstanbul between 2015 and 2016 that yielded yeast on culture, *Candida* species were detected in 1,332 (92.3%) samples, *T. asahii* in 106 samples (7.3%) and other yeasts in four samples (0.2%).

Identification was performed using the matrix-assisted laser desorption ionization-time of flight mass spectrometry-ITEK MS IVD V.2 (Bio-Mérieux, Marcy l'Etoile, France) automated system-as well as conventional methods (macroscopic and microscopic morphologies, appearance on corn meal agar with Tween 80, and urease positivity). Isolates were stored at -80 °C until the time of analysis and revived with two passages in Sabouraud dextrose agar.

Broth microdilution is standardized only for *Candida* and *Cryptococcus* species on CLSI M27-A3, which is intended for antifungal susceptibility testing; however, similar to previous studies, (3,4,9) our study investigated the *in vitro* susceptibility profiles of *T. asahii* for antifungal agents according to CLSI M27-A3 (17). Only the minimum inhibitory concentration (MIC) values obtained were specified because clinical thresholds of antifungals for the genus *Trichosporon* are still unestablished. Antifungal agents used in the study included amphotericin B (Sigma Chemical Co., St. Louis, MO, USA), fluconazole (Sigma Chemical Co.), voriconazole (Sigma Chemical Co.), itraconazole (Sigma Chemical Co.), posaconazole (Sigma Chemical Co.), and isavuconazole (Toronto Research). Microdilution plates were prepared with a final antifungal concentration of 32-0.06 µg/L for fluconazole; 16-0.03 µg/L for amphotericin B and itraconazole; 8-0.015 µg/L for voriconazole, posaconazole, and micafungin; and 4-0.008 µg/L for isavuconazole. The experiment was repeated twice for each strain. *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as quality control strains. The yeast suspensions resulted in concentrations of 2.5×10^3 cells/mL, and MIC was defined as the lowest antifungal concentration capable of promoting a 50% inhibition for azoles and 90% for amphotericin B at the end of 24 and 48 hours.

RESULTS

Among the 100 *T. asahii* strains isolated from the urine samples, 68 (68%) were isolated from male and 32 (32%) from female patients, and 66 (66%) of the total were from patients aged ≥70 years. The mean age of the patients was 69.94 (±20.30164) years. Among the 82 patients admitted in the ICU, 56 (68%) were male and 26 were female, and 68% were aged 70 and above. Table 1 presents the MIC ranges, MIC_{50}, MIC_{90}, and geometric mean values of isolates against amphotericin B, micafungin, and the five azole antifungal agents. The growth evaluation at 24 h revealed that most of the strains (89%) had a MIC value of ≥1 µg/mL for amphotericin B, while the rate was 96% at 48 h. Azole antifungal agents, including voriconazole, itraconazole, posaconazole, and isavuconazole, showed similar and low

Table 1. Results of *in vitro* susceptibility tests for *Trichosporon* asahii strains

	MIC (µg/mL) at 24 h				MIC (µg/mL) at 48 h			
Drug	MIC range	MIC_{50}	MIC_{90}	GM	MIC range	MIC_{50}	MIC_{90}	GM
Amphotericin B	0.25-2	1	2	0.99	0.5-4	2	2	1.81
Fluconazole	0.25-16	2	8	1.93	0.25-32	2	8	2.1
Voriconazole	≤0.015-1	0.06	0.12	0.05	≤0.015-1	0.06	0.12	0.06
Itraconazole	0.03-2	0.12	0.25	0.13	0.012-2	0.12	0.25	0.14
Posaconazole	≤0.015-0.5	0.25	0.5	0.18	0.012-1	0.25	1	0.18
Isavuconazole	≤0.008-0.5	0.12	0.25	0.09	≤0.008-2	0.12	0.25	0.12
Micafungin	>8	>8	>8	-	>8	>8	>8	-

GM: Geometric mean, MIC: Minimal inhibitory concentration

MIC values at both time points, while the MIC values for fluconazole were higher than those for other azole agents. The MIC values of all strains for micafungin were >8 mg/L Overall, *T. asahii* colonies became more prominent, and MIC values were more accurately determined at 48 h.

DISCUSSION

Infections caused by *Trichosporon* species often arise from endogenous flora, and the risk of such infections increases especially in patients with immunosuppression or in patients admitted in the ICU due to facilitating factors such as microbial translocation through the gastrointestinal mucosa and presence of vascular or urinary catheters (1). In a previous study, the prevalence of UTIs caused by *Trichosporon* in the ICU in a two-year period was 6% and the mortality rate was 20%. The prevalence was higher among men (65%) and individuals aged >70 years (55%) (6).

Among *Trichosporon* species, *T. asahii* is the most common cause of UTIs (4,9). *T. asahii* is an emergent pathogen in older patients with urinary catheter (12). Our study also identified *T. asahii* as the most commonly isolated species, with prevalence being higher among patients in the ICU (82%) and in male patients (68%). Furthermore, 66% of such patients were ≥70 years. Although UTIs are typically more common in women because of their anatomical structure (short urethra, vagina-anus proximity, etc.), (6) those caused by the genus *Trichosporon* were more common among men in our patient population, consistent with some other studies (6,12).

Triazole antifungal agents and amphotericin B are usually used for the treatment of *Trichosporon* infections (1). Previous studies have reported that amphotericin B has inadequate fungicidal activity against some *Trichosporon* strains and has limited *in vivo* activity along with evidence of *in vitro* resistance (11). Susceptibility test results vary from study to study. There are reports of low MIC (0.06-1) values, (8) as well as high MIC values (14). Although the fungus appears to be susceptible to amphotericin B *in vitro*, *in vivo* resistance may develop through a biofilm layer formed by the *Trichosporon* species; as a result, the desired effect is not observed (13). In our study, the MIC value for amphotericin B was ≥1 µg/mL in 89% and 96% of the strains at 24 h and 48 h, respectively.

Studies have reported that triazole antifungal agents, particularly voriconazole, are superior to amphotericin B in terms of efficacy in trichosporonosis treatment, and this group of agents are more commonly preferred for treatment (13,16). That said, there are reports of fatal pediatric cases (18) and treatment failures due to *T. asahii* infection, despite treatment with amphotericin B and voriconazole (19,20). In addition to the antifungal susceptibility of the agent, the patient's immunity system and neutrophil count play an important role in treatment success (21).

The 2014 clinical guidelines for the diagnosis and management of rare invasive yeast infections drawn up by the European Society for Clinical Microbiology and Infectious Diseases recommends the use of triazoles, particularly voriconazole, for the treatment of invasive infections caused by *T. asahii*. (15) Studies that compared the *in vitro* efficacy of triazole antifungal agents against *T. asahii* strains have reported fluconazole as the triazole antifungal agent with the lowest activity, whereas voriconazole demonstrated the highest activity. Other triazole antifungal agents, such as itraconazole, posaconazole, and isavuconazole, showed comparable activity (3,4,14,16,22,23) The findings of the present study were consistent with the results of such studies. To the best of our knowledge, only a few studies have investigated the susceptibility of the genus *Trichosporon* to isavuconazole, which is the newest member of triazole antifungals. The MIC_{50}-MIC_{90} values of

Table 2. ***In vitro*** **antifungal susceptibility test results of** ***Trichosporon asahii*** **isolates (µg/mL, 48 h), as reported by previous studies**

Authors	Number of isolates	Drugs	MIC range	MIC_{50}	MIC_{90}	GM
Montaya et al. (14)	39	AMB	0.25->16	2	4	1.84
		FLZ	0.12-16	0.5	1	0.78
		VOR	0.03-1	0.03	0.03	0.04
		POS	0.03-0.5	0.06	0.25	0.08
		MICA	>8	>8	>8	ND
Francisco et al. (4)	273	AMB	0.032-64	2	32	ND
		FLZ	0.25-64	2	8	ND
		VOR	0.03-2	0.06	0.125	ND
		POS	0.03-2	0.25	0.5	ND
Hazırolan et al. (22)	90	FLZ	0.5-16	4	8	3.24
		ITR	0.12-1	0.25	1	0.37
		VOR	≤0.015-0.25	0.06	0.12	0.06
		POS	0.06-1	0.25	0.5	0.25
		ISA	≤0.015-0.5	0.12	0.25	0.1
Kalkanci et al. (23)	87	FLZ	4-64	8	16	13.66
		ITR	0.25-2	1	2	0.985
Guo et al. (3)	108	AMB	0.125-4	1	2	1.36
		FLZ	0.5-512	4	8	3.56
		ITR	0.25-32	0.5	1	0.48
		VOR	0.03-16	0.064	0.25	0.09
		MICA	>8	>8	>8	>8
		CAS	>8	>8	>8	>8

FLZ: Fluconazole, ITR: Itraconazole, VOR: Voriconazole, POS: Posaconazole, ISA: Isavuconazole, CAS: Caspofungin, MICA: Micafungin, AMB: Amphotericin B, ND: Not determined, GM: Geometric mean, MIC: Minimal inhibitory concentration

clinical *T. asahii* isolates for isavuconazole, were found to be as follows: Hazirolan et al. (22) (n=90), 0.125-0.25 µg/mL; Thompson et al. (24) (n=40), 0.125 µg/mL; and the present study, 0.12-0.25 µg/mL, which is consistent with the previous data. Table 2 presents the *in vitro* susceptibility test results of *T. asahii* strains to several antifungal agents after 48 h of incubation, as reported in various studies.

Echinocandins, which are another group of antifungal agents, have demonstrated limited and inadequate *in vitro* activity against *Trichosporon* species (15,25). The MIC values for all strains against micafungin were >8 mg/L in the present study. Patients developing breakthrough invasive trichosporonosis while undergoing echinocandin therapy were also reported. Therefore, the risk of breakthrough infections of *Trichosporon* should not be ignored in patients with high risk status undergoing empirical or prophylactic therapy with echinocandins (16,25,26).

The generalization of the study's results is limited by the lack of differentiation between infection and colonization in patients; as a result, isolates deemed as potential causes are being considered as "related to the clinical picture." Standardization is required to differentiate between colonization and infection by *Trichosporon* species, particularly among patients in the ICU.

CONCLUSION

In conclusion, previous studies have identified various susceptibilities to antifungal agents and have shown that *in vitro* activity does not always correlate with efficacy *in vivo*. Our study established that voriconazole, an azole antifungal agent, was the most effective antifungal against *T. asahii* isolates *in vitro*. Considering the high MIC values, breakthrough infections of *Trichosporon* should be considered in patients with high risk status receiving

empirical or prophylactic therapy of echinocandin or fluconazole.

ETHICS

Ethics Committee Approval: The study were approved by the Haydarpasa Numune Education and Research Hospital of Local Ethics Committee (Protocol number: HNHEAH-KAEK 2019/103-955).

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

Authorship Contributions

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

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

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

REFERENCES

1. Colombo AL, Padovan AC, Chaves GM. Current knowledge of Trichosporon spp. and Trichosporonosis. Clin Microbiol Rev 2011;24:682-700.
2. Liu XZ, Wang QM, Göker M, Groenewald M, Kachalkin AV, Lumbsch HT, et al. Towards an integrated phylogenetic classification of the Tremellomycetes. Stud Mycol 2015;81:85-147.
3. Guo LN, Yu SY, Hsueh PR, Al-Hatmi AMS, Meis JF, Hagen F, et al. Invasive Infections Due to Trichosporon: Species Distribution, Genotyping, and Antifungal Susceptibilities from a Multicenter Study in China. J Clin Microbiol 2019;57:e01505-18.
4. Francisco EC, de Almeida Junior JN, de Queiroz Telles F, Aquino VR, Mendes AVA, de Andrade Barberino MGM, et al. Species distribution and antifungal susceptibility of 358 Trichosporon clinical isolates collected in 24 medical centres. Clin Microbiol Infect 2019;25:909.e1-909.e5.
5. Urs TA, Kadiyala V, Deepak S, Karthik MK. Catheter associated urinary tract infections due to Trichosporon asahii. J Lab Physicians 2018;10:464-70.
6. Mattede Md, Piras C, Mattede KD, Ferrari AT, Baldotto LS, Assbu MS. Urinary tract infections due to Trichosporon spp. in severely ill patients in an intensive care unit. Rev Bras Ter Intensiva 2015;27:247-51.
7. Bentubo HD, Gompertz OF. Effects of temperature and incubation time on the in vitro expression of proteases, phospholipases, lipases and DNases by different species of Trichosporon. Springerplus 2014;3:377.
8. Sun W, Su J, Xu S, Yan D. Trichosporon asahii causing nosocomial urinary tract infections in intensive care unit patients: genotypes, virulence factors and antifungal susceptibility testing. J Med Microbiol 2012;61:1750-7.
9. Almeida AA, Crispim Bdo A, Grisolia AB, Svidzinski TI, Ortolani LG, Oliveira KM. Genotype, antifungal susceptibility, and biofilm formation of Trichosporon asahii isolated from the urine of hospitalized patients. Rev Argent Microbiol 2016;48:62-6.
10. Iturrieta-González IA, Padovan AC, Bizerra FC, Hahn RC, Colombo AL. Multiple species of Trichosporon produce biofilms highly resistant to triazoles and amphotericin B. PLoS One 2014;9:e109553.
11. Walsh TJ, Melcher GP, Rinaldi MG, Lecciones J, McGough DA, Kelly P, et al. Trichosporon beigelii, an emerging pathogen resistant to amphotericin B. J Clin Microbiol 1990;28:1616-22.
12. Treviño M, García-Riestra C, Areses P, García X, Navarro D, Suárez FJ, et al. Emerging Trichosporon asahii in elderly patients: epidemiological and molecular analysis by the DiversiLab system. Eur J Clin Microbiol Infect Dis 2014;33:1497-503.
13. Tanyildiz HG, Yesil S, Toprak S, Candir MO, Sahin G. Two Case Presentations Infected by Trichosporon asahii and Treated with Voriconazole Successfully. Case Rep Infect Dis 2015;2015:651315.
14. Montoya AM, Sánchez González A, Palma-Nicolás JP, Gómez-Treviño A, González JG, González GM. Genotyping, extracellular compounds, and antifungal susceptibility testing of Trichosporon asahii isolated from Mexican patients. Med Mycol 2015;53:505-11.
15. Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, et al. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. Clin Microbiol Infect 2014;20 Suppl 3:76-98.
16. de Almeida Júnior JN, Hennequin C. Invasive Trichosporon Infection: a Systematic Review on a Re-emerging Fungal Pathogen. Front Microbiol 2016;7:1629.
17. CLSI. Reference method for broth dilution antifungal susceptibility testing of yeasts Approved standard-third edition. Wayne, PA: Clinical and Laboratory Standards Institute; April 2008.
18. Thibeault R, Champagne M, de Repentigny L, Fournet JC, Tapiero B, Moghrabi A, et al. Fatal disseminated Trichosporon asahii infection in a child with acute lymphoblastic leukemia. Can J Infect Dis Med Microbiol 2008;19:203-5.
19. Kurnaz F, Kaynar L, Doğan S, Eser B, Metan G. Treatment Failure of Disseminated Trichosporon asahii Infection with Voriconazole in a Patient with Acute Myeloid Leukemia. Acta Oncol Turc 2010;43:32-5.
20. Chen J, Chen F, Wang Y, Yang LY, Miao M, Han Y, et al. Use of combination therapy to successfully treat breakthrough Trichosporon asahii infection in an acute leukemia patient receiving voriconazole. Med Mycol Case Rep 2014;6:55-7.
21. Hosokawa K, Yamazaki H, Mochizuki K, Ohata K, Ishiyama K, Hayashi T, et al. Successful treatment of Trichosporon fungemia in a patient with refractory acute myeloid leukemia using voriconazole combined with liposomal amphotericin B. Transpl Infect Dis 2012;14:184-7.
22. Hazirolan G, Canton E, Sahin S, Arikan-Akdagli S. Head-to-head comparison of inhibitory and fungicidal activities of fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole against clinical isolates of Trichosporon asahii. Antimicrob Agents Chemother 2013;57:4841-7.
23. Kalkanci A, Sugita T, Arikan S, Yucesoy M, Ener B, Otag F, et al. Molecular identification, genotyping, and drug susceptibility of the basidiomycetous yeast pathogen Trichosporon isolated from Turkish patients. Med Mycol 2010;48:141-6.
24. Thompson GR 3rd, Wiederhold NP, Sutton DA, Fothergill A, Patterson TF. In vitro activity of isavuconazole against Trichosporon, Rhodotorula, Geotrichum, Saccharomyces and Pichia species. J Antimicrob Chemother 2009 Jul;64:79-83.
25. Yang MF, Gao H, Li LL . A fatal case of Trichosporon asahii fungemia and pneumonia in a kidney transplant recipient during caspofungin treatment. Ther Clin Risk Manag 2014;10:759-62.
26. Liao Y, Hartmann T, Zheng T, Yang RY, Ao JH, Wang WL. Breakthrough trichosporonosis in patients receiving echinocandins: case report and literature review. Chin Med J (Engl) 2012;125:2632-5.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:135-141
DOI: 10.4274/BMJ.galenos.2021.19970

Research

The Effect of Nurse Telephone Consultation After Coronary Artery Bypass on the Autonomy Level of Elderly Patients: A Quasi-Experimental Study

Koroner Arter Bypass Sonrası Telefonla Hemşire Danışmanlığının Yaşlı Hastaların Otonomi Düzeyine Etkisi: Yarı Deneysel Çalışma

Figen Dığın[1], Ümmü Yıldız Fındık[2]

[1]Kırklareli University Health High School, Department of Midwife, Kırklareli, Turkey
[2]Trakya University Faculty of Health Sciences, Department of Surgical Nursing, Edirne, Turkey

ABSTRACT

Objective: The effect of nurse telephone consultations was determined on the autonomy levels of elderly patients after coronary artery bypass surgery in this quasi-experimental study.

Methods: This study was conducted as a quasi-experimental research investigation from December 25, 2015, to January 10, 2017, at the cardiovascular surgery clinic of a university hospital and included 64 patients (32 cases, 32 control group patients). The data were collected using the "patient descriptive form," "The Functional Autonomy Measurement System," "Discharge Information Guide," and "The Telephone Consultation Follow-Up Form." The patients in the case group were provided with nurse telephone consultations six weeks after discharge. The Functional Autonomy Measurement System was re-administered to all patients at the end of six weeks. The necessary ethical and institutional approvals were obtained before the study.

Results: The mean age of the patients was 69.96 (±4.94) years, 76.56% (n=49) were males, 85.9% (n=55) were married, and 73.43% (n=47) were primary school graduates. The mean autonomy score (-4.04±2.52) and the autonomy level of daily life activities of the patients (-2.20±1.71) in the case group (p=0.000) were significantly higher at the end of six weeks (p<0.05).

Conclusion: Nurse telephone consultations increased the autonomy level of elderly patients undergoing coronary artery bypass surgery.

Keywords: Autonomy, telehealth, coronary artery bypass, elderly, nursing

ÖZ

Amaç: Bu yarı deneysel çalışmada koroner arter bypass sonrası telefonla hemşire danışmanlığının yaşlı hastaların otonomi düzeyine etkisi belirlendi.

Gereç ve Yöntem: Çalışma 25 Aralık 2015-10 Ocak 2017 tarihleri arasında bir üniversite hastanesi Kalp Damar Cerrahisi Kliniği'nde koroner arter bypass ameliyatı olan 64 (32 deney ve 32 kontrol) yaşlı hastanın katılımı ile yarı deneysel olarak yapıldı. Veriler "Hasta Tanıtım Formu", "Otonomi Değerlendirme Ölçeği", "Taburculuk Bilgilendirme Rehberi" "Telefon Danışmanlığı İzlem Formu" kullanılarak toplandı. Deney grubundaki hastalara taburculuk sonrası 6 hafta süresince telefonla hemşire danışmanlığı yapıldı. Hastaların tamamına 6 haftanın sonunda Otonomi Değerlendirme Ölçeği tekrar uygulandı. Çalışmaya başlamadan once gerekli etik ve kurum izni alındı.

Bulgular: Hastaların yaş ortalamalarının 69,95±4,94, %76,6'sının (n=49) erkek, %85,9'unun (n=55) evli ve %73,4'ünün (n=47) ilköğretim mezunu olduğu belirlendi. Deney grubundaki hastaların 6 hafta sonundaki otonomi puan ortalamasının (-4,04±2,52) ve günlük yaşam aktiviteleri alt boyutu otonomi puan ortalamasının (-2,20±1,71) kontrol grubuna göre yüksek olduğu bulundu (p=0,000) (p<0,05).

Sonuç: Telefonla hemşire danışmanlığının koroner arter bypass sonrası yaşlı hastaların otonomi düzeyini artırdığı belirlendi.

Anahtar Kelimeler: Otonomi, tele sağlık, koroner arter bypass, yaşlı, hemşirelik

Address for Correspondence: Figen Dığın, Kırklareli University Health High School, Department of Midwife, Kırklareli, Turkey
Phone: +90 505 646 19 29 E-mail: fgndgn2013@gmail.com ORCID ID: orcid.org/0000-0003-1861-0221

Cite as: Dığın F, Yıldız Fındık Ü. The Effect of Nurse Telephone Consultation After Coronary Artery Bypass on the Autonomy Level of Elderly Patients: A Quasi-Experimental Study. Med J Bakırköy 2021;17:135-141

Received: 20.10.2020
Accepted: 16.06.2021

INTRODUCTION

Basic changes in the body caused by old age indicate that chronic diseases replace acute diseases. One of the most significant chronic diseases is coronary artery disease, the most common cause of mortality and morbidity in the elderly (1). It leads to decreased physical, social, and mental functions, deterioration of health perception, and decreased the quality of life of patients with coronary artery disease. These patients undergo coronary artery bypass surgery, which provides blood flow to the myocardium to prolong life and reduce symptoms (2,3). The literature suggests that recovery after coronary artery bypass surgery is a very complicated process that includes achieving physical, psychological, and social health to attain overall recovery (2,3). Good preoperative preparation and effective postoperative care should be provided to perform surgery safely on elderly patients and reduce complications and mortality (4). The physiological changes that occur in elderly patients, the surgical methods and techniques applied, the duration of the surgery, and the length of stay in the intensive care unit and the hospital all affect the autonomy level of patients. Moreover, postoperative complications, susceptibility to chronic diseases, previous diseases, and social support systems also contribute positively or negatively to the autonomy level of patients (5). In particular, functional losses occur in the elderly after surgery due to age, depression, inadequate social support, lack of mobility, and cognitive disorders (6). During the 6-8 week postdischarge recovery period, patients experience pain management, wound care, respiratory and cardiac problems, nutritional deficiency, diarrhea, constipation, depression, and edema (6,7). These issues lead to decreased autonomy levels of elderly patients after coronary artery bypass surgery (8). Therefore, elderly patients suffer temporary or permanent loss of autonomy after surgery (9,10). During the recovery process, the autonomy levels of patients should be supported by home care practices (5). In line with recent technological developments, new methods are starting to deliver home care services. One of these is telehealth services. Telehealth is sharing health-related information using interactive audiovisual tools for health care applications, diagnosis, consultation, and treatment. One of the most frequently used technological methods in the telehealth system is communication by telephone (3). In telehealth services, telephone use is recommended to establish an emotional connection between the hospital and the home, support the functional independence of elderly patients, and improve the quality of care (10-12). Nurses play an active role in-home care services offered to elderly individuals. Nurses use different technological applications to follow-up with patients at home after discharge. A nurse telephone consultation, one of these applications, provides accessibility regarding the care of the elderly patient at home and maintaining that care (11). Nurse telephone consultations are highly beneficial in supporting functional independence levels of elderly patients by enabling them to participate in-home care after coronary artery bypass surgery (7). After coronary artery bypass surgery, it is recommended that nurse telephone consultation be used effectively in-home care processes for elderly patients whose autonomy levels have decreased due to both surgery and age (7,13,14).

This study will determine the effectiveness of nurse telephone consultation on the autonomy level of elderly patients after coronary artery bypass surgery. It will help maintain the focus of the agenda by attracting patient and health care workers' attention. This study aims to determine the effect of nurse telephone consultation on the autonomy level of elderly patients after coronary artery bypass surgery.

METHODS

The randomized controlled study was conducted from December 25, 2015, to January 4, 2017, at the cardiovascular surgery department of a university hospital with 64 elderly patients who underwent coronary artery bypass. Based on the findings (the Functional Autonomy Measurement System (SMAF) score: -7.60±10.21) in the work titled "The validity and reliability study of the "Functional Autonomy Measurement System" among 65 years and over age group" by Tuna and Çelik (5) in 2012 the sample size was calculated as 64 at a 95% confidence interval and a 5% margin of error. The sample included patients who volunteered to participate in the study, were aged 65 and over, were undergoing coronary artery bypass surgery, had no communication problems (i.e., no vision or hearing problems), and had no mental problems (i.e., dementia, amnesia). Also, patients (or relatives) who could communicate by telephone, were willing to volunteer to participate in the study, accepted the randomization, and had at most two chronic diseases (diabetes and/or hypertension). Patients with reoperated coronary artery bypass surgery and patients with operated coronary artery bypass surgery and valve surgery were excluded from the study.

The group to be provided telephone consultation by the researcher was determined as the case group. The other patients were included in the control group. In the study, patient in the case and control groups were followed simultaneously. The randomization was determined by the simple random randomization method with a total of

64 patients in the case (32) and control groups (32) (Figure 1). The study sample was randomly allocated into two groups. Patients were numbered according to the order of hospitalization in the cardiovascular surgery clinic to avoid bias while performing simple randomization in the study. Even-numbered patients were included in the case group, and odd-numbered patients were included in the control group by the researcher (Figure 1).

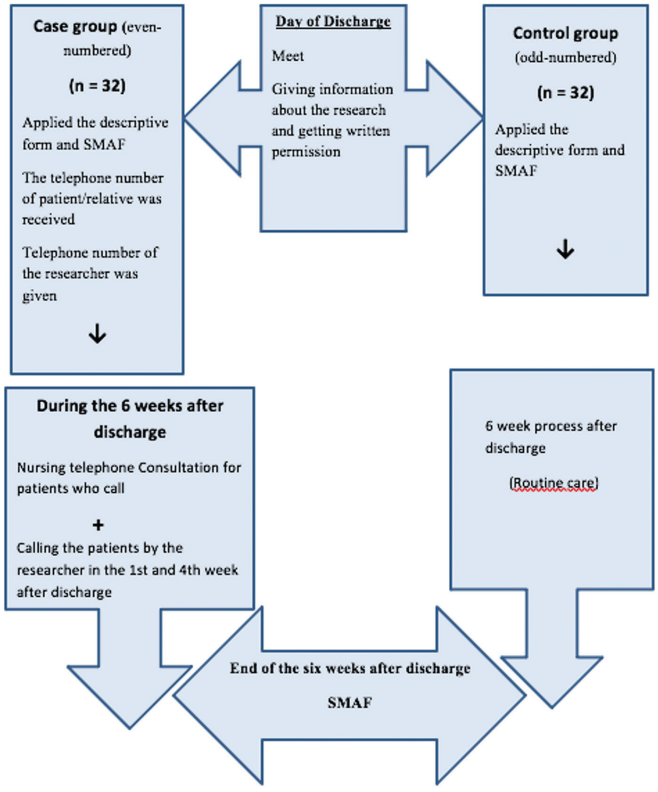

Figure 1. Flow chart of the study
SMAF: Functional Autonomy Measurement System.

The patient descriptive form consisted of 14 questions prepared by the researchers who questioned the individual patients.

The SMAF was developed in 1984 by Hebert et al. (15) to determine the level of functional independence of patients aged 65 or older. Its reliability and validity studies were performed by Tuna and Çelik (5). Each item can receive a score of between 0 and -3, and the total dependency score of the adapted scale was -75 points. Thus, the highest possible patient score is 0, and the lowest score is -75. The level of autonomy increases as the patient's score approaches 0. In the Turkish version of the scale, Cronbach's α coefficient for the SMAF was 0.95. In this study, Cronbach's α coefficient was 0.85 before discharge and 0.82 after discharge.

The Discharge Information Guide was prepared by the researcher according to the literature. The literature was reviewed to determine the educational protocols for patients undergoing coronary artery bypass surgery before discharge (2,16,17).

The Telephone Consultation Follow-up form included questions about the date of the patient interview, the reason for the interview, and the proposed interventions/recommendations. In addition, it was used to record the telephone conversations that the patients in the case group had with the researcher six weeks after discharge.

Before starting the data collection, 64 patients who were assessed for eligibility were prospectively identified. No patient was excluded from the study. After providing verbal and written information to all patients who underwent coronary artery bypass, they were asked to sign the informed consent form. During the study, the researcher collected information. The data was recorded by the researcher in line with the answers of the patients.

The patient descriptive form and the SMAF were administered to the patients in the case group who planned to be discharged within 24 hours by the face-to-face interview method in the patient's room. The telephone number of each patient/relative in the case group was received, and the telephone number of the researcher was given to them. The patients were informed that they could receive a telephone consultation from the researcher concerning the problems they might experience during the home care process. According to the Discharge Information Guide, the researcher called and consulted patients in the case group during the first and fourth weeks after discharge. Besides, telephone consultations were provided to the patients who called the researcher for six weeks. Fourteen patients received counseling services by phone. Some patients called on two consecutive days, whereas other patients called once a week. It was seen that some of the patients received counseling for a few problems in one call. It was determined that the patients who received nurse counseling over the phone received consultancy for back, shoulder, waist, and leg pain for the first time, respiratory distress for the second, and wound healing for the third time. The information provided to patients by telephone consultation was recorded in the Telephone Consultation Follow-up Form. There was no time limit on-call hours for the telephone consultation provided to the patients. The researcher provided telephone consultation at every hour for patients. The researcher re-administered the SMAF to the patient in the case group by calling on the telephone at the end of the six weeks after discharge (Figure 1).

The patient descriptive form and SMAF were administered to the patients in the control group who were planned to be discharged within 24 hours by the face-to-face interview method in the patient's room. In addition, the telephone number of each patient/relative of the control group was received. Finally, the researcher re-administered the SMAF to the patients in the control group by calling on the telephone at the end of the six weeks after discharge (Figure 1).

Statıstcal Analysis

In the study, data of 64 patients were analyzed using the Statistical Package for Social Sciences (SPSS) 21.0 program. Collected data were expressed using descriptive statistics, such as number, percentage, arithmetic mean, and standard deviation. In addition, Fisher's Exact, chi-square, independent samples t-test, the Wilcoxon signed-rank test, and Mann-Whitney U test were used to compare the autonomy level of the 64 patients. The statistical significance value was accepted as $p<0.05$.

Written permission was obtained from the general directorship of the hospital and the Trakya University Medical Faculty Scientific Research Ethics Committee. Verbal and written consent was obtained from the patients participating in the study, regarding their volunteer participation and the study process, by reading the voluntary consent form. The patients participating in the study were informed that their decision to participate was their free will, and the information obtained during the study process would remain confidential and used only for this study.

RESULTS

The mean age of the patients was 69.96 (±4.94) years, 76.56% (n=49) were males, 73.43% (n=47) were primary school graduates (Table 1). While the case and control groups were similar regarding sociodemographic characteristics, a higher number of patients living in urban areas in cases and a higher number of patients not using alcohol in the control group were statistically significant (p=0.008, p=0.039) (Table 1).

In the sixth week after discharge, the mean autonomy scale score of case group patients was significantly higher than control group patients (Z=-5.565, p=0.000) (Table 2). In addition, the daily life activities sub-dimension mean autonomy scale score in case group patients in the sixth week after discharge was significantly higher than control group patients (Z=-5.778, p=0.000) (Table 2).

In the sixth week after discharge, there was no significant difference between the mean scores of mental functions and communication in the case and control groups (Z=-1.732, p=0.083; Z=0.000, p=1.000) (Table 2).

DISCUSSION

In this study, the patients given telephone consultations had a high autonomy level six weeks after discharge. Similarly, Tuna and Çelik. (5,17) found a high level of autonomy in patients who received professional support and nurse consultations. Bikmordi et al. (18) reported that telephone counseling after coronary artery bypass effectively improved patients' quality of life. Moon et al. (19) found that nurse telephone consultation improved the self-care power of patients in heart failure patients. Furuya et al. (7) determined that telephone consultation was used for cardiovascular disease care, postoperative complications care, and self-care. Also, the study by Schulz et al. (14) determined that a tele-follow-up by a nurse effectively prevented delays in postoperative recovery. Furthermore, Kleinpell and Avitall (20) stated that at home tele-follow-ups with at-risk patients undergoing coronary artery bypass surgery positively affect symptom management. A study by Lallement et al. (21) stated that 24% of elderly patients discharged after surgery experience loss of autonomy, affecting the mortality and morbidity of patients. Melholt et al. (22) stated that using telehealth applications for patient education and cardiac rehabilitation in cardiac patients is beneficial. This study and other similar studies reveal that elderly patients experience autonomy problems during the postoperative period. Our study observed that nurse telephone consultations significantly contribute to the autonomy level of patients, helping them cope with these problems. As a result, the autonomy level of patients who received nurse telephone consultations was higher.

In this study, the daily life activities sub-dimension mean autonomy scale score in the case group patients in the sixth week after discharge was significantly higher than in control group patients. Decreases in physical and psychological capacity, and aging, cause problems for the elderly about fulfilling their daily life functions. The elderly experience difficulties with daily life activities, such as bathing, dressing, urinary control, preparing food, dishwashing, laundering, shopping, house cleaning, and transportation (23). However, coronary artery bypass surgery affects the autonomy of elderly patients in performing their daily life activities (5,17). A study stated that the elderly are dependent regarding daily life activities during the home care process and should be followed up (24). A study conducted by Akay and Akyol (25) stated that the daily life activities of patients who were followed up using the tele-follow-up method after

Table 1. Demographic characteristics of the patients (n=64)

Sociodemographic characteristics	Case group (n=32)		Control group (n=32)		Test p
	n	%	n	%	
Age (Mean ± SD)	69.37±4.94		70.56±4.94		t=-0.986* p=0.328
Gender					
Female	7	21.9	8	25.0	χ^2=0.087**
Male	25	78.1	24	75.0	p=0.768
Marital status					
Married	27	84.4	28	87.5	χ^2=0.129**
Single	5	15.6	4	12.5	p=0.719
Education					
Illiterate	1	3.10	5	15.6	
Literate	2	6.30	1	3.10	
Primary school	23	71.9	24	75.0	χ^2=4.989**
High school	2	6.30	1	3.10	p=0.284
University	4	12.5	1	3.10	
Working status					
Working	4	12.5	2	6.30	χ^2=0.736**
Not working	28	87.5	30	93.8	p=0.672
Area of residence					
Urban area	26	81.3	16	50.0	χ^2=6.926†
Rural area	6	18.8	16	50.0	p=0.008
Smoking status					
Yes	1	3.10	4	12.5	
No	12	37.5	13	40.6	χ^2=2.175***
Quit	19	59.4	15	46.9	p=0.359
Alcohol status					
Yes	8	25.0	2	6.30	
No	16	50.0	25	78.1	χ^2=6.128***
Quit	8	25.0	5	15.6	p=0.039
Number of people living together					
Lives alone	2	6.30	3	9.40	χ^2=0.217**
Two people and above	30	93.8	29	90.6	p=0.641
Education level of the person living together (n=59)					
Illiterate	2	6.70	6	20.7	
Literate	2	6.70	1	3.40	
Primary school	16	53.3	19	65.5	χ^2=6.620***
High school	6	20.0	1	3.40	p=0.142
University	4	13.3	2	6.90	
Level of support received from the social environment					
Very good	11	34.3	8	25.0	χ^2=1.266***
Good	19	59.4	23	71.9	p=0.555
Poor/No support	2	6.30	1	3.10	

*Independent sample t-test, **Pearson chi-square, ***Fisher's Exact, n: Number of patients SD: Standard deviation

coronary artery bypass surgery showed improvement. Also, Rantanen et al. (3) observed that using the tele-follow-up method improved the postoperative daily life activities of patients who undergo coronary artery bypass surgery. Lafaro et al. (26) stated that telehealth perioperative physical activity intervention is feasible and acceptable for elderly patients. Also, Dinesen et al. (27) determined that telehealth applications increase the sense of autonomy and motivation of cardiac patients. Moreover, studies have found that patient counseling after discharge increased the activity, independence level, and self-care ability in patients who undergo coronary artery bypass surgery (28). Results

Table 2. Changes in autonomy scores for both groups (n=64)

SMAF	Case group (n=32)		Control group (n=32)		Changes	
	Day of discharge	After discharge 6 weeks	Day of discharge	After discharge 6 weeks	Day of discharge	After discharge 6 weeks
	Mean ± SD				Test p	
Activities of daily living	-20.17±6.78	-2.20±1.71	-22.07±9.69	-8.06±4.40	Z=-0.524 p=0.600	Z=-5.778** p=0.000
Communication	-0.96±0.82	-0.96±0.82	-1.03±0.82	-1.03±0.82	Z=-0.413 p=0.679	Z=-0.413** p=0.679
Mental functions	-0.96±1.17	-0.87±1.03	-1.00±1.16	-0.96±1.14	Z=-0.122 p=0.903	Z=-2.216** p=0.829
Total	-22.10±7.02	-4.04±2.52	-24.10±9.81	-10.06±4.40	Z=-0.638 p=0.523	Z=-5.565** p=0.000

n: Number of patients, SD: Standard deviation, SMAF: Functional Autonomy Measurement System.

* Wilcoxon signed-rank t-test, **Mann-Whitney U test

NOTE: No statistical comparison was made on the day of discharge or six weeks after discharge because communication sub-dimension scores were the same

of studies show that nurse telephone consultation after coronary artery bypass surgery increased the autonomy level of elderly patients concerning daily life activities.

Study Limitations

These results cannot be generalized because they provide a single-center experience. At this point, multicenter studies should be performed.

CONCLUSION

In this study, there was a statistically significant difference between the functional autonomy level in patients in the case and control groups. Furthermore, it was revealed that nurse telephone consultations increased independence and autonomy, especially regarding the daily life activities of elderly patients who had undergone coronary artery bypass surgery. In light of these results, we recommend nurse telephone consultations to increase the autonomy level of elderly patients undergoing coronary artery bypass surgery.

ACKNOWLEDGMENTS

We would like to thank all the patients who took part in the study.

ETHICS

Ethics Committee Approval: The study were approved by the Trakya University Medical Faculty Scientific Research Ethics Committee (Protocol number: B.30.2.T RK.0.20.05.04/050.04.02).

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

Authorship Contributions

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

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

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

REFERENCES

1. Graf C. Functional decline in hospitalized older adults. Am J Nurs 2006;106:58-67.
2. Korkmaz FD, Alcan AO, Aslan FE, Çakmakçı H. An evaluation of quality of life following coronary artery bypass graft surgery. Turk Gogus Kalp Damar 2015;23:285-94.
3. Rantanen A, Tarkka MT, Kaunonen M, Tarkka M, Sintonen H, Koivisto AM, et al. Health-related quality of life after coronary artery bypass grafting. J Adv Nurs 2009;65:1926-36.
4. Aygin D. Yaşlı cerrahisinde bakım. Aslan FE (Ed). Cerrahi bakım vaka analizleri ile birlikte. Ankara: Akademisyen Tıp Kitabevi, 2017:181-247.
5. Tuna Z, Çelik SS. The validity and reliability study of the "functional autonomy measurement system" among 65 years and over age group. European Geriatric Medicine 2013;4(supplement 1):97.
6. Mohanty S, Rosenthal RA, Russell MM, Neuman MD, Ko CY, Esnaola NF. Optimal Perioperative Management of the Geriatric Patient: A Best Practices Guideline from the American College of Surgeons NSQIP and the American Geriatrics Society. J Am Coll Surg 2016;222:930-47.

7. Furuya RK, Mata LR, Veras VS, Appoloni AH, Dantas RA, Silveira RC, et al. Original research: telephone follow-up for patients after myocardial revascularization: a systematic review. Am J Nurs 2013;113:28-31.
8. Demir N. Determining functional autonomy level of elderly patients in the preoperative period (thesis). Ankara, Gazi University, Health Science Institute, 2014.
9. Malani PN, Vaitkeviciu PV, Orringer MB. Perioperative Evaluation and Management. (In: Halter JB, Ouslander JG, Tinetti ME, Studenski S, Hıgh KP, Asthana S. (ed). Hazzard's Geriatric Medicine And Gerontology. Mc Graw Hill Medical, Sixth Edition, 2009, 407-38.
10. De Cola MC, Maresca G, D'Aleo G, Carnazza L, Giliberto S, Maggio MG, et al. Teleassistance for frail elderly people: A usability and customer satisfaction study. Geriatr Nurs 2020;41:463-7.
11. Hintistan S, Çilingir D. A current approach in nursing practice: telephone usage. Journal of Education and Research in Nursing 2012;9:30-5.
12. Guidelines for Telenursing Practice. 2002. Registered Nurses' Association of Nova Scotia, www.rnans.ns.ca. Accessed April, 2020.
13. Mols RE, Hald M, Vistisen HS, Lomborg K, Maeng M. Nurse-led Motivational Telephone Follow-up After Same-day Percutaneous Coronary Intervention Reduces Readmission and Contacts to General Practice. J Cardiovasc Nurs 2019;34:222-30.
14. da Silva Schulz R, Santana RF, Dos Santos CTB, Faleiro TB, do Amaral Passarelles DM, Hercules ABS, et al. Telephonic nursing intervention for laparoscopic cholecystectomy and hernia repair: A randomized controlled study. BMC Nurs 2020;19:38.
15. Hebert R, Carrier R, Bilodeau A. The Functional Autonomy Measurement System (SMAF): description and validation of an instrument for the measurement of handicaps. Age Ageing 1988;17:293-302.
16. Dal Ü, Bulut H, Demir SG. The problems experienced by the patients at home after surgery. Medical Journal of Bakırköy 2012;8:34-40.
17. Tuna Z, Çelik SS. Discharge training and counseling: Functional autonomy and post-discharge problems of elderly patients undergoing coronary artery bypass graft surgery. Turk Gogus Kalp Damar 2014;22:570-6.
18. Bikmoradi A, Masmouei B, Ghomeisi M, Roshanaei G, Masiello I. Impact of telephone counseling on the quality of life of patients discharged after coronary artery bypass grafts. Patient Educ Couns 2017;100:2290-6.
19. Moon MK, Yim J, Jeon MY. The Effect of a Telephone-Based Self-management Program Led by Nurses on Self-care Behavior, Biological Index for Cardiac Function, and Depression in Ambulatory Heart Failure Patients. Asian Nurs Res (Korean Soc Nurs Sci) 2018;12:251-7.
20. Kleinpell RM, Avitall B. Integrating telehealth as a strategy for patient management after discharge for cardiac surgery: results of a pilot study. J Cardiovasc Nurs 2007;22:38-42.
21. Lallement M, Maulat C, Suc B, Péré G, Lozano S, Bérard E, et al. Short-term autonomy and survival after hepatectomy in the elderly. J Visc Surg 2020;157:378-86.
22. Melholt C, Joensson K, Spindler H, Hansen J, Andreasen JJ, Nielsen G, et al. Cardiac patients' experiences with a telerehabilitation web portal: Implications for eHealth literacy. Patient Educ Couns 2018;101:854-61.
23. Karadakovan A, Çelebioğlu A, Sert ZE, Gündüzoğlu ÇN, Topçu S, Sağkal T, Bozkurt S. Determing the social support needs of elders. Journal of Ege University Nursing Faculty 2017;33:64-75.
24. Şahbaz M, Tel H. Determination of the relationship between the dependence status on daily living activities and home accidents among 65 years of age and older individuals living at home. Turkish Journal of Geriatrics 2006;9:85-93.
25. Akay B, Akyol AD. Investigation of the effect of tele monitoring on the self care agency in patients with chronic heart failure. Turk J Card Nur 2014;5:75-88.
26. Lafaro KJ, Raz DJ, Kim JY, Hite S, Ruel N, Varatkar G, et al. Pilot study of a telehealth perioperative physical activity intervention for older adults with cancer and their caregivers. Support Care Cancer 2020;28:3867-76.
27. Dinesen B, Nielsen G, Andreasen JJ, Spindler H. Integration of Rehabilitation Activities Into Everyday Life Through Telerehabilitation: Qualitative Study of Cardiac Patients and Their Partners. J Med Internet Res 2019;21:13281.
28. Aydın A, Gürsoy A. The care needs and care dependency of coronary artery bypass graft (CABG) patients after hospital discharge. Journal of Education and Research in Nursing 2019;16:8-14.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:142-148
DOI: 10.4274/BMJ.galenos.2021.30075

Research

The Effects of an Absorbable Hemostat Produced From Oxidized Regenerated Cellulose on Adhesion Formation in a Rat Model

Oksitlenmiş Rejenere Selülozdan Üretilen Absorbe Edilebilir Bir Hemostatın Rat Modelinde Adezyon Oluşumu Üzerine Etkisi

Adem Yavuz[1], Gökalp Öner[2], Mustafa Taş[3], Selim Çınaroğlu[4]

[1]Niğde Ömer Halisdemir University Faculty of Medicine, Department of Obstetrics and Gynecology, Niğde, Turkey
[2]Acıbadem Kayseri Hospital, Clinic of Obstetrics and Gynecology, Kayseri, Turkey
[3]Acıbadem Mehmet Ali Aydınlar University, Department of Obstetrics and Gynecology, İstanbul, Turkey
[4]Niğde Ömer Halisdemir University Faculty of Medicine, Department of Anatomy, Niğde, Turkey

ABSTRACT

Objective: This study aimed to analyze the effect of an absorbable hemostat produced from oxidized regenerated cellulose (ORC) on pelvic adhesion formation in a rat model using an adhesion scoring system and immunohistochemical staining.

Methods: This randomized, controlled experimental study included 20 female Wistar-Albino rats that were equally divided into the following groups: control and absorbable hemostat groups. The uterine horns of all the rats were exposed by laparotomy and using 10 W bipolar cautery. Five standard lesions were applied to the antimesenteric areas of each uterine horn. The experimental group received an absorbable hemostat to the traumatized uterine surfaces, whereas the control group did not. After a 28-day follow-up period, a relaparotomy was performed, and adhesions were evaluated based on an adhesion scoring system, and histological sections from areas with adhesion were obtained for immunohistochemical staining. Immunohistochemical staining included analysis of Ki-67 (proliferation index), CD-31 (neovascularization index), and Masson Trichrome [(MTC) fibrosis and collagen formation index]. Additionally, acute and chronic inflammation indices were determined via polymorphonuclear leukocytes (PMNL) and mononuclear leukocytes (MNL), respectively.

Results: The intensity and scope of adhesion and overall adhesion ratings were substantially higher in the absorbable hemostat group than the control group (2.8±0.85 vs. 2.2±0.53, 0.92±0.26 vs. 0.61±0.25, and 3.72±0.96 vs. 2.81±0.75, respectively). Staining results for Ki-67, CD-31, MTC, PMNL, and MNL were also significantly higher in the absorbable hemostat group than in the control group ($p<0.05$ for all).

Conclusion: The obtained results suggest that the use of ORC-based absorbable hemostats in pelvic surgery may increase adhesion formation on peritoneal surfaces by increasing inflammation, vascularity, and collagen formation.

Keywords: Pelvic, adhesion, surgical hemostasis, rats

ÖZ

Amaç: Bu çalışmanın amacı, okside rejenere sellülozdan (ORC) üretilen absorbe edilebilir bir hemostatın rat modelinde pelvik adezyon oluşumu üzerindeki etkisini adezyon skorlama sistemi ve immünohistokimyasal boyama kullanarak değerlendirmektir.

Gereç ve Yöntem: Çift kör, randomize, kontrollü bir deneysel çalışma tasarlandı. Yirmi dişi Wistar-Albino rat eşit olarak kontrol ve absorbe edilebilir hemostat gruplarına ayrıldı. Tüm ratların uterin hornları laparotomi ile ortaya çıkarıldı ve her uterus hornunun antimezenterik yüzeyine 10 W bipolar koter kullanılarak beş standart lezyon uygulandı. Deney grubunda travmatize olmuş uterin yüzeylere absorbe edilebilir hemostat uygulanırken, kontrol grubuna herhangi bir müdahale yapılmadı. Yirmi sekiz günlük bir takip süresinin ardından tekrar laparotomi yapıldı ve adezyon skorlama sistemine göre adezyonlar değerlendirildi ve immünohistokimyasal boyama için adezyonlu alanlardan histolojik kesitler alındı. İmmünohistokimyasal boyama, Ki-67 (proliferasyon indeksi), CD-31 (neovaskülarizasyon indeksi) ve Masson Trikrom [(MTC), fibrozis ve kollajen oluşum indeksi] analizini içeriyordu. Ek olarak, sırasıyla polimorfonükleer lökositler (PMNL) ve mononükleer lökositler (MNL) aracılığıyla akut ve kronik enflamasyon indeksleri belirlendi.

Address for Correspondence: Adem Yavuz, Niğde Ömer Halisdemir University Faculty of Medicine, Department of Obstetrics and Gynecology, Niğde, Turkey
Phone: +90 537 822 34 25 E-mail: ademyavuz@ohu.edu.tr ORCID ID: orcid.org/0000-0003-4191-4004

Cite as: Yavuz A, Öner G, Taş M, Çınaroğlu S. The Effects of an Absorbable Hemostat Produced From Oxidized Regenerated Cellulose on Adhesion Formation in a Rat Model. Med J Bakırköy 2021;17:142-148

Received: 23.12.2020
Accepted: 25.06.2021

Bulgular: Adezyon şiddeti ve yaygınlığı ile toplam adezyon skorları, kontrollere kıyasla absorbe edilebilir hemostat grubunda anlamlı düzeyde daha yüksekti (sırasıyla: 2,8±0,85'e karşı 2,2±0,53, 0,92±0,26'ya karşı 0,61±0,25 ve 3,72±0,96'ya karşı 2,81±0,75). Ki-67, CD-31, MTC, PMNL ve MNL için boyama sonuçları da emilebilir hemostat grubunda kontrol grubuna göre anlamlı düzeyde daha yüksekti (tümü için $p<0,05$).

Sonuç: Bulgularımız, pelvik cerrahide ORC bazlı absorbe edilebilir hemostat kullanımının enflamasyon, vaskülarite ve kollajen oluşumunu artırarak peritoneal yüzeylerde adezyon oluşumunu artırabileceğini düşündürmektedir.

Anahtar Kelimeler: Pelvik, adezyon, cerrahi hemostaz, sıçanlar

INTRODUCTION

Generally, the most serious complication following abdominal and pelvic surgery is adhesion formation. This postoperative problem has been linked with numerous morbidities, including small bowel obstruction, increased risk for inadvertent bowel injury in later surgeries, increased operation time, chronic abdominal pain, decreased likelihood of pregnancy, increased fertility treatments, and intraoperative complications (1). Although the pathogenesis of adhesion formation is still not well known, it is well recognized that an imbalance between fibrin deposition and fibrinolysis is the keystone of adhesion formation. Several elements such as tissue hypoxia and elevated inflammation, however, lead to a cytokine-rich setting that blocks the deposited fibrin lysis. As a result, fibrin clots remain organized and are usually degraded within a few days, and the infiltration of fibroblasts and other cells allows the clot to be reorganized into healthy connective tissues (2).

Topical hemostatic agents produced from oxidized regenerated cellulose (ORC), when applied dry, have a greater hemostatic effect and can be easily and firmly attached to bleeding tissues until hemostasis is achieved (3). ORC facilitates hemostasis by activating coagulation on the collagen surface, and since it has a lower pH, it plays a role as a caustic hemostatic agent. Thus, it is favored instead of gelatin foam in contaminated locations (4,5). Despite these well-established advantages, low pH can cause tissue infection and delay recovery. This is particularly important since some materials may remain within the tissues for a long time (up to several months or years), although the majority is absorbed within 7 to 14 days (4,6). In line with the advantages listed before, studies have shown that the status of topical hemostatic agents in minimally invasive gynecologic operations has not been specifically identified, but topical hemostatic agents that originated from ORC can efficaciously achieve hemostasis, decrease blood loss, minimize operative times, and reduce transfusion needs (7). Numerous animal studies with different methodologies have been performed to elucidate the effects of ORC-based absorbable hemostats on adhesion formation in rat models, but the results have been inconsistent (8-12).

This experimental work was prepared to elucidate the effects of an ORC-based absorbable hemostat on adhesion formation in uterine horn lesions of rats, as measured by a clinical adhesion scoring system and immunohistochemical staining for Ki-67, CD-31, masson trichrome (MTC), polymorphonuclear leukocytes (PMNL), and mononuclear leukocytes (MNL).

METHODS

In our study, experimental animals were used for research in Niğde Ömer Halisdemir University Laboratories according to the approval of the Local Animal Studies Ethics Committee (date: July 20, 2020, approval number: 2020/09).

A total of 20 female Wistar-Albino rats, aged 20-24 weeks and weighing 180-210 g, were kept in standard-bedding cages with controlled environment temperatures (22°C±2°C). Day/night cycles were mimicked with a 12/12 h light/dark period, and adequate food and water were provided ad libitum. The animals were divided into two groups as follows: control (sham operation) and intervention groups (absorbable hemostat recipients). They were evaluated twice a week for weight changes and behavioral characteristics by a research team member who did not partake in later procedures or measurements. The veterinary staff of the facility performed routine checks on the animals every day. Any notable changes were reported to the principal researcher and dealt with according to a unanimous decision from the researchers.

Procedures for both groups were carried out in the same fashion. Anesthesia was performed using 50 mg/kg ketamine hydrochloride (Ketalar®, flakon, Eczacibasi, İstanbul, Turkey) and 7 mg/kg xylazine hydrochloride (Rompun®, Bayer, Germany) via intraperitoneal administration. The abdominal skin of the animals was sanitized with a 10% povidone-iodine solution. The uterine horns were exposed by laparotomy access via a 3 cm midline incision, and five standard lesions were applied in the antimesenteric surfaces of each uterine horn using a 10 W bipolar cautery (applied for 1 s), as previously described (13). The lesion-forming procedure was performed meticulously to avoid damaging tissues in other sites. The traumatized uterine surfaces of the absorbable hemostat group were covered with a 1

cm^2-sized ORC-based absorbable hemostat (Surgicel®, Ethicon SARL, Neuchatel, Switzerland), whereas there was no application of medication to the control group. The incision was covered in two strata with a 4-0 Prolene suture (Ethicon, Inc., Somerville, NJ, USA) for the peritoneum and 3-0 Prolene suture for the dermis. The same researcher (S.C.) conducted all laparotomy processes and was given closed envelopes containing the description of the final intervention to be performed after completing all other steps of the laparotomy. No antibiotic prophylaxis was given during the research. A total of two rats died within 24 h after the procedure because of anesthesia complications (one from each group). Two days after the procedure, the abdominal wall scars of each rat were inspected for signs of infection.

After a 28-day follow-up period, all rats (n=18) underwent relaparotomy with the same anesthesia procedures applied in the first laparotomy. The 28-day duration was determined based on the absorption characteristics of ORC (beginning within 24 h and usually dissolving completely within 2-6 weeks) (6,14,15). Adhesion scoring was performed, and biopsy samples were taken from adhesion sites for histological examination. Adhesion scores were classified according to Linsky et al. (16) clinical adhesion scoring system and performed by a different researcher (A.Y.) who was blinded to the rat groups. The degree of adhesion was measured as follows: 0=no adhesion, 1=25% of the surface covered, 2=50% of the surface covered, and 3=fully covered. Adhesion intensity was calculated as follows: 0=no resistance to separation, 0.5=some resistance, and 1=need for sharp dissection. The overall score was obtained by summarizing these two different scores.

The biopsy samples taken were fixed with formalin, embedded in paraffin blocks, divided into about 4 µm thickness, and, after immunohistochemical coloring, examined with a Zeiss Scope A1 microscope (Germany) by a pathologist (Y.P.) irrelevant to the study. While evaluating the results, the pathologist assessed frequently addressed features such as collagen formation, fibrosis, inflammation, neovascularization, and cellular proliferation in histological evaluations. Ki-67 staining was used (NCL-L-Ki67-MM1; Leica, New Castle, UK) for the proliferation index, CD-31 staining (NCL-CD31-1A10; Leica) for the neovascularization index, and MTC staining (Bio Optica, Milan, Italy) for the fibrosis and collagen formation index. The Ki-67 marker is a proliferation marker that has gained unanimous acceptance for its role in quantifying the expression of mitotic cells, and it has been demonstrated to be associated with adhesion formation (17,18). CD-31 is an angiogenesis marker found to have increased expression during the development of new blood vessels (neovascularization). It has been correlated positively with adhesion formation in previous studies (19,20). Collagen formation can be measured by MTC, a marker that is important in fibrosis and may be related to the degree of fibrosis (19). In addition, PMNL was evaluated for the acute inflammation index after staining with hematoxylin-eosin and MNL for the chronic inflammation index. To assess the immunohistochemical staining ratings, an updated scoring method was used as follows: 0=no expression, 1=mild, 2=moderate, and 3=intense staining (8).

Statistical Analyses

SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was utilized to evaluate the statistical analyses in the study. The comparison of nonnormally distributed quantitative and normally distributed variables were assessed using the Mann-Whitney U and Student's t-tests, respectively. Statistical significance was defined as $p<0.05$. All values were described as mean ± standard deviation, regardless of the actual statistical method used for comparisons (parametric or nonparametric). The Sigma-Stat 3.5 software was used for power analysis. These values are listed under the tables.

RESULTS

A total of 18 rats survived until the end of the study and were included in the final analyses. The animals in both groups were statistically similar in terms of weight gain, activity, appetite, and water intake. The mean adhesion severity and adhesion extent scores were 2.2±0.53 and 0.61±0.25 in the control group and 2.8±0.85 and 0.92±0.26 in the intervention group, respectively. The total adhesion scores were 2.81±0.75 and 3.72±0.96, respectively. All scores were significantly higher in the intervention group than in the control group ($p<0.05$ for all; Table 1).

The evaluation of immunohistochemical parameters demonstrated that adhesion-related findings were

Table 1. Adhesion scores of the groups

	Group 1 (Control)	Group 2 (Absorbable hemostat)	p
Severity	2.20±0.53[a]	2.80±0.85[b]	<0.05
Extent	0.61±0.25[a]	0.92±0.26[b]	<0.05
Total score	2.81±0.75[a]	3.72±0.96[b]	<0.05

Statistically significant difference is not present in groups sharing the same letter. Data are expressed as mean ± standard deviation. All data sets of power of the performed test with alpha 0.050: 0.834-1.000.

significantly increased in the absorbable hemostat recipients than the controls ($p<0.05$ for all; Table 2).

Figure 1 shows the adhesion formation procedure and compares the macroscopic appearances of adhesion in both groups (1 month after the procedure). Cystic formation accompanied adhesion in absorbable hemostat recipients. Figure 2 shows the immunohistochemical screening of PMNL, MNL, vascular proliferation, and fibrosis. Figure 3 displays the Ki-67 scores of the groups.

DISCUSSION

The current study demonstrated that ORC-based absorbable hemostats (Surgicel®) may increase the severity and extent of adhesion formation after pelvic surgery. Macroscopic evaluations were consistent with the results obtained via immunohistochemistry and adhesion scores. Although the intraoperative and postoperative advantages of ORC-based hemostats cannot be questioned, the results

Table 2. Immunohistochemical scores of the adhesion areas in the groups

Parameters	Group 1 (control)	Group 2 (absorbable hemostat)	p
PMNL index (acute inflammation)	2.15±0.51[a]	2.32±0.61[b]	<0.05
MNL index (chronic inflammation)	2.10±0.80[a]	2.62±0.81[b]	<0.05
Ki-67 index (proliferation marker)	2.07±0.42[a]	2.25±0.66[b]	<0.05
CD-31 index (neovascularization marker)	1.92±0.5[a]	2.32±0.45[b]	<0.05
Masson Trichrome index (fibrosis marker)	2.02±0.54[a]	2.25±0.56[b]	<0.05

Statistically significant difference is not present in groups sharing the same letter. Note: Data is expressed as mean ± SD. PML: Polymorphonuclear leucocytes MNL: Mononuclear leucocytes

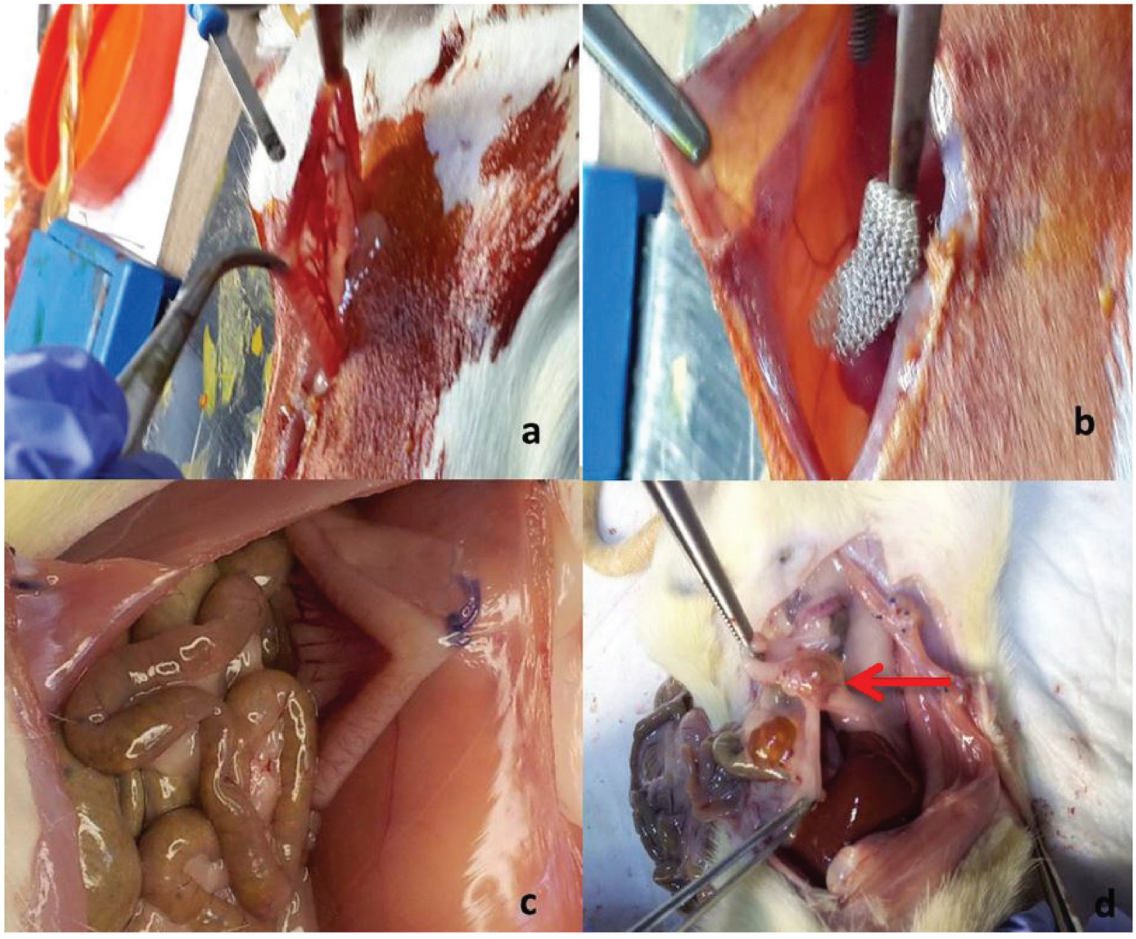

Figure 1. The macroscopic appearance of adhesion formation during and after 1 month. (a) and (c) Control group. (b) and (d) Absorbable hemostatic group and thickness showing cystic formations (a) Control group adhesion formation procedure and (b) absorbable hemostat group adhesion formation procedure. (c) Control group 1 month after surgery. (d) Absorbable hemostat group 1 month after surgery. Thickness showing cystic formation

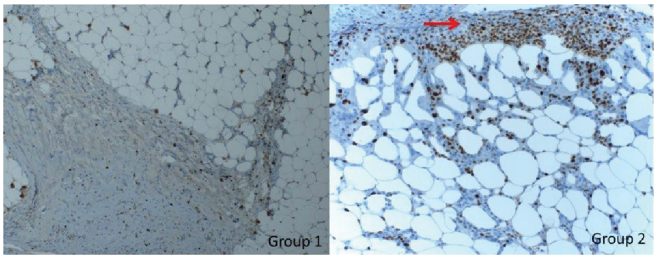

Figure 2. Immunohistochemical studies of the groups. Thickness showing aggregation of inflammatory cells

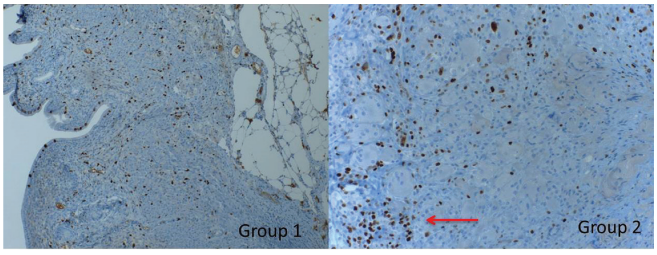

Figure 3. Ki-67 staining procedure for groups. Thickness showing proliferation of adhesion formation

of the present study indicated that these materials should be utilized sparingly, considering the risks imposed by their application.

As a consequence of pelvic inflammation, endometriosis, or direct trauma, pelvic adhesions may develop during operation. Reports utilizing second-look laparoscopy have shown that 25% to 92% of individuals may develop pelvic adhesion after laparoscopy (21,22). Because of their ease of use, biocompatibility, bactericidal effects, and ultimate tissue absorption, ORC-based hemostatic materials have gained popularity in various fields of surgery (23). However, their utility in gynecology surgical practice has not been conclusively determined.

Numerous animal studies with different methodologies and results have been conducted to elucidate the effects of Surgicel® on adhesion development in experimental rat models. McGaw et al. (24) sacrificed all rats on the 7th day after the operation and reported that Surgicel® use reduced abdominal adhesion formation, whereas Hoffman et al. (10) reported that both Surgicel® recipients and the control groups were similar in terms of adhesion scores. In a recent study, Güney et al. (11) investigated Surgicel and quercetin (again for 14 days) in relation to pelvic adhesion development in a rat model. In this study, all parameters examined in our study (adhesion score and subscores and immunohistochemistry for Ki-67, CD-31, MTC, PMNL, and MNL) were significantly higher in the Surgicel® group than in the control group. Considering that ORC was completely resolved within 2-6 weeks (6,14,15), the most important reason we obtained different results from the majority of previous studies (8,11,24,25) may be that we analyzed the effect of Surgicel® on pelvic adhesion formation on the 28th postoperative day. Importantly, our findings suggest coherently that the use of Surgicel® may increase pelvic adhesion formation by causing local chronic pelvic inflammation.

Localized suppression of trauma-induced peritoneal fibrinolysis causes the development of early fibrinous adhesions, whereas an infestation of fibroblasts and blood vessels (that develop shortly after) may cause lasting adhesions with vascular features (26). Carboxyl groups on oxidized cellulose, which act as a matrix for solid fibrin clot development when added to the bleeding area, can lower the pH (27). Although low pH has theoretical advantages, such as potential antimicrobial and caustic action leading to potentiation of hemostasis and clotting, it also has disadvantages, such as the inactivation of biologically active coagulants, elevation of local inflammation, and prolongation of the normal healing process (28). Mesothelial cells respond to acidification by rising plasminogen activator inhibitor type-1 (PAI-1) (29). PAI-1 is the primary inhibitor of tissue plasminogen activator in the peritoneal cavity; therefore, its increase will downregulate fibrinolysis and could increase postoperative adhesion formation (30,31). Furthermore, it has been shown that most of the cells seen in the 2nd week of adhesion are fibroblasts, and in particular locations, macrophages and lymphocytes. An increased collagen volume is formed over a span of 2 weeks to 2 months, and the cellular adhesion material steadily becomes less concentrated, accompanied by small blood vessel formation (32). These physiological alterations cannot be accounted for within 2 weeks, suggesting that studies evaluating adhesion development should involve a longer follow-up period.

The most important limitation of our study may be the low number of rats used, and the other can be the method of lesion formation (via cautery). It is clearly understood that normal peritoneal mesothelium has fibrinolytic activity on the wound surface, gradually increasing from the 2nd to the 8th day (25). This effect of the peritoneal mesothelium, which may act reciprocally to the decrease in fibrinolytic activity caused by Surgicel®, may be affected by cautery use. Therefore, our results may be in relation to this specific injury type; however, it is evident that such injuries may be frequently encountered in surgical practice.

This is the first study to examine the impacts of an ORC-based absorbable hemostat material (Surgicel®) on pelvic adhesion formation at the end of the postoperative 4th week

in a rat model. Our results, with an extensive set of analyses, including immunohistochemical staining methods and adhesion scoring, indicate that Surgicel® strongly increased adhesion scores, as well as the quantity and proportion of cells that tested positive for Ki-67, CD-31, and MTC, and the number of PMNL and MNL in specimens obtained from ORC-administered rats. These findings suggest that Surgicel® may have proliferative and inflammatory effects after the early period in which various studies have suggested protective effects against adhesion. In addition, the macroscopic evaluation showed cystic formation in the Surgicel® group. This finding supports that Surgicel®, although considered biocompatible, can trigger a foreign body reaction (15).

Conclusion

ORC-based hemostats can lead to increased severity and extent of adhesion, possibly caused by local pockets of chronic inflammation and foreign body reaction. However, further studies, which would benefit from utilizing other methods of lesion formation, are needed to ascertain the effects of ORC on adhesion development with regard to its clinical use.

Acknowledgment: We want to express our deepest thanks to Dr. Yalçın Polat for his efforts on pathological assessments.

ETHİCS

Ethics Committee Approval: The study were approved by the Niğde Ömer Halisdemir University Animal Experimentation Local Ethics Committee (protocol number: 2020/09).

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

Authorship Contributions

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

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

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

REFERENCES

1. ten Broek RP, Issa Y, van Santbrink EJ, Bouvy ND, Kruitwagen RF, Jeekel J, et al. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. BMJ 2013;347:5588.
2. Herrmann A, De Wilde RL. Adhesions are the major cause of complications in operative gynecology. Best Pract Res Clin Obstet Gynaecol 2016;35:71-83.
3. Achneck HE, Sileshi B, Jamiolkowski RM, Albala DM, Shapiro ML, Lawson JH. A comprehensive review of topical hemostatic agents: efficacy and recommendations for use. Ann Surg 2010;251:217-28.
4. Mudge MC. Chapter 4 - Hemostasis, Surgical Bleeding, and Transfusion. In: Auer JA, Stick JA, editors. Equine Surgery (Fourth Edition). Saint Louis: W.B. Saunders, 2012, 35-47.
5. Spangler D, Rothenburger S, Nguyen K, Jampani H, Weiss S, Bhende S. In vitro antimicrobial activity of oxidized regenerated cellulose against antibiotic-resistant microorganisms. Surg Infect (Larchmt) 2003;4:255-62.
6. Sileshi B, Achneck HE, Lawson JH. Management of surgical hemostasis: topical agents. Vascular. 2008;16 Suppl 1:S22-8.
7. Ito TE, Martin AL, Henderson EF, Gaskins JT, Vaughn VM, Biscette SM, et al. Systematic Review of Topical Hemostatic Agent Use in Minimally Invasive Gynecologic Surgery JSLS 2018;22:2018.00070.
8. Larsson B, Nisell H, Granberg I. Surgicel--an absorbable hemostatic material--in prevention of peritoneal adhesions in rats. Acta Chir Scand 1978;144:375-8.
9. Shimanuki T, Nishimura K, Montz FJ, Nakamura RM, diZerega GS. Localized prevention of postsurgical adhesion formation and reformation with oxidized regenerated cellulose. J Biomed Mater Res 1987;21:173-85.
10. Hoffmann NE, Siddiqui SA, Agarwal S, McKellar SH, Kurtz HJ, Gettman MT, et al. Choice of hemostatic agent influences adhesion formation in a rat cecal adhesion model. J Surg Res 2009;155:77-81.
11. Güney G, Kaya C, Oto G, Yıldırım S, Özdemir H, Tokmak A. Effects of quercetin and surgicel for preventing adhesions after gynecological surgery: A rat uterine horn model. J Obstet Gynaecol Res 2017;43:179-84.
12. Altun I. An Experimental Study of Histopathologic Effects of Hemostatic Agents Used in Spinal Surgery. World Neurosurg 2016;90:147-53.
13. Kaya C, Sever N, Cengiz H, Yıldız Ş, Ekin M, Yaşar L. A randomized controlled study of the efficacy of misoprostol and hyaluronic acid in preventing adhesion formation after gynecological surgery: a rat uterine horn model. Eur J Obstet Gynecol Reprod Biol 2014;176:44-9.
14. Barnard J, Millner R. A review of topical hemostatic agents for use in cardiac surgery. Ann Thorac Surg 2009;88:1377-83.
15. Tompeck AJ, Gajdhar AUR, Dowling M, Johnson SB, Barie PS, Winchell RJ, et al. A comprehensive review of topical hemostatic agents: The good, the bad, and the novel. J Trauma Acute Care Surg 2020;88:e1-21.
16. Linsky CB, Diamond MP, Cunningham T, Constantine B, DeCherney AH, diZerega GS. Adhesion reduction in the rabbit uterine horn model using an absorbable barrier, TC-7. J Reprod Med 1987;32:17-20.
17. Jean RA, O'Neill KM, Pei KY, Davis KA. Impact of hospital volume on outcomes for laparoscopic adhesiolysis for small bowel obstruction. J Surg Res 2017;214:23-31.
18. Sharma JB, Malhotra M. Topical oxidized cellulose for tubal hemorrhage hemostasis during laparoscopic sterilization. Int J Gynaecol Obstet 2003;82:221-2.
19. Kansra S, Yamagata S, Sneade L, Foster L, Ben-Jonathan N. Differential effects of estrogen receptor antagonists on pituitary lactotroph proliferation and prolactin release. Mol Cell Endocrinol 2005;239:27-36.
20. Sitruk-Ware R, Bricaire C, De Lignieres B, Yaneva H, Mauvais-Jarvis P. Oral micronized progesterone. Bioavailability pharmacokinetics,

pharmacological and therapeutic implications--a review. Contraception 1987;36:373-402.

21. Ahmad G, Kim K, Thompson M, Agarwal P, O'Flynn H, Hindocha A, et al. Barrier agents for adhesion prevention after gynaecological surgery. Cochrane Database Syst Rev 2020;3:CD000475.
22. Okabayashi K, Ashrafian H, Zacharakis E, Hasegawa H, Kitagawa Y, Athanasiou T, et al. Adhesions after abdominal surgery: a systematic review of the incidence, distribution and severity. Surg Today 2014;44:405-20.
23. Franceschini G. Internal surgical use of biodegradable carbohydrate polymers. Warning for a conscious and proper use of oxidized regenerated cellulose. Carbohydr Polym 2019;216:213-6.
24. McGaw T, Elkins TE, DeLancey JO, McNeeley SG, Warren J. Assessment of intraperitoneal adhesion formation in a rat model: can a procoagulant substance prevent adhesions? Obstet Gynecol 1988;71:774-8.
25. Raftery AT. Regeneration of peritoneum: a fibrinolytic study. J Anat 1979;129(Pt 3):659-64.
26. Penzias A, Bendikson K, Falcone T, Gitlin S, Gracia C, Hansen K et al. Postoperative adhesions in gynecologic surgery: a committee opinion. Fertility and sterility 2019:1;112:458-63.
27. Zhang S, Li J, Chen S, Zhang X, Ma J, He J. Oxidized cellulose-based hemostatic materials. Carbohydr Polym 2020;230:115585.
28. Pereira BM, Bortoto JB, Fraga GP. Topical hemostatic agents in surgery: review and prospects. Rev Col Bras Cir 2018;45:e1900.
29. Bergström M, Falk P, Holmdahl L. Effect of acidosis on expression of mesothelial cell plasminogen activator inhibitor type-1. Surg Endosc 2006;20:1448-52.
30. Falk K, Björquist P, Strömqvist M, Holmdahl L. Reduction of experimental adhesion formation by inhibition of plasminogen activator inhibitor type 1. Br J Surg 2001;88:286-9.
31. Holmdahl L, Eriksson E, Al-Jabreen M, Risberg B. Fibrinolysis in human peritoneum during operation. Surgery 1996;119:701-5.
32. Milligan DW, Raftery AT. Observations on the pathogenesis of peritoneal adhesions: a light and electron microscopical study. Br J Surg 1974;61:274-80.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:149-153
DOI: 10.4274/BMJ.galenos.2021.39358

Research

The Gastroscopic Findings of the Pediatric Patients With Hematemesis

Çocuk Acil Polikliniğine Hematemesis Şikayetiyle Başvuran Hastaların Gastroskopik Bulguları

Sinem Oral Cebeci[1], Hasret Ayyıldız Civan[2]

[1]Istanbul University-Cerrahpaşa Faculty of Medicine Department of Pediatric Emergency, Istanbul, Turkey
[2]University of Health Sciences Turkey, İstanbul Dr. Sadi Konuk Training and Research Hospital, Clinic of Pediatric Gastroenterology, İstanbul, Turkey

ABSTRACT

Objective: Hematemesis is a common symptom of upper gastrointestinal bleeding, bleeding from the mouth that appears fresh red or looks like "coffee grounds." It is a rare, life-threatening emergency condition and potentially requires emergency intervention in children. Our study aims to evaluate the gastroscopy findings of pediatric patients who presented with vomiting of blood.

Methods: Seventy children and adolescents (35 females, 35 males) presented with hematemesis were evaluated retrospectively. Patients' demographic characteristics, gastroscopic findings, pre- and post-operative laboratory results were compared and evaluated.

Results: Male patients (6.91±5.21) were significantly younger than female patients (10.51±4.79) at the time of diagnosis (p=0.005). Of these, 92.9% had no symptoms. According to the gastroscopy findings performed at the time of diagnosis, the underlying pathology was detected in 65.7% of cases. Esophagitis was the most common pathology with a rate of 52.2% and followed by pangastritis (30.4%). As a result of post-treatment gastroscopy, 21.7% (n=15) of cases had pathological findings. Moreover, the mean hemoglobin value measured during diagnosis was significantly lower in cases with underlying pathology according to post-treatment gastroscopy results (p=0.025).

Conclusion: Hematemesis was observed at an early age in male children with a higher rate of underlying pathology after treatment. In addition, the significantly low hemoglobin levels reported in the cases with positive gastroscopy have reaffirmed the diagnostic and therapeutic importance of gastroscopy. It has also highlighted the requirement for immediately monitoring vital symptoms following the patient's admission.

Keywords: Upper gastrointestinal bleeding, hematemesis, gastroscopy, children, adolescents

ÖZ

Amaç: Hematemez, kanın taze kırmızı veya "kahve telvesi" şeklinde ağızdan geldiği, üst gastrointestinal sistem kanamasının yaygın bir semptomudur. Hayatı tehdit edebilecek acil bir durum olarak tanımlanır ve potansiyel olarak çocuklarda acil müdahale gerektirir. Çalışmamızda kanlı kusma ile başvuran çocuk hastaların gastroskopi bulgularını değerlendirmeyi amaçladık.

Gereç ve Yöntem: Hematemez ile başvuran 70 çocuk ve ergen (35 kız, 35 erkek) geriye dönük olarak değerlendirildi. Hastaların demografik özellikleri, gastroskopik bulguları, ameliyat öncesi ve sonrası laboratuvar sonuçları karşılaştırıldı ve değerlendirildi.

Bulgular: Tanı anında erkek hastalar (6,91±5,21) kadın hastalardan (10,51±4,79) anlamlı olarak daha gençti (p=0,005). Hastaların %92.9'unda herhangi bir belirti yoktu. Tanı anında yapılan gastroskopi bulgularına göre; vakaların %65,7'sinde altta yatan patoloji tespit edildi. Özofajit %52,2 ile en sık görülen patoloji olup, onu pangastrit (%30,4) izledi. Tedavi sonrası gastroskopi sonucunda olguların %21,7'sinde (n=15) patolojik bulgular vardı. Ayrıca tanı sırasında ölçülen ortalama hemoglobin değerinin altta yatan patolojisi olan olgularda tedavi sonrası gastroskopi sonuçlarına göre istatistiksel olarak daha düşük olduğu tespit edildi (p=0,025).

Sonuç: Tedavi sonrası altta yatan patoloji oranı daha yüksek olan erkek çocuklarda daha erken yaşta hematemez görüldü. Ek olarak, gastroskopi bulgusu pozitif olan olgularda bildirilen önemli ölçüde düşük hemoglobin seviyeleri, gastroskopinin tanısal ve terapötik önemini yeniden teyit etmiştir. Ayrıca, hastanın yatışını takiben yaşamsal semptomların derhal izlenmesinin gerekliliğini vurgulamıştır.

Anahtar Kelimeler: Üst gastrointestinal kanama, hematemez, gastroskopi, çocuklar, ergenler

Address for Correspondence: Hasret Ayyıldız Civan, University of Health Sciences Turkey, İstanbul Dr. Sadi Konuk Training and Research Hospital, Clinic of Pediatric Gastroenterology, İstanbul, Turkey
Phone: +90 505 747 97 65 E-mail: hasretayyildiz@yahoo.com ORCID ID: orcid.org/0000-0002-5604-9722

Cite as: Ayyıldız Civan H, Oral Cebeci S. The Effect of Nurse Telephone Consultation After Coronary Artery Bypass on The Autonomy Level Of Elderly Patients: Quasi - Experimental Study. Med J Bakırköy 2021;17:149-153

Received: 14.03.2021
Accepted: 16.06.2021

INTRODUCTION

In children, upper gastrointestinal bleeding (UGIB) is a rare condition. Its reported an annual incidence is 1-2/10,000 with high mortality risk (1). Hematemesis is a relatively common symptom of a UGIB, and defined as bleeding from the mouth that appears fresh red or looks like "coffee grounds." Although hematemesis most frequently occurs due to benign causes, it carries a serious mortality potential that may require urgent intervention (2).

The etiology of hematemesis is heterogeneous and varies with age, comorbidity, and geographic location in children. In Western countries, Mallory-Weiss tears, gastric and duodenal ulcers, esophagitis, gastritis, and esophageal-gastric varices are the most common underlying etiology of UGIB in children (1,2). Hematemesis and/or melena typically accompany UGIB. Hematemesis usually indicates that the lesion causing the bleeding is above the Treitz ligament. Fresh hematemesis is a reliable marker of active bleeding. Laboratory tests seldomly contribute to the diagnosis. However, laboratory tests are necessary for controlling thrombocytopenia and coagulopathy (3). After detailed anamnesis and physical examination, endoscopy is the gold standard diagnostic and therapeutic tool. The bleeding focus can be identified, and re-bleeding can be prevented via endoscopy (3,4).

In the clinical management of the patient, resuscitation should be evaluated primarily by focusing on their hemodynamic stability by monitoring vital symptoms, such as heart rate, blood pressure, and capillary refill time. Then, the patient should be symptomatically treated until the diagnostic processes are completed (5). In our study, we aim to evaluate the gastroscopy findings of pediatric patients who presented with vomiting of blood.

METHODS

Sample

This study was performed with the Institutional Review Board protocol approval date July 20, 2020, and number 2020/15 in İstanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital between April 1, 2017, and April 1, 2020. In this study, 70 children and adolescents aged between 0 and 18 years who presented with hematemesis were evaluated retrospectively. Exclusion criteria for the current study were the presence of any known esophageal, gastric, duodenal, and chronic liver diseases.

Measures

A cell blood count analysis was performed on patients' venous blood samples. Hematological parameters were analyzed using a hematology analyzer (Cell-Dyne 3700, Abbott, Abbott Park, IL, USA). Biochemical analysis performed from serum samples by electro-chemiluminescence immunoassay on Beckman Coulter Unicel DXI 800 analyzer. The blood prothrombin time (PT) and international normalized ratio (INR) were measured by automated coagulation analyzer CS2100i (Sysmex Corporation, Kobe, Japan).

Patients' demographic characteristics, gastroscopic findings, and pre- and post-operative laboratory results were recorded and evaluated.

Statistical Analysis

All the data were analyzed with Statistical Package for the Social Sciences (SPSS) software for Windows (v21.0; IBM, Armonk, NY, USA). Individual and aggregate data were summarized using descriptive statistics, including means, standard deviations, medians (min-max), frequency distributions, and percentages. The normality of data distribution was verified by the Kolmogorov-Smirnov test. Comparison of the variables with normal distribution was made with Student's t-test. The variables that were not normally distributed were compared using the Mann-Whitney and Kruskal-Wallis tests. Categorical variables were evaluated by the chi-square test. P-values of <0.05 were considered statistically significant.

RESULTS

This study included 70 hematemesis cases, of which 35 were females (50.0%), 35 were males (50.0%), and the mean age was 8.71±5.29 (range: 0-18) years. In addition, the mean age of female patients (10.51±4.79 years) was significantly higher than male patients (6.91±5.21 years) (p=0.017) (Table 1).

While 92.9% (n=65) of patients had no complaints, two patients presented with abdominal pain and melena, and one patient with pallor. However, a history of drug use was reported by two patients (2.9%); no oral findings were found in any patient time of diagnosis, the underlying pathology was detected in 65.7% (n=46) of cases. Esophagitis was the most common pathology with a rate of 52.2% (n=24) and was followed by pangastritis (30.4%) (n=14). Of these

Table 1. The mean age and gender analysis of the cases

	n (%)	Age (month) (mean ± SD)	p
Male	35 (50.0%)	6.91 ±5.21	
Female	35 (50.0%)	10.51±4.79	0.005*
Total	**70 (100%)**	**8.71±5.29**	

SD: Standard deviation, *p<0.05 statistically significant

patients, 68.6% (n=48) received treatment. The proton pump inhibitor (PPI)-sucralfate was the most common treatment protocol, with a rate of 29.2% (n=14). It was followed by a PPI-sucralfate-domperidone treatment protocol with a rate of 27.1% (n=13) (Table 2).

As a result of the gastroscopy performed after treatment, the pathological findings were determined in 21.7% (n=15) of cases, and no pathology was found in 54 cases (78.3%). According to the evaluation of laboratory findings at the time of diagnosis; the mean values of platelets were 312.2±133.1×109/L, hemoglobin was 11.29±2.47 g/dL, hematocrit was 34.67±6.97, INR was 1.12±0.21, and PT was 14.02±2.28 seconds in our study. No statistically significant differences were found according to the laboratory results and the mean age between the patients with negative and positive gastroscopy findings (p>0.05) (Table 3). However, the mean hemoglobin value measured during diagnosis was significantly lower in cases with underlying pathology according to the post-treatment gastroscopy results (p=0.025) (Table 4) (Figure1).

Moreover, a significantly higher rate of underlying pathology was detected in patients who received treatment (29.8%, n=14) than patients without treatment (4.5%, n=1). In addition, males (31.4%, n=11) had a significantly higher rate of underlying pathology compared with females (11.8%, n=4) (p=0.048) after treatment. However, the presence of symptoms and the history of drug use during diagnosis were not correlated with post-treatment gastroscopy findings (p-values=0.204 and 0.390, respectively).

DISCUSSION

Hematemesis in children often requires medical intervention and treatment regardless of underlying etiology, in addition to causing serious anxiety in parents. It has been reported in published data that the risk of UGIB increases with

Table 2. Treatment protocols and frequencies

Treatment	n	%
Proton pump inhibitor	4	8.3
Na-aliginate + Na-bicarbonate	1	2.1
PPI - Domperidone	7	14.6
Na-aliginate + Na-bicarbonate - Domperidone	3	6.3
PPI - Sucralfate	14	29.2
PPI - Sucralfate - Domperidone	13	27.1
PPI - Na-aliginate + Na-bicarbonate - Domperidone	6	12.5

PPI: Proton pump inhibitor

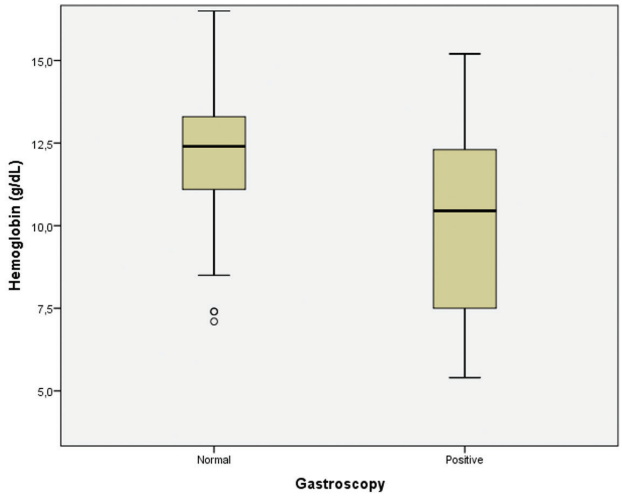

Figure 1. The comparison of mean hemoglobin values according to the post-treatment gastroscopy results

increasing age in children. In a study conducted with 613 patients with hematemesis, Freedman et al. (6) reported that cases with severe hemorrhage had a significantly higher mean age than cases with mild symptoms (mean age 9.7 vs. 2.9 years; p<0.001). Mihai et al. (7) documented a mean age of 9.7 years in a study consisting of 66 males and 63 females, 95 of whom presented with hematemesis. Similarly, Nasher et al. (8) reported a significantly higher mean age in female patients (8.2±6.0 years) than male patients (5.9±5.5 years) in 32 children (17 males, 15 females) who presented with hematemesis (59.3%) and hematemesis + melena (15.6%). In accordance with published data, the mean age was 8.71±5.29 years in our sample group. In addition, male patients were significantly younger than females. This finding of our study is rather remarkable. To our knowledge, young age and male gender have not been evaluated as risk factors for hematemesis in published data. In this regard, further research should be performed with larger study groups. Results of these studies might enable understanding risk factors in children with hematemesis.

In children, the causes of UGIB usually vary according to geography and age groups. In neonates, coagulation disorders associated with vitamin K deficiency and milk protein intolerance take the first place. In older children, the most common causes of UGIB include swallowing a foreign body in children aged between one month and one year, erosive esophagitis and gastritis in children aged between one and five years, coagulation disorders in children aged between five and 18, and gastritis and erosive esophagitis (9) . In a study consisting of 1,218 UGIB patients who presented with hematemesis (59.3%), melena (22.6%), and hematemesis + melena (18.1%), Yu et al. (10)

Table 3. The comparison of laboratory findings and gastroscopy results

	Gastroscopy negative (mean ± SD)	Gastroscopy positive (mean ± SD)	Total (mean ± SD)	p
Age	7.33±5.56	9.43±5.05	14.02±2.28	0.118
INR	1.06±0.28	1.14±0.17	1.12±0.21	0.738
PT (sn)	14.62±1.72	13.78±2.46	14.02±2.28	0.290
Hemoglobin (g/dL)	10.85±2.47	11.46±2.48	11.29±2.47	0.570
Hematocrit (%)	33.39±6.74	35.14±7.09	34.67±6.97	0.554
Thrombocyte (×10⁹/L)	312.5±113.5	312.1±141.3	312.2±133.1	0.992

INR: International normalized ratio, PT: Prothrombin time, SD: Standard deviation

Table 4. The comparison of laboratory findings and post-treatment gastroscopy results

Laboratory findings	Gastroscopy negative (mean ± SD)	Gastroscopy positive (mean ± SD)	Total (mean ± SD)	p
PT (sn)	14.01±2.42	14.04±2.03	14.02±2.28	0.971
INR	1.10±0.23	1.16±0.14	1.12±0.21	0.519
Hemoglobin (g/dL)	11.82±2.12	9.97±2.88	11.29±2.47	0.025*
Hematocrit (%)	36.16±5.52	31.02±8.97	34.67±6.97	0.075
Thrombocyte (×10⁹/L)	295.0±124.8	353.7±151.9	312.2±133.1	0.174

*p<0.05 statistically significant, INR: International normalized ratio, PT: Prothrombin time, SD: Standard deviation

documented erosive gastritis (33.5%) as the most common endoscopic finding, followed by duodenal ulcer (23.2%) and gastric ulcer (9.0%). In another study consisting of 80 pediatric patients with hematemesis, the most commonly reported endoscopic findings were esophageal varices at a rate of 30.0%, followed by gastritis (26.3%) and duodenitis ulcer (25.0%) (11). Similarly, Freedman et al. (6) reported the most common endoscopic finding as esophageal/gastric varies at a rate of 26% in their study conducted with 613 hematemesis patients. In our study, the underlying pathology was detected in 65.7% of cases. Esophagitis was the most common pathology with a rate of 52.2% and was followed by pangastritis (30.4%).

Although aspirin and other non-steroidal anti-inflammatory drugs have been associated with UGIB in children in some published studies, the etiological value of drugs for UGIB is not entirely understood because the data was obtained from a few studies with small sample group sizes and case reports (12). Mazigh et al. (13) reported that gastro-toxic drug utilization is an independent risk factor [Odds ratio (OR): 1.3; 95% confidence invertal (CI): 0.8-2.3] for bleeding by multivariate logistic regression analysis in their study conducted with 489 pediatric patients with hematemesis. Also, Grimaldi-Bensouda et al. (12) associated NSAID utilization, such as ibuprofen and aspirin, with UGIB in a 2-year study consisting of 177 children. In our study, while the history of drug use was found only in 2.9% of cases, the history of drug use during diagnosis was not significantly associated with post-treatment gastroscopy findings.

In children, a diagnostic approach for hematemesis is usually provided by the data obtained from the studies related to adult diagnoses. Currently, the key point in the diagnostic approach is the evaluation of laboratory and endoscopy findings combined with detailed anamnesis and physical examination. However, a differential diagnosis should be applied for lower gastrointestinal bleeding and non-GI sources (4,13). There are limited published data comparing laboratory outcomes between positive and negative endoscopy results in children with hematemesis. Cleveland et al. (14) performed endoscopy in 2569 UGIB cases, 73.4% of whom presented with hematemesis. Researchers reported a bleeding source in 57% and no underlying pathology in 11.4% of cases. In addition, 29.7% of cases were documented as suspicious positive. However, no significant differences were found regarding the mean hemoglobin values between the negative pathology (12.0±2.3 g/dL) and suspicious positive (11.6±2.3 g/dL) groups. Also, researchers reported significantly lower hemoglobin levels in the cases with duodenal erosion/ulceration (8.3±2.8 g/dL) and varices (7.7±2.3 g/dL) (14). Similarly, our study found no significant differences between patients with negative and positive gastroscopy findings at the time of diagnosis

according to the mean hemoglobin values. However, as a result of the control gastroscopy performed after treatment, the underlying pathology was determined in 21.7% of cases. According to the post-treatment gastroscopy results, the mean hemoglobin value measured during diagnosis was statistically lower in the cases with underlying pathology.

CONCLUSION

In conclusion, observing hematemesis at early ages in male children with a higher rate of underlying pathology is remarkable. Whether early age is a risk factor for male children should be re-evaluated by comparing the findings of our study in further research studies with larger sample groups. In addition, the significantly low hemoglobin levels reported in cases with positive gastroscopy have reaffirmed the diagnostic and therapeutic importance of gastroscopy. It has also highlighted the requirement for immediately monitoring vital symptoms following the patient's admission. Therefore, considering the high risk of morbidity in cases with hematemesis, a detailed history, comorbid diseases, drug use, bleeding characteristics, and the physical examination should be evaluated rapidly and carefully using a multidisciplinary approach.

ACKNOWLEDGMENTS

The authors declare that they have no conflict of interest.

ETHICS

Ethics Committee Approval: This study was performed with the Institutional Review Board protocol approval date July 20, 2020, and number 2020/15 in İstanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital between April 1, 2017, and April 1, 2020.

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

Authorship Contributions

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

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

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

REFERENCES

1. Nasher O, Devadason D, Stewart RJ. Upper Gastrointestinal Bleeding in Children: A Tertiary United Kingdom Children's Hospital Experience. Children (Basel) 2017;4:95.
2. Villa X. Approach to upper gastrointestinal bleeding in children. UpToDate [enlínea][consultado el 16/11/2013]. Disponibleen: www.uptod a te. com/contents/approach-to-upper-gastrointestinal-bleeding-in-children. 2017.
3. Lirio RA. Management of Upper Gastrointestinal Bleeding in Children: Variceal and Nonvariceal. Gastrointest Endosc Clin N Am 2016;26:63-73.
4. Shahraki T, Shahraki M, Miri-Aliabad G. The importance of endoscopy as a useful method for diagnosis of upper gastrointestinal bleeding in children. J Comp Ped 2018;9:e55022.
5. Mittal SK, Bhattacharya M. Upper gastrointestinal bleeding in children. Indian Journal of practical Pediatrics 2018;20:184-92.
6. Freedman SB, Stewart C, Rumantir M, Thull-Freedman JD. Predictors of clinically significant upper gastrointestinal hemorrhage among children with hematemesis. J Pediatr Gastroenterol Nutr 2012;54:737-43.
7. Mihai L, Balasa A, Mihai CM, Stroia V, Cuzic V, Frecus C, Stoicescu R. 795 Etiology of Upper Gastrointestinal Bleeding in Children. Pediatric Research 2010;68:401.
8. Nasher O, Devadason D, Stewart RJ. Upper Gastrointestinal Bleeding in Children: A Tertiary United Kingdom Children's Hospital Experience. Children (Basel) 2017;4:95.
9. Owensby S, Taylor K, Wilkins T. Diagnosis and management of upper gastrointestinal bleeding in children. J Am Board Fam Med 2015;28:134-45.
10. Yu Y, Wang B, Yuan L, Yang H, Wang X, Xiao Y, et al. Upper Gastrointestinal Bleeding in Chinese Children: A Multicenter 10-Year Retrospective Study. Clin Pediatr (Phila) 2016;55:838-43.
11. Fatma A, Esraa TA, Faddan MD. Clinical Audit on Management of Hematemesis in Children Admitted to Pediatric Gastroenterology and Hepatology Unit of Assiut University Children Hospital. The Medical Journal of Cairo University 2018;86:4531-6.
12. Grimaldi-Bensouda L, Abenhaim L, Michaud L, Mouterde O, Jonville-Béra AP, Giraudeau B, et al. Clinical features and risk factors for upper gastrointestinal bleeding in children: a case-crossover study. Eur J Clin Pharmacol 2010;66:831-7.
13. Mazigh S, Boukthir S, Hachicha S, Brini I, Sammoud A. Les facteurs cliniques prédictifs de lesions endoscopiques à haut risqué de saignement chez un enfant présentant une hématémèse [Clinical predictors of high risk bleeding endoscopic lesions in children with hematemesis]. Tunis Med 2015;93:454-7.
14. Cleveland K, Ahmad N, Bishop P, Nowicki M. Upper gastrointestinal bleeding in children: an 11-year retrospective endoscopic investigation. World J Pediatr 2012;8:123-8.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:154-160
DOI: 10.4274/BMJ.galenos.2021.48568

Research

Comparison of Use of Steroid Alone or in Combination With Cyclophosphamide For the Initial Therapy of Idiopathic Membranous Nephropathy

İdiyopatik Membranöz Nefropatinin Başlangıç Tedavisinde Steroidin Tek Başına veya Siklofosfamid ile Kombine Kullanımının Karşılaştırılması

Sibel Yücel Koçak[1], Özlem Harmankaya[2], Arzu Özdemir Kayalar[1], Mürvet Yılmaz[1], Süheyla Apaydin[1]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Education Training and Research Hospital, Clinic of Nephrology, İstanbul, Turkey
[2]Biruni University Medical Faculty, Department of Internal Medicine, Division of Nephrology, İstanbul, Turkey

ABSTRACT

Objective: Idiopathic membranous nephropathy (IMN) is one of the major causes of adult-onset nephrotic syndrome. Despite treatment, approximately 30%-40% of the patients can develop end-stage renal disease (ESRD) within 10-15 years. The objective of this study is to evaluate the efficacy and safety of using oral steroid monotherapy and compare it with steroid and cyclophosphamide (CTX) combination treatment in patients with IMN.

Methods: All native biopsies (n=509) performed at our center between 2006 and 2018 were retrospectively examined. In total, 83 (16.3%) patients were diagnosed with biopsy-proven IMN and 47 patients with IMN presented with nephrotic syndrome. Clinical remission and renal progressions of 20 patients receiving oral steroid monotherapy and 27 patients treated with oral steroid and CTX were evaluated.

Results: All patients in the oral steroid-receiving group achieved remission [n=12, 60% complete; n=8, 40% partial remissions (PR)] as compared to the combination therapy-receiving group (n=9, 33.3%, complete; n=18, 66.7% PR). Steroid monotherapy and steroid + CTX-induced similar complete remission rates (60% vs 33.3%; p>0.05). Relapse occurred in 6 and 10 patients in the steroid-receiving group (30.0%) and the steroid + CTX-receiving group (37%), respectively (p>0.05). Proteinuria (g/day) at baseline, at six months, and at the end of the treatment were not different between the groups [7.58±4.10 vs 8.74±4.02, p=0.34 (baseline); 1,452±1,579 vs 2,682±2,730, p=0.059 (six months); 362±416 vs. 220±274, p=0.115 (at the end of treatment)].

Conclusion: This study's results suggest that the application of oral steroid monotherapy can function as an alternative therapeutic regimen for patients with nephrotic IMN. The short-term efficiency and patient tolerability of both regimens were found to be acceptable. Further randomized controlled trials with more subjects are needed to clarify the exact benefits of oral steroid monotherapy in patients with IMN.

Keywords: Cyclophosphamide, membranous nephropathy, nephrotic syndrome, steroid monotherapy, immunosuppression

ÖZ

Amaç: İdiyopatik membranöz nefropati (İMN), erişkin nefrotik sendromun başlıca nedenlerinden biridir. Tedaviye rağmen, hastaların yaklaşık % 30-40'ında 10-15 yıl içinde son dönem böbrek hastalığı gelişebilir. Bu çalışmanın amacı, İMN hastalarında oral steroid monoterapisinin etkinliğini ve güvenliğini değerlendirmek ve steroid/siklofosfamid kombinasyon tedavisi ile karşılaştırmaktır.

Gereç ve Yöntem: Merkezimizde 2006-2018 yılları arasında yapılan tüm biyopsiler (n=509) geriye dönük olarak incelendi. Seksen üçüne (%16,3) biyopsi ile İMN tanısı kondu. Kırk yedisi İMN hastası nefrotik sendrom ile başvurdu. Oral steroid monoterapisi alan 20 hasta ile oral steroid/siklofosfamid tedavisi alan 27 hastanın klinik remisyon ve renal progresyonları değerlendirildi.

Bulgular: Oral Steroid tedavisi alan grubun 12'sinde (%60) tam, 8'inde (%40) kısmi remisyon sağlanırken; kombinasyon tedavisi ile 9 (%33,3) tam 18 (%66,7) kısmi remisyon sağlandı. Steroid monoterapi ve steroid/siklofosfamid kombinasyon tedavisi ile tam remisyon oranları benzer bulundu (%60; %33,3, p>0,05). Steroid alan grupta 6 hastada (%30,0), steroid/siklofosfamid alan grupta 10 hastada (%37) relaps gelişti (p>0,05). Başlangıçta, altı ayda ve tedavinin sonunda proteinüride (g/gün) gruplar arasında fark yoktu (7,58±4,10, 8,74±4,02, p=0,34; 1.452±1.579, 2.682±2.730, p=0,059; 362±416, 220±274, p=0,115, sırasıyla).

Address for Correspondence: Sibel Yücel Koçak, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Nephrology, İstanbul, Turkey
Phone: +90 505 583 08 60 E-mail: fsibelkocak@yahoo.com ORCID ID: orcid.org/0000-0003-3984-4043

Cite as: Yücel Koçak S, Harmankaya Ö, Özdemir Kayalar A, Yılmaz M, Apaydin S. Comparison of Use of Steroid Alone or in Combination With Cyclophosphamide For the Initial Therapy of Idiopathic Membranous Nephropathy. Med J Bakırköy 2021;17:154-160

Received: 15.03.2021
Accepted: 18.06.2021

Sonuç: Bu çalışma, oral steroid monoterapisinin nefrotik İMN'li hastalar için alternatif bir terapötik rejim olabileceğini düşündürmektedir. Her iki rejimin kısa vadeli etkinliği ve hasta tolere edilebilirliği kabul edilebilir bulunmuştur. İMN hastalarında oral steroid monoterapisinin kesin faydasını açıklığa kavuşturmak için daha fazla denek ile daha fazla randomize kontrollü çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Siklofosfamid, membranöz nefropati, nefrotik sendrom, steroid monoterapisi, immünosupresyon

INTRODUCTION

Globally, membranous glomerulonephritis (GNP) or idiopathic membranous nephropathy (IMN) is one of the most common GNP; however, its treatment is still debatable. In most patients, it initially appears as a primary renal disease but in 20% of cases, it can be associated with some systemic conditions such as systemic lupus erythematosus, infections, cancer, or drug exposure (1,2). Age (older than 50 years), male gender, renal insufficiency at first diagnosis, and persistent level of proteinuria (above 8-10 g/day) during the first year of the disease are recognized as risk factors for developing end-stage renal disease (ESRD). If patients do not receive any treatment, then about 25%-30% of patients with primary IMN undergo spontaneous remissions and the other 30%-50% show progression toward ESRD (3-5). To avoid progression into ESRD, patients can be treated with immunosuppressive drugs. "Who?," "when to treat?," and "whom to treat with potentially toxic therapies?" are the basic questions that need to be answered. The optimal immunosuppressive regimen for patients with IMN remains controversial (6,7). Data relating to the efficacy of different immunosuppressive protocols still need detailed clarification; in particular, there are little data available on the efficacy or benefits of a corticosteroid-based regimen. The objective of the study is to evaluate the efficiency and safety of applying oral steroid monotherapy for treating nephrotic IMN, and compare the results with the combination of steroid and cyclophosphamide (CTX) therapy.

METHODS

Patient Selection

All native biopsies (n=509) performed at our center between 2006 and 2018 were retrospectively examined. In total, 83 participants were histologically diagnosed with IMN. All patients were screened for the secondary causes of MN such as hepatitis B and C virus, malignancies, systemic lupus erythematosus, and medication-related consequences. Patients with secondary MN, IMN without nephrotic syndrome, patients who rejected treatment or could not be followed up in our center, and patients who took immunosuppressive therapy before the biopsies were excluded and the remaining 47 patients were included in this study.

Therapeutic Regimen

Immunosuppressive therapy was started only in patients with nephrotic syndrome and when at least one of the following conditions was observed:

1. The urinary protein excretion persistently exceeds 4 g/d and remains at over 50% of the baseline value;

2. If the patient does not show a progressive decline during antihypertensive and antiproteinuric therapies during an observation period of at least six months; and

3. The presence of severe, disabling, or life-threatening symptoms and serum creatinine (SCr) levels had risen by 30% or more within 6-12 months from the time of diagnosis and the estimated glomerular filtration (eGFR) is not less than 30 mL/min per 1.73 m^2 and this change is not explained by superimposed complications.

In total, 47 patients were categorized into 2 groups based on the treatment with immunosuppressors:

1. Oral steroid monotherapy group (Group 1): Patients received oral steroid monotherapy at an initial dose of 1 mg/kg/day for 8 weeks, and the dose was reduced by 5 mg every 2 weeks to 30 mg/day. Then, it was reduced by 5 mg every 4 weeks to 5 mg/day for 6 months.

2. Monthly intravenous CTX and oral steroid group (Group 2): Patients received intravenous CTX at a dose of 0.5-0.75 g/m^2 once in every month initially for 6 months, the regimen was combined with a steroid (prednisone: 1 mg/kg/day), and the prednisone dose was reduced by 5 mg every week. It was withheld temporarily when the patients' leucocyte counts fell below 3,500/mm^3 or in case of any infection or other adverse effects. We restarted intravenous CTX administration after the adverse effects had recovered; in these cases, the treatment duration was prolonged. The target blood pressure was less than 130/80 mmHg during the follow-up. In addition to supportive care, all patients received angiotensin-converting enzyme (ACE) inhibitor/angiotensin II receptor blocker (ACEI/ARB), diuretics, and lipid-lowering agents. All patients were observed for the treatment effects, recurrence, and side effects.

Clinical Parameters and Definitions

Nephrotic syndrome was defined with proteinuria >3.5 g/day and plasma albumin concentrations <2.5 g/dL. We adopted the definition of The Kidney Disease: Improving

Global Outcomes (KDIGO) practice guidelines on glomerulonephritis, presented in 2012. Complete remission (CR) was defined as urine protein and creatinine ratio (UPCR) <300 mg/g or <0.3 g/day accompanied by normal serum concentrations of albumin and SCr. Partial remission (PR) was defined as UPCR ≤3,500 mg/g or <3.5 g/day or a decrease in proteinuria by at least 50% from the initial value and <3.5 g/day for at least 2 weeks, accompanied by either improvement or the normalization of serum albumin concentration and a stable level of SCr. No remission was defined as no improvement in the urinary protein excretion and serum albumin levels. Patients who did not meet the definitions above were considered to be unresponsive. The remissions were recorded at six months after histological diagnosis. Relapse was defined as nephrotic proteinuria (>3.5 g/day) after a period of remission. The relapse rates from patients with remissions were also recorded. The demographic characteristics and initial laboratory data were recorded. These data included age, sex, blood urea nitrogen, SCr, serum albumin, total cholesterol, and 24-h urinary protein excretion. The date of achieving the first remission for each patient was recorded. The primary outcome was the number of CR or PR in proteinuria. Other outcomes included the time for remission, deterioration of renal function, and adverse effects.

Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee (2021/61-15.02.2021) approved this study's protocol.

Statistical Analysis

Categorized variables are represented as numbers; percentages and the mean values were presented with ± standard deviation or median (minimum-maximum). The differences were compared by independent samples t-test. All statistics were performed using SPSS 23 software. A value of p<0.05 was considered significant for this study.

RESULTS

The data of 83 patients with MN were retrieved retrospectively, and 47 patients with IMN presented with nephrotic syndrome [20 of them were in the oral steroid monotherapy group (mean age: 49.5±16.1 years) and 27 of them were in the steroid + CTX group (mean age: 45.6±18.0 years)] were included in the study. There were no significant differences in the means of average age and the distribution of sex between the groups. The follow-up time was 53.00±39.88 (6-150) months, but the average length of follow-up in the steroid group was longer than that of the second group (63.10±40.99 months and 45.52±38.06 months, respectively) with statistically insignificant difference (p>0.05). At the baseline, there was no significant difference in SCr, serum albumin, and daily proteinuria between the groups (p>0.05). Proteinuria (g/day) levels at baseline, at six months, and at the end of the treatment were not different between the groups [7.58±4.10 vs 8.74±4.02, p=0.34 (baseline); 1.452±1.579 vs. 2.682±2.730, p=0.059 (six months); 362±416 vs. 220±274, p=0.115 (at the end of treatment)]. Table 1 shows the demographic features and laboratory parameters of the study population.

Patients were followed until March 2018 or until the occurrence of one of the endpoints of ESRD or death.

The median time to achieve CR was similar [7.78±5.26 (2-16 months) vs. 8.22±3.15 (6-15 months), p=0.72, respectively]. The average duration time of remissions in patients with CR was 51.89±23.57 (24-144) months in the steroid group, whereas it was 35.78±33.74 (12-108) months in the combination group (p=0.36). After the initial treatment, all patients achieved either CR or PR. In the oral steroid monotherapy group, 12 patients (60.0%) had CR, whereas it was only 9 (33.3%) in the other group (p=0.069). In total, 8 patients in the oral steroid group and 18 patients in steroid + CTX group achieved PR (p=0.69). Table 2 presents a

Table 1. Laboratory parameters and baseline characteristics of patients

	Steroid (n=20)	Steroid + CTX (n=27)	p
Age (years)	49.5±16.1	45.6±18.0	0.33
Gender (female/male)	10/10	16/11	0.52
Urea (mg/dL)	30.15±11.71	32.74±10.91	0.83
sCreatinine (mg/dL)	0.9±0.44	0.85±0.36	0.84
sAlbumin (g/dL)	2.65±0.67	2.4±0.63	0.64
Proteinuria (before therapy) (g/day)	7.58±4.1	8.74±4.02	0.96
Proteinuria (6 months) (g/day)	1.45±1.57	2.68±2.73	0.12
Proteinuria (at end follow-up) (g/day)	0.36±0.41	0.22±0.27	0.16

CTX: Cyclophosphamide. Independent sample t-test; Pearson chi-square test; p<0.05

detailed description of remission times for both groups. The relapse rates were 30% and 37% in the steroid group and the steroid + CTX group, respectively (Table 3). The difference was not statistically significant (p=0.615). Proteinuria was significantly decreased and the serum albumin was significantly increased after immunosuppressive treatment in both groups (p<0.001). No patients developed renal failure during the follow-up, whereas two new onset cases of diabetes mellitus in the steroid group and two cases with pulmonary infection in the combined corticosteroid and CTX group were noted.

DISCUSSION

The natural course of IMN is variable. The conventionally accepted clinical course is the "rule of thirds": in untreated patients, the spontaneous CR of proteinuria occurs in 5%-30% of patients at 5 years, spontaneous PR in 25%-40% of patients at 5 years, and progression to ESRD in 41% of patients at 5 years (8). Therefore, predicting the outcome is important for deciding which patient will receive benefits from immunosuppressive therapy. A better risk prediction is based on the clinical parameters of proteinuria and creatinine clearance over a fixed period of time. Male sex, old age (>50 years), hypertension, massive proteinuria (>10 g/24 h), and elevated SCr concentrations at the time of renal biopsies are poor prognostic factors for IMN. The occurrence of relapse or persistence of proteinuria exert a negative impact on renal survival in patients with IMN and nephrotic syndrome. The serum albumin level at diagnosis is the strongest prognostic factor for progression into NS (9,10). The aim in the management of persistent proteinuric disorders is the prevention of renal function deterioration and progression to ESRD (11,12). Declining proteinuria in IMN with the subsequent attainment of a CR or PR correlates with a better renal survival (13,14). Patients can be categorized into three groups according to risk prediction: low-risk, medium-risk, and high-risk groups. The normalization of blood pressure and serum cholesterol levels are important in all the groups.

Patients in the low-risk group (normal SCr level and proteinuria <4 g/day over 6 months of observation) should be treated with diet, ACE inhibitors, and/or angiotensin II type 1 receptor blockers (ARB) instead of aggressive immunosuppressive treatment. In recent years, some studies have shown that ACE inhibitors and ARB combinations are ineffective for treating nephrotic proteinuria, especially >5 gr/day (15). Therefore, immunosuppressive treatment should be initiated in patients with proteinuria between 4 and 8 g/day. If proteinuria persists despite conservative treatment, there is a progression of renal insufficiency accompanied by the development of complications of NS (16,17). In contrast, patients with nephrotic proteinuria and poor prognostic factors should be treated with immunosuppressive drugs. Patients in the high-risk group have persistent proteinuria (>8 g/day) and/or a deteriorating kidney function (16). In patients with IMN and nephrotic proteinuria, the risk of progression to kidney failure should be balanced against the risks and benefits of immunosuppressive therapy (18). The KDIGO guidelines recommend using alternating monthly cycles of oral and intravenous corticosteroids and oral alkylating agents (chlorambucil and CTX) (the Ponticelli regimen) or cyclosporine combination with prednisone. Alkylating agents are the gold standard for the treatment. These all treatments predispose to opportunistic infections and they can even increase the cancer risk threefold. The guideline also suggests the observation without administering immunosuppression for six months because the spontaneous remission rate is already mentioned to be over 30% (19-21). Alternative regimens for the initial

Table 3. Patients who were followed up until March 2018 or until the occurrence of end-stage renal disease

	Steroid (n=20)	Steroid + CTX (n=27)	p
Complete remission	60% (12)	33.3% (9)	0.069
Partial remission	40% (8)	66.7% (18)	0.069
No relapse	70% (n=14)	63% (n=17)	0.615

CTX: Cyclophosphamide, Pearson chi-square test; p<0.05

Table 2. Time to remission according to therapy of patients followed up

	Steroid (n=20)	Steroid + CTX (n=27)	p
Mean time to complete remission (mo)	7.78±5.26 (2-16)	8.22±3.15 (6-15)	0.72
Duration of complete remission (mo)	51.89±23.57 (24-144)	35.78±33.74 (12-108)	0.36
Mean time to partial remission(mo)	5.42±3.52 (1-15)	5.71±2.04 (3-8)	0.26
Duration of partial remission (mo)	38.42±39.46 (5-131)	32.65±39.04 (3-114)	0.88
Follow-up time (mo)	63.1±40.99 (11-150)	45.52±38.06 (6-134)	0.13

CTX: Cyclophosphamide, Months (mo). Independent samples t-test; p<0.05

therapy for IMN are calcineurin inhibitors (CNI) such as cyclosporine A and tacrolimus, anti-proliferative agents such as mycophenolate mofetil, azathioprine, and rituximab (22). Mycophenolate mofetil monotherapy appears to be ineffective, but may be beneficial when administered together with steroids (23). The effects of steroids on IMN are controversial. KDIGO guidelines for glomerulonephritis suggest that corticosteroid monotherapy should not be used for initial therapy. Because the efficacy of corticosteroid monotherapy is still being debated, new studies should be designed to study the efficacy of steroid monotherapy in patients who do not respond to antiproteinuric treatment with RAS blockers over six months. In some of studies, the long-term, alternate-day steroid treatment resulted in a significant reduction in proteinuria and the rate of progression to renal failure (24). An early study reported that a two- to three-month course of high-dose, alternate-day prednisone administration resulted in a significant reduction in progression to kidney failure; however, there was no sustained effect on proteinuria (25). Our study demonstrated that steroid monotherapy was as effective as the combined steroid and cytotoxic drug therapy in the reduction of proteinuria and preservation of renal function. These results showed that steroid monotherapy can be used as an alternative treatment for patients with IMN. In a study conducted in Asia, 949 patients with IMN were divided into three groups based on the type of treatment: the steroid group, the combined corticosteroid and CTX group, and the supportive therapy group. Importantly, more than 80 patients in all groups reached CR or PR. This study showed that immunosuppressive drug treatment and the achievement of CR or PR affects renal survival but it must be noted that the proportion of RAS blocker use was only 10% among the patients. The authors believe that the clinical outcome varies among different races and geography. Steroid therapy, which has not been recommended for IMN in most review articles, appears to be useful at least for Japanese patients (26). Recently, a retrospective study was performed and enrolled patients were divided into two groups based on the interval from biopsy to the initiation of immunosuppression. The patients who received immunosuppressive agents within six months of diagnosis and those who did not receive treatment were compared. In contrast to Western countries, patients with IMN who were treated with any steroid monotherapy may have a better renal preserve and high remission rate in the first year (27).

Recently, a network meta-analysis of RCTs (36 trials, 2018 patients) had been performed and 11 kinds of immunosuppressives were included in the therapies. A meta-analysis showed that a combination of alkylating agents and corticosteroids reduced the risk of ESRD. The total remission rate was 59.2% in the patients treated with immunosuppressive therapy and 32.4% in patients treated with non-immunosuppressive agents. Patients with IMN in whom immunosuppressive therapy is warranted, treatment with either an alkylating agent combined with prednisone or cyclosporine is recommended by the KDIGO GN guidelines (28). Patients with IMN diagnosed since 2006 were enrolled in our study. KDIGO GN guideline was presented in 2012, and it was decided to compare oral steroid monotherapy for patients with nephrotic IMN with the protocol of CTX combined with oral steroids. Although Ponticelli et al. (29) showed that the remission rate was 48.1% in patients treated with steroids and alkylating agents at the ten-year follow-up, our study demonstrated a higher rate of CR in the steroid monotherapy group (60%). Despite similar baseline characteristics of our study population, the combined therapy group had a higher PR rate (66.7%) and a lower CR rate (33.3%). Clinical trials using the cyclical treatment of alternating steroids and alkylating agents or CNI in IMN have shown an excellent kidney survival in those subjects with CR or PR, even in the long-term. However, the relapses of nephrotic syndrome occur in 25%-30% of patients within 5 years of discontinuation of the therapy with alkylating agents and 40%-50% of patients within 1 year of discontinuation of CNI (30). One study reported that 76% of 39 patients who received immunosuppressive achieved at least one PR in 5 years after diagnosis, whereas 32.8% experienced a relapse. The relapse rate was similar to that in our study (30% in monotherapy group vs 37% in combined therapy group) (31). Older patients tend to develop a complication of NS and infection because of immunosuppressive treatment. In a study conducted in Japan, older patients were divided into three groups: the prednisolone monotherapy group (n=35), the combined cyclosporine group (n=66), and the supportive therapy group (n=70). Moreover, the frequency of nephrotic syndrome and infection were compared among the groups. The proportion of patients achieving a 30% decrease in eGFR was not significantly different among the three groups, whereas the proportion of patients achieving CR and the rate of hospitalization due to infection were significantly higher in the immunosuppressive therapy groups than the supportive group (32). Remission may be delayed for as long as 18-24 months. In a recent study, the meantime to remission was 14.7±11.4 months following the third presentation, whereas the meantime to CR was 8.26±4.04 (2-16) months in our study. It is better to wait to see the long-term response unless there is a deterioration of renal function or decrease in serum albumin level. In this study, there was no difference in SCr levels between the

groups. None of the patients developed renal insufficiency during/in the follow-up period. Significantly decreased proteinuria and elevated serum albumin levels at 6 months of the treatment were observed in both the groups. Two patients exhibited steroid-induced diabetes in the steroid group and two developed infectious diseases in the combination group. All these results showed that the steroid monotherapy induced similar clinical outcomes and side effect profiles compared to the combined therapy group.

CONCLUSION

It was found that the steroid monotherapy had a beneficial effect on patients with IMN who presented with nephrotic proteinuria. It induced a higher CR rate and had a favorable effect on the survival rate. It showed an acceptable short-term efficiency and patient tolerability. Oral steroid monotherapy may be an alternative therapeutic regimen for patients with nephrotic IMN, but further randomized controlled trials are needed to clarify the benefits of early oral steroid monotherapy in patients with IMN.

Footnotes: This study has been presented at the 56th European RenalAssociation - European Dialysis and Transplant Association Congress (ERA/EDTA Congress), 13-16 June 2019, Budapest, Hungary.

ETHICS

Ethics Committee Approval: The study protocol was approved by Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee (2021/61-15.02.2021).

Informed Consent: Informed consent was not obtained since the study is retrospective.

Authorship Contributions

Concept: S.K.Y., O.H., M.Y., Design: S.K.Y., O.H., A.O., Data Collection or Processing: S.K.Y., A.O., M.Y., Analysis or Interpretation: O.H., S.A., Literature Search: S.K.Y., O.H., A.O., S.A., Writing: O.H., M.Y., S.A.,

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

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

REFERENCES

1. Makker SP, Tramontano A. Idiopathic membranous nephropathy: an autoimmune disease. Semin Nephrol 2011;31:333-40.
2. Glassock RJ. The pathogenesis of idiopathic membranous nephropathy: a 50-year odyssey. Am J Kidney Dis 2010;56:157-67.
3. Polanco N, Gutiérrez E, Covarsí A, Ariza F, Carreño A, Vigil A et al. Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy. Journal of the American Society of Nephrology 2010 Apr 1;21:697-704.
4. van den Brand JA, Hofstra JM, Wetzels JF. Low-molecular-weight proteins as prognostic markers in idiopathic membranous nephropathy. Clin J Am Soc Nephrol 2011;6:2846-53.
5. du Buf-Vereijken PW, Branten AJ, Wetzels JF. Idiopathic membranous nephropathy: outline and rationale of a treatment strategy. Am J Kidney Dis 2005;46:1012-29.
6. Ponticelli C, Glassock RJ. Glomerular diseases: membranous nephropathy--a modern view. Clin J Am Soc Nephrol 2014;9:609-16.
7. Waldman M, Austin HA 3rd. Treatment of idiopathic membranous nephropathy. J Am Soc Nephrol 2012;23:1617-30.
8. Jha V, Ganguli A, Saha TK, Kohli HS, Sud K, Gupta KL, et al. A randomized, controlled trial of steroids and cyclophosphamide in adults with nephrotic syndrome caused by idiopathic membranous nephropathy. J Am Soc Nephrol 2007;18:1899-904.
9. Sprangers B, Bomback AS, Cohen SD, Radhakrishnan J, Valeri A, Markowitz GS, et al. Idiopathic membranous nephropathy: clinical and histologic prognostic features and treatment patterns over time at a tertiary referral center. Am J Nephrol 2012;36:78-89.
10. Huh H, Lee H, Lee JP, Kim DK, Oh S, Oh YK, et al. Factors affecting the long-term outcomes of idiopathic membranous nephropathy. BMC Nephrol 2017;18:104.
11. Pisoni R, Aros C, Ruggenenti P, Remuzzi G. Mechanisms of progression of chronic renal disease. Saudi J Kidney Dis Transpl 2002;13:250-6.
12. Ruggenenti P, Schieppati A, Remuzzi G. Progression, remission, regression of chronic renal diseases. Lancet 2001;357:1601-8.
13. Troyanov S, Wall CA, Miller JA, Scholey JW, Cattran DC; Toronto Glomerulonephritis Registry Group. Idiopathic membranous nephropathy: definition and relevance of a partial remission. Kidney Int 2004;66:1199-205.
14. Ponticelli C, Passerini P, Altieri P, Locatelli F, Pappalettera M. Remissions and relapses in idiopathic membranous nephropathy. Nephrol Dial Transplant 1992;7 Suppl 1:85-90.
15. Praga M, Hernández E, Montoyo C, Andrés A, Ruilope LM, Rodicio JL. Long-term beneficial effects of angiotensin-converting enzyme inhibition in patients with nephrotic proteinuria. Am J Kidney Dis 1992;20:240-8.
16. Alfaadhel T, Cattran D. Management of Membranous Nephropathy in Western Countries. Kidney Dis (Basel) 2015 Sep;1:126-37.
17. Radhakrishnan J, Cattran DC. The KDIGO practice guideline on glomerulonephritis: reading between the (guide)lines--application to the individual patient. Kidney Int 2012;82:840-56.
18. Hofstra JM, Fervenza FC, Wetzels JF. Treatment of idiopathic membranous nephropathy. Nat Rev Nephrol 2013;9:443-58.
19. Hofstra JM, Wetzels JF. Management of patients with membranous nephropathy. Nephrol Dial Transplant 2012;27:6-9.
20. Cattran DC. Mycophenolate mofetil and cyclosporine therapy in membranous nephropathy. Semin Nephrol 2003;23:272-7.
21. Ponticelli C, Altieri P, Scolari F, Passerini P, Roccatello D, Cesana B, et al. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. J Am Soc Nephrol 1998;9:444-50.
22. Remuzzi G, Chiurchiu C, Abbate M, Brusegan V, Bontempelli M, Ruggenenti P. Rituximab for idiopathic membranous nephropathy. Lancet 2002;360:923-4.
23. Choi JY, Kim DK, Kim YW, Yoo TH, Lee JP, Chung HC, et al. The Effect of Mycophenolate Mofetil versus Cyclosporine as Combination Therapy with Low Dose Corticosteroids in High-risk

Patients with Idiopathic Membranous Nephropathy: a Multicenter Randomized Trial. J Korean Med Sci 2018;33:e74.

24. Bolton WK, Atuk NO, Sturgill BC, Westervelt FB Jr. Therapy of the idiopathic nephrotic syndrome with alternate day steroids. Am J Med 1977;62:60-70.
25. Cattran DC, Delmore T, Roscoe J, Cole E, Cardella C, Charron R, et al. A randomized controlled trial of prednisone in patients with idiopathic membranous nephropathy. N Engl J Med 1989;320:210-5.
26. Shiiki H, Saito T, Nishitani Y, Mitarai T, Yorioka N, Yoshimura A, et al. Prognosis and risk factors for idiopathic membranous nephropathy with nephrotic syndrome in Japan. Kidney Int 2004;65:1400-7.
27. Chan CK, Lai TS, Chen PM, Chou YH, Wu CF, Chiu YL, et al. Early initiation of immunosuppressive treatment in membranous nephropathy patients. J Formos Med Assoc 2017;116:266-75.
28. Ren S, Wang Y, Xian L, Toyama T, Jardine M, Li G, et al. Comparative effectiveness and tolerance of immunosuppressive treatments for idiopathic membranous nephropathy: A network meta-analysis. PLoS One 2017;12:e0184398.
29. Ponticelli C, Zucchelli P, Passerini P, Cesana B, Locatelli F, Pasquali S, et al. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. Kidney Int 1995;48:1600-4.
30. Waldman M, Austin HA 3rd. Controversies in the treatment of idiopathic membranous nephropathy. Nat Rev Nephrol 2009;5:469-79.
31. McQuarrie EP, Stirling CM, Geddes CC. Idiopathic membranous nephropathy and nephrotic syndrome: outcome in the era of evidence-based therapy. Nephrol Dial Transplant 2012;27:235-42.
32. Yamaguchi M, Ando M, Yamamoto R, Akiyama S, Kato S, Katsuno T, et al. Patient age and the prognosis of idiopathic membranous nephropathy. PLoS One 2014;9:e110376.

Medical Journal of Bakırköy

Med J Bakırköy 2021;17:161-166
DOI: 10.4274/BMJ.galenos.2021.12599

Research

The Relationship Between Cyclo-Oxygenase-2 *-1195A/G* Gene Polymorphism and Renal Cell Carcinoma

Siklooksijenaz-2 *-1195 A/G* Gen Polimorfizmi ile Böbrek Hücreli Karsinom Arasındaki İlişki

İlknur Bingül[1], Canan Küçükgergin[1], Selçuk Erdem[2], Tzevat Tefik[2], Öner Şanlı[2], Şule Seçkin[1]

[1]İstanbul University-İstanbul Faculty of Medicine, Department of Medical Biochemistry, İstanbul, Turkey
[2]İstanbul University-İstanbul Faculty of Medicine, Department of Urology, İstanbul, Turkey

ABSTRACT

Objective: This study was aimed to evaluate the association of cyclo-oxygenase 2 (COX-2) -1195A/G polymorphism with initiation and progression of renal cell carcinoma (RCC) and interaction with smoking in RCC patients in a Turkish population.

Methods: The *COX-2 -1195A/G* gene polymorphism was analyzed by method of polymerase chain reaction in DNA samples of 154 healthy controls and 114 patients with RCC.

Results: No significant variation in terms of age, sex, body mass index (BMI) or smoking between RCC patients and controls was observed. There was no statistical significance between *COX-2 -1195A/G* gene polymorphism and onset or progression of RCC in patients and controls (p>0.05). In addition, no relationship was identified regarding high stage and poorly differentiated RCC risk after adjusting for age, sex, BMI and smoking status. Furthermore, this polymorphism was not significantly associated with development of RCC when accompanied by smoking status.

Conclusion: Our results showed that the COX-2 -1195A/G polymorphism does not seem to be a major risk factor for both the onset and progression of RCC in a Turkish population.

Keywords: Cyclo-oxygenase-2, single nucleotide polymorphism, renal cell carcinoma

ÖZ

Amaç: Bu çalışmada, Türk toplumundaki böbrek hücreli karsinom (BHK) hastalarında siklooksijenaz 2 *(COX-2) -1195A/G* gen polimorfizminin BHK'nin başlangıcı ve ilerlemesi ile ilişkisi ve sigara kullanımı ile etkileşimi araştırılmıştır.

Gereç ve Yöntem: *COX-2 -1195A/G* gen polimorfizmi, 154 sağlıklı kontrol ve 114 BHK tanısı alan hastaların DNA örneklerinde polimeraz zincir reaksiyonu yöntemi ile incelenmiştir.

Bulgular: BHK'li hastalar ve kontrol grubu arasında yaş, cinsiyet, vücut kitle indeksi ve sigara kullanımı açısından anlamlı bir fark bulunmadı. Hatsa ve kontrol gruplarında *COX-2 -1195A/G* gen polimorfizmi ile BHK'nin oluşumu ve gelişimi arasında istatistiksel olarak anlamlı bir fark saptanmadı (p>0,05). Buna ek olarak, yaş, cinsiyet, vücut kitle indeksi ve sigara kullanımına göre düzeltme yapıldıktan sonra da yüksek evre ve yüksek dereceli BHK riski ile ilgili bir farklılık bulunmadı. Ayrıca, *COX-2 -1195A/G* gen polimorfizmi ile sigara kullanımının eşlik ettiği BHK gelişimi arasında bir ilişki de saptanmadı.

Sonuç: Sonuçlarımıza göre *COX-2 -1195A→G* gen polimorfizminin Türk toplumunda BHK'nin başlangıcı ve ilerlemesi bakımından majör bir risk faktörü olmadığı ileri sürülebilir.

Anahtar Kelimeler: Siklooksijenaz-2, tek nükleotid polimorfizmi, böbrek hücreli karsinom

Address for Correspondence: İlknur Bingül, İstanbul University-İstanbul Faculty of Medicine, Department of Medical Biochemistry, İstanbul, Turkey
Phone: +90 533 358 33 04 E-mail: ilknur.bingul@İstanbul.edu.tr ORCID ID: orcid.org/0000-0002-6432-3541

Cite as: Bingül İ, Küçükgergin C, Erdem S, Tefik T, Şanlı Ö, Seçkin Ş. The Relationship Between Cyclooxygenase-2 *-1195A→G* Gene Polymorphism and Renal Cell Carcinoma in A. Med J Bakırköy 2021;17:161-166

Received: 01.05.2021
Accepted: 22.06.2021

INTRODUCTION

Renal cell carcinoma (RCC) is a common and deadly disease, accounting for about 2% of all cancer diagnoses and 90% of all kidney cancers in adults (1). According to Global Cancer Observatory data, RCC is the seventh most common cancer in the developed world, with more than 400,000 new cases and around 175,000 deaths in 2018 (1,2). Epidemiological researches have suggested that the development of RCC is associated with multiple genetic and environmental factors including age, sex, race, hypertension, obesity, smoking, diet, occupational exposure, and drugs (1,3). In addition, recent studies have reported that genetic variations especially single nucleotide polymorphisms (SNPs) may also be involved in the development of various types of cancer (4,5).

Cyclo-oxygenases (COXs), the key enzymes in conversion of arachidonic acid to prostaglandins, are also known as prostaglandin-endoperoxide synthases (6). COXs consist of two isoforms named COX-1 and COX-2. While COX-1 is constitutively expressed in various tissues and maintains homeostasis of various physiological functions, *COX-2* is an inducible form and expressed in response to various factors such as tumorigenic, inflammatory and growth factors (6,7). Therefore, overexpression of COX-2 may contribute to carcinogenesis by increasing cell proliferation, angiogenesis, and inflammation and suppressing apoptosis (7,8,).

The COX-2 gene is located on q25.2-25.3 chromosome 1, including 10 exons and 9 introns with a total length of approximately 8.3 kb (6,7,8). SNP in the *COX-2* gene may affect the activity of that enzyme and consequently alter an individual's susceptibility to different types of cancer. Available data suggested that COX-2 -1195A/G gene polymorphism is associated with the initiation of various cancers including lung cancer (9), epithelial ovarian carcinoma (10), gastrointestinal system cancer (11), and hepatocellular carcinoma (12). However, no association has been found between this polymorphism and development of cancers such as lung (13), oral (14), and RCC (15) in recent studies conducted in different ethnic groups.

Therefore, in the present study, the possible association of the *COX-2 -1195A/G* gene polymorphism on the initiation and progression of RCC in a Turkish population was examined.

METHODS

A total of 114 patients with histopathologically confirmed RCC who experienced radical or partial nephrectomy at the Urology Department of İstanbul Faculty of Medicine were included in this study. All study subjects completed a questionnaire with detailed information. The control group was made up of 154 healthy individuals who were eligible by age, sex, and smoking status, and had no previous or present history of cancer. All individuals were classified as either smokers or non-smokers. The tumor-node-metastasis (TNM) classification system of the American Joint Committee on Cancer (AJCC) and Fuhrman et al. (16) grading system were used to determine of tumor staging and grading, respectively. Based on TNM staging, the patients were assigned into two groups: localized group (Stage I and II) and advanced group (Stage III and IV). They were also divided into two groups using Fuhrman et al. (16) grade: low grade (Grade I and II) and high grade (Grade III and IV). Ethical approval for this study was obtained from the Ethics Committee of İstanbul Faculty of Medicine and informed consent was signed by each participant.

Peripheral blood samples from each RCC patient and each control subject were taken into tubes containing EDTA, and genomic DNA was isolated by using a commercially available kit (Roche Diagnostics, Mannheim, Germany), and stored at -20 0C.

The gene polymorphism of COX-2 -1195A→G (rs rs689466) was genotyped using PCR-RFLP. 5'-CCCTGAGCACTACCCATGAT-3' and 5'-GCCTTCATAGGAGATACTGG -3' were used as forward and reverse primers, respectively, for PCR analysis. The PCR reactions were carried out to amplify the COX-2 gene of the subjects. The amplified PCR products were digested by the PvuII restriction enzyme (Thermo Scientific), and visualized on 3% agarose gel stained with ethidium bromide. Fragment sizes of the COX-2 genotypes were AA (273 bp), AG (273 bp, 220 bp,53 bp) and GG (220 bp, 53 bp).

Statistical Analyses

Data analyses were performed using the Statistical Package for the Social Science (21.0; SPSS Inc., Chicago, IL, USA). For all statistical analyses, $p<0.05$ was considered statistically significant. Mean values were compared between controls and RCC patients by Mann-Whitney U test. Chi-square tests were used to analyze the distributions of genotypes and allele frequencies between patients and controls. Odds ratios (ORs) were determined together with their corresponding 95% confidence intervals (95% CI) by using logistic regression analyses. The chi-squared test was used to test whether the genotype distributions corresponded to the Hardy Weinberg equilibrium (HWE). The power of the study was calculated as 84% with NCSS 2000 statistical

package (NCSS Inc.; Kaysville, UT) to detect an effect size (w) of 0.20 using 2 degrees of freedom (α: 0.05).

RESULTS

The demographic parameters of all subjects and clinicopathological characteristics of the patients are demonstrated in Table 1. There was no statistically significant variation in age, sex, BMI, and smoking status between patients with RCC and controls. The grade distribution of the RCC was low grade (I-II) for 65.8% in patients with RCC and high grade (III-IV) for 34.2% in patients with RCC. In addition, 65.8% of patients with RCC were low stage (I-II), and 34.2% of patients with RCC were high stage (III-IV). Most of the patients with RCC included the low stage and grade.

Genotype distribution of the *COX-2* gene polymorphism was consistent with HWE in both patients (p=0.096) and control groups (p=0.935).

In *COX-2 -1195A→G* polymorphism, the AA, AG, and GG genotypes were detected in 86.0%, 12.3%, and 1.8% among patients with RCC, respectively. In the control group, the distributions of the COX-2 genotypes were 77.9% for AA, 20.8% for AG, and 1.3% for GG, respectively (Table 2).

On the other hand, the possible variation in the distributions of genotypes and allele frequencies between smokers and non-smokers was evaluated for RCC susceptibility. No association was observed between *COX-2* gene polymorphism and RCC risk in smokers or non-smokers (Table 3).

In addition, no relationship was found between *COX-2* gene polymorphism and clinicopathological characteristics of RCC (Table 4).

DISCUSSION

The clinical and experimental studies supported the notion that COX-2 has an important role in carcinogenesis by promoting tumor growth, angiogenesis, invasion and metastasis, and inhibiting apoptosis (6,14).

COX-2 is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandin H2, the precursor

Table 1. General characteristics of the controls and patients with renal cell carcinoma (mean ± SD)

Parameters	Controls (n=154)	Patients (n=114)	[a]p
Age (years) (mean ± SD)	56.8±10.7	55.1±9.72	0.070
BMI (kg/m^2) (mean ± SD)	26.7±3.01	27.2±3.50	0.057
Sex (%) (female /male)	41(26.6)/113 (73.4)	43 (37.7)/71(62.3)	0.053
Smoking status (%) (never/current)	65.6/34.4	55.3/44.7	0.086
Grade			
I	-	22 (19.3)	-
II	-	53 (46.5)	-
III	-	27 (23.7)	-
IV	-	12 (10.5)	-
Stage			
I	-	72 (63.2)	-
II	-	3 (2.6)	-
III	-	33 (28.9)	-
IV	-	6 (5.3)	-

[a]p from Pearson's χ^2 test for categorical variables and the Mann-Whitney U or Student's t-tests for continuous variables, SD: Standard deviation, BMI: Body mass index

Table 2. The distribution of genotypes and alleles in controls and patients with renal cell carcinoma

	Controls n (%)	Patients n (%)	p	OR[a] (95% CI)
COX-2-1195A→G				
AA	120 (77.9)	98 (86.0)	-	1.00*
AG	32(20.8)	14 (12.3)	0.077	0.43 (0.17-1.09)
GG	2 (1.3)	2 (1.8)	0.595	1.32 (0.47-3.63)
AG + GG	34 (22.1)	16 (14.0)	0.094	0.57 (0.30-1.10)
Allele				
A	272 (88.3)	210 (92.1)	-	1.00*
G	36 (11.7)	18 (7.9)	0.149	0.64 (0.35-1.17)

[a]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex, BMI, and smoking status

*: Reference genotype, BMI: Body mass index

Table 3. Impact of smoking status on the distribution of genotypes and alleles for patients with renal cell carcinoma

		Controls n (%)	Patients n (%)	p	OR[a] (95% CI)
Non-smokers	**COX-2-1195A→G**				
	AA	79 (77.5)	53 (84.1)	-	1.00*
	AG	22 (21.5)	9 (14.3)	0.231	0.44 (0.12-1.66)
	GG	1 (1.0)	1 (1.6)	0.470	1.71 (0.39-7.31)
	AG + GG	24 (22.5)	10 (15.9)	0.297	0.64 (0.28-1.47)
	Allele				
	A	180 (88.2)	115 (91.3)	-	1.00*
	G	24 (11.8)	11 (8.7)	0.384	0.71 (0.33-1.52)
Smokers	**COX-2-1195A→G**				
	AA	42 (79.2)	45 (88.2)	-	1.00*
	AG	10 (18.9)	5 (9.8)	0.326	0.52 (0.41-1.91)
	GG	1 (1.9)	1(2.0)	0.982	1.01 (0.24-4.16)
	AG + GG	11 (20.8)	6 (11.8)	0.206	2.80 (0.53-14.6)
	Allele				
	A	94 (88.7)	95 (93.1)	-	1.00*
	G	12 (11.3)	7 (6.9)	0.344	2.08 (0.52-8.33)

[a]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex and BMI;

*: Reference genotype, BMI: Body mass index

Table 4. The distribution of genotypes and alleles in patients with renal cell carcinoma according to the grade and stage of the disease

	Low grade[a] n (%)	High grade[b] n (%)	p	OR[e] (95% CI)
COX-2-1195A→G				
AA	63 (84.0)	35 (89.7)		1.00*
AG	11 (14.7)	3 (7.7)	0.229	0.25 (0.02-2.35)
GG	1 (1.3)	1 (2.6)	0.795	1.21 (0.28-5.12)
AG + GG	12 (16.0)	4 (10.3)	0.402	0.60 (0.18-2.00)
Allele				
A	137 (91.3)	73 (93.6)	-	1.00*
G	13 (8.7)	5 (6.4)	0.548	0.72 (0.24-2.10)
	Low stage[c] n (%)	High stage[d] n (%)	p	OR[e] (95% CI)
COX-2-1195A→G				
AA	64 (85.3)	34 (87.1)	-	1.00*
AG	10 (13.3)	4 (10.3)	0.781	0.78 (0.13-4.47)
GG	1 (1.4)	1 (2.6)	0.843	1.15 (0.27-4.85)
AG + GG	11 (14.7)	5 (12.9)	0.787	0.85 (0.27-2.66)
Allele				
A	138 (92)	72 (92.3)	-	1.00*
G	12 (8)	6 (7.7)	0.934	0.95 (0.34-2.65)

[a]Low grade (I-II), [b]High grade (III-IV), [c]Low stage (I-II), [d]High stage (III-IV), [e]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex, BMI, and smoking status

*: Reference genotype, BMI: Body mass index

of pro-inflammatory mediators such as thromboxane, prostaglandin E2, and prostaglandin I2. Typically, COX-2 expression is often undetectable in normal tissue, however pro-inflammatory stimuli and growth factors induce the expression of COX-2. Therefore, it was proposed that overexpression of COX-2 influenced immune response, cell growth, and proliferation, apoptosis, and promoted tumorigenesis via complex mechanisms (6-8,11).

In a study related to RCC, Cho et al. (17) proposed that the increased expression of COX-2 was associated only with tumor size but not be an effective factor for initiation of RCC. Miyata et al. (18) also demonstrated COX-2 expression was related to tumor status including tumor size and grade. Yoshimura et al. (19) suggested that COX-2 expression was not associated with stage or tumor grade in patients with RCC. In addition, Güçer et al. (20) reported that there was no relationship between COX-2 expression or clinicopathological parameters of RCC. These conflicting results indicate that the underlying mechanism of the regulation of *COX-2* gene expression has yet to be fully explored, and may be affected by genetic variations.

Association of various *COX-2* gene polymorphisms with susceptibility to tumorigenesis has so far been investigated in many published studies. According to these studies, it is generally considered that *COX-2* gene mutations are strongly related to the various types of cancer such as hepatocellular carcinoma, ovarian, lung, and esophagus cancer (9,10,12,21). However, the relationship between this polymorphism and RCC is still unclear.

The *COX-2 -1195A/G* gene polymorphism is a functional SNP resulting from the change of adenine to guanine at position -1195 in the promoter region of this gene. Recent studies have shown that the nucleotide base change of -1195 G to A generates a binding site for c-MYB in the *COX-2* gene promoter region leading to the higher transcriptional activity of this gene. c-MYB, a transcription factor, targets a variety of genes to coordinate the balance between cell division, differentiation, and survival. Therefore, it is suggested that the -1195A→G polymorphism may influence an individual's susceptibility to any type of cancer (11,13,22-24).

This is the only study in the literature that investigated COX-2 -1195A/G polymorphism in RCC performed by Chang et al. (15) in Taiwan, 2014. A total of 92 phenomena with RCC and 580 healthy controls were included in this study. It was reported that the distributions of the genotype of this polymorphism did not differ between the two groups.

In our study we investigated the effect of *COX-2 -1195A/G* gene polymorphism in Turkish patients with RCC and no significant association was identified between this polymorphism or initiation and progression of RCC. In addition, no association was detected between this polymorphism and tumor grade and stage or smoking as well. In the present study, our sample size may be considered as a limiting factor. For this reason, the statistical power of the results may be increased by conducting studies with higher sample numbers.

The variation of ethnicity, control population and sample size may lead to obtaining conflicting results in studies examining the relationship between the gene polymorphism of COX-2 and different types of cancer.

CONCLUSION

In conclusion, this study indicated that *COX-2 -1195A/G* gene polymorphism was not associated with initiation or progression of RCC in the Turkish population. Further functional investigations based on a larger sample size are required in order to clarify the relationship between the COX-2 -1195A→G polymorphism and RCC.

ETHICS

Ethics Committee Approval: This study was approved by the Ethics Committee of İstanbul Faculty of Medicine (date:14.9.2018; number:1254).

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

Authorship Contributions

Concept: Ö.Ş., Ş.S. Design: Ö.Ş., Ş.S. Data Collection or Processing: S.E., T.T. Analysis or Interpretation: İ.B., C.K. Literature Search: İ.B., C.K.; Writing: İ.B., C.K., Ş.S.

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

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

REFERENCES

1. Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, et al. Epidemiology of Renal Cell Carcinoma. World J Oncol 2020;11:79-87.
2. Linehan WM, Ricketts CJ. The Cancer Genome Atlas of renal cell carcinoma: findings and clinical implications. Nat Rev Urol 2019;16:539-52.
3. Petejova N, Martinek A. Renal cell carcinoma: Review of etiology, pathophysiology and risk factors. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2016;160:183-94.
4. Hemminki K, Bermejo JL. Relationships between familial risks of cancer and the effects of heritable genes and their SNP variants. Mutat Res 2005;592:6-17.
5. Bernig T, Chanock SJ. Challenges of SNP genotyping and genetic variation: its future role in diagnosis and treatment of cancer. Expert Rev Mol Diagn 2006;6:319-31.

6. Rizzo MT. Cyclooxygenase-2 in oncogenesis. Clin Chim Acta 2011;412:671-87.
7. Fosslien E. Molecular pathology of cyclooxygenase-2 in neoplasia. Ann Clin Lab Sci 2000;30:3-21.
8. Trifan OC, Hla T. Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. J Cell Mol Med 2003;7:207-22.
9. Coskunpinar E, Eraltan IY, Turna A, Agachan B. Cyclooxygenase-2 gene and lung carcinoma risk. Med Oncol 2011;28:1436-40.
10. Agachan Cakmakoglu B, Attar R, Kahraman OT, Dalan AB, Iyibozkurt AC, Karateke A, et al. Cyclooxygenase-2 gene and epithelial ovarian carcinoma risk. Mol Biol Rep 2011;38:3481-6.
11. Zhang XW, Li J, Jiang YX, Chen YX. Association between COX-2 -1195G>A polymorphism and gastrointestinal cancer risk: A meta-analysis. World J Gastroenterol 2017;23:2234-45.
12. Chen Z, Zhu J, Huang C, Lian F, Wu G, Zhao Y. The association between three cyclooxygenase-2 polymorphisms and hepatocellular carcinoma risk: a meta-analysis. PLoS One 2015;10:e0118251.
13. Moraes JL, Moraes AB, Aran V, Alves MR, Schluckbier L, Duarte M, et al. Functional analysis of polymorphisms in the COX-2 gene and risk of lung cancer. Mol Clin Oncol 2017;6:494-502.
14. Li D, Hao SH, Sun Y, Hu CM, Ma ZH, Wang ZM, et al. Functional Polymorphisms in COX-2 Gene Are Correlated with the Risk of Oral Cancer. Biomed Res Int 2015;2015:580652.
15. Chang WS, Liao CH, Miao CE, Wu HC, Hou LL, Hsiao CL, et al. The role of functional polymorphisms of cyclooxygenase 2 in renal cell carcinoma. Anticancer Res 2014;34:5481-6.
16. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982;6:655-63.
17. Cho DS, Joo HJ, Oh DK, Kang JH, Kim YS, Lee KB, et al. Cyclooxygenase-2 and p53 expression as prognostic indicators in conventional renal cell carcinoma. Yonsei Med J 2005;46:133-40.
18. Miyata Y, Koga S, Kanda S, Nishikido M, Hayashi T, Kanetake H. Expression of cyclooxygenase-2 in renal cell carcinoma: correlation with tumor cell proliferation, apoptosis, angiogenesis, expression of matrix metalloproteinase-2, and survival. Clin Cancer Res 2003;9:1741-9.
19. Yoshimura R, Matsuyama M, Kawahito Y, Tsuchida K, Kuratsukuri K, Takemoto Y, et al. Study of cyclooxygenase-2 in renal cell carcinoma. Int J Mol Med 2004;13:229-33.
20. Güçer H, Şahan E, Akyıldız-İğdem A, Tetikkurt ÜS, Erdoğan N. Cox-2 Expression and Microvessel Density in Clear Cell Type Renal Cell Carcinoma. Turkish J Pathol 2009;25:13-9.
21. Hu HM, Kuo CH, Lee CH, Wu IC, Lee KW, Lee JM, et al. Polymorphism in COX-2 modifies the inverse association between Helicobacter pylori seropositivity and esophageal squamous cell carcinoma risk in Taiwan: a case control study. BMC Gastroenterol 2009;9:37.
22. Zhang X, Miao X, Tan W, Ning B, Liu Z, Hong Y, et al. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. Gastroenterology 2005;129:565-76.
23. Tang Z, Nie ZL, Pan Y, Zhang L, Gao L, Zhang Q, et al. The Cox-2 -1195 G > A polymorphism and cancer risk: a meta-analysis of 25 case-control studies. Mutagenesis 2011;26:729-34.
24. Ramsay RG, Barton AL, Gonda TJ. Targeting c-Myb expression in human disease. Expert Opin Ther Targets 2003;7:235-48.