ISSN: 1305-9319 | e-ISSN: 1305-9327 | Number: 2 | Volume: 18 | Year: 2022

MEDICAL JOURNAL OF BAKIRKÖY

www.bakirkoytip.org

ISSN: 1305-9319 | e-ISSN: 1305-9327 | Number: 2 | Volume: 18 | Year: 2022

MEDICAL JOURNAL OF BAKIRKÖY

Medical Journal of Bakırköy 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
Gülay Saday
Gülşah Özgül

Finance Coordinator
Sevinç Çakmak
Emre Kurtulmuş

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

Research&Development
Nihan Karamanlı
Melisa Yiğitoğlu

Digital Marketing Specialist
Ümit Topluoğlu

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: June 2022
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

Researches

Contents

Contents

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:113-120
DOI: 10.4274/BMJ.galenos.2021.2021.10-14

Research

Comparison of the Sweet Taste Receptor (TAS1R2) Polymorphism and Nutrient Intakes in Adults

Yetişkinlerde Tatlı Tat Reseptör (TAS1R2) Polimorfizmi ile Besin Ögesi Alımlarının Karşılaştırılması

Kübra Karadeniz[1], Serap Andaç Öztürk[2,3], Ahu Soyocak[4]

[1]Istanbul Aydın University, Institute of Graduate Studies, Department of Nutrition and Dietetic, Istanbul, Turkey,
[2]Istanbul Sabahattin Zaim University Faculty of Health Sciences, Department of Nutrition and Dietetic, Istanbul, Turkey
[3]Istanbul Aydın University Faculty of Health Sciences, Department of Nutrition and Dietetic, Istanbul, Turkey
[4] Istanbul Aydın University Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey

ABSTRACT

Objective: It is known that genetic variations in the mechanism of taste perception play an essential role in food intake. In this study, we investigated the SNP rs35874116 polymorphism in the sweet taste receptor (TAS1R2) gene and nutrient intakes of adults.

Methods: The study conducted with 95 volunteers. Food consumption records of participants obtained by 24-h recall method, and analyzed by Computer-Aided Nutrition Software, Nutrition Information Systems 8.1 Package Software (BeBiS). Venous blood samples of the participants were collected to determine genotype distribution, and genotype distributions were determined using the Kompetitive Allele-Specific PCR (KASP) method.

Results: The genotype examination of participants revealed that the percentage of individuals with GA, AA, and GG genotypes were 67.3%, 26.3%, and 6.3% respectively. Daily total carbohydrate and sucrose intakes were found as the highest in individuals with GG genotype (145.55±56.69 g and 28.66±26 g, respectively), but without statistical difference.

Conclusion: According to our knowledge the study is the first to examine TAS1R2 polymorphism and nutrient intake in the Turkish population. We did not find any difference between TAS1R2 (rs35874116) polymorphism and nutrient intake; however, the study may serve as a preliminary result. Studies with a wider sample may help to enhance our understanding of the TAS1R2 and nutrient intake.

Keywords: TAS1R2, nutrient intake, carbohydrate, polymorphism

ÖZ

Amaç: Tat algısı mekanizmasındaki genetik varyasyonların besin alımında önemli rol oynadığı bilinmektedir. Bu çalışmada tatlı tat reseptörü (TAS1R2) SNP rs35874116 polimorfizmi ve yetişkin bireylerin besin ögesi alımı incelenmiştir.

Gereç ve Yöntem: Mevcut çalışma 95 gönüllü birey ile yürütülmüştür. Katılımcılardan 24 saatlik geri çağırma tekniği ile besin tüketim kayıtları toplanmış ve ilgili veriler bilgisayar destekli beslenme programı, Beslenme Bilgi Sistemi (BeBiS), 8.1 Paket programında analiz edilmiştir. Genotip dağılımı Kompetitif Allel-Spesifik PCR yöntemi ile belirlenmiş olup, bunun için araştırmaya katılan bireylerden venöz kan örnekleri toplanmıştır.

Bulgular: Katılımcılar genotiplerine göre incelendiğinde GA, AA ve GG genotiplerine sahip bireylerin yüzdesi sırasıyla %67,3, %26,3 ve %6,3'tür. Günlük toplam karbonhidrat ve sükroz alımları GG genotipli bireylerde daha yüksek (sırasıyla 145,55±56,69 g ve 28,66±26 g) bulunmuştur, ancak sonuçlar istatistiksel olarak anlamlı farklılık göstermemiştir.

Sonuç: Bilgimiz dahilinde mevcut çalışma, Türk popülasyonunda TAS1R2 polimorfizmi ve makro besin alımını inceleyen ilk çalışmadır. TAS1R2 (rs35874116) polimorfizm ve makro besin alımı arasında herhangi bir fark bulunamamış olmakla birlikte mevcut sonuçlar bir ön sonuçtur olarak literatüre katkıda bulunabilir. Daha geniş örneklemli çalışmalar, TAS1R2 ve gıda alımı konusundaki rolünün anlaşılmasında yardımcı olabilir.

Anahtar Kelimeler: TAS1R2, besin ögesi alımı, karbonhidrat, polimorfizm

This article was derived from the Master's thesis by Kübra Karadeniz in March 2021.

Address for Correspondence: Serap Andaç Öztürk, Istanbul Sabahattin Zaim University Faculty of Health Sciences, Department of Nutrition and Dietetic, Istanbul, Turkey
Phone: +90 444 97 98 E-mail: serandac@yahoo.com ORCID ID: orcid.org/0000-0002-6253-4118

Cite as: Karadeniz K, Andaç Öztürk S, Soyocak A. Comparison of the Sweet Taste Receptor (TAS1R2) Polymorphism and Nutrient Intakes in Adults. Med J Bakirkoy 2022;18:113-120

Received: 01.11.2021
Accepted: 17.12.2021

INTRODUCTION

Taste is considered the primary determinant of food preference and intake. Given that taste can affect nutritional preferences, understanding the factors that mediate differences in taste function and their effect on food preference and consumption is important (1). Many factors affect individuals' food choices, including physiological, nutritious, environmental, socio-cultural, and genetic factors (2). The number of studies on taste genetics and biology is increasing day by day as the genetic factors underlying individual differences in the ability to perceive tastes, which can affect eating behavior and food intake (3).

Taste perception in the taste sensory system occurs through specialized taste receptor cells (TRCs) found in the taste buds of the tongue (4). TRCs are indirectly or directly stimulated, detecting different tastes. G protein-coupled receptors (GPCRs) trigger indirect stimulation, whereas ion channels trigger direct stimulation (5). GPCRs mediate the perception of sweet, bitter, and umami taste, whereas the specific membrane channels mediate sour and salty taste (6). The GPCR protein family consists of 3 different taste receptor type 1 (T1R) and approximately 30 different taste receptor type 2 (T2R) members. Sweet taste ligands are bound to heterodimeric T1R2/T1R3 receptor, umami tastes ligands with heterodimeric T1R1/T1R3 receptor, and bitter taste ligands with T2R receptors to detect different flavors (7-9).

The T1R protein family that detects sweet and umami flavors are encoded by the *TAS1R1*, *TAS1R2*, and *TAS1R3* genes (10). T1R2, a sweet taste receptor protein, is synthesized from the *TAS1R2* gene discovered in 1999 (11,12). The *TAS1R2* gene is located on chromosome 1p36.13 and consists of six exons and produces a protein with 839 amino acids (2,13). Genetic diversity of sweet taste receptor genes has been shown to have a role in sweet taste sensitivity in adults (9,14,15).

Studies reported that the single nucleotide polymorphisms (SNP) of these chemosensory genes of the taste-sensing mechanism may be associated with food preferences and consumption (9,16). TAS1R2 is a highly polymorphic gene, and this high polymorphic ratio is assumed to be associated with variations in sweet taste perception. One of the *TAS1R2* gene SNPs occurs by nucleotide replacement of Adenine/Guanine (A571G, rs35874116) in the base 571 of the exon 3. This change alters the triplet codon sequence, causing the isoleucine/valine amino acid conversion in position 191 (3,17). A study investigated the relationship of the Ile191Val variations with carbohydrate intake and revealed that individuals with Val/Val genotype were associated with high carbohydrate intake (17). Another study investigated the relationship between sugar consumption of individuals with and without diabetes and variations of Ile191Val and reported that this variation may affect sugar consumption habits (9). Another study revealed that children with TT genotype in the TAS1R2 rs35874116 locus (Ile191Val) mostly preferred sweet foods and consumed desserts mostly in the evening (18). Additionally, according to another study, variation in TAS1R2 affects food consumption including cruciferous vegetables and foods with an umami taste (19).

The genetic background of food consumption has been widely evaluated, but individual food choices could be affected by genetic variations. Thus, we investigated the effect of SNP rs35874116 polymorphism in the sweet taste receptor (*TAS1R2*) gene on the nutrient intake to contribute to the enlightenment of factors involved in food preference and consumption.

METHODS

Study Group

This study included 95 volunteers between 21 and 60 years old. Our study was approved by Istanbul Aydın Universiry Non-Interventional Clinical Research Ethics Committee on 09/10/2019 with the decision number 2019/115. Volunteers were included in the research by obtaining written and oral consent.

Food Consumption Record/Nutrient Intake Analysis

The daily energy and food intakes of individuals were evaluated by a 24-hour recall food consumption record. The food consumption records of participants were analyzed using the "Computer-Aided Nutrition Software, Nutrition Information Systems 8.1 Package Software (BeBiS)," and nutrient intake was calculated.

Blood Sample Collection and Deoxyribonucleic Acid (DNA) Isolation

Venous blood samples of the participants were stored into tubes containing 2 mL of ethylene diamine tetraacetic acid. DNA isolation from the samples was performed using a commercial kit (EZ-10 Spin Column Genomic DNA Kit, Bio Basic Inc., Markham, Canada) using the spin column method. The purity and quantity determinations of DNA samples were spectrophotometrically performed (Thermo Scientific Multiskan Go, Thermofisher, USA), and samples with a measurement rate of A260/A280 ≅ 1.8 at 260 and 280 nm absorbency were considered pure. DNA amounts for the Kompetitive Allele-Specific PCR (KASP) method were ensured to be 10 ng/µL. Of each sample, 2 µL, whose purity

and quantity was determined, were used for the KASP genotype reaction mixture.

KASP Genotyping

The KASP™ method was used for TAS1R2 (rs35874116) genotyping. The KASP reaction mixture (10 µL) contained 5 µL of 2× KASP master mix, 0.14 µL of KASP primer assay mix, and 5 µL of DNA template (1 µL of PCR product/DNA extract + sterile water of 4 µL). There were Primer Allele A (FAM) (CAGCTGCACCATGGCCTCGAT) and Primer Allele G (HEX) (GCTGCACCATGGCCTCGAC) and primer common (CACCCAGCGCCGACCACCA) sequences specific to SNP rs35874116 within the KASP primer assay. KASP conditions were 1 cycle of 30 °C/1 min and 1 cycle of 94 °C/15 min, followed by 10 cycles of 94 °C/20 s and 61 °C/1 min, and 26 cycles of 94 °C/20 s and 55 °C/1 min. Finally, fluorescent endpoint readings were performed at 30 °C/1 min using the Applied Biosystems Step One Plus Real-Time PCR Systems (Foster City, CA, USA).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences 25.0 software. The relationship between genotypes and categorical variables was evaluated using the Fisher's exact test. The Kruskal-Wallis test was used to compare the values of genotypes that did not match the normal distribution. Binary comparisons of statistically significant variables were evaluated using the Mann-Whitney U test and Bonferroni correction. Additionally, p-values of <0.05 were considered statistically significant.

RESULTS

The genotype and allele frequency distributions of participants are summarized in Table 1.

According to the dominant model, the average energy and nutrient intake of participants were summarized in Table 2. No statistically significant difference was found between the median values of measurements according to the dominant model (p>0.05). The odds ratio could not be calculated because the number of samples in the dominant model was insufficient (Table 2).

Participants' energy and nutrient intake are presented in Table 3. No statistical difference was found between genotypes and median values (p>0.05).

DISCUSSION

This study aimed to investigate the TAS1R2 rs35874116 (A > G, Ile191Val) sweet taste receptor polymorphism, and nutrient intake of adult individuals.

Taste perception plays an important role in determining individual food preferences and eating habits. Genetic diversity of sweet taste receptor genes has played a role in sweet taste sensitivity in adults (9,14,15). TAS1R2 is a highly polymorphic gene and this high polymorphism is assumed to be associated with variations in sweet taste perception (3,17). Therefore, genetic variations in the TAS1R2 receptor may contribute to the differences between individual dietary intake (17,20). Eny et al. (9) investigated the effect of TAS1R2 Ile191Val (rs35874116) polymorphism on sugar intake with two different populations including 1,037 individuals without diabetes and 100 individuals with type 2 diabetes. They found a significant relationship between Ile191Val and body mass index (BMI) in terms of sugar consumption in 1,037 individuals without diabetes, and individuals with the Val allele (103±6 g sugar/day) in at least one locus consumed less sugar than individuals with homozygote Ile/Ile genotype (122±6 g sugar/day). A study that investigated the effects of polymorphisms in TAS1R2 receptor (rs9701796, rs35874116) on chocolate powder consumption and dietary fiber intake in obese children revealed that the rs9701796 variant in obese children was associated with high chocolate powder consumption and rs35874116 variant was associated with low dietary fiber intake. They revealed that the daily carbohydrate consumption was 258±36 g for Ile homozygotes and 248±41 g for the Val carriers. Daily sugar intake was determined as 58 g in Ile homozygotes and 51 g in Val carriers. Val allele in the variant rs35874116 was found to be associated with low fiber consumption in children and adolescents with obesity (2). Ramos-Lopez et al. (17), investigated the polymorphism of the TAS1R2 (Ile191Val) gene and revealed that Val/Val carriers consumed

Table 1. Genotype and allele frequency distributions of participants

TAS1R2 rs35874116	n	%
GG genotype	6	6.3
GA genotype	64	67.4
AA genotype	25	26.3
Allel frequency		
G	76	40
A	114	60
Dominant model		
AA + GA vs	89	93.7
GG	6	6.3
Recessive model		
GG + GA vs	70	73.7
AA	25	26.3

Table 2. Amount of nutrients and energy intake of individuals according to the dominant model

	Dominant model	n	Median	p-value		Dominant model	n	Median	p-value
Energy	AA + GA	89	1228.37 (518.08-3734.30)	0.32	Vit E (mg)	AA + GA	89	8.67 (1.5-53.57)	0.963
	GG	6	1374.235 (1125.09-2386.64)			GG	6	8.815 (3.08-19.35)	
Protein (g)	AA + GA	89	49.44 (15.26-153.32)	0.215	Vit B1 (mg)	AA + GA	89	0.67 (0.2-3.02)	0.233
	GG	6	69.81 (31.31-102.40)			GG	6	0.795 (0.5-1.21)	
Protein (%)	AA + GA	89	17 (9-29)	0.89	Vit B2 (mg)	AA + GA	89	1.08 (0.29-2.68)	0.869
	GG	6	16.00 (11-28)			GG	6	1.485 (0.96–2)	
Fat (g)	AA + GA	89	55.82 (13-248.52)	0.375	Vit B6 (mg)	AA + GA	89	1.03 (0.20-3.15)	0.4
	GG	6	67.75 (33.29-116.01)			GG	6	1.09 (0.59-2.85)	
Fat percentage (%)	AA + GA	89	42 (21-75)	0.824	Folate (µg)	AA + GA	89	228.81 (70.6-795.05)	0.963
	GG	6	43 (26-47)			GG	6	176.375 (107.7-480.8)	
CHO (g)	AA + GA	89	119.18 (8.12-289.6)	0.543	Vit C (mg)	AA + GA	89	64.32 (1.11-246.94)	0.624
	GG	6	136.04 (87.86-249.55)			GG	6	68.765 (41.71-329.59)	
CHO (%)	AA + GA	89	41 (6-66)	0.963	Sodium (mg)	AA + GA	89	2339.9 (435.3-53137.75)	0.818
	GG	6	40 (28-57)			GG	6	2878.68 (924.67-5602.04)	
Fiber	AA + GA	89	15.4 (1.88-49.1)	0.951	Potassium (mg)	AA + GA	89	1972.68 (354.95-5101.6)	0.463
	GG	6	13.51 (6.27-29.17)			GG	6	2191.24 (922.7-4188.43)	
Alcohol (g)	AA + GA	89	0.00 (0-52.27)	0.278	Calcium (mg)	AA + GA	89	504.74 (84.2-1255.26)	0.491
	GG	6	0.00 (0-0)			GG	6	595.65 (199.76-991.64)	
Alcohol (%)	AA + GA	89	0.00 (0-19)	0.513	Magnesium (mg)	AA + GA	89	201.86 (50.74-644.6)	0.223
	GG	6	0.00 (0-0)			GG	6	246.265 (129.5-600.25)	
MUFA (g)	AA + GA	89	19.97 (4-92.41)	0.571	Phosphorus (mg)	AA + GA	89	816.1 (267.3-2431.65)	0.284
	GG	6	22.735 (13.45-39.44)			GG	6	1057.15 (530.46-1387.18)	
SFA (g)	AA + GA	89	22.91 (6.39-65.27)	0.641	Iron (mg)	AA + GA	89	7.24 (1.6-20.98)	0.071
	GG	6	25.26 (11.38-59.21)			GG	6	9.47 (5.73-18.6)	
PUFA (g)	AA + GA	89	7.5 (1.45-76.28)	0.482	Zinc (mg)	AA + GA	89	7.6 (1.95-26.24)	0.15
	GG	6	10.69 (4.83-27.43)			GG	6	10.66 (4.86-19.69)	
Cholesterol (mg)	AA + GA	89	232 (9-1079.4)	0.233	Sucrose (g)	AA + GA	89	13.03 (0.41-76.61)	0.32
	GG	6	376.025 (35.8-859.1)						
Carotene (mg)	AA + GA	89	1.93 (0.1-32.16)	0.379		GG	6	23.81 (2.71-84.76)	
	GG	6	1.275 (0.33-12.34)						

Mann-Whitney U test, CHO: Carbohydrate, MUFA: Monounsaturated fatty acids, SFA: Saturated fatty acids, PUFA: Polyunsaturated fatty acids

332.7±102.6 g of carbohydrates, Ile/Ile carriers consumed 273±102.4 g, and Ile/Val carriers consumed 265.2±98.1 g of carbohydrates daily. The difference in carbohydrate intake between genotypes was found to be statistically significant (p=0.01), and Val/Val genotype of TAS1R2 was associated with higher carbohydrate intake. Chamoun et al. (18) investigated the effect of taste genetics on snack consumption habits in preschoolers and revealed that children with the TT genotype TAS1R2 rs35874116 consumed more sugary and high-calorie snacks than children with the C allele. Additionally, children with TT genotypes were significantly more likely to opt for sugary snacks in the evening. Han et al. (16) evaluated the relationship between TAS1R2 rs38574116 polymorphism and carbohydrate intake and found that C alleles (CC and CT) were associated with higher sugary food consumption than TT allele. Contrarily, Hwang et al. (21) found no association between sugar/sweet food intake and TAS1R2 (rs35874116). Our study revealed that daily total carbohydrate and sucrose intakes were the highest in individuals with GG genotype (145.55±56.69 g and 28.66±26 g, respectively) but results were not statistically different between daily carbohydrate and sucrose intake (p>0.05) according to the genotypes of participants (Table 2).

Dias et al. (1) investigated the relationship of TAS1R2 sweet taste receptor polymorphisms (rs12033832, rs12137730, rs35874116, rs3935570, rs4920564, rs4920566, rs7513755, and rs9701796) with sweet taste threshold and sugar intake and revealed that individuals with the GG/GA genotype consumed more sugar compared to individuals with the AA allele in a BMI of ≥25, and individuals with the GG/GA genotype with BMI of <25 consume less sugar compared to individuals with the AA genotype (1). Accordingly, TAS1R2 rs12033832 polymorphism was found to be associated with individuals' sweet taste threshold and sugar consumption, but this relationship differed according to the BMI. Additionally, the association of the polymorphism of rs35874116 with the BMI and the threshold of sweet taste was not found. However, the polymorphism rs35874116 was found to be associated with differences in carbohydrate and sugar intake, regardless of taste perception. This difference was assumed to be related to the expression of TAS1R2 in the small intestine, and this effect can be created by a post-digestive mechanism (1). We also investigated the relationship of sugar and carbohydrate consumption with BMI, thus we divided the participants into two groups, BMI of <25 kg/m^2 and BMI of ≥25 kg/m^2, but no difference was found between the groups' sugar and carbohydrate intake (data not shown).

In the literature, studies on TAS1R2 variations mainly focused on carbohydrate and sweet consumption. However, some studies also provide a perspective on TAS1R2 and other nutrient intakes. Of which, one study that evaluated TAS1R2 (rs7534618) and dietary intake revealed that genetic variation had an association with food intake, including total grain and bread consumption. Additionally, the authors revealed that in the Korean female population, compared to those with the rs7534618 A allele, (AA and AC genotypes), having the CC genotype, which corresponds to the rs12033832 AA genotype, seemed to be associated with decreased carbohydrates but increased fat intake (22). Choi et al. (19) found that TAS1R2 polymorphisms affected cruciferous vegetables, citrus fruit, fatty, and umami food intake. The TAS1R2 rs9701796 variant allele was associated with decreased cruciferous vegetable consumption in males. TAS1R1 rs34160967, diplotype, and TAS1R2 rs35874116 exhibited differential umami foods intake by genotype (19). In the studies that investigated the TAS1R2 rs38574116, some studies also examined the relationship between participants' food consumption records and TAS1R2 rs38574116 genotypes GA (Ile/Val, CT), AA (Ile/Ile, TT), and GG (Val/Val, CC). Ramos-Lopez et al. (17) revealed no difference between the daily average calorie, protein (%), protein (g), fat (%), and fat (g) consumption of individuals with Ile/Ile, Ile/Val, and Val/Val genotypes. Contrarily, daily carbohydrate (g) and fiber (g) consumption of individuals with the Val/Val genotype was significantly higher than Ile/Val and Ile/Ile genotypes (17). Similar to that study, Han et al. (16) found no differences between total energy, carbohydrate (g), protein (g), and fat (g) consumption according to the TT and CC/CT genotypes. Sweet (g) consumption was significantly higher in CC/CT genotype and dietary protein (%) was higher in the TT genotype (16). Our study revealed no significant difference in the participants in terms of energy, protein (g), protein (%), carbohydrate (g), carbohydrate (%), fat (g), fat (%), and sucrose consumption according to their genotypes (Table 3).

CONCLUSION

Elucidating the relationship between individuals' food consumption and genetic basis will contribute to creating individual-specific nutrition programs and maintaining and improving health. We investigated the effect of SNP rs35874116 polymorphism in the sweet taste receptor (*TAS1R2*) gene on the nutrient intake to contribute to the enlightenment of factors food preference and consumption. Contrary to the literature, we found no difference between genotypes and nutrient intake, especially in sweet/sugar

Table 3. Amount of nutrients and energy intake of individuals according to the genotype

	Genotype	n	Median (min-max)	p-value		Genotype	n	Median (min-max)	p-value
Energy	AA	25	1138.52 (562.36-3734.30)	0.533	Alcohol (g)	AA	25	0.00 (0.00-1.20)	0.400
	GA	64	1258.06 (518.08-3273.24)			GA	64	0.00 (0.00-52.27)	
	GG	6	1374.24 (1125.09-2386.64)			GG	6	0.00 (0.00-0.00)	
Protein (g)	AA	25	46.20 (15.26-153.32)	0.269	Alcohol (%)	AA	25	0.00 (0-1)	0.645
	GA	64	51.98 (16.54-112.99)			GA	64	0.00 (0-19)	
	GG	6	69.81 (31.31-102.40)			GG	6	0.00 (0-0)	
Protein (%)	AA	25	17.00 (9-26)	0.515	MUFA (g)	AA	25	19.58 (4.00-92.41)	0.851
	GA	64	17.00 (11-29)			GA	64	20.29 (5.52-75.65)	
	GG	6	16.00 (11-28)			GG	6	22.74 (13.45-39.44)	
Fat (g)	AA	25	52.87 (13-248.52)	0.650	SFA (g)	AA	25	22.57 (6.60-61.26)	0.660
	GA	64	56.54 (18.09-191.30)			GA	64	23.24 (6.39-65.27)	
	GG	6	67.75 (33.29-116.01)			GG	6	25.26 (11.38-59.51)	
Fat percentage (%)	AA	25	41.00 (21-59)	0.948	PUFA (g)	AA	25	9.56 (1.45-76.28)	0.644
	GA	64	42.00 (21-75)			GA	64	7.08 (2.28-60.34)	
	GG	6	43.00 (26-47)			GG	6	10.69 (4.83-27.43)	
CHO (g)	AA	25	118.85 (58.36-221.11)	0.744	Cholesterol (mg)	AA	25	201.80 (9.0-670.35)	0.397
	GA	64	121.28 (8.12-289.60)			GA	64	253.20 (10.98-1079.40)	
	GG	6	136.04 (87.86-249.55)			GG	6	376.02 (35.80-859.10)	
CHO (%)	AA	25	41.00 (24-66)	0.966	Carotene (mg)	AA	25	2.97 (0.50-32.16)	0.083
	GA	64	40.50 (6-60)			GA	64	1.81 (0.10-23.35)	
	GG	6	40.00 (28-57)			GG	6	1.28 (0.33-12.34)	
Fiber	AA	25	14.91 (5.17-42.45)	0.992	Vit E (mg)	AA	25	7.57 (1.85-53.57)	0.935
	GA	64	15.56 (1.88-49.10)			GA	64	8.84 (1.50-50.46)	
	GG	6	13.51 (6.27-29.17)			GG	6	8.82 (3.08-19.35)	
Vit B1 (mg)	AA	25	0.63 (0.29-3.02)	0.484	Calcium (mg)	AA	25	421.45 (84.20-1134.15)	0.321
	GA	64	0.68 (0.20-2.68)			GA	64	557.06 (94.40-1255.26)	
	GG	6	0.78 (0.50-1.21)			GG	6	595.65 (199.76-991.64)	
Vit B2 (mg)	AA	25	1.02 (0.31-1.86)	0.113	Magnesium (mg)	AA	25	179.05 (110.00-644.60)	0.424
	GA	64	1.11 (0.29-2.68)			GA	64	206.88 (50.74-606.85)	
	GG	6	1.48 (0.96-2.00)			GG	6	246.26 (129.50-600.25)	
Vit B6 (mg)	AA	25	1.04 (0.32-3.15)	0.695	Phosphorus (mg)	AA	25	810.89 (290.30-2056.10)	0.361
	GA	64	1.02 (0.2-2.55)			GA	64	891.18 (267.30-2431.65)	
	GG	6	1.09 (0.59-2.85)			GG	6	1057.15 (530.46-1387.18)	

Table 3. Continued

	Genotype	n	Median (min-max)	p-value		Genotype	n	Median (min-max)	p-value
Folate (µg)	AA	25	211.85 (70.60-639.80)	0.836	Iron (mg)	AA	25	7.63 (2.72-16.36)	0.196
	GA	64	243.10 (71.30-795.05)			GA	64	7.22 (1.60-20.98)	
	GG	6	176.38 (107.10-480.80)			GG	6	9.47 (5.73-18.60)	
Vit C (mg)	AA	25	64.58 (12.25-215.66)	0.714	Zinc (mg)	AA	25	7.35 (2.53-20.25)	0.354
	GA	64	63.84 (1.11-246.94)			GA	64	7.61 (1.95-26.24)	
	GG	6	68.76 (41.71-329.59)			GG	6	10.66 (4.86-19.69)	
Sodium (mg)	AA	25	2827.80 (543.30-5744.20)	0.971	Sucrose (g)	AA	25	14.83 (1.06-54.81)	0.601
	GA	64	2292.06 (435.30-53137.75)			GA	64	11.84 (0.41-79.61)	
	GG	6	2878.68 (924.67-5602.04)						
Potassium (mg)	AA	25	1864.90 (897.30-5101.60)	0.763					
	GA	64	40.50 (6-60)			GG	6	23.81 (2.71-84.76)	
	GG	6	40.00 (28-57)						

Kruskal-Wallis test, CHO: Carbohydrate, MUFA: Monounsaturated fatty acids, SFA: Saturated fatty acids, PUFA: Polyunsaturated fatty acids, min: Minimum, max: Maximum, vit: Vitamin

consumption. Considering the interpretation of these results, our sample size was small and food consumption is affected by cultural and individual perceptions. Thus, this study may serve as a preliminary result for our country.

ETHICS

Ethics Committee Approval: Our study was approved by Istanbul Aydın University Non-Interventional Clinical Research Ethics Committee on 09/10/2019 with the decision number 2019/115.

Informed Consent: Volunteers were included in the research by obtaining written and oral consent.

Authorship Contributions

Concept: S.A.Ö., A.S., Design: K.K., S.A.Ö., A.S., Data Collection or Processing: K.K., S.A.Ö., A.S., Analysis or Interpretation: K.K., S.A.Ö., A.S., Literature Search: K.K., S.A.Ö., A.S., Writing: K.K., S.A.Ö., A.S.

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

Financial Disclosure: This research was supported by a grant from the Research Foundation of Istanbul Aydın University, Turkey (project no: 2018/09).

REFERENCES

1. Dias AG, Eny KM, Cockburn M, Chiu W, Nielsen DE, Duizer L, et al. Variation in the TAS1R2 Gene, Sweet Taste Perception and Intake of Sugars. J Nutrigenet Nutrigenomics 2015;8:81-90.
2. Pioltine MB, de Melo ME, Santos AS, Machado AD, Fernandes AE, Fujiwara CT,et al. Genetic Variations in Sweet Taste Receptor Gene Are Related to Chocolate Powder and Dietary Fiber Intake in Obese Children and Adolescents. J Pers Med 2018;8:7.
3. Chamoun E, Mutch DM, Allen-Vercoe E, Buchholz AC, Duncan AM, Spriet LL, et al. A review of the associations between single nucleotide polymorphisms in taste receptors, eating behaviors, and health. Crit Rev Food Sci Nutr 2018;58:194-207.
4. Bachmanov AA, Beauchamp GK. Taste receptor genes. Annu Rev Nutr 2007;27:389-414.
5. Roper SD, Chaudhari N. Taste buds: cells, signals and synapses. Nat Rev Neurosci 2017;18:485-497.
6. Spaggiari G, Di Pizio A, Cozzini P. Sweet, umami and bitter taste receptors: State of the art of in silico molecular modeling approaches. Trends Food Sci Technol 2020;96:21-9.
7. Habberstad C, Drake I, Sonestedt E. Variation in the Sweet Taste Receptor Gene and Dietary Intake in a Swedish Middle-Aged Population. Front Endocrinol (Lausanne) 2017;8:348.
8. Melo SV, Agnes G, Vitolo MR, Mattevi VS, Campagnolo PDB, Almeida S. Evaluation of the association between the TAS1R2 and TAS1R3 variants and food intake and nutritional status in children. Genet Mol Biol 2017;40:415-20.
9. Eny KM, Wolever TM, Corey PN, El-Sohemy A. Genetic variation in TAS1R2 (Ile191Val) is associated with consumption of sugars in overweight and obese individuals in 2 distinct populations. Am J Clin Nutr 2010;92:1501-10.
10. Treesukosol Y, Smith KR, Spector AC. The functional role of the T1R family of receptors in sweet taste and feeding. Physiol Behav 2011;105:14-26.

11. Joseph PV. Sucrose thresholds and genetic polymorphisms of sweet and bitter taste receptor genes in children [dissertation]. University of Pennsylvania; 2015.
12. Reed DR, McDaniel AH. The human sweet tooth. BMC Oral Health 2006;6 Suppl 1:S17.
13. Kim UK, Wooding S, Riaz N, Jorde LB, Drayna D. Variation in the human TAS1R taste receptor genes. Chem Senses 2006;31:599-611.
14. Fushan AA, Simons CT, Slack JP, Drayna D. Association between common variation in genes encoding sweet taste signaling components and human sucrose perception. Chem Senses 2010;35:579-92.
15. Fushan AA, Simons CT, Slack JP, Manichaikul A, Drayna D. Allelic polymorphism within the TAS1R3 promoter is associated with human taste sensitivity to sucrose. Curr Biol 2009;19:1288-93.
16. Han P, Keast RSJ, Roura E. Salivary leptin and TAS1R2/TAS1R3 polymorphisms are related to sweet taste sensitivity and carbohydrate intake from a buffet meal in healthy young adults. Br J Nutr 2017;118:763-70.
17. Ramos-Lopez O, Panduro A, Martinez-Lopez E, Roman S. Sweet Taste Receptor TAS1R2 Polymorphism (Val191Val) Is Associated with a Higher Carbohydrate Intake and Hypertriglyceridemia among the Population of West Mexico. Nutrients 2016;8:101.
18. Chamoun E, Hutchinson JM, Krystia O, Mirotta JA, Mutch DM, Buchholz AC, Duncan AM, Darlington G, Haines J, Ma DWL; Guelph Family Health Study. Single Nucleotide Polymorphisms in Taste Receptor Genes Are Associated with Snacking Patterns of Preschool-Aged Children in the Guelph Family Health Study: A Pilot Study. Nutrients 2018;10:153.
19. Choi JH, Lee J, Choi IJ, Kim YW, Ryu KW, Kim J. Variations in TAS1R taste receptor gene family modify food intake and gastric cancer risk in a Korean population. Mol Nutr Food Res 2016;60:2433-45.
20. Bachmanov AA, Bosak NP, Floriano WB, Inoue M, Li X, Lin C, et al. Genetics of sweet taste preferences. Flavour Fragr J 2011;26:286-94.
21. Hwang LD, Lin C, Gharahkhani P, Cuellar-Partida G, Ong JS, An J, et al. New insight into human sweet taste: a genome-wide association study of the perception and intake of sweet substances. Am J Clin Nutr 2019;109:1724-37.
22. Choi JH. TAS1R2 sweet taste receptor genetic variation and dietary intake in Korean females. Appetite 2021;164:105281.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:121-126
DOI: 10.4274/BMJ.galenos.2022.2021.12-16

Research

The Comparison of TOAST, CCS, and ASCO Etiological Classifications in Ischemic Stroke Patients

İskemik İnme Hastalarında TOAST, CCS ve ASCO Etiyolojik Sınıflamalarının Karşılaştırılması

Anıl Özkaya[1], Hacı Ali Erdoğan[2], İbrahim Acır[2], Murat Çabalar[3], Vildan Yayla[2]

[1]Mardin State Hospital, Clinic of Neurology, Mardin, Turkey
[2]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Neurology, Istanbul, Turkey
[3]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Neurology, Istanbul, Turkey

ABSTRACT

Objective: Classifications of stroke etiology is generally based on lesion pathology and ischemic stroke or hemorrhage were two main groups. Nowadays, most common classification is Trial of Org 10172 in Acute Stroke Treatment (TOAST) which was published in 1993. After the TOAST classification system, Causative Classification of Stroke System (CCS), Atherothrombosis, Small vessel disease, Cardiac causes, and Other uncommon causes (ASCO) classification systems were developed. The purpose of the study is to discuss whether CCS or ASCO systems can be replaced for TOAST classification system which is most commonly used to determine the stroke etiology. And to determine whether the number of patients can be decreased in an unidentified cause of the etiology group according to TOAST by these CCS or ASCO systems.

Methods: Three hundred acute ischemic stroke patients hospitalized at Bakırköy Dr. Sadi Konuk Training and Research Hospital between 01.01.2016 and 30.06.2016 were evaluated retrospectively and they were assessed based on their neurological examination, laboratory findings and sub-types of stroke were identified according to TOAST, CCS, and ASCO etiology classifications. Results were compared based on TOAST, CCS, and ASCO. For comparison, obvious reasons for TOAST, obvious and potential reasons for CCS, and first and second evidence were used.

Results: The mean age of 300 patients (176 male-124 female) was 67.13±14.19 years. No significant differences were detected between TOAST, CCS and ASCO systems for detecting etiological subtypes; large artery atherosclerosis, small artery disease, other determined causes or undetermined causes but significant differences were found between ASCO and CCS in determining cardioembolic subtype (p=0.002). Correlation analysis showed high and significant correlations between TOAST and CCS (r=0.765) and TOAST and ASCO (r=0.731). The correlation between CCS and ASCO (r=0.928).

Conclusion: The traditional TOAST classification system, in use for a long time in determining stroke etiology, cannot be updated but it still maintains its practicality and availability compared to the new classification systems CCS and ASCO. There was no significant decrease in the number of patients in an undetermined etiology subgroup by CCS and ASCO compared to TOAST. As far as our results are concerned, CCS and ASCO systems have not superiority on TOAST.

Keywords: Ischemic stroke, etiological classification, TOAST

ÖZ

Amaç: İnme etiyolojisine yönelik sınıflamalar, genellikle lezyonun patolojisine göre yapılmış ve tüm inmeler iskemi veya hemoraji olmak üzere iki ana gruba ayrılmıştır. Günümüzde en yaygın kullanılan sınıflama 1993 yılında yayınlanan Akut İnme Tedavisinde Organizasyon Çalışması 10172 (TOAST) sınıflamasıdır. TOAST sınıflamasından hareketle daha sonraki dönemde İnme Sisteminin Nedensel Sınıflandırması (CCS), Aterotromboz, Küçük damar hastalığı, Kardiyak sebepler ve Diğer nadir sebepler (ASCO) sistemleri geliştirilmiştir. Bu çalışmanın amacı, inmenin etiyolojik nedenini belirlerken yaygın olarak kullanılan TOAST sınıflama sistemi yerini daha sonradan yapılan CCS veya ASCO sistemlerinin alıp alamayacağını göstermek ve TOAST'nin zayıf yönü olan nedeni belirlenemeyen etiyoloji grubundaki hasta sıklığını azaltıp azaltmadığını belirlemektir.

Gereç ve Yöntem: Akut inme tanısı ile Bakırköy Dr Sadi Konuk Eğitim ve Araştırma Hastanesi'ne 01.01.2016-30.06.2016 tarihleri arasında yatışı yapılan 300 hasta epikrizleri ve tetkikleriyle birlikte değerlendirildi ve TOAST, CCS ve ASCO etiyolojik sınıflamalarına göre gruplandırılmış, inme

Address for Correspondence: İbrahim Acır, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Neurology, Istanbul, Turkey
Phone: +90 544 449 86 78 E-mail: xiacir33@gmail.com ORCID ID: orcid.org/0000-0002-9650-8022

Cite as: Özkaya A, Erdoğan HA, Acır İ, Çabalar M, Yayla V. The Comparison of TOAST, CCS, and ASCO Etiological Classifications in Ischemic Stroke Patients. Med J Bakirkoy 2022;18:121-126

Received: 20.12.2021
Accepted: 19.01.2022

alt tipleri belirlenmiş ve çıkan sonuçlar birbirleri ile karşılaştırılmıştır. Karşılaştırmalar için TOAST sınıflamasında bariz nedenler, CCS sınıflamasında bariz ve olası nedenler, ASCO sınıflaması için birinci ve ikinci derecede kanıtlar alındı.

Bulgular: Değerlendirmeye alınan 300 hastanın (176 erkek, 124 kadın) yaş ortalaması 67,13±14,19 yıl idi. İnme etiyolojik alt tiplerinden büyük arter aterosklerozu, küçük damar hastalığı, diğer etiyolojiler ve nedeni belirlenemeyen etiyolojileri belirlemede TOAST, CCS ve ASCO arasında anlamlı farklılık saptanmadı. Kardiyoembolik alt sınıfı belirlenmesinde ASCO ve CCS arasında anlamlı farklılık mevcuttu (p=0,002). Korelasyon analizleri ile TOAST ile CCS arasında ileri düzeyde (r=0,765), TOAST ile ASCO arasında ileri düzeyde uyumluluk (r=0,731) gözlenirken CCS ve ASCO arasındaki ilişki mükemmele yakın düzeyde anlamlı idi (r=0,928).

Sonuç: İnme etiyolojisini belirlemede uzun süreden beri kullanılan geleneksel TOAST sınıflama sistemi, daha yeni sistemler olan CCS ve ASCO ile karşılaştırıldığında güncelleme imkanı olmadığı halde pratikliğini ve kullanılabilirliğini koruduğu düşünüldü. TOAST ile nedeni belirlenemeyen etiyoloji alt grubundaki hasta sayısında CCS ve ASCO ile anlamlı bir azalma gözlenmedi. Sonuçlarımıza bakıldığında CCS ve ASCO sistemi TOAST'ye göre bir üstünlük getirememiştir.

Anahtar Kelimeler: İskemik inme, etiyolojik sınıflama, TOAST

INTRODUCTION

One of the traditional and most frequently used methods for stroke etiological classification, Trial of ORG 10172 in Acute Stroke Treatment (TOAST) sub-divides clinical data of stroke according to the formation mechanisms. In this classification, because it is impossible to determine which group is appropriate for patients who have more than one reason for stroke, many patients are evaluated within the undetermined group. The Causative Classification of Stroke (CCS), a web-based system organized by Harvard University, was created to reduce the limitations of the traditional TOAST system and reduce the unclassified group rate (1,2). It also creates a common language among physicians while determining the etiology and has a high level of compatibility among practitioners. Atherothrombosis, Small vessel disease, Cardiac causes, and Other uncommon causes (ASCO) classification is a phenotypic system that classifies stroke patients according to the composition of their etiological characteristics. Also, the ASCO system lists all etiologies that may be responsible for stroke pathophysiology (1-4). This study aims to compare the subtypes of these three classifications with each other, to prevent the inclusion of many patients to the undetermined group and to investigate whether the number of patients in this group will be affected.

METHODS

In our study, patients who were followed up at Bakırköy Dr. Sadi Konuk Training and Research Hospital Neurology Clinic between January 1, 2016 and June 30, 2016 with the diagnosis of ischemic stroke were included. Anamnesis, histories, physical and neurological examination findings, imaging methods, and all other examinations performed for etiology were analyzed for all 300 patients. All patients participated in the study and their legal heirs were informed and consent were obtained. Exclusion criteria were; hemorrhagic stroke, patients under 18 years, and the doubt in the diagnosis of ischemic stroke. This study, with protocol number 2017/144, was approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee decision no 2017-06-32 (date: 19.06.2017).

Routine biochemical tests, hemogram, blood sedimentation rate and C-reactive protein, brain computed tomography and diffusion magnetic resonance (MR) imaging, electrocardiogram (ECG), transthoracic echocardiography (TTE), bilateral carotid-vertebral artery Doppler ultrasonography, cranial-cervical MR and MR angiographies have been selected for almost all patients. Transesophageal echocardiography (TEE), 24-hour rhythm holter examination, vasculitis tests, thrombophilia panel were performed on patients who needed advanced diagnosis. It was determined which subtypes of TOAST, CCS and ASCO etiological classifications are suitable for each patient. Obvious reasons for TOAST classification, obvious and possible reasons for CCS classification, first and second-degree evidence for ASCO classification were obtained for comparing classifications to each other. If any patient had two first-degree evidence in the ASCO classification, these patients were included in the group whose etiology could not be determined. If any patient had two or more definite etiologies according to the TOAST classification, these patients were also included in the group whose cause was not found. There are 4 subtypes normally defined in the ASCO classification, and the condition defined as ASCO-0 for cases in which the etiological cause cannot be determined. In our study, ASCO-0 was taken as the 5th subtype for comparison with TOAST and CCS.

Statistical Analysis

Frequency and percentage were calculated for qualitative variables, and mean and standard deviation were calculated for quantitative variables. The Marginal Homogeneity test was used to compare the TOAST, CCS, and ASCO classification methods for classifying possible etiology subgroups of stroke. Bonferroni Corrected McNemar test was used for pairwise comparisons between methods. Spearman correlation analysis was performed for the correlation and Cramer's V correlation coefficient (r) was calculated.

RESULTS

A total of 300 patients were included in the study and 124 patients were women (41.33%) and 176 patients (58.67%) were men. The mean age of the patients was found as 67.13±14.19 years. Hypertension in 191 (63.6%) of 300 patients, diabetes mellitus in 110 (36.6%), atrial fibrillation (AF) in 32 (10.6%), a history of stroke or transient ischemic attack (TIA) in 48 (16%), hyperlipidemia in 63 (21%), a history of coronary artery disease, metallic heart valve or heart failure in 89 (29.6%), a smoking history in 81 (27%), and other risk factors in 35 (11%, 6) were available. After imaging methods, 5 (1.6%) of 300 patients had the anterior cerebral artery area infarcts, 112 (37.3%) patients had the middle cerebral artery area infarcts, 21 (7%) patients had the posterior cerebral artery area infarcts, 68 (22.6%) patients had the vertebrobasilar area infarcts. And 30 (10%) patients had lacunar infarcts, 39 (13%) patients had multiple areas infarcts, and 17 (5.6%) patients had border zone infarcts. The TIA clinic was present in 8 patients, and no infarct area was observed in neuroimaging.

TOAST system was used when evaluating cardioembolic risk factors. Eighty nine patients with isolated AF, 22 patients with other major cardiac risks (dilated cardiomyopathy, atrial thrombus, mechanical heart valve, infective endocarditis, mitral stenosis, etc.) and 54 patients with minor cardiac risks (patent foramen ovale, mitral valve prolapse, hypokinetic left ventricular segment, bioprosthetic heart valve, etc.) was detected.

There was no significant difference between TOAST and CCS methods in determining the etiological subgroups of ischemic stroke (p=0.115) (Table 1).

No significant difference was found between TOAST and ASCO methods in determining the etiological subgroups of ischemic stroke (p=0.803) (Table 2).

There was a significant difference between CCS and ASCO methods in determining the etiological subgroups of ischemic stroke (p=0.033) (Table 3).

A significant difference was found between CCS and ASCO classification methods for cardioembolic etiologic causes of ischemic stroke (p=0.002) (Table 4).

One patient (2.8%) who was included in the large artery atherosclerosis subgroup in ASCO classification was included in the group of undetermined etiology in the CCS classification. Ten patients (9.71%) who was included in the cardioembolism subgroup in ASCO classification was included in the group of undetermined etiology in the CCS classification. Five patients (4.5%) were in the undetermined etiology subgroup according to ASCO classification were included in the small vessel disease group in the CCS classification (Table 5).

DISCUSSION

TOAST classification system is the most widely used system in clinics thanks to its practicality, on the other hand, its important disadvantage is that includes patients with more than one etiology into the undetermined group. Nowadays with current technology, more than one possible etiology in the stroke mechanism can be determined, so approximately half of the stroke patients are included in the undetermined etiology group. Another disadvantage of this classification is, when a positive etiology is found, the TOAST etiological subclass of the patient is determined, other investigations are not performed so maybe a possible second cause is missed. Here, the etiological subtypes in the TOAST system are determined at a low-reliability level (1,2).

The web-based CCS system, which is one of the modern classification systems, has been developed to reduce the

Table 1. Comparison of TOAST and CCS etiological classification systems

	TOAST		CCS		
	n	%	n	%	p*
1	54	18	47	15.67	0.115
2	99	33	93	31	-
3	23	7.67	31	10.33	-
4	11	3.67	12	4	-
5	113	37.67	117	39	-

*Marginal homogeneity test, TOAST: Trial of Org 10172 in Acute Stroke Treatment, CCS: Causative Classification of Stroke System, 1- Large artery atherosclerosis, 2- Cardioembolism, 3- Small vessel disease, 4- Other etiology, 5- Undetermined etiology

Table 2. Comparison of TOAST and ASCO etiological classification systems

	TOAST		ASCO		
	n	%	n	%	p*
1	54	18	48	16	0.803
2	99	33	103	34.33	-
3	23	7.67	26	8.67	-
4	11	3.67	12	4	-
5	113	37.67	111	37	-

*Marginal homogeneity test, TOAST: Trial of Org 10172 in Acute Stroke Treatment, ASCO: Atherothrombosis, Small vessel disease, Cardiac causes, and Other uncommon causes, 1- Large artery atherosclerosis, 2- Cardioembolism, 3- Small vessel disease, 4- Other etiology, 5- Undetermined etiology

limitations of the TOAST system and to reduce the rate of undetermined groups. Reliability studies conducted by Ay et al. (2,3) and by Arsava et al. (4) showed that especially the CCS classification was used safely in stroke patients.

The ASCO classification makes a phenotypic classification by grading all causes of stroke, and thus all etiological causes are classified according to the degree of evidence. The reliability of the ASCO system has not been well-known yet (5).

The fact that TOAST and CCS systems depend on the reason but the ASCO system is a phenotypic system creates difficulties in terms of comparison with each other. To make a meaningful comparison, 'obvious and clear causes' etiologies in CCS (possible etiologies were not taken), first and second-degree evidence in ASCO were taken (3rd-degree evidences were not obtained). Similarly, the inclusion criteria were limited in previous studies, which makes it possible to compare the classifications with each other (6,7).

In our study, there was no significant difference between subtypes of CCS and TOAST such as large artery atherosclerosis (p=0.189), cardioembolism (p=0.146), small vessel disease (p=0.02), other etiologies (p=1.00) and undetermined etiologies (p=0.572). The fact that being no significant difference between undetermined etiologies contradicts the purpose of the CCS system. Because the CCS classification has been created with the aim of reducing the undetermined etiology of the TOAST system. Our study showed that instead of the TOAST system, also the CCS system can be applied in daily practice. Lanfranconi and Markus (8), by comparing TOAST and CCS systems in 2012, found both classification systems in high compatible with each other, as we also found.

When comparing TOAST and ASCO etiological classifications, no significant difference was found between subgroups. The 103 patients (34.33%) in the study were included in the cardioembolic subgroup in the ASCO classification, and this rate is slightly above the rate of cardioembolism etiology (20%-30%) mentioned in the literature (9). The reason for this is the higher number of coronary artery diseases in our patient population with

Table 3. Comparison of CCS and ASCO etiological classification systems

	CCS		ASCO		
	n	%	n	%	p*
1	47	15.7	48	16	0.033
2	93	31	103	34.3	-
3	31	10.3	26	8.67	-
4	12	4	12	4	-
5	117	39	111	37	-

*Marginal homogeneity test, CCS: Causative Classification of Stroke System, ASCO: Atherothrombosis, Small vessel disease, Cardiac causes, and Other uncommon causes, 1- Large artery atherosclerosis, 2- Cardioembolism, 3- Small vessel disease, 4- Other etiology, 5- Undetermined etiology

Table 4. Binary comparisons between CCS and ASCO classifications

	CCS		ASCO			
	n	%	n	%	% Dif.	p*
1	47	15.7	48	16	0.33	1
2	93	31	103	34.3	3.33	**0**
3	31	10.3	26	8.67	-1.66	0.06
4	12	4	12	4	0	1
5	117	39	111	37	-2	0.21

*McNemar test with Bonferroni Correction, CCS: Causative Classification of Stroke System, ASCO: Atherothrombosis, Small vessel disease, Cardiac causes, and Other uncommon causes, Dif.: Difference, 1- Large artery atherosclerosis, 2- Cardioembolism, 3- Small vessel disease, 4- Other etiology, 5- Undetermined etiology

Table 5. Cross comparison of CCS and ASCO

		ASCO									
		1		2		3		4		5	
		n	%	n	%	n	%	n	%	n	%
CCS	1	47	97.92	0	0	0	0	0	0	0	0
	2	0	0	93	90.29	0	0	0	0	0	0
	3	0	0	0	0	26	100	0	0	**5**	**4.5**
	4	0	0	0	0	0	0	12	100	0	0
	5	**1**	**2.08**	**10**	**9.71**	0	0	0	0	106	95.5

CCS: Causative Classification of Stroke System, ASCO: Atherothrombosis, Small vessel disease, Cardiac causes, and Other uncommon causes, 1- Large artery atherosclerosis, 2- Cardioembolism, 3- Small vessel disease, 4- Other etiology, 5- Undetermined etiology

a mean age of 67.13±14.19 years. As a result, ejection fractions were below 35% in TTE and ASCO classification have included this in the cardioembolism group as first-degree evidence. Montero et al. (10), in their recent study, emphasized that the rate of undetermined stroke decreased and the frequency of cardioembolism increased in ASCO classification compared to TOAST, and this is also supporting our study.

A significant difference was found between CCS and ASCO etiological classifications in terms of the cardioembolic subgroup (p=0.002). ASCO evaluates "ejection fraction below 35%" as first-degree evidence, "having apical akinesia in the left ventricle despite ejection fraction above 35%" as second-degree evidence, "having a history of myocardial infarction and multiple infarcts on both anterior and posterior systems" as another evidence. All these expanded criteria may have led to this significant difference. These criteria are not categorized as 'obvious or clear' according to CCS but are categorized as 'possible'. As stated before in our study, the first and second-degree evidence levels of ASCO and the obvious and clear degrees of CCS were accepted as etiological reasons for each group.

Cardioembolic etiology was found in 99 patients (33.00%) according to TOAST, 93 patients (31.00%) according to CCS and 103 patients (34.33%) according to ASCO among 300 patients who were evaluated. The reason for the higher rate of cardioembolic etiology compared to the literature may be the increased tests performed for cardiac examination thus more cardiac reasons may be determined. For example, 24-hour rhythm holter examination and transesophageal echocardiography are now more frequently performed in patients who were evaluated only with ECG and TTE in the past. In our study, paroxysmal AF was detected in 12 patients with the 24-hour rhythm holter and atrial thrombus was detected in 2 patients with TEE, and they were included in the cardioembolic subgroup instead of the undetermined group.

Twenty three cases (7.67%) according to TOAST, 31 cases (10.33%) according to CCS, 26 cases (8.67%) according to ASCO were classified as small vessel disease, and a significant correlation between them was detected. The presence of hypertension in patients with infarction of deep branch arteries, is a criterion of small artery disease according to TOAST. In our study, lacunar or deep branch infarcts were found in some patients without hypertension. The lower number of patients in TOAST can be explained by this.

To the undetermined etiology group, 113 patients (37.67%) according to TOAST, 117 patients (39.00%) according to CCS, 111 patients (37.00%) according to ASCO were included, and no significant difference was observed between them in paired comparisons. In our study, among the etiological subtypes, 'undetermined etiology group' was the group with the most patients. It was thought that the reason for this might be that the etiological examinations were normal at approximately 25%, two or more etiologies were found that could cause stroke in 6%-7% of the patients, and some patients were transferred to the intensive care unit or lost during follow-up.

CONCLUSION

Compared to the newer systems CCS and ASCO, the TOAST classification system maintains its practicality and usability. According to the statistical results, CCS and ASCO systems could not provide any innovation according to TOAST. Considering the importance of the stroke etiology subtype in the clinical follow-up and in determining the treatment strategy, maybe different classifications will be needed.

ETHICS

Ethics Committee Approval: This study, with protocol number 2017/144, was approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee decision no 2017-06-32 (date: 19.06.2017).

Informed Consent: All patients participated in the study and their legal heirs were informed and consent were obtained.

Authorship Contributions

Surgical and Medical Practices: A.Ö., Concept: V.Y., Design: V.Y., M.Ç., Data Collection or Processing: A.Ö., H.A.E., Analysis or Interpretation: H.A.E., İ.A., Literature Search: M.Ç., İ.A., Writing: A.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. Ay H. Etiologic Classification in Ischemic Stroke. Turk J Neurol 2011;17:1-6.
2. Ay H, Benner T, Arsava EM, Furie KL, Singhal AB, Jensen MB, et al. A computerized algorithm for etiologic classification of ischemic stroke: the Causative Classification of Stroke System. Stroke 2007;38:2979-84.
3. Ay H, Furie KL, Singhal A, Smith WS, Sorensen AG, Koroshetz WJ. An evidence-based causative classification system for acute ischemic stroke. Ann Neurol 2005;58:688-97.
4. Arsava EM, Ballabio E, Benner T, Cole JW, Delgado-Martinez MP, Dichgans M, et al. The Causative Classification of Stroke system:

an international reliability and optimization study. Neurology 2010;75:1277-84.

5. Saatci I. İnmede görüntüleme yöntemleri. Balkan S. (Editör) Serebrovasküler hastalıklar'da. Ankara: Güneş Kitabevi; 2005. p. 207-42.
6. Marnane M, Duggan CA, Sheehan OC, Merwick A, Hannon N, Curtin D, et al. Stroke subtype classification to mechanism-specific and undetermined categories by TOAST, A-S-C-O, and causative classification system: direct comparison in the North Dublin population stroke study. Stroke 2010;41:1579-86.
7. Desai JA, Abuzinadah AR, Imoukhuede O, Bernbaum ML, Modi J, Demchuk AM, et al. Etiologic classification of TIA and minor stroke by A-S-C-O and causative classification system as compared to TOAST reduces the proportion of patients categorized as cause undetermined. Cerebrovasc Dis 2014;38:121-6.
8. Lanfranconi S, Markus HS. Stroke subtyping for genetic association studies? A comparison of the CCS and TOAST classifications. Int J Stroke 2013;8:626-31.
9. Özeren A, Kutluk K (ed). Klinik yaklaşım ve sınıflama. İskemikinme. Nobel Kitabevi: 2004. p. 61-73.
10. Montero MV, Pastor AG, Cano BC, García PS, Mohedano AI, Otero FD, et al. The A-S-C-O classification identifies cardioembolic phenotypes in a high proportion of embolic stroke of undetermined source (ESUS). J Neurol Sci 2016;367:32-3

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:127-134
DOI: 10.4274/BMJ.galenos.2022.2021.12-17

Research

The Role of Neutrophil Gelatinase-associated Lipocalin as a Predictive Biomarker of Acute Kidney Injury in Patients Undergoing Major Abdominal Surgery

Majör Abdominal Cerrahi Geçiren Hastalarda Akut Böbrek Hasarının Prediktif Biyobelirteci Olan Nötrofil Gelatinaz İlişkili Lipokalinin Rolü

Aynur Mustafayeva, Meliha Orhon Ergün, Seniyye Ülgen Zengin

Marmara University Faculty of Medicine, Department of Anesthesiology and Reanimation, Istanbul, Turkey

ABSTRACT

Objective: The current study investigates the development of acute kidney injury (AKI) in patients undergoing major abdominal surgery via earlier determination of the rise in neutrophil gelatinase-associated lipocalin (pNGAL) compared to that in creatinine.

Methods: In this prospective observational study, 60 patients aged between 18 and 65 undergoing major abdominal surgery were selected for the investigation after obtaining ethics committee approval. Patients who did not meet the age criteria, had liver and kidney failures, severe cardiac and respiratory distress, used angiotensin-converting enzyme inhibitors and nonsteroidal anti-inflammatory drugs, developed postoperative respiratory failure, cardiogenic and septic shock, relapse in the 1st 24 h, and those who underwent emergency surgery were excluded from the study. Leukocyte and creatinine values (preoperatively and at 24 and 48 h postoperatively), pNGAL (at 0 h intraoperatively and 6 h and 24 h postoperatively), lactate values (at 0 h intraoperatively and 24 h and 48 h postoperatively), and urine output value (at 24 and 48 h postoperatively) were evaluated.

Results: Of the 60 patients, 9 (15%) showed increased pNGAL values at 6 h postoperatively; however, increased creatinine values and decreased urine output values were observed at 48 h postoperatively. Thus, AKI development was detected at an early stage. The change in leukocyte and lactate values was not statistically significant.

Conclusion: pNGAL was proven to be an early predictive biomarker of AKI in patients undergoing major abdominal surgery.

Keywords: Major abdominal surgery, acute kidney injury, plasma NGAL (neutrophil gelatinase-associated lipocalin), creatinine

ÖZ

Amaç: Çalışmanın amacı majör abdominal cerrahi geçiren 60 hastada plazma nötrofil jelatinaz ilişkili lipokalin (pNGAL) değerinin kreatinin değerinden daha erken dönemde akut böbrek hasarının (ABH) geliştiğini belirlemesini araştırmaktır.

Gereç ve Yöntem: Etik kurul onayı alınan, 18-65 yaş arası, Amerikan Anesteziyoloji Derneği I-III, majör abdominal cerrahi geçiren 60 hasta seçildi. Preoperatif, postoperatif 24. ve 48. saatte lökosit, kreatinin; intraoperatif 0. saat, postoperatif 6. ve 24. saatte pNGAL; intraoperatif 0. saat, postoperatif 24. ve 48. saatte laktat değerlerine bakıldı, postoperatif 24. ve 48. saatlerde saatlik idrar çıkışları değerlendirildi.

Bulgular: Altmış hastadan 9'unda (%15) plasma NGAL seviyesi postoperatif 6. saatte yükselmiş olsada, kreatinin değerleri postoperatif 48. saatte artmış oldu, postoperatif saatlik idrar çıkışları 0,5 cc/kg altına düşmüş oldu. Böylece, gelişen ABH erken dönemde tespit edilmiş oldu. Lökosit ve laktat değerlerindeki değişim istatistiksel olarak anlamlı bulunmadı.

Sonuç: Majör abdominal cerrahi geçiren hastalarda plasma NGAL'nin akut böbrek hasarının en erken prediktif biyobelirteci olduğu kanıtlanmıştır. Postoperatif 2 aylık takip süresinde hastalarda renal replasman terapisi veya ölüm insidansı saptanmamıştır.

Anahtar Kelimeler: Majör abdominal cerrahi, akut böbrek hasarı, plazma NGAL (nötrofil getalinaz ilişkili lipokalin), kreatinin

Address for Correspondence: Seniyye Ülgen Zengin, Marmara University Faculty of Medicine, Department of Anesthesiology and Reanimation, Istanbul, Turkey
Phone: +90 505 714 24 43 E-mail: ulgen_t@yahoo.com ORCID ID: orcid.org/0000-0003-1795-6597

Cite as: Mustafayeva A, Orhon Ergün M, Ülgen Zengin S. The Role of Neutrophil Gelatinase-associated Lipocalin as a Predictive Biomarker of Acute Kidney Injury in Patients Undergoing Major Abdominal Surgery. Med J Bakirkoy 2022;18:127-134

Received: 20.12.2021
Accepted: 28.01.2022

INTRODUCTION

Major abdominal surgery is a procedure performed via the intraperitoneal approach under general anesthesia (1). The pathogenesis of postoperative acute kidney injury (AKI) after major abdominal surgery is complex and differs from that observed after cardiac or vascular surgery. It involves fluid loss, neuroendocrine response to general anesthesia and surgery itself, injury-induced inflammation, urinary tract obstruction, and intra-abdominal pressure (2).

Surgery is the leading cause of AKI in hospitalized patients and may account for 40% of AKI cases (3). AKI is most common after cardiac surgery, followed by general and thoracic surgeries (4). The incidence of AKI in patients undergoing major abdominal surgery may reach 35% (4).

AKI is defined as a rapid deterioration of kidney function, with an increase in serum creatinine or a decrease in the urine output (5). AKI is common in hospitalized patients and has a significant impact on in-hospital mortality, length of hospital stay, healthcare costs, chronic kidney disease (CKD) progression, and increased risk of cardiovascular disease (6,7). Recently, although there has been a decrease in mortality rates, mortality increases as the severity of AKI increases, and it can reach up to 60% in critically ill patients (8).

The most common cause of AKI is sepsis, whereas the second most common cause is surgery (9). Chronic diseases, such as CKD, diabetes mellitus, and chronic obstructive pulmonary disease are associated with the development of AKI (10).

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein that is synthesized in the bone marrow during granulocyte maturation (9). NGAL is also an early indicator of tubular damage (11). This study aimed to detect AKI in the early stage of its development by evaluating NGAL and show the potential of NGAL as an early biomarker of AKI in patients undergoing major abdominal surgery.

The incidence of AKI varies between 1% and 7% in all hospitalized patients and 30% and 50% in intensive care patients. Especially in intensive care patients, the mortality rate is very high (up to 50%). Moreover, 20%-50% of surviving patients with AKI may subsequently develop CKD, 5% may develop end-stage renal disease, and finally may develop the need for renal replacement therapy (RRT) (12).

In patients with impaired renal autoregulation, an intraoperative mean arterial pressure of 60 mmHg may lead to decreased renal perfusion and AKI development (13). Intraoperative hypotension lasting for more than 1 min significantly increases the risk of postoperative AKI (13).

NGAL, also known as lipocalin-2, siderocalin, oncogeneprotein 24p33, uterocalin, and alpha-2 microglobulin-associated protein, is a 25-kD protein comprising 178 amino acids. It was first identified as a protein covalently bound to the gelatinase of neutrophils (14). NGAL is expressed in insignificant amounts in many human tissues, including the kidney tissue, but increases markedly because of epithelial damage (15). In the body, NGAL mRNA is normally found in many tissues such as those of the bone marrow, uterus, prostate, salivary gland, stomach, colon, trachea, lung, liver, and kidney (16).

NGAL can also be markedly increased in most types of cancer (28) and has bacteriostatic properties (17).

METHODS

After obtaining the approval of the ethics committee (Marmara University Faculty of Medicine Clinical Research Ethics Committee- protocol code: 09.2021.74, date: 08.01.2021) and obtaining written consent from the patients our study included 60 patients [American Society of Anesthesiology (ASA) status I-III] aged between 18 and 65 who underwent major abdominal surgery under general anesthesia.

Patients having an age beyond the specified age range, liver and kidney failures, severe cardiac and respiratory distress, using angiotensin-converting enzyme inhibitors and nonsteroidal anti-inflammatory drugs, those who developed postoperative respiratory failure, cardiogenic and septic shock, and relapsed in the 1st 24 h were excluded from the study.

All the patients included in our study underwent the same anesthesia induction procedure (2 mg/kg propofol/1-2 mcg/kg remifentanil/0.6 mg/kg rocuronium bromide). Additionally, desflurane inhalation and intravenous remifentanil infusion were used for anesthesia maintenance. Maintenance fluid was administered to the patients intravenously at a rate of 10-12 mLl/kg/h without fluid restriction.

Creatinine, lactate, and leukocyte values were measured preoperatively and postoperatively at 24 and 48 h using the blood samples of the patients. For measuring NGAL values, approximately 3 mL of venous blood sample was collected preoperatively at 0 h and postoperatively at 6 and 24 h into an ethylenediaminetetraacetic acid-containing tube and centrifuged for 15 min at 3000 rpm in a centrifuge (Nüve NF800, REF: Z10.NF 800). The plasma portion of the

centrifuged blood was placed in an Eppendorf tube and stored in a -20 °C freezer until plasma NGAL value analysis was conducted.

The hourly urine output was measured at 24 and 48 h postoperatively, and the glomerular filtration rate (GFR) and creatinine clearance (CrCl) value of the patients were calculated.

Patients' age, gender, body weight, ASA status, lactate, creatinine, leukocyte, NGAL, Kidney Disease Improving Global Outcomes (KDIGO) stages and postoperative hourly urine output were recorded in the patient evaluation form.

Statistical Analysis

Minimum sample size was calculated for obtaining the area under the receiver operating characteristic (ROC) curve. While an area under the ROC curve for a variable at the value of 0.500 indicates that the variable has no discriminative power, a value of ≥0.800 indicates excellent discrimination. In this context, it is assumed that the minimum area value required for statistical significance is 0.800. For this calculation, the ratio of subjects developing/not developing an incident is also important. Based on the study conducted by Shavit et al. (18), this ratio was accepted as 10/64. On the basis of these values, it was calculated that at the value of α=0.05, the minimum sample size required to reach 80% power should be 45.

RESULTS

Baseline and Intraoperative Characteristics

Table 1 shows the patients mean age, weight and gender. The increase in the urine output observed at 48 h postoperatively was statistically significant compared to that of the subjects at 24 h postoperatively.

Table 2 shows that the change in the creatinine values of the subjects was statistically significant (p=0.001). Because of the evaluations, the decrease observed in the values at 24 h postoperatively compared to those observed preoperatively and the increase in the values at 48 h postoperatively compared to those at 24 h postoperatively were found to be statistically significant (p<0.001, p=0.033, respectively).

The change observed in the GFR values of the patients over time was statistically significant (p=0.001). The increase observed in the values at 24 h postoperatively compared to those observed preoperatively and the decrease in the values at 48 h postoperatively compared to those at 24 h postoperatively were found to be statistically significant (p<0.001, p=0.036, respectively).

The change observed in the CrCl values of the subjects was found to be statistically significant (p=0.001). The increase in the values observed at 24 h postoperatively compared to those observed preoperatively was statistically significant (p<0.001, p<0.001, respectively).

The change observed in the lactate values of the subjects was found to be statistically significant (p=0.003). The analyses showed that the increase observed in the values at 24 and 48 h postoperatively values compared to those at 0 h intraoperatively was statistically significant (p=0.002, p=0.020, respectively).

Even though we had only one group in our study, we decided conditionally to divide the patients into two groups according to pNGAL value. Table 3 shows that the subjects with an NGAL value <90 ng/mL at postoperative 6 h and 24 h were found to have normal creatinine values. All the subjects with an NGAL value >90 ng/mL at postoperative 6 h and 24 h were classified as AKI class I and II based on their creatinine values. Patients with NGAL values >90 ng/mL had a higher tendency to be included in AKI class I and II based on their creatinine values (p<0.001).

Furthermore, 3.9% of the subjects with an NGAL value <90 ng/mL had hourly urine output values <0.5 cc/kg/h, whereas all cases with an NGAL value >90 ng/mL had hourly urine output values <0.5 cc/kg/h. It was found that the percentage of subjects with hourly urine output values <0.5 cc/kg/h was higher than that of those with NGAL values >90 ng/mL (p<0.001). In those with NGAL values < 90 ng/mL, the increase in the values observed at 48 h postoperatively compared to those at 24 h postoperatively was statistically significant (p=0.010). In those with NGAL values >90 ng/mL, the change observed in the urine output values at 48 h postoperatively compared to that at 24 h postoperatively was not statistically significant (p>0.05).

Table 1. Data on identifying features

(n=60)	Minimim-maximum	Mean ± SD
Age (years)	20-65	50.77±12.44
Weight (kg)	43-90	68.25±9.70
	n	**%**
Gender		
Female (number)	24	40
Male (number)	36	60
ASA (number =60)		
I	7	11.6
II	42	70
III	11	18.4

ASA: American Society of Anesthesiologists, SD: Standard deviation

Table 2. Data on laboratory results

	WBC ($x10^3/\mu L$)	CRE (mg/dL)	GFR (mL/minute/1.73 m^2)	CrCl (mL/minute)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Preoperatively	7.54±3.59	0.75±0.21	102.15±20.77	112.47±35.88
Postoperative 24	10.5±4	0.68±0.23	107.32±21.19	124.97±38.24
Postoperative 48	9.68±3.58	0.75±0.32	101.8±25.62	117.98±40.08
Test value, p	**F=14.883, p<0.001**	**F=7.831, p=0.001**	**F=7.510, p=0.001**	**F=6.779, p=0.001**

WBC: White blood cell, CRE: Creatinine, GFR: Glomerular filtration rate, CrCl: Creatinine clearance, SD: Standard deviation

Table 3. Relationship between NGAL and creatinine values and hourly urine output

	NGAL <90 ng/mL	NGAL> 90 ng/mL	Test value, p
	n (%)	n (%)	
Creatinine			**χ^2=52.941, p<0.001**
Normal	51 (100)	0	
AKI I & II	0	9 (100)	
Hourly urine output (cc/kg/h)			**χ^2=47.166, p<0.001**
Postop 24 h	0.74±0.35	0.62±0.06	**t=1.011, p=0.314**
Postop 48 h	0.9±0.46	0.48±0.01	**t=2.713, p=0.008**
Normal	49 (96.1)	0	
<0.5 cc/kg/h	2 (3.9)	9 (100)	

NGAL: Neutrophil gelatinase-associated lipocalin, Fisher's Exact test: p<0.05

In those with NGAL values <90 ng/mL and those with NGAL values >90 ng/mL, the change observed in creatinine values over time was statistically significant (p<0.001, p<0.001, respectively) (Table 4).

In those with NGAL values <90 ng/mL and those with NGAL values >90 ng/mL, the change observed in CrCl values was found to be statistically significant (p<0.001, p<0.001, respectively). The decrease in the values observed at 48 h postoperatively compared to those observed preoperatively and at 24 h preoperatively was found to be statistically significant (p<0.001, p<0.001, respectively) (Table 5).

In those with NGAL values <90 ng/mL; the change in lactate values was found to be statistically significant (p=0.012). The increase in the values observed at 24 h postoperatively compared to those observed at 0 h intraoperatively was statistically significant (p=0.006). In those with NGAL values >90 ng/mL, the change observed in lactate values over time was not statistically significant (p>0.05).

The graph of the change in plasma NGAL values of the patients over time shows that the values tended to increase at 6 and 24 h postoperatively compared to 0 h (Figure 1).

Concerning the change in the creatinine values of the patients over time, they decreased at 24 h postoperatively in 60 patients, but they tended to increase at 48 h postoperatively (Figure 2).

When NGAL and creatinine values of all subjects were compared, it was found that NGAL values increased after 6 h postoperatively and creatinine values increased at 48 h postoperatively (Figure 3).

DISCUSSION

This study investigated the efficacy of plasma NGAL in predicting AKI, hemodialysis, and mortality in patients undergoing major abdominal surgery. It was found that NGAL values measured at 6 and 24 h postoperatively were higher than those measured at 0 h intraoperatively and postoperative creatinine values increased later. Therefore, it has been proven that NGAL has a more predictive value in diagnosing AKI in patients undergoing major abdominal laparotomy surgery than creatinine. Additionally, it enables AKI detection at the early stage (at 6 h postoperatively).

In another study by Teixeira et al. (19) that included 450 patients undergoing major nonvascular abdominal

surgery, the incidence of postoperative AKI in the first 48 h after surgery was 22.4% and AKI was associated with the incidence of in-hospital mortality. In our study, AKI was observed in 15% of the 60 patients; however, RRT was not performed in these patients.

We conducted this study because the relationship between postoperative AKI and the need for long-term dialysis and/or decreased kidney function has only been investigated in patients who had undergone prior hepato-biliary, pancreatic, and cardiac surgeries (20) but not in those who had undergone major abdominal surgery.

In AKI, the initiating mechanism and subsequent response to injury decrease the recovery of the basal structure and function of the kidneys (21). In our study, it was proven that AKI can also develop after major abdominal surgery.

AKI is one of the most common critical diseases with high morbidity and poor prognosis. Its causes are extremely complicated, and it is linked to conditions such as hypovolemia, decreased cardiac output, nephrotoxic drug use and urinary tract obstruction (5). In our study, to protect the patients from hypovolemia, a pleth variablitiy index (PVI) device was used. The PVI value was kept between 5 and 13, and intravenous fluids were administered at a rate of 10-12 cc/kg/h.

Hypoxia caused by by different reasons can lead to inflammation, oxidative stress, immune system activation, and cell death (22). AKI is a common complication that may occur after abdominal surgery. AKI development after abdominal surgery causes a 3.5-fold increase in mortality in patients (23). Although AKI developed in 9 of the 60 patients included in our study, the incidence of death was not observed during the 2-month postoperative follow-up.

Numerous studies in the literature have shown that the most important risk factor for AKI development after abdominal surgery is preoperative renal failure. Other risk factors include dehydration, intra-abdominal hypertension, blood transfusion, and nephrotoxic drug use (24). We took care that the patients included in our study did not have kidney failure and did not use any nephrotoxic drug.

High lactate values in the blood reflect tissue microcirculation failure. Previous studies have shown that high lactate values are a risk factor in critically ill patients (25). Lactate values in the blood may be affected by conditions such as intraoperative tissue ischemia, postoperative coagulation, acute inflammatory response, and sepsis (26). In our study, although the intraoperative lactate values of the patients were not high, the change observed over time was found to be statistically significant. The analyses showed that the increase observed in the values at 24 and 48 h postoperatively compared to those at 0 h intraoperatively was statistically significant (p=0.002, p=0.020, respectively). Conversely, the change observed in the values at 48 h postoperatively compared to those at 24 h postoperatively was not significant (p>0.05).

The endotoxin load released because of the occurrence of intestinal ischemia, visceral perfusion disorder, and portal endotoxemia during abdominal surgery activates the proinflammatory response, resulting in endothelial damage,

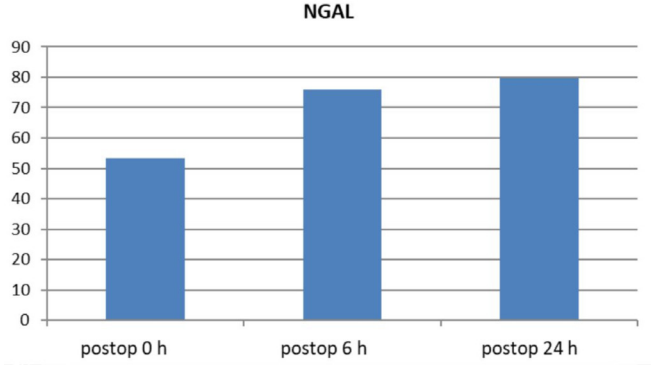

Figure 1. Change in plasma NGAL values over time
NGAL: Neutrophil gelatinase-associated lipocalin

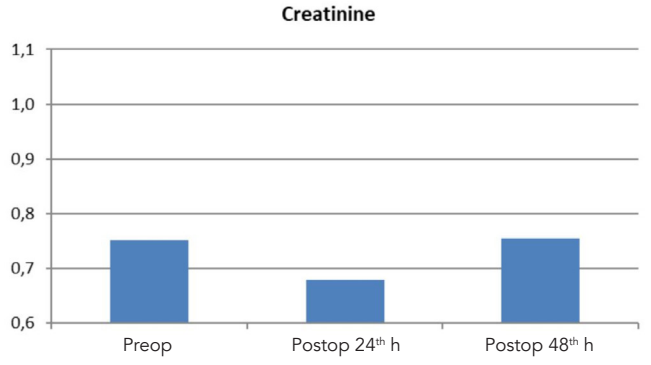

Figure 2. Change in serum creatinine value over time

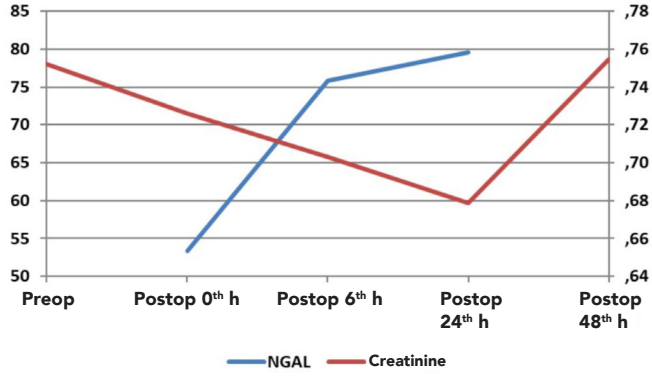

Figure 3. Comparison of the changes in plasma NGAL and creatinine values over time
NGAL: Neutrophil gelatinase-associated lipocalin

Table 4. Relationship between NGAL values and change in creatinine

Creatinine (mL/minute)	NGAL <90 ng/mL	NGAL> 90 ng/mL	Test value, p
	Mean ± SD	Mean ± SD	
Preoperatively	0.75±0.22	0.75±0.13	**t=-0.073, p=0.942**
Postop 24 h	0.68±0.24	0.7±0.16	**t=0.265, p=0.791**
Postoperative 48 h	0.65±0.22	1.32±0.24	**t=8.406, p<0.001**
Test value, p	**F=11.550, p<0.001**	**F=152.943, p<0.001**	

NGAL: Neutrophil gelatinase-associated lipocalin, SD: Standard deviation, Generalized linear mixed model: p<0.05

Table 5. Comparison of NGAL and CrCl

CrCl (mL/minute)	NGAL<90 ng/mL	NGAL>90 ng/mL	Test value, p
	Mean ± SD	Mean ± SD	
Preoperatively	110.69±36.57	122.56±31.67	**t=0.837, p=0.404**
Postoperative 24 h	122.82±35.68	137.11±51.28	**t=1.090, p=0.277**
Postoperative 48 h	126.53±36.28	69.56±22.67	**t=-4.518, p<0.001**
Test value, p	**F=10.776, p<0.001**	**F=61.923, p<0.001**	

NGAL: Neutrophil gelatinase-associated lipocalin, SD: Standard deviation, CrCl: Creatinine clearance, Generalized linear mixed model: p<0.05

followed by vasoconstriction, microvascular occlusion, and leukocyte congestion. Immune activation resulting from AKI causes systemic inflammatory changes (27). In our study, the change observed over time in white blood cell counts of the patients with AKI was not found to be statistically significant (p>0.05).

In their randomized study, Gözce et al. (28) reported that monitoring biomarkers in the blood of critically ill patients undergoing major noncardiac surgery significantly reduced the incidence of class II and severe AKI. In our study, according to the creatinine values, 10% (n=6) of the cases had class I AKI, 5% (n=3) had class II AKI, and 15% (n=10) patients in total were found to develop AKI. It was observed that in 15% (n=9) of the patients, the cut-off value of NGAL increased (>90 units).

In severe AKI cases, RRT may be necessary to maintain blood volume, electrolytes, and acid-base balance. The use of RRT in critically ill patients is increasing over time. It has been observed that 10%-15% of critically ill patients require RRT, resulting in increased incidence of mortality and length of hospital stay (29). In our study, RRT was not performed, and none of the patients had died during the 2-month postoperative follow-up.

NGAL is synthesized by intestinal, liver, and lung tissue and can be detected in blood plasma. In healthy adult individuals, the normal plasma NGAL value is considered as 50-90 ng/mL (20,30). Although there are studies investigating different cut-off values of NGAL in the literature, the cut-off value for NGAL values measured in our study was deemed >90 ng/mL, and AKI developed in 9 (15%) out of 60 patients.

Mishra et al. (31) founded that plasma and urine NGAL values increased within 2 h postoperatively in 71 pediatric patients who had undergone open heart surgery and cardiopulmonary bypass, and AKI developed in patients within 24-72 h postoperatively. In our study, NGAL values were normal in 60 patients at 0 h intraoperatively and increased above 90 ng/mL at 6 and 24 h postoperatively in 9 patients. Creatinine and hourly urine output values at 48 h postoperatively were evaluated by the KDIGO classification and indicated class I and II AKI. Thus, it has been proven that NGAL values show an early response (at 6 h postoperatively) compared to creatinine values.

Study Limitations

The survival of the patients included in our study was evaluated at 48 h postoperatively, and patient follow-up was conducted until 2 months postoperatively, which could have been conducted for a longer time. In our study, NGAL values were measured at 0 h intraoperatively and 6 and 24 h postoperatively; however, there are some studies in the literature that have used different intervals for performing measurements like at 0 h intraoperatively; at 2h, 6 h, 24h, 48 h, and 72 h postoperatively. Since our study was conducted

in a single center, it limited the social evaluation of how effective a major laparoscopic surgery is on AKI. The lack of a definite cut-off value for NGAL in studies in the literature can be cited as another limitation. Furthermore, our study was conducted on patients undergoing major abdominal surgery, but it could also have included patients undergoing more elaborate surgeries. Not measuring intra-abdominal pressure during surgery can also be considered another limitation.

The most important advantage of our study is that plasma NGAL enabled the detection of AKI at an early stage. Measuring the plasma NGAL values at 0 h and considering it as a baseline value is another advantage. The third advantage of our study is that it was done by a single team.

CONCLUSION

It has been proven that compared to creatinine, NGAL is more efficient in predicting the development of AKI in patients undergoing major abdominal laparotomy. Additionally, it predicts AKI development in the early stage (at 6 h postoperatively).

ETHICS

Ethics Committee Approval: Approval for this study was obtained from the Clinical Research Ethics Committee of Marmara University Faculty of Medicine (protocol code: 09.2021.74, date: 08.01.2021).

Informed Consent: Written informed consent was obtained from the patients.

Authorship Contributions

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

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. Gameiro J, Fonseca JA, Marques F, Lopes JA. Management of Acute Kidney Injury Following Major Abdominal Surgery: A Contemporary Review. J Clin Med 2020;9:2679.
2. Vaara ST, Bellomo R. Postoperative renal dysfunction after noncardiac surgery. Curr Opin Crit Care 2017;23:440-6.
3. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA 2005;294:813-8.
4. Grams ME, Sang Y, Coresh J, Ballew S, Matsushita K, Molnar MZ, et al. Acute Kidney Injury After Major Surgery: A Retrospective Analysis of Veterans Health Administration Data. Am J Kidney Dis 2016;67:872-80.
5. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. Lancet 2019;394:1949-64.
6. Pannu N, James M, Hemmelgarn B, Klarenbach S; Alberta Kidney Disease Network. Association between AKI, recovery of renal function, and long-term outcomes after hospital discharge. Clin J Am Soc Nephrol 2013;8:194-202.
7. Thongprayoon C, Hansrivijit P, Kovvuru K, Kanduri SR, Torres-Ortiz A, Acharya P, et al. Diagnostics, Risk Factors, Treatment and Outcomes of Acute Kidney Injury in a New Paradigm. J Clin Med 2020;9:1104.
8. Srisawat N, Sileanu FE, Murugan R, Bellomod R, Calzavacca P, Cartin-Ceba R, et al. Variation in risk and mortality of acute kidney injury in critically ill patients: a multicenter study. Am J Nephrol 2015;41:81-8.
9. Saadat-Gilani K, Zarbock A, Meersch M. Perioperative Renoprotection: Clinical Implications. Anesth Analg 2020;131:1667-8.
10. Biteker M, Dayan A, Tekkeşin Aİ, Can MM, Taycı İ, İlhan E, et al. Incidence, risk factors, and outcomes of perioperative acute kidney injury in noncardiac and nonvascular surgery. Am J Surg 2014;207:53-9.
11. Cruz DN, de Cal M, Garzotto F, Perazella MA, Lentini P, Corradi V, et al. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. Intensive Care Med 2010;36:444-51.
12. Aydoğdu M, Gürsel G, Sancak B, Yeni S, Sarı G, Taşyürek S, et al. The use of plasma and urine neutrophil gelatinase associated lipocalin (NGAL) and Cystatin C in early diagnosis of septic acute kidney injury in critically ill patients. Dis Markers 2013;34:237-46.
13. An R, Pang QY, Liu HL. Association of intra-operative hypotension with acute kidney injury, myocardial injury and mortality in non-cardiac surgery: A meta-analysis. Int J Clin Pract 2019;73:e13394.
14. Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 1993;268:10425-32.
15. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2007;18:407-13.
16. Mishra J, Mori K, Ma Q, Kelly C, Yang J, Mitsnefes M, et al. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2004;15:3073-82.
17. Devarajan P. Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. Biomark Med 2010;4:265-80.
18. Shavit L, Dolgoker I, Ivgi H, Assous M, Slotki I. Neutrophil gelatinase-associated lipocalin as a predictor of complications and mortality in patients undergoing non-cardiac major surgery. Kidney Blood Press Res 2011;34:116-24.
19. Teixeira C, Rosa R, Rodrigues N, Mendes I, Peixoto L, Dias S, et al. Acute kidney injury after major abdominal surgery: a retrospective cohort analysis. Crit Care Res Pract 2014;2014:132175.
20. Cho E, Kim SC, Kim MG, Jo SK, Cho WY, Kim HK. The incidence and risk factors of acute kidney injury after hepatobiliary surgery: a prospective observational study. BMC Nephrol 2014;15:169.
21. Lopes JA, Fernandes P, Jorge S, Gonçalves S, Alvarez A, Costa e Silva Z, et al. Acute kidney injury in intensive care unit patients: a comparison between the RIFLE and the Acute Kidney Injury Network classifications. Crit Care 2008;12:R110.

22. Hultström M, Becirovic-Agic M, Jönsson S. Comparison of acute kidney injury of different etiology reveals in-common mechanisms of tissue damage. Physiol Genomics 2018;50:127-41.
23. Kim M, Brady JE, Li G. Variations in the risk of acute kidney injury across intraabdominal surgery procedures. Anesth Analg 2014;119:1121-32.
24. An Y, Shen K, Ye Y. Risk factors for and the prevention of acute kidney injury after abdominal surgery. Surg Today 2018;48:573-83.
25. Bou Chebl R, El Khuri C, Shami A, Rajha E, Faris N, Bachir R, et al. Serum lactate is an independent predictor of hospital mortality in critically ill patients in the emergency department: a retrospective study. Scand J Trauma Resusc Emerg Med 2017;25:69.
26. Pucino V, Bombardieri M, Pitzalis C, Mauro C. Lactate at the crossroads of metabolism, inflammation, and autoimmunity. Eur J Immunol 2017;47:14-21.
27. Welborn MB, Oldenburg HS, Hess PJ, Huber TS, Martin TD, Rauwerda JA, et al. The relationship between visceral ischemia, proinflammatory cytokines, and organ injury in patients undergoing thoracoabdominal aortic aneurysm repair. Crit Care Med 2000;28:3191-7.
28. Göcze I, Jauch D, Götz M, Kennedy P, Jung B, Zeman F, et al. Biomarker-guided Intervention to Prevent Acute Kidney Injury After Major Surgery: The Prospective Randomized BigpAK Study. Ann Surg 2018;267:1013-20.
29. Hoste EAJ, Kellum JA, Selby NM, Zarbock A, Palevsky PM, Bagshaw SM, et al. Global epidemiology and outcomes of acute kidney injury. Nat Rev Nephrol 2018;14:607-25.
30. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008;36:1297-303.
31. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005;365:1231-8.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:135-140
DOI: 10.4274/BMJ.galenos.2022.2022.1-5

Research

Can Isokinetic Testing Lead to More Precise Monitoring of Chondromalacia Patella? Prospective, Cross-sectional Study

İsokinetik Test, Kondromalazi Patellanın Daha Kesin Monitorizasyonunu Sağlayabilir mi? Prospektif, Çapraz-kesitsel Çalışma

İlknur Saral[1], Hande Başat[1], Mehmet Ağırman[2], Esra Tekeci[3], Engin Çakar[4]

[1]Bahçeşehir University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Turkey
[2]Istanbul Medipol University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Istanbul, Turkey
[3]Memorial Hospital, Clinic of Physical Medicine and Rehabilitation, Istanbul, Turkey
[4]Üsküdar University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Turkey

ABSTRACT

Objective: Chondromalacia patella, one of the most common musculoskeletal problems encountered in clinical practice. The isometric test using the isokinetic dynamometer can be used for a valid assessment and follow-up of knee strength during the rehabilitation of chondromalacia patella. Our study investigates the effectiveness of the isokinetic dynamometer as a monitoring tool for individuals with chondromalacia patella.

Methods: Twenty-eight patients with chondromalacia patella (mean age 39±10.84 years, mean symptom duration 11.75±7.38 months) were included in the study. All patients had physical therapy and rehabilitation program that was applied for 15 sessions, 5 days a week during 3 weeks. Knee flexion and extension strengths were measured at 60°/second and 180°/second (5 sets) angular velocity with an isokinetic dynamometer system before and after physical therapy and rehabilitation program. Visual analog scale (VAS)-pain value was used for clinical assessment. Modifies magnetic resonance imaging (MRI) staging system was used in the staging of chondromalacia patella. The relationship between VAS pain value and isokinetic dynamometer parameters was analyzed, as well as correlation in radiological MRI staging, treatment effectiveness, symptom duration. A value of $p<0.05$ was considered significant.

Results: Statistically significant improvement was observed in the knee flexion and extension muscle strengths at 60°/second and the 180°/the second angular velocity (PTE_60, PTF_60, PTE_180, PTF_180) after the physical therapy and rehabilitation program in affected knees ($p=0.0001$ for all). Analysis of correlation and interaction of chondromalacia patella MR stages, VAS pain value, body mass index, duration of symptoms, and treatment efficacy was also statistically significant ($p<0.05$).

Conclusion: Our study results suggest that physical therapy and rehabilitation program improves the strength of both knee extensors and flexors, and isokinetic parameters have a significant effect on the treatment effectiveness. The isokinetic dynamometer may provide a precise monitoring to assess treatment efficacy of patients with chondromalacia patella. More studies are needed for further scientific evidence.

Keywords: Chondromalacia patella, muscle strength, the dynamometer, isokinetic testing, physical therapy and rehabilitation

ÖZ

Amaç: Kondromalazi patella, klinik pratikte en sık karşılaşılan kas-iskelet sistemi problemlerinden biridir. İzokinetik dinamometre kullanılarak yapılan izokinetik testleme, kondromalazi patellanın rehabilitasyonu sırasında diz kuvvetinin objektif değerlendirmesi ve takibi için kullanılabilir. Bu çalışma, kondromalazi patellalı bireylerde izleme aracı olarak izokinetik dinamometrenin etkinliğini araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Kondromalazi patellası olan yirmi sekiz hasta (ortalama yaş 39±10,84 yıl, ortalama semptom süresi 11,75±7,38 ay) çalışmaya dahil edildi. Tüm hastalara 3 hafta boyunca, haftada 5 gün, 15 seans fizik tedavi ve rehabilitasyon programı uygulandı. Program öncesi ve sonrası izokinetik dinamometre ile 60°/saniye ve 180°/saniye (5 set) açısal hızda diz fleksiyon ve ekstansiyon kas kuvvetleri ölçüldü. Klinik değerlendirme için VAS-ağrı skoru kullanıldı. Kondromalazi patella evrelemesinde Modifiye MRG Evreleme Sistemi kullanıldı. Vizüel analog skala (VAS)-ağrı

Address for Correspondence: İlknur Saral, Bahçeşehir University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Turkey
Phone: +90 532 318 05 52 E-mail: ilknurbsaral@gmail.com ORCID ID: orcid.org/0000-0001-7245-6304

Cite as: Saral İ, Başat H, Ağırman M, Tekeci E, Çakar E. Can Isokinetic Testing Lead to More Precise Monitoring of Chondromalacia Patella? Prospective, Cross-sectional Study. Med J Bakirkoy 2022;18:135-140

Received: 10.01.2022
Accepted: 10.02.2022

skoru ile izokinetik dinamometre parametreleri arasındaki ilişkinin yanı sıra radyolojik manyetik rezonans görüntüleme (MRG) evreleme, tedavi etkinliği ve semptom süresindeki korelasyon analiz edildi. P<0,05 değeri, istatistiksel anlamlı kabul edildi.

Bulgular: Etkilenen dizlerde fizik tedavi ve rehabilitasyon programı sonrası 60°/saniye ve 180°/saniye açısal hızlarda (PTE_60, PTF_60, PTE_180, PTF_180), diz fleksiyon ve ekstansiyon kas kuvvetlerinde istatistiksel olarak anlamlı iyileşme gözlendi (p=0,001). Kondromalazi patella MRG evreleri, VAS-ağrı skoru, vücut kitle indeksi, semptom süresi ve tedavi etkinliği arasındaki korelasyon ve etkileşim analizi de istatistiksel olarak anlamlı bulundu (p<0,05).

Sonuç: Çalışma sonuçlarımız, fizik tedavi ve rehabilitasyon programının hem diz ekstansörlerinin hem de fleksörlerinin kuvvetini geliştirdiğini ve izokinetik parametrelerin tedavi etkinliği üzerinde önemli bir etkiye sahip olduğunu göstermektedir. İzokinetik dinamometre, kondromalazi patellalı hastaların tedavi etkinliğini değerlendirmek için, kesin bir izleme aracı olarak kullanılabilir. Daha ileri bilimsel kanıtlar için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Kondromalazi patella, kas kuvveti, dinamometre, izokinetik test, fizik tedavi ve rehabilitasyon

INTRODUCTION

Chondromalacia patella is a commonly diagnosed disease at physical rehabilitation and arthrology clinics (1-7) and is a common cause of anterior knee pain among young individuals (8). This pain recurs after descending hills or stairs, knee-bend, 90° of knee flexion for an extended period of time (e.g. prolonged sitting), and repeated exercises of flexion/extension with extreme loading (1,2). Besides pain at knee cap area, and physical examination may reveal snapping or grinding sound during affected joint's movement (7). Histopathological examination ascertains hyaline cartilage damage overlying the patella (2).

Chondromalacia patella, also known as runner's knee, is a disease of sportive individuals such as runners, athletes, soccer players, bikers, skiers, gymnasts predominantly. Patellar trauma, patellofemoral instability, anatomic variations of bones, patellar kinematic abnormalities and occupation hazards are involved in the etiology. When there is extensive, and long-term bending, flexion, twist, constraint to the knee joint, overutilization and repetitive microtrauma occurs at the knee cap. As a result, intraarticular cartilage is degenerated, chondromalacia patella develops (1,3,7,8).

Noteworthily, the backbone of treatment for chondromalacia patella is physical therapy. Principal reasons for the use of physical therapy include decreasing patellofemoral pain, regaining of patellar alignment. It frequently involves exercises that focus on muscle strengthening (e.g. quadriceps, vastus medialis, hamstring); mobilization (e.g. patella); ice or heat packs for pain, soreness; therapeutic ultrasound; immobilizing cast; foot orthotics and/or devices that help walking. Besides this physical therapy, various modalities that does not involve the operation exists, ranging from immobilization to medicinal drugs. When aforementioned non-operative management fails, surgery can be an option (1,9,10).

Although magnetic resonance imaging (MRI) is the most frequently used non-invasive tool for diagnosis or differential diagnosis or monitorization the effects of therapy in patients with chondromalacia patella (2-5), isokinetic dynamometer could be used for monitorization of knee disorders (11-13).

The study aimed to analyze the efficacy of isokinetic dynamometer as a monitoring tool for individuals with chondromalacia patella. In this study, isokinetic testing was used to monitor changes in knee muscle strength before and after physical therapy. The relationship between pain and isokinetic strength test was analyzed, as well as changes in radiological MR stages in patients with chondromalacia patella. Through the study, we also compared the changes of the muscle strength of the affected knee and the healthy knee before and after physical therapy.

METHODS

Research subjects selected for this study were twenty-eight patients with chondromalacia patella, twenty males and eight females (mean age 39±10.84; age interval 21-62). Sixteen subjects presented chondromalacia patella for the left knee, and twelve subjects for the right knee. The reported mean duration of symptoms was 11.75±7.38 months. The study protocol was approved by Istanbul Medipol University Non-Invasive Clinical Research Ethics Committee (decision no: 1146, date: 25.11.2021), and written informed consent was obtained from each patient. The study was conducted in compliance with the principles of the Declaration of Helsinki.

Thirty-five consecutive patients who had clinical signs and symptoms of chondromalacia patella were referred to the radiology department for the diagnosis and MRI staging of chondromalacia patella. Modified MRI Staging System was used in the staging of chondromalacia patella. In this staging system, stage 0 is normal cartilage; stage 1 is softening or edema without contour irregularities in cartilage; stage 2 is fragmentation in cartilage, fissuring or focal defects below 50%; stage 3 is fragmentation, fissuring or defects above 50%; stage 4 is full-thickness cartilage lesions. Among these patients, a total of twenty-eight patients met the eligibility criteria were included in the study. The inclusion criteria were as follows: (i) aged 18-50 years; (ii) diagnosis of

chondromalacia patella according to modified MRI staging system. Exclusion criteria were as follows: (i) presence of neuromuscular and rheumatological disease; (ii) history of knee surgery; (iii) presence of severe hearing and vision impairment; (iv) presence of uncontrolled hypertension; and (v) pregnancy. Use of non-steroidal anti-inflammatory drug medications, incomplete follow up and inability to complete the isokinetic test is excluded from the study.

Demographic characteristics and past medical history of patients were obtained at the baseline assessment. A summary of age, height, weight, body mass index (BMI), duration of knee symptoms, the affected knee, chondromalacia patella stage by MRI, and gender is seen in Table 1.

In the study, isokinetic tests were performed with isokinetic dynamometer (HUMAC NORM, Version: 10.000.0039, CSMi, USA) to assess the bilateral knee flexor and extensor group muscles' strength (peak torque). Specifically, 60 PT_E (Concentric Peak Torque for Extension); 60 PT_F (Concentric Peak Torque for Flexion); 180 PT_E and 180 PT_F was recorded. None of the subjects had previously undergone isokinetic testing. Patients were seated upright and fixed with pelvic and distal thigh belts. They were allowed to hold on both sides of the chair with their hands. Muscle strength was measured concentrically at two angular velocities of 60°/s and 180°/s with five sets at each velocity. Subjects performed trial repetitions before each set and a 20 s resting interval was provided between the two sets. The muscle contraction variables measured were total extensor peak torque, total flexor peak torque, extensor peak torque difference and flexor peak torque difference (11-13).

All patients with chondromalacia patella had a physical therapy program including transcutaneous electrical nerve stimulation (TENS; 20 min, 60-120 Hz), cold pack therapy (15 min), therapeutic ultrasound (0.5 watt/cm^2, 50% intermittent, 5 min), quadriceps muscle-strengthening exercises, stretching, and isokinetic exercise by the isokinetic dynamometer for 15 sessions, 5 days a week during 3 weeks.

Visual analog scale (VAS)-pain value, and isokinetic dynamometer scores before and after physical therapy were recorded for each patient at baseline and after 3 weeks.

Statistical Analysis

The SPSS statistical package version 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. We applied the Kolmogorov-Smirnov assessment for testing normality of statistical distribution. Mean and standard deviation were used for continuous data. The association between categorical variables were determined using Pearson's correlation coefficient, Paired samples t-test, and general linear model univariate analysis of covariance (GLM ANCOVA). A value of $p<0.05$ was considered significant.

RESULTS

Twenty-eight patients with chondromalacia patella (20 males and 8 females, mean age 39±10.84 years (minimum: 21, maximum: 62 years), mean symptom duration 11.75±7.38 months, mean BMI 24.67±3.18 were included in the study. In our study population, all participants had unilateral chondromalacia patella [12 (42.86%) on the right side and 16 (57.14%) on the left side]. According to modified MRI staging system, 12 (42.9%) of the patients was stage 1, 9 of patients (32.1%) was stage 2, and 7 (25%) of patients was stage 3 (Table 1).

When we considered VAS-pain values, mean VAS-pain value was 5.39±1.85 at the beginning and 2.11±1.16 after 15 sessions. The VAS-pain values decreased after 15 sessions of physical therapy (-3.28 units) and this value was statistically significant ($p<0.0001$, Table 2).

A statistically significant, very high positive correlation was found between the MRI staging and the duration of symptoms ($r=0.89$; $p=0.0001$). The duration of symptoms of patients with stage 1 was 6.42±1.44, stage 2 was 10.89±2.85 and stage 3 was 22.00±7.05 months.

Pre-treatment and post-treatment of isokinetic test values in the affected knee and improvement compared to healthy knee are seen in Table 3.

Table 1. Baseline characteristics of chondromalacia patella patients

		Mean	Standard deviation
Age (years)		39.00	10.84
Height (cm)		175.04	11.03
Weight (kg)		76.46	17.00
Body mass index		24.67	3.18
Duration of symptoms (months)		11.75	7.38
		Number	**Percentage (%)**
Affected knee	Right	12	42.9%
	Left	16	57.1%
Chondromalacia patella magnetic resonance stage	Stage 1	12	42.9%
	Stage 2	9	32.1%
	Stage 3	7	25.0%
Gender	Male	20	71.4%
	Female	8	28.6%

When we considered affected knees, post-treatment power torque 60-degree extensor (PTE_60) and power torque 180-degree extensor (PTE_180) mean values were statistically significantly improved compared to pre-treatment value (both p=0.0001 for the difference). Additionally, when we compared affected knees with the healthy knees, improvement was also statistically significant for PTE_60 and PTE_180 (p values were 0.008 and 0.006 respectively) (Table 3).

Besides, power torque 60-degree flexor (PTF_60) and power torque 180-degree flexor (PTF_180) difference between pre-treatment and post-treatment was also statistically significant in affected knees (both p=0.0001 for the difference). Nevertheless, PTF_60 mean value of affected knees was statistically significantly improved compared to healthy knees (p=0.001; Table 3). However, when we considered PTF_180 improvement compared to healthy knee, it was not statistically significant (p=0.170; Table 3).

We also analyzed correlation and interaction between chondromalacia patella MRI stages, VAS pain value, BMI, duration of symptoms, and treatment efficacy. It is shown in Table 3, column at the end of right side. We observed that isokinetic parameters of PTE_60, PTF_60, PTE_180 has a significant effect on the treatment effectiveness (p values were 0.029; 0.018; and 0.017 respectively). Besides, a statistically significant relationship was found between VAS pain value and isokinetic parameters of PTE_60, PTE_180, and PTF_60 (r=-0.41; p=0.03). If VAS pain value improved, treatment effectiveness was also improved.

Table 2. VAS pain score of chondromalacia patella patients (pre-treatment and post-treatment)

	Mean	Standard deviation
VAS pain pre-treatment	5.39	1.85
VAS pain post-treatment	2.11*	1.16

*p<0.0001 versus VAS pain pre-treatment, VAS: Visual analog scale

DISCUSSION

Chondromalacia patella is the most common lower limb problem encountered in clinical practice (14). According to the studies, a commonly used tool for assessing muscle strength of lower limb is isokinetic systems due to its inherent patient safety, objectivity, and reproducibility in testing measures (14,15).

As explained by Keating and Matyas (16), in previous studies, the influence of impairments on dynamometric measurements has been investigated by comparing measurements for an affected limb with that for a contralateral healthy limb. These studies have found that measurements for affected limbs to be lower than those for healthy limbs. The results of these investigations therefore support the notion that dynamometric measurements can reflect anticipated weakness in an injured limb (16). In our study, we used an isokinetic dynamometer as a monitoring tool for individuals with chondromalacia patella, and we compared affected limbs with contralateral healthy limbs.

Table 3. Pre-treatment and post-treatment of isokinetic dynamometer values in affected knee and improvement compared to healthy knee

Parameter	Treatment	Affected knee			Improvement compared to healthy knee			
		Mean ± SD	Difference	Correlation	Mean ± SD	Difference	Correlation	TE*MR*VAS*BMI*SS
60 power torque extensor	Pre-	94.86±51.82	**0.0001**	0.86	15.79±26.77	**0.008**	0.78	**0.020**
	Post-	127.64±57.86			6.68±19.53			
60 power torque flexor	Pre-	49.82±25.28	**0.0001**	0.78	23±20.67	**0.001**	0.42	**0.018**
	Post-	67.68±27.66			6.61±21.61			
180 power torque extensor	Pre-	61.93±30.48	**0.0001**	0.83	12.07±24.84	**0.006**	0.75	**0.017**
	Post-	87.57±36.29			2.68±18.27			
180 power torque flexor	Pre-	37.32±13.98	**0.0001**	0.65	12.14±16.5	0.170	0.12	0.636
	Post-	49.64±20.08			6.86±13.23			
TWDE	Pre-	1001.39±536.34	**0.0001**	0.83	11.5±27.06	0.086	0,51	0.092
	Post-	1460.07±602.58			3.61±16.84			
TWDF	Pre-	501.93±304.76	**0.001**	0.60	17.18±31.96	0.34	0.25	0.178
	Post-	751.11±419.55			10.54±26.9			

TE: Treatment efficacy, SS: Symptom duration, MR: Magnetic resonance, VAS: Visual analog scale, BMI: Body mass index, SD: Standard deviation, TWDE: Total work deficit extensor, TWDF: Total work deficit flexor

We think that our approach is consistent with the previous studies (16) because isokinetic muscle strength results of the affected knees were lower than healthy knees in our 28-patient study population.

In a case study, Lennington and Yanchuleff (17) examined the use of isokinetic system for treating chondromalacia patella. They used Cybex II (Cybex, Division of Lumex Inc., Ronkonkoma, NY) as a tool, and physical therapy. They found that the use of Cybex II isokinetic strengthening of quadriceps in short arch fashion appeared to be a reasonable treatment for chondromalacia patella (17). In concordance with this study, we found statistically significant improvement in chondromalacia patients with isokinetic strengthening and physical therapy.

In another study, Elton et al. (18), compared clinical examination findings (history, physical, and isokinetics) and arthroscopic findings in patients with chondromalacia patella (n=20). They found that physical examination including Cybex II isokinetic testing results was not statistically significant (18). In discordance with the study by Elton et al. (18), we found statistically significant correlation and interaction between chondromalacia patella MRI stages, VAS pain value, BMI, duration of symptoms, and treatment efficacy. We observed that isokinetic parameters of PTE_60, PTF_60, PTE_180 has a significant effect on the treatment effectiveness (p values were 0.029; 0.018; and 0.017 respectively). Besides, a statistically significant relationship was found between VAS pain value and isokinetic parameters of PTE_60, PTE_180, and PTF_60 (r=-0.41; p=0.03). If VAS pain value improved, treatment effectiveness was also improved.

In a recent study, Ozel et al. (19), evaluated the correlation between clinical and magnetic resonance findings of chondromalacia patella. They found that there was a positive correlation between age and MRI staging, and the highest coefficient obtained with patellar cartilage score (19). In our study, there are some similarities with this study because we found also a positive correlation with MRI staging and VAS-pain value, in addition to a positive correlation with MRI staging and duration of symptoms.

In a 2021 study, Ouazzani et al. (20), investigated isokinetic profile of knee extensor and flexor muscles in patellofemoral pain syndrome patients (n=58). They found that pain values were not correlated with isokinetic measurements (20). In discordance with this study, we found that there was a positive correlation between isokinetic measurements and VAS-pain value.

This study's small sample size limits the generalization of these outcomes to a larger population. A major limitation of our study is the lack of the control group, we only compared affected knees with healthy knees in the same population. Also, the follow-up period of the study was 3 weeks and was not long enough. However, the strengths of our study were the isokinetic measurements of the patients before and after treatment, staging of all patients with MRI. Literature with isokinetic dynamometer in the treatment follow-up of patients with chondromalacia patella is relatively poor. In this respect, our study contributes to the literature.

CONCLUSION

The results of this study showed that the isokinetic dynamometer may be a precise monitoring tool to assess treatment efficacy of patients with chondromalacia patella. More studies are needed for further scientific evidence.

ETHICS

Ethics Committee Approval: The study protocol was approved by Istanbul Medipol University Non-Invasive Clinical Research Ethics Committee (decision no: 1146, date: 25.11.2021).

Informed Consent: Written informed consent was obtained from each patient.

Authorship Contributions

Surgical and Medical Practices: E.T., E.Ç., Concept: H.B., Design: H.B., Data Collection or Processing: E.T., Analysis or Interpretation: M.A., Literature Search: H.B., Writing: İ.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. McMullen W, Roncarati A, Koval P. Static and isokinetic treatments of chondromalacia patella: a comparative investigation. J Orthop Sports Phys Ther 1990;12:256-66.
2. Özgen A, Taşdelen N, Fırat Z. A new MRI grading system for chondromalacia patellae. Acta Radiol 2017;58:456-63.
3. Gagliardi JA, Chung EM, Chandnani VP, Kesling KL, Christensen KP, Null RN, et al. Detection and staging of chondromalacia patellae: relative efficacies of conventional MR imaging, MR arthrography, and CT arthrography. AJR Am J Roentgenol 1994;163:629-36.
4. Krieger EAG, Karam FC, Soder RB, da Silva JLB. Prevalence of patellar chondropathy on 3.0 T magnetic resonance imaging. Radiol Bras 2020;53:375-80.
5. Mete BD, Gursoy M, Kocyigit H. Magnetic resonance imaging of the patellofemoral joint. Turk J Phys Med Rehab 2015;61:261-71.
6. OUTERBRIDGE RE. The etiology of chondromalacia patellae. J Bone Joint Surg Br 1961;43-B:752-7.
7. Hauser RA, Sprague IS. Outcomes of prolotherapy in chondromalacia patella patients: improvements in pain level and function. Clin Med Insights Arthritis Musculoskelet Disord 2014;7:13-20.

8. Zheng W, Li H, Hu K, Li L, Bei M. Chondromalacia patellae: current options and emerging cell therapies. Stem Cell Res Ther 2021;12:412.
9. Crossley K, Bennell K, Green S, Cowan S, McConnell J. Physical therapy for patellofemoral pain: a randomized, double-blinded, placebo-controlled trial. Am J Sports Med 2002;30:857-65.
10. McCONNELL J. The management of chondromalacia patellae: a long term solution. Aust J Physiother 1986;32:215-23.
11. de Araujo Ribeiro Alvares JB, Rodrigues R, de Azevedo Franke R, da Silva BG, Pinto RS, Vaz MA, et al. Inter-machine reliability of the Biodex and Cybex isokinetic dynamometers for knee flexor/extensor isometric, concentric and eccentric tests. Phys Ther Sport 2015;16:59-65.
12. de Amorim Aquino M, Leme LE. Isokinetic dynamometry in elderly women undergoing total knee arthroplasty: a comparative study. Clinics (Sao Paulo) 2006;61:215-22.
13. Yildiz Y, Aydin T, Sekir U, Cetin C, Ors F, Alp Kalyon T. Relation between isokinetic muscle strength and functional capacity in recreational athletes with chondromalacia patellae. Br J Sports Med 2003;37:475-9.
14. Weng P, Janssen J, Richards JD, Selfe J. Validity of two clinical knee strength assessments compared to the reference standard. International Journal of Physiotherapy and Research 2015;3:1264-70.
15. Wilk KE, Romaniello WT, Soscia SM, Arrigo CA, Andrews JR. The relationship between subjective knee scores, isokinetic testing, and functional testing in the ACL-reconstructed knee. J Orthop Sports Phys Ther 1994;20:60-73.
16. Keating JL, Matyas TA. The influence of subject and test design on dynamometric measurements of extremity muscles. Phys Ther 1996;76:866-89.
17. Lennington KR, Yanchuleff TT. The Use of Isokinetics in the Treatment of Chondromalacia Patellae: A Case Report. J Orthop Sports Phys Ther 1983;4:176-8.
18. Elton K, McDonough K, Savinar-Nogue E, Jensen GM. A Preliminary Investigation: History, Physical, and Isokinetic Exam Results versus Arthroscopic Diagnosis of Chondromalacia Patella *. J Orthop Sports Phys Ther 1985;7:115-23.
19. Ozel D, Kir MC, Oncu M. Evaluation of correlation between clinical and magnetic resonance findings of patellar chondromalacia. Haydarpasa Numune Med J 2020;60:221-5.
20. Ouazzani H, Ngandzali DD, Abdelfettah Y. Isokinetic profile of knee extensor and flexor muscles in patellofemoral pain syndrome patients. SAS J Med 2021;7:130-8.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:141-145
DOI: 10.4274/BMJ.galenos.2022.2022.1-14

Research

Novel Once-daily Extended-release Tacrolimus Versus Twice-daily Tacrolimus in *De Novo* Kidney Transplant Recipients During the Early Posttransplant Period

Erken Posttransplant Dönemde Hızlı Salınımlı Takrolimus ve Uzun Salınımlı Takrolimusun *De Novo* Kullanımı

Gülay Yılmaz[1], Ebru Özdemir[2], Murat Yıldar[2], Abdülhak Hamit Karayağız[3], İbrahim Berber[4], Ülkem Çakır[5]

[1]Acıbadem International Hospital, Clinic of Nephrology and Transplantation, Istanbul, Turkey
[2]Acıbadem International Hospital, Clinic of Transplantation, Istanbul, Turkey
[3]Istanbul Esenyurt Necmi Kadıoğlu State Hospital, Clinic of General Surgery, Istanbul, Turkey
[4]Acıbadem University Faculty of Medicine, Department of General Surgery, Istanbul, Turkey
[5]Acıbadem University Faculty of Medicine, Department of Internal Medicine, Division of Nephrology, Istanbul, Turkey

ABSTRACT

Objective: Tacrolimus is used in more than 80% of kidney transplant recipients due to its ability to avoid rejection. Irregularities in tacrolimus level may affect clinical outcomes by subjecting patients to adverse events associated with graft rejection or immunosuppressive therapy. There are two forms of tacrolimus; immediate-release (IR-T) and prolonged release (PR-T). This study is designed to compare the clinical follow-up of kidney transplant patients who are receiving prolonged-release (PR-T; Advagraf) and immediate-release (IR-T; Prograf) tacrolimus in the posttransplant first week.

Methods: This study included 78 *de novo*, adult kidney transplant patients to prolonged-release tacrolimus 0.15 mg/kg/day (group 1, n=39) and immediate-release tacrolimus 0.15 mg/kg/day (group 2, n=39) for the first week in the posttransplant period. Demographic features, whole blood tacrolimus levels and kidney function were compared between the two groups. The presence of acute rejection and adverse events, antihypertensive drug use and arterial blood pressure of all patients were considered. Drug doses were determined according to the previously targeted tacrolimus level. SPSS 22 for Windows was used for statistical analysis.

Results: Acute rejection was not seen in any patient and there were no adverse events in the posttransplant first week. However, group 2 patients were found to have higher tacrolimus levels on the posttransplant 1st, 4th and 7th days (p=0.02, p=0.009 and p=0.013 respectively). Serum creatinine levels were significantly increased in group 2 patients on the posttransplant 7th day (p=0.02). Systolic, diastolic or mean arterial blood pressure were not different between the groups.

Conclusion: Prolonged-release tacrolimus is effective in preventing acute rejection when adequate blood levels are maintained, and appears promising as it makes it possible to avoid interindividual variation in absorption and early calcineurin inhibitor toxicity.

Keywords: Immediate-release tacrolimus, kidney transplantation, prolonged-release tacrolimus

ÖZ

Amaç: Takrolimus, rejeksiyonu önleme yeteneği nedeniyle böbrek nakli alıcılarının %80'inden fazlasında kullanılmaktadır. Takrolimusun hem düşük hem de aşırı dozu, hastaları greft reddi veya immünosüpresif tedaviyle ilişkili advers olaylara maruz bırakarak klinik sonuçları etkileyebilir. Takrolimus, hızlı salınımlı (IR-T) ve yavaş salınımlı (PR-T) olmak üzere iki formda bulunmaktadır. Bu çalışma, posttransplant birinci haftada, yavaş salınımlı (PR-T; Advagraf) ve hızlı salınımlı (IR-T; Prograf) takrolimus alan böbrek nakli alıcılarımızın klinik takibini karşılaştırmak için tasarlanmıştır.

Address for Correspondence: Gülay Yılmaz, Acıbadem International Hospital, Clinic of Nephrology and Transplantation, Istanbul, Turkey
E-mail: drgulaytastan@hotmail.com ORCID ID: orcid.org/0000-0001-6565-8063

Cite as: Yılmaz G, Özdemir E, Yıldar M, Karayağız AH, Berber İ, Çakır Ü. Novel Once-daily Extended-release Tacrolimus Versus Twice-daily Tacrolimus in *De Novo* Kidney Transplant Recipients During the Early Posttransplant Period. Med J Bakirkoy 2022;18:141-145

Received: 01.02.2022
Accepted: 11.02.2022

Gereç ve Yöntem: Bu çalışma, nakil sonrası yedi gün boyunca yavaş salınımlı takrolimus 0,15 mg/kg/gün (grup 1, n=39) ve hızlı salınımlı takrolimus 0,15 mg/kg/gün (grup 2, n=39) alan 78 *de novo*, yetişkin böbrek nakli alıcılarını içermektedir. Takrolimus düzeyi ve böbrek fonksiyonu, demografik özellikler ve klinik parametreler iki grup arasında karşılaştırıldı. Tüm hastalarda akut rejeksiyon ve advers olay varlığı, antihipertansif ilaç kullanımı ve arteriyel kan basıncı araştırıldı. Önceden hedeflenen takrolimus düzeyine göre ilaç dozları belirlendi. İstatistiksel analiz için Windows için SPSS 22 kullanıldı.

Bulgular: Hiçbir hastada akut rejeksiyon olmadı ve nakil sonrası ilk haftada herhangi bir yan etki görülmedi. Ancak, grup 2 hastalarında nakil sonrası 1., 4. ve 7. günlerde tam kan takrolimus düzeyleri daha yüksek bulundu (sırasıyla p=0,02, p=0,009 ve p=0,013). Ayrıca grup 2 hastalarında transplantasyon sonrası 7. günde serum kreatinin seviyeleri anlamlı olarak arttı (p=0,02). Sistolik, diyastolik veya ortalama arteriyel kan basıncı gruplar arasında farklı değildi.

Sonuç: Yavaş salınımlı takrolimus, yeterli kan seviyeleri sağlandığında akut rejeksiyonu önlemede etkilidir, ayrıca emilim ve erken kalsinörin inhibitörü toksisitesinde bireyler arası varyasyondan kaçınmayı mümkün kılması nedeni ile de umut verici görünmektedir.

Anahtar Kelimeler: Hızlı salınımlı takrolimus, böbrek nakli, yavaş salınımlı takrolimus

INTRODUCTION

Tacrolimus (TAC) is a macrolide immunosuppressive isolated from *Streptomyces tsukubaensis* in 1984 (1). It is used in over 80% of kidney transplant patients due to its ability to prevent rejection. Metabolism of TAC exists in the liver by cytochrome p450 3A4 and cytochrome p3A5 and 1% of TAC is excreted in urine (2). Oral bioavailability of TAC exhibits large variability, ranging 5% to 90%. Drugs that induce or inhibit CYP3A enzymes interact with TAC blood level. Also, gut motility affects absorption and high blood levels of TAC can be seen in diarrhea because of decreased p-glycoprotein activity. TAC binds to FK-binding protein and TAC- FK-binding protein complex interferes with calcineurin. After the inhibition of calcineurin, dephosphorylation of nuclear factor of activated T cells does not occur and T cell proliferation is stopped. TAC has a narrow therapeutic index so the drug concentration must be monitored. After the transplant, blood TAC level is kept between 5 and 15 ng/mL (3). Irregularities in TAC level may affect clinical outcomes by subjecting patients to adverse events associated with graft rejection or immunosuppressive therapy. Acute and chronic nephrotoxicity, hypertension, hyperlipidemia, electrolyte imbalance, diarrhea, posttransplant diabetes mellitus (PTDM) and neurotoxicity are common advers events of TAC (4).

There are two forms of TAC; immediate-release (IR-T) and prolonged release (PR-T). While the excipient of IR-T is croscarmellose, excipient of PR-T is ethylcellulose and this slows down the diffusion of the drug. While IR-T is taken twice a day, PR-T is taken once a day. After oral administration IR-T reaches a maximum plasma concentration in about 174 min, PR-T reaches in about 300 min. Conversion from IR-T to PR-T in the early period of posttransplant or using *de novo* PR-T in kidney transplantation has been studied in many studies. In Osaka Trial, PR-T regimen showed similar efficacy to IR-T regimen (5). The effectiveness and safety of both TAC preparation is well known and similar, but PR-T has an advantage of single daily dose. Nonadherence to immunosuppressive drugs in renal transplant patients is a immense problem. Nonadherence to immunosuppressive drugs is a serious cause of rejection. A study that compares adherence to IR-T and PR-T in renal transplant patients found that adherence was higher in the IR-T group than the PR-T group (6). The other problem about TAC is to reach steady-state trough levels. Intra and interpatient variability of TAC level is reported to be higher in IR-T than PR-T. High intra- and interpatient variability is related to graft loss, kidney dysfunction and acute rejection (7). Due to pharmacokinetic characteristics of PR-T, it reduces intra- and interpatient instability.

PR-T has been used safely in kidney transplantation for less than a decade. Our experience with switching form IR-T to PR-T goes along with the successful results reported in the literature. However since we were not so sure about using *de novo* PR-T in kidney transplant patients we designed a prospective randomized study to compare the clinical follow-up of kidney transplant patients who are receiving PR-T and IR-T in the first week of posttransplant period.

METHODS

This study included 78 *de novo* adult kidney transplant patients (38 male, 30 female; age ranging from 14 to 66 years) receiving PR-T 0.15 mg/kg/day (group 1, n=39) and IR-T 0.15 mg/kg/day (group 2, n=39) in the posttransplant first week. Both forms of TAC were started on the day before the operation. All the patients had low immunological risk and all the patients received anti-thymocyte globulin 2.5 mg/kg/day for 3 days according to our treatment protocol for kidney transplant patients. Demographic features, whole blood TAC levels and kidney function were compared between the groups. Adverse events, acute rejection, antihypertensive drug use and arterial blood pressure of all patients were considered. Drug doses were determined according to the previously targeted TAC level. The demographic and clinical data were obtained from the files of the patients. Written informed consent was obtained

from all patients. The study protocol was approved by the Ethics Committee of Acıbadem University on 02.09.2021 with approval number 2021-16/01.

Statistical Analysis

The results were analyzed by use of the Statistical Package for the Social Sciences, version 22 (SPSS, Chicago, Ill, United States). Values displaying a normal distribution are defined as mean ± standard deviation. Differences between numeric variables were tested with the use of independent-samples Student t-test or Mann-Whitney U test, that was appropriate. Chi-square tests were used to compare categorical variables. The degree of relation between two parametric values was analyzed with Pearson correlation test while Spearman correlation test was preferred for nonparametric values. A value was considered statistically significant at $p<0.05$.

RESULTS

Data regarding demographic and clinical aspects such as age, gender, diagnosis of diabetes mellitus, hypertension and presence of acute rejection episodes were described.

This study included 78 *de novo* adult kidney transplant patients (38 male, 30 female; age ranging from 14 to 66 years) receiving PR-T 0.15 mg/kg/day (group 1, n=39) and IR-T 0.15 mg/kg/day (group 2, n=39) in the posttransplant first week. Mean age of group 1 was 44±13.6 and mean age of group 2 was 43.9±13.7. No age difference was seen between the two groups (p=0.980), but male predominance was present in group 1 (p=0.02) (Table 1). Creatinine levels on the postoperative first 6 days were comparable in both groups, but serum creatinine levels of the postoperative 7th day were lower in group 1 (p=0.025) (Table 2). Diabetic or hypertensive nephropathy prevalence as the etiology of chronic kidney disease in each group was comparable. Also, none of these diseases were seen in the early posttransplant period. Regarding TAC levels, target trough values (8-12 ng/mL) were achieved in two groups, however mean TAC levels on days 1, 4, 5, and 7 were higher in group 2 (p=0.020 p=0.009 p=0.013 p=0.013 respectively) (Table 3). Neither acute rejection episodes nor graft loss was seen in both groups. There was no statistically significant difference between the two groups in terms of systolic, diastolic or mean arterial blood pressure (Table 4).

DISCUSSION

Since TAC is the cornerstone of the immunosuppressive therapy, its appropriate use in kidney transplant patients plays an imperative role in determining graft and patient survival. Adjustment of the optimal TAC dose for not only preventing acute rejection, but also avoiding the adverse effects is an important parameter leading to reach high survival rates.

Table 1. Demographic features

	Group 1	Group 2	p
Gender	19 males (48.7%)	29 males (48.7%)	0.020
	20 females (51.3%)	10 females (25.6%)	
Age (years)	44.0±13.6	43.9±13.7	0.980

Table 2. Creatinine levels on postoperative days 1-7

	Group 1		Group 2		
Serum creatinine (mg/dL)	**Mean ± SD**	**Min-max (median)**	**Mean ± SD**	**Min-max (median)**	**p**
Day 1	2.13±1.00	0.58-5.22 (1.99)	2.34±0.93	0.88-4.26 (2.12)	0.348
Day 2	1.17±0.57	0.46-2.85 (1.09)	1.28±0.52	0.59-2.53 (1.16)	0.259
Day 3	0.99±0.43	0.42-2.27 (0.89)	1.01±0.38	0.1-2.03 (0.91)	0.532
Day 4	1.01±0.45	0.41-2.88 (0.94)	1.06±0.33	0.59-2.08 (0.99)	0.296
Day 5	1.02±0.41	0.46-2.55 (0.98)	1.03±0.31	0.17-1.69 (0.98)	0.407
Day 6	1.03±0.42	0.46-2.56 (0.99)	1.10±0.33	0.6-2.01 (1.05)	0.407
Day 7	1.02±0.38	0.45-2.3 (0.99)	1.17±0.33	0.59-2.11 (1.12)	0.025

SD: Standard deviation, min: Minimum, max: Maximum

Conversion from IR-T to PR-T has been evaluated in many studies, but *de novo* use of PR-T and its effects are still unclear. In Osaka study, which compared *de novo* use of PR-T and IR-T outcomes in both groups were evaluated. Patient and graft survival was similar in all groups (4). Kolonko et al. (8) enrolled 72 kidney transplant patients and converted from IR-T to PR-T. They found that the conversion from IR-T to PR-T was followed by advancement in kidney graft function, but this improvement was not associated with TAC blood trough levels (8). In an overview of phase 3 and 4 studies in *de novo* patients, it was shown that PR-T and IR-T had a similar effect to avoid graft dysfunction and rejection (9).

Table 3. Tacrolimus levels on postoperative days 1-7

	Group 1		Group 2		
TAC (ng/mL)	Mean ± SD	Min-max (median)	Mean ± SD	Min-max (median)	p
Day 0	9.2±5.0	2-27.6 (8.6)	7.4±3.5	3.1-18.4 (6.6)	0.115
Day 1	11.3±5.4	2.6-29.3 (10.7)	18.2±14.1	4.5-56.4 (13)	0.020
Day 2	14.1±6.1	7.5-29.5 (12.8)	14.8±7.1	6.9-44.2 (12.8)	0.904
Day 3	11.4±4.1	7.1-20.5 (10.1)	12.5±4.5	0.2-21.1 (13.4)	0.377
Day 4	12.9±4.7	6.8-27.8 (11.8)	15.8±5.1	6.6-32.4 (14.2)	0.009
Day 5	11.6±3.7	6.2-19.8 (10.8)	14.5±4.1	9-27.5 (14)	0.013
Day 6	16.8±23.0	7-108 (11.9)	12.7±3.7	1.3-23.4 (12.1)	0.352
Day 7	11.3±4.3	6-23.5 (10.7)	13.4±4.5	8.3-27.4 (12)	0.013

SD: Standard deviation, min: Minimum, max: Maximum, TAC: Tacrolimus

Table 4. Blood pressure levels in both groups

		Group 1		Group 2		
		Mean ± SD	Min-max (median)	Mean ± SD	Min-max (median)	p
Systolic blood pressure	Day 1	138.7±16.2	106-177 (140)	141.8±15.0	100-170 (140)	0.370
	Day 2	140.5±19.9	100-190 (140)	144.6±16.4	118-186 (149)	0.320
	Day 3	141.0±20.9	90-189 (140)	143.7±18.0	110-190 (140)	0.512
	Day 4	140.2±14.6	110-170 (140)	139.7±16.3	100-180 (140)	0.876
	Day 5	132.6±16.5	92-170 (132.5)	137.8±14.8	110-180 (140)	0.137
	Day 6	131.2±15.6	100-160 (130)	137.6±18.9	110-190 (135)	0.211
	Day 7	127.8±17.4	80-162 (130)	133.7±19.4	100-180 (130)	0.378
Diastolic blood pressure	Day 1	83.3±11.5	56-104 (80)	86.6±10.1	60-103 (90)	0.237
	Day 2	82.9±10.7	60-108 (80)	85.2±11.5	70-116 (80)	0.577
	Day 3	84.5±11.8	50-110 (85)	85.7±10.8	65-110 (90)	0.791
	Day 4	83.6±10.4	70-109 (80)	84.2±10.5	60-103 (80)	0.660
	Day 5	81.9±9.8	60-100 (80)	83.7±10.1	70-110 (80)	0.490
	Day 6	80.4±10.7	50-100 (80)	82.3±11.1	70-120 (80)	0.734
	Day 7	77.6±10.6	50-97 (80)	80.7±12.0	60-104 (80)	0.439
Mean arterial pressure	Day 1	101.8±11.3	72.7-123.3 (101.7)	105.0±10.1	80-120 (106.7)	0.198
	Day 2	102.1±12.0	73.3-123.3 (103.3)	105.0±11.8	86.7-139.3 (105)	0.286
	Day 3	103.3±13.5	63.3-130.3 (104.7)	105.0±11.8	80-136.7 (106.7)	0.652
	Day 4	102.5±10.7	84.7-125 (100)	102.7±11.2	73.3-120 (100)	0.729
	Day 5	96.3±19.3	0-119 (98.7)	101.8±9.8	83.3-126.7 (100.3)	0.205
	Day 6	94.8±19.0	0-116.7 (96.7)	100.7±12.8	83.3-140 (100)	0.364
	Day 7	84.6±31.2	0-116 (93.3)	78.2±42.1	0-126.7 (93.3)	0.976

SD: Standard deviation, min: Minimum, max: Maximum

Our study involved 78 *de novo*, adult kidney transplant patients receiving PR-T and IR-T in the posttransplant period. Target trough levels of TAC (8-12 ng/mL) were reached in both groups. Lower mean levels of TAC with lower serum creatinine levels on the postoperative 7th day were remarkable in patients with the PR-T groups immunosuppression is the main risk factor for PTDM. TAC decreases insulin release from pancreatic beta cells (10) and PTDM enhances the risk of cardiovascular disease, kidney graft loss and death. Regarding PTDM, Silva et al. (11) found that the incidence of PTDM was lower with PR-T than IR-T (11), however we did not observe any difference in the early posttransplant period.

As it is known 18%-30% of renal transplant patients die from cardiovascular diseases (12). Hypertension is one of the most important causes of cardiovascular diseases. Hypertension is seen with the prevalence of 80% in kidney transplant patients in the early posttransplant period and it reduces to 50% at the end of the first year. So, blood pressure control is critical for renal transplant patients. TAC use is one of the reasons for hypertension in renal transplant recipients. PR-T and IR-T were compared in many studies for developing hypertension. Guirado et al. (13) did not find any statistical significance when compared the two forms of TAC in developing uncontrolled hypertension and we also did not observe any difference between IR-T and PR-T groups in terms of arterial blood pressure.

CONCLUSION

Our results suggesting lower levels of TAC with lower serum creatinine levels in the PR-T group seem safe and effective in terms of preventing acute rejection when effective blood level is provided and it also protects from calcineurin inhibitor toxicity.

ETHICS

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Acıbadem University on 02.09.2021 with approval number 2021-16/01.

Informed Consent: Written informed consent was obtained from all patients.

Authorship Contributions

Surgical and Medical Practices: E.Ö., M.Y., İ.B., Concept: G.Y., İ.B., Ü.Ç., Design: G.Y., A.H.K., İ.B., Data Collection or Processing: E.Ö., M.Y., Analysis or Interpretation: E.Ö., M.Y., Ü.Ç., Literature Search: G.Y., A.H.K., Writing: G.Y., Ü.Ç.

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. Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. J Antibiot (Tokyo) 1987;40:1256-65.
2. Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug Metab Pharmacokinet 2007;22:328-35.
3. Chapman JR. The KDIGO clinical practice guidelines for the care of kidney transplant recipients. Transplantation 2010;89:644-5.
4. Lancia P, Aurich B, Ha P, Maisin A, Baudouin V, Jacqz-Aigrain E. Adverse Events under Tacrolimus and Cyclosporine in the First 3 Years Post-Renal Transplantation in Children. Clin Drug Investig 2018;38:157-71.
5. Albano L, Banas B, Klempnauer JL, Glyda M, Viklicky O, Kamar N; Optimising immunoSuppression After Kidney transplantation with ADVAGRAF Study Group. OSAKA trial: a randomized, controlled trial comparing tacrolimus QD and BD in kidney transplantation. Transplantation 2013;96:897-903.
6. Kuypers DR, Peeters PC, Sennesael JJ, Kianda MN, Vrijens B, Kristanto P, et al. Improved adherence to tacrolimus once-daily formulation in renal recipients: a randomized controlled trial using electronic monitoring. Transplantation 2013;95:333-40.
7. Shuker N, Shuker L, van Rosmalen J, Roodnat JI, Borra LC, Weimar W, et al. A high intrapatient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation. Transpl Int 2016;29:1158-67.
8. Kolonko A, Chudek J, Wiecek A. Improved kidney graft function after conversion from twice daily tacrolimus to a once daily prolonged-release formulation. Transplant Proc 2011;43:2950-3.
9. McCormack PL. Extended-release tacrolimus: a review of its use in de novo kidney transplantation. Drugs 2014;74:2053-64.
10. Øzbay LA, Smidt K, Mortensen DM, Carstens J, Jørgensen KA, Rungby J. Cyclosporin and tacrolimus impair insulin secretion and transcriptional regulation in INS-1E beta-cells. Br J Pharmacol 2011;162:136-46.
11. Silva HT Jr, Yang HC, Abouljoud M, Kuo PC, Wisemandle K, Bhattacharya P, et al. One-year results with extended-release tacrolimus/MMF, tacrolimus/MMF and cyclosporine/MMF in de novo kidney transplant recipients. Am J Transplant 2007;7:595-608.
12. Collins AJ, Foley RN, Chavers B, Gilbertson D, Herzog C, Ishani A, et al. US Renal Data System 2013 Annual Data Report. Am J Kidney Dis 2014;63(1 Suppl):A7.
13. Guirado L, Cantarell C, Franco A, Huertas EG, Fructuoso AS, Fernández A, et al. Efficacy and safety of conversion from twice-daily to once-daily tacrolimus in a large cohort of stable kidney transplant recipients. Am J Transplant 2011;11:1965-71.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:146-150
DOI: 10.4274/BMJ.galenos.2022.2022.2-8

Research

Is There a Relation Between Pretreatment CONUT Score and Neoadjuvant Chemotherapy Response in Breast Cancer Patients?

Meme Kanserinde Tedavi Öncesi CONUT Skoru Neoadjuvan Kemoterapi Yanıtı ile İlişkili mi?

Ayşe İrem Yasin, Atakan Topçu

Bezmialem Vakıf University Faculty of Medicine, Department of Medical Oncology, Istanbul, Turkey

ABSTRACT

Objective: To evaluate the relationship between pre-treatment nutritional status and pathological complete response (pCR) rates in patients with breast cancer.

Methods: The study group consisted of 109 female patients. Clinicopathologic factors, pathological response status, and pre-treatment laboratory values of the patients were recorded retrospectively. CONUT score, consisting of total cholesterol, serum albumin, and total lymphocyte, was calculated to assess the nutritional status. Factors affecting pCR were evaluated, and the relationship between pCR and CONUT was analyzed.

Results: The mean age was 49.78±10.92. Thirty-two (29.4%) patients had pCR. The rate of pCR in the hormone-negative group was significantly higher than that in the hormone-positive group (p<0.001). Additionally, the pCR rate in the HER2+ group was significantly higher than that in the HER2- group (p<0.001). Patients with pCR had a significantly higher Ki67 index (p<0.001). There was no significant difference when the pCR rates of patients with high and low CONUT scores were compared.

Conclusion: This is the first study evaluating the association between pre-treatment CONUT score and pCR in breast cancer patients. There is a need for comprehensive studies to reveal the relationship more clearly.

Keywords: Breast cancer, CONUT score, neoadjuvant chemotherapy, nutrition, pathological complete response

ÖZ

Amaç: Neoadjuvan kemoterapi ile tedavi edilen meme kanseri hastalarında tedavi öncesi beslenme durumu ile patolojik tam yanıt (pTY) arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntem: Çalışmaya 109 kadın meme kanseri hastası dahil edildi. Hastalara ait klinikopatolojik faktörler, patolojik yanıt durumları ve tedavi öncesi laboratuvar değerleri hasta dosyalarından taranarak kaydedildi. Toplam kolesterol, serum albümin ve toplam lenfosit değerlerinden oluşan ve nutrisyonel durumu değerlendiren CONUT skoru valide edilmiş skorlama sistemine göre hesaplandı. pTY'yi etkileyen faktörler değerlendirildi. pTY ve CONUT arasındaki ilişki analiz edildi.

Bulgular: Ortalama yaş 49,78±10,92 idi. Otuz iki (%29,4) hastada pTY saptandı. Hormon negatif gruptaki pTY oranı, hormon pozitif gruptan anlamlı derecede yüksekti (p<0,001). Ek olarak, HER2+ grubundaki pTY oranı, HER2- grubundan anlamlı olarak yüksekti (p<0,001). pTY'li hastalarda Ki67 indeksi anlamlı olarak daha yüksekti (p<0,001). Hastalar yüksek ve düşük CONUT skoru gruplarına ayrıldığında gruplar arasında pTY açısından anlamlı fark yoktu.

Sonuç: Bu çalışma, meme kanserli hastalarda tedavi öncesi CONUT skoru ile pTY arasındaki ilişkiyi değerlendiren ilk çalışmadır. Geniş hasta gruplarında yapılacak kapsamlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Meme kanseri, CONUT skoru, neoadjuvan kemoterapi, beslenme, patolojik yanıt

Address for Correspondence: Ayşe İrem Yasin, Bezmialem Vakıf University Faculty of Medicine, Department of Medical Oncology, Istanbul, Turkey
E-mail: ayseiremyasin@gmail.com ORCID ID: orcid.org/0000-0002-1528-8065

Cite as: Yasin Aİ, Topçu A. Is There a Relation Between Pretreatment CONUT Score and Neoadjuvant Chemotherapy Response in Breast Cancer Patients?. Med J Bakirkoy 2022;18:146-150

Received: 17.02.2022
Accepted: 06.03.2022

INTRODUCTION

Neoadjuvant chemotherapy (NAC), which allows breast-conserving surgery and predicts chemotherapy response, is frequently used in breast cancer patients, the most common cancer among women (1-3). After NAC, a pathological complete response (pCR) is a validated prognostic survival factor (4). Survival times are longer in patients with pCR, particularly in triple-negative and HER2+ breast cancer (4). Response to NAC provides clinicians with prognostic information and guidance for further treatment options (5). Therefore, it is essential to define the factors affecting the NAC response.

Perioperative immune-nutritional status is considered a prognostic factor in different tumor types, including breast cancer (6). There are several scoring systems that evaluate the nutritional status of cancer patients (7). The prognostic nutritional index (PNI), one of these nutritional scoring systems, was predictive of pCR in breast cancer patients (8). Controlling nutritional status (CONUT), consisting of total cholesterol, total lymphocyte count, and serum albumin level, is another comprehensive scoring system that evaluates nutritional status (9). When CONUT and PNI were compared, CONUT was more predictive of the survival times of cancer patients (10). Several studies have demonstrated the impact of CONUT on survival outcomes in many types of cancer, including gastric cancer, lung cancer, esophageal cancer, mesothelioma, ovarian cancer, sarcoma, renal cell carcinoma, and hepatocellular carcinoma (9). Two recent studies from China identified that a high CONUT score was related to a worse prognosis in surgically treated breast cancer patients (10,11). Furthermore, preoperative high CONUT score was related to lower pCR in gastric cancer patients (12). This is the first study evaluating the relationship between pCR and CONUT score in patients with breast cancer.

METHODS

Patients

The data of patients followed up with breast cancer diagnosis in our Medical Oncology Clinic between January 2012-December 2021 were evaluated retrospectively. Of the 1,853 patients, 264 patients were treated with NAC. All biopsies and post-NAC surgical materials of the patients included in the study were evaluated at our center by experienced pathologists. Pre-treatment cholesterol, albumin, and lymphocyte values of 109 patients were obtained and recorded. All patients were female, aged between 18 and 80 years, had stage 2 or 3 breast cancer and underwent surgery. The tumor-node-metastasis staging was performed before NAC according to American Joint Committee on Cancer (13). NAC regimens they received before surgery were four cycles of doxorubicin and cyclophosphamide or epirubicin and cyclophosphamide every 21 days, followed by paclitaxel for twelve weeks. Trastuzumab was added to this regimen if the HER2 receptor was positive. HER2 positivity is defined as a 3+ score by immunochemistry or a 2+ score by immunochemistry and fluorescence *in situ* hybridization positivity (14). Scarff-Bloom-Richardson scheme was used for tumor grading (15). Estrogen (ER) and/or progesterone receptor positivity >1% in tumor nuclei by immunochemistry are accepted as hormone-positive disease (16). Miller-Payne criteria was used to assess the chemotherapy response, and no invasive disease was accepted as pCR (17). All demographic and clinicopathologic information of patients were recorded from the archive files. Two groups were created: pCR (+) and pCR (-). CONUT score was calculated according to the scoring system (Table 1) (18). Patients with a score ≥3 were defined in the high-CONUT group, and patients with a score <2 in the low-CONUT group (19). The data collection process was conducted according to the Helsinki Declaration. The study was approved by the Bezmialem

Table 1. CONUT scoring system

	Degree of malnutrition			
Parameter	**Normal**	**Mild**	**Moderate**	**Severe**
Serum albumin (g/dL)	≥3.50	3.00-3.49	2.50-2.99	<2.50
Albumin score	0	2	4	6
Total lymphocyte (/mm^3)	≥1,600	1,200-1,599	800-1,199	<800
Lymphocyte score	0	1	2	3
Total cholesterol (mg/dL)	≥180	140-179	100-139	<100
Cholesterol score	0	1	2	3

CONUT score = Albumin score + Total lymphocyte score + Cholesterol score, CONUT: Controlling nutrional status

Vakıf University Ethics Committee (no: 2021-393, date: 08.02.2022).

Statistical Analysis

The data were analyzed with SPSS 22 Statistics program (IBM Corporation, NY, USA) for Windows (Microsoft Corporation, WA, USA). Descriptive statistics are shown as mean ± standard deviation for variables with a normal distribution. Nominal variables were demonstrated as the number of cases and percentage. Two independent groups were compared with the independent sample t-test. Categorical variables were analyzed using the chi-square test or Fisher's Exact test. Results with $p<0.05$ were accepted as statistically significant.

RESULTS

One hundred nine female breast cancer patients were included in the study. The mean age was 49.78±10.92 in the patient group. Seventy five (68.8%) patients had a hormone-positive disease, and 34 (31.2%) patients had a hormone-negative disease. The number of HER2- patients was 82 (75.2%), and the number of HER2+ patients was 27 (24.8%). pCR was detected in 32 (29.4%) patients. The rate of pCR in the hormone-negative group was significantly higher than that in the hormone-positive group ($p<0.001$). Additionally, the pCR rate in the HER2+ group was significantly higher than that in the HER2- group ($p<0.001$). Ki67 proliferation index was significantly high in the patient group with pCR ($p<0.001$) (Table 2).

According to the CONUT scoring system, the scores were calculated. Patients with a score ≥3 were defined in the high-CONUT group and patients with a score <2 in the low-CONUT group. Eleven (10.1%) patients were in the high-CONUT group, and 98 (89.9%) patients were in the low-CONUT group. When the pCR rates of the two groups were compared, there was no significance (Table 3).

DISCUSSION

This is the first study evaluating the relationship between pre-treatment CONUT score and pCR in breast cancer patients. The results demonstrated no significant difference in pCR rates between the high-CONUT and low-CONUT groups. However, hormone receptor negativity, HER2 positivity, and a high proliferation index were related to high pCR rates.

Growing evidence revealed that tumor progression, treatment tolerance, and survival of cancer patients are closely related to the nutritional and immune-inflammatory status (20). Albumin is a reliable serum marker for nutritional status and the immune-inflammatory system (21). Low albumin levels in cancer patients are associated with poor survival and an increased risk of cancer-related death (22). Cholesterol plays an essential role in the cell membrane and in biochemical reactions related to the immune response (23). The correlation of low cholesterol levels with poor prognosis was demonstrated in various types of cancer (24). Furthermore, the host immune response was insufficient in patients with low peripheral lymphocyte count (25,26).

CONUT score consists of serum albumin, total cholesterol, and lymphocyte count in peripheral blood (18). Recently, in two studies, CONUT was found to be predictive of prognosis in breast cancer patients (10,11). In these studies, patients did not receive NAC, and the relationship between CONUT and pCR was not assessed. However, in gastric cancer patients who received NAC, high CONUT was associated with low pCR (12). This can be explained by the undernutrition degree of patients with gastrointestinal

Table 2. Clinicopathological factors affecting pathological complete response

	Patients with pathologic complete response (n=34)	Patients without pathologic complete response (n=75)	p
Age (mean ± SD)	47.91±10.98	50.56±10.86	0.952
Hormone receptor (ER/PR) status			
Positive	10 (31.2%)	65 (84.4%)	<0.001*
Negative	22 (68.8%)	12 (15.6%)	
HER2 receptor status			
Positive	16 (50%)	11 (14.3%)	<0.001*
Negative	16 (50%)	66 (85.7%)	
Ki67, % (mean ± SD)	52.39±26.87	30.51±21.8	<0.001*

*Significant results, SD: Standard deviation, ER: Estrogen, PR: Progesterone

Table 3. The association between pre-treatment CONUT score and pCR

	pCR + patients (n=34)	pCR - patients (n=75)	p
CONUT score			0.874
High	5	6	
Low	29	69	

CONUT: Controlling nutritional status, pCR: Pathologic complete response

system cancers being higher than that of patients with other types of cancer (27). Additionally, breast cancer patients are relatively in the younger age group (3), so they have better performance and fewer comorbidities, causing impaired nutritional and immune-inflammatory status.

PNI, another scoring system for nutritional status, was found to be predictive of pCR in breast cancer patients (8). CONUT is considered a more comprehensive scoring system with an additional parameter, total cholesterol, and was superior to PNI for predicting nutritional status (11). However, the role of cholesterol in breast cancer patients is controversial (28). The risk of developing breast cancer is related to various commodities such as metabolic syndrome (28). Hyperlipidemia is a common entity in this metabolic syndrome (29). It has been shown that oxysterol plays a mitogenic role in ER-positive breast cancer, and that low-density lipoprotein receptors are upregulated in cancer cells (30). Moreover, there are some differences in cholesterol function between hormone positive and negative breast cancer subgroups (31). This complex role of cholesterol in the pathophysiology of breast cancer may have influenced the relationship between the CONUT score and pathological response. However, significance might be observed when the histological subgroups were evaluated separately.

There were some limitations to our study. First, we calculated the CONUT score based on the pre-treatment laboratory values of the patients. However, side effects during NAC might impair the nutritional status, and preoperative values after NAC may yield different results. Second, breast cancer patients are a heterogeneous patient group because of their different receptor status (1). Because we had a relatively small sample size, we could not perform subgroup analyses of different histological breast cancer types.

CONCLUSION

It is important to discover markers that predict pCR associated with longer survival times in breast cancer. Although CONUT was not associated with pCR in this study, further investigation into different histological subtypes is needed to clarify the impact of immune-nutritional status on treatment response and prognosis of patients. Thus, the way for personalized treatment options will be paved.

ETHICS

Ethics Committee Approval: The study was approved by the Bezmialem Vakıf University Ethics Committee (no: 2021-393, date: 08.02.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: A.İ.Y., A.T., Concept: A.İ.Y., A.T., Design: A.İ.Y., A.T., Data Collection or Processing: A.İ.Y., A.T., Analysis or Interpretation: A.İ.Y., A.T., Literature Search: A.İ.Y., A.T., Writing: A.İ.Y., A.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. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Charfare H, Limongelli S, Purushotham AD. Neoadjuvant chemotherapy in breast cancer. Br J Surg 2005;92:14-23.
3. von Minckwitz G, Blohmer JU, Costa SD, Denkert C, Eidtmann H, Eiermann W, et al. Response-guided neoadjuvant chemotherapy for breast cancer. J Clin Oncol 2013;31:3623-30.
4. Spring LM, Fell G, Arfe A, Sharma C, Greenup R, Reynolds KL, et al. Pathologic Complete Response after Neoadjuvant Chemotherapy and Impact on Breast Cancer Recurrence and Survival: A Comprehensive Meta-analysis. Clin Cancer Res 2020;26:2838-48.
5. Wang H, Mao X. Evaluation of the Efficacy of Neoadjuvant Chemotherapy for Breast Cancer. Drug Des Devel Ther 2020;14:2423-33.
6. Sun K, Chen S, Xu J, Li G, He Y. The prognostic significance of the prognostic nutritional index in cancer: a systematic review and meta-analysis. J Cancer Res Clin Oncol 2014;140:1537-49.
7. Castillo-Martínez L, Castro-Eguiluz D, Copca-Mendoza ET, Pérez-Camargo DA, Reyes-Torres CA, Ávila EA, et al. Nutritional Assessment Tools for the Identification of Malnutrition and Nutritional Risk Associated with Cancer Treatment. Rev Invest Clin 2018;70:121-5.
8. Büyükşimşek M, Oğul A, Mirili C, Paydaş S. Inflammatory Markers Predicting Pathological Complete Response in Cases with Breast Cancer Treated by Neoadjuvant Chemotherapy. Eur J Breast Health 2020;16:229-34.
9. Kheirouri S, Alizadeh M. Prognostic Potential of the Preoperative Controlling Nutritional Status (CONUT) Score in Predicting Survival of Patients with Cancer: A Systematic Review. Adv Nutr 2021;12:234-50.
10. Li W, Li M, Wang T, Ma G, Deng Y, Pu D, et al. Controlling Nutritional Status (CONUT) score is a prognostic factor in patients with resected breast cancer. Sci Rep 2020;10:6633.
11. Huang ZZ, Song CG, Huang JJ, Xia W, Bi XW, Hua X, et al. Prognostic significance of the Controlling Nutritional Status

(CONUT) score in surgically treated breast cancer patients. Gland Surg 2020;9:1370-9.

12. Jin H, Zhu K, Wang W. The Predictive Values of Pretreatment Controlling Nutritional Status (CONUT) Score in Estimating Short- and Long-term Outcomes for Patients with Gastric Cancer Treated with Neoadjuvant Chemotherapy and Curative Gastrectomy. J Gastric Cancer 2021;21:155-68.
13. Sahin AA, Gilligan TD, Caudell JJ. Challenges With the 8th Edition of the AJCC Cancer Staging Manual for Breast, Testicular, and Head and Neck Cancers. J Natl Compr Canc Netw 2019;17:560-4.
14. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ; Panel members. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011;22:1736-47.
15. Bansal C, Singh US, Misra S, Sharma KL, Tiwari V, Srivastava AN. Comparative evaluation of the modified Scarff-Bloom-Richardson grading system on breast carcinoma aspirates and histopathology. Cytojournal 2012;9:4.
16. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med 2010;134:e48-72.
17. Ogston KN, Miller ID, Payne S, Hutcheon AW, Sarkar TK, Smith I, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. Breast 2003;12:320-7.
18. Ignacio de Ulíbarri J, González-Madroño A, de Villar NG, González P, González B, Mancha A, et al. CONUT: a tool for controlling nutritional status. First validation in a hospital population. Nutr Hosp 2005;20:38-45.
19. Toyokawa T, Kubo N, Tamura T, Sakurai K, Amano R, Tanaka H, et al. The pretreatment Controlling Nutritional Status (CONUT) score is an independent prognostic factor in patients with resectable thoracic esophageal squamous cell carcinoma: results from a retrospective study. BMC Cancer 2016;16:722.
20. Mantzorou M, Koutelidakis A, Theocharis S, Giaginis C. Clinical Value of Nutritional Status in Cancer: What is its Impact and how it Affects Disease Progression and Prognosis? Nutr Cancer 2017;69:1151-76.
21. Krishnasamy K, Li Yoong T, Mei Chan C, Peng Choong L, Chinna K. Identifying Malnutrition: Nutritional Status in Newly Diagnosed Patients With Cancer. Clin J Oncol Nurs 2017;21:E23-9.
22. Lis CG, Grutsch JF, Vashi PG, Lammersfeld CA. Is serum albumin an independent predictor of survival in patients with breast cancer? JPEN J Parenter Enteral Nutr 2003;27:10-5.
23. Strasak AM, Pfeiffer RM, Brant LJ, Rapp K, Hilbe W, Oberaigner W, et al. Time-dependent association of total serum cholesterol and cancer incidence in a cohort of 172,210 men and women: a prospective 19-year follow-up study. Ann Oncol 2009;20:1113-20.
24. Mayengbam SS, Singh A, Pillai AD, Bhat MK. Influence of cholesterol on cancer progression and therapy. Transl Oncol 2021;14:101043.
25. Vicente Conesa MA, Garcia-Martinez E, Gonzalez Billalabeitia E, Chaves Benito A, Garcia Garcia T, Vicente Garcia V, et al. Predictive value of peripheral blood lymphocyte count in breast cancer patients treated with primary chemotherapy. Breast 2012;21:468-74.
26. Hoskin PJ, Rojas AM, Peiris SN, Mullassery V, Chong IY. Pre-treatment haemoglobin and peripheral blood lymphocyte count as independent predictors of outcome in carcinoma of cervix. Clin Oncol (R Coll Radiol) 2014;26:179-84.
27. Deftereos I, Kiss N, Isenring E, Carter VM, Yeung JM. A systematic review of the effect of preoperative nutrition support on nutritional status and treatment outcomes in upper gastrointestinal cancer resection. Eur J Surg Oncol 2020;46:1423-34.
28. Nazih H, Bard JM. Cholesterol, Oxysterols and LXRs in Breast Cancer Pathophysiology. Int J Mol Sci 2020;21:1356.
29. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA 1999;282:1523-9.
30. Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, et al. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. Nat Med 2007;13:1185-92.
31. Torres-Luquis O, Madden K, N'dri NM, Berg R, Olopade OF, Ngwa W, et al. LXR/RXR pathway signaling associated with triple-negative breast cancer in African American women. Breast Cancer (Dove Med Press) 2018;11:1-12.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:151-156
DOI: 10.4274/BMJ.galenos.2022.2022.1-16

Research

The Effect of WT-1 Positivity on Survival Outcomes in Patients with High-grade Serous Epithelial Ovarian Cancer

High-grade Seröz Epitelyal Over Kanserli Hastalarda WT-1 Pozitifliğinin Sağkalım Sonuçlarına Etkisi

Sema Karakaş[1], Aysun Fendal Tunca[1], Serdar Altınay[2], Güneş Özlem Yıldız[3], Murat Ekin[1]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Gynecology and Obstetrics, Istanbul, Turkey
[2]University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital, Clinic of Pathology, Istanbul, Turkey
[3]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Anesthesiology and Reanimation, Istanbul, Turkey

ABSTRACT

Objective: This study investigated the significance WT-1 positivity on survival outcomes in patients with high-grade serous epithelial ovarian cancer (HGSOC).

Methods: The medical records of the women who underwent surgery due to HGSOC between January 2015 and January 2020 were recorded. The patients' basic characteristic, serum tumor markers, disease stage, chemotherapeutic response, overall survival (OS), and progression-free survival (PFS) were recorded. WT-1 immune-staining was performed for all tissue samples.

Results: The median age of the patients in the WT-1 (+) group was 59 (40-88) years, and it was 52 (41-78) in the WT-1 (-) group that was significantly higher in the WT-1 (+) group (p=0.007). The median Ca125 level was significantly higher in the WT-1 (+) group than the WT-1 (-) group [634 (7-12,580) vs. 274 (12-2,856), p=0.03]. The PFS [22 (8-54) vs. 12 (0-48), p<0.001] and OS [30 (8-56) vs. 16 (2-70), p<0.001] rates were significantly higher in the WT-1 (-) group than the WT-1 (+) group in Cox regression analysis, Ca125 was significantly associated with OS in univariate analysis (HR:1, 95% confidence interval:1-1.001, p=0.005).

Conclusion: The patients with WT-1 positivity was associated with significantly shorter PFS and OS. Besides the patients in the WT-1 (+) group was older and had higher Ca125 levels than the WT-1 (-) group. The number of postmenopausal patients was higher in the WT-1 (+) group.

Keywords: Ovarian cancer, prognosis, survival, tumor marker, SWT-1

ÖZ

Amaç: Bu çalışma, high-grade seröz epitelyal over kanserli (HGSOC) hastalarda WT-1 pozitifliğinin sağkalım sonuçlarına etkisini araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Ocak 2015-Ocak 2020 tarihleri arasında HGSOC nedeniyle ameliyat olan hastaların tıbbi kayıtları değerlendirildi. Hastaların temel özellikleri, serum tümör belirteçleri, hastalık evresi, kemoterapötik yanıt, genel sağkalım (OS) ve progresyonsuz sağkalım (PFS) parametreleri analiz edildi. Tüm doku örnekleri için WT-1 immün boyama yapıldı.

Bulgular: WT-1 (+) grubundaki hastaların ortanca yaşı 59 (40-88) yıl olarak saptandı. WT-1 (-) grubunda ortanca yaş 52 (41-78) olup, WT-1'de anlamlı olarak daha yüksekti. Medyan Ca125 düzeyi, WT-1 (+) grubunda WT-1 (-) grubuna [634 (7-12.580) ve 274 (12-2.856), p=0,03] göre anlamlı derecede yüksekti. PFS [22 (8-54) vs 12 (0-48), p<0,001] ve OS [30 (8-56) vs 16 (2-70), p<0,001] oranları diğer grupta anlamlı olarak daha yüksekti. WT-1 (-) grubu, WT-1 (+) grubuna göre Cox regresyon analizinde, Ca125, tek değişkenli analizde OS ile anlamlı şekilde ilişkiliydi (HR:1, %95 güven aralığı:1-1,001, p=0,005).

Sonuç: WT-1 pozitifliği olan hastalar, önemli ölçüde daha kısa PFS ve OS ile ilişkilendirildi. Ayrıca WT-1 (+) grubundaki hastalar WT-1 (-) grubuna göre daha yaşlı ve Ca125 düzeyleri daha yüksekti. WT-1 (+) grubunda postmenapozal hasta sayısı daha fazlaydı.

Anahtar Kelimeler: Over kanseri, prognoz, sağkalım, tümör belirteci, SWT-1

Address for Correspondence: Sema Karakaş, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Gynecology and Obstetrics, Istanbul, Turkey
Phone: +90 532 709 03 99 E-mail: semaakyildiz@hotmail.com ORCID ID: orcid.org/0000-0002-2795-4766

Cite as: Karakaş S, Fendal Tunca A, Altınay S, Yıldız GÖ, Ekin M. The Effect of WT-1 Positivity on Survival Outcomes in Patients with High-grade Serous Epithelial Ovarian Cancer. Med J Bakirkoy 2022;18:151-156

Received: 01.02.2022
Accepted: 08.04.2022

INTRODUCTION

Ovarian cancer (OC) is with a high mortality rate and accounts for the most common gynecological malignancy. The increased mortality depends on the lack of symptoms in the early stage of the disease and insufficient diagnostic tools. The reported overall survival (OS) is quite low at 29%-51% in advanced-stage disease. The higher rates of recurrences, residual disease, and chemoresistance also reduce the expected OS rates (1,2).

The *Wilms' tumor-1 (WT-1)* gene is a well-known tumor suppressor gene mainly associated with WT development (3). The expression of the *WT-1* gene is found in the kidney, ovary, testis, spleen, endothelial cells, and mesothelium (3,4). Regarding OC, WT-1 expression is used to differentiate serous phenotypes from endometrial type (5,6). A moderate-to-strong expression of WT-1 was reported in more than 50% of the tumor cells in 94.7% of patients with ovarian serous carcinoma (5-7).

Previous studies have evaluated the relation between the expression rate of WT-1 and OS in various neoplasms (5,8). In a study by Miyoshi et al. (8), the high expression of WT-1 was associated with a poor prognosis in patients with breast adenocarcinoma. However, to date, there is a lack of knowledge regarding the effect of WT-1 positivity and survival rates in women with HGSOCs.

Therefore, this study evaluated the effect of preoperative WT-1 positivity on the survival outcomes of the patients with HGSOCs.

METHODS

A retrospective cohort study was conducted with the medical records of 77 patients out of 205 serous OC patients who underwent surgery with WT-1 immune-staining evaluation and confirmed diagnosis of HGSOC between January 2015 and January 2020 in a tertiary referral hospital. After maintaining the Bakirkoy Dr. Sadi Konuk Training and Research Hospital Ethics Committee approval (decision no: 2021-03-02, date: 15.02.2021), Helsinki guidelines and ethical considerations on human studies were maintained. Informed consent was not obtained since the medical record of the women were used anonymously.

The data of women who had a pathological diagnosis of HGSOC with a regular clinical follow-up and WT-1 immune-staining results were included. Any women with an active infection, hematologic, liver or kidney neoplasms, and autoimmune disorders were excluded.

The basic characteristic such as age, body mass index (BMI), menopausal status, Ca125 levels, FIGO stage, the presence of residual disease, number of surgically removed pelvic and para-aortic lymph nodes, response to chemotherapy, time interval of follow-up, the length of hospitalization was recorded. The last recruited patient record was obtained in November 2020. The patients had a routine gynecological examination, computed tomography scan, and Ca-125 evaluation every 2-3 months in the first two years and then every 4-6 months. R0 was defined as no residual tumor on the main evaluation after the operation. Chemosensitivity was determined as the time interval between terminating the last chemotherapy dose and the presence of recurrence after more than 6 months. OS was defined as the time between treatment initiation till to death or the last follow-up. The term progression-free survival (PFS) was used for the treatment initiation time until the date when recurrence or progression consisted.

WT-1 Analysis

Formalin-fixed paraffin-embedded tissues were sliced at a thickness of 4 microns. Predilute ready-to-use (Cell marqure, 6F-H2, USA, Lot V0002192) primary antibodies were used, and immunohistochemical stainings were performed in a Ventana BenchMark XT (Roche, Mannheim, Germany) instrument following protocols proposed by the manufacturer (Figure 1-3).

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Descriptive data were presented as mean, standard deviation, median (minimum-maximum), number, and percentages. The Shapiro-Wilk test was used to evaluate the distribution of the continuous data. Student t-test or Mann-Whitney U tests were used to evaluate the quantitative data. The chi-square test or Fisher's Exact test was used to examine qualitative data.

Kaplan-Meier test was used to evaluate OS and PFS rates. Univariate and multivariate Cox regression model was used to define the prognostic factors that could affect OS. The potential factors that could affect OS, such as age, FIGO tumor stage, the presence of residual disease, chemotherapeutic response, Ca-125, WT-1 positivity, menopausal status, were used in regression analysis. A p-value of <0.05 was considered statistically significant.

RESULTS

The median age of the patients in the WT-1 (+) group was 59 (40-88) years, and it was 52 (41-78) in the WT-1 (-) group that was significantly higher in the WT-1 (+) group ($p=0.007$).

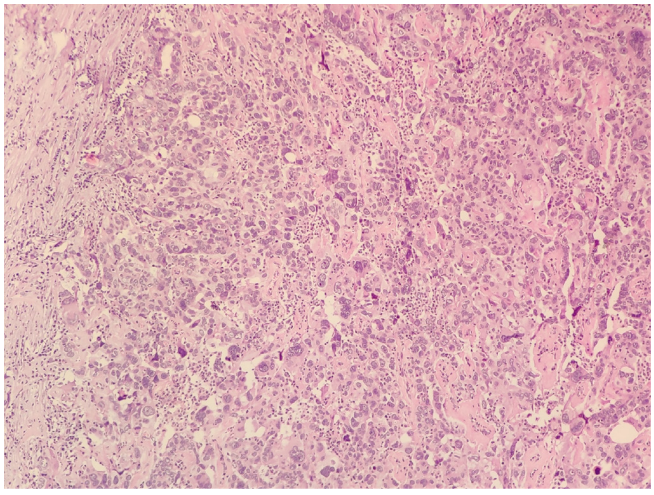

Figure 1. The picture shows atypical tumor cells with pleomorphic vesicular nuclei (H&Ex200)

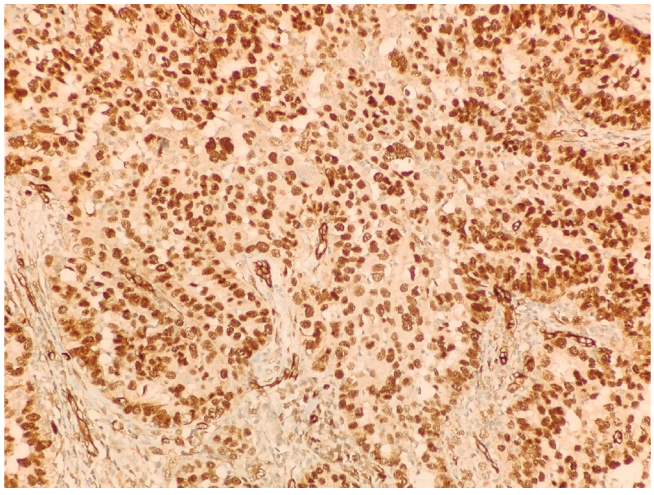

Figure 2. High nuclear positive staining with WT-1 in tumor cells (WT-1 immune-staining x200)
WT: Wilms' tumor

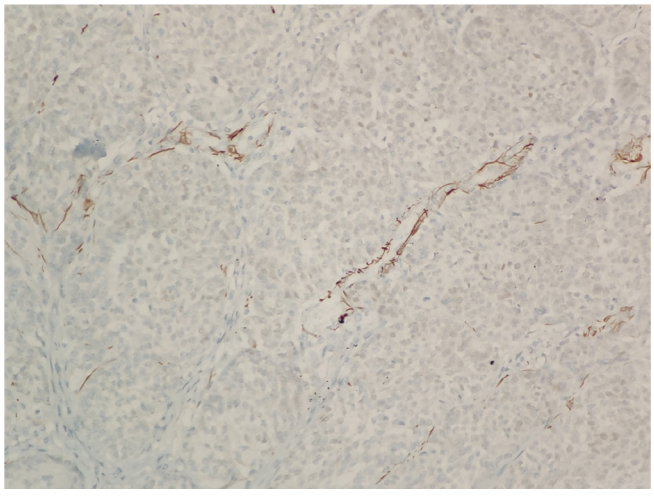

Figure 3. Negative staining with WT-1 in tumor cells (WT-1 immune-staining x200)
WT: Wilms' tumor

No significant differences were observed between the study groups regarding BMI, number of pelvic and para-aortic lymph nodes, and hospital stay length ($p>0.05$, for all comparisons). The median Ca125 level was significantly higher in the WT-1 (+) group than the WT-1 (-) group [634 (7-12,580) vs. 274 (12-2,856), $p=0.03$]. The PFS [22 (8-54) vs. 12 (0-48), $p<0.001$) and OS [30 (8-56) vs. 16 (2-70), $p<0.001$] rates were significantly higher in the WT-1 (-) group than the WT-1 (+) group (Table 1).

There was no significant difference between the study groups regarding FIGO stage, chemosensitivity, the presence of metastatic pelvic para-aortic lymph nodes, recurrence, residual disease ($p>0.05$, for all comparisons). The number of postmenopausal patients was significantly higher in the WT-1 (+) group than the WT-1 (-) group [43 (89.6%) vs. 18 (62.1%), $p=0.004$] (Table 2). There were six deaths in the WT-1 (+) group, and no death was observed in the WT-1 (-) group. In Cox regression analysis, Ca125 was significantly related to univariate analysis (HR:1, 95% confidence interval:1-1.001, $p=0.005$). However, no significance was observed in multivariate analysis for the Ca125 level. The HR was 71.6 for WT-1 positivity considering OS. However, this was not reached statistical significance. Besides, no significance was observed regarding age, FIGO stage, residual disease, chemotherapy response, menopausal status in univariate Cox regression analysis (Table 3).

DISCUSSION

The patients with WT-1 positivity were associated with significantly shorter PFS and OS. The patients in the WT-1 (+) group were older and had higher Ca125 levels than the WT-1 (-) group. The number of postmenopausal patients was higher in the WT-1 (+) group.

Since OC remains one of the prevalent gynecological malignancies with higher mortality rates, recent studies have focused on a noninvasive marker to aid early cancer diagnosis (9). In this context, it was reported that WT-1 expression could be characteristic for the differential diagnosis of serous OCs. Its expression is much lesser in other OC subtypes (9). Al-Hussaini et al. (10) reported a 94.7% of WT-1 expression in serous OCs. They concluded that WT-1 positivity could be associated with poorly differentiated ovarian neoplasm morphology.

The presence of WT-1 was investigated to differentiate it from the tumors with adenocarcinoma morphology arising at other sites (5-7). Şakirahmet Şen et al. (11) conducted a study to evaluate the benefits of WT-1 antigen. The authors concluded that the ovarian adenocarcinoma slides were positively stained with WT-1 in 82.5% of the patients

(11). In another study, Hylander et al. (12) reported that WT-1 could significantly predict the prognosis of ovarian adenocarcinoma. Moreover, they concluded that WT-1 expression could be correlated with tumor grade and tumor stage, but they found no relationship with the survival (12).

In previous studios WT-1 was significantly associated serous OCs, especially in high-grade cancers sensitivity varying between 64-96% (13-17). Moreover, it was reported that serous borderline ovarian tumors related to early-stage OCs were negative or weak expression of WT-1 (10). In our study, shorter PFS and OS rates were observed in patients WT-1 positivity. We may speculate that WT-1 could be presented in high-grade tumors due to increased affinity to endothelial tissue since positive staining of WT-1 was noted in endothelial cells (5).

Table 1. Comparison of the study groups regarding basic characteristic and survival rates

		WT-1 negative (n=29)	WT-1 positive (n=48)	p-value
Age (years)		52 (41-78)	59 (40-88)	**0.007**
BMI (kg/m^2)		30.7 (16-49)	32.5 (23-53)	0.63
Menopause	No	11 (37.9%)	5 (10.4%)	**0.004**
	Yes	18 (62.1%)	43 (89.6%)	
Preoperative Ca125 U/mL		274 (12-2,856)	634 (7-12,580)	**0.03**
Number of pelvic lymph node		17 (0-35)	14 (0-38)	0.65
Number of paraaortic lymph nodes		17 (0-35)	16 (0-46)	0.27
PFS (months)		22 (8-54)	12 (0-48)	**<0.001**
OS (months)		30 (8-56)	16 (2-70)	**<0.001**
Mean follow-up (months)		24 (8-56)	16 (2-70)	**0.001**
Hospitalization (days)		10 (7-18)	10.5 (3-30)	0.18

BMI: Body mass index, PFS: Progression-free survival, OS: Overall survival, WT: Wilms' tumor

Table 2. Comparison of the study groups regarding surgical outcomes and response to the treatment

		WT-1 negative (n=29)	WT-1 positive (n=48)	p-value
FIGO stage	I	6 (20.7%)	4 (8.3%)	0.17
	II	3 (10.3%)	5 (10.4%)	
	III	19 (65.5%)	38 (79.2%)	
	IV	1 (3.4%)	1 (2.1%)	
Chemotherapeutic response	Sensitive	29 (100%)	43 (89.6%)	0.08
	Resistant	0 (0)	5 (10.4%)	
The presence of metastatic pelvic lymph node	No	20 (69%)	35 (72.9%)	0.71
	Yes	9 (31%)	13 (27.1%)	
The presence of metastatic paraaortic lymph node	No	19 (65.5%)	33 (68.8%)	0.71
	Yes	10 (34.5%)	15 (31.3%)	
Recurrence	No	18 (62.1%)	32 (66.7%)	0.68
	Yes	11 (37.9%)	16 (33.3%)	
Residual disease	No	24 (82.8%)	42 (87.5%)	0.39
	Yes	5 (17.2%)	6 (12.5%)	
Mortality	No	29 (100%)	42 (87.5%)	0.052
	Yes	0 (0)	6 (12.5%)	

WT: Wilms' tumor

Table 3. Cox regression analysis considering the variables that could affect on OS

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (years)	1.02	0.94-1.09	0.69	-	-	-
FIGO stage	2.6	0.45-14.83	0.28	-	-	-
WT-1 positivity	71.6	0.09-533	0.21	-	-	-
Residual disease	0.03	0.1-62	0.51	-	-	-
Chemotherapeutic response	2.7	0.31-23.21	0.36	-	-	-
Menopause	-	-	-	-	-	-
Preoperative Ca125 U/mL	1	1-1.001	**0.005**	1	1-1.001	0.13

OS: Overall survival, HR: Hazard ratio , CI: Confidence interval, WT: Wilms' tumor

Study Limitations

The limitations of our study can be considered its retrospective design and the small number of patients. Besides, we could not report the expression percentage of WT-1. Our study presented the results of a single oncology center, and thus further multicenter studies could confirm our results. However, this is the first study that has evaluated the relation between WT-1 positivity and survival rates in patients with HGSOC.

CONCLUSION

In conclusion, the patients with WT-1 positivity were with significantly shorter PFS and OS. Besides, the patients in the WT-1 (+) group were older and had higher Ca125 levels. In regression analysis only Ca125 was associated with OS.

ETHICS

Ethics Committee Approval: Approval for this study was obtained from the Clinical Research Ethics Committee of Bakırköy Dr. Sadi Konuk Training and Research Hospital (decision no: 2021-03-02, date: 15.02.2021).

Informed Consent: Informed consent was not obtained since the medical record of the women were used anonymously.

Authorship Contributions

Surgical and Medical Practices: S.K., S.A., Concept: S.K., M.E., Design: S.K., Data Collection or Processing: S.A., Analysis or Interpretation: S.A., Literature Search: A.F.T., G.Ö.Y., Writing: S.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. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin 2018;68:284-96.
2. Ogino S, Galon J, Fuchs CS, Dranoff G. Cancer immunology--analysis of host and tumor factors for personalized medicine. Nat Rev Clin Oncol 2011;8:711-9.
3. Pritchard-Jones K, Hastie ND. Wilms' tumour as a paradigm for the relationship of cancer to development. Cancer Surv 1990;9:555-78.
4. Charles AK, Mall S, Watson J, Berry PJ. Expression of the Wilms' tumour gene WT1 in the developing human and in paediatric renal tumours: an immunohistochemical study. Mol Pathol 1997;50:138-44.
5. Goldstein NS, Bassi D, Uzieblo A. WT1 is an integral component of an antibody panel to distinguish pancreaticobiliary and some ovarian epithelial neoplasms. Am J Clin Pathol 2001;116:246-52.
6. Goldstein NS, Uzieblo A. WT1 immunoreactivity in uterine papillary serous carcinomas is different from ovarian serous carcinomas. Am J Clin Pathol 2002;117:541-5.
7. Lee BH, Hecht JL, Pinkus JL, Pinkus GS. WT1, estrogen receptor, and progesterone receptor as markers for breast or ovarian primary sites in metastatic adenocarcinoma to body fluids. Am J Clin Pathol 2002;117:745-50.
8. Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, et al. High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. Clin Cancer Res 2002;8:1167-71.
9. Shimizu M, Toki T, Takagi Y, Konishi I, Fujii S. Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. Int J Gynecol Pathol 2000;19:158-63.
10. Al-Hussaini M, Stockman A, Foster H, McCluggage WG. WT-1 assists in distinguishing ovarian from uterine serous carcinoma and in distinguishing between serous and endometrioid ovarian carcinoma. Histopathology 2004;44:109-15.
11. Şakirahmet Şen D, Gökmen Karasu AF, Özgün Geçer M, Karadayı N, Ablan Yamuç E. Utilization of Wilms' tumor 1 antigen in a panel for differential diagnosis of ovarian carcinomas. Turk J Obstet Gynecol 2016;13:37-41.
12. Hylander B, Repasky E, Shrikant P, Intengan M, Beck A, Driscoll D, et al. Expression of Wilms tumor gene (WT1) in epithelial ovarian cancer. Gynecol Oncol 2006;101:12-7.
13. Acs G, Pasha T, Zhang PJ. WT1 is differentially expressed in serous, endometrioid, clear cell, and mucinous carcinomas of the

peritoneum, fallopian tube, ovary, and endometrium. Int J Gynecol Pathol 2004;23:110-8.

14. Wang X, Dupont J, Marshall DS. WT1 expression in ovarian and endometrial carcinomas using tissue microarrays. Modern Pathol 2003;16:213A-4.
15. Zhang PJ, Williams E, Pasha T, Acs G. WT1 is expressed in serous, but not in endometriod, clear cell or mucinous carcinomas of the peritioneum, Fallopian tube, ovaries and endometrium. Modern Pathol 2003;16:216a.
16. Hashi A, Yuminamochi T, Murata S, Iwamoto H, Honda T, Hoshi K. Wilms tumor gene immunoreactivity in primary serous carcinomas of the fallopian tube, ovary, endometrium, and peritoneum. Int J Gynecol Pathol 2003;22:374-7.
17. Rekhi B, Deodhar KK, Menon S, Maheshwari A, Bajpai J, Ghosh J, et al. Napsin A and WT 1 are useful immunohistochemical markers for differentiating clear cell carcinoma ovary from high-grade serous carcinoma. APMIS 2018;126:45-55.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:157-163
DOI: 10.4274/BMJ.galenos.2022.2021.12-10

Research

Evaluation of White Blood-cell-based Inflammatory Markers in Gestational Diabetes Mellitus

Gestasyonel Diabetes Mellitusta Beyaz Kan Hücresi Temelli Enflamatuvar Belirteçlerin Değerlendirilmesi

Zeynep Levent Cirakli[1], Nuray Gulec[2]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Medical Biochemistry Laboratory, Istanbul, Turkey
[2]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Medical Microbiology Laboratory, Istanbul, Turkey

ABSTRACT

Objective: It was aimed to evaluate the white blood-cell-based inflammatory markers in the normal pregnancy reference value range in terms of the presence of low-grade systemic inflammation in gestational diabetes mellitus (GDM).

Methods: A total of 690 subjects were included in the study. GDM was defined according to the criteria of the international association of diabetes and pregnancy study groups. Four hundred seventy six pregnant women with normal glucose tolerance (NGT) and 214 pregnant women with GDM were included in the control and the case groups, respectively.

Results: Age was statistically significantly higher in the GDM group than the NGT group (p=0.030). Hemoglobin levels, mean corpuscular volume levels and the mean hematocrit level were statistically significantly higher with no clinical significance in the NGT group, compared to the GDM group; (p=0.024), (p<0.001) and (p=0.008) respectively. Fasting plasma levels of glucose and post 75 g load glucose (oral glucose tolerance test); 1 h and 2 h plasma glucose were significantly higher in the GDM group than in the NGT group (p<0.001). No statistically significant differences in white blood cell count, neutrophil count, lymphocyte count, platelet count, mean platelet volume value, neutrophil-to-lymphocyte ratio value, the platelet-to-lymphocyte ratio value and the systemic immune- inflammation index value were found between the two groups.

Conclusion: There was no statistically significant difference between white blood cell-based inflammatory markers in normal pregnancy reference values between pregnant women with GDM and pregnant women with NGT in this study. White blood cell-based inflammatory markers can be useful in understanding the pathophysiology of GDM and future studies may provide further evidence on the role of white blood cell-based inflammatory markers as an indicator of subclinical inflammation in GDM.

Keywords: Gestational diabetes, inflammation, oral glucose tolerance test, platelet, white blood cells

ÖZ

Amaç: Gestasyonel diabetes mellitusta (GDM) düşük dereceli sistemik enflamasyon varlığı açısından gebelerde, normal gebelik referans değer aralığında, beyaz kan hücresi temelli enflamatuvar belirteçlerin değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Çalışmaya toplam 690 gebe dahil edildi. GDM, uluslararası diyabet ve gebelik çalışma grupları derneği kriterlerine göre tanımlandı. Normal glukoz toleransı (NGT) olan 476 gebe kontrol grubuna ve GDM olan 214 gebe olgu grubuna dahil edildi.

Bulgular: GDM grubunda yaş, NGT grubuna göre istatistiksel olarak anlamlı daha yüksekti (p=0,030). Hemoglobin, ortalama eritrosit hacmi ve ortalama hematokrit seviyesi, GDM grubuna kıyasla NGT grubunda klinik olarak anlamlı olmaksızın istatistiksel olarak anlamlı derecede yüksekti; sırasıyla (p=0,024), (p<0,001) ve (p=0,008). Açlık plazma glikoz seviyeleri ve 75 g yükleme sonrası glikoz (oral glukoz tolerans testi); 1 saat ve 2 saat plazma glikozu GDM grubunda NGT grubuna göre anlamlı derecede yüksekti (p<0,001). İki grup arasında beyaz kan hücresi sayısı, nötrofil sayısı, lenfosit sayısı, trombosit sayısı, ortalama trombosit hacmi değeri, nötrofil-lenfosit oranı değeri, trombosit-lenfosit oranı değeri ve sistemik immün enflamasyon indeksi değerinde istatistiksel olarak anlamlı fark bulunmadı.

Address for Correspondence: Zeynep Levent Çıraklı, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Medical Biochemistry Laboratory, Istanbul, Turkey
Phone: +90 121 414 71 71 (5070) E-mail: zlcirakli@gmail.com ORCID ID: orcid.org/0000-0001-9104-599X

Cite as: Cirakli ZL, Gulec N. Evaluation of White Blood-cell-based Inflammatory Markers in Gestational Diabetes Mellitus. Med J Bakirkoy 2022;18:157-163

Received: 30.09.2020
Accepted: 11.04.2022

Sonuç: Bu çalışmada, GDM'li gebeler ile NGT olan gebeler arasında normal gebelik referans değerlerinde beyaz kan hücresi temelli enflamatuvar belirteçler arasında istatistiksel olarak anlamlı bir fark yoktu. Beyaz kan hücresi temelli enflamatuvar belirteçler, GDM'nin patofizyolojisinin anlaşılmasında yararlı olabilir ve gelecekteki çalışmalar, GDM'de subklinik enflamasyonun bir göstergesi olarak beyaz kan hücre temelli enflamatuvar belirteçlerin rolü hakkında daha fazla kanıt sağlayabilir.

Anahtar Kelimeler: Gebelik diyabeti, enflamasyon, oral glukoz tolerans testi, trombosit, beyaz kan hücreleri

INTRODUCTION

Gestational diabetes mellitus (GDM) is a substantial and growing health concern in many parts of the world and is defined as carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or the first recognition during pregnancy (1). The condition is present when blood glucose values are above normal but still below those diagnostic of diabetes (2). Gestational diabetes is associated with both short- and long-term complications in both the mother and the child. Additionally, women with GDM are at increased risk of the development of type 2 diabetes mellitus (T2DM), after pregnancy (3).

The white blood cell (WBC) count increases during pregnancy, and both the lower and upper limit of the reference range during pregnancy are quite high, with a typical reference range of 6 10^9-16×10^9/L (4). To assist the survival of the fetus pregnancy is associated with normal physiological changes. During normal pregnancy, the innate immune system is activated but the adaptive immune system is suppressed. Peripheral circulation of pregnancy is characterized by an increased percentage of granulocytes (5) and particularly in the third trimester platelet (PLT) count decreases during pregnancy (6). Recently, inflammation play a role in GDM pathogenesis (7). Compared with normal pregnancy, GDM is characterized by increased insulin resistance and although the association between inflammation and GDM is a new discovery, the connection between inflammation and insulin resistance is well known. This is also supported by clinical as well as epidemiologic data. Women with GDM have even higher inflammatory markers such as tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) compared to women with a normal pregnancy (8).

Recently, several new WBC-based inflammatory markers have been introduced as prognostic markers: the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR) and the systemic immune-inflammation index (SII index) and they have been considered systemic inflammatory response markers. These are also defined as WBC-based inflammatory biomarkers and calculated from complete blood count (CBC). They have been reported to be useful in the diagnosis, follow up and survey of many systemic inflammatory processes and as a markers of increased immune response with chronic inflammation (9).

In this study, we evaluate for any low-grade systemic inflammation presence in GDM at normal pregnancy WBC range and whether white blood-cell-based inflammatory markers that are simple, accessible and cost-effective have any value in the prediction of gestational diabetes.

METHODS

This retrospective case-control study was conducted in Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey, with the approval of the Local Ethics Committee (decision no: 2019-08-04, date: 22.04.2019) in compliance with the Helsinki Declaration.

The study was designed as a retrospective, laboratory information system (LIS) based study of pregnant women who underwent their GDM screening by oral glucose tolerance test (oGTT) from August 2015 to January 2018.

A total of 690 subjects age within 18-47 years were included in the study. GDM was defined according to the criteria of the international association of diabetes and pregnancy study groups (IADPSG) (10). Four hundred seventy six pregnant women with normal glucose tolerance (NGT) and 214 pregnant women with GDM were included in the control and case groups, respectively.

The inclusion criteria were 1. Pregnant women who completed all tests required in this study and applied by 3-point 75 g of glucose 2-h oGTT between 24th and 28th week of pregnancy.

Exclusion criteria were as 1. Unavailable at least a data which planned to work from LIS, 2. Pregestational type 1 diabetes mellitus, 3. Pregestational T2DM, 4. Pregnant women evaluated as having overt diabetes mellitus according to the IADPSG criteria, 5. Pregnant women with systemic diseases or any gestational disease throughout the pregnancy period (preeclampsia, eclampsia) and with acute or chronic infections, 6. Total blood WBC count <6x10^9/L and >16x10^9/L (4).

All pregnant women who included in this study were screened for GDM using a 3-point 75 g of glucose 2-h oGTT between 24th and 28th week of pregnancy. The oGTT used standard procedures (2). Briefly, the test was performed in the morning after a 12 h overnight fast and collected fasting, 1-hour and 2-hour samples were from an antecubital vein in a sitting position. Cut-offs of IADPSG criteria are as follows:

Fasting plasma glucose (FPG)≥92 mg/dL, 1-hr post-load glucose: ≥180 mg/dL, 2-hr postload glucose: ≥153 mg/dL. A pregnant woman with one abnormal value was diagnosed with GDM using the IADPSG criteria.

The CBC parameters such as WBC, neutrophil count, lymphocyte count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), PLT, mean platelet volume (MPV), and the screening test glucose results were obtained from LIS. CBC parameters were the results measured in fasting samples taken just before the oGTT test was performed at the same time as which between 24th and 28th week of pregnancy. Glucose was measured by hexokinase-based enzymatic method using Roche Cobas C501 chemistry analyzer and commercial reagents (Roche Diagnostics GmbH, Mannheim, Germany) and calculated plasma level. CBC was performed using CELL-DYN RUBY analyzer (Abbott Diagnostic, Abbott Park, IL, USA).

The NLR was calculated on the basis of absolute peripheral neutrophil (N×10^9/L) and lymphocyte (L×10^9/L) blood counts, using the formula: NLR=N/L. The PLR was calculated on the basis of peripheral PLT (P×10^9/L) and lymphocyte (L×10^9/L) blood counts, using the formula: PLR=P/L. The SII index was calculated on the basis of peripheral PLT (P×10^9/L), neutrophil (N×10^9/L) and lymphocyte (L×10^9/L) blood counts, using the following formula: SII=P×N/L (9).

Statistical Analysis

All the data were collected in a computerized database for statistical analysis. Error control was done. Descriptive statistics [mean, standard deviation (SD), median, minimum, maximum, number] were generated for the two groups then monitored for conformity to the normal distribution by the Kolmogorov-Smirnov test. Mean SD was used for parameters that were normally distributed. Median was used for groups that were not distributed normally. The Mann-Whitney U test was used for the comparison of two groups in the variables that did not realize the normal distribution. Independent t-test analysis was used to determine correlational relationships between variables that provided the normal distribution. Spearman correlation analysis was used to determine correlational relationships between variables that did not provide the normal distribution. Receiver operating characteristic (ROC) curve analysis was performed to find the sensitivity and specificity of NLR, PLR and SI index in the prediction of normal pregnancy and GDM results.

Diagnostic performance (sensitivity, specificity, and positive and negative predictive values) at the best cut off point for NLR, PLR and SI index was calculated.

The analysis were performed using the NCSS11 (Number Cruncher Statistical System, 2017 Statistical Software) program and Med Calc Statistical Software version18 (Med Calc Software bvba, Ostend, Belgium; http://www.medcalc.org;2018).

A p-value ≤0.05 was set as statistically significant.

RESULTS

Characteristics of 690 pregnant women who were included in this study are shown in Table 1. According to the criteria of the IADPSG, pregnant women with NGT and with GDM were included in the control (n=476) and case (n=214) groups, respectively.

We found a significantly higher level of the GDM group compared to age matched NGT groups 31 (18-45), 29 (18-47) respectively; (p=0.030).

Hb levels were significantly higher in the NGT group, compared to GDM group 11.5 (8.4-15.0) g/dL, 11.4 (7.3-14.1) g/dL respectively; (p=0.024). Also, the mean Hct level in the NGT group was significantly higher, compared to GDM group 34.39±2.81% (mean ± SD), 33.75±3.16% respectively; (p=0.008).

Compared with the GDM group, MCV levels were statistically significantly higher in NGT group 87 (56-98) fL, 89 (62-105) fL, respectively; (p<0.001).

FPG levels were significantly higher in the GDM group than NGT group 97 (65-133) mg/dL, 83 (50-91) mg/dL, respectively; (p<0.001).

Post 75 g load glucose (oGTT); 1 h plasma glucose levels were significantly higher in the GDM group, compared to NGT group 151 (62-196) mg/dL, 141 (64-179) mg/dL, respectively; (p<0.001). Also, 2 h plasma glucose levels were significantly higher in the GDM group, compared to NGT group 135 (51-198) mg/dL, 116 (50-152) mg/dL, respectively; (p<0.001).

No statistically significant differences in WBC count, neutrophil count, lymphocyte count, PLT count, MPV value, NLR value, PLR value and SII Index value were found between the two groups (p>0.05).

The results of the ROC curve analysis for the diagnostic performance of gestational diabetes with NLR, PLR and SII index are presented in Figure 1 and in Table 2.

At a cut-off level of 3.6, NLR accurately diagnosed GDM [area under the curve (AUC)=0.538, 95% confidence interval 0.500-0.575, p=0.098] with sensitivity and specificity rates of 64% and 47% and positive and negative predictive values of 35.6% and 74.8%, respectively. At a cut-off level of 132 PLR,

Table 1. Characteristics of study subjects

	NGT n= 476	GDM n=214	p
Age	29 (18-47)	31 (18-45)	0.030
WBC count, (x10^9/L)	10.31 (6-16)	10.46 (6.34-16)	0.911
Neutrophil count, (x10^9/L)	7.45 (3.87-12.9)	7.64 (4.12-12.8)	0.513
Lymphocyte count, (x10^9/L)	2 (0.97-4.25)	1.95 (0.86-3.96)	0.317
Haemoglobin (g/dL)	11.5 (8.4-15.0)	11.4 (7.3-14.1)	0.024
Haematocrit (%)	[34.39±2.81]**	[33.75±3.16]**	0.008*
MCV (fL)	89 (62-105)	87 (56-98)	<0.001
Platelet count (x10^9/L)	236 (104-547)	236 (106-459)	0.933
MPV (fL)	8.54 (4.93-15.8)	8.61 (5.29-15.6)	0.645
NLR	3.73 (1.73-7.91)	3.94 (1.94-6.29)	0.113
PLR	114 (49-238)	118 (44-253)	0.257
SII	850 (297-1959)	897 (331-1898)	0.193
oGTT (24-28th week of gestation)			
FPG (mgL/dL)	83 (50-91)	97 (65-133)	<0.001
1-h post 75 g load	141 (64-179)	151 (62-196)	<0.001
2-h post 75 g load	116 (50-152)	135 (51-198)	<0.001

Data expressed as a median (IQR) or proportions. Differences evaluated by nonparametric Mann-Whitney U test or *Independent t-test, respectively. **[mean ± SD], p-value ≤0.05 was accepted as statistically significant.

NGT: Normal glucose tolerance, GDM: Gestational diabetes mellitus, WBC: White blood cell, MCV: Mean corpuscular volume, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune index, oGTT: Oral glucose tolerance test, FPG: Fasting plasma glucose

Table 2. Diagnostic performance of gestational diabetes with NLR, PLR and SII

Group	Cut-off	Sensitivity %	Specificity %	AUC	p-value	CI	PPV	NPV
NLR	3.6	64	47	0.538	0.098	0.500-0.575	35.6	74.8
PLR	132	37	71	0.527	0.267	0.489-0.565	37	71.7
SII	831	62	47	0.531	0.191	0.493-0.569	34.3	73.1

NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune index, CI: Confidence interval, AUC: Area under the curve, PPV: Positive predictive values, NPV: Negative predictive values

accurately diagnosed GDM [AUC=0.527, 95% confidence interval 0.489-0.565, p=0.267] with sensitivity and specificity rates of 37% and 71% and positive and negative predictive values of 37% and 71.7%, respectively. At a cut-off level of 831 SII index accurately diagnosed GDM [AUC=0.531, 95% confidence interval 0.493-0.569, p=0.191] with sensitivity and specificity rates of 62% and 47% and positive and negative predictive values of 34.3% and 73.1%, respectively.

DISCUSSION

In this retrospective case-control study, WBC-based inflammatory markers were evaluated at normal gestational WBC range for any low-grade systemic inflammation presence in GDM. Maternal age has been found to be a risk factor for GDM in many studies (11,12). The mean age of the pregnant women with GDM was significantly higher than the pregnant women with NGT in this study.

The total blood volume increases by about 1.5 L to supply the needs of the new vascular bed during pregnancy. Red cell mass also increases by 10%-20% but the net result is that Hb concentration falls (13). Consequently, Hb concentrations and Hct values decrease in pregnancy. In addition MCV is normally slightly increased (4). In this study, the levels of Hb, Hct and MCV were statistically significantly higher in the pregnant women with NGT, compared with the pregnant women with GDM.

The IADPSG criteria was developed based on the results of the Hyperglycemia and Adverse Pregnancy Outcomes study, which is a large multinational and multicenter study.

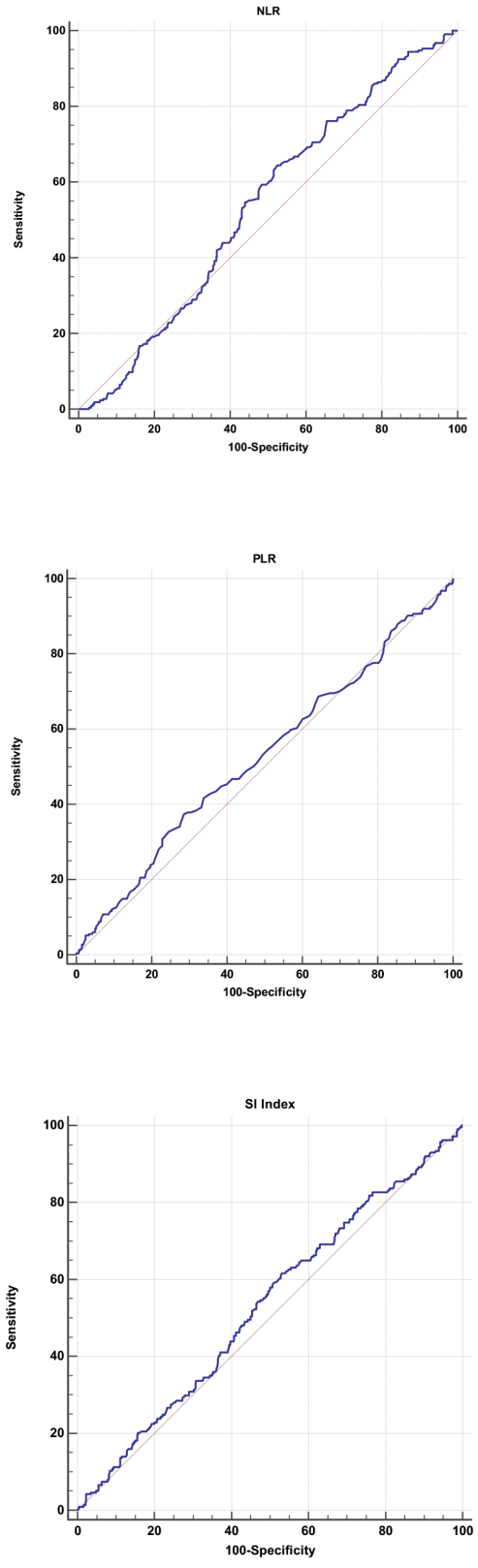

Figure 1. ROC curves of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index
PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, SI: Systemic immune-inflammation, ROC: Receiver operating characteristic

In accordance with the criteria of the IADPSG, independent of the other two values of the 75 g oGTT FPG≥92 mg/dL confirms GDM (10). FPG levels were found to be significantly higher in the GDM group than in the NGT group ($p<0.001$) in this study.

The immune system and metabolic system highly interact with each other to keep the body function properly (14). Additionally, studies have showing that metaflammation develops when this interaction is impaired (15). Recently, some evidence suggested that immune system disorders play a key role during the development of inflammation and that chronic low-grade inflammation is a major factor in the etiology of insulin resistance (16). With a general activation of the innate immune system T2DM is a chronic state of low-grade inflammation (17) and during normal pregnancy, the innate immune system is activated likewise (5). According to some authors, GDM represents an early stage in the natural history of T2DM (18). Inflammation may represent the pathophysiological link between GDM and the risk of future T2DM. Recently, some authors have reported that increased early pregnancy leukocyte count was associated with the results of GDM screening tests and increased risk of developing GDM. They also concluded that women who developed GDM display increased inflammation during early pregnancy approximately 20 weeks before the GDM was diagnosed. Physiological increase in insulin resistance associated with normal pregnancy and subclinical inflammation together manifest as GDM probably (19). There was no significant difference between the NGT group and GDM groups concerning WBC count and both neutrophil and lymphocyte counts in this study. In accordance with the purpose of the study, limitation of leukocyte level at normal pregnancy level may have affected the results.

In pregnancy, the PLT count is usually within normal, except during the third trimester when benign gestational thrombocytopenia can be observed (20). PLT volume is measured using MPV is a marker of PLT activation and function. In a study, while no statistically significant difference was observed in the PLT count, mean MPV value of the GDM group was evaluated to be significantly higher than the mean MPV value of the healthy pregnancy group in the last trimester (32-36 weeks) (21). This finding was further supported by another study (22). However in a recent study mean MPV value was observed to be lower in GDM and mean NLR and mean PLR values were statistically not different in patients with GDM as compared with healthy pregnant women (23). No statistically significant differences in PLT count and MPV value were found between the GDM group and NGT groups in this study.

Some studies have been conducted to assess the utility of NLR and PLR as inflammation biomarkers in GDM. In a study the mean NLR level and PLR level were significantly higher in pregnant women with GDM (24) and the other study the mean NLR level was significantly higher in pregnant women with GDM (25). Additionally NLR and PLR were evaluated in association with GDM in a recent study (26). In another study conducted in the first trimester of pregnancy, while mean NLR level and mean PLR level was similar in pregnant women with GDM and healthy pregnant group, mean MPV level was higher in pregnant women with GDM (27). It was shown that there was no relation between NLR and PLR with GDM in another study but the leukocyte counts were significantly higher during the second and early third trimesters in the women with GDM and the authors did not recommend use blood NLR and PLR as biomarkers for GDM screening (28). Similarly, no statistically significant differences in NLR and PLR and SII index were found between the GDM group and NGT group in this study. The results of the ROC curve analysis for the diagnostic performance of gestational diabetes with NLR, PLR and SII index were evaluated. The AUC values of them were considered to have poor predictive capabilities as screening tests and poor diagnostic capabilities as biomarkers according to the cut off values, which determined in this study. There is no literature on the relationship between GDM and the SII index. Here, the current study is the first study to investigate the relationship between GDM and SII index in normal pregnancy WBC reference value.

Screening and management can make better outcomes for women with GDM and their babies. Unfortunately, screening and diagnostic standards are not the same worldwide, so this might lead to underdiagnosis and undermanagement of the disease (29). There is no international consensus on the screening and diagnostic criteria for GDM currently. Additionally it was reported that because of large changes in inflammatory mediators during normal pregnancy, a comparison between studies is challenging. Meanwhile identifying circulating biomarkers that could lead to better tests to predict, diagnose, and monitor the progression of this important disease is essential (30).

This study has some limitations because of its retrospective nature. Due to this retrospective design, we do not have data on some parameters as body mass index and parity of women and data relating to the insulin levels or insulin resistance and inflammatory cytokines as IL-6 and TNF-α.

CONCLUSION

There was no statistically significant difference between WBC-based inflammatory markers in normal pregnancy reference values between pregnant women with gestational diabetes mellitus and pregnant women with NGT in this study. Additionally, they have poor predictive capabilities as screening tests and poor diagnostic capabilities as biomarkers. WBC-based inflammatory markers can be useful in understanding the pathophysiology of GDM and future studies may provide further evidence on the role of WBC-based inflammatory markers as an indicator of subclinical inflammation in GDM.

ETHICS

Ethics Committee Approval: This retrospective case-control study was conducted in Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey, with the approval of the Local Ethics Committee (decision no: 2019-08-04, date: 22.04.2019) in compliance with the Helsinki Declaration.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: Z.L.C., N.G., Concept: Z.L.C., Design: Z.L.C., N.G., Data Collection or Processing: Z.L.C., N.G., Analysis or Interpretation: Z.L.C., Literature Search: Z.L.C., Writing: Z.L.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. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1:Diagnosis and Classification of Diabetes Mellitus. Geneva, World Health Organization; 1999 (WHO/NCD/NCS/99.2).
2. World Health Organization. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva; 2013. Available from: https://apps.who.int/iris/handle/10665/85975
3. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet 2009;373:1773-9.
4. Pavord S, Hunt B eds. The Obstetric Hematology Manual. Cambridge University Press: 2010.
5. Luppi P, Haluszczak C, Trucco M, Deloia JA. Normal pregnancy is associated with peripheral leukocyte activation. Am J Reprod Immunol 2002;47:72-81.
6. Boehlen F, Hohlfeld P, Extermann P, Perneger TV, de Moerloose P. Platelet count at term pregnancy: a reappraisal of the threshold. Obstet Gynecol 2000;95:29-33.
7. Gomes CP, Torloni MR, Gueuvoghlanian-Silva BY, Alexandre SM, Mattar R, Daher S. Cytokine levels in gestational diabetes mellitus:

a systematic review of the literature. Am J Reprod Immunol 2013;69:545-57.

8. Richardson AC, Carpenter MW. Inflammatory mediators in gestational diabetes mellitus. Obstet Gynecol Clin North Am 2007;34:213-24, viii.
9. Fest J, Ruiter R, Ikram MA, Voortman T, van Eijck CHJ, Stricker BH. Reference values for white blood-cell-based inflammatory markers in the Rotterdam Study: a population-based prospective cohort study. Sci Rep 2018;8:10566.
10. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676-82.
11. Di Cianni G, Volpe L, Lencioni C, Miccoli R, Cuccuru I, Ghio A, et al. Prevalence and risk factors for gestational diabetes assessed by universal screening. Diabetes Res Clin Pract 2003;62:131-7.
12. Erem C, Kuzu UB, Deger O, Can G. Prevalence of gestational diabetes mellitus and associated risk factors in Turkish women: the Trabzon GDM Study. Arch Med Sci 2015;11:724-35.
13. Taylor DJ, Lind T. Red cell mass during and after normal pregnancy. Br J Obstet Gynaecol 1979;86:364-70.
14. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860-7.
15. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011;29:415-45.
16. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med 2012;18:1407-12.
17. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004;27:813-23.
18. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Del Prato S. Intermediate metabolism in normal pregnancy and in gestational diabetes. Diabetes Metab Res Rev 2003;19:259-70.
19. Wolf M, Sauk J, Shah A, Vossen Smirnakis K, Jimenez-Kimble R, Ecker JL, Thadhani R. Inflammation and glucose intolerance: a prospective study of gestational diabetes mellitus. Diabetes Care 2004;27:21-7.
20. Hellgren M. Hemostasis during normal pregnancy and puerperium. Semin Thromb Hemost 2003;29:125-30.
21. Bozkurt N, Yilmaz E, Biri A, Taner Z, Himmetoğlu O. The mean platelet volume in gestational diabetes. J Thromb Thrombolysis 2006;22:51-4.
22. Iyidir OT, Degertekin CK, Yilmaz BA, Toruner FB, Akturk M, Arslan M. Elevated mean platelet volume is associated with gestational diabetes mellitus. Gynecol Endocrinol 2014;30:640-3.
23. Mertoglu C, Gunay M, Gungor M, Kulhan M, Kulhan NG. A study of inflammatory markers in gestational diabetes mellitus. Gynecol Obstet Reprod Med 2019;2:1-5.
24. Aktulay A, Engin-Ustun Y, Ozkan MS, Erkaya S, Kara M, Kaymak O, et al. Gestational diabetes mellitus seems to be associated with inflammation. Acta Clin Croat 2015;54:475-8.
25. Yilmaz H, Celik HT, Namuslu M, Inan O, Onaran Y, Karakurt F, et al. Benefits of the neutrophil-to-lymphocyte ratio for the prediction of gestational diabetes mellitus in pregnant women. Exp Clin Endocrinol Diabetes 2014;122:39-43.
26. Sahbaz A, Cicekler H, Aynioglu O, Isik H, Ozmen U. Comparison of the predictive value of plateletcrit with various other blood parameters in gestational diabetes development. J Obstet Gynaecol 2016;36:589-93.
27. Vural Yılmaz Z, Akkaş Yılmaz E, Icer B, Kucukozkan T. Association of Complete Blood Count Parameters with Gestational Diabetes Mellitus. Gynecol Obstet Reprod Med 2017;23:65-9.
28. Sargın MA, Yassa M, Taymur BD, Celik A, Ergun E, Tug N. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios: are they useful for predicting gestational diabetes mellitus during pregnancy? Ther Clin Risk Manag 2016;12:657-65.
29. Reece EA, Leguizamón G, Wiznitzer A. Gestational diabetes: the need for a common ground. Lancet 2009;373:1789-97.
30. Lekva T, Norwitz ER, Aukrust P, Ueland T. Impact of Systemic Inflammation on the Progression of Gestational Diabetes Mellitus. Curr Diab Rep 2016;16:26.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:164-171
DOI: 10.4274/BMJ.galenos.2022.2021.12-9

Research

A Retrospective Evaluation of Children Diagnosed with Dermatomyositis: A Single-center Study

Dermatomiyozit Tanılı Çocuk Hastaların Retrospektif Değerlendirmesi: Tek Merkezli Bir Çalışma

Mahmut Aslan[1], Serdal Güngör[1], Yılmaz Tabel[2], Bilge Özgör[1], Serkan Kırık[1]

[1]İnönü University Faculty of Medicine, Department of Pediatric Neurology, Malatya, Turkey
[2]İnönü University Faculty of Medicine, Department of Pediatric Rheumatology, Malatya, Turkey

ABSTRACT

Objective: Juvenile idiopathic inflammatory myopathies are systemic autoimmune disorders that are characterized by chronic skeletal muscle inflammation, skin rashes and other systemic involvements. We analyzed the clinical findings, laboratory values at admission and treatment protocols and treatment responses of patients who were followed up with a diagnosis of juvenile dermatomyositis (JDM) in a department of pediatric neurology and rheumatology clinics.

Methods: Fifteen patients who were referred to the department of pediatric neurology and pediatric rheumatology clinics, diagnosed with JDM between 2010 and 2017 were evaluated retrospectively via their medical records.

Results: Of the study sample, 12 (80%) of the patients were female and 3 (20%) were male, and their mean age was 9.26±3.21 years. The mean time between complaint and diagnosis was 7.8±6 months, and the patients were followed up for 24.93±15.28 months after their diagnosis. The mean creatine kinase levels of the patients were 1.354±840 U/L. Fifteen (100%) of the patients had muscle weakness, 14 (93.3%) had Gottron's papules and 12 (80%) patients had a heliotrope rash. Ten (66.6%) underwent muscle biopsy, 9 (60%) underwent electromyography and 5 (33.3%) patients underwent muscle magnetic resonance imaging. All the patients were treated with corticosteroids and immunosuppressive agents.

Conclusion: JDM is a rare inflammatory myopathy observed during childhood. Better responses can be achieved by early diagnosis, intensive immunosuppressive therapy and effective physical therapy.

Keywords: Juvenile dermatomyositis, steroid, creatine kinase, muscle biopsy

ÖZ

Amaç: Juvenil dermatomiyozit deri bulguları, kas tutulumu ve diğer sistemik tutulum ile seyreden enflamatuvar otoimmün bir hastalıktır. Bu çalışmada çocuk nörolojisi ve romatoloji kliniklerince juvenil dermatomiyozit (JDM) tanısı ile izlenen hastaların klinik bulguları, laboratuvar değerleri, başvuru ve tedavi protokolleri ve tedavi yanıtlarını analiz etmeyi amaçladık.

Gereç ve Yöntem: 2010-2017 yılları arasında çocuk nöroloji ve çocuk romatoloji kliniğine sevk edilen ve JDM tanısı alan 15 hasta tıbbi kayıtlarla geriye dönük olarak değerlendirildi.

Bulgular: Çalışma örnekleminin 12'si (80%) kadın, 3'ü (%20) erkekti ve ortalama yaş 9,26±3,21 yıldı. Şikayet ile tanı arasındaki ortalama süre 7,8±6 aydı ve hastalar tanıdan sonra 24,93±15,28 ay takip edildi. Hastaların ortalama kreatinin kinaz seviyeleri 1,354±840 U/L idi. Hastaların tamamında kas güçsüzlüğü, 14'ünde (%93,3) Gottron papülleri ve 12'sinde (%80) heliotrope döküntüleri vardı. On (%66,6) hastaya kas biyopsisi, 9 (%60) hastaya elektromiyografi ve 5 (%33,3) hastaya kas manyetik rezonans görüntüleme uygulandı. Tüm hastalar kortikosteroidler ve immünosüpresif ajanlarla tedavi edildi.

Sonuç: JDM, çocukluk çağında görülen nadir bir enflamatuvar miyopatidir. Erken teşhis, yoğun immünosüpresif tedavi ve etkili fizik tedavi ile daha iyi yanıtlar elde edilebilir.

Anahtar Kelimeler: Juvenil dermatomiyozit, steroid, kreatin kinaz, kas biyopsisi

Address for Correspondence: Serkan Kırık, İnönü University Faculty of Medicine, Department of Pediatric Neurology, Malatya, Turkey
Phone: +90 505 577 14 80 E-mail: srknkrk@hotmail.com ORCID ID: orcid.org/0000-0002-8658-2448

Cite as: Aslan M, Güngör S, Tabel Y, Özgör B, Kırık S. A Retrospective Evaluation of Children Diagnosed with Dermatomyositis: A Single-center Study. Med J Bakirkoy 2022;18:164-171

Received: 21.04.2021
Accepted: 11.04.2022

INTRODUCTION

Juvenile idiopathic inflammatory myopathies are systemic autoimmune disorders that are characterized by chronic skeletal muscle inflammation, skin rashes and other systemic involvement. The most common form of these disorders observed during childhood is juvenile dermatomyositis (JDM), while polymyositis is relatively more common in adults (1). The etiology of JDM is still unclear, although it has been associated with environmental triggers, immune dysfunction and specific tissue response in the muscles, skin and small vessel endothelium in genetically susceptible individuals (1,2). No remarkable familial transmission has been demonstrated. The mean age of onset is 7-9 years, and it is twice as common in girls as in boys. The incidence of JDM is estimated to be between 0.19 and 4.1 per million (3).

The disease is characterized by proximal muscle weakness and cutaneous findings such as heliotrope rash, Gottron's papules and calcinosis, and it can also affect such internal organs as the lungs and heart. The disease is still diagnosed and classified according to the criteria established by Bohan and Peter (4) in 1975. These criteria include symmetrical proximal muscle weakness and characteristic rashes, as well as the demonstration of elevated serum muscle enzyme levels, electromyographical changes and inflammatory myositis in muscle biopsy. In addition to the characteristic skin rashes, at least two of these findings should be present for diagnosis, although the pediatric suitability of these criteria is limited, as some pediatric patients may have normal electromyography (EMG) and muscle biopsy findings, despite the presence of typical rashes (5). These are referred to as amyopathic dermatomyositis cases (1). Amyopathic dermatomyositis manifests only with skin findings, with patients presenting no other finding of muscle involvement. However, a detailed investigation of muscle strength and muscle biopsy often show muscular involvement (4).

Corticosteroids are the gold standard for treating JDM, reducing both acute and long-term morbidity. Most patients respond well to therapy, although the presence of calcinosis, lipodystrophy and contractures, as treatment-resistant factors, can lead to poor prognostic (1,6). New advanced therapeutic options, such as biological agents and autologous stem cell transplantations, have recently been shown to be useful, particularly in refractory patients with poor prognosis (1).

In this study, we evaluated the demographic characteristics, clinical findings, laboratory values at admission, treatment protocols and treatment responses of those patients who were followed up with a diagnosis of JDM in a department of pediatric neurology and pediatric rheumatology clinics.

METHODS

The medical records from the JDM registry of Inonu University Hospital in Turkey were retrospectively reviewed for the period between January 2010 and December 2017. A total of 20 patients were included in this study, but five patients were excluded due to missing data in their medical records. Therefore, 15 patients who were diagnosed with JDM and were followed up in pediatric neurology and pediatric rheumatology clinics, as the regional reference center in the eastern side of Turkey, between 2010 and 2017, were evaluated retrospectively. Their demographic characteristics, clinical findings, laboratory values at admission, treatment protocols and treatment responses were examined, and the findings of a detailed physical and neurological examination were reviewed. The creatine kinase (CK), aspartate transaminase (AST), alanine transaminase, lactate dehydrogenase, platelet (PLT), white blood cell, hemoglobin (HBG), C-reactive protein (CRP) levels and erythrocyte sedimentation rates (ESR) measured at the time of diagnosis were evaluated. The results of an antinuclear antibody (ANA) test, double-stranded DNA (dsDNA) tests, muscle biopsy, EMG and echocardiography performed for diagnostic and differential diagnostic purposes were reviewed. The treatments used during the acute phase of the disease and during maintenance treatments, as well as the responses of the patients to the treatment, were also evaluated. This study was approved by the institutional review board.

Statistical Analysis

All statistical analyses were performed using the SPSS statistics 22 software. All quantitative data are expressed as mean ± standard deviation. All categorical variables are expressed as number and percentage (n, %).

Approval this study was obtained by the Inonu University Institutional Ethics Committee (decision no: 2018/9-7, date: 24.04.2018). Informed consent was obtained from the parents of all the patients. The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

In total, 12 (80%) patients were female and three (20%) were male. The age range was 3 to 14 years and the mean age was 9.3±3.2 years. The time between the onset of symptoms and diagnosis ranged from 1 to 24 months, with a mean

value of 7.8±6 months. Patients were followed up for 10-60 months after being diagnosed, and the mean duration of follow-up was 24.9±15.3 months (Tables 1, 2).

Among the most common findings of JDM are those related to the skin. In our study, Gottron's papules (Figure 1) and heliotrope rashes (Figure 2) were present in 14 (93.3%) and 12 (80%) patients, respectively, while three patients (20%) were seen to have alopecia, and hand calcinosis and hand ulcers were observed in three patients (20%) and a single patient (6.6%), respectively, in their follow-up process, although these were not present at the time of diagnosis. The musculoskeletal system is often affected by JDM, and in this study, six patients (40%) had contractures of various joints, seven patients (46.6%) had arthritis/arthralgia, three patients (20%) had osteoporosis and a single patient (6.6%) had scoliosis. Furthermore, one (6.6%) patient had type 1 diabetes mellitus, two patients (13.3%) had repetitive pneumonia, a single patient (6.6%) had tuberculosis and a single patient (6.6%) died of sepsis. Gastrointestinal involvement is also common in patients with JDM. In this study, five patients (33.3%) were observed to have a gastrointestinal involvement, and of those, three had dysphagia, one had hepatomegaly and one had gastritis. Raynaud phenomenon was also noted in three (20%) patients. No ocular complications developed in any patient (Table 3).

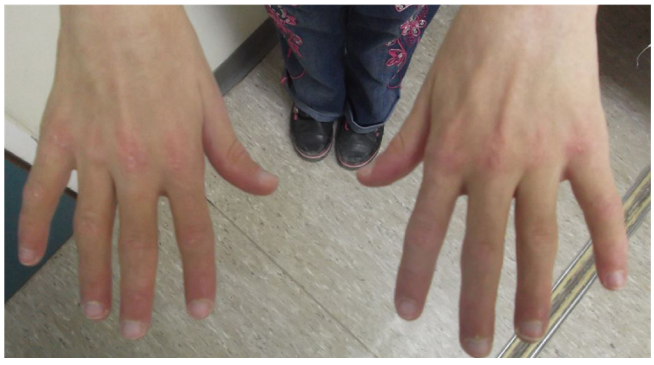

Figure 1. 7th patient's Gottron's papules

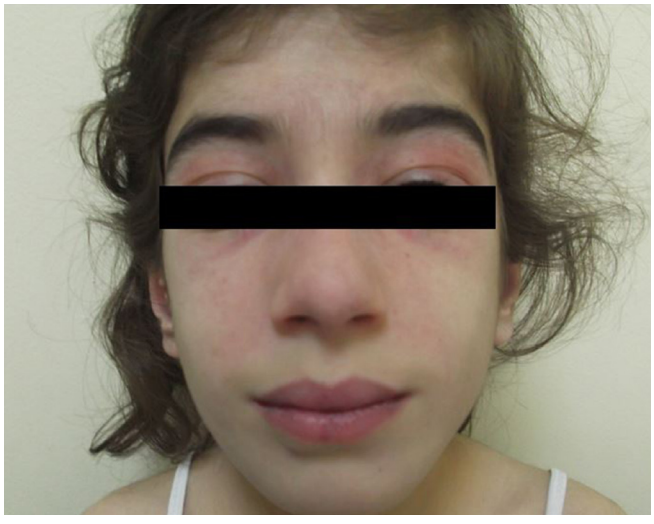

Figure 2. 7th patient's Heliotrope rashes

Patients with JDM almost always present with neurological involvements (5). The neurological examination findings of the patients at the time of diagnosis were reviewed. All the patients had varying degrees of muscular strength loss, particularly in the proximal muscles, eight (53.3%) had Gowers' sign and deep tendon reflexes were decreased in seven (46.6%) patients. During the follow-up period, a generalized tonic-clonic seizure was observed in a single patient (6.6%) and antiepileptic therapy was initiated, whereas two patients (13.3%) developed neuropathies. Chronic findings such as scapular winging and lumbar lordosis developed particularly in those patients with a protracted period between symptom onset and diagnosis (patients number 1, 4 and 13), and one patient (patient number 10) had no muscular involvement and was being followed up with a diagnosis of amyopathic JDM (Table 3).

Laboratory measurements obtained at the time of diagnosis are reviewed (Table 1). At the time of diagnosis, only 12 (80%) patients had an ESR higher than 20 mm and 11 (73.4%) patients had positive CRP measurements. Antinuclear antibodies were positive in eight (53.3%) patients; six (40%) patients were ANA positive, and two (13.3%) patients were positive for both ANA and anti-dsDNA (Tables 1, 2).

Of the 15 patients, 10 (66.6%) underwent muscle biopsy. Consistent with JDM, all biopsy procedures revealed mild diameter differences in muscles, atrophy in some perifascicular fibers and degenerated fibers with basophilic staining. Other tests were performed on patients who could not undergo such invasive procedures, such as muscle biopsies, to support their diagnosis. A standard magnetic resonance imaging (MRI) of the gluteal or gastrocnemius muscle was performed in five (33.3%) of the 15 patients. All patients had T2 and a flair intensity increase in the present muscle structures, consistent with the JDM diagnosis. Varying degrees of myofibril irritation (fibrillation potentials, repetitive complex discharges and sharp positive waves) were detected in eight of the nine patients who underwent EMG, which was interpreted in favor of myositis. An echocardiography was performed on eight (53.3%) patients, and no pathology was observed (Table 2).

During the acute phase, patients were administered pulse steroids and maintenance steroid therapy. Of all patients receiving steroid therapy, 12 (80%) responded during the acute phase, while three (20%) were nonresponsive. Following the acute phase, no recurrence was observed in

three (20%) of the 15 patients, and they were considered cured. Well-implemented physical therapy facilitates and supports the treatment process in JDM (5). All patients received physical therapy in this study, and of the total, six (40%) were therapy-resistant, and nine (60%) were treatment-resistant and experienced intermittent episodes of muscle weakness. These treatment-resistant patients were started on methotrexate following the steroid therapy. Patients who experienced recurrences despite methotrexate treatment were given other immunosuppressive therapies (cyclophosphamide, IVIG, hydroxychloroquine) (Table 2). Mortality is a rare finding of JDM. In this study, frequent pulmonary infections, ulcers and dysphagia developed in patient number 8, and in the 18th month of follow-up, this patient died from sepsis and intracranial bleeding (Table 3).

DISCUSSION

Although the incidence of JDM is low during the childhood period, it is still the most common inflammatory myopathy seen in children (5). The first clinical signs of JDM include skin and muscle involvement, while heart, lung, and gastrointestinal involvement are rare (7). The diagnostic criteria suggested by Bohan and Peter (4) in 1975 are still the standard means of diagnosis of JDM, although recently developed autoantibodies, biological agents and imaging methods can also contribute, and calcinosis and findings of myositis in MRI have also emerged as diagnostic criteria (8,9).

JDM is more common among girls, with previous studies in the literature reporting F/M ratios of 2-3:1 in Europe and the United States (10,11). In the study by Barut et al. (12), the F/M ratio in the western regions of Turkey was reported to be 2.3:1, while this ratio was 4:1 in this study. This observed difference may be attributed to the small number of cases evaluated in this study, though regional or genetic factors may also be effective. The mean age of the patients included in this study was 9.2 years, the mean duration between the complaint and diagnosis was 7.8 months and the mean follow-up duration was 24.9 months. In the literature, the mean age of diagnosis was reported to be 7.5 years in the study by Malek et al. (13), 7.1 years in the study by Gowdie et al. (9) and 6.75 years in the study by Okong'o et al. (14) The mean time between symptom onset and diagnosis was reported to be between 4 and 6 months (12,13). The higher mean age of diagnosis in this study indicates a delay in the diagnosis. This was likely due to the location of our clinic in the eastern part of Turkey. The number of specialized physicians like pediatric neurologists and rheumatologists, is limited in this region. Patients with widespread systemic findings may be followed up by outpatient clinics having

Table 1. Patients' results of demographic findings, laboratory findings

Patient	n (%)
Gender	
Female	12 (80)
Male	3 (20)
Age (average ± SD)	3-14 years (9.26±3.21 years)
Time interval between complaint and diagnosis (months)	1-24 month (7.8±6 months)
Length of follow-up (months)	10-60 month (24.93±15.28 month)
Laboratory tests (average ± SD)	
CK	625-4,000 U/L (1,354±840 U/L)
AST	62-216 U/L (94±40 U/L)
ALT	44-163 U/L (84±32 U/L)
LDH	192-824 U/L (443±207 U/L)
WBC	12.3±1.2 g/dL
HBG	9.9±2.7 10^9/L
PLT	310±118 10^9/L
ESR	2-38 mm (25.4±10.1 mm)
CRP	0.3-3 mg/dL (1.58±1.03 mg/dL)

SD: Standard deviation, CK: Creatine kinase, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, PLT: Platelet, WBC: White blood cell, HBG: Hemoglobin, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate

Table 2. Patients' neurological findings at the first diagnosis, and systemic complications on follow-up

Findings	1. Patient	2. Patient	3. Patient	4. Patient	5. Patient	6. Patient	7. Patient
Cutaneous	Gottron Heliotrope	Gottron Calcinosis	Calcinosis Heliotrope	Gottron Heliotrope	Gottron Heliotrope	Gottron Heliotrope	Gottron Heliotrope
Muscle	Scoliosis	Contracture	Osteoporosis	Contracture Art/Art	-	Osteoporosis	Contracture
Endocrine	-	-	DM	-	-	-	-
Respiratory	Tuberculosis	-	-	-	-	Recurrent respiratory tract infection	-
GIS	Gastritis	-	-	HM	-	-	-
Eye	-	-	-	-	-	-	-
CV	-	-	-	-	-	-	Raynaud's phenomenon
Infection	Tuberculosis	-	-	-	-	-	-
	Neurological examination (first diagnosis)						
Muscle force							
-Upper -Lower	2/5 3/5	5/5 4/5	4/5 3/5	3/5 3/5	4/5 5/5	3/5 4/5	5/5 4/5
DTR							
-Upper -Lower	Hypoactive Hypoactive	N N	Hypoactive Hypoactive	Hypoactive Hypoactive	N N	Hypoactive Hypoactive	N N
Neuropathy	+	-	+	-	-	-	-
Seizure	-	-	-	-	-	-	-
Gowers' Sign	+	-	+	+	-	+	-
Others	Scapular winging Lumber lordosis	-	-	Scapular winging	-	-	-

CV: Cardiovascular, DTR: Deep tendon reflex, GIS: Gastrointestinal system, DM: Diabetes mellitus, HM: Hepatomegaly, N: Normal, IKH: Intracranial hemorrhage, ex: Exitus, GTC: Generalized tonic clonic, Art/Art: Arthritis/arthralgia.

received a different diagnosis due to difficulties in accessing physicians, and this leads to diagnostic delay.

CK elevation is the most important laboratory parameter for a JDM diagnosis (12). The incidence of elevated CK is reported to be between 87% and 100% (12,13). In this study, all patients had elevated CK levels at the time of diagnosis, and the mean CK level was found to be 1,354±840 U/L. Elevated CK levels may lead to confusion in the form of differential diagnoses, as some patients were being followed up by pediatricians with a preliminary diagnosis of muscular dystrophy (MD), based on their elevated CK. However, a detailed evaluation of skin findings, which are reported in almost all cases in different studies, may contribute to an accurate diagnosis. In the presence of dermatomyositis, ESR and CRP elevations are significant findings in their indication of inflammation. In the study by Barut et al. (12), the mean ESR level was 35±22.1 mm and CRP positivity was reported in 38% of the cases. In this study, the mean ESR value was 25.4±10.1 mm and 80% of the patients were CRP positive. ANA positivity is important for JDM follow-up, while also being a marker of morbidity (13). In the literature, rates of ANA positivity of 68% and 60-70% have been reported, while in this study, 12 (53.3%) of the patients were found to be ANA positive, and better results were achieved in 10 of them during both the acute and long-term periods. ANA positivity can be considered a good predictive factor for treatment response (12,15).

8. Patient (ex)	9. Patient	10. Patient	11. Patient	12. Patient	13. Patient	14. Patient	15. Patient
Gottron Heliotrope Alopecia Calcinosis Ulcer	Gottron Heliotrope	Gottron Heliotrope	Gottron Heliotrope	Gottron	Gottron Heliotrope Alopecia	Gottron Heliotrope	Gottron Alopecia
Art/Art Contracture	Contracture Art/Art	-	Contracture Art/Art	-	Osteoporosis	Art/Art	-
-	-	-	-	-	-	-	-
Recurrent respiratory tract infection	-	-	-	-	-	-	-
Dysphagia	-	-	-	-	Dysphagia	-	Dysphagia
-	-	-	-	-	-	-	-
Raynaud's phenomenon	-	-	-	-	Raynaud's phenomenon	-	-
Sepsis (ex)	-	-	-	-	-	-	-
5/5 4/5	4/5 4/5	5/5 5/5	5/5 4/5	4/5 4/5	4/5 4/5	5/5 4/5	4/5 4/5
N N	Hypoactive Hypoactive	N N	N N	Hypoactive Hypoactive	N N	N N	Hypoactive Hypoactive
-	-	-	-	-	-	-	-
+ (GTC)	-	-	-	-	-	-	-
-	+	-	+	+	+	-	-
	-	-	-	-	Lumber lordosis	-	-

Proximal muscle weakness and typical skin rashes are the pathognomonic clinical findings of JDM (16). In the study by Gowdie et al. (9), 95% of the patients had muscle weakness, 91% had Gottron's papules and 71% had a heliotrope rash. Barut et al. (12), on the other hand, reported a heliotrope rash in 100% of cases, Gottron's papules in 96% and muscle weakness in 90%. Concurring with the findings in the literature, muscle weakness, Gottron's papules and heliotrope rash were present in 100%, 93.3% and 80% of the patients in this study, respectively. Supporting this data, the presence of skin findings in almost all JDM cases admitted with muscle weakness would appear to be a valid aid to clinicians when making a definite diagnosis, although it should be kept in mind that amyopathic dermatomyositis may also be observed, particularly in pediatric patients (1). Muscle findings are one of the most important clinical manifestations of the disease. Proximal muscle weakness, particularly at the hip and shoulder intersections, is the most important clinical finding. Affected children experience limited movement, may experience difficulties in climbing stairs and are frequently positive for Gower's sign (17). In this study, 14 of the patients had muscle involvement as the most common complaint at the time of admission, with lower and upper extremity involvement noted in 86.6% and 60% of the patients, respectively. One patient (patient number 10), on the other hand, had no muscular involvement, and this patient was followed up with a diagnosis of JDM. When presenting with CK elevation, this finding causes cases to be

followed erroneously with a diagnosis of MD, although AST levels may help differentiate such cases from MD, as such cases do not present with the elevated AST levels seen in MD cases, despite findings of myopathy. Our case only had a mild CK elevation. Duchenne muscular dystrophy (DMD) is the most common type of MD seen during childhood. While differentiating this condition from DMD, the dominance of the female sex in JDM should be considered, although symptomatic female DMD carriers should also be kept in mind.

While the leading means of diagnosis of JDM is standard muscle biopsy, an EMG and muscle MRI may also be helpful (18,19). In the study by Malek et al. (13), the findings of myositis in EMG was reported to be 96% and myositis in muscle biopsy was reported to be 93.7%. In the study by Okong'o et al. (14), a muscle MRI was performed in 36% of the patients, and the findings favored inflammation in 88.8% of the cases. In this study, muscle biopsy was carried out on the 66.6% of patients who agreed to the procedure, while a muscle MRI was performed on the remaining 33.4% of the patients. In both groups, 60% of the patients underwent an EMG. The findings pointed to myositis in 100% of the patients who underwent muscle biopsy, 88.8% of the patients who underwent an EMG and 100% of the patients who underwent an MRI. In our study, 33.4% of the patients underwent an MRI, and inflammation was noted in 100% of cases. Although muscle biopsy is the leading method of diagnosis, a muscle MRI can also be safely used as a non-invasive procedure.

Recent recommendations for treating JDM suggest the use of corticosteroids and methotrexate in combination (intravenous methylprednisolone 15-30 mg/kg/g and 1-2 mg/kg/g prednisolone+15-20 mg/m^2/patient methotrexate for three consecutive days). A more conventional treatment approach is the use of intravenous methylprednisolone at 15-30 mg/kg/day and maintenance prednisolone at 1-2 mg/kg/g. Our treatment protocol was solely based on the use of steroids in the first stage, and treatment-resistant patients were administered methotrexate following steroid therapy. Other immunosuppressive therapies were given to patients who encounter episodes despite the methotrexate treatment, and well-implemented physical therapy facilitates and supports this treatment process (12). All the patients in this study underwent physical therapy and immunosuppressive therapy. A complete response to treatment was noted in 12 (80%) of the 15 patients during the acute phase, and in the long-term, six (40%) patients followed a treatment-resistant disease course and required multiple immunosuppressive therapies.

Table 3. Patients' positive examination findings of

Physical examination	n (%)
Skin	
Gottron's papules	14 (93.3)
Heliotrope rash	12 (80)
Calcinosis	3 (20)
Alopecia	3 (20)
Muscle-skeleton	
Contracture	6 (40)
Arthritis/arthralgia	5 (33.3)
Osteoporosis	3 (20)
Endocrine	
Diabetes mellitus	1 (6.6)
Respiratory	
Recurrent respiratory tract infection	2 (13.3)
Tuberculosis	1 (6.6)
Gastrointestinal	
Dysphagia	3 (20)
Eye	-
Cardiovascular	
Raynaud's phenomenon	3 (20)
Infection	
Sepsis	1 (6.6)
Neurological	
Lower extremity involvement	13 (86.6)
Upper extremity involvement	9 (60)
DTR hypoactive	7 (46.6)
Gowers' sign	8 (53.3)
Scapular winging	2 (13.3)
Lumber lordosis	2 (13.3)

DTR: Deep tendon reflex

Calcinosis is one of the most significant complications of JDM, being seen in 18-27.7% of children with JDM, as well as being one of the major complications affecting morbidity. It may already be present at disease onset or it may develop in later stages (11). In the study by Barut et al. (12), calcinosis was observed in 38% of the patients, while calcinosis was noted in only three (20%) patients in this study. During long-term follow-up, the involvement of the musculoskeletal or gastrointestinal systems can seriously affect the quality of life of patients with JDM (12,13). In our study, gastrointestinal involvement and musculoskeletal

system involvement was observed in five and eight patients, respectively. JDM is also associated with mortality; with one patient (6.6%) in our study dying from intracranial bleeding and sepsis, while in the study by Okong'o et al. (14), the mortality rate was reported to be 8%.

CONCLUSION

In conclusion, JDM is a rare inflammatory myopathy seen during childhood, and it has a high rate of morbidity and low mortality. Better responses can be achieved with early diagnosis, intensive immunosuppressive therapy and effective physical therapy.

Acknowledgments: Special thanks to Dr. M. Ayse Selimoglu and other members of pediatric departments pediatric neurology and pediatric rheumatology team at the İnönü University Turgut Özal Medical Center who managed most of the cases and documented the findings that formed our most important source of information for this study. Thanks to the members of the Radiology and Pediatric Cardiology Departments for reporting this study.

ETHICS

Ethics Committee Approval: The ethics committee decision regarding our study was taken from the Ethics Committee of Inonu University Faculty of Medicine with the decision reference number 2018/9-17 (date: 24.04.2018).

Informed Consent: We received informed consent from all the patients' families.

Authorship Contributions

Surgical and Medical Practices: M.A., S.G., Y.T., B.Ö., S.K., Concept: S.G., Y.T., Design: M.A., S.G., Y.T., Data Collection or Processing: M.A., B.Ö., S.K., Analysis or Interpretation: M.A., S.G., Y.T., S.K., Literature Search: M.A., S.G., Y.T., B.Ö., S.K., Writing: M.A., B.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. Pagnini I, Vitale A, Selmi C, Cimaz R, Cantarini L. Idiopathic Inflammatory Myopathies: an Update on Classification and Treatment with Special Focus on Juvenile Forms. Clin Rev Allergy Immunol 2017;52:34-44.
2. Liu SW, Velez NF, Lam C, Femia A, Granter SR, Townsend HB, et al. Dermatomyositis induced by anti-tumor necrosis factor in a patient with juvenile idiopathic arthritis. JAMA Dermatol 2013;149:1204-8.
3. Cassidy JT, Petty RE. Textbook of Pediatric Rheumatology. New York: Elsewier Saunders Company; 2005.
4. Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292:403-7.
5. Rider LG, Katz JD, Jones OY. Developments in the classification and treatment of the juvenile idiopathic inflammatory myopathies. Rheum Dis Clin North Am 2013;39:877-904.
6. Carstens PO, Schmidt J. Diagnosis, pathogenesis and treatment of myositis: recent advances. Clin Exp Immunol 2014;175:349-58.
7. Shah M, Mamyrova G, Targoff IN, Huber AM, Malley JD, Rice MM, et al. The clinical phenotypes of the juvenile idiopathic inflammatory myopathies. Medicine (Baltimore) 2013;92:25-41.
8. Brown VE, Pilkington CA, Feldman BM, Davidson JE; Network for Juvenile Dermatomyositis, Paediatric Rheumatology European Society (PReS). An international consensus survey of the diagnostic criteria for juvenile dermatomyositis (JDM). Rheumatology (Oxford) 2006;45:990-3.
9. Gowdie PJ, Allen RC, Kornberg AJ, Akikusa JD. Clinical features and disease course of patients with juvenile dermatomyositis. Int J Rheum Dis 2013;16:561-7.
10. Mendez EP, Lipton R, Ramsey-Goldman R, Roettcher P, Bowyer S, Dyer A, et al. US incidence of juvenile dermatomyositis, 1995-1998: results from the National Institute of Arthritis and Musculoskeletal and Skin Diseases Registry. Arthritis Rheum 2003;49:300-5.
11. Ruperto N, Pistorio A, Oliveira S, Zulian F, Cuttica R, Ravelli A, et al. Prednisone versus prednisone plus ciclosporin versus prednisone plus methotrexate in new-onset juvenile dermatomyositis: a randomised trial. Lancet 2016;387:671-8.
12. Barut K, Aydin PO, Adrovic A, Sahin S, Kasapcopur O. Juvenile dermatomyositis: a tertiary center experience. Clin Rheumatol 2017;36:361-6.
13. Malek A, Raeeskarami SR, Ziaee V, Aghighi Y, Moradinejad MH. Clinical course and outcomes of Iranian children with juvenile dermatomyositis and polymyositis. Clin Rheumatol 2014;33:1113-8.
14. Okong'o LO, Esser M, Wilmshurst J, Scott C. Characteristics and outcome of children with juvenile dermatomyositis in Cape Town: a cross-sectional study. Pediatr Rheumatol Online J 2016;14:60.
15. Prasad S, Misra R, Agarwal V, Lawrence A, Aggarwal A. Juvenile dermatomyositis at a tertiary care hospital: is there any change in the last decade? Int J Rheum Dis 2013;16:556-60.
16. Huber A, Feldman BM. An update on inflammatory myositis in children. Curr Opin Rheumatol 2013;25:630-5.
17. Maddison PJ, Isenberg DA, Woo P, Glass DN. Oxford Textbook of Rheumatology. London: Oxford University Pres; 2004
18. Kolasinski SL, Chi AS, Lopez-Garib AJ. Current Perspectives on Imaging for Systemic Lupus Erythematosus, Systemic Sclerosis, and Dermatomyositis/Polymyositis. Rheum Dis Clin North Am 2016;42:711-32.
19. Elessawy SS, Abdelsalam EM, Abdel Razek E, Tharwat S. Whole-body MRI for full assessment and characterization of diffuse inflammatory myopathy. Acta Radiol Open 2016;5:2058460116668216.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:172-176
DOI: 10.4274/BMJ.galenos.2022.2021.11-12

Research

Gallbladder Cancer Incidentally Detected in the Histopathological Analysis of Patients Undergoing Cholecystectomy: The Case of Turkey

Kolesistektomi Yapılan Hastaların Histopatolojik İncelemesinde İnsidental Saptanan Safra Kesesi Kanseri: Türkiye Örneği

Hüseyin Aslan, Sadettin Er

[1]Sakarya University of Applied Sciences Faculty of Health Sciences, Department of Health Management, Sakarya, Turkey
[2]University of Health Sciences Turkey, Ankara City Hospital, Clinic of General Surgery, Ankara, Turkey

ABSTRACT

Objective: Gallbladder cancer is a type of cancer that is difficult to diagnose. Although its prevalence is low, it may differ from one society to another. The current study evaluates whether the histopathological evaluation of specimen should be performed after cholecystectomy.

Methods: This research was designed as a retrospective, cross-sectional registry study. The diagnosis-related group data of 112,884 patients who underwent cholecystectomy in 2016 in hospitals affiliated with the Turkish Ministry of Health were used in the study. Descriptive statistics were used in the analysis of demographic data, diagnosis and types of procedures, and the chi-square analysis was used to compare the distribution of gallbladder cancer.

Results: Approximately 19% of the patients who underwent cholecystectomy were in the age group of 65 years and over. Approximately 75% were women. The rate of laparoscopic cholecystectomy was found to be higher in female patients. Because of the histopathological examination of the cholecystectomy specimen, 0.10% of the patients were diagnosed with gallbladder cancer. The incidence of benign neoplasms of the gallbladder was equal in men and women. Gallbladder cancer was seen at a higher rate in men than in women and in the ≥65-year group compared to the <65-year group. The incidence of gallbladder cancer according to the type of procedure was determined to be 16 times higher in patients who underwent open cholecystectomy and 17 times higher in cases converted to open cholecystectomy compared to laparoscopic cholecystectomy.

Conclusion: During the detection of gallbladder cancer, a thorough evaluation of the risk factors of patients involving detailed and careful macroscopic examinations can prevent unnecessary histological examinations. Thus, resources allocated to health services can be used more efficiently by reducing unnecessary health expenditures.

Keywords: Gallbladder cancer, cholecystectomy, histopathology

ÖZ

Amaç: Safra kesesi kanserleri tanı konulması zor olan kanser türlerinden biridir. Prevelansı düşük olduğu bilinmekle birlikte, prevelansı topluma göre farklılık gösterebilmektedir. Bu araştırmanın amacı kolesistektomi sonrası spesmenin histopatolojik değerlendirme yapılmasının gerekip gerekmediğini değerlendirmekti.

Gereç ve Yöntem: Araştırma geriye dönük, kesitsel bir kayıt araştırmasıdır. Araştırmada Türkiye Sağlık Bakanlığı hastanelerinde, 2016 yılında kolesistektomi işlemi yapılan 112.884 hastanın tanı ilişkili gruplar verileri kullanıldı. Demografik verilerin, tanı ve işlem türlerinin analizinde tanımlayıcı istatistikler; safra kesesi kanserlerinin dağılımının karşılaştırmasında ki-kare analizi kullanıldı.

Bulgular: Kolesistektomi yapılan hastaların yaklaşık %19'u 65 ve üzeri yaş grubundaydı. Yaklaşık %75'i kadınlardan oluşmaktaydı. Kadın hastalarda laparoskopik kolesistektomi yapılma oranı daha yüksek bulundu. Kolesistektomi spesmenin histopatolojik incelmeleri sonucunda hastaların toplam %0,10'unda safra kesesi kanseri teşhis edildi. Safra kesesinin selim neoplazm görülme oranı kadınlar ve erkeklerde eşit bulundu. Safra kesesi kanseri ≥65 yaş ve erkeklerde karşıt gruplarına göre daha yüksekti. İşlem türüne göre safra kesesi kanseri görülme oranı; laparoskopik

Address for Correspondence: Hüseyin Aslan, Sakarya University of Applied Sciences Faculty of Health Sciences, Department of Health Management, Sakarya, Turkey
Phone: +90 554 381 44 44 E-mail: huseyinaslan@subu.edu.tr ORCID ID: orcid.org/0000-0001-8963-7638

Cite as: Aslan H, Er S. Gallbladder Cancer Incidentally Detected in the Histopathological Analysis of Patients Undergoing Cholecystectomy: The Case of Turkey. Med J Bakirkoy 2022;18:172-176

Received: 02.10.2021
Accepted: 11.04.2022

kolesistektomi hastalarına göre açık kolesistektomi işlemi yapılan hastalarda 16 kat, açığa dönen kolesistektomi işlemi yapılan hastalarda 17 kat daha yüksek bulundu.

Sonuç: Safra kesesi kanserlerini tespit etmek için risk faktörlerinin iyi değerlendirilmesi, ayrıntılı ve dikkatli makroskobik incelemelerle gereksiz histopatolojik incelemelerin önüne geçilebilir. Böylece gereksiz sağlık harcamaları azaltılarak sağlık hizmetlerine ayrılan kaynakların daha etkin kullanılabileceğini söyleyebiliriz.

Anahtar Kelimeler: Safra kesesi kanseri, kolesistektomi, histopatoloji

INTRODUCTION

Gallbladder cancer is a type of cancer that is difficult to manage (1). It is a very aggressive malignancy with rapid progression and high mortality rate, and usually detected in advanced ages when it spreads to other organs (2,3). The prevalence of gallbladder cancer is low, but it occurs in approximately 1%-2% of all cholecystectomy patients (4). Although it is less common in developed countries, ethnicity is also known to be one of the risk factors, and its prevalence varies considerably (5). Age, gender, ethnicity, gallstones, chronic infection, obesity, environmental exposures, gallbladder polyps, and genetic predisposition increase the risk of gallbladder cancer (5). It is also more common in women than in men (6).

The treatment of the disease is surgery, and a pathological analysis is not performed in every patient (5,7). However, a histological examination has become routine practice in some countries (8,9). The diagnosis of gallbladder cancer can be made based on a macroscopic pathological examination (9). Some studies claim that a histopathological examination does not change the results obtained from macroscopic examinations during and after surgery (1,10). Therefore, significant savings in health expenditures can be achieved by eliminating unnecessary histopathological examinations (8,11,12).

Early-stage gallbladder cancer generally shows the same characteristics as cholecystitis (5,6,13), and among the patients who underwent laparoscopic cholecystectomy, the rate gallbladder cancer was reported to be 0.3% based on a histopathological examination (5). Studies indicate that it is more effective to decide whether to send gallbladder material for a histological examination according to the evaluations made at the end of the macroscopic examination since the rate of gallbladder cancer detected because of a histological examination is low (1,8). This will ensure the more efficient use of resources, such as time, space, human power, and health technologies.

This study examined the necessity of a histopathological evaluation of the specimen after cholecystectomy.

METHODS

Data

In this study, a total of 112,884 patients who underwent cholecystectomy in 2016 in public hospitals affiliated with the Turkish Ministry of Health were screened. The data of the study were obtained from the General Directorate of Health Services of the Turkish Ministry of Health with permission (dated 06.07.2018 and numbered 23642684-010.99). The research protocol was approved by the Non-Interventional Clinical Research Ethics Committee of Hacettepe University with the decision dated 24.08.2017 and numbered GO 17/709-32.

Patient data were obtained from the clinical coding data of diagnosis-related groups (DRG). The demographic data and pathology results of the patients were obtained retrospectively from the electronic records. Gender, age (<65 and ≥65 years) were used as demographic data. All patients diagnosed with gallbladder cancer were included in the study. Patients diagnosed with any other cancer were excluded from the sample. The diagnosis of gallbladder cancer was based on the pathology laboratory examinations of the patients' cholecystectomy procedure materials. In public hospitals in Turkey, cholecystectomy materials are routinely subjected to a histological examination, and gallbladder cancer is diagnosed on the basis of the results of this examination.

Statistical Analysis

Microsoft Office Excel 2016 was used to organize the data of the patients, and the analysis of the data was performed using IBM SPSS Statistics v. 23 software package (IBM Corp., Armonk, NY, 2016). Descriptive statistics were given for data on gender, age, gallbladder malignant neoplasms, benign gallbladder neoplasms, and cholecystectomy procedure type. The chi-square analysis was used to examine the distribution of gallbladder cancer by gender, age, and cholecystectomy procedure type and compare them variable groups. The confidence interval was set as 95% and the significance value as $p<0.05$.

RESULTS

According to the results of the study, approximately 19% of the patients who underwent cholecystectomy were in the age group of ≥65 years. It was observed that approximately 68% of this age group consisted of women. The incidence of malignant neoplasms of the gallbladder was approximately two times higher in male patients than in females. The incidence of benign neoplasms of the gallbladder was similar between the genders. In the female patients, the rate of laparoscopic cholecystectomy was higher but the rate of conversion from laparoscopic to open cholecystectomy was lower compared to the male patients (Table 1).

Although the number of female patients who underwent cholecystectomy was three times higher than that of male patients, the rate of gallbladder cancer detection in the male patients was approximately two times greater compared to the female patients. The detection rate of gallbladder cancer in patients aged 65 years and over was approximately five times higher than the group under 65 years, and this difference was found to be statistically significant ($p<0.001$). While gallbladder cancer was detected to be very low at 0.04% in laparoscopic cholecystectomy, this rate was approximately 19 times higher in patients undergoing open cholecystectomy and 16 times higher in cases converted from laparoscopic to open cholecystectomy ($p<0.001$ for both) (Table 2).

DISCUSSION

This study is critical since, among the studies conducted to date, it included the highest number of patients undergoing cholecystectomy and diagnosed with gallbladder cancer in Turkey.

In this study, we determined that the number of women who underwent cholecystectomy was approximately three times higher than that of men. Women having a cholecystectomy indication rate is similar to literature studies (5,8,9,11-15). However, contrary to the literature, we found the incidence of gallbladder cancer to be approximately twice greater than in men than in women. In other studies, the gallbladder cancer detection rate has been reported to be higher in female patients (8,14). In this study, the rate of benign gallbladder neoplasm detection was found to be equal between the male and female patients.

According to our results, the rate of gallbladder cancer detection in the ≥65-year group was approximately five times higher than the <65-year group. Similarly, other studies have also shown that the rate of gallbladder cancer detection is higher in elderly patients (9,11,12).

In our study, the rate of gallbladder cancer was found to be 0.10% according to the histopathological examination results of 112,884 patients. In a study by Bazoua et al. (12) with 2,890 patients who underwent cholecystectomy, the gallbladder cancer detection rate was reported to be 0.17%. In another study, van Vliet et al. (11) suspected gallbladder cancer in the macroscopic examinations of approximately 14% of patients who underwent cholecystectomy and detected gallbladder cancer in 6% of these patients after a histopathological examination. In the same study, according to the results of the histopathological examination, gallbladder cancer was not present in any patient without an abnormal finding in the macroscopic examination. Siddiqui et al. (13), evaluating 220 patients who underwent cholecystectomy, found the gallbladder cancer detection rate as 2.8%. In a study by Lundgren et al. (7), the rate of gallbladder cancer detection

Table 1. Distribution of age, neoplasm and cholecystectomy procedure type by gender in cholecystectomy patients

		Gender (n/%)		Total
		Male	Female	
Age	<65	21.611/76.2	69.918/82.7	91.529/81.1
	≥65	6.768/23.8	14.587/17.3	21.355/18.9
Malignant neoplasm of the gallbladder	No	28.334/99.8	84,434/99.9	112.768/99.9
	Yes	45/0.2	71/0.1	116/0.1
Benign neoplasm of the gallbladder	No	28.340/99.9	84.411/99.9	112.751/99.9
	Yes	39/0.1	94/0.1	133/0.1
Cholecystectomy type	Laparoscopy	24.403/86.0	77.958/92.3	102.361/90.7
	Laparoscopy converted to open	517/1.8	573/0.7	1.090/1.0
	Open	3.459/12.2	5.974/7.1	9.433/8.4
Total		28.379/25.1	84.505/74.9	112.884/100.0

Table 2. Gender and age group comparison of gallbladder cancer diagnosis

		Malignant neoplasm of the gallbladder (n/%)		p
		No	Yes	
Gender	Male	28.334/99.84	45/0.16	0.001*
	Female	84.434/99.92	71/0.08	
Age	<65	91.476/99.94	53/0.06	<0.001*
	≥65	21.292/99.70	63/0.30	
Cholecystectomy type	Laparoscopy	102.325/99.96	36/0.04	<0.001*
	Laparoscopy converted to open	1.083/99.36	7/0.64	
	Open	9.360/99.23	73/0.77	
Total		112.775/99.90	109/0.10	

*p<0.05, Pearson's chi-square test results

among cholecystectomy patients was 0.26%. Emmett et al. (8) reported that gallbladder cancer could be successfully diagnosed through a careful macroscopic evaluation and the incidence of undetectable gallbladder cancer during the macroscopic examination was very low. Therefore, the authors suggested that it was not necessary to send the gallbladder material of every patient for a histopathological examination (1,8,10). For patients with no clinical or imaging suspicion of gallbladder carcinoma (GBC) and no evident abnormalities on gross examination, there is no consensus on a uniform pathological examination protocol. It has been reported that in areas with high GBC prevalence, microscopic evaluation can be performed for at least three random areas and cystic duct margins in the gallbladder, which appears normal on examination (16). Evidence of dysplasia or neoplasia on the initial random sampling ensures complete sampling of the gallbladders in many countries, microscopic examination is not recommended or performed in these situations (17).

GBC has been associated with choledochal cysts, abnormal union of the pancreatobiliary ducts, and primary sclerosing cholangitis, particularly the presence of polyps larger than 1 cm detected preoperatively (16,18). A more comprehensive examination of the gallbladder is required in such circumstances. Additionally, cases of hyalinized cholecystitis with minimal or no calcification (incomplete porcelain gallbladder) tend to have a high prevalence of mild invasive cancer and should be thoroughly explored (19).

In our study, according to the results of the histological examination, the rate of gallbladder cancer was 0.04% for the patients who underwent laparoscopic cholecystectomy, 0.64% for the cases in which laparoscopic surgery was converted to open surgery, and 0.77% for those that underwent open cholecystectomy. In a study by Yamamoto et al. (20) with 1,829 patients who underwent cholecystectomy, gallbladder cancer was reported because of a histological examination in 0.54% of those who underwent laparoscopic cholecystectomy. In another study, the rate of gallbladder cancer detection was reported to be 0.5% among patients who underwent laparoscopic cholecystectomy (4). Kalita et al. (15), who performed laparoscopic cholecystectomy, determined this as 0.44%. In the same study, the authors stated that gallbladder cancer was detected at a rate of 0.1% in the histopathological examination of 9,991 patients without suspicious findings in the macroscopic examination.

This study has several limitations. First, this research is a retrospective cross-sectional study. Second, only public hospital patient data were used in the study. Research data were obtained from the Turkish DRG system. Therefore, the accuracy of the data is limited by the accuracy of the data in the DRG system.

CONCLUSION

During the detection of gallbladder cancer, a thorough evaluation of the risk factors of patients involving detailed and careful macroscopic examinations can prevent unnecessary histological examinations. Thus, resources allocated to health services can be used more efficiently by reducing unnecessary health expenditures.

ETHICS

Ethics Committee Approval: The research protocol was approved by the Non-Interventional Clinical Research Ethics Committee of Hacettepe University with the decision dated 24.08.2017 and numbered GO 17/709-32.

Informed Consent: Retrospective study.

Authorship Contributions

Concept: H.A., S.E., Design: H.A., S.E., Data Collection or Processing: H.A., Analysis or Interpretation: H.A., S.E., Literature Search: H.A., S.E., Writing: H.A., S.E.

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

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

REFERENCES

1. Darmas B, Mahmud S, Abbas A, Baker AL. Is there any justification for the routine histological examination of straightforward cholecystectomy specimens? Ann R Coll Surg Engl 2007;89:238-41.
2. Björk D, Bartholomä W, Hasselgren K, Edholm D, Björnsson B, Lundgren L. Malignancy in elective cholecystectomy due to gallbladder polyps or thickened gallbladder wall: a single-centre experience. Scand J Gastroenterol 2021;56:458-62.
3. Shrestha R, Tiwari M, Ranabhat SK, Aryal G, Rauniyar SK, Shrestha HG. Incidental gallbladder carcinoma: value of routine histological examination of cholecystectomy specimens. Nepal Med Coll J 2010;12:90-4.
4. Lundberg O, Kristoffersson A. Open versus laparoscopic cholecystectomy for gallbladder carcinoma. J Hepatobiliary Pancreat Surg 2001;8:525-9.
5. Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol 2014;6:99-109.
6. Gourgiotis S, Kocher HM, Solaini L, Yarollahi A, Tsiambas E, Salemis NS. Gallbladder cancer. Vol. 196, American Journal of Surgery. Elsevier; 2008. p. 252-64.
7. Lundgren L, Muszynska C, Ros A, Persson G, Gimm O, Valter L, et al. Are Incidental Gallbladder Cancers Missed with a Selective Approach of Gallbladder Histology at Cholecystectomy? World J Surg 2018;42:1092-9.
8. Emmett CD, Barrett P, Gilliam AD, Mitchell AI. Routine versus selective histological examination after cholecystectomy to exclude incidental gallbladder carcinoma. Ann R Coll Surg Engl 2015;97:526-9.
9. Koppatz H, Nordin A, Scheinin T, Sallinen V. The risk of incidental gallbladder cancer is negligible in macroscopically normal cholecystectomy specimens. HPB (Oxford) 2018;20:456-61.
10. Dix FP, Bruce IA, Krypcyzk A, Ravi S. A selective approach to histopathology of the gallbladder is justifiable. Surgeon 2003;1:233-5.
11. van Vliet JL, van Gulik TM, Verbeek PC. Is it necessary to send gallbladder specimens for routine histopathological examination after cholecystectomy? The use of macroscopic examination. Dig Surg 2013;30:472-5.
12. Bazoua G, Hamza N, Lazim T. Do we need histology for a normal-looking gallbladder? J Hepatobiliary Pancreat Surg 2007;14:564-8.
13. Siddiqui FG, Memon AA, Abro AH, Sasoli NA, Ahmad L. Routine histopathology of gallbladder after elective cholecystectomy for gallstones: waste of resources or a justified act? BMC Surg 2013;13:26.
14. Zhang WJ, Xu GF, Zou XP, Wang WB, Yu JC, Wu GZ, et al. Incidental gallbladder carcinoma diagnosed during or after laparoscopic cholecystectomy. World J Surg 2009;33:2651-6.
15. Kalita D, Pant L, Singh S, Jain G, Kudesia M, Gupta K, et al. Impact of routine histopathological examination of gall bladder specimens on early detection of malignancy - a study of 4,115 cholecystectomy specimens. Asian Pac J Cancer Prev 2013;14:3315-8.
16. Aloia TA, Járufe N, Javle M, Maithel SK, Roa JC, Adsay V, et al. Gallbladder cancer: expert consensus statement. HPB (Oxford) 2015;17:681-90.
17. Renshaw AA, Gould EW. Submitting the entire gallbladder in cases of dysplasia is not justified. Am J Clin Pathol 2012;138:374-6.
18. Goldin RD, Roa JC. Gallbladder cancer: a morphological and molecular update. Histopathology 2009;55:218-29.
19. Patel S, Roa JC, Tapia O, Dursun N, Bagci P, Basturk O, et al. Hyalinizing cholecystitis and associated carcinomas: clinicopathologic analysis of a distinctive variant of cholecystitis with porcelain-like features and accompanying diagnostically challenging carcinomas. Am J Surg Pathol 2011;35:1104-13.
20. Yamamoto H, Hayakawa N, Kitagawa Y, Katohno Y, Sasaya T, Takara D, et al. Unsuspected gallbladder carcinoma after laparoscopic cholecystectomy. J Hepatobiliary Pancreat Surg 2005;12:391-8.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:177-188
DOI: 10.4274/BMJ.galenos.2021.2021.11-2

Research

A Valid and Reliable Tool to Assess Nursing Professional Competences: The Nursing Professional Competence Scale

Hemşirelik Mesleki Yeterliklerini Değerlendirmede Geçerli ve Güvenilir Bir Araç: Hemşirelik Mesleki Yeterlik Ölçeği

Figen Çalışkan[1], Emine Şenyuva[2]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, In-service Training Unit, Turkey
[2]Istanbul University-Cerrahpaşa Florence Nightingale Faculty of Nursing, Department of Nursing Education, Istanbul, Turkey

ABSTRACT

Objective: Nursing competence is a basic skill to fulfill the professional roles and responsibilities. Therefore, there is a need for studies to develop the valid and reliable measurement tools to identify the professional competences and competence levels expected from the nurses and evaluate them in the regular intervals. This study aims to develop a valid and reliable tool that could be used to assess the professional competence of the nurses.

Methods: The study has a methodological design. The population of this study included 3,133 nurses who worked at three training and research hospitals, one university hospital, and one private hospital in the Istanbul, and its sample included 902 nurses. The study used a Nurse Information Form and Nursing Professional Competence Scale Draft Form.

Results: The item-total score correlations ranged from 0.56-0.90. In the exploratory factor analysis, the Kaiser-Meyer-Olkin value was calculated as 0.970, and the Bartlett's test of Sphericity result was also significant [x^2 (2211)=43301.45; p<0.01]. The Cronbach's alpha of the Nursing Professional Competence scale was calculated as 0.98.

Conclusion: The study found that the Nursing Professional Competence scale is a valid and reliable tool to use. The scale developed in this context could be used in the researches to determine the professional competences of the nurses, to identify the variables affecting the professional competences of nurses, and to examine the effects of nursing education programs on professional competences of the nurses.

Keywords: Nursing, competence, nursing professional competence, scale development

ÖZ

Amaç: Hemşirelik yeterliği, mesleki rol ve sorumlulukları yerine getirmek için gerekli olan temel bir beceridir. Bu nedenle hemşirelerden beklenen mesleki yeterliklerin ve yeterlik düzeylerinin belirlenmesine ve düzenli aralıklarla değerlendirilmesine yönelik geçerli ve güvenilir ölçme araçlarının geliştirilmesine yönelik araştırmalara ihtiyaç vardır. Bu çalışma hemşirelerin mesleki yeterliklerini değerlendirmede kullanılabilecek geçerli ve güvenilir bir araç geliştirmeyi amaçlamaktadır.

Gereç ve Yöntem: Araştırma bir metodolojik araştırma tasarımındadır. Bu araştırmanın evrenini İstanbul'da bulunan üç eğitim ve araştırma hastanesi, bir üniversite hastanesi ve bir özel hastanede görev yapan 3.133 hemşire, örneklemini ise 902 hemşire oluşturmuştur. Araştırmada Hemşire Bilgi Formu ve Hemşirelik Mesleki Yeterlik Ölçeği Taslak Formu kullanılmıştır.

Bulgular: Madde toplam puan korelasyonları 0,56 ile 0,90 arasında değişmektedir. Açımlayıcı faktör analizinde Kaiser-Meyer-Olkin değeri; 0,970 olarak hesaplanmıştır ve Bartlett Küresellik testi sonucu da anlamlı bulunmuştur [x^2 (2211)=43301.459; p<0,01]. Hemşirelik Mesleki Yeterlik ölçeğinin Cronbach alfa değeri 0,98 olarak hesaplanmıştır.

Sonuç: Araştırma, Hemşirelik Mesleki Yeterlik ölçeğinin geçerli ve güvenilir bir araç olduğunu bulmuştur. Bu kapsamda geliştirilen ölçek, hemşirelerin mesleki yeterliklerini belirlemek, hemşirelerin mesleki yeterliklerini etkileyen değişkenleri belirlemek ve hemşirelik eğitim programlarının hemşirelerin mesleki yeterlikleri üzerindeki etkilerini ortaya koymaya yönelik araştırmalarda kullanılabilir.

Anahtar Kelimeler: Hemşirelik, yeterlik, hemşirelik mesleki yeterlik, ölçek geliştirme

Address for Correspondence: Emine Şenyuva, Istanbul University-Cerrahpaşa Florence Nightingale Faculty of Nursing, Department of Nursing Education, Istanbul, Turkey
Phone: +90 (212) 440 00 00 (27066) E-mail: esenyuva@iuc.edu.tr ORCID ID: orcid.org/0000-0001-8855-6692

Cite as: Çalışkan F, Şenyuva E. A Valid and Reliable Tool to Assess Nursing Professional Competences: The Nursing Professional Competence Scale. Med J Bakirkoy 2022;18:177-188

Received: 03.11.2021
Accepted: 27.12.2021

INTRODUCTION

Today, there are rapid and significant changes and developments in scientific, technological, sociodemographic, and economic fields; affecting the social, cultural, and economic aspects of a life. In addition to these changes and developments, factors including the increased chronic diseases, emergence of new diseases, diagnosis techniques, and treatment methods, shortened hospital stay, and growing need for the home care services also affect the healthcare systems (1,2).

Being competent means having a special knowledge, capacity, and ability to fulfill one's duty, adequateness (3) and being sufficiently qualified, skilled, or effective (4,5). Competence is a set of demonstrable characteristics and skills that enable a person to perform professional tasks in accordance with the relevant standards (6).

Nursing competence is the combination of a nurse's knowledge, skills, attitudes, values, and abilities required to perform professional nursing roles, and their ability to adapt that knowledge and those skills to a different circumstance (2,7). Nursing competence is considered acceptable professional performance on knowledge, attitude, and psychomotor levels according to the World Health Organization (WHO) (8), and effective application of a combination of the knowledge, skills and professional decisions in a professional performance and daily practices according to the International Council of Nurses (ICN) (9). Takase and Teraoka (10) defined nursing competence as a nurse's ability to effectively demonstrate a set of attributes such as: personal characteristics, professional attitude, values, knowledge, and skills, and to fulfill their professional responsibility through a practice.

Nursing competence helps to guarantee the high quality and effectiveness of healthcare and protect the social values and status of the nursing profession. Nursing core competencies include qualifications such as: willingness to serve, observation, judgment, and responsibility, as well as the basic behavioral characteristics such as care, communication, and collaboration, management, self-development, innovation and research, stress management, and mastery of the practical skills (7). Nursing competence also contains complex processes including the performance, leadership, professional development, diagnosis, planning, observation, motivation, cognitive ability (critical thinking, decision-making, analysis, judgment, thinking ability, etc.), social participation, communication, assertiveness, and personal perception (11,12). Nevertheless, nursing competence, which covers many features/skills, is influenced by the several factors including knowledge, skills, attitudes, behaviors, and individual characteristics required for the effective performance of the nurses in a professional life and various clinical practices. Therefore, nurses should have the necessary individual characteristics (understanding, self-control, critical thinking, problem solving ability), professional attitudes and behaviors (assuming the professional responsibilities, being autonomous, being aware of the own limits, respecting the patient rights, promoting the continuous learning and following current knowledge and skills), and the ability to provide care based on a professional knowledge, skills and values (cooperating with the other healthcare professionals, developing an interpersonal relationships, education and training, managing nursing care, ensuring nursing safety and quality, and increasing nursing capacity), to be a competent professional member, and fulfill the professional roles and responsibilities (10,13).

Nursing competence is a basic skill to fulfill the professional roles and responsibilities. Additionally, it is notable for nurses to take their place effectively in the healthcare systems of the future and use their professional competences in the different application environments and situations to increase the quality of a nursing care (1,14-18). Therefore, it is important to determine the development process of the nursing competences for continuous professional development after obtaining a nursing license (7,13,16,19-21). In this context, there is a need for studies to develop valid and reliable measurement tools to identify the professional competences and competence levels expected from the nurses and evaluate them in the regular intervals.

METHODS

Aim

The purpose of this study is to develop a valid and reliable tool that may be used to evaluate the professional competences among the nurses.

Design

This is a methodological study.

Participants and Sampling

The population of the study consisted of 3,133 nurses working at three research and training hospitals, one university hospital, and one private hospital with the highest bed capacity in the Istanbul, Turkey. Studies report that a ratio of five or ten participants per item is sufficient to evaluate the validity and reliability of the assessment tool (22). In this study, the sample size was calculated based on the number of scale items, whereby the participant/item

ratio was determined as 10/1. In this context, the sample of the study consisted of 902 nurses who agreed to participate in the study and were selected using a random sampling method.

Instruments

The data were collected using the Nurse Information Form and the Nursing Professional Competence Draft scale.

Nurse Information Form: The form was developed by the researchers in line with the literature (1,10,15,23,24). It consisted of of ten questions, including four questions about the nurses' sociodemographic characteristics (age, gender, marital status, education level) and the six questions about their professional characteristics (work place, work unit, nursing work experience, working time in current work place, current position, and working time).

Nursing Professional Competence Draft Scale: The draft scale was developed by the researchers at the two stages. First, an item pool was formed, and s draft scale was created. In this context, nurses' essential roles stipulated by the Nursing Regulations, published in the Turkish Official Gazette dated 08.03.2010 and numbered 27515; the nursing competences, roles and responsibilities published by ICN and WHO, and the international and national studies on the professional competences of the nurses were examined, and the expressions that could be used as scale items regarding the professional competences that the nurses should have were determined (1,9,10,15,18,23,25,26). Thus, an item pool with the 125 items reflecting the professional competences of the nurses was created. In the pool, there were 11 items for the "Care Needs" factor, 28 items for the "Care Planning, Implementation and Evaluation" factor, 11 items for the "Professional Ethical Practice" factor, 8 items for the "Teamwork" factor, 15 items for the "Professional Development" factor, 16 items for the "Communication" factor, 19 items for the "Health/Patient Education" factor, 7 items for the "Research and Development" factor, and 10 items for the "Critical Thinking and Analysis" factor. The numbers of items in the factors were determined considering the characteristics of each factor rather than the equality in quantity.

A total of 26 experts including the 16 nurse lecturers, three nurse educators, one nurse manager, and six clinic nurses were asked to evaluate the draft scale in items of both the linguistic validity and the content validity. To get their opinions, one four-degree rating system (unsuitable, slightly suitable, suitable, and very suitable) was used for linguistic validity, and one two-degree rating system (unsuitable, suitable) was used for content validity (27). Additionally, a "recommendation" section was added for each item in terms of both the linguistic validity and content validity.

According to the feedback obtained from the experts; each item of the draft scale was examined by the researchers. It was determined how many experts approved the options of each item. The minimum (min) content validity ratio for each item was accepted as 0.80 (27). Considering the content validity calculations for each item included in the draft scale, 42 items with content validity ratios <0.80 were removed from the draft scale, and five items were edited in terms of a language and expression, by considering the experts' recommendations. As a result, the total number of items in the draft scale was reduced from 125 to 83.

A pilot study was conducted having 100 nurses to evaluate the scale items in terms of a linguistic validity and content validity of the draft scale that was edited according to the expert opinions. The nurses were asked to evaluate the items using a four-point Likert type rating system [Always (4), Often (3), Sometimes (2), Never (1)]. No item was changed, eliminated, or deleted in line with the data obtained. The Nursing Professional Competence Draft scale, which was prepared as a four-point Likert type scale and consisted of 83 items, was made ready for the implementation (stage II) among the nurses who participated in the study. All the items in the scale are positive, and there is no reversely scored items. The increase in the mean score of the scale indicates the increase in the professional competences of the nurses.

Secondly, the validity and reliability of the Nursing Professional Competence Draft scale was tested. In this regard, the draft scale was applied to 902 nurses working at three research and training hospitals, one university hospital, and one private hospital in the Istanbul. Then, the validity and reliability study and psychometric evaluation of the draft scale were performed. The item-item and item-total correlations of the draft scale were analyzed with an item analysis using the data obtained from this evaluation. After the item analysis, exploratory factor analyses were performed to determine the scale factors. Internal consistency analysis was conducted to evaluate the internal consistency of the items related to the factors, that were identified in the factor analysis.

Data Collection

Data were collected between February and May 2019. A total of 26 experts were asked to evaluate the Nursing Professional Competence Draft scale in terms of a linguistic validity and content validity. They were interviewed to receive their expert opinions, whereby they were explained

the purpose of the study, and sent the draft scale via E-mail. After they evaluated the draft scale in terms of a content validity and the linguistic validity, it was sent back to the researchers via E-mail. A pilot study for the draft scale, in which necessary arrangements were made in line with the expert opinions, was carried out with the 100 nurses by the researcher. The data regarding the Nursing Professional Competence Draft scale were collected by the researchers from the nurses who agreed to participate in the study.

Ethical Considerations

For conducting the study, ethical approval was obtained from the Social Sciences and Humanities Ethics Committee at Istanbul University (number: 2018/46, date: 05.03.2018), and institutional permissions where the study was conducted were received. The nurses who agreed to participate in the study were explained about the purpose of the study, emphasizing that the research data would not be used for any other purpose, and not be shared with the third parties, and then, their verbal and written consents were obtained.

Statistical Analysis

The data were analyzed using a Number Cruncher Statistical System 2007 (Kaysville, Utah, USA). Descriptive statistical methods [frequency, percentage, mean, standard deviation, median, min-maximum (max) values] were used to demonstrate the nurses' sociodemographic characteristics, professional characteristics, professional development, and distribution of the scale items. Item Analysis, Exploratory Factor Analysis, Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy, and Bartlett's Test of Sphericity were performed to evaluate the validity and reliability of the Nursing Professional Competence Draft scale. Pearson's correlation analysis was performed to assess the relationship between the scale scores (22).

RESULTS

Sociodemographic and Professional Characteristics of the Nurses

The mean age of nurses was 28.94±7.8 (min: 19 and max: 59) years, 83.9% of them were female, 59.8% were single, and 60.8% had the undergraduate degrees. The mean work experience of the nurses was 7.04±7.46 years, whereby they had been working for 4.54±5.44 years at the same institution. Among the nurses, 46.7% were employed at the internal medicine units, 38.1% were employed at the surgical units, 76.3% were nurses in service, 12.1% were special branch nurses, and 8.8% were service charge nurses. The nurses had been working in the same position for 4.39±5.20 years.

Validity and Reliability Studies of the Nursing Professional Competence Draft Scale

The results regarding the validity and reliability study of the Nursing Professional Competence Draft scale are presented under the headings of an Item Analysis (Table 1), Exploratory Factor Analysis (Table 2), KMO Measure of Sampling Adequacy, and Bartlett's test of Sphericity, Scale Factor Item Distributions (Naming) and Variance Values (Table 3), Internal Consistency (Table 3), and Scale Total and Factors Score Distribution.

Item Analysis

An item analysis was performed to examine all the items in a draft scale and eliminate those exhibited a relatively low correlation with the total scale score (22). In this analysis, the item-total score correlation was calculated for each item. Accordingly, the item-total score correlations ranged from 0.56-0.90 (Table 1).

Exploratory Factor Analysis

An exploratory factor analysis with the varimax rotation method was performed to determine the factor structure of a draft scale. The acceptable level for the scale items was set to be >0.40 (28).

At the first stage, a total of 12 items (8, 22, 23, 28, 29, 30, 72, 73, 74, 77, 78, 79) including those with the factor loading values <0.40 or those with the nursing professional competence scalehe factor loading values that were close to each other in the multiple factors (difference <0.10) were removed from the draft scale.

At the second stage, another exploratory factor analysis was applied for the remaining 71 items of the scale, and a total of four items (20, 45, 46, 52) including those with the factor loading values <0.40 or those with factor loading values that were close to each other in the multiple factors (difference <0.10) were also removed from the draft scale.

At the third stage, an exploratory factor analysis was applied once again for the remaining 67 items of the scale, whereby the items were divided into seven factors that accounted for 59.24% of the total variance, and whose factor loadings ranged from 0.422-0.741 (Table 2).

The eigenvalue was calculated as 26.673 for Factor 1, 3.361 for Factor 2, 3.051 for Factor 3, 2.034 for Factor 4, 1.696 for Factor 5, 1.529 for Factor 6, and 1.348 for Factor 7.

In the exploratory factor analysis, the KMO value was calculated as 0.970, and the Bartlett's test of Sphericity result was also significant [x^2 (2211)-= 43301.459; p<0.01].

Table 1. Nursing Professional Competence Draft scale Item-total score correlations (n=902)

Factor number and item	Mean	SD	Item-total score correlation			
			Subgroup		Total scale	
			R	p	R	p
Factor 1	3.53	0.51	-	-	0.762	0.001**
I1	3.62	0.66	0.697	0.001**	0.485	0.001**
I2	3.56	0.62	0.830	0.001**	0.611	0.001**
I3	3.43	0.72	0.832	0.001**	0.648	0.001**
I4	3.34	0.78	0.771	0.001**	0.579	0.001**
I5	3.64	0.61	0.704	0.001**	0.528	0.001**
I6	3.60	0.63	0.797	0.001**	0.617	0.001**
I7	3.54	0.63	0.706	0.001**	0.601	0.001**
Factor 2	3.57	0.46	-	-	0.851	0.001**
I8	3.58	0.64	0.757	0.001**	0.646	0.001**
I9	3.60	0.61	0.767	0.001**	0.635	0.001**
I10	3.59	0.63	0.769	0.001**	0.603	0.001**
I11	3.48	0.67	0.718	0.001**	0.644	0.001**
I12	3.61	0.61	0.734	0.001**	0.608	0.001**
I13	3.56	0.62	0.722	0.001**	0.611	0.001**
I14	3.62	0.57	0.753	0.001**	0.630	0.001**
I15	3.60	0.62	0.773	0.001**	0.639	0.001**
I16	3.65	0.54	0.684	0.001**	0.556	0.001**
I17	3.62	0.56	0.651	0.001**	0.548	0.001**
I18	3.41	0.74	0.684	0.001**	0.608	0.001**
I19	3.53	0.63	0.756	0.001**	0.697	0.001**
I20	3.60	0.62	0.775	0.001**	0.681	0.001**
Factor 3	3.35	0.57	-	-	0.895	0.001**
I21	3.19	0.87	0.596	0.001**	0.536	0.001**
I22	3.21	0.77	0.677	0.001**	0.615	0.001**
I23	3.36	0.71	0.736	0.001**	0.689	0.001**
I24	3.46	0.71	0.742	0.001**	0.657	0.001**
I25	3.38	0.74	0.784	0.001**	0.673	0.001**
I26	3.40	0.71	0.798	0.001**	0.709	0.001**
I27	3.38	0.75	0.810	0.001**	0.713	0.001**
I28	3.32	0.76	0.804	0.001**	0.711	0.001**
I29	3.36	0.74	0.817	0.001**	0.704	0.001**
I30	3.29	0.78	0.806	0.001**	0.681	0.001**
I31	3.34	0.77	0.827	0.001**	0.718	0.001**
I32	3.36	0.75	0.829	0.001**	0.737	0.001**
I33	3.37	0.72	0.784	0.001**	0.717	0.001**
I34	3.46	0.68	0.689	0.001**	0.729	0.001**

Table 1. Continued

Factor number and item	Mean	SD	Item-total score correlation			
			Subgroup		Total scale	
			R	p	R	p
Factor 4	3.30	0.65	-	-	0.664	0.001**
I35	3.07	1.00	0.822	0.001**	0.516	0.001**
I36	3.25	0.81	0.875	0.001**	0.568	0.001**
I37	3.45	0.69	0.805	0.001**	0.539	0.001**
I38	3.43	0.74	0.718	0.001**	0.527	0.001**
Factor 5	3.55	0.51	-	-	0.767	0.001**
I39	3.57	0.60	0.807	0.001**	0.634	0.001**
I40	3.64	0.57	0.758	0.001**	0.561	0.001**
I41	3.55	0.62	0.842	0.001**	0.609	0.001**
I42	3.50	0.66	0.865	0.001**	0.677	0.001**
I43	3.49	0.66	0.815	0.001**	0.652	0.001**
Factor 6	3.55	0.43	-	-	0.897	0.001**
I44	3.51	0.64	0.712	0.001**	0.687	0.001**
I45	3.54	0.62	0.770	0.001**	0.741	0.001**
I46	3.55	0.60	0.748	0.001**	0.694	0.001**
I47	3.48	0.65	0.738	0.001**	0.726	0.001**
I48	3.62	0.58	0.749	0.001**	0.665	0.001**
I49	3.64	0.55	0.750	0.001**	0.651	0.001**
I50	3.63	0.56	0.748	0.001**	0.663	0.001**
I51	3.53	0.58	0.704	0.001**	0.626	0.001**
I52	3.55	0.56	0.726	0.001**	0.601	0.001**
I53	3.58	0.58	0.727	0.001**	0.592	0.001**
I54	3.59	0.57	0.717	0.001**	0.589	0.001**
I55	3.42	0.70	0.563	0.001**	0.508	0.001**
I56	3.58	0.60	0.673	0.001**	0.577	0.001**
I57	3.53	0.61	0.686	0.001**	0.610	0.001**
I58	3.51	0.63	0.719	0.001**	0.655	0.001**
I59	3.54	0.62	0.744	0.001**	0.675	0.001**
I60	3.53	0.62	0.698	0.001**	0.631	0.001**
Factor 7	3.32	0.58	-	-	0.744	0.001**
I61	3.28	0.80	0.740	0.001**	0.514	0.001**
I62	3.42	0.68	0.662	0.001**	0.544	0.001**
I63	2.91	1.04	0.687	0.001**	0.594	0.001**
I64	3.41	0.70	0.799	0.001**	0.686	0.001**
I65	3.42	0.71	0.827	0.001**	0.667	0.001**
I66	3.47	0.65	0.797	0.001**	0.649	0.001**
I67	3.36	0.78	0.798	0.001**	0.564	0.001**

**p<0.001, I: Item, r: Spearman's correlation coefficient, SD: Standard deviation

Table 2. Psychometric properties of the Nursing Professional Competence Draft scale (n=902)

Item	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	T-value
	EFA	EFA	EFA	EFA	EFA	EFA	EFA	
I1	0.636							20.65
I2	0.724							28.70
I3	0.696							28.43
I4	0.668							23.85
I5	0.555							20.78
I6	0.609							25.66
I7	0.454							21.53
I8		0.609						25.93
I9		0.641						26.32
I10		0.691						25.89
I11		0.541						23.04
I12		0.602						24.00
I13		0.621						23.14
I14		0.655						24.97
I15		0.690						25.99
I16		0.628						21.39
I17		0.547						19.77
I18		0.511						20.95
I19		0.581						25.34
I20		0.647						26.03
I21			0.438					16.93
I22			0.480					20.77
I23			0.533					23.81
I24			0.634					24.42
I25			0.705					26.51
I26			0.704					27.87
I27			0.717					28.66
I28			0.696					28.40
I29			0.739					29.41
I30			0.737					28.38
I31			0.722					29.84
I32			0.701					30.11
I33			0.630					27.58
I34			0.426					22.71
I35				0.632				24.11
I36				0.741				29.99
I37				0.712				24.59
I38				0.563				19.04

Table 2. Continued

Item	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	T-value
	EFA	EFA	EFA	EFA	EFA	EFA	EFA	
I39					0.588			25.18
I40					0.563			22.31
I41					0.690			27.64
I42					0.664			30.17
I43					0.586			26.91
I44						0.442		24.06
I45						0.501		27.23
I46						0.487		25.84
I47						0.424		25.74
I48						0.593		25.95
I49						0.624		25.80
I50						0.588		25.95
I51						0.571		22.99
I52						0.669		23.44
I53						0.694		23.12
I54						0.680		22.70
I55						0.422		15.99
I56						0.610		20.79
I57						0.611		21.18
I58						0.590		23.03
I59						0.572		24.74
I60						0.553		22.25
I61							0.633	17.94
I62							0.482	16.77
I63							0.597	14.51
I64							0.650	31.65
I65							0.707	33.49
I66							0.678	29.90
I67							0.729	27.17

I: Item, EFA: Exploratory Factor Analysis

Scale Factor Item Distributions (Naming) and Variance Values

The first factor consisted of seven items whose factor loadings varied between 0.454 and 0.724. It explained 39.810% of the total variance. The items in this factor were about the "nurses' roles in learning a patient history, determining the patients' needs, and getting opinions of the other healthcare team members." Therefore, the factor was named as "Diagnosis" (Table 3).

The second factor consisted of the 13 items whose factor loadings varied between 0.511 and 0.691. It explained 5.016% of the total variance. The items in this factor were about the "nurses' functions in making diagnosis, creating care plan, implementing care plan, ensuring patient safety, and evaluating care efficacy." Therefore, the factor was named as "Implementation of Nursing Process" (Table 3).

The third factor consisted of 14 items whose factor loadings varied between 0.426 and 0.739. It explained 4.554% of

Table 3. Internal consistency, factor item distributions, and variance values of the Nursing Professional Competence Draft scale

Factor	Items	Variance %	Total variance %	Cronbach's alpha
Diagnosis	1, 2, 3, 4, 5, 6, 7	39.810	39.810	0.88
Implementation of nursing process	9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 21, 24, 25	5.016	44.826	0.93
Health/patient education	12, 26, 27, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 57	4.554	49.380	0.95
Professional development	41, 42, 43, 44	3.035	52.415	0.82
Ethical practice	47, 48, 49, 50, 51	2.531	54.946	0.88
Critical thinking and teamwork	53, 54, 55, 56, 58, 59, 60, 61, 62, 63, 64, 65, 69, 70, 71, 75, 76	2.283	57.228	0.94
Research and development	66, 67, 68, 80, 81, 82, 83	2.012	59.240	0.88
Scale total				0.98

the total variance. The items in this factor were about the "nurses' responsibilities for planning the care with the patients and relatives and evaluating the patient satisfaction, determining and planning the training needs of healthy/ill individuals and providing and evaluating health/patient education." Therefore, the factor was named as "Health/Patient Education" (Table 3).

The fourth factor consisted of four items whose factor loadings varied between 0.563 and 0.741. It explained 3.035% of the total variance. The items in this factor were about the "nurses' contribution to the education of other healthcare team members, students, and new recruits." Therefore, the factor was named as "Professional Development" (Table 3).

The fifth factor consisted of five items whose factor loadings varied between 0.563 and 0.690. It explained 2.531% of the total variance. The items in this factor were about the "nurses' responsibilities of determining ethical dilemmas in patient care, acting in accordance with the patient rights, and taking precautions regarding patient rights violations." Therefore, the factor was named as "Ethical Practice" (Table 3).

The sixth factor consisted of 17 items whose factor loadings varied between 0.442 and 0.694. It explained 2.283% of the total variance. The items in this factor were about the "nurses' interpretation, critical thinking and analysis of patient needs, awareness of their own knowledge and skills, cooperation, emphasis on a teamwork in the professional practices, adaptation of lifelong learning and communication." Therefore, the factor was named as "Critical Thinking and Teamwork" (Table 3).

The seventh factor consisted of seven items whose factor loadings varied between 0.482 and 0.729. It explained 2.012% of the total variance. The items in this factor were about the "nurses' involvement in research activities, use of research outcomes in nursing care, and participation in educational programs that support their professional development." Therefore, the factor was named as "Research and Development" (Table 3).

Internal Consistency

The item-total score correlations and the Cronbach's alpha internal consistency values were calculated for the factors in the seven-factor structure scale created after the exploratory factor analysis is being done.

An acceptable value for the internal consistency of the scale was determined as the 0.70 (29). The internal consistency analysis revealed that the item-total correlation coefficients and Cronbach's alpha reliability values of both the total scale and the factors were high.

The Cronbach's alpha values showing the internal consistency of the Nursing Professional Competence Draft scale were calculated as 0.98 for the total scale, 0.88 for diagnosis, 0.93 for implementation of the nursing process, 0.95 for health/patient education, 0.82 for a professional development, 0.88 for ethical practice, 0.94 for critical thinking and teamwork, and 0.88 for research and development (Table 3).

Scale Total and Factors Score Distribution

The items of the Nursing Professional Competence Draft scale were prepared in four-point Likert type form. The scale included three intervals between 1 and 4. Each interval was scored dividing the number of the intervals by the number of items (22,30), which was formulated as 3:4=0.75, suggesting that each interval should cover a range of 0.75 points. Accordingly, the score intervals were as follows:

- 1-1.75: Never,
- 1.76-2.50: Sometimes,
- 2.51-3.25: Often,
- 3.26-4.00: Always.

DISCUSSION

An item analysis is performed to eliminate the scale items that exhibit relatively low correlation compared to the total scale. In the literature, it is desirable for a correlation between the variables not to be negative or low, as low item-total score correlation value decreases the scale reliability. Studies argue that a correlation value <0.30 indicates that the items are insufficient, whereas a value >0.40 indicates that the items have a good distinguishing feature (31). In the study, the item-total score correlation was calculated for an each item (Table 1), and this result suggests that the distinguishing feature of all scale items is good.

In the literature, an exploratory factor analysis is recommended to create a small number of conceptually meaningful new factors by bringing many interrelated variables together (32). Although there is no definite limit for the factor load values that explain the relationship of the items with a factor, Akgül (33) states that the lowest acceptable factor load value is 0.30, whereas the factor load values between 0.30-0.59 are accepted as a moderate and the values of ≥0.60 are considered as high. Büyüköztürk (32) qualifies a factor load value of ≥0.45 as a good criterion. In the study, the factor load value of all items was ≥0.45, and 16 items with a factor load <0.40 were removed from the draft scale (Table 2).

In the present study, a principal component analysis, which is the most common and widely used technique for an exploratory data analysis in the literature and is relatively easy to interpret, was used in the exploratory factor analysis. In addition, axis rotation was performed to provide an independence during the factor analysis and clarity in the interpretation. Varimax Rotation Technique, one of the most frequently used vertical rotation techniques, was also used in the study (32). The Varimax Rotation Technique prioritizes the factor loading column to reach the simple structure and significant factors, whereby a rotation is performed so that the factor variances are at the highest level with the fewer variables. As a result of the analysis, the higher the total variance explained by the factors, the stronger the factor structures of the scale. The total variance explained is expected to be at least 30% in the single-factor scales and to be higher (<40% and 60%) in the multi-factor scales (28). In the study, the total variance explained by all the scale items was 59.24% (Table 2). Therefore, the scale had a strong factor structure.

As a result of the factor analysis, it is aimed to find a small number of independent, conceptually meaningful factors among the many related original variables that are difficult to interpret (28). Therefore, the researchers decided that the scale consisted of the seven factors, by combining the items that were conceptually close to each other and had a highly positive and statistically significant relationship ($p<0.001$) (Table 2).

In the literature, it is recommended to use the different analyzes in a scale development studies to evaluate whether the sample has a sufficient size for the data analysis. The KMO sample adequacy test was used in the present study. Studies argue that the KMO value should be between 0-1, whereby factor analysis could be applied if the KMO test result is >0.50, and state that a KMO value between 0.70-0.80 indicates a moderate sampling adequacy, the value between 0.80-0.90 indicates a good sampling adequacy, and the value >0.90 indicates an excellent sampling adequacy. A significance result of the Barlett's test, another indicator for a sample suitability, suggests that the correlation matrix of the items in the draft scale is suitable for the factor analysis, in other words, reveals whether the correlation between the items in the draft scale is sufficient (31). In the present study, the KMO value and the Bartlett's test of the Sphericity results suggest that the sample size was sufficient for the factor analysis, whereby the correlation matrix of the items was excellent.

The naming of the factors emerging because of the exploratory factor analysis depends on the theoretical expectations and interpretations. Therefore, it is important to benefit from the opinions of the experts on the subject (33). The experts consulted while creating an item pool in the study were also interviewed to name the factors of the scale, and the relevant literature on this subject was examined and observed (1, 10, 15, 17, 18). In the study, nine of the factors predicted at the end of the literature review about the professional competences of the nurses were included in a draft scale. However, because of the analysis, it was found appropriate to combine the factors of Critical Thinking, Analysis, and Teamwork (17 items) under one factor. The draft scale has become a scale with seven factors (diagnosis, implementation of a nursing process, health/patient education, professional development, ethical practice, critical thinking and teamwork, and research and development) (Table 3). Similar results of this factor were reported in studies of Notarnicola et al. (1), Nilsson et al. (18) and Juntasopeepun et al. (20). This result is compatible with a theoretical framework.

Cronbach's alpha coefficient is a measure of the internal consistency and the homogeneity of the scale items. A scale consisting of the items with a high correlation with

each other also has a higher Cronbach's alpha coefficient (32). A Cronbach's alpha coefficient of $0.0<\alpha<0.39$ shows that the scale is not reliable, $0.40<\alpha<0.59$ indicates a low scale reliability, $0.60<\alpha<0.79$ indicates that the scale is reliable, and the $0.80<\alpha<1.00$ shows a high scale reliability (22). This value should be as close to 1 as possible (32). The Cronbach's alpha coefficients, which showed that the internal consistency of the Nursing Professional Competence scale in the study, were calculated as 0.98 for the total scale (Table 3). The acceptable value for the internal consistency of the scale is determined as 0.70 (29). This result, which shows that the total scale and factors' Cronbach' alpha values were >0.70, indicates that the total scale and factors are consistent within themselves, whereby the internal consistency was excellent, suggesting a high scale reliability.

Study Limitations

Nurses may answer items of the scale as they think they should answer rather than they respond from their owm experiences.

CONCLUSION

The Nursing Professional Competence scale was found to be a valid and reliable tool for the assessment of nurses' professional competences. The scale is a tool that could be easily applied by the researcher nurses and manager nurses. It could be used in research to determine the nurses' professional competences and its associated variables. Furthermore, it may also be used to examine the effects of nursing education programs on the professional competences of nurses in several areas including the diagnosis, implementation of nursing process, health/patient education, ethical practice critical thinking and teamwork. It is thought that this scale will be useful in determining the areas of the nurses' professional competence that are not sufficient or need to be improved.

Acknowledgments: We are grateful for the contributions from all the participants.

ETHICS

Ethics Committee Approval: For conducting the study, ethical approval was obtained from the Social Sciences and Humanities Ethics Committee at Istanbul University (number: 2018/46, date: 05.03.2018), and institutional permissions where the study was conducted were received.

Informed Consent: The nurses who agreed to participate in the study verbal and written consents were obtained.

Authorship Contributions

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

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

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

REFERENCES

1. Notarnicola I, Stievano A, De Jesus Barbarosa MR, Gambalunga F, Iacorossi L, Petrucci C, et al. Nurse Competence Scale: psychometric assessment in the Italian context. Ann Ig 2018;30:458-69.
2. Tzeng HM. Nurses' self-assessment of their nursing competencies, job demands and job performance in the Taiwan hospital system. Int J Nurs Stud 2004;41:487-96.
3. Türk Dil Kurumu. Yeterli olmak. Erişim linki: sozluk.gov.tr (Erişim tarihi: 16 July 2020)
4. Brunt BA. Identifying performance criteria for staff development competencies. J Nurses Staff Dev 2002;18:314-21.
5. Clinton M, Murrells T, Robinson S. Assessing competency in nursing: a comparison of nurses prepared through degree and diploma programmes. J Clin Nurs 2005;14:82-94.
6. Gruppen LD, Mangrulkar RS, Kolars JC. The promise of competency-based education in the health professions for improving global health. Hum Resour Health 2012;10:43.
7. Chen YC. [Essential professional core competencies for nurses]. Hu Li Za Zhi 2010;57:12-7.
8. World Health Organization Regional Office for Europe. (2001). Nurses and midwives for health, WHO European strategy for nursing and midwifery education section 1-8 guidelines for member states on the implementation of the strategy, World Health Organization. Available from: https://www.euro.who.int/__data/assets/pdf_file/0005/125744/E72918.pdf (accessed 16 July 2020).
9. International Council of Nurses. (2009). ICN framework of competencies for the nurse specialist. Available from: https://siga-fsia.ch/files/user_upload/08_ICN_Framework_for_the_nurse_specialist.pdf (accessed 16 July 2020).
10. Takase M, Teraoka S. Development of the Holistic Nursing Competence Scale. Nurs Health Sci 2011;13:396-403.
11. Fukada M. Nursing Competency: Definition, Structure and Development. Yonago Acta Med 2018;61:1-7.
12. Sroczynski M, Gravlin G, Route PS, Hoffart N, Creelman P. Creativity and connections: the future of nursing education and practice: the Massachusetts Initiative. J Prof Nurs 2011;27:e64-70.
13. Karasuda S, Tsumoto Y, Uchida H. Relationship between clinical competence of new graduate nurses and instructive support of colleagues—from the survey result at the time of one year after graduation. Shimane Daigaku Igakubu Kiyo 2014;37:27-36.
14. Bathish M, Wilson C, Potempa K. Deliberate practice and nurse competence. Appl Nurs Res 2018;40:106-9.
15. Cowan DT, Jenifer Wilson-Barnett D, Norman IJ, Murrells T. Measuring nursing competence: development of a self-assessment tool for general nurses across Europe. Int J Nurs Stud 2008;45:902-13.

16. Feliciano EE, Boshra AY, Mejia PCG, Feliciano AZ, Maniago JD, Alsharyah HM, et al. Understanding Philippines nurses' competency in the delivery healthcare services. Journal of Patient Care 2019;5:1-6.
17. Meretoja R, Leino-Kilpi H, Kaira AM. Comparison of nurse competence in different hospital work environments. J Nurs Manag 2004;12:329-36.
18. Nilsson J, Engström M, Florin J, Gardulf A, Carlsson M. A short version of the nurse professional competence scale for measuring nurses' self-reported competence. Nurse Educ Today 2018;71:233-9.
19. Fan JY, Wang YH, Chao LF, Jane SW, Hsu LL. Performance evaluation of nursing students following competency-based education. Nurse Educ Today 2015;35:97-103.
20. Juntasopeepun P, Turale S, Kawabata H, Thientong H, Uesugi Y, Matsuo H. Psychometric evaluation of the Nurse Competence Scale: A cross-sectional study. Nurs Health Sci 2019;21:487-93.
21. Wu XV, Enskär K, Lee CC, Wang W. A systematic review of clinical assessment for undergraduate nursing students. Nurse Educ Today 2015;35:347-59.
22. Karagöz Y. SPSS 21.1 Uygulamalı Biyoistatistik. 1st ed. Nobel; 2014.
23. Aydin A, Hiçdurmaz D. Holistic nursing competence scale: Turkish translation and psychometric testing. Int Nurs Rev 2019;66:425-33.
24. Hsu LL, Hsieh SI. Development and psychometric evaluation of the competency inventory for nursing students: a learning outcome perspective. Nurse Educ Today 2013;33:492-7.
25. Hemşirelik Yönetmeliği. 2010. Erişim linki: https://www.mevzuat.gov.tr/zuat?MevzuatNo=13830&MevzuatTur=7&MevzuatTertip=5 (Erişim tarihi: 16 July 2020).
26. World Health Organization. Nurse educator core competencies. Available from: https://www.who.int/hrh/nursing_midwifery/nurse_educator050416.pdf (accessed 16 July 2020).
27. Davis LL. Instrument review: getting the most from a panel of experts. Applied Nursing Research 1992;5:194-7.
28. Tavşancıl E. Tutumların Ölçülmesi ve SPSS ile Veri Analizi. 5th ed. Nobel; 2014.
29. Seçer I. SPSS ve LISREL ile Pratik Veri Analizi. Anı; 2013.
30. Uysal M, Öztürk H, Döş İ. Eğitimde ölçme ve değerlendirme. Ankara: Nobel Akademik Yayıncılık; 2013.
31. Polit FD, Beck CT. Nursing research principles and method. 6th ed. Lippincott Williams & Wilkins; 2012.
32. Büyüköztürk Ş. Sosyal Bilimler için Veri Analizi Kitabı. 15th ed. Pegem; 2011.
33. Akgül A. Faktör Analizi. İçinde: Tıbbi Araştırmalarda İstatistiksel Analiz Teknikleri SPSS Uygulamaları. 2nd ed. Emek; 2005.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:189-194
DOI: 10.4274/BMJ.galenos.2022.2022.3-13

Research

Skin Biopsy Results of Geriatric Patients Over a 5-year Period and the Frequency of Skin Diseases Before and After COVID-19 Pandemic

Geriatrik Hastalarda 5 Yıllık Süreçte Alınan Deri Biyopsisi Sonuçları, COVID-19 Pandemi Öncesi ve Sonrasında Görülen Farklar

Dilek Bıyık Özkaya[1], Gamze Erfan[2], Yıldız Okuturlar[3], Zeynep Tosuner[4], Deniz Demircioğlu[5], Özgür Timurkaynak[2]

[1]Acıbadem University Atakent Hospital, Clinic of Dermatology, Istanbul, Turkey
[2]Acıbadem University Altunizade Hospital, Clinic of Dermatology, Istanbul, Turkey
[3]Acıbadem University Atakent Hospital, Clinic of Internal Medicine, Istanbul, Turkey
[4]Acıbadem University Atakent Hospital, Clinic of Pathology, Istanbul, Turkey
[5]Acıbadem University Maslak Hospital, Clinic of Dermatology, Istanbul, Turkey

ABSTRACT

Objective: The number of older adults has increased throughout the world. Aging affects all the organs and creates psychological, physiologic and anatomic changes. One of the most important organs of the human body is the skin, which shows the effects of aging the most. This study aims to determine whether age, gender, and season of biopsy play a significant role in skin biopsy results. Additionally, the study investigates whether the frequency of skin diseases differs before and after the coronavirus disease-2019 (COVID-19) pandemic.

Methods: We conducted a retrospective study on the histopathology results of patients over 65 years old between June 2016 and September 2021. The histopathology results were obtained from the Acibadem Pathology Department.

Results: Of the 677 patients, 310 (45.8%) were male and 367 (54.2%) were female. The most common disease in all patients were benign cutaneous neoplasms (23%), followed by eczematous disease (18.5%) and epithelial cutaneous cancers (16.8%). We divided the results into 12 groups: group 1: Urticaria, erythema and purpuras, group 2: Papulosquamous and eczematous diseases, group 3: Infectious diseases, group 4: Rheumatologic diseases and alopecia, group 5: Benign cutaneous neoplasms, group 6: Precancerous lesions, group 7: Basal cell carcinoma, squamous cell carcinoma, group 8: Cutaneous metastasis and other skin cancers, group 9: Pigmentation disorders, group 10: Pschycology related dermatological disorders, group 11: Granulomatous dermatitis, group 12: Bullous dermatitis. Before the COVID-19 pandemic, the most prevalent results were group 2 (21.3%), followed by group 5 (20.4%) and group 7 (16.7%) whereas, after the COVID-19 pandemic, the most frequent results were group 5 (28.4%) followed by group 7 (17.1%), and group 6 (14.9%). In terms of seasons, the most common diseases were group 5 (24.1%) in winter, group 2 (21.6%) in spring, group 5 (30.0%) in summer, and group 2 (18.9%) in autumn. Before the COVID-19 pandemic, the most common result was group 2 (21.3%), followed by group 5 (20.4%) and group 7 (16.7%), and during the COVID-19 pandemic, the most common result was group 5 (28.4%), followed by group 7 (17.1%), group 6 (14.9%).

Conclusion: Many skin diseases affect the geriatric population. Geriatric patients face challenges such as multiple drug use, comorbidities, mobility problems and cognitive disorders. In our study, the most common diseases in all patients were benign cutaneous neoplasms (23%), followed by eczematous diseases (18.5%), and epithelial cutaneous cancers (16.8%). Knowing about the frequency of skin diseases is critical for the early detection of precancerous and cancerous lesions.

Keywords: Skin, geriatric, skin biopsy, elderly, skin disease

Address for Correspondence: Dilek Bıyık Özkaya, Acıbadem University Atakent Hospital, Clinic of Dermatology, Istanbul, Turkey
Phone: +90 533 350 69 43 E-mail: dilekozkaya@gmail.com ORCID ID: orcid.org/000-0002-5003-5346

Cite as: Bıyık Özkaya D, Erfan G, Okuturlar Y, Tosuner Z, Demircioğlu D, Timurkaynak Ö. Skin Biopsy Results of Geriatric Patients Over a 5-year Period and the Frequency of Skin Diseases Before and After COVID-19 Pandemic. Med J Bakirkoy 2022;18:189-194

Received: 16.03.2022
Accepted: 18.04.2022

ÖZ

Amaç: Tüm dünyada yaşlı nüfusta artış görülmektedir. Yaşlanma tüm organları etkilemekte ve psikolojik, fizyolojik ve anatomik değişikliklere sebep olmaktadır. Vücudumuzun en önemli organlarından olan deri yaşlanma etkilerinin en çok görüldüğü organlardan biridir. Bu çalışmada 65 yaş üstü hastalarda alınmış deri biyopsisi sonuçlarını incelemeyi, yaş, cinsiyet ve biyopsinin alındığı mevsimin hastalık sıklığına etkisi olup olmadığını araştırmayı amaçladık. Aynı zamanda koronavirüs hastalığı-2019 (COVID-19) pandemi döneminin biyopsi ile tanı konmuş hastalık sıklığına etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Haziran 2016 ile Eylül 2021 tarihleri arasında Acıbadem Üniversitesi Atakent Acıbadem, Maslak Acıbadem, Altunizade Acıbadem Hastaneleri Dermatoloji kliniklerine başvurmuş olan hastaların biyopsi sonuçları retrospektif olarak tarandı. Sonuçlar 12 grupta incelendi.

Bulgular: Biyopsi sonucu taranan 677 hastanın 310'u (%45,8) erkek, 367'si (%54,2) kadındı. Tüm hastalarda en sık rastlanan hastalık grubu benin deri tümörleriydi (%23). Sonrasında sırayla ekzematöz deri hastalıkları (%18,5) ve epitelyal deri kanserleri (%16,8) saptandı. Sonuçları 12 gruba böldük. Grup 1: Ürtiker, eritemli hastalıklar ve purpuralar, grup 2: Papüloskuamöz hastalıklar ve ekzematöz hastalıklar, grup 3: Enfeksiyöz hastalıklar, grup 4: Romatolojik hastalıklar ve alopesi, grup 6: Benin kutanöz tümörler, grup 6: Prekanseröz lezyonlar, grup 7: Bazal hücreli karsinom, skuamöz hücreli karsinom, grup 8: Kutanöz metastaz ve digger deri kanserleri, grup 9: Pigmentasyon bozuklukları, grup 10: Psikoloji ilişkili dermatolojik hastalıklar, grup 11: Granulomatöz dermatitler, grup 12: Büllöz hastalıklar. COVID-19 pandemisi öncesinde en sık rastlanan hastalık grupları 2 (%21,3), 5 (%20,4), 7 (%16,7) iken COVID-19 pandemi döneminde en sık rastlanan gruplar 5 (%28,4), 7 (%17,1) ve 6 (%14,9) olarak saptandı.

Sonuç: Geriatrik populasyonda birçok deri hastalığı gözlenmektedir. Geriatrik hastalarda çoklu ilaç kullanımı, eşlik eden komorbiditeler, hareket etmekteki sıkıntılar, bilişsel bozukluklar tanı ve tedavide zorluklara sebep olmaktadır. Çalışmamızda geriatrik hastalarda en sık görülen deri biyopsisi sonuçlarını benign deri tümörleri (%23), ekzematöz hastalıklar (%18,5) ve epitelyal deri kanserleri (%16,8) olarak saptadık. Geriatrik hastalardaki deri hastalıkları sıklıklarını bilmek prekanseröz ve kanseröz deri hastalıkları konusunda bilinçli davranmak ve geriatrik hastalara multidisipliner yaklaşmak konusunda önemlidir.

Anahtar Kelimeler: Deri, geriatrik, deri biyopsisi, yaşlı, deri hastalıkları

INTRODUCTION

The geriatric population is defined as people aged 65 and over. The number of older adults is increasing all around the world. The skin is a vital organ of the body. It covers all the surfaces of our body and provides a physical barrier. It has various functions such as protecting from losing water and electrolytes, protecting from infections, regulating the body temperature, feeling the touches, pressure, pain, itching by the nerves and sensory receptors. Skin aging occurs by intrinsic and extrinsic factors. Thinning of the epidermis and dermis, decrease in elastin and collagen fibers, decrease in vascularity and supporting structures in the dermis, impaired immune response, impaired neurologic responses, atrophy of sweat glands, decreased stratum corneum lipids, and a decrease in melanocytes are all signs of skin aging (1). Intrinsic aging is a natural process that occurs over time and is related to genetics. Over time, the function of keratinocytes, fibroblasts decrease, loss of telomeres, mitochondrial damage affects the intrinsic aging. Environmental factors affect our skin and can cause aging. UV, air pollution, smoking, sun exposure, poor diet, stress are the extrinsic causes of skin aging. Environmental factors cause free radicals, DNA damage, glycation, inflammation, and this causing cellular damage and skin aging (2). With the effects of intrinsic and extrinsic factors, dermal atrophy decreased collagen, decreased elasticity, due to increased melanogenesis pigmentation disorders, roughness, wrinkling, laxity of the skin occurs (3,4).

Aging affects all the organs and creates psychologic, physiologic and anatomic changes. Older patients have multiple diseases, such as diabetes, heart disease, hypertension and generally use multiple drugs (5). In this study, we investigated whether age, gender, and season play a significant role in skin biopsy results. Additionally, the study investigated whether the frequency of skin diseases differs before and after the coronavirus disease-2019 (COVID-19) pandemic.

METHODS

We retrospectively examined the histopathology results of patients over 65 years old between June 2016 and September 2021. The histopathology results were obtained from three centres: Acibadem University Atakent Acibadem, Maslak Acibadem, Altunizade Acibadem Dermatology clinics the age groups were divided into 3 groups: 65-75, 75-85 and >85. We investigated whether sex and age play a significant role in skin diseases. Season may also affect the skin. In winter, xerosis increases and this may cause pruritis and eczematous changes. Therefore, we also found out whether seasons play a significant role in skin diseases. The first COVID infection was reported on 11.03.2020 in Turkey and since then the population referring to outpatient clinics has changed. We revealed whether there is a significant difference between the results before and after COVID-19 infections.

All procedures carried out in studies involving human participants were in accordance with the ethical standards

of the institutional (Acibadem University-decision no: 2022-04/07, date: 25.02.2022) and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statistical Analysis

Data obtained were analyzed using SPSS 24 software (IBM Corp, Armonk, NY). During the evaluation of study variables, descriptive statistical methods (mean, standard error, rate) were used. Data were analyzed using Student's t-test, Mann-Whitney U tests chi-square test and Fisher's Exact tests as appropriate. A value of $p<0.05$ was considered as statistically significant.

RESULTS

There were 677 patients in the study; 310 (45.8%) of them were male and 367 (54.2%) were female (Table 1). The mean age of the male patients was 73.45±6.58 median 72 (65-94) and the mean age of the female patients was 72.53±6.27 [median 71 (minimum-maximum 65-91)]. There wasn't a statistical difference between the mean age of female and male cases. In all patients, the most frequently seen 10 diseases were seborrheic keratosis (15%), basal cell carcinoma (BCC) (13%), eczematous dermatitis (9%), actinic keratosis (AK) (8%), human papilloma virus (HPV) related lesions (5%), drug reactions (3.5%), Bowen (3%), squamous cell carcinoma (2.7%), lichen plan (2.7%) and bullous pemphigoid (2.2%). We divided the results into 12 groups (Table 1).

The most common disease in all patients were benign cutaneous neoplasms (23%), followed by eczematous diseases (18.5%), and epithelial cutaneous cancers (16.8%). The most common group among the female patients was group 5 (24.6%), followed by group 7 (16.9%), and group 2 (15%) respectively. The most common group among the male patients was group 2 (22.6%), followed by group 5 (21.9%) and group 7 (16.8%) respectively (Table 1). In group 2, the most common disease was contact dermatitis (18%); in group 5, seborrheic keratosis (65%), in group 6, AK (72%), and in group 7, BCC was the most common lesion (71%).

When we divided the ages into 3 groups 65-74,75-84, >85, the most common disease was group 5 (22%), followed by group 5 (26.3%) and group 2 (22.2%) respectively. When the data before and after the COVID-19 pandemic were analyzed, it was seen that there was no significant difference between the female and male patients (p=0.274). The mean age during the COVID-19 pandemic was 73.00, whereas it was 72.64 before the pandemic. This shows that there is no statistically significant difference in terms of the mean age. When we analyzed the seasonal effects, there was no statistically significant difference between the female and male patients (p=0.483) Before the COVID-19 pandemic, the most common result was group 2 (21.3%), followed by group 5 (20.4%) and group 7 (16.7%), and during the COVID-19 pandemic, the most common result was group 5 (28.4%), followed by group 7 (17.1%), group 6 (14.9%). When we looked into seasonal effects, the most common diseases

Table 1. Groups and male, female biopsy results

		Male		Female	
Group		Frequency	Percent	Frequency	Percent
1	Urticaria, erythemas and purpuras	23	7.4	26	7.1
2	Papulosquamous and eczematous diseases	70	22.6	55	15.0
3	Infectious diseases	29	9.4	21	5.7
4	Rheumatologic diseases and alopecia	8	2.6	15	4.1
5	Benign cutaneous neoplasms	68	21.9	88	24.0
6	Precanserous lesions	26	8.4	50	13.6
7	BCC, SCC	52	16.8	62	16.9
8	Cutaneous metastasis and other skin cancers	14	4.5	23	6.3
9	Pigmentation disorders	5	1.6	7	1.9
10	Pschyco related dermatological disorders	4	1.3	7	1.9
11	Granuloumatous dermatitis	4	1.3	4	1.1
12	Bullous dermatitis	7	2.3	9	2.5
	Total	310	100.0	367	100.0

BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma

were in group 5 (24.1%) in winter, group 2 (21.6%) in spring, group 5 (30.0%) in summer, group 2 (18.9%) in autumn.

DISCUSSION

The geriatric population is increasing day by day. Nevertheless, the dermatologic diseases of geriatric patients are still a less researched area. To our knowledge, there is no study on skin biopsy results. Studies from Turkey show that eczematous diseases are the most common disease in the geriatric population (6-8).

The results of our study illustrate that the most common disease is a benign cutaneous neoplasm. In outpatient clinics, a dermatologic examination is enough for the diagnosis of most of the diseases. Eczematous dermatitis, pruritus, fungal infections are the most common diseases in the elderly population, but they rarely require biopsy for diagnosis (9,10).

In our study, benign cutaneous neoplasms accounted for 23% of all cases. Grover and Narasimhalu (11) investigated 200 elderly patients and 537 (74.5%) benign cutaneous neoplasms. Liao et al. (9) reported benign tumors 12.8% in a study from Taiwan. Yaldiz (7) reported benign tumor 7.19%. Yalçin et al. (6) reported 1.7% benign cutaneous neoplasms. Seborrheic keratosis in 15% of the study population and 65% of the benign cutaneous neoplasms in our study. Grover and Narasimhalu (11) reported 43% seborrheic keratosis. Cvitanović et al. (12) reported 18.9% seborrheic keratosis in the elderly population. Our results were similar to Cvitanović et al.'s (12) study.

Seborrheic keratoses are very common benign neoplasms, and the incidence increases with age. It can be difficult to diagnose using the naked eye because it can resemble various lesions such as basal cell carcinoma, pigmented Bowen's disease, melanoma, common warts, and acanthosis nigricans. Dermoscopy increases the chance of accurate diagnosis and helps differentiate from malign neoplasms (13).

In our study, the most common group among the female patients was 5 while group 2 was the most common among the male patients. Liao et al. (9) found benign tumors more frequent in male patients than in female patients. Yaldiz (7) determined benign neoplasm 7.19%, female patients 7.78% and, male patients 6.67% and in Yalçin et al.'s (6) study, there was no significant difference between sex and the benign neoplasm (1.7%).

Makrantonaki et al. (14) discovered that benign neoplasms accounted for 20% of females and 14% of males. This appears similar to our results. Women place a higher value on their appearance and take greater care of their skin. The excess of women, in our opinion, is due to women's skincare habits (14).

In our study, the second most common group was eczematous diseases (18.5%). The findings of Yildiz's (8) study indicated that the most common disease in elderly patients was contact dermatitis (n=1380, 15.2%) whereas the study of Smith and Leggat (15) in southern Taiwan at a nursing home showed that dermatitis was the third (7.3%) common disease. Yaldiz (7) found that eczematous dermatitis was the most common skin disease (24.3%) in his study from Turkey. Grover and Narasimhalu (11) found eczema 39% in the elderly population in his study from Bangalore. In addition to these studies, Thaipisuttikul (16) found eczema 22.8% in their study from Bangkok, and Yalçin et al. (6) found 20.4% in their study from Turkey. Our results were similar to Yalçin et al.'s (6) results.

Epidermal barrier function deteriorates with age. A washing product not irrits at a younger age may easily become irritant at an older age. The immune system changes with age, and Th1 decreases and Th2 dominance makes the person more allergic. Dryness, a common condition in older skin, facilitates itching and facilitates contact dermatitis. The comorbidities and systemic diseases increase at advanced ages. Because of these conditions, patients must use multiple drugs. These drugs may cause reactions or make the skin dry, and thus eczema may occur easily. Generally, in outpatient clinics, physicians give treatments without taking biopsy. Therefore, eczematous disease belongs to the second group (17).

In our study, cutaneous epithelial neoplasms were the third most common group (16.8%) and BCCs were the most common malignant tumor (71%). The frequency was 42.7% in women and 57.3% in men. The difference was not statistically significant. BCCs are the most common skin cancers, accounting for 70%-80% of non-melanoma skin cancers (18). According to the study of Kumar et al. (10), skin cancers account for 5.2% of all cases, and they point out that the frequency may be low in their country because of the Fitzpatrick skin type 4 and 5. Smith and Leggat (15) reported only one BCC (0.3%) in their study and they mentioned that BCC rates are rare in Taiwan. Liao et al. (9) reported 29.8% BCC in their study from Taiwan. Makrantonaki et al. (14) found epithelial skin cancers 13.3% in the female, and 34% in the male population. Yalçin et al. (6) found 5.2% and Yaldiz (7) found 9.2% precancerous and malignant skin neoplasias from Turkey (10). Since the patients in our study had biopsies and the reports in the other studies were from outpatient clinics, our results were higher than theirs. Life expectancy

has increased worldwide, and BCC cases are increasing with age. Additionally, the cumulative effect of the sun and the decrease in DNA repair capacity facilitate the formation of skin cancers (19). When we analyzed the relationship between season and skin diseases, we found that benign cutaneous neoplasms (24.1%) were most common in winter, eczematous dermatosis 21.6% in spring, benign cutaneous neoplasms (30%) in summer and eczematous dermatosis 18.9% in autumn. Especially in winter, dryness of the skin increases and we expect eczematous diseases to be seen more in the wintertime. Yaldiz (7) and Yalçin et al. (6) reported similar findings: in the winter, the most common diseases were eczematous diseases, xerosis, and pruritus, while in the summer, fungal infections were the most common. Since we reported on biopsy reports in the past, our findings do not reflect the true prevalence of diseases. Therefore, we were unable to find a link between the season and the biopsy results.

Especially in the first months of COVID-19 pandemics in our outpatient clinics, the number of patients decreased. Patients went to policlinic only in emergencies. When we investigated whether there was a difference in the results before and after the pandemic, we found that after the COVID-19 pandemic, the most common groups were group 5 (28.4%), group 7 (17.1%) and group 6 (14.9%). The eczematous group was the most common group before the pandemic. However, the percentage of the precancerous group increased after the pandemic. In this group, 72% of the lesions were AK (20). Templier et al. (20) reported that skin cancers accounted for 4.9% and 1/3 of the cases and their study group had AK. Makrantonaki et al. (14) found precancerous skin lesions 6.7% in the female population and 20% in the male population.

AK has a worldwide prevalence of 11%-25%. When individuals above 70 years were observed, this rate increased to 34.1% and 18.2% in men and women, respectively. At 60-69 years of age, the rates were 83% and 64%. In our study, AK was 30.9% in women and 69.1% in men. This discrepancy between the genders has been attributed to the differences in sun exposure (21).

The main causative agent of AK is UV radiation. With UV, DNA damage, free reactive oxygen species, inhibition of p53 may cause AK. AK also occurs frequently in immunosuppressant patients such as organ transplant patients and patients with HIV/AIDS. And HPV infection may have a related to AK (21).

CONCLUSION

Aging changes our skin. Therefore, the geriatric population suffers from various skin diseases. Besides, they face challenges such as multiple drug use, comorbidities, mobility problems and cognitive disorders. Knowing about the frequency of skin diseases is critical for the early detection of precancerous and cancerous lesions. The dermatologic diseases of geriatric patients are still a less researched area, therefore, close collaboration between geriatricians and dermatologists, as well as additional research, is crucial for the early diagnosis and treatment of skin cancers.

ETHICS

Ethics Committee Approval: The study was approved by the Acibadem University Ethics Committee (decision no: 2022-04/07, date: 25.02.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: D.B.Ö., Y.O., Design: D.B.Ö., G.E., Y.O., Data Collection or Processing: D.B.Ö., G.E., Z.T., D.D., Ö.T., Analysis or Interpretation: D.B.Ö., Y.O., Literature Search: D.B.Ö., G.E., Writing: D.B.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. Farage MA, Miller KW, Berardesca E, Maibach HI. Clinical implications of aging skin: cutaneous disorders in the elderly. Am J Clin Dermatol 2009;10:73-86.
2. Baumann L. How to Use Oral and Topical Cosmeceuticals to Prevent and Treat Skin Aging. Facial Plast Surg Clin North Am 2018;26:407-13.
3. Sunder S. Relevant Topical Skin Care Products for Prevention and Treatment of Aging Skin. Facial Plast Surg Clin North Am 2019;27:413-8.
4. Khavkin J, Ellis DA. Aging skin: histology, physiology, and pathology. Facial Plast Surg Clin North Am 2011;19:229-34.
5. Scheinfeld N, Yu T, Weinberg J, Gordon M, Silapunt S, Norman RA, et al. Cutaneous oncologic and cosmetic surgery in geriatric patients. Dermatol Clin 2004;22:97-113.
6. Yalçin B, Tamer E, Toy GG, Oztaş P, Hayran M, Alli N. The prevalence of skin diseases in the elderly: analysis of 4099 geriatric patients. Int J Dermatol 2006;45:672-6.
7. Yaldiz M. Dermatological diseases in the geriatric age group: Retrospective analysis of 7092 patients. Geriatr Gerontol Int 2019;19:582-5.
8. Yildiz H. Prevalence of Geriatric Dermatoses Among Elderly Patients Treated at the Dermatology Outpatient Clinic in Eskisehir, Turkey. South. Clin. Ist. Euras 2019;30:47-51.
9. Liao YH, Chen KH, Tseng MP, Sun CC. Pattern of skin diseases in a geriatric patient group in Taiwan: a 7-year survey from the outpatient clinic of a university medical center. Dermatology 2001;203:308-13.
10. Kumar D, Das A, Bandyopadhyay D, Chowdhury SN, Das NK, Sharma P, et al. Dermatoses in the Elderly: Clinico-Demographic

Profile of Patients Attending a Tertiary Care Centre. Indian J Dermatol 2021;66:74-80.

11. Grover S, Narasimhalu CR. A clinical study of skin changes in geriatric population. Indian J Dermatol Venereol Leprol 2009;75:305-6.
12. Cvitanović H, Knezević E, Kuljanac I, Jancić E. Skin disease in a geriatric patients group in outpatient dermatologic clinic Karlovac, Croatia. Coll Antropol 2010;34 Suppl 2:247-51.
13. Wollina U. Recent advances in managing and understanding seborrheic keratosis. F1000Res. 2019;8:F1000 Faculty Rev-1520. doi: 10.12688/f1000research.18983.1.
14. Makrantonaki E, Steinhagen-Thiessen E, Nieczaj R, Zouboulis CC, Eckardt R. Prevalence of skin diseases in hospitalized geriatric patients : Association with gender, duration of hospitalization and geriatric assessment. Z Gerontol Geriatr 2017;50:524-31.
15. Smith DR, Leggat PA. Prevalence of skin disease among the elderly in different clinical environments. Australasian Journal on Ageing 2005;24:71-6.
16. Thaipisuttikul Y. Pruritic skin diseases in the elderly. J Dermatol 1998;25:153-7.
17. Berger TG, Steinhoff M. Pruritus in elderly patients--eruptions of senescence. Semin Cutan Med Surg 2011;30:113-7.
18. Ciążyńska M, Narbutt J, Woźniacka A, Lesiak A. Trends in basal cell carcinoma incidence rates: a 16-year retrospective study of a population in central Poland. Postepy Dermatol Alergol 2018;35:47-52.
19. Kasumagic-Halilovic E, Hasic M, Ovcina-Kurtovic N. A Clinical Study of Basal Cell Carcinoma. Med Arch 2019;73:394-8.
20. Templier C, Boulanger E, Boumbar Y, Puisieux F, Dziwniel V, Mortier L, et al. Systematic skin examination in an acute geriatric unit: skin cancer prevalence. Clin Exp Dermatol 2015;40:356-60.
21. de Oliveira ECV, da Motta VRV, Pantoja PC, Ilha CSO, Magalhães RF, Galadari H, et al. Actinic keratosis - review for clinical practice. Int J Dermatol 2019;58:400-7.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:195-201
DOI: 10.4274/BMJ.galenos.2022.2022.3-8

Research

May Hepatic Steatosis Be Associated with Gynecomastia and Epicardial Fat? A Retrospective Study of 599 Male Patients

Hepatik Steatoz Jinekomasti ve Epikardiyal Yağlanma ile İlişkili Olabilir mi? 599 Erkek Hastanın Retrospektif Analizi

Süleyman Hilmi Aksoy[1], Selami Aydın[2], Işıl Yurdaışık[3], Orhan Dalkılıç[4]

[1]Hisar Intercontinental Hospital, Clinic of Radiology, Istanbul, Turkey
[2]Hisar Intercontinental Hospital, Clinic of Internal Medicine, Istanbul, Turkey
[3]Istinye University, Medical Park Gaziosmanpaşa Hospital, Department of Radiology, Istanbul, Turkey
[4]Hisar Intercontinental Hospital, Clinic of Chest Diseases, Istanbul, Turkey

ABSTRACT

Objective: There is no study in the literature investigating the association of hepatic steatosis both gynecomastia and epicardial fat thickness together. We determined the correlations between hepatic steatosis through liver density, gynecomastia and epicardial fat thickness in patients undergoing computed tomography (CT) scans due to suspected coronavirus disease-2019 (COVID-19) symptoms.

Methods: A total of 599 male patients who underwent chest CT scans because of a presumed diagnosis of COVID-19 in our radiology clinic were included in the study. Patients' age, diameters of the subareolar glandular tissues of the right and left breasts, the right retroareolar fatty tissue, liver and spleen density, epicardial fat thickness and biochemical parameters were recorded and analyzed. Laboratory analyses were performed according to the standard methods.

Results: The mean age of the patients was 47.21±15.00 years. The left subareolar tissue thickness and the right retroareolar fatty tissue thickness that are used to indicate gynecomastia were significantly correlated with liver density in the negative direction (r=-0.137, p<0.001; r=-0.172, p<0.001; respectively). Epicardial fat thickness was statistically significantly correlated with right subareolar tissue thickness (r=0.085, p=0.037), left subareolar tissue thickness (r=0.101, p=0.014) and right retroareolar fatty tissue thickness (r=0.148, p<0.001).

Conclusion: The results of this study showed that gynecomastia was significantly correlated with both age and hepatic steatosis. Epicardial fat thickness is also associated with hepatic steatosis. We demonstrated the significant correlations between epicardial fat thickness and gynecomastia for the first time. Nevertheless, our results need to be confirmed by further comprehensive studies.

Keywords: Hepatic steatosis, gynecomastia, epicardial fat, computed tomography

ÖZ

Amaç: Literatürde hepatik steatozun hem jinekomasti hem de epikardiyal yağ kalınlığı ile ilişkisini araştıran bir çalışma bulunmamaktadır. Şüpheli koronavirüs hastalığı-2019 (COVID-19) semptomları nedeniyle bilgisayarlı tomografi (BT) taraması yapılan hastalarda karaciğer yoğunluğu, jinekomasti ve epikardiyal yağ kalınlığı ile hepatik steatoz arasındaki ilişkileri belirlemeyi amaçladık.

Gereç ve Yöntem: Radyoloji kliniğimizde COVID-19 ön tanısı ile akciğer tomografisi çekilen toplam 599 erkek hasta çalışmaya dahil edilmiştir. Hastaların yaşı, sağ ve sol meme subareolar glandüler doku çapları, sağ retroareolar yağ dokusu, karaciğer ve dalak yoğunluğu, epikardiyal yağ kalınlığı ve biyokimyasal parametreleri kaydedilerek analiz edilmiştir. Laboratuvar analizleri standart yöntemlere göre yapılmıştır.

Bulgular: Hastaların yaş ortalaması 47,21±15,00 yıldır. Jinekomasti varlığını belirtmek için kullanılan sol subareolar doku kalınlığı ve sağ retroareolar yağ dokusu kalınlığı negatif yönde karaciğer yoğunluğu ile anlamlı olarak ilişkili bulundu (sırasıyla r=-0,137, p<0,001; r=-0,172, p<0,001). Epikardiyal yağ kalınlığı, sağ subareolar doku kalınlığı (r=0,085, p=0,037), sol subareolar doku kalınlığı (r=0,101, p=0,014) ve sağ retroareolar yağ dokusu kalınlığı (r=0,148, p<0,001) ile istatistiksel olarak anlamlı korelasyon göstermiştir.

Address for Correspondence: Süleyman Hilmi Aksoy, Hisar Intercontinental Hospital, Clinic of Radiology, Istanbul, Turkey
Phone: +90 505 188 78 04 E-mail: mdshilmiaksoy@gmail.com ORCID ID: orcid.org/0000-0002-2356-0268

Cite as: Aksoy SH, Aydın S, Yurdaışık I, Dalkılıç O. May Hepatic Steatosis Be Associated with Gynecomastia and Epicardial Fat? A Retrospective Study of 599 Male Patients. Med J Bakirkoy 2022;18:195-201

Received: 22.03.2022
Accepted: 19.04.2022

Sonuç: Bu çalışmanın sonuçları, jinekomastinin hem yaş hem de karaciğer yağlanması ile önemli ölçüde ilişkili olduğunu göstermiştir. Epikardiyal yağ kalınlığı da karaciğer yağlanması ile ilişkilidir. Epikardiyal yağ kalınlığı ile jinekomasti arasındaki anlamlı korelasyonu ilk kez gösterdik. Bununla birlikte, sonuçlarımızın daha kapsamlı çalışmalarla doğrulanması gerekmektedir.

Anahtar Kelimeler: Hepatik steatoz, jinekomasti, epikardiyal yağ, bilgisayarlı tomografi

INTRODUCTION

Hepatic steatosis, also known as fatty liver disease, refers to the accumulation of triglycerides within cytoplasmic vesicles of hepatocytes. Hepatic steatosis is associated with liver damage ranging from simple steatosis to liver fibrosis, cirrhosis and hepatocellular carcinoma (1). Numerous risk factors of developing hepatic steatosis have been identified, including diabetes mellitus, insulin resistance, obesity, dyslipidemia, hypertension and alcohol overuse (2). The prevalence of hepatic steatosis has been reported 3-39% due to variable and subjective diagnostic criteria (3,4). Several imaging modalities such as ultrasound, computed tomography (CT) and magnetic resonance imaging can show changes in hepatic steatosis in a non-invasive manner. CT can provide more objective measurement of liver density, thus hepatic steatosis (5). Non-contrast CT scan can detect hepatic steatosis with a sensitivity of 82% and specificity of 100% (6).

Gynecomastia is a benign proliferation of breast glandular tissue in men. It is the most common male breast abnormality and is thought to develop due to a hormonal imbalance through multiple mechanisms (7). Gynecomastia can be physically disturbing, causing psychological distress and may have negative effects on body image and self-confidence. Glandular tissue of ≥2 cm in the subareolar area is generally accepted as gynecomastia (8). The prevalence of gynecomastia has been reported to be between 32-65% depending on the diagnostic method, age and lifestyle (9). However, the actual incidence of gynecomastia in the general population is unclear because most of these patients are asymptomatic and breast screening is not routinely performed in men. The widespread use of CT for other indications results in gynecomastia to is reported commonly as an incidental finding on thoracic CT (10). Some studies have reported that hepatic liver disease is among the risk factors of developing gynecomastia (11). Patients with hepatic steatosis often have high estrogen and low testosterone levels that clinically manifest as testicular atrophy, palmar erythema and gynecomastia (12). However, the exact mechanism through which hepatic steatosis can contribute to the development of gynecomastia has yet to be clarified.

However, the severity of hepatic steatosis has been associated with epicardial fat thickness, which is a marker of visceral fat (13). Hepatic steatosis may coexist and interplay with epicardial fat. Quantification of epicardial fat thickness can be easily obtained using multislice CT. Measurement of the epicardial fat thickness on CT is performed using regions of interest on short axis views (14). To the best of our knowledge, there is no study in the literature investigating the association of hepatic steatosis with both gynecomastia and epicardial fat thickness. In this study, we determined the correlations between hepatic steatosis through liver density, gynecomastia and epicardial fat thickness in patients undergoing CT scans due to suspected coronavirus disease-2019 (COVID-19) symptoms.

METHODS

Before the beginning, the study protocol was approved by the local ethics committee of Istinye University with the 05/08/2021 dated and (2017-KAEK-120)/2/2021.G-111 numbered decision. The study was conducted in accordance with the ethical principles of 1964 Declaration of Helsinki and its later amendments.

A total of 599 male patients who underwent chest CT scans due to a presumed diagnosis of COVID-19 in our radiology clinic between 2018 and 2021 were included in the study. Female patients, those with established heart disease, hepatic failure, renal failure, active infection, malignancy, chronic systemic inflammatory disease and patients with missing data were excluded from the study. Patients' age, diameters of the subareolar glandular tissues of the right and left breasts, diameter of the right retroareolar fatty tissue, liver density, spleen density, epicardial fat thickness and biochemical parameters including fasting blood glucose, the levels of cholesterol, triglycerides, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase and gamma-glutamyltransferase were recorded and analyzed.

Blood samples were collected from the patients after a 12-h fasting for laboratory analysis of fasting blood glucose, cholesterol, triglycerides and liver enzymes. Laboratory analyses were performed according to the standard methods.

CT Examinations

All images were taken using a 160-slices 320-row area detector CT device (Aquilion Prime ONE 320, Toshiba Medical Systems). CT protocols were performed at 120

kV, 100-200 mA and reconstructed at a slice thickness of 1 mm. The diagnosis of gynecomastia was established as a glandular tissue diameter (long axis of the area showing increased density) ≥2 cm at the nipple level in the axial plane or a glandular tissue diameter between 1 and 2 cm accompanied by vertical growth consistent with gynecomastia. Measurements of axial diameters were made separately for both breasts (Figure 1). Hepatic steatosis was defined as a liver density at least 10 Hounsfield Units (HU) smaller than the spleen density or a liver density <40 HU as measured on CT images (Figure 2). Epicardial fat thickness was measured from the visceral epicardium to the outside of the myocardium, and perpendicular to the surface of the heart on the axial section in millimeters (Figure 3).

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 (SPSS, Statistical Package for Social Sciences, IBM Inc., Armonk, NY, USA) software. Continuous variables were expressed as mean ± standard deviation, while categorical variables were given as frequency (n) and percentage (%). The normal distribution of the data was evaluated with Kolmogorov-Smirnov and Shapiro-Wilk tests. Statistical correlations between hepatic steatosis, gynecomastia, epicardial fat and laboratory parameters were examined with Pearson's correlation analysis (r).

RESULTS

A total of 599 male patients with a diagnosis of gynecomastia were included in the study. The mean age of the patients was 47.21±15.00 years. The age distribution of the gynecomastia patients is shown in Figure 4.

When parameters of epicardial fat were examined; the mean diameter of right subareolar granular tissue was measured as 12.27±5.95 mm, the mean diameter of left subareolar granular tissue as 14.70±7.07 mm and the mean diameter of right retroareolar fatty tissue as 18.29±9.55 mm.

The mean liver density of the patients was measured as 51.03±10.85 HU and the mean spleen density as 48.12±8.06 HU. The mean epicardial fat thickness was measured as 5.91±3.88 mm. Laboratory findings of the biochemical parameters are shown in Table 1.

The correlations between gynecomastia, hepatic steatosis, epidural fat thickness and biochemical parameters were evaluated with Pearson's correlation analysis. Accordingly, age was positively correlated with the left subareolar glandular tissue diameter (r=0.113, p=0.05), and right retroareolar fatty tissue diameter (r=0.116, p=0.04), and epicardial fat thickness (r=0.189, p<0.001) and negatively correlated with spleen density (r=-0.125**, p=0.002). Statistically significant strong correlations were found between right subareolar tissue thickness, left subareolar tissue thickness, right retroareolar fatty tissue thickness, liver density, spleen density and epicardial fat thickness (Table 2).

However, liver density showed a strong positive correlation with cholesterol (r=0.262, p<0.00) and a strong negative correlation with triglycerides (r=-0.122, p=0.004), while spleen density was strongly correlated with cholesterol (r=0.194, p<0.001), AST (r=0.235, p<0.001) and ALT (r=0.232, p<0.001) in the positive direction. No statistically

Table 1. Biochemical parameters of the patients

Biochemical parameter	Mean	± SD
Cholesterol	122.13	33.59
Triglycerides	130.27	80.78
Fasting blood glucose	113.85	40.78
AST	37.37	268.74
ALT	50.45	310.62
ALP	88.51	147.88
GGT	66.30	112.73

AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyltransferase, SD: Standard deviation

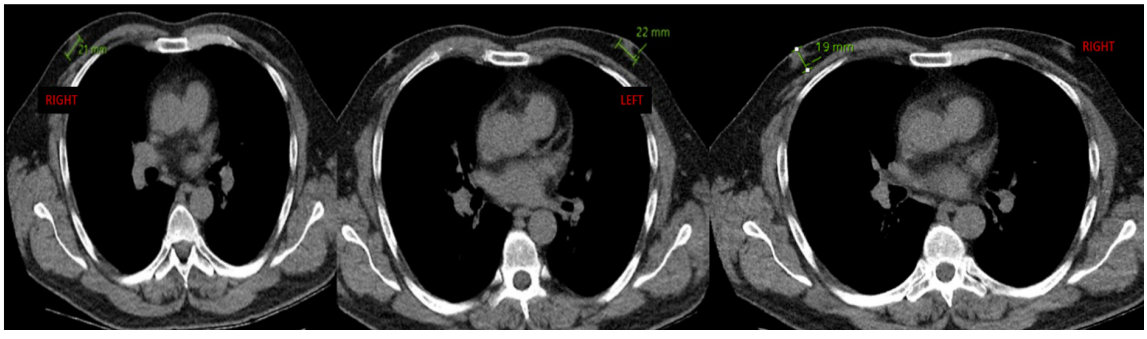

Figure 1. Measurements of gynecomastia parameters on CT sections: right subareolar glandular tissue diameter (left), left subareolar glandular tissue diameter (middle) and right retroareolar glandular tissue diameter (right)
CT: Computed tomography

Table 2. Correlations between the study parameters

		Right subareolar tissue thickness	Left subareolar tissue thickness	Right retroareolar fatty tissue thickness	Liver density	Spleen density	Epicardial fat thickness
Right subareolar tissue thickness	r	1	**0.524****	**0.304****	0.004	-0.032	**0.085***
	p	-	**<0.001**	**<0.001**	0.929	0.441	**0.037**
Left subareolar tissue thickness	r	**0.524****	1	-0.43	**-0.137****	-0.009	**0.101***
	p	**<0.001**	-	0.299	**0.001**	0.827	**0.014**
Right retroareolar fatty tissue thickness	r	**0.304****	-0.43	1	**-0.172****	-0.064	**0.148****
	p	**<0.001**	0.299	-	**<0.001**	0.116	**<0.001**
Liver density	r	0.004	**-0.137****	**-0.172****	1	**0.278****	**0.178****
	p	0.929	**0.001**	**<0.001**	-	**<0.001**	**<0.001**
Spleen density	r	-0.032	-0.009	-0.064	**0.278****	1	-0.003
	p	0.441	0.827	0.116	**<0.001**	-	0.947
Epicardial fat thickness	r	**0.085***	**0.101***	**0.148****	**0.178****	-0.003	1
	p	**0.037**	**0.014**	**<0.001**	**<0.001**	0.947	-

*Statistically significant, **Statistically highly significant

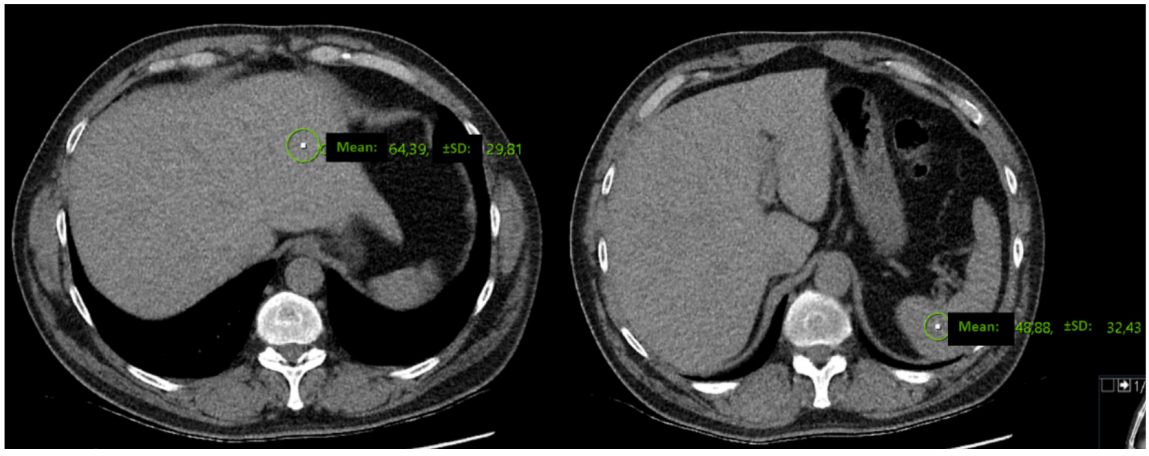

Figure 2. Measurements of hepatic steatosis parameters on CT images in Hounsfield Units: liver density (left), spleen density (right)
CT: Computed tomography

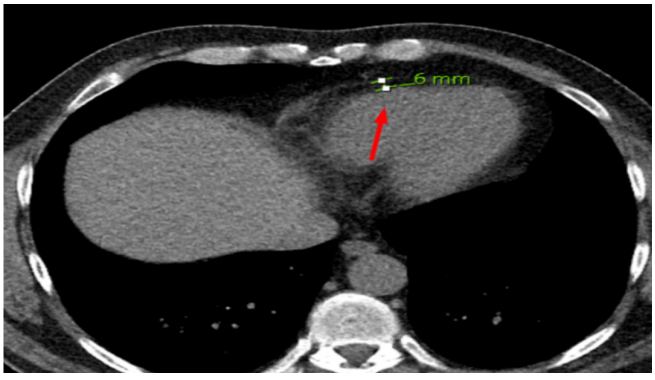

Figure 3. Measurement of epicardial fat thickness from the visceral epicardium to the outside of the myocardium

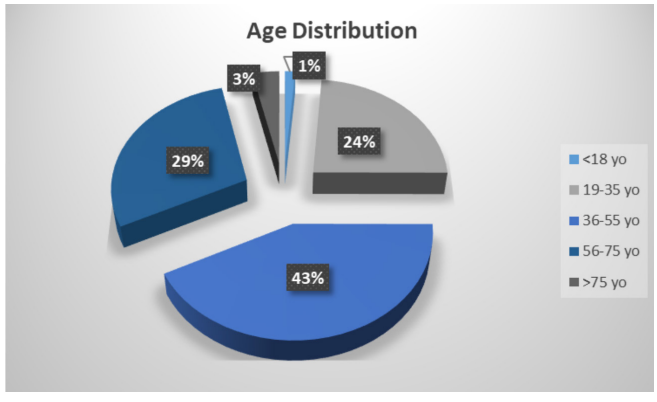

Figure 4. Distribution of the patients by age groups

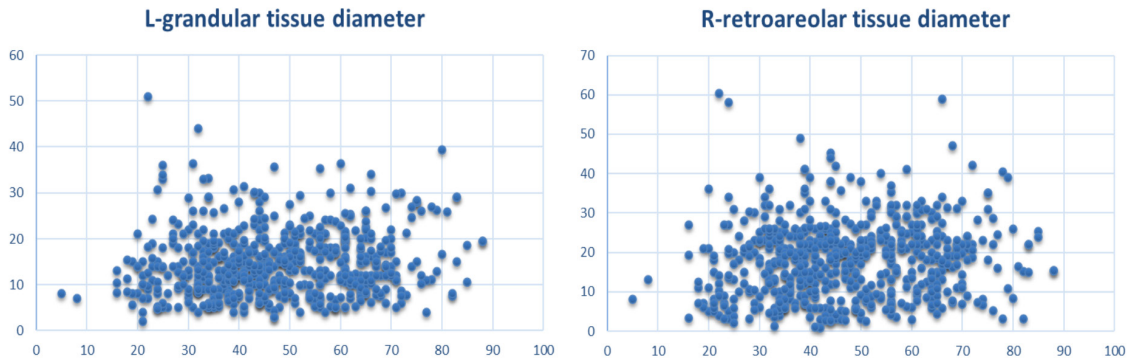

Figure 5. Correlations between age and glandular tissues of the left and right breasts

significant correlation was observed between the other study parameters.

DISCUSSION

The currently ongoing COVID-19 pandemic, has increased the number of CT scans performed due to a presumed diagnosis of pneumonia worldwide (8). This has caused various diseases and/or medical conditions to be noticed incidentally in CT examinations (15-17). However, this situation could be seen as an opportunity to retrospectively examine the relationships between various parameters through these CT scans with more objective measurements. In this study, we investigated the correlations between hepatic steatosis as determined on CT images of male patients who underwent chest imaging due to suspected COVID-19, gynecomastia, and epicardial fat thickness.

In this study, the mean age of patients with gynecomastia was found as 47.21 years. Kim et al. (9) reported the mean age as 56.99 years in 650 male patients with gynecomastia. Aslan et al. (8) reported the most common age group with gynecomastia as 30-39 years. This range was 36-55 years in our study, although age ranges were different between the two studies, general ranges were similar. Similar to our study, Aslan et al. examined CT images of male patients who were admitted during the COVID-19 pandemic to determine the prevalence of gynecomastia. The authors found significant correlations between age, right breast glandular tissue diameter ($r=0.235$, $p<0.001$) and left breast glandular tissue diameter ($r=0.219$, $p<0.001$) (8). In this study, age was positively correlated with the left subareolar glandular tissue diameter ($r=0.113$, $p=0.05$), and right retroareolar fatty tissue diameter ($r=0.116$, $p=0.04$) (Figure 5). These findings suggest a relationship between age and gynecomastia, possibly due to decreased testosterone levels with aging.

Studies investigating the relationship between liver disease and gynecomastia have reported conflicting results. Hepatic steatosis has been associated with low testosterone and high estrogen levels, diminished libido and gynecomastia, which is the most common benign condition characterized by enlargement of the male breast (18). Furthermore, there are studies reported that testosterone administration to cirrhotic patients has decreased the prevalence of gynecomastia (19). However, others could not find such a relationship and proposed that breast tissue sensitivity to a raised ratio of estrogen/testosterone is highly variable (11). In this study, the left subareolar tissue thickness and the right retroareolar fatty tissue thickness that are used indicate gynecomastia were significantly correlated with liver density in the negative direction ($r=-0.137$, $p<0.001$; $r=-0.172$, $p<0.001$; respectively) suggesting a relationship between hepatic steatosis and gynecomastia. Nevertheless, there is no sufficient data in the literature to draw a definitive conclusion on this issue and mechanisms of such a potential correlation are still to be clarified.

Epicardial fat accounts for 20% of heart weight and constitutes 80% of the heart's surface. Although it is a relatively neglected component of the heart, it has been proposed as a marker of cardiovascular risk (20,21). However, previous studies have reported the relationship between epicardial fat thickness and various diseases and medical conditions. Shemirani and Khoshavi (22) reported a correlation between epicardial fat thickness and the severity of coronary artery disease. Metwalley et al. (23) observed elevated epicardial fat thickness in pediatric patients with congenital adrenal hyperplasia. According to Petta et al. (13), in patients with non-alcoholic fatty liver disease, a higher epicardial fat thickness is associated with the severity of the disease. Iacobellis et al. (24) reported that epicardial fat thickness is a good indicator of hepatic steatosis in obese patients. Results of our study indicated a strong correlation between epicardial fat thickness and liver

density, which is used to determine the presence of hepatic steatosis (r=0.178, p<0.01).

Although epicardial fat thickness is closely associated with fat deposition, no study could be found to investigate its correlation with fat proliferation in men's breasts, namely, gynecomastia. In this study, epicardial fat thickness was statistically significantly correlated with right subareolar tissue thickness (r=0.085, p=0.037), left subareolar tissue thickness (r=0.101, p=0.014) and right retroareolar fatty tissue thickness (r=0.148, p<0.001). However, the mechanisms underlying these correlations are yet to be clarified.

Study Limitations

The major limitations of this study include its retrospective design and being performed in a single center. Additionally, the same correlations could be investigated between obese and non-obese patients as epicardial fat thickness is closely associated with obesity. Finally, our results on the correlation between epicardial fat thickness and gynecomastia could not be compared due to the lack of similar studies. However, the number of our patients is relatively large and we examined the associations between hepatic steatosis, gynecomastia and epicardial fat thickness together, for the first time in the literature.

CONCLUSION

The results of this study showed that gynecomastia was significantly correlated with both age and hepatic steatosis. However, currently there is no clear data especially on the relationship between hepatic steatosis and gynecomastia. Additionally, epicardial fat thickness was also associated with hepatic steatosis. However, we demonstrated the significant correlations between epicardial fat thickness and gynecomastia for the first time. Nevertheless, our results need to be confirmed by further comprehensive multi-center studies with a larger series of patients.

Publication: This article has been published as pre-print on a website (researchsquare) as it can be seen in the similarity report. The link to the pre-print version is below: https://www.researchsquare.com/article/rs-1063300/v1

ETHICS

Ethics Committee Approval: Before the beginning, the study protocol was approved by the local ethics committee of Istinye University with the 05/08/2021 dated and (2017-KAEK-120)/2/2021.G-111 numbered decision.

Informed Consent: This was a retrospective study.

Authorship Contributions

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

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. Zhu Y, Yang Q, Lv F, Yu Y. The Effect of Hepatosteatosis on Response to Antiviral Treatment in Patients with Chronic Hepatitis B: A Meta-Analysis. Gastroenterol Res Pract 2017;2017:1096406.
2. Boyce CJ, Pickhardt PJ, Kim DH, Taylor AJ, Winter TC, Bruce RJ, et al. Hepatic steatosis (fatty liver disease) in asymptomatic adults identified by unenhanced low-dose CT. AJR Am J Roentgenol 2010;194:623-8.
3. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387-95.
4. Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med 2005;143:722-8.
5. Strauss S, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. AJR Am J Roentgenol 2007;189:W320-3.
6. Lawrence DA, Oliva IB, Israel GM. Detection of hepatic steatosis on contrast-enhanced CT images: diagnostic accuracy of identification of areas of presumed focal fatty sparing. AJR Am J Roentgenol 2012;199:44-7.
7. Sansone A, Romanelli F, Sansone M, Lenzi A, Di Luigi L. Gynecomastia and hormones. Endocrine 2017;55:37-44.
8. Aslan Ö, Bayraktaroğlu S, Çinkooğlu A, Ceylan N, Savaş R, Oktay A. Prevalence of Incidental Gynecomastia by Chest Computed Tomography in Patients with a Prediagnosis of COVID-19 Pneumonia. Eur J Breast Health 2021;17:173-9.
9. Kim MS, Kim JH, Lee KH, Suh YJ. Incidental Gynecomastia on Thoracic Computed Tomography in Clinical Practice: Characteristics, Radiologic Features, and Correlation With Possible Causes in South Korean Men. Am J Mens Health 2020;14:1557988320908102.
10. Gossner J. Gynecomastia on Computed Tomography of The Chest -Prevalence in A Clinical Population and An Analysis of Possible Causes. Eur J Breast Health 2018;15:67-8.
11. Cavanaugh J, Niewoehner CB, Nuttall FQ. Gynecomastia and cirrhosis of the liver. Arch Intern Med 1990;150:563-5.
12. Deng N, Mallepally N, Peng FB, Kanji A, Marcelli M, Hernaez R. Serum testosterone levels and testosterone supplementation in cirrhosis: A systematic review. Liver Int 2021;41:2358-70.
13. Petta S, Argano C, Colomba D, Cammà C, Di Marco V, Cabibi D, et al. Epicardial fat, cardiac geometry and cardiac function in patients with non-alcoholic fatty liver disease: association with the severity of liver disease. J Hepatol 2015;62:928-33.
14. Gorter PM, van Lindert AS, de Vos AM, Meijs MF, van der Graaf Y, Doevendans PA, et al. Quantification of epicardial and peri-coronary fat using cardiac computed tomography; reproducibility and relation with obesity and metabolic syndrome in patients suspected of coronary artery disease. Atherosclerosis 2008;197:896-903.

15. Ramanan RV, Joshi AR, Venkataramanan A, Nambi SP, Badhe R. Incidental chest computed tomography findings in asymptomatic Covid-19 patients. A multicentre Indian perspective. Indian J Radiol Imaging 2021;31(Suppl 1):S45-52.
16. Kwee TC, Kwee RM. Chest CT in COVID-19: What the Radiologist Needs to Know. Radiographics 2020;40:1848-65.
17. Franceschi AM, Clifton M, Ahmed O, Matthews R, Franceschi D. Incidental PET/CT Findings of Suspected COVID-19 in a Region of High Prevalence. Cureus 2020;12:e9716.
18. Saeki C, Tsubota A. Influencing Factors and Molecular Pathogenesis of Sarcopenia and Osteosarcopenia in Chronic Liver Disease. Life (Basel) 2021;11:899.
19. Swerdloff RS, Ng CM. Gynecomastia: Etiology, Diagnosis, and Treatment. 2019 Jul 7. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, Dungan K, Hershman JM, Hofland J, Kalra S, Kaltsas G, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, Morley JE, New M, Purnell J, Sahay R, Singer F, Sperling MA, Stratakis CA, Trence DL, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000–.
20. Rabkin SW. Epicardial fat: properties, function and relationship to obesity. Obes Rev 2007;8:253-61.
21. Bertaso AG, Bertol D, Duncan BB, Foppa M. Epicardial fat: definition, measurements and systematic review of main outcomes. Arq Bras Cardiol 2013;101:e18-28.
22. Shemirani H, Khoshavi M. Correlation of echocardiographic epicardial fat thickness with severity of coronary artery disease-an observational study. Anadolu Kardiyol Derg 2012;12:200-5.
23. Metwalley KA, Farghaly HS, Abdelhamid A. Epicardial Fat Thickness in Children with Classic Congenital Adrenal Hyperplasia. J Clin Res Pediatr Endocrinol 2019;11:61-9.
24. Iacobellis G, Barbarini G, Letizia C, Barbaro G. Epicardial fat thickness and nonalcoholic fatty liver disease in obese subjects. Obesity (Silver Spring) 2014;22:332-6.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:202-208
DOI: 10.4274/BMJ.galenos.2022.2022.2-22

Research

The Potential Protective Effects of Ginkgo Biloba on Bilirubin Cytotoxicity in Newborn Rat

Yenidoğan Ratlarda Ginkgo Bilobanın Bilirubin Sitotoksisitesi Üzerindeki Potansiyel Koruyucu Etkisi

Özlem Şahin[1], Hacer Ergin[2], Aydın Demiray[3], Mehmet Bülent Özdemir[4], Hakan Akça[3], Çiğdem Yenisey[5]

[1]University of Health Sciences Turkey, Umraniye Training and Research Hospital, Neonatal Intensive Care Unit, Istanbul, Turkey
[2]Pamukkale University Faculty of Medicine, Department of Pediatrics, Neonatal Intensive Care Unit, Denizli, Turkey
[3]Pamukkale University Faculty of Medicine, Department of Medical Genetic, Denizli, Turkey
[4]Pamukkale University Faculty of Medicine, Department of Anatomy, Denizli, Turkey
[5]Aydın Adnan Menderes University Faculty of Medicine, Department of Biochemistry, Aydın, Turkey

ABSTRACT

Objective: The mechanism of neurotoxicity associated with high serum bilirubin concentrations is still not fully elucidated. The cytotoxic effect of bilirubin has been demonstrated in various cell types, including astrocytes and neurons. The protective effect of Ginkgo biloba (EGB-761), which has antioxidant, anti-inflammatory, and anti-apoptotic effects, against neurotoxicity due to hyperbilirubinemia is not known. This study aimed to investigate the effect of EGB-761 in neonatal rat astrocyte cell cultures with hyperbilirubinemia-induced cytotoxicity.

Methods: Astrocyte cell culture was obtained from one-day-old Wistar albino rats using the modified Cole and de Vellis method. Indirect bilirubin was found to be toxic to 50% of astrocyte cells at a dose of 10 µM (TC_{50}). Bilirubin-induced apoptotic cell death was evaluated using the TUNEL staining method. EGB-761 increased cell viability by 100% and 110% at 10 µg/mL and 0.5 µg/mL concentrations, respectively. No drug was administered to the control group. In the study group, for the protective effect, 10 µM bilirubin was administered to the astrocyte cell culture 4 hours after 10 µg/mL EGB-761 was administered in the ginkgo^{10}+bilirubin^{10} group, and for therapeutic effect, 10 µg/mL EGB-761 was administered 4 hours after 10 µM bilirubin was administered in the bilirubin^{10}+ginkgo^{10} group, for a duration of 48 hours. Cell viability and apoptosis were evaluated in both prophylaxis and treatment groups after the procedure.

Results: There was a 50% decrease in cell viability and a five-fold increase in apoptosis in the bilirubin^{10} group compared with the control group ($p<0.001$, $p<0.001$). EGB-761 given for prophylaxis and treatment increased cell viability ($p<0.001$, $p<0.001$) and reduce apoptosis ($p<0.001$, $p<0.001$) compared with the control group.

Conclusion: In this *in vitro* study, it was shown that bilirubin has a cytotoxic effect on astrocyte cells, and EGB-761 used for prophylaxis and treatment reduced the cytotoxic effects of bilirubin.

Keywords: Bilirubin, Ginkgo biloba, neurotoxicity, newborn

ÖZ

Amaç: Yüksek serum bilirubin konsantrasyonu ile ilişkili nörotoksisitenin mekanizması günümüzde hala tam olarak açıklanamamıştır. Bilirubinin sitotoksik etkisi, astrositler ve nöronları da içeren değişik hücre tiplerinde gösterilmiştir. Antioksidan, antienflamatuvar, antiapoptotik etkileri olduğu bilinen Ginkgo bilobanın (EGB-761), hiperbilirubinemiye bağlı nörotoksisitedeki koruyucu etkisi bilinmemektedir. Bu çalışmada hiperbilirubinemiye bağlı sitotoksisite oluşturulmuş yenidoğan rat astrosit hücre kültüründe EGB-761'in etkisinin araştırılması hedeflendi.

Gereç ve Yöntem: Bir günlük Wistar albino ratlardan modifiye Cole ve de Vellis yöntemi ile astrosit hücre kültürü elde edildi. İndirekt bilirubinin 10 µM dozunda (TC_{50}) astrosit hücrelerinin %50'sine toksik etkili olduğu saptandı. TUNEL boyama yöntemiyle bilirubine bağlı apopitotik hücre

The article we sent to the journal is the thesis prepared by Özlem Şahin at Pamukkale University Faculty Hospital.

Address for Correspondence: Özlem Şahin, University of Health Sciences Turkey, Umraniye Training and Research Hospital, Neonatal Intensive Care Unit, Istanbul, Turkey
Phone: +90 505 373 77 65 E-mail: colkozlem@yahoo.com ORCID ID: orcid.org/0000-0001-9951-8624

Cite as: Şahin Ö, Ergin H, Demiray A, Özdemir MB, Akça H, Yenisey Ç. The Potential Protective Effects of Ginkgo Biloba on Bilirubin Cytotoxicity in Newborn Rat. Med J Bakirkoy 2022;18:202-208

Received: 01.04.2022
Accepted: 07.05.2022

ölümü değerlendirildi. EGB-761'in 10 µg/mL, 0,5 µg/mL konsantrasyonlarda hücre canlılığını sırasıyla %100 ve %110 artırdığı saptandı. Kontrol grubuna ilaç uygulanmadı. Çalışma grubunda, koruyucu etki için astrosit hücre kültürüne ginkgo^{10}+bilirubin^{10} grubunda 10 µg/mL EGB-761 uygulandıktan 4 saat sonra 10 µM bilirubin, tedavi edici etki için bilirübin^{10}+ginkgo^{10} grubunda 10 µM bilirubin uygulandıktan 4 saat sonra 10 µg/mL EGB-761 48 saat süreyle uygulandı. İşlem sonrasında hem profilaksi hem tedavi grubunda hücre canlılığı ve apoptozis değerlendirildi.

Bulgular: Kontrol grubuna göre bilirubin^{10} grubunda hücre canlılığında yaklaşık %50 oranında azalma, apoptozisde beş kat artış saptandı (p<0,001, p<0,001). Profilaksi ve tedavi amacıyla verilen EGB-761'in kontrol grubuna göre hücre canlılığını artırdığı (p<0,001, p<0,001); apoptozisi azalttığı saptandı (p<0,001, p<0,001).

Sonuç: *İn vitro* olarak yapılan bu çalışmada bilirubinin astrosit hücrelerine sitotoksik etkili olduğu, profilaksi ve tedavi amacıyla verilen EGB-761'in bilirubinin sitotoksik etkilerini azalttığı gösterildi.

Anahtar Kelimeler: Bilirubin, Ginkgo biloba, nörotoksisite, yenidoğan

INTRODUCTION

Despite the developments in neonatology, neurotoxicity caused by hyperbilirubinemia is still an important problem in newborns. High bilirubin levels cause encephalopathy and kernicterus, low bilirubin levels oxidative damage in newborns. In newborns, the increase in bilirubin and insufficient enterohepatic circulation cause high serum levels of indirect bilirubin, which is dissolved in fat and can easily pass cross the blood-brain barrier. Many mechanisms have been suggested to explain the neurotoxic effect of bilirubin. The basic cellular mechanism is the inhibition of oxidative phosphorylation in neurons (1-3). High bilirubin levels induced oxidative stress by increasing the formation of free radicals in the brain. The harmful effects of the free radicals continuously formed in biological systems are prevented by neutralizing the effect of antioxidant defense mechanisms. In newborns the insufficient antioxidant defense mechanisms contribute to the development of cerebral ischemia, excitotoxicity and neurodegenerative processes in the nervous system (3). Several mechanisms have been reported for bilirubin toxicity. High bilirubin levels have been shown to increase apoptosis (2).

In bilirubin toxicity, the primary targets are glial cells and neurons. Among nerve cells, neurons were shown to be more susceptible to the toxic effects of bilirubin than astrocytes (3,4). Astrocytes, the most intense cell group in the brain, are critically important in protection of the central nervous system as they provide metabolic and trophic support to neurons, which also contribute to form blood-brain barrier. Astrocytes are the first cells effected by bilirubin, which eventually causes blood-brain barrier damage (4,5). Astrocytes have been reported to be more resistant to bilirubin-associated oxidative damage, firstly increased expression of the pump, which removes bilirubin out of the cell, and by their high antioxidant capacities (4). Astrocytes have been used in many studies as an experimental kernicterus model (3,4,6). Astrocytes are also thought to play an important role in encephalopathy developed during severe hyperbilirubinemia, and are potential targets in the future treatment models. In this study, we used astrocytes to assess the toxic effects of bilirubin.

Ginkgo biloba is an agent derived from the dried leaves of this plant. Ginkgo biloba has been traditionally used in China and Western countries for the treatment of cerebrovascular diseases. The neuroprotective effects of plants have were shown in numerous *in vivo* and *in vitro* studies. Ginkgo biloba and its metabolites can cross the blood-brain barrier, which provides healing in different types of neurological damages, without side effects (7-10). Ginkgo biloba extract shows its effect through its flavonoid (22-27%) and terpenoid (5-7%) content (8). Many mechanisms explaining the neuroprotective effect of Ginkgo biloba were suggested in *in vivo* and *in vitro* studies; these mechanisms are protection of mitochondrial ATP synthesis, inhibition of apoptotic damage, suppression of hypoxia induced membrane damage in the brain, and increased expression of mitochondrial DNA encoding COX III subunitin of cytochrome c oxidase and NF I subunitin of NADH dehydrogenase (11,12). In animal studies, Ginkgo biloba extract (EGB-761) was reported to have protective effects against oxidative damage by removing free radicals. Moreover, Ginkgo biloba increases the activities of antioxidant enzymes such as superoxide dismutase, catalase by the flavonoid fraction of the EGB-761 extract. EGB-761 was reported that nitric oxide (NO) production decrease by suppressing inducible NO synthase as well as inhibiting malondialdehyde (MDA) (7,8,12).

Therefore, discovery of new agents that will decrease the toxic effects of bilirubin has gained importance. Although, most of these agents have given favorable results in experimenter studies, only a few of them could be used for clinic use. Although the antioxidant, antiapoptotic, vasorelaxant, antiaggregant, anti-inflammatory effects of EGB-761 have been shown, its effect on bilirubin toxicity in astrocytes remain unclear. We started this study with the hypothesis that Ginkgo biloba might be effective against bilirubin neurotoxicity. This study investigates the effects of Ginkgo biloba extract on newborn rat primary astrocyte cell culture by modeling hyperbilirubinemia-associated neurotoxicity.

METHODS

Study Groups

Group I, the control group (n=6): No drug was administered.

Group II, bilirubin^{10} group (n=6): 10 µM bilirubin (Sigma Aldrich, B 4126-1G, St. Louis, MO, USA) was applied to the astrocyte cell culture for 48 hours.

Group III, ginkgo^{10} group (n=6): 10 µg/mL ginkgo alkaloid (EGB-761) (Ginkgo biloba Hevert injekt. Dil. D3 2 mL, Hevert-Arzneimittel GmbH & Co. KG Nussbaum, Deutschland) was applied to the astrocyte cell culture for 48 hours.

Group IV, ginkgo$^{0.5}$ group (n=6): 0.5 µg/mL EGB-761 was applied to astrocyte cell culture for 48 hours.

Group V, four hours after 10 µM bilirubin was added to the astrocyte cell culture, 10 µg/mL EGB-761 was added and applied for 48 hours.

Group VI, bilirubin^{10}+ginkgo$^{0.5}$ group (n=6): Four hours after 10 µM bilirubin was added to the astrocyte cell culture, 0.5 µg/mL EGB-761 was added and applied for 48 hours.

Group VII, ginkgo^{10}+bilirubin^{10} group (n=6): Four hours after the addition of 10 µg/mL EGB-761 to the astrocyte cell culture, 10 µM bilirubin was added and administered for 48 hours.

Group VIII, ginkgo$^{0.5}$+bilirubin^{10} group (n=6): Four hours after 0.5 µg/mL EGB-761 was added to the astrocyte cell culture, 10 µM bilirubin was added and administered for 48 hours.

Cell Cultures

The study was launched with the Pamukkale University Ethics Committee's approval, dated 19.08.2011, and numbered 2011/031. The study was conducted in accordance with the Declaration of Helsinki. Astrocyte cell cultures were prepared from the brains of 1-day-old Wistar albino rat pups using a modified version of Cole and de Vellis' shake off method (13,14). Following decapitation, brains were extracted and meninges were fully trimmed. The brains were mechanically minced and dissociated before being sieved through a nylon mesh (pore size of 70 m; Millipore). Cells were spun at 1500 rpm/min for 5 minutes using a benchtop centrifuge, and the cell pellets were resuspended in Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) (DMEM/Ham's F-12 1:1, 500 mL, Biochrom, Berlin, Germany), which contained 10% heat inactivated fetal bovine serum (FBS) (Hyclone, 100 mL, Thermo Scientific, Cromlington, UK), 500 µl gentamisin (Gentamisin, 10 mg/10 mL, Sigma Aldrich, St Louis, USA) and 5 mL fungisone (Gibco Antibiotic-Antimycotic, 25 µg/mL amphoterisin B, 100x/100 mL, Invitrogen, New York, USA). For primary neuron cell culture, the resuspended cells were seeded in 75-cm^2 flasks previously coated with 10 µg/mL of poly-D-lisine (Sigma-Aldrich, St Louis, USA). Cells were incubated in a humidified CO_2 incubator at 370C, 5% CO_2, and 95% humidity, with medium changes every 3 days (Figure 1). Macrophages and loosely attached cells were removed from the astrocyte monolayer after 8-10 days of culture by shaking cultures at 150 rpm for 1 hour (Figure 1). The oligodendrocytes on top of a confluent monolayer of astrocytes were then dislodged by orbital shaking at 150 rpm for 24 hours. The media containing floating cells, microglia, and oligodendrocytes were transferred to separate flasks. At the bottom of the flasks, astrocyte cells were collected and the cell pellets were resuspended in astrocyte medium [DMEM/F12 containing 500 µL gentamisin, 5 mL fungizone, 15% FBS, 5 mL L-glutamine (Gibco L-Glutamine-200 mM, 100x/100 mL, Invitrogen, New York, USA)] and 500 µL insulin (Human insulin <rh>, 0.5 mg/mL, Biochrom, Berlin, Germany). The method for preparing astrocytes is been estimated to produce cultures with a purity of approximately 95% astrocytes. Stock unconjugated bilirubin (UCB) was prepared in 0.1 N NaOH and stored at 40 °C in the dark before being used. Under sterile conditions, the stock UCB solution was further diluted with astrocyte medium and added to cultures at various concentrations. Ginkgo biloba (EGB-761) was purchased from Biochrom.

Determination of Astrocyte Cell Viability and UCB and Ginkgo Biloba Concentrations

Cells were seeded in 96-well plates (3x10^4 cells/well). After 24 hours, the cells were treated with UCB at the following concentrations: 400 µM, 200 µM, 100 µM, 80 µM, 60 µM, 40 µM, 20 µM, 10 µM, 8 µM, 5 µM, 4 µM, 2 µM, 1 µM, and 0.5 µM for 48 hours. Astrocyte cells were also treated with EGB-761 at the following concentrations: 60 µg/mL, 40 µg/mL, 20 µg/mL, 10 µg/mL, 8 µg/mL, 6 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, and 0.5 µg/mL. Cell viability was measured using the luminetric method by Becerir et al. (15).

Apoptosis Evaluation

To investigate any protective and/or curative effects, cells were treated with IC_{50} values of bilirubin before or after 4-h treatment with EGB-761. The cells were washed with phosphate-buffered saline and trypsinized after 24 hours of incubation. Apoptosis was determined using the deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) method. TUNEL staining was performed using the ApopTag plus Peroxidase *in situ*

Apoptosis Detection (Millipore) kit as per the manufacturer's instructions. Each sample had at least five random microscopic fields counted, and the mean values were expressed as a percentage of apoptotic nuclei (16).

Statistical Analysis

Statistical packages for social sciences (SPSS) (SPSS for Windows 17.0; SPSS, Chicago, Illinois, USA) software was used to computation the data on a computer. The statistical significance of the study groups was determined using both parametric (paired samples t-test) and non-parametric tests (Kruskal-Wallis and Mann-Whitney U). Data were tested for conformity to the normal distribution. ANOVA and posthoc tests were used among the parametric tests when comparing means in the data conforming to a normal distribution. All data were presented in the form of mean ± standard deviation. Statistical significance was set at $p<0.05$.

RESULTS

Determining the Bilirubin and Ginkgo Alkaloid Concentrations for Testing

Cell vitality was measured in 48-hour astrocyte cultures after different concentrations of bilirubin were administered. Figure 2 depicts the results as a percentage of cell vitality ± standard deviation at the studied bilirubin concentration. The results showed that increasing bilirubin concentrations concentration-dependently reduced cell viability. The concentration of indirect bilirubin that has a toxic effect on 50% of astrocyte cells (TC_{50}) was determined to be 10 µM and was used in cytotoxicity and apoptosis tests.

Cell vitality was determined after administration of different concentrations of EGB-761 in 48 hour-cultured astrocytes. Figure 3 depicts the results as a percentage of cell vitality ± standard deviation for each EGB-761 concentration administered. The concentrations of ginkgo alkaloid

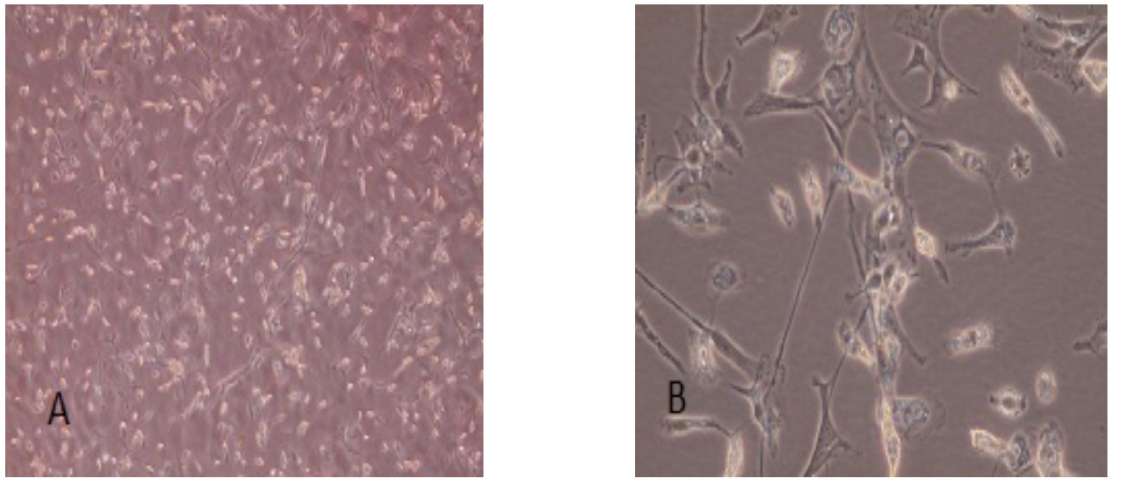

Figure 1. Neuron cell (A), astrocyte cell (B)

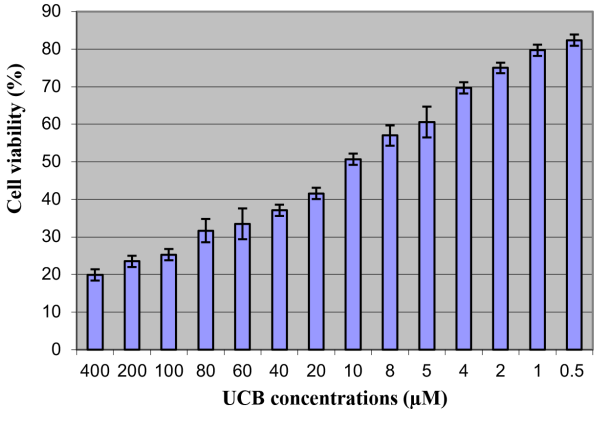

Figure 2. Concentration-response curve of UCB
UCB: Unconjugated bilirubin

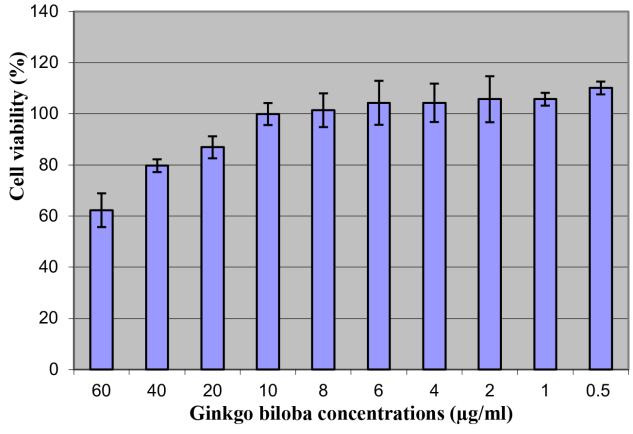

Figure 3. Concentration-response curve of Ginkgo biloba

that increase cell vitality by 100% and most (110%) were determined to be 10 µg/mL and 0.5 µg/mL, respectively, and these concentrations were used in cytotoxicity measurements. EGB-761 at 10 µg/mL concentration was found to be the most effective ginkgo alkaloid concentration for apoptosis and was used in apoptosis tests.

Cytotoxicity Assessment

Cell vitality of the groups was evaluated in 48-hour cultures. At the outset, cell vitality was assumed to be 100%. Cell vitality reached 147.1±25.2% in the control group, and 69.9±5.7% in the B^{10} group compared to the outset. The reduction in the cell vitality in the B^{10} group compared to the control group was found to be statistically significant ($p<0.001$). Cell vitality was observed as 151.5±14.8% in the G^{10} group, and 162.7±10.3% in the $G^{0.5}$ group. Increases in cell vitality in the control, G^{10} and $G^{0.5}$ groups compared with the B^{10} group were found to be statistically significant ($p<0.001$). Compared to the onset, cell vitality was observed as 117.9±16.4% in the $B^{10}+G^{10}$ group, 105.5±12.3% in $B^{10}+G^{0.5}$ group, 134.4±18.8% in $G^{0.5}+B^{10}$ group, and 147.2±10.2% in the $G^{10}+B^{10}$ group. In $G^{10}+B^{10}$ and $G^{0.5}+B^{10}$ groups, the increase in cell vitality was not different compared to the control groups ($p>0.05$). A significant reduction was observed in cell vitality in the $B^{10}+G^{10}$ and $B^{10}+G^{0.5}$ groups ($p=0.039$, $p=0.001$, respectively) compared to the control group (Figure 4).

Apoptosis Detection

Apoptosis in astrocyte cells was assessed in 48-hour cultures. Apoptosis was found to be 4.1±0.6% in the control group (Figure 5), 19.1±2.3% in the B^{10} group (Figure 5), 5.2±0.9% in the G^{10} group (Figure 5), 9.2±1.6% in the $G^{10}+B^{10}$ group (Figure 5), and 10.7±1.6% in the $B^{10}+G^{10}$ group (Figure 5). Apoptosis was significantly higher in the B^{10} group than in the other groups ($p<0.001$). Apoptosis was also higher with combined bilirubin and Ginkgo biloba administration compared to Ginkgo biloba alone.

DISCUSSION

Primary cell culture systems are very useful for toxicological and neurotoxicological studies, and have been used in the assessment of susceptibility of different neuron cells to toxins (17). Astrocytes, which provide metabolic, trophic support to the neurons, which are of critical importance in protection of the central nervous system, and are more resistant to oxidative damage than neurons, protect neurons from toxic damage in case of damage of blood-brain barrier (4,5). Astrocytes are thought to play an important role in encephalopathy developing during severe hyperbilirubinemia, and to be potential targets in the future treatment models (5,17). In bilirubin toxicity, main target is glial cells and neurons (3,4).

It was shown that bilirubin-associated damage is more persistent in neurons compared to astrocytes, of which the damage is mostly reversible. Silva et al. (4) reported that neurons are more susceptible to bilirubin toxicity than astrocytes.

In *in vitro* studies, threshold value for the neurotoxic effect of UCB was shown to be within a broad range, starting from as low as 70 nM (1,18,19). The fact that differences in toxic bilirubin concentrations are likely resulted from different methods, cell function and maturation and variation in the duration of bilirubin exposure (1). In the study by Tastekin et al. (20), TC_{50} concentration in primary cerebellar cell culture was found as 10 µM. In our study, indirect bilirubin

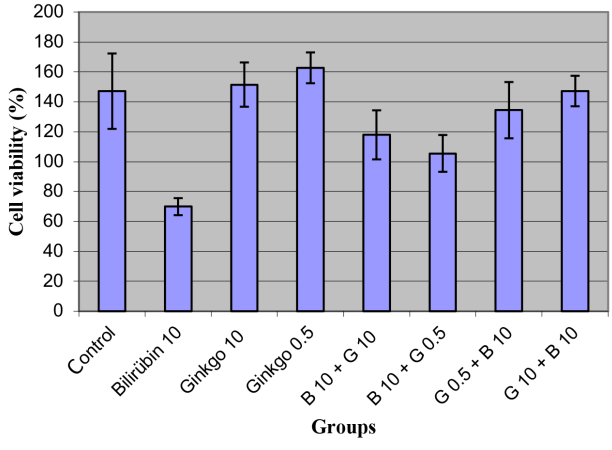

Figure 4. Alterations of the astrocyte cell viability in the groups (%)
*Decrease of the cell viability of the group B^{10} compared to the control, $G^{0.5}$, G^{10} group ($p<0.001$)

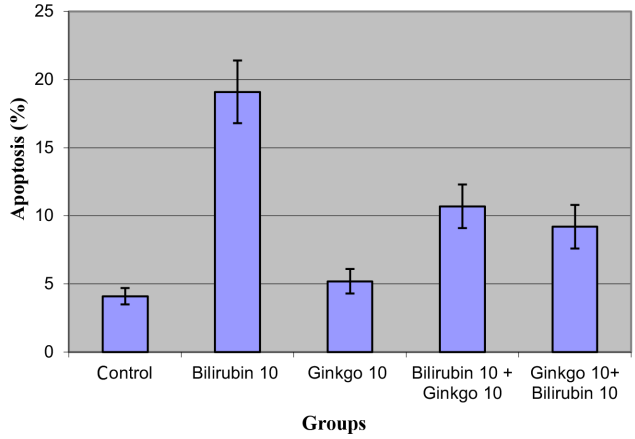

Figure 5. Evaluation of apoptosis in the groups
*Apoptosis of the B^{10} compared to the other group ($p<0.001$)

was administered at the concentrations of 0.5-400 μM to primary astrocyte cell culture, and TC_{50} was found at 10 μM, as in the studies by Berns et al. (21) and Becerir et al. (15). Hence, bilirubin at 10 μM concentration was used in cytotoxicity and apoptosis tests. In our experiments, the cell death rate was higher at increased bilirubin concentrations as was reported by Ostrow et al. (1), Kumral et al. (5) and Becerir et al. (15).

Our study found that EGB-761 administered both prophylactically and therapeutically decreased bilirubin cytotoxicity by leading to a significant increase in cell vitality and a significant decrease in apoptosis in astrocyte cell culture. Many studies have been conducted for the prevention of bilirubin neurotoxicity using different agents NMDA channel antagonist MK-801, L-carnitin, glycoursodeoxicolic acid, taurine acting by blocking intracellular calcium increase, minocycline (20,22,23) that all have protective effects in neurons against oxidative damage associated with bilirubin.

This study investigated the bilirubin anti-neurotoxicity and therapeutic effects of Ginkgo biloba, which has previously shown to have neuroprotective effects on bilirubin neurotoxicity (7,9). In our study, approximately 100% cell vitality was attained with 10 μg/mL dose of Ginkgo biloba, and 110% with 0.5 μg/mL. This dose is reported in the literature to be within the effective dose interval (7). Bastianetto et al. (9) showed that, in beta amyloid-induced neurotoxicity in mixed hippocampal cell culture, 10 μg/mL and 100 μg/mL doses of EGB-761 prevented apoptosis. In our study, Ginkgo biloba increased cell vitality up to 162.7±10.3% while bilirubin decreased cell vitality to (69.9±5.7%). Pre and post applications of EGB-761 increased cell viability in bilirubin-treated cells. Preadministration of Ginkgo biloba had showed slightly better cell viability compared to the treatment group. In conclusion, EGB-761 was shown to provide a neuroprotective effect through administration both prophylactically and therapeutically.

The study by Oyama et al. (24) demonstrated decreased formation of hydrogen peroxide and reactive oxygen radicals in Ginkgo biloba treated cerebellar neuron cells in dose-dependent way. It is reported that Ginkgo biloba decreases intracellular calcium concentrations associated with the glutamate receptor agonist kainate in rat cerebellar neurons (25) and decreases calcium dependent oxidative metabolism (26). Ginkgo biloba substantially improves cell viability in hydrogen peroxide applied neuron cells (27).

Bilirubin-associated apoptotic cell death is thought to develop due to excitotoxicity occurring as a result of NMDA receptor activation, the disruption of mitochondrial functions, proapoptotic Bax translocation, decrease in Na-K ATPaz activity, increase in intracellular calcium level, intracellular cytochrome c increase, and disruption of cytoskeleton, lipid peroxidation and protein oxidation associated with oxidative stress (2,4,6,18,20,28). EGB-761 prevents bilirubin-associated neurotoxicity by protecting mitochondrial functions, preventing the cells from oxidative damage by increasing antioxidant enzyme activities, and decreasing proapoptotic caspase-3, Bax, c-Myc, and p-53, increasing antiapoptotic Bcl-2 activity (10,11,29,30). In our study, Ginkgo biloba was found to reduce bilirubin-induced apoptosis by half prophylactically and therapeutically.

EGB-761 may have neuroprotective effects by preventing cell neurons from oxidative, nitrosative damage and by its antiapoptotic properties.

This study clearly shows that bilirubin has neurotoxic effects on astrocytes *in vitro* and that Ginkgo biloba prophylactically and therapeutically substantially decreases the neurotoxic effects of bilirubin. The protective effects of Ginkgo biloba may occur through its antioxidant, antiapoptotic, anti-inflammatory, anti-nitrosative and anti-excitotoxicity effects. Further studies must elucidate the exact mechanisms of Ginkgo biloba, which may have future potential in use of treatment of bilirubin-associated neurotoxicity in newborns.

CONCLUSION

Despite the developments in neonatology, neurotoxicity caused by hyperbilirubinemia is still an important problem in newborns. The mechanism of neurotoxicity associated with high serum bilirubin concentrations is still not fully elucidated. The cytotoxic effect of bilirubin has been demonstrated in various cell types, including astrocytes and neurons. Our study found that EGB-761 administered both prophylactically and therapeutically decreased bilirubin cytotoxicity by leading to a significant increase in cell vitality and a significant decrease in apoptosis in astrocyte cell culture. Further studies must elucidate the exact mechanisms of Ginkgo biloba, which may have future potential in use of treatment of bilirubin-associated neurotoxicity in newborns.

ETHICS

Ethics Committee Approval: The study was launched with the Pamukkale University Ethics Committee's approval, dated 19.08.2011, and numbered 2011/031.

Informed Consent: Animal experiment study.

Authorship Contributions

Concept: Ö.Ş., H.E., A.D., M.B.Ö., H.A., Ç.Y., Design: Ö.Ş., H.E., A.D., M.B.Ö., H.A., Ç.Y., Data Collection or Processing: Ö.Ş., A.D., Analysis or Interpretation: Ö.Ş., H.E., A.D., H.A., Literature Search: Ö.Ş., H.E., Writing: Ö.Ş., H.E., A.D., H.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. Ostrow JD, Pascolo L, Tiribelli C. Reassessment of the unbound concentrations of unconjugated bilirubin in relation to neurotoxicity in vitro. Pediatr Res 2003;54:98-104.
2. Ostrow JD, Pascolo L, Brites D, Tiribelli C. Molecular basis of bilirubin-induced neurotoxicity. Trends Mol Med 2004;10:65-70.
3. Brito MA, Rosa AI, Falcão AS, Fernandes A, Silva RF, Butterfield DA, et al. Unconjugated bilirubin differentially affects the redox status of neuronal and astroglial cells. Neurobiol Dis 2008;29:30-40.
4. Silva RF, Rodrigues CM, Brites D. Rat cultured neuronal and glial cells respond differently to toxicity of unconjugated bilirubin. Pediatr Res 2002;51:535-41.
5. Kumral A, Genc S, Genc K, Duman N, Tatli M, Sakizli M, et al. Hyperbilirubinemic serum is cytotoxic and induces apoptosis in murine astrocytes. Biol Neonate 2005;87:99-104.
6. Silva RF, Rodrigues CM, Brites D. Bilirubin-induced apoptosis in cultured rat neural cells is aggravated by chenodeoxycholic acid but prevented by ursodeoxycholic acid. J Hepatol 2001;34:402-8.
7. Ahlemeyer B, Krieglstein J. Neuroprotective effects of Ginkgo biloba extract. Cell Mol Life Sci 2003;60:1779-92.
8. Liu KX, Wu WK, He W, Liu CL. Ginkgo biloba extract (EGb 761) attenuates lung injury induced by intestinal ischemia/reperfusion in rats: roles of oxidative stress and nitric oxide. World J Gastroenterol 2007;13:299-305.
9. Bastianetto S, Ramassamy C, Doré S, Christen Y, Poirier J, Quirion R. The Ginkgo biloba extract (EGb 761) protects hippocampal neurons against cell death induced by beta-amyloid. Eur J Neurosci 2000;12:1882-90.
10. Xin W, Wei T, Chen C, Ni Y, Zhao B, Hou J. Mechanisms of apoptosis in rat cerebellar granule cells induced by hydroxyl radicals and the effects of EGb761 and its constituents. Toxicology 2000;148:103-10.
11. Defeudis FV. Bilobalide and neuroprotection. Pharmacol Res 2002;46:565-8.
12. Park YM, Won JH, Yun KJ, Ryu JH, Han YN, Choi SK, et al. Preventive effect of Ginkgo biloba extract (GBB) on the lipopolysaccharide-induced expressions of inducible nitric oxide synthase and cyclooxygenase-2 via suppression of nuclear factor-kappaB in RAW 264.7 cells. Biol Pharm Bull 2006;29:985-90.
13. McCarthy KD, de Vellis J. Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J Cell Biol 1980;85:890-902.
14. Shahar A, de Vellis J, Vernadakis A, Haken B . A dissection and tissue culture manual of the nervous system, 1st ed.New York: Wiley-Liss; 1989. p. 203-6.
15. Becerir C, Kılıç İ, Sahin Ö, Özdemir Ö, Tokgün O, Özdemir B, et al. The protective effect of docosahexaenoic acid on the bilirubin neurotoxicity. J Enzyme Inhib Med Chem 2013;28:801-7.
16. Negoescu A, Lorimier P, Labat-Moleur F, Drouet C, Robert C, Guillermet C, et al. In situ apoptotic cell labeling by the TUNEL method: improvement and evaluation on cell preparations. J Histochem Cytochem 1996;44:959-68.
17. Silva RF, Falcão AS, Fernandes A, Gordo AC, Brito MA, Brites D. Dissociated primary nerve cell cultures as models for assessment of neurotoxicity. Toxicol Lett 2006;163:1-9.
18. Grojean S, Koziel V, Vert P, Daval JL. Bilirubin induces apoptosis via activation of NMDA receptors in developing rat brain neurons. Exp Neurol 2000;166:334-41.
19. Ostrow JD, Pascolo L, Shapiro SM, Tiribelli C. New concepts in bilirubin encephalopathy. Eur J Clin Invest 2003;33:988-97.
20. Tastekin A, Gepdiremen A, Ors R, Buyukokuroglu ME, Halici Z. Protective effect of L-carnitine against bilirubin-induced neuronal cell death. Brain Dev 2006;28:436-9.
21. Berns M, Toennessen M, Koehne P, Altmann R, Obladen M. Ibuprofen augments bilirubin toxicity in rat cortical neuronal culture. Pediatr Res 2009;65:392-6.
22. Shapiro SM, Sombati S, Geiger A, Rice AC. NMDA channel antagonist MK-801 does not protect against bilirubin neurotoxicity. Neonatology 2007;92:248-57.
23. Geiger AS, Rice AC, Shapiro SM. Minocycline blocks acute bilirubin-induced neurological dysfunction in jaundiced Gunn rats. Neonatology 2007;92:219-26.
24. Oyama Y, Ueha T, Hayashi A, Chikahisa L, Noda K. Flow cytometric estimation of the effect of Ginkgo biloba extract on the content of hydrogen peroxide in dissociated mammalian brain neurons. Jpn J Pharmacol 1992;60:385-8.
25. Kanada A, Nishimura Y, Yamaguchi JY, Kobayashi M, Mishima K, Horimoto K, et al. Extract of Ginkgo biloba leaves attenuates kainate-induced increase in intracellular Ca2+ concentration of rat cerebellar granule neurons. Biol Pharm Bull 2005;28:934-6.
26. Oyama Y, Hayashi A, Ueha T. Ca(2+)-induced increase in oxidative metabolism of dissociated mammalian brain neurons: effect of extract of ginkgo biloba leaves. Jpn J Pharmacol 1993;61:367-70.
27. Oyama Y, Chikahisa L, Ueha T, Kanemaru K, Noda K. Ginkgo biloba extract protects brain neurons against oxidative stress induced by hydrogen peroxide. Brain Res 1996;712:349-52.
28. Brito MA, Brites D, Butterfield DA. A link between hyperbilirubinemia, oxidative stress and injury to neocortical synaptosomes. Brain Res 2004;1026:33-43.
29. Martínez-Solís I, Acero N, Bosch-Morell F, Castillo E, González-Rosende ME, Muñoz-Mingarro D, et al. Neuroprotective Potential of Ginkgo biloba in Retinal Diseases. Planta Med 2019;85:1292-303.
30. Guo M, Suo Y, Gao Q, Du H, Zeng W, Wang Y, et al. The protective mechanism of Ginkgolides and Ginkgo flavonoids on the TNF-α induced apoptosis of rat hippocampal neurons and its mechanisms in vitro. Heliyon. 2015;1:e00020.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:209-217
DOI: 10.4274/BMJ.galenos.2022.2022.3-18

Research

Comparison of Perioperative Results of the Use of a Double-Lumen Tube and Laryngeal Mask Airway in Video-assisted Thoracoscopic Wedge Resection: A Retrospective Clinical Study

Video Yardımlı Torakoskopik Wedge Rezeksiyonunda Çift Lümenli Tüp ve Laringeal Maske Hava Yolu Kullanımının Perioperatif Sonuçlarının Karşılaştırılması; Retrospektif Klinik Çalışma

Selçuk Köse[1], Gökhan Sertçakacılar[2,3]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Thoracic Surgery, Istanbul, Turkey
[2]Cleveland Clinic, Anesthesiology Institute, Department of Outcomes Research, Cleveland, OH, USA
[3]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Anesthesiology and Reanimation, Istanbul, Turkey

ABSTRACT

Objective: To evaluate the feasibility and safety of double-lumen tube (DLT) endotracheal intubation and laryngeal mask airway (LMA) use in the airway management of patients undergoing general anesthesia in thoracoscopic surgery of primary spontaneous pneumothorax.

Methods: For this retrospective observational study, patients were assigned to the LMA group (n=68) and DLT group (n=52) according to the airway method used. Perioperative clinical parameters of patients in both groups were collected through an institutional computer-based documentation system. The SPSS 22.00 program was used for data analysis.

Results: There was no statistically significant difference between the groups in terms of demographic and surgical data (p>0.05). After induction, heart rate (p=0.026) and mean blood pressure (p=0.019) were found to be statistically higher in the DLT group than the LMA group in the T_2 period when patients were treated with LMA or DLT. When the mechanical ventilation data of both groups were compared, plateau pressure, peak pressure, driver pressure, compliance, and calculated mechanical power values were statistically similar between the two groups. The number of patients experiencing sore throats during the postoperative period was significantly lower in the LMA group (n=11, 16.17%) than that in the DLT group (n=12, 23.07%) (p=0.034).

Conclusion: Compared with DLT, the use of an LMA in patients for airway management in video assisted thoracoscopic wedge resection can be performed safely and successfully, providing more stable hemodynamics and similar mechanical ventilation parameters.

Keywords: Laryngeal mask airway, endotracheal intubation, double-lumen tube, wedge resection, primary spontaneous pneumothorax

ÖZ

Amaç: Bu çalışma, primer spontan pnömotoraks tedavisi için torakoskopik cerrahi geçiren hastalarda anesteziye bağlı hava yolu yönetiminde çift lümenli tüp (ÇLT) ile endotrakeal entübasyon ve laringeal maske hava yolunun (LMA) uygulanabilirliğini ve güvenliğini değerlendirmekte ve karşılaştırmaktadır.

Gereç ve Yöntem: Retrospektif gözlemsel çalışma desenindeki bu çalışma için hastalar başvuru sırasına göre ardışık olarak, LMA grubuna (n=68) ve ÇLT grubuna (n=52) atandı. Her iki gruptaki hastalardan perioperatif klinik parametreler kurumsal bilgisayar tabanlı dokümantasyon sistemi aracılığıyla toplandı. Verilerin analizi için SPSS 22.00 programı kullanıldı.

Address for Correspondence: Gökhan Sertçakacılar, Cleveland Clinic, Anesthesiology Institute, Department of Outcomes Research, Cleveland, OH, USA
Phone: +1-216-983-9862 E-mail: gokhansertcakacilar@hotmail.com ORCID ID: orcid.org/0000-0002-4574-0147

Cite as: Köse S, Sertçakacılar G. Comparison of Perioperative Results of the Use of a Double-Lumen Tube and Laryngeal Mask Airway in Video-assisted Thoracoscopic Wedge Resection: A Retrospective Clinical Study. Med J Bakirkoy 2022;18:209-217

Received: 22.03.2022
Accepted: 10.05.2022

Bulgular: Gruplar arasında demografik ve cerrahi veriler açısından istatistiksel olarak anlamlı fark saptanmadı (p>0.05). İndüksiyon sonrası LMA veya ÇLT ile hastalara müdahale yapıldığı T_2 periyodunda kalp hızı (p=0.026) ve ortalama kan basıncı (p=0.019) ÇLT grubunda istatistiksel olarak yüksek saptandı. Her iki grupta mekanik ventilasyon verileri karşılaştırıldığında ise plato basıncı, tepe basıncı, sürücü basıncı, kompliyans ve hesaplanan mekanik güç değerleri iki grup arasında istatistiksel olarak benzerdi. Postoperatif dönemde boğaz ağrısı yaşayan hasta sayısı LMA grubunda (n=11, %16.17) ÇLT grubuna (n=12, %23.07) göre anlamlı olarak daha düşüktü (p=0.034).

Sonuç: ÇLT ile karşılaştırıldığında video yardımlı torakoskopik wedge rezeksiyonu cerrahisinde hava yolu yönetimi için hastalara LMA uygulanması daha stabil hemodinami ve benzer mekanik ventilasyon parametreleri sağlayarak güvenli ve başarılı şekilde gerçekleştirilebilir.

Anahtar Kelimeler: Laringeal maske hava yolu, endotrakeal entübasyon, çift lümenli tüp, wedge rezeksiyon, primer spontan pnömotoraks

INTRODUCTION

Primary spontaneous pneumothorax (PSP) is a disease characterized by the partial or total collapse of the lung, which is particularly common in young and tall men (1). It is usually possible to expand the lung using tube thoracostomy, but surgery is required for treating prolonged or recurrent PSP cases. Video-assisted thoracoscopic wedge resection is a promising surgical treatment for prolonged PSP (2). In wedge resection procedures in video-assisted thoracoscopic surgery (VATS), one-lung ventilation (OLV) with the operated lung collapsed is considered mandatory to create a surgical field. To provide this condition, general anesthesia with double-lumen endobronchial intubation is applied. However, possible complications of double-lumen endobronchial intubation have been reported in the literature (3,4). Problems such as sore throat and hoarseness are common in the postoperative period because double-lumen tubes (DLTs) are more difficult to manipulate during intubation and have a larger diameter than single-lumen tubes (5). Tracheal rupture is the most serious complication that can be experienced. In addition, barotrauma and volutrauma may be seen due to OLV (6,7). In addition, the bronchoscopy performed for the duration of the procedure and the localization control of the tube can prolong the anesthesia period of the patient.

In this minimally invasive surgical technique, an alternative method has been sought to prevent complications caused by DLT, and laryngeal mask airways (LMA) have been tried in different studies for airway management in VATS. The results obtained show that the use of LMA for airway management is promising (8). However, these studies were conducted on a small number of patients and compared with single-lumen endotracheal tubes (ETT). However, the use of LMAs in thorax surgery is still very limited.

There is no study in the literature examining the effectiveness of DLT and LMA in thoracoscopic wedge resection procedures. Our hypothesis was hat LMA would provide a more comfortable postoperative period for patients. This study provided additional evidence on this issue and to evaluate the intraoperative efficacy, safety, and postoperative results of LMA use in this procedure through comparison with DLTs.

METHODS

Study Design and Patients

Approval this study was approved by the Clinical Research Ethics Committee of Bakırköy Dr. Sadi Konuk Training and Research Hospital (decision no: 2022-04, date: 21.02.2022). For the study, the records of patients who underwent wedge resection with the VATS technique between January 1st, 2020, and December 31st, 2021, were reviewed, retrospectively.

The inclusion criteria were defined as patients with unilateral PSP requiring thoracoscopic wedge resection, aged 18 years and over, American Society of Anesthesiologists physical status I-II, and mallampati score I-II. The exclusion criteria were data loss, body mass index >30 kg/m^2, and history of previous thoracic surgery. The institutional computer-based documentation system and patient follow-up forms were used as data collection tools. A total of 120 consecutive patients who met the inclusion criteria were analyzed in either the DLT or LMA groups according to the airway control method used. All surgeries were performed by the same surgeon (S.K.) and anesthesiologist (G.S.). The study was conducted in accordance with the principles set in the Declaration of Helsinki (as revised in 2013).

General Anesthesia Technique

Before surgery, 22-gauge vascular access was established in all patients, and an infusion with 2-4 mL/kg/st Ringer's lactate solution was initiated for rehydration. Standard monitoring was achieved using electrocardiography, noninvasive blood pressure, and peripheral oxygen saturation. After monitoring, 0.03-0.05 mg/kg midazolam was administered to all patients for premedication. In the DLT group, after induction was achieved using 2 mcg/kg fentanyl, 3 mg/kg propofol, and 0.8 mg/kg intravenous rocuronium, the patients were intubated with a left-sided DLT suitable for their weight and height. The position of the DLT was confirmed through auscultation and fiberoptic bronchoscopy. In the LMA group, after induction was achieved with 1.5 µg/kg fentanyl, 2 mg/kg propofol, and

0.2 mg/kg intravenous rocuronium, a number 3 LMA was placed for women and number 4 for men. The preferred LMA was i-gel (Intersurgical Ltd, Berkshire, UK®).

During OLV, mechanical ventilation of the lung was adjusted to maintain normocapnia and prevent hypoxemia (tidal volume: 3-4 mL/kg, inspiratory, and expiratory ratio: 1:1-1:2, frequency: 15-18, end-tidal carbondioxide ($ETCO_2$) concentration, 35-45 mm Hg), a peak airway pressure of more than 30 cm H_2O was not desired. Anesthesia was maintained with 60-100% oxygen at a flow rate of 3 L/min, and 0.8-1% MAC sevoflurane and IV remifentanil (0.05-0.1 mcg/kg/min) infusion. When the surgical field was closed, all patients were administered 0.7 mg/kg IV meperidine hydrochloride for postoperative analgesia and 4 mg ondansetron for nausea and vomiting prophylaxis. At the end of the surgery, all patients were followed up in the recovery room with mask O_2 (2 L/min) support after extubating. Those with a modified Aldrete score ≥9 were sent to the ward. To provide analgesia in the postoperative period, tenoxicam 20 mg IV twice per day and paracetamol 1g IV three times per day were administered to all patients. Tramadol hydrochloride (1 mg/kg) was administered as rescue analgesia to patients with pain scores ≥4.

Data Collection

Hemodynamic data, mechanical ventilation settings, airway pressures, and compliance of the patients when the patient was monitored (T_1), immediately after intubation (T_2), after clamping in the DLT group, after carbondioxide (CO_2) insufflation in the LMA group (T_3), when the surgical field was closed (T_4), at the end of surgery (T_5), and after extubation (T_6) were recorded. Only hemodynamic data were recorded because the patient was awake during the T_1 and T_6 periods. Driving pressure was calculated using the formula [P plateau- positive end-expiratory pressure (PEEP)], and mechanical power (J/minute) was calculated using the formula [0.098 x tidal volume x respiratory rate x (Ppeak-1/2 x driving pressure)]. In addition, patients were evaluated in terms of duration of anesthesia, surgical duration, length of hospital stay, and postoperative complications.

Surgical Technique

Under general anesthesia, the patient was placed in the lateral decubitus position in the DLT group and the semilateral decubitus position in the LMA group, and three port incisions were made on the upper side of the patient where the surgery was planned. The lung that underwent surgery in the DLT group collapsed by blocking the airway. In the LMA group, after the port placement, CO_2 gas was insufflated into the thoracic cavity at a pressure of 8-10 cm H_2O and the lung collapsed. Surgical exploration was performed by placing 30° optics through the lowest port.

After detecting the bullous area, which is generally located in the upper lobe of the lung, air leak control is performed. Wedge resection was performed on the ruptured and non-ruptured bulla area using an endoscopic stapler. After the wedge resection, air leakage control was repeated. A partial pleurectomy was performed on the apicolateral region of the parietal pleura. At the end of the surgery, 32-French thoracic drains were placed through the port incisions and the procedure was completed. All surgeries were performed by the same surgeon (S.K.).

Outcome Measurements

The primary outcome of the study was to determine the mechanical power values. These are predictors of mechanical ventilation-associated lung injury (VILI) that may develop due to airway dynamics in the DLT and LMA groups. Secondary outcomes were the determination of intraoperative hemodynamic and airway pressure parameters. In addition, the length of hospital stay and postoperative pulmonary complications were recorded.

Statistical Analysis

The G*Power 3.1.9.2 program was used to calculate the sample size of the study. A pilot retrospective study was conducted with ten patients from each group to determine the minimal sample size for the primary outcome. The mean mechanic power value was 7.25±1.16 in the group LMA and 8.47±1.85 in the group DLT. An effect size 0.790 and α error=0.05 with a power of 95% was assumed so that each group had at least 43 participants. We included 68 patients in group LMA and 52 patients in group DLT due to the possibility of dropouts. Patient data from the pilot study were not included in the main study.

The data collected in the study were evaluated using the SPSS 22.00 program for Windows 10. The Kolmogorov-Smirnov test was used to check the normality of the data distributions. For descriptive statistics, categorical variables are given as percentages (%) and numerical variables as mean ± standard deviation. In the comparison of the quantitative data of the two groups when the normality conditions were met, the two-sample independent t-test was used, and Fisher's Exact test was used when the variables were qualitative. The Mann-Whitney U test was used for quantitative variable data comparisons where normality conditions were not met. The statistical significance level of alpha was set as $p<0.05$.

RESULTS

The files of 158 patients who had undergone thoracoscopic wedge resection were retrospectively analyzed for the study. Due to missing data and other exclusion criteria, 22 patients from the LMA group and 16 patients from the DLT group were excluded from the study. In total, the data of 120 patients were analyzed (Figure 1). The demographic and surgical data of the study are summarized in Table 1. There was no statistically significant difference between the groups in terms of age, sex, duration of anesthesia and surgery, and the side of the surgery ($p>0.05$).

When the intraoperative haemodynamic data of the patients were compared, heart rate and mean blood pressure were found to be statistically higher in the DLT group in the T_2 period when the LMA was placed after induction or intubation with DLT was performed ($p<0.05$) (Table 2). When the mechanical ventilation data in both types of airway management types were compared, no statistically significant difference was found in terms of applied PEEP, tidal volume, respiratory rate, and a fraction of inspired oxygen values (Table 3). Similarly, airway pressures and compliance were compared according to the airway technique used. There was no difference between the two groups in terms of plateau pressure, peak pressure, driver pressure, and compliance values. The mechanical power values calculated according to the applied tidal volume, respiratory rate, and formed airway pressures were also statistically similar between the two groups (Table 4). When switching to OLV in the DLT group, 43 patients had excellent operative lung collapse and five patients had good. In the other four patients, the lungs did not collapse. The patients were re-evaluated with a bronchoscope, necessary manipulations were made, and the lungs were colocated. In the LMA group, ventilation was not sufficient in two patients after LMA placement, and they were changed to a higher numbered LMA. In both groups, the surgical procedure was completed with the VATS technique. There was no difference between the groups in terms of postoperative complications. The recurrence of pneumothorax occurred in two patients in the LMA group, expansion returned limited in two patients in the DLT group, and pneumothorax recurred in a patient after surgery. Chest drainage time, and hospital stay were also similar between the groups. The number of patients experiencing sore throat during the postoperative period was significantly lower in the LMA group (Table 1).

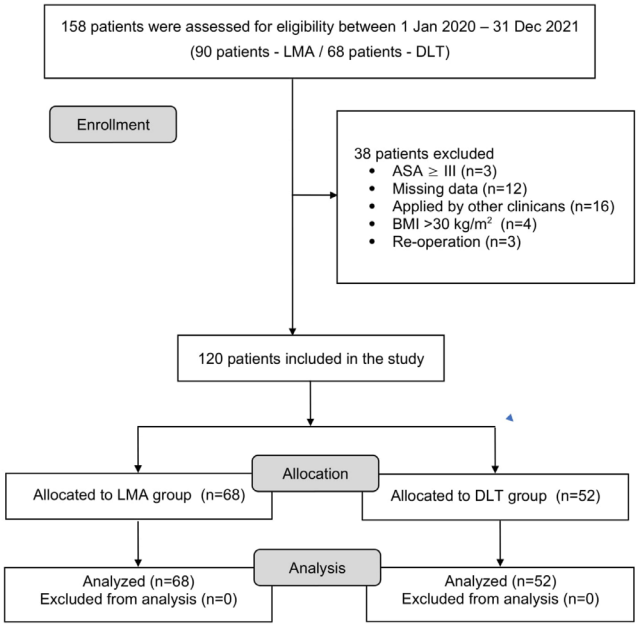

Figure 1. CONSORT flow chart of the study
LMA: Laringeal mask airway, DLT: Double-lumen tube, ASA: American Society of Anesthesiologists, BMI: Body mass index

DISCUSSION

Today, the thoracoscopic wedge resection technique performed for treating prolonged PSP is commonly performed with DLT intubation and OLV. In this study, we compared the efficacy, safety, and perioperative results of the use of LMAs in the surgical treatment of PSP with the use of DLTs to prevent complications of traditional airway management (4-7). Intraoperative and postoperative results from airway management with LMAs provided results similar to those of intubation with DLTs. Therefore, our study shows that thoracoscopic wedge resection can also be performed safely under the LMA.

In traditional thoracoscopy procedures, OLV with DLT is applied to provide the surgical field (9). However, strategies applied during OLV can cause barotrauma and volutrauma. In addition, DLTs themselves can lead to lung injury due to mechanical strain and lower respiratory tract infections (10,11). In addition, various complications such as airway spasm, vocal cord paralysis, laryngeal edema and tracheal stenosis, can be seen more frequently with the use of DLT (12). ETT or cuff erosion damage can result in tracheal stenosis. An overinflated endotracheal cuff may develop during pressure necrosis of the tracheal mucosa, resulting in tracheal constriction. Following DLT intubation, stenosis is defined as thickening of the tracheal wall and accompanying luminal narrowing, most typically in the left or right major bronchial region at the level of the ETT balloon (13).

Another airway method that can be used as an alternative to DLTs during general anesthesia is LMA. Cases in which LMAs

Table 1. Demographic, anesthetic and surgical data

	LMA group (n=68)	DLT group (n=52)	p
Age (year)	27±6.18	28±6.55	0.672
Sex			
Female	8 (11.8%)	8 (15.4%)	0.753
Male	60 (88.2%)	44 (84.6%)	
Height (cm)	176.76±.4.28	179.85±7.31	0.159
Weight (kg)	63.88±8.41	67.69±7.89	0.217
BMI (kg/m^2)	20.85±2.60	20.89.8±1.80	0.963
ASA			
I	36 (52.9%)	24 (46.2%)	0.713
II	32 (47.1%)	28 (53.8%)	
History of nicotine dependence	36 (52.9%)	28 (53.8%)	0.961
Duration of anesthesia (min)	63.41±10.94	83.08±12.67	<0.001*
Duration of surgery (min)	51.65±9.12	55.38±12.15	0.344
Operation side			
Right	52 (76.5%)	28 (53.8%)	0.193
Left	16 (23.5%)	24 (46.2%)	
ARISCAT score	24.65±2.66	29.08±8.43	0.066
Hypoxemia	-	-	n/a
Postop pulmoner complication	2 (11.8%)	2 (15.4%)	0.862
Sore throat	11 (16.17%)	12 (23.07%)	0.034*
Chest drainage time (day)	2 (1-3)	2 (1-3)	0.561
Lenght of hospital stay (day)	3.17±0.38	3.32±0.63	0.095

*p<0.05 shows statistical significance. Data are presented as the mean and standard deviation, median or number of patients (%) in each group. ASA: American Society of Anesthesiologists, BMI: Body mass index, ARISCAT score: Assess respiratory risk in surgical patients in Catalonia score, DLT: Double-lumen tube, LMA: Laringeal mask airway

were successfully used in surgeries such as spontaneous pneumothorax and Nuss procedures performed with the VATS technique have been shown in the literature (8,14-16).

Compared with DLT, the use of LMA in thoracic surgery has many advantages. It is easy to use, does not require a laryngoscope for its insertion, usually does not require muscle relaxants, is successfully inserted in a short time, and postoperative sore throat is rare. In our study, the LMA was placed in a very short time, around 10-20 seconds, allowing the surgery to start quickly.

In DLT, on the other hand, there was a delay while waiting for the muscle relaxant effect, and the placement of the tube after intubation was confirmed by both auscultation and using a bronchoscope. Therefore, although the duration of surgery was similar in both groups, the duration of anesthesia was found to be significantly longer in the DLT group.

Tracheal intubation with DLT requires satisfactory mouth opening and full suppression of airway reflexes during tube manipulation because the tube diameter is larger and advanced to the main bronchus. For this reason, the use of deep anesthesia and high-dose muscle relaxants in patients using DLT may cause the recovery time and thus the anesthesia time to be prolonged, especially in short cases such as wedge resection. LMA, on the other hand, is placed by advancing from the oropharynx to the larynx, and because it does not contact the epiglottis and trachea, it stimulates the airway reflexes less than DLT. Therefore, muscle relaxant agents are generally not needed in patients using LMAs. In the presented study, the muscle relaxant dose used in the LMA group was one-quarter of that used in the DLT group. In addition, the infusion dose of the opioid analgesic agent (remifentanil) used for anesthesia maintenance was also statistically significantly lower.

Table 2. Comparison of hemodinamic data between groups

	LMA group (n=68)	DLT group (n=52)	p
SpO_2			
T_1	99.00±0.86	99.46±0.96	0.107
T_2	98.82±1.33	98.08±2.39	0.579
T_3	98.59±1.17	97.69±2.72	0.606
T_4	98.88±1.05	98.46±2.02	0.929
T_5	98.80±1.26	99.50±0.75	0.189
T_6	98.41±1.41	98.62±1.04	0.795
HR			
T_1	81.06±14.15	85.15±9.45	0.351
T_2	72.65±16.18	84.54±9.39	0.026*
T_3	71.65±12.97	72.00±13.39	0.942
T_4	70.82±13.13	71.00±13.91	0.972
T_5	67.60±10.52	69.38±4.89	0.659
T_6	84.76±9.37	87.69±13.98	0.498
MAP			
T_1	65.76±10.74	66.92±14.77	0.805
T_2	66.94±16.28	78.31±7.77	0.019*
T_3	65.06±12.41	63.38±10.36	0.698
T_4	64.65±8.47	59.62±9.33	0.134
T_5	63.00±7.47	58.63±5.20	0.157
T_6	73.88±15.22	76.08±9.55	0.633

*p<0.05 shows statistical significance. Data are expressed as the mean and standart deviation in each group. LMA: Laringeal mask airway, DLT: Double-lumen tube, SpO_2: Peripheral oxygen saturation, HR: Heart rate, MAP: Mean arterial pressure, T_1: Monitoring time, T_2: After intubation, T_3: After clamping in the DLT group, after carbondioxide insufflation in the LMA group, T_4: Closing time, T_5: End of surgery, T_6: After extubation

Table 3. Comparison of mechanic ventilation parameters between groups

	LMA group (n=68)	DLT group (n=52)	p
PEEP			
T_2	5 (3-5)	5 (3-5)	0.699
T_3	5 (3-6)	5 (3-7)	0.857
T_4	5 (3-6)	5 (3-6)	0.382
T_5	5 (5-6)	5 (5-5)	0.317
Respiratory rate			
T_2	15 (12-19)	17 (13-19)	0.132
T_3	15 (12-18)	17 (12-19)	0.063
T_4	15 (12-18)	16 (12-19)	0.460
T_5	15 (12-17)	13 (12-18)	0.667
Tidal volume			
T_2	479.35±46.84	488.31±32.36	0.561
T_3	426.41±73.43	381.54±82.44	0.127
T_4	421.12±78.57	398.85±67.06	0.420
T_5	433.47±72.31	486.50±9.19	0.487
FiO_2			
T_2	50 (40-60)	50 (40-70)	0.427
T_3	45 (40-65)	50 (35-80)	0.454
T_4	45 (40-60)	50 (35-80)	0.659
T_5	50 (40-55)	47 (40-55)	0.621
$ETCO_2$			
T_2	40 (32-47)	41 (36-47)	0.454
T_3	40 (35-48)	40 (31-48)	0.721
T_4	40 (35-44)	38 (29-43)	0.159
T_5	39 (34-44)	34 (32-43)	0.074

Data are presented as the mean and standart deviation, median or number of patients (%), PEEP: Positive end-expiratory pressure, FiO_2: Fraction of inspired oxygen, $ETCO_2$: End-tidal carbondioxide, LMA: Laringeal mask airway, DLT: Double-lumen tube, T_2: After intubation, T_3: After clamping in the DLT group, after carbondioxide insufflation in the LMA group, T_4: Closing time, T_5: End of surgery

The i-gel used in the study is a new-generation LMA, and thanks to its soft silicone structure, it interacts with body temperature, fits more comfortably in the laryngopharynx, and causes less mechanical trauma than traditional LMAs (17,18). Generally because LMAs are non-invasive, different respiratory complications such as sore throat, bronchospasm, laryngeal edema, recurrent laryngeal nerve palsy, vocal cord injury, and tracheal rupture are prevented in patients (19). In this study, the incidence of sore throat was found to be higher in the DLT group, albeit not statistically significant.

When the hemodynamic data of both groups were compared, we found that hemodynamic parameters such as mean arterial pressure and heart rate during airway intervention were lower in the LMA group. This indicates that patients were exposed to less airway stimulation during the LMA procedure. The main reason for this is that the chin-hanging maneuver is not used because the laryngoscope is not used when placing the LMA, and the LMA is not inserted into the trachea. In the presented study, although the dose of muscle relaxants, hypnotic, and analgesic agents used during induction was lower, the hemodynamic parameters of the patients remained more stable while the LMA was placed.

The most important difference between our study and other studies on this subject is that the pressure parameters of

Table 4. Airway pressures data

	LMA group (n=68)	DLT group (n=52)	p
Ppeak			
T_2	14.18±2.81	16.23±5.23	0.177
T_3	17.88±4.36	20.85±6.66	0.152
T_4	17.00±4.50	20.54±6.51	0.089
T_5	15.53±3.62	17.25±3.99	0.308
P plato			
T_2	13.12±2.84	14.23±3.85	0.370
T_3	15.94±3.83	17.62±5.00	0.308
T_4	15.53±4.03	17.31±5.51	0.316
T_5	13.87±2.87	14.88±4.19	0.502
Driving pressure			
T_2	8.24±2.79	9.23±3.85	0.419
T_3	10.94±3.47	12.69±4.60	0.245
T_4	10.53±3.69	12.23±5.19	0.303
T_5	8.73±2.84	10.25±4.02	0.303
Dynamic complians			
T_2	68.21±21.90	69.00±22.77	0.923
T_3	65.55±27.48	51.98±13.16	0.113
T_4	59.25±20.88	53.91±18.13	0.522
T_5	58.59±14.02	55.73±19.71	0.770
Mechanical power			
T_2	6.4±2.1	5.1±2.7	0.451
T_3	7.5±1.8	8.6±3.5	0.242
T_4	7.4±2.4	8.7±3.4	0.221
T_5	7.8±1.5	8.1±3.1	0.914

Data are expressed as the mean and standart deviation in each group. LMA: Laringeal mask airway, DLT: Double-lumen tube, Ppeak: Peak pressure, P plato: Plato pressure, LMA: Laringeal mask airway, DLT: Double-lumen tube, T_2: After intubation, T_3: After clamping in the DLT group, after carbondioxide insufflation in the LMA group, T_4: Closing time, T_5: End of surgery

the airway are discussed in detail. This is because non-physiologic increases in transpulmonary pressure during ventilation cause VILI. In studies on VILI, it has been determined that respiratory rate and inspiratory flow rate are the main factors, except for tidal volume, plateau pressure, and PEEP, which are the static components of respiration, because the number of times per minute a potential volutrauma or barotrauma is delivered to the lungs is related to the respiratory rate. Similarly, the rate at which this potentially occurring trauma is performed is a concept related to the inspiratory flow rate. In isolated lung and animal studies, it has been shown that reducing the respiratory rate helps prevent the formation of VILI (20,21). The concept in which the basic mechanical factors (volume, pressure, velocity, and flow) of mechanical ventilation are gathered under a single definition is mechanical power (energy/time unit). The equation describing the power is simply defined as [0.098 x tidal volume x respiratory rate x (Ppeak-1/2 x driving pressure)] (22).

Particularly in thoracic surgery, respiratory rate is increased during OLV to avoid hypoxemia and hypercarbia in patients. In addition, the drive pressure due to the OLV also increases and can eventually increase the calculated mechanical power, resulting in VILI.

However, it is unclear to what extent the airway device used affects airway pressures and its effect on the mechanical power value. Therefore, in our study, we compared the mechanical power values and other airway pressures calculated from the patients in the LMA and DLT groups, using the same MV mode and providing optimum airway care. According to the results of the study, there was no significant difference between the airway pressures and mechanical power values in all periods between the two groups. In parallel, similar results were obtained for both groups in terms of postoperative pulmonary complications.

On the other hand, the use of LMA may cause some problems during thoracic surgery. Undesirable hypercarbia may develop in patients because the operative lungs are deflated by CO_2 insufflation. This metabolic state, which is easily noticed by the increase in $ETCO_2$ level, may increase sympathetic activity in patients, causing tachycardia, arrhythmia, myocardial ischemia, and cerebral vasodilation to increase cerebrospinal fluid pressure and intracellular acidosis, leading to a narcotic effect. In our study, although $ETCO_2$ levels of the patients in the LMA group were higher than those in the DLT group, no statistically significant difference was found between the groups. We think that hypercarbia did not develop in the patients in the LMA group because of the young age of the patients, the absence of chronic lung diseases, and the short surgical time. In addition, the location of the LMA may change depending on the position during surgery, resulting in airway leakage or a sudden increase in airway pressure. However, in the i-gel LMA type used in this study, these possibilities are minimized due to the silicon structure. In the presented study, no such problems were experienced in the patients. The patients were required to fast for 8 hours before surgery because LMAs generally increase the risk of aspiration, and LMAs were not used in patients with gastroesophageal reflux.

This study has some limitations. Randomization could not be performed because it was a retrospective observational study. Although patients were included in the trial sequentially according to the surgery date, this may have biased patient selection and management. In addition, because the study included patients from a single-center, the results obtained might not reflect the general population. Although the sample size is more significant than in previous studies, it is still not sufficient for a retrospective study. Surgeon's comfort may also change when DLT is compared with LMA. But surgeon's satisfaction or comfort with surgical conditions was not evaluated in this study.

CONCLUSION

The use of LMA provided similar respiratory parameters to DLTs and more stable hemodynamics. The data obtained from the study show that PSP treatment can be performed safely and successfully using LMAs for airway management in thoracoscopic wedge resection surgery. However, larger studies are needed to carry out our limited experience on this subject further and to enable large-scale thoracic surgeries such as lobectomy and pneumonectomy to be performed with LMA.

ETHICS

Ethics Committee Approval: Approval this study was approved by the Clinical Research Ethics Committee of Bakırköy Dr. Sadi Konuk Training and Research Hospital (decision no: 2022-04, date: 21.02.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: S.K., G.S., Concept: S.K., G.S., Design: S.K., G.S., Data Collection or Processing: S.K., G.S., Analysis or Interpretation: S.K., G.S., Literature Search: S.K., G.S., Writing: S.K., G.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. Huang H, Peng X, Zhang H, Li W, Wang C. A Retrospective Case-Control Study on the Chest Wall and Lung Characteristics in Patients with Primary Spontaneous Pneumothorax. Med Sci Monit 2019;25:8482-91.
2. Yoak MB, Lambert CJ Jr. Video-assisted thoracoscopic surgery for management of spontaneous pneumothorax. W V Med J 1997;93:176-8.
3. Cools E, Neyrinck AP. Left mainstem bronchus rupture due to a left-sided double lumen tube. Acta Anaesthesiol Belg 2015;66:31-3.
4. Schneider T, Storz K, Dienemann H, Hoffmann H. Management of iatrogenic tracheobronchial injuries: a retrospective analysis of 29 cases. Ann Thorac Surg 2007;83:1960-4.
5. Knoll H, Ziegeler S, Schreiber JU, Buchinger H, Bialas P, Semyonov K, et al. Airway injuries after one-lung ventilation: a comparison between double-lumen tube and endobronchial blocker: a randomized, prospective, controlled trial. Anesthesiology 2006;105:471-7.
6. Lohser J, Slinger P. Lung Injury After One-Lung Ventilation: A Review of the Pathophysiologic Mechanisms Affecting the Ventilated and the Collapsed Lung. Anesth Analg 2015;121:302-18.
7. Güldner A, Braune A, Ball L, Silva PL, Samary C, Insorsi A, et al. Comparative Effects of Volutrauma and Atelectrauma on Lung Inflammation in Experimental Acute Respiratory Distress Syndrome. Crit Care Med 2016;44:e854-65.
8. Ambrogi MC, Fanucchi O, Gemignani R, Guarracino F, Mussi A. Video-assisted thoracoscopic surgery with spontaneous breathing laryngeal mask anesthesia: preliminary experience. J Thorac Cardiovasc Surg. 2012;144:514-5.
9. Mavi J, Moore DL. Anesthesia and analgesia for pectus excavatum surgery. Anesthesiol Clin 2014;32:175-84.
10. Li Bassi G, Torres A. Ventilator-associated pneumonia: role of positioning. Curr Opin Crit Care 2011;17:57-63.
11. Bouadma L, Wolff M, Lucet JC. Ventilator-associated pneumonia and its prevention. Curr Opin Infect Dis 2012;25:395-404.
12. Cook TM, MacDougall-Davis SR. Complications and failure of airway management. Br J Anaesth 2012;109 Suppl 1:i68-i85.
13. Silva CIS, Müller LN. In Teaching Files in Radiology, The Teaching Files: Chest. Silva CIS, Müller LN, Editors. Case 120. W.B. Saunders; 2010. p. 240-1.
14. Cai K, Wang X, Ye J, Diao D, He J, Liu J, et al. [Laryngeal mask anesthesia in video-assisted thoracoscopic surgery for pulmonary bulla: comparison with intubation anesthesia]. Nan Fang Yi Ke Da Xue Xue Bao 2013;33:756-60.
15. Du X, Mao S, Cui J, Ma J, Zhang G, Zheng Y, et al. Use of laryngeal mask airway for non-endotracheal intubated anesthesia for patients with pectus excavatum undergoing thoracoscopic Nuss procedure. J Thorac Dis 2016;8:2061-7.
16. Li P, Liang W, Gu H. One-lung ventilation using Proseal laryngeal mask airway and Arndt endobronchial blocker in paediatric scoliosis surgery. Br J Anaesth 2009;103:902-3.
17. Kalra N, Gupta A, Sood R, Kaur M. Comparison of Proseal Laryngeal Mask Airway with the I-Gel Supraglottic Airway During the Bailey Manoeuvre in Adult Patients Undergoing Elective Surgery. Turk J Anaesthesiol Reanim 2021;49:107-13.
18. Singh I, Gupta M, Tandon M. Comparison of Clinical Performance of I-Gel with LMA-Proseal in Elective Surgeries. Indian J Anaesth 2009;53:302-5.
19. Taheri A, Hajimohamadi F, Soltanghoraee H, Moin A. Complications of using laryngeal mask airway during anaesthesia in patients undergoing major ear surgery. Acta Otorhinolaryngol Ital 2009;29:151-5.
20. Cressoni M, Gotti M, Chiurazzi C, Massari D, Algieri I, Amini M, et al. Mechanical Power and Development of Ventilator-induced Lung Injury. Anesthesiology 2016;124:1100-8.
21. Hotchkiss JR Jr, Blanch L, Murias G, Adams AB, Olson DA, Wangensteen OD, et al. Effects of decreased respiratory frequency

on ventilator-induced lung injury. Am J Respir Crit Care Med 2000;161(2 Pt 1):463-8.

22. Gattinoni L, Tonetti T, Cressoni M, Cadringher P, Herrmann P, Moerer O, et al. Ventilator-related causes of lung injury: the mechanical power. Intensive Care Med 2016;42:1567-75.a

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:218-224
DOI: 10.4274/BMJ.galenos.2022.2022.3-3

Research

The Effect of Comorbidities and Obesity on Postoperative Outcomes

Obezite ve Komorbiditelerin Postoperatif Sonuçlara Etkisi

Güneş Özlem Yıldız[1], Gökhan Sertçakacılar[1,2], Furkan Tontu[3], Duygu Akyol[4]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Anesthesiology and Reanimation, Division of Intensive Care, Istanbul, Turkey
[2]Cleveland Clinic, Anesthesiology Institute, Department of Outcomes Research, Cleveland, OH, USA
[3]Ağrı Training and Research Hospital, Clinic of Anesthesiology and Reanimation, Division of Intensive Care, Ağrı, Turkey
[4]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Anesthesiology and Reanimation, Division of Intensive Care, Istanbul, Turkey

ABSTRACT

Objective: Obesity is a major public health issue worldwide. Obesity, along with its associated comorbidities, may impact surgical outcomes. Obesity is linked to wound infection, thromboembolic complications, and respiratory difficulties in surgical patients. Obesity may or may not have a negative impact on postoperative outcomes.

Methods: Patients over the age of 18 who had endometrial cancer surgery were screened retrospectively. Patient data from the hospital database were retrieved in terms of demographics, comorbidities, American Society of Anesthesiologists (ASA) score, Charlson Comorbidity index (CCI) score, duration of operation and anesthesia, postoperative intensive care admission, complications, and length of hospital stay. They were also reviewed and recorded from the patient file.

Results: The subjects' mean age was 57.2±9.8 years (range: 31-84 years). In patients with endometrial cancer, American Society of Anesthesiologist (ASA) (p=0.001), Charlson Comorbidity Index (CCI) (p=0.001) scores, operation time, postoperative complication risk (p=0.004), and hospital stay increase (p=0.029) as we progress from the normal group to the obese and morbidly obese groups.

Conclusion: Obesity increases the risk of postoperative complications such as wound infection, sepsis, acute renal damage, evisceration, and hospital stay in patients who have had endometrial cancer surgery. The ASA and CCI scores were related to postoperative complications, with the CCI score also being related to the length of hospital stay. The fact that these scores provide information about the postoperative outcomes of patients from the preoperative period emphasizes the significance of these scores once more.

Keywords: Charlson comorbidity index, endometrial cancer, intensive care, obesity

ÖZ

Amaç: Obezite tüm dünyada önemli bir halk sağlığı sorunudur. Obezite, eşlik eden komorbiditeleri ile birlikte cerrahi sonuçları etkileyebilir. Obezite cerrahi hastalarda yara enfeksiyonu, tromboembolik komplikasyonlar ve solunum güçlükleri ile bağlantılıdır. Obezite ameliyat sonrası sonuçlar üzerinde olumsuz bir etkiye sahip olabilir veya olmayabilir.

Gereç ve Yöntem: On sekiz yaş üstü endometriyal kanser cerrahisi geçiren hastalar retrospektif olarak tarandı. Hastane veri tabanından hasta verileri demografik özellikler, komorbiditeler, Amerikan Anesteziyoloji Derneği (ASA) skoru, Charlson Komorbidite indeksi (CCI) skoru, operasyon ve anestezi süresi, postoperatif yoğun bakıma yatış, komplikasyonlar ve hastanede kalış süresi açısından gözden geçirildi ve kaydedildi.

Bulgular: Hastaların ortalama yaşı 57.2±9.8 yıldı (aralık: 31-84 yıl). Endometriyum kanserli hastalarda normal gruptan obez ve morbid obez gruplara doğru ilerledikçe ASA (p=0.001), CCI (p=0.001) skorları, operasyon süresi, postoperatif komplikasyon riski (p=0.004) ve hastanede kalış süresi (p=0.029) artmaktadır.

Sonuç: Obezite, endometriyum kanseri ameliyatı geçirmiş hastalarda yara enfeksiyonu, sepsis, akut böbrek hasarı, eviserasyon ve hastanede kalış gibi postoperatif komplikasyon riskini artırır. ASA ve CCI skorlarının postoperatif komplikasyonlarla ilişkili olduğu, ek olarak CCI skorunun

Address for Correspondence: Güneş Özlem Yıldız, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Anesthesiology and Reanimation, Division of Intensive Care, Istanbul, Turkey
Phone: +90 212 414 71 71 E-mail: gunesozlemyildiz@gmail.com ORCID ID: orcid.org/0000-0002-4557-9517

Cite as: Yıldız GÖ, Sertçakacılar G, Tontu F, Akyol D. The Effect of Comorbidities and Obesity on Postoperative Outcomes. Med J Bakirkoy 2022;18:218-224

Received: 18.03.2022
Accepted: 11.05.2022

hastanede kalış süresi ile de ilişkili olduğu bulundu. Bu skorların hastaların ameliyat öncesi dönemdeki ameliyat sonrası sonuçları hakkında bilgi vermesi, bu skorların önemini bir kez daha vurgulamaktadır.

Anahtar Kelimeler: Charlson komorbidite indeksi, endometriyal kanser, yoğun bakım, obezite,

INTRODUCTION

Obesity is a major public health issue worldwide (1,2). Endometrial cancer, one of the most common cancers worldwide, is strongly linked to obesity (3,4). Endometrial cancer is primarily treated through surgery (5). Obesity, along with its associated comorbidities, is thought to impact surgical outcomes. Obesity is associated with wound infection, thromboembolic problems, and respiratory problems in patients undergoing surgery, but whether obesity causes a negative postoperative outcome is debatable (6,7). As a result, studying the effect of obesity on postoperative outcomes is critical for predicting morbidity and hospital stay in patients undergoing surgery. Because of the numerous comorbid diseases that accompany obese patients, a practical preoperative evaluation is essential (8,9).

For more than sixty years, the American Society of Anesthesiologists (ASA) score has been the most commonly used score for preoperative patient evaluation by anesthesiologists. When combined with other factors (for example, the type of surgery), the score, which was last updated in 2020, is extremely useful in estimating perioperative risk (10). Obese patients [body mass index (BMI); 30-40] with no other comorbidities, for example, were classified as ASA-II; morbidly obese patients (BMI>40) were classified as ASA-III. In other words, according to the ASA score, the presence/increase of obesity alone raises the perioperative risk. However, the ability of this score to predict postoperative risk is unclear. The management of obese patients is difficult because of accompanying metabolic dysfunction, impaired glucose intolerance, and respiratory and cardiovascular diseases (11).

The Charlson Comorbidity index (CCI) was developed to assess the one-year mortality risk by allocating specific points to each of the seventeen comorbidities (12). CCI is a simple and easily applicable method for estimating the risk of death from comorbid disease (13). This index was later adapted for use with the International Classification of Diseases nine and ten, and it was used to estimate mortality (14,15). There are also studies on the use of CCI to predict postoperative outcomes in the literature (16,17). Obesity is not listed as a risk factor in CCI. Obesity, on the other hand, can be accompanied by diseases such as diabetes mellitus and stroke. In studies, the accuracy of the hypothesis is that as obesity increases, so do comorbid disorders and, consequently, CCI has not been fully clarified (18). CCI studies in obese patients are also important in testing this hypothesis.

The purpose of this retrospective study was to assess the effect of obesity on postoperative complications in endometrial cancer patients, including the role of two commonly used scoring systems (ASA, CCI) in predicting postoperative complications.

METHODS

Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee approval was obtained (protocol no: 2020/221, date: 08.06.2020). Patients over the age of 18 who had endometrial cancer surgery between January 2018 and April 2020 were screened retrospectively. Demographic information, comorbidities, ASA score, CCI score, duration of operation, duration of anesthesia, hospitalization in the postoperative intensive care unit (ICU), complications, and length of hospital stay of patients included in the study. In terms of time, patient data were analyzed and recorded from the hospital database and patient file. Patients who presented to the gynecological oncology clinic with the diagnosis of endometrial cancer were also included. Patients with missing data were barred from participation in the study.

All patients undergoing endometrial ca are subjected to a standard anesthesia protocol developed in our clinic. Patients who undergo a preoperative anesthesia examination have detailed physical examination and laboratory results, ASA scores based on comorbidities, and anesthesia risks recorded. The ASA standard (electrocardiogram, peripheral oxygen saturation, and non-invasive blood pressure) is used to monitor each patient brought into the operating room. An infusion of 4-6 mL/kg/h crystalloid fluid is initiated, and antibiotic therapy is initiated. Following anesthesia induction with 2 mcg/kg iv fentanyl, 2-3 mg/kg propofol, and 0.6 mg/kg rocuronium, patients are intubated and connected to the anesthesia device in volume control mode. The tidal volume is 6-8 mL/kg, and the respiratory frequency is 12 beats per minute. End-tidal CO_2 is adjusted to stay between 35 and 45 mmHg. For anesthesia maintenance, sevoflurane (MAC 0.8-1) and remifentanil (0.05-0.2 mcg/kg/min) are used. When the surgical field was being closed, the patients were given paracetamol 1 g and tramadol 1 mg/kg iv as analgesics. In the postoperative recovery room, patients with numerical

rating scale pain scores greater than 4 are given pethidine hydrochloride 0.3 mg/kg as rescue analgesia.

The need for postoperative intensive care is assessed following the operation, and admission to the ICU is determined based on the patients' comorbidities, the preoperative hemodynamic course, respiratory distress, and the need for vasopressor/inotrope. Patients whose general condition and hemodynamics are stable are extubated and taken to the recovery room. Patients who have a modified Aldrete score of more than 8 in their follow-up are referred to the service.

Statistical Analysis

Data were analyzed using SPSS 20 for Windows (IBM Corp., Armonk, NY, USA). The normal distribution of the data was evaluated using the Kolmogorov-Smirnov test. The normally distributed variables were presented as the mean ± standard deviation, while the non-normally distributed variables were presented as the median (interquartile range: 25-75 percentiles). Categorical variables were presented as numbers and percentages. ANOVA test (post-hoc: Bonferroni correction) was used for the group comparison of the normally distributed variables and the Kruskal-Wallis H test (post-hoc: Dunn's correction) was used for the intergroup comparison of the non-normally distributed variables. The chi-square and Fisher Exact tests were used for the intergroup comparison of the categorical variables. Possible risk factors for developing postoperative complications were determined by Cox regression analysis. Variables related to operation time and complication development time were analyzed by Spearman correlation analysis. $P<0.05$ was accepted as statistically significant.

RESULTS

The mean age of the subjects was 57.2±9.8 years (range: 31-84 years), and BMI was 35.1±8.8 kg/m^2. According to ASA classification the percentage of ASA II patients' were 54.7% (n=70) and ASA III patients' were 45.3% (n=58). In addition, 35.9% of patients had normal BMI levels. While 32.8% patients were obese, 31.3% were morbidly obese. Comorbidities were present in 67.2% of the patients. Postoperative complications developed in 16.4% of the patients. The median length of hospital stay was five days, and the mortality rate was 1.6%. The comorbidity ratio and ASA-III rate ratio were higher in the morbid obese group compared to the other groups. The highest value of median CCI scores was in the morbid obesity group. The complication ratio was higher in the morbid obese group than in the other groups, and higher in the obese group than the normal weight. The duration of hospital stay was longer in the morbid obese group compared to the other groups, while it was similar in the obese group and normal weight (Table 1).

The factors associated with the risk of complications are shown in Table 2. Increased BMI, morbid obesity, the presence of respiratory diseases, ASA-III score, increased duration of operation, and hospitalization in the ICU are associated with the risk complications (Table 2). In the regression model, including all risk factors, the independent risk factors that increased the risk of complications were morbid obesity [hazard ratio (HR)= 6.03, p=0.008] and hospitalization in ICU (HR=10.2, p=0.001). A positive correlation was found between BMI level and length of hospital stay (r=0.336, p=0.007), and a negative correlation was found between the time of complication development (r=-0.328, p=0.013) (Table 3).

Compared with the normal BMI group, ASA ($p<0.001$) and CCI ($p<0.001$) scores, operation time, length of hospital stay (p=0.029) and postoperative complication risk (p=0.004) were increased in the obese and morbidly obese patient group.

DISCUSSION

The current study assessed the effect of obesity on postoperative complications in patients undergoing endometrial cancer surgery, as well as the ability of ASA, CCI, one of the widely used scoring systems, to predict postoperative outcome. Our secondary goal was to assess the impact of obesity on these patients' length of hospital stay and the ability of the scores to indicate this.

In our study, it was found that as BMI increased in patients with endometrial cancer, the rate of comorbidity increased (p=0.005), the CCI score increased ($p<0.001$) and the length of hospital stay was prolonged. Obesity is associated with a number of comorbid diseases, independent of endometrial cancer. Insulin resistance, type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, stroke and sleep apnea are the most serious ones (19,20). In our study, we observed a significant increase in hypertension, diabetes and respiratory tract diseases as BMI increased ($p<0.05$). While the presence of respiratory tract diseases increased the risk of postoperative complications in our patients ($p<0.05$), the presence of diabetes mellitus and hypertension did not increase the risk of complications. In addition to the increase in comorbid diseases seen in obese patients, chronic inflammation and dysmetabolism have been shown to adversely affect postoperative outcomes (7,21). However, there are also studies stating that the risk

Table 1. Demographic, clinical and laboratory findings

Variables	Normal n=46	Obesity n=42	Morbid obesity n=40	p
Age, years	55.3±0.6	57.5±11.0	58.7±7.2	0.242
BMI, kg/m^2	**26.7±2.1**	**34.5±2.8**	**45.5±6.7**	<0.001*
Co-morbidity, n (%)				
No	22 (47.8)	**14 (33.3)**	**6 (15.0)**	0.005*
Yes	24 (52.2)	**28 (66.7)**	**34 (85.0)**	
Hypertension	12 (26.1)	11 (26.2)	**20 (50.0)**	0.035*
Diabetes mellitus	**2 (4.3)**	11 (26.2)	11 (27.5)	0.004*
Cerebrovascular event	1 (2.2)	1 (2.4)	3 (7.5)	0.444
Thyroid disorders	2 (4.3)	4 (9.5)	6 (15.0)	0.220
Respiratory diseases	6 (13.0)	7 (16.7)	**15 (37.5)**	0.021*
Rheumatological diseases	2 (4.3)	2 (4.8)	2 (5.0)	0.999
Psychological diseases	1 (2.2)	1 (2.4)	1 (2.5)	0.999
ASA, n (%)				
2	37 (79.6)	33 (78.6)	-	<0.001*
3	9 (19.6)	9 (21.4)	40 (100.0)	
CCI score	**3 (3-4)**	4.5 (3-8)	5 (3-8)	<0.001*
Duration of operation, min	**180 (160-300)**	240 (190-300)	240 (180-280)	<0.001*
Duration of anesthesia, min	220 (200-315)	300 (210-350)	267 (205-318)	0.173
Hospitalization in ICU, n (%)	2 (4.3)	5 (11.9)	6 (15.0)	0.071
Complication, n (%)				
No	**42 (93.3)**	**37 (88.1)**	**27 (67.5)**	0.004*
Yes	**3 (6.7)**	**5 (11.9)**	**13 (32.5)**	
Wound infection	3 (6.5)	3 (7.1)	4 (10.0)	0.844
Acute renal failure	-	1 (2.4)	3 (7.5)	0.073
DVT	-	-	1 (2.5)	0.312
Evisceration	-	-	1 (2.5)	0.312
Ilius	-	-	1 (2.5)	0.312
Incisional hernia	-	-	1 (2.5)	0.312
Pneumonia	-	1 (2.4)	-	0.641
VTE	-	-	1 (2.5)	0.312
Sepsis	-	-	1 (2.5)	0.312
Mortality, n (%)	-	-	2 (5.0)	0.202
Duration of stay in hospital, days	5 (4-6)	5 (4-6)	**8 (5-15)**	0.029*

Numerical variables with normal distribution were shown as mean ± standard deviation. Numerical variables that do not show normal distribution are shown as median (IQR). Categorical variables were shown as numbers (%). *p<0.05 shows statistical significance. Bold characters represent the group that differs in significance. ASA: American Society of Anesthesiologists, BMI: Body mass index, ICU: Intensive care unit, CCI: Charlson Comorbidity index, DVT: Deep vein thrombosis, VTE: Venous thromboembolism, IQR: Interquartile range

Table 2. Factors associated with risk of complications

Variables	Complication		Univariable regression		
	No n=107	Yes n=21	HR	95% CI	p
Age, years	56.7±10	59.1±9	1.02	0.98-1.07	0.336
BMI, kg/m^2	34.1±8.6	40.3±8.2	1.06	1.02-1.10	0.003*
Normal	43 (40.2)	3 (14.3)	ref		
Obesity	37 (34.6)	5 (23.8)	1.93	0.46-8.09	0.367
Morbid obesity	27 (25.2)	13 (61.9)	6.15	1.75-21.70	0.005*
Co-morbidity, n (%)					
No	38 (35.5)	4 (19.0)	ref		
Yes	69 (64.5)	17 (81.0)	2.21	0.74-6.56	0.155
Hypertension	35 (32.7)	8 (38.1)	1.26	0.52-3.05	0.602
Diabetes mellitus	21 (19.6)	3 (14.3)	0.74	0.22-2.51	0.627
SVO	3 (2.8)	2 (9.5)	2.74	0.64-11.78	0.175
Thyroid disorders	9 (8.4)	3 (14.3)	1.76	0.52-6.00	0.364
Respiratory diseases	19 (17.8)	9 (42.9)	2.93	1.23-6.96	0.015*
Rheumatological diseases	5 (4.7)	1 (4.8)	0.95	0.13-7.06	0.958
ASA, n (%)					
II	78 (72.9)	5 (23.8)	ref		
III	29 (27.1)	16 (76.2)	7.26	2.65-19.32	<0.001*
Duration of operation, min	200 (180-300)	250 (200-360)	1.05	1.01-1.10	0.026*
Duration of anesthesia, min	250 (200-320)	285 (250-410)	1.01	0.98-1.09	0.095
Hospitalization in ICU, n (%)	3 (2.8)	9 (42.9)	10.75	4.42-26.15	<0.001*
Mortality, n (%)	-	2 (9.5)	-	-	-
Duration of stay in hospital, days	5 (4-6)	14 (10-21)	-	-	-

Numerical variables with normal distribution are shown as mean ± standard deviation. Numerical variables that do not show normal distribution are shown as medians (minimum-maximum). Categorical variables are shown as numbers (%). *p<0.05 shows statistical significance. ASA: American Society of Anesthesiologists, BMI: Body mass index, ICU: Intensive care unit, CCI: Charlson Comorbidity index, DVT: Deep vein thrombosis, VTE: Venous thromboembolism, HR: Hazard ratio, CI: Confidence intervals

of complications (22) and mortality (23,24) is significantly lower in obese patients as an obesity paradox. In addition to obesity, it has been shown that the risk of postoperative complications is higher in patients with high ASA and CCI, long operative time and preoperative respiratory disorders (25-29). In our study, it has been shown that the ASA scores of the patients are high, CCI scores increase as BMI increases (p<0.05), the operation time is longer (p<0.05) and the need for ICU hospitalization is higher, supporting the literature. These scores and associated comorbid diseases explain the relationship between scores and postoperative complications as obesity rises. From the preoperative period, these two scores must predict the postoperative outcome. In our study, while the increase in CCI score was associated with the length of hospital stay (p<0.05), the same correlation was not found in the ASA score. We think that this may be due to the study population being limited to ASA-II and III patients. There are studies in the literature supporting the prognostic importance of CCI in terms of postoperative complications. Our study results were in agreement with the results of previous studies in predicting the postoperative outcome of obesity and CCI by cross-sectional design (30,31). A study of patients undergoing radical cystectomy found that demographic factors and comorbidity indices such as ASA, BMI, and CCI had poor discriminating ability for adverse events (sepsis, wound infection, hospitalization, and prolonged hospital stay) (32). The ASA independently estimated the duration of surgery, length of hospital stay, and hospital cost in a study of total knee arthroplasty patients; BMI has been associated

Table 3. Parameters related to the length of hospital stay and complication development time

Variables	Duration of stay in hospital		Complication onset time	
	r	p	r	p
Age	0.327	0.019*	0.083	0.352
BMI	0.336	0.007*	-0.328	0.013*
ASA score	0.178	0.403	-0.315	0.016*
CCI score	0.345	0.002*	0.117	0.449
Duration of operation	0.315	0.020*	0.107	0.496
Duration of anesthesia	0.304	0.030*	0.106	0.503

*p<0.05 shows statistical significance. ASA: American Society of Anesthesiologists, BMI: Body mass index, CCI: Charlson Comorbidity index

with intraoperative time (33). According to the findings of this study, there is no statistically significant increase in postoperative intensive care admissions as we approach the morbidly obese group. However, as one progresses from the normal BMI group to the obese and morbidly obese groups, the hospitalization rate in the postoperative ICU increases by 4.3, 11.9, and 15.0 percent, respectively. As a result, it is expected that increasing the number of patients in the groups will result in statistical significance. In our study, the factors affecting the length of hospital stay were found to be age, BMI, CCI score, and operation time. In direct proportion to the increase in these factors, the length of stay in the hospital also increased.

Study Limitations

The number of patients in the groups was insufficient because of the retrospective nature of the presented study. Furthermore, whether the patients' operations were performed laparoscopically or openly may have affected postoperative complications and hospital stay, but this was not investigated in our study.

CONCLUSION

Obesity increases the risk of postoperative complications such as wound infection, sepsis, acute renal damage, evisceration, and hospital stay in patients who have had endometrial cancer surgery. Obesity is an effective risk factor for developing endometrial cancer, and it is associated with a poor postoperative outcome in patients who have this cancer and have undergone surgery. Furthermore, in our study, ASA and CCI scores were linked to postoperative complications. Simultaneously, the CCI score was linked to the length of hospital stay. The fact that these scores, which are widely used in routine practice, provide us with information about patients' postoperative outcomes from the preoperative period emphasizes the importance of these scores once more.

ETHICS

Ethics Committee Approval: Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee approval was obtained (proctocol no:2020/221, date: 08.06.2020).

Informed Consent: This was a retrospective study.

Authorship Contributions

Surgical and Medical Practices: G.Ö.Y, G.S., Concept: G.Ö.Y., G.S., F.T., D.A., Design: G.Ö.Y., G.S., F.T., D.A., Data Collection or Processing: D.A., Literature Search: G.S., F.T., Writing: G.Ö.Y., G.S., F.T., D.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. Wyatt SB, Winters KP, Dubbert PM. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. Am J Med Sci 2006;331:166-74.
2. Sabaz MS, Aşar S, Sertçakacılar G, Sabaz N, Çukurova Z, Hergünsel GO. The effect of body mass index on the development of acute kidney injury and mortality in intensive care unit: is obesity paradox valid? Ren Fail 2021;43:543-55.
3. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. Cancer Epidemiol Biomarkers Prev 2002;11:1531-43.
4. Schmandt RE, Iglesias DA, Co NN, Lu KH. Understanding obesity and endometrial cancer risk: opportunities for prevention. Am J Obstet Gynecol 2011;205:518-25.
5. Giede C, Le T, Power P; SOGC-GOC-SCC POLICY AND PRACTICE GUIDELINES COMMITTEE; SPECIAL CONTRIBUTORS. The role of surgery in endometrial cancer. J Obstet Gynaecol Can 2013;35:370-1.
6. Flancbaum L, Choban PS. Surgical implications of obesity. Annu Rev Med 1998;49:215-34.
7. Doyle SL, Lysaght J, Reynolds JV. Obesity and post-operative complications in patients undergoing non-bariatric surgery. Obes Rev 2010;11:875-86.
8. Ortiz VE, Kwo J. Obesity: physiologic changes and implications for preoperative management. BMC Anesthesiol 2015;15:97.
9. Smith NA, Martin G, Marginson B. Preoperative assessment and prehabilitation in patients with obesity undergoing non-bariatric surgery: A systematic review. J Clin Anesth 2022;78:110676.
10. American Society of Anesthesiologists. ASA Physical Status Classification System. Available from: https://www.asahq.org/standards-and-guidelines/asa-physical-status-classification-system.
11. Hergünsel GO, Sabaz MS. The effect of obesity on mortality in geriatric patients followed in the intensive care unit. Turkish Journal of Geriatrics 2021;24:315-29.

12. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
13. Sabaz MS, Aşar S. Association of Charlson Comorbidity and Pneumonia Severity Indices with Mortality in Patients with Coronavirus Disease-2019 in the Intensive Care Unit.Turk J Intensive Care 2021;19:33-41.
14. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. J Clin Epidemiol 1992;45:613-9.
15. Sundararajan V, Henderson T, Perry C, Muggivan A, Quan H, Ghali WA. New ICD-10 version of the Charlson comorbidity index predicted in-hospital mortality. J Clin Epidemiol 2004;57:1288-94.
16. Wang MY, Green BA, Shah S, Vanni S, Levi AD. Complications associated with lumbar stenosis surgery in patients older than 75 years of age. Neurosurg Focus 2003;14:e7.
17. Arrigo RT, Kalanithi P, Cheng I, Alamin T, Carragee EJ, Mindea SA, et al. Charlson score is a robust predictor of 30-day complications following spinal metastasis surgery. Spine (Phila Pa 1976) 2011;36:E1274-80.
18. Afolabi HA, Zakariya Zb, Ahmed Shokri AB, Mohammad Hasim MNB, Vinayak R, Afolabi-Owolabi OT, et al. The relationship between obesity and other medical comorbidities. Obesity Medicine 2020;17:100164.
19. Khaodhiar L, McCowen KC, Blackburn GL. Obesity and its comorbid conditions. Clin Cornerstone 1999;2:17-31.
20. Anderson JW, Konz EC. Obesity and disease management: effects of weight loss on comorbid conditions. Obes Res 2001;9 Suppl 4:326S-34S.
21. Bamgbade OA, Rutter TW, Nafiu OO, Dorje P. Postoperative complications in obese and nonobese patients. World J Surg 2007;31:556-60; discussion 561.
22. Tjeertes EK, Hoeks SE, Beks SB, Valentijn TM, Hoofwijk AG, Stolker RJ. Obesity--a risk factor for postoperative complications in general surgery? BMC Anesthesiol 2015;15:112.
23. Mullen JT, Moorman DW, Davenport DL. The obesity paradox: body mass index and outcomes in patients undergoing nonbariatric general surgery. Ann Surg 2009;250:166-72.
24. Valentijn TM, Galal W, Hoeks SE, van Gestel YR, Verhagen HJ, Stolker RJ. Impact of obesity on postoperative and long-term outcomes in a general surgery population: a retrospective cohort study. World J Surg 2013;37:2561-8.
25. Maradit Kremers H, Visscher SL, Kremers WK, Naessens JM, Lewallen DG. Obesity increases length of stay and direct medical costs in total hip arthroplasty. Clin Orthop Relat Res 2014;472:1232-9.
26. Bradley BM, Griffiths SN, Stewart KJ, Higgins GA, Hockings M, Isaac DL. The effect of obesity and increasing age on operative time and length of stay in primary hip and knee arthroplasty. J Arthroplasty 2014;29:1906-10.
27. Terada T, Johnson JA, Norris C, Padwal R, Qiu W, Sharma AM, et al. Severe Obesity Is Associated With Increased Risk of Early Complications and Extended Length of Stay Following Coronary Artery Bypass Grafting Surgery. J Am Heart Assoc 2016;5:e003282.
28. Poelemeijer YQM, Lijftogt N, Detering R, Fiocco M, Tollenaar RAEM, Wouters MWJM. Obesity as a determinant of perioperative and postoperative outcome in patients following colorectal cancer surgery: A population-based study (2009-2016). Eur J Surg Oncol 2018;44:1849-57.
29. Shinonara K, Ugawa R, Arataki S, Nakahara S, Takeuchi K. Charlson comorbidity index is predictive of postoperative clinical outcome after single-level posterior lumbar interbody fusion surgery. J Orthop Surg Res 2021;16:235.
30. Cannata F, Laudisio A, Ambrosio L, Vadala G, Russo F, Zampogna B, et al. The Association of Body Mass Index with Surgical Time Is Mediated by Comorbidity in Patients Undergoing Total Hip Arthroplasty. J Clin Med 2021;10:5600.
31. Nuttall M, van der Meulen J, Emberton M. Charlson scores based on ICD-10 administrative data were valid in assessing comorbidity in patients undergoing urological cancer surgery. J Clin Epidemiol 2006;59:265-73.
32. Meng X, Press B, Renson A, Wysock JS, Taneja SS, Huang WC, et al. Discriminative Ability of Commonly Used Indexes to Predict Adverse Outcomes After Radical Cystectomy: Comparison of Demographic Data, American Society of Anesthesiologists, Modified Charlson Comorbidity Index, and Modified Frailty Index. Clin Genitourin Cancer 2018;16:e843-50.
33. Hinton ZW, Fletcher AN, Ryan SP, Wu CJ, Bolognesi MP, Seyler TM. Body Mass Index, American Society of Anesthesiologists Score, and Elixhauser Comorbidity Index Predict Cost and Delay of Care During Total Knee Arthroplasty. J Arthroplasty 2021;36:1621-5.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:225-229
DOI: 10.4274/BMJ.galenos.2022.2022.3-17

Research

Evaluation of 846 Liver Transplant Patients Infected with COVID-19 in Turkey

Türkiye'de COVID-19 ile Enfekte olan 846 Karaciğer Nakilli Hastanın Değerlendirilmesi

Yusuf Yavuz[1], Himmet Durgut[2]

[1]Health Services General Directorate, Department of Health Services Planning, Ankara, Turkey
[2]Health Services General Directorate, Department of Blood and Blood Products, Ankara, Turkey

ABSTRACT

Objective: The clinical course of coronavirus infection in liver transplant patients is not known accurately. The aim of this study was to examine the epidemiological incidence and outcomes of liver transplant patients after coronavirus disease-2019 (COVID-19) infection who have been registered in the data system of the Tissue, Organ Transplant and Dialysis Services Department.

Methods: In this study, which was designed non-interventional, retrospective, and observational; the demographic information, clinical and radiological parameters, lifetime, hospital service and intensive care requirements and length of stay of the patients who were recorded in the information systems of the Ministry of Health, have were examined. A total of 3,426 liver transplant patients who were admitted to the hospital with suspected COVID-19 in Turkey between April 2020 and April 2021 were included in the study.

Results: Between April 2020-April 2021, 3,426 cases of liver transplant who admitted to hospitals with symptoms of COVID-19 infection in Turkey were examined. The ratio of patients diagnosed with COVID-19 infection was 24.69% (846), with a mean age of 52.3%. The 13.48% (462 people) of 3,426 people who had liver transplants were hospitalized. The mean age of the hospitalized patients was 46.6, and the average length of hospital stay was 8.64 days. When the thorax computed tomography scans of 3,426 people with suspected COVID-19 and liver transplant were examined, pneumonia was detected in 344 (10%) people and they were treated as an inpatient. The mean age of the patients with pneumonia was 59 years. The number of liver transplant patients who died was 108 (3.1%), with a mean age of 65 years. The ratio of follow-up in the intensive care unit for organ transplant recipients was 0.32%, and 0.26% of them were intubated patients.

Conclusion: Despite the use of immunosuppressive drugs in patients with liver transplant, the requirement for intensive care and the length of stay in the intensive care unit was found to be low, and the importance of strict follow-up and treatment in such patients was recognized once again.

Keywords: COVID-19, liver transplant, mortality

ÖZ

Amaç: Karaciğer nakli olan hastalarda koronavirüs enfeksiyonunun klinik seyri tam olarak bilinmemektedir. Bu çalışmadaki amaç; Türkiye'de Doku, Organ Nakli ve Diyaliz Hizmetleri Başkanlığı veri sisteminde kayıtlı karaciğer nakilli hastaların koronavirüs hastalığı (COVID-19) enfeksiyonu sonrası epidemiyolojik insidans ve sonuçlarının incelenmesidir.

Gereç ve Yöntem: Girişimsel olmayan retrospektif gözlemsel dizayn edilen bu çalışmada; Hastaların Sağlık Bakanlığı bilgi sistemlerine kaydedilmiş, demografik bilgileri, klinik ve radyolojik parametreleri, yaşam süresi, hastane servis ve yoğun bakım gerekliliği ve kalış süreleri incelenmiştir. Çalışmaya, Nisan 2020-Nisan 2021 tarihleri arasında Türkiye'de COVID-19 şüphesiyle hastaneye başvuran toplam 3.426 karaciğer nakilli kişi dahil edilmiştir.

Bulgular: Nisan 2020-Nisan 2021 tarihleri arasında Türkiye'de COVID-19 enfeksiyonu belirtileriyle hastanelere başvuran karaciğer nakli olmuş 3.426 olgu incelenmiştir. COVID-19 enfeksiyonu tanısı konan hasta oranı %24,69 (846) olup ortalama yaşları 52,3 çıkmıştır. Karaciğer nakilli 3.426 kişinin %13,48'i (462 kişi) hastaneye yatırılmıştır. Hastaneye yatırılan kişilerin ortalama yaşı 46,6 olup hastanede kalış süreleri ortalama 8,64 gün olarak saptanmıştır. 3.426 COVID-19 şüpheli karaciğer nakilli kişinin toraks bilgisayarlı tomografi görüntülemeleri incelendiğinde 344 kişide (%10)

Address for Correspondence: Yusuf Yavuz, Health Services General Directorate, Department of Health Services Planning, Ankara, Turkey
Phone: +90 541 458 17 78 E-mail: dryusufyavuz@hotmail.com ORCID ID: orcid.org/0000-0001-8031-2992

Cite as: Yavuz Y, Durgut H. Evaluation of 846 Liver Transplant Patients Infected with COVID-19 in Turkey. Med J Bakirkoy 2022;18:225-229

Received: 23.03.2022
Accepted: 12.05.2022

pnömoni saptanmış olup, yatarak tedavi altına alınmışlardır. Pnömoni saptanan hastaların yaş ortalaması 59 olarak belirlenmiştir. Karaciğer nakilli hastalarda ölen hasta sayısı 108 (%3,1) olup ortalama yaşları 65 çıkmıştır. Organ nakli olanların yoğun bakım ünitesinde takip oranı %0,32 olup, %0,26'sı entübe hastalardan oluşmaktadır.

Sonuç: Karaciğer nakli olan hastalarda immünosüpresif ilaçlar kullanılmasına rağmen yoğun bakım ihtiyacı ve yoğun bakımda kalış sürelerinin düşük olduğu saptanmış ve bu tür hastalarda sıkı takip ve tedavinin önemi bir kez daha anlaşılmıştır.

Anahtar Kelimeler: COVID-19, karaciğer nakli, mortalite

INTRODUCTION

Coronaviruses (CoV) are a large family of viruses that are frequently seen in society and can cause self-limiting mild infections, such as the common cold, to more serious infections such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). In December 2019, the World Health Organization (WHO) reported cases of pneumonia of unknown etiology in Wuhan, China. In January 2020, WHO first defined the causative agent as a novel coronavirus (2019-nCoV), and later acknowledged the name of the disease as coronavirus disease-2019 (COVID-19). WHO first described the COVID-19 outbreak as an "international public health emergency" and then declared it a pandemic on March 11, after COVID-19 cases were seen in many countries outside China, where the epidemic started. The studies on COVID-19 in our country started in January, and the first COVID-19 case was seen on March 11 (1).

Common manifestations of COVID-19 infection include respiratory symptoms, fever, cough and dyspnea. The symptoms such as headache, sore throat, runny nose, myalgia, arthralgia, asthenia, newly emerging anosmia and ageusia, diarrhea can also be seen. Although the majority of patients can be asymptomatic, in severe cases, pneumonia, severe acute respiratory tract infection, kidney failure, multi-organ failure may develop and death may occur. While the mortality rate was 11% in the SARS epidemic, 35-50% in MERS-CoV, the COVID-19 mortality rate has been reported as 3.8%. In our country, this rate was determined 2.6% as of May 2020 (1,2). However, immunosuppressed patients older than 60 years of age are at higher risk of 2019-nCoV infection and may have prolonged viral clearance (2,3). Liver transplant patients and other solid organ transplant patients are more prone to viral infections such as COVID-19 since their immunity is suppressed due to the use of immunosuppression (4).

It is been known that COVID-19 infection affects the gastrointestinal system less than the respiratory tract; the information on how it affects the liver in the gastrointestinal system is still insufficient. However, it is estimated that the main mechanism of liver damage is due to the binding of the virus to the angiotensin converting enzyme 2 receptor, which is present at a high rate in the bile ducts. It is also considered to affect other vital organs such as the heart, pancreas, kidneys and intestines in a similar manner (5,6).

The aim of this study was to examine the epidemiological incidence and outcomes of liver transplant patients after COVID-19 infection who have been registered in the data system of the Ministry of Health, Tissue, Organ, Transplantation and Dialysis Services Department in Turkey.

METHODS

Our study was designed as non-invasive, retrospective, and observational. The study was conducted using the data registered in the Tissue, Organ Transplantation and Dialysis Services Department of the Ministry of Health. Necessary permissions were obtained from the Ministry of Health for the use of the data. Demographic information, clinical and radiological parameters, life time, hospital service and intensive care requirements of the patients and accordingly, the length of stay was examined. In the study 3,426 people were included who had previously undergone liver transplantation and admitted to public, university and private hospitals in Turkey with the suspected COVID-19 between April 2020 and April 2021. As a result of these tests, 846 liver transplant patients diagnosed with COVID-19 have been evaluated. In the study for the diagnosis of patients considered as COVID-19 those with positive real-time fluorescent real-time polymerase chain reaction (RT-PCR) detection of 2019-nCoV nucleic acid or those with positive serum 2019-nCoV-specific IgM antibodies were acknowledged as infected by COVID-19 infection. It was not considered COVID-19 if two consecutive tests of 2019-nCoV nucleic acid (sampling time at least 24 hours apart) were negative and 7 days later, the IgM/IgG antibodies specific for 2019-nCoV were still negative. 2019-nCoV nucleic acid detection; it was preferred in

nasopharyngeal swabs, sputum, other lower respiratory tract secretions (sputum or airway extracts).

The study was initiated after obtaining the approval of the Ankara City Hospital Non-interventional Clinical Research Ethics Committee with the decision number E2-21-244 (date: 10.03.2021). Our study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments.

Statistical Analysis

Liver transplant patients statistics for each year were recorded on the tabulation software and the frequency changes were calculated using the statistical formulas of the tabulation software. Patient consent was waived because of the study.

RESULTS

Between April 2020 and April 2021, 3,426 liver transplant patients with COVID-19 symptoms or suspected COVID-19 in Turkey were analysed. Among these cases, 1,576 (46%) were female and 1,850 (54%) were male. The mean age of men was 48.3, the mean age of women was 46.6. Among these liver-transplanted cases, 24.69% (846) were diagnosed with COVID-19 infection with positivity of real-time fluorescent RT-PCR detection or serum 2019-nCoV-specific IgM antibodies.

Among the patients diagnosed with COVID-19 infection, 338 (39.9%) were female, 508 (59.5%) were male, with a mean age of 52.3. The 53.9% (456 people) of 846 liver transplant patients diagnosed with COVID-19 infection were hospitalized. The mean age of the patients who were diagnosed with COVID-19 infection and then hospitalized and followed up for the disease was 48.6 years, and 286 (62.2%) of these patients were male and 170 (37.8%) were female. The hospital stay of liver transplant patients was between 3 and 35 days, with an average of 8.64 days.

When the thorax computed tomography (CT) scans of 846 liver transplant patients diagnosed with COVID-19 were examined, pneumonia was detected in 347 (41%) patients and they were treated as inpatients (Figure 1). Among these patients diagnosed with pneumonia, 202 (58.21%) were male and 145 (41.79%) were female. In addition, the mean age of these patients with pneumonia was 58.3 years.

Among the patients diagnosed with COVID-19 infection and hospitalized because of the disease, the number of patients followed up in the intensive care unit was 94 (11.1%). The mean length of stay in the intensive care unit was 7.88 days, and the mean age of these patients was 63 (Figure 2).

Among the 94 patients hospitalized in the intensive care unit, 67 (71%) were intubated. The length of stay in the intensive care unit of these intubated patients was 7.88 days. When all the patients hospitalized in the intensive care unit were examined, there were only 2 patients under the age of 50 and 22 patients over the age of 70.

The number of patients who died in the intensive care unit or in the emergency and service follow-ups was 108, and the mean age of these patients was 64.4. This ratio was 8.1% among patients diagnosed with COVID-19. Among the patients who died, 62 (57.4%) were male and 46 (42.6%) were female.

DISCUSSION

Although data on liver transplant patients are limited, there is a concern that immunosuppressed patients are at a higher risk of morbidity and mortality because of COVID-19 infection. It has been estimated that transplant recipients may have a greater viral load and have more infections (4-7).

While in our country the rate of patients diagnosed with COVID-19 infection in the general population was 8.1%, within the same period the rate of patients diagnosed with COVID-19 infection among the liver transplant patients was found to be 24.69%, which was significantly higher than the general population.

Similar to the study by Colmonero et al. (7) in Spain, there was a homogeneous distribution of cases in the geographical regions. Also, in the same study, although 111 (0.82%) of 13.450 liver transplant patients in Spain were diagnosed with COVID-19, 846 (0.80%) of 10,520 liver transplant patients were diagnosed with COVID-19 in our country (7).

Lung CT is the recommended imaging procedure for diagnosing pneumonia. The imaging findings of COVID-19 are similar to those of other viral pneumonias. In the early stage, interstitial changes with multiple small patchy shadows and prominent extrapulmonary bands are seen. Multiple ground-glass shadows and lung consolidation occur in the progression phase. Compared to the general population, liver transplant recipients have more widespread lung lesions, multiple lesions, and more lower lobe involvement (8).

When the data of the Ministry of Health were examined, the average of COVID-19 pneumonia was 4.8% in the general population, however, within the same period when the thorax CT scans of liver transplant patients were examined, pneumonia was detected as 10%. In the study by Lee et al. (8), although the hospitalization rate of COVID-19 patients with liver transplant was determined as 33%, in our country,

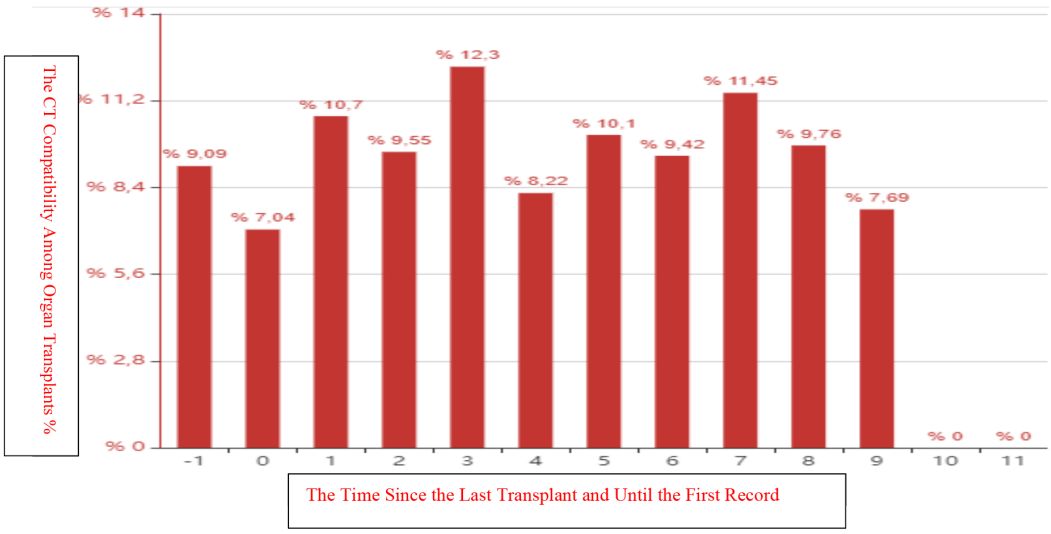

Figure 1. The CT compatibility % among organ transplants. The time since the last transplant and until the first record
CT: Computed tomography

this rate was determined to be higher than 54% because of the strict follow-up of liver transplant patients with COVID-19 infection. While all liver transplant patients diagnosed with COVID-19 in our country were hospitalized or followed up regardless of their symptoms, it has been reported in the literature that the duration and rates of hospitalization are higher in elderly liver transplant patients with COVID-19 (4). While the number of patients who died among liver transplant patients was 108 (3%), the rate of follow-up in the intensive care unit was 0.31%, and the rate of intubated patients was 0.28%, when the data of the Ministry of Health for the same period was considered, the mortality rate of COVID-19 in the general population was 2.6%, the rate of follow-up in intensive care units was 0.78%, and the rate of intubated patients was 0.56% (2-4).

When the published reports on COVID-19 were reviewed, among the risk factors for mortality; while advanced age and male gender were present, immunosuppression is not mentioned and deaths have not been reported to be correlated with other conditions requiring transplantation or immunosuppressive therapy (9,10).

In the study by Lee et al. (8), although it was stated that immunosuppression treatment would make a positive contribution by reducing the inflammatory response, they have mentioned that it may be more mortal in terms of causing hepatitis-related liver degeneration, fungi and susceptible conditions for other infections that will occur over time.

However, in the study by Gavriilidis and Pai (11), it was considered that immunosuppression may be effective for reducing mortality rates, and in accordance with our study,

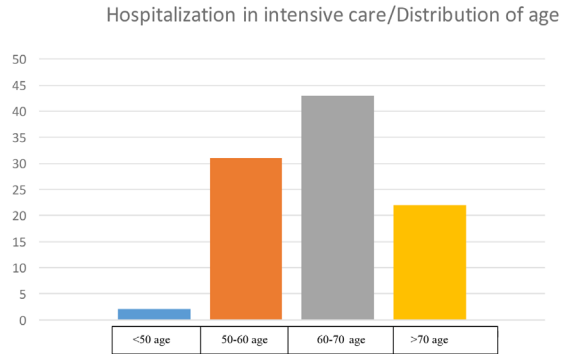

Figure 2. Under the age of 50 years, 50-60 years of age, 50-60 years of age, over the age of 70 years

it was observed that the mortality rate was lower in liver transplant patients compared with the normal population with COVID-19.

Although the susceptibility to infection has increased among liver transplant patients with COVID-19 who were immunosuppressed and under follow-up, as indicated by Choudhury et al. (12), with good care and strict follow-up there was no significant increase in the risk of death compared to the general population.

According to the study by Belli et al. (9), mortality rates in liver transplant patients with COVID-19 were more common in male patients over 60 years of age. In our study, the mean age of 108 deceased patients was 64, and 19 these patients were over 70 years of age, and 14 of them were male. The study by Verma et al. (10) indicated that the incidence of COVID-19 in liver transplant patients was lower than the normal population. In our study and many studies in the literature, among liver transplant patients like other transplant patients COVID-19 infection, COVID-19

pneumonia, and correlated hospitalization rates were higher than the general population in the world and in our country (4,13,14). When the studies on organ transplantation in many countries were examined; although the risk of contracting the disease, COVID-19 pneumonia case rates, and hospitalization rates being higher compared to the general population is a common outcome, the facts that distinguish our study from other studies were less mortality, less need for intensive care and shorter length of stay in the intensive care unit in our country.

CONCLUSION

Although the risk of transmission of COVID-19, PCR positivity and the presence of pneumonia were higher in liver transplant patients compared to the general population, the patients who were followed up and treated regularly in liver transplant centres in our country because they had been kept under control in a hospital environment or had strict follow-up and successful treatment process regardless of their symptoms, it has been observed that the need for intensive care and the length of stay in the intensive care unit in these patients diagnosed with COVID-19 was less compared to the patients in the general population.

As a result, it has been concluded that, if necessary, follow-up is made and precautions are taken this disease despite the use of immunosuppression can be kept under control in liver transplant patients.

Acknowledgments: This study was derived from the corresponding author's dissertation (date: July 2014. "The Evaluation of 846 Liver Transplant Patients Infected with COVID-19 in Turkey")

ETHICS

Ethics Committee Approval: The study was initiated after obtaining the approval of the Ankara City Hospital Non-interventional Clinical Research Ethics Committee with the decision number E2-21-244 (date: 10.03.2021). Our study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments.

Informed Consent: Patient consent was waived because of the study.

Authorship Contributions

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

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. Sağlık bakanlığı, Halk Sağlığı Genel Müdürlüğü COVID-19, SARS-CoV-2 Enfeksiyonu, genel bilgiler, epidemiyoloji ve tanı. Erişim linki: https://covid19.saglik.gov.tr/Eklenti/39551/0/covid-19 rehberi genel bilgiler epidemiyoloji ve tanı pdf.pdf
2. Türkiye Cumhuriyeti Sağlık Bakanlığı COVID-19 Bilgilendirme Platformu. Erişim linki: https://covid19.saglik.gov.tr/ (Erişim tarihi: 18.12.2020)
3. Chai X, Hu L, Zhang Y, Han W, Lu Z, Ke A, et al. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. bioRxi 2020. Doi: 10.1101/2020.02.03.931766
4. Canbaz H, Beştemir A, Surel A, Aydinli B, Yekeler E, Eldegez U, et al. Investigation of emergency service and hospital applications of solid organ transplant patients infected with covid-19 in turkey. Tıbbi Sosyal Hizmet Dergisi 2021;18:67-81.
5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
6. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507-13.
7. Colmenero J, Rodríguez-Perálvarez M, Salcedo M, Arias-Milla A, Muñoz-Serrano A, Graus J, et al. Epidemiological pattern, incidence, and outcomes of COVID-19 in liver transplant patients. J Hepatol 2021;74:148-55.
8. Lee BT, Perumalswami PV, Im GY, Florman S, Schiano TD; COBE Study Group. COVID-19 in Liver Transplant Recipients: An Initial Experience From the US Epicenter. Gastroenterology 2020;159:1176-8.e2.
9. Belli LS, Duvoux C, Karam V, Adam R, Cuervas-Mons V, Pasulo L, et al. COVID-19 in liver transplant recipients: preliminary data from the ELITA/ELTR registry. Lancet Gastroenterol Hepatol 2020;5:724-5.
10. Verma A, Khorsandi SE, Dolcet A, Prachalias A, Suddle A, Heaton N, et al. Low prevalence and disease severity of COVID-19 in post-liver transplant recipients-A single centre experience. Liver Int 2020;40:1972-6.
11. Gavriilidis P, Pai M. The Impact of COVID-19 Global Pandemic on Morbidity and Mortality of Liver Transplant Recipients Children and Adults: A Systematic Review of Case Series. J Clin Med Res 2020;12:404-8.
12. Choudhury A, Reddy GS, Venishetty S, Pamecha V, Shasthry SM, Tomar A, et al. COVID-19 in Liver Transplant Recipients - A Series with Successful Recovery. J Clin Transl Hepatol 2020;8:467-73.
13. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020. DOI: 10.1056/NEJMoa2002032
14. Alberici F, Delbarba E, Manenti C, Econimo L, Valerio F, Pola A, et al. A single center observational study of the clinical characteristics and short-term outcome of 20 kidney transplant patients admitted for SARS-CoV2 pneumonia. Kidney Int 2020;97:1083-8.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:230-237
DOI: 10.4274/BMJ.galenos.2022.2022.3-22

Research

Management of Acute Mastoiditis and Accompanying Complications in Pediatric Patients: Single Center Experience

Pediatrik Hastalarda Akut Mastoidit ve Eşlik Eden Komplikasyonların Yönetimi: Tek Merkez Deneyimi

Mehmet Akif Abakay[1], Hüseyin Avni Ulusoy[2], Mehmet Yiğitbay[1], Pınar Sayın[3], Filiz Gülüstan[1], Nevin Hatipoğlu[4]

[1]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Otolaryngology Head and Neck Surgery, Istanbul, Turkey
[2]Çankırı State Hospital, Clinic of Otolaryngology Head and Neck Surgery, Çankırı, Turkey
[3]University of Health Sciences Turkey, Şişli Etfal Training and Research Hospital, Clinic of Anesthesiology and Reanimation, Istanbul, Turkey
[4]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Pediatric Infection, Istanbul, Turkey

ABSTRACT

Objective: Acute mastoiditis (AM) is the most common complication associated with acute otitis media and plays a key role in the development of further complications. We identify the clinical characteristics, management, and outcome of patients with AM and to investigate the relationship between the development of recurrence and the treatment protocol of the first episode.

Methods: Children hospitalized in our clinic due to a diagnosis of AM were retrospectively reviewed. Demographic data, disease-related symptoms, types of complications accompanied by AM, medical/surgical treatment modalities, culture results, laboratory findings, and presence of recurrence were screened. Laboratory findings were compared between those with and without accompanying complications, and between those with and without accompanying intracranial complications.

Results: Of the 58 patients with AM, 24 (41.3%) had isolated AM. Complications associated with AM include subperiosteal abscess (61.7%), sigmoid sinus thrombosis (11.7%), facial paralysis (8.8%), petrositis with subperiosteal abscess (5.8%), meningitis (5.8%), facial paralysis with subperiosteal abscess (2.9%) and, epidural abscess with sigmoid sinus thrombosis (2.9%). Seven patients developed intracranial complications (12%), of whom one had more than one complication. Hemoglobin and hematocrit levels were found to be significantly lower in the presence of accompanying complications (p values, respectively p=0.000, p=0.000). C-reactive protein (CRP) levels were found to be significantly higher in the presence of intracranial complications (p=0.028). Recurrent AM developed in 9 patients (15.5%) during follow-up. There was no statistically significant relationship between the treatment protocol of the first episode and the development of recurrence (p=0.332).

Conclusion: A conservative approach may be preferred for surgical treatment in patients without accompanying intratemporal or intracranial complications. Low hemoglobin and hematocrit levels, with the symptoms and imaging of the patients, may be a warning of the development of accompanying complications, and high CRP values may be a warning of accompanying intracranial complications.

Keywords: Accompanying complications, mastoiditis, otitis media, recurrence

ÖZ

Amaç: Akut mastoidit (AM), akut otitis media ile ilişkili en sık görülen komplikasyondur ve daha ileri komplikasyonların gelişmesinde anahtar rol oynar. AM'li hastaların klinik özelliklerini, yönetimini ve sonuçlarını incelemeyi ve ayrıca nüks gelişimi ile ilk epizodun tedavi protokolü arasındaki ilişkiyi araştırmayı amaçlıyoruz.

Gereç ve Yöntem: AM tanısı ile kliniğimize yatırılan çocuklar geriye dönük olarak incelendi. Demografik veriler, hastalığa bağlı semptomlar, AM'nin eşlik ettiği komplikasyon türleri, tıbbi/cerrahi tedavi modaliteleri, kültür sonuçları, laboratuvar bulguları ve nüks varlığı tarandı. Laboratuvar bulguları eşlik eden komplikasyonu olanlar ve olmayanlar ile intrakraniyal komplikasyonu olan ve olmayan gruplar arasında karşılaştırıldı.

Address for Correspondence: Filiz Gülüstan, University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Otolaryngology Head and Neck Surgery, Istanbul, Turkey
Phone: +90 212 414 71 71 E-mail: filizyirci@hotmail.com ORCID ID: orcid.org/0000-0003-4857-214X

Cite as: Abakay MA, Ulusoy HA, Yiğitbay M, Sayın P, Gülüstan F, Hatipoğlu N. Management of Acute Mastoiditis and Accompanying Complications in Pediatric Patients: Single Center Experience. Med J Bakirkoy 2022;18:230-237

Received: 04.04.2022
Accepted: 20.05.2022

Bulgular: Elli sekiz hastanın 24'ünde (%41,3) izole AM vardı. AM ile ilişkili komplikasyonlar arasında subperiostal apse (%61,7), sigmoid sinüs trombozu (%11,7), fasiyal paralizi (%8,8), subperiostal apse ile beraber petrosit (%5,8), menenjit (%5,8), subperiostal apse ile beraber yüz felci (%2,9) ve sigmoid sinüs trombozu ile beraber epidural apse (%2,9) yer aldı. Yedi hastada intrakraniyal komplikasyon gelişti (%12) ve bunlardan birinde birden fazla komplikasyon görüldü. Hemoglobin ve hematokrit düzeyleri eşlik eden komplikasyonu olanlarda anlamlı olarak düşük bulundu (p değerleri sırasıyla p=0,000, p=0,000). C-reaktif protein (CRP) düzeyleri intrakraniyal komplikasyon varlığında anlamlı olarak yüksek bulundu (p=0,028). Takipte 9 hastada (%15,5) nüks AM gelişti. İlk epizodun tedavi protokolü ile nüks gelişimi arasında istatistiksel olarak anlamlı bir ilişki saptanmadı (p=0,332).

Sonuç: İntratemporal veya intrakraniyal komplikasyonun eşlik etmediği hastalarda tedavide konservatif cerrahi yaklaşım tercih edilebilir. Düşük hemoglobin ve hematokrit düzeyleri, hastaların semptom ve görüntülemeleri ile birlikte değerlendirildiğinde eşlik eden komplikasyonların varlığı için, yüksek CRP değerleri ise eşlik eden intrakraniyal komplikasyonların varlığı için bir uyarı olabilir.

Anahtar Kelimeler: Eşlik eden komplikasyon, mastoidit, orta kulak iltihabı, nüks

INTRODUCTION

Acute mastoiditis (AM) is the most common complication associated with acute otitis media (AOM), develops when the infection spreads to the mastoid process of the temporal bone and plays a key role in the development of further complications (1,2). In complicated AOM, the purulent infection first causes AM, which leads to further intratemporal and intracranial complications (ICCs), such as sub-periosteal abscess (SPA), facial paralysis (FP), labyrinthitis, petrositis, meningitis, sigmoid sinus thrombosis (SST), and intracranial abscess (2-4). Despite the rational use of antibiotics and advances in imaging and surgical techniques, AM-led complication rates are reported to be between 13% and 38% (5,6). Given the serious nature of intratemporal and but particularly ICCs, their early diagnosis and effective treatment is vital, since delays in their diagnosis and treatment are related to increased morbidity and mortality, and those commonly used antibiotics can suppress the classic signs and symptoms of ICCs (3-7). Temporal bone computed tomography (CT) with intravenous contrast was found to be highly sensitive (97% sensitivity) in evaluating the complications of AOM (4,8). However, the treatment is rather a center-and clinician-dependent; there is still no consensus for managing pediatric AM. Also, management depends on the patient's presentation but may include intravenous antibiotics alone or along with myringotomy and/or ventilation tube (VT) insertion, drainage of the abscess, and cortical mastoidectomy (CM) (2,9,10).

In this study, we aimed to identify the clinical characteristics, management, and outcome of patients with AM, and to investigate differences between laboratory findings between those with and without accompanying complications. Also, we determine the clinical characteristics of patients with recurrent AM, to investigate the relationship between the development of recurrence and the treatment protocol used in the first episode.

METHODS

A retrospective study was conducted at our tertiary referral hospital between January 2014 and June 2020 over six years. The study protocol was approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital's local ethics board (decision no: 2020-18-07, date: 07.09.2020). The medical charts of children hospitalized in our Otorhinolaryngology clinic due to AM were retrospectively reviewed. The diagnosis of AM is based on clinical signs of AOM (ongoing or within 14 days) associated with retro-auricular signs of infection: swelling, erythema, tenderness, protrusion of the auricle, and decrease in the posterior upper wall of the external ear canal. Also, the diagnosis of AM was confirmed by imaging methods, CT scan of the temporal bone with fine cuts in all patients, and magnetic resonance imaging testing for patients with suspected ICCs. The demographic data of the patients, disease-related symptoms, complications accompanied by AM, the month of application, laboratory data [white blood cell count (WBC), C-reactive protein (CRP), red blood cell count, hematocrit (Hct), neutrophil count, lymphocyte count, neutrophil-to-lymphocyte ratio, microbiological cultures], length of hospital stay, medical and/or surgical treatments, and outcome were recorded.

Children with immunodeficiencies or craniofacial malformations and children presenting with AM found to be because of underlying chronic otitis media with/without cholesteatoma were excluded from the study.

On admission, we used sterile swab sticks to collect a specimen of the ear discharge. In cases of surgical intervention, we collected the pus during surgery. All patients received empiric intravenous antibiotics shortly after the admission and supportive therapy if needed. When there was no response to iv antibiotics for 24/48 hours surgical intervention was performed in patients with no accompanying complications and performed in all patients in the presence of accompanying complications (Figure 1). Medical treatment was performed in all patients collaboration with the pediatrics department. If there was suspicion of ICCs, a multidisciplinary approach was used for

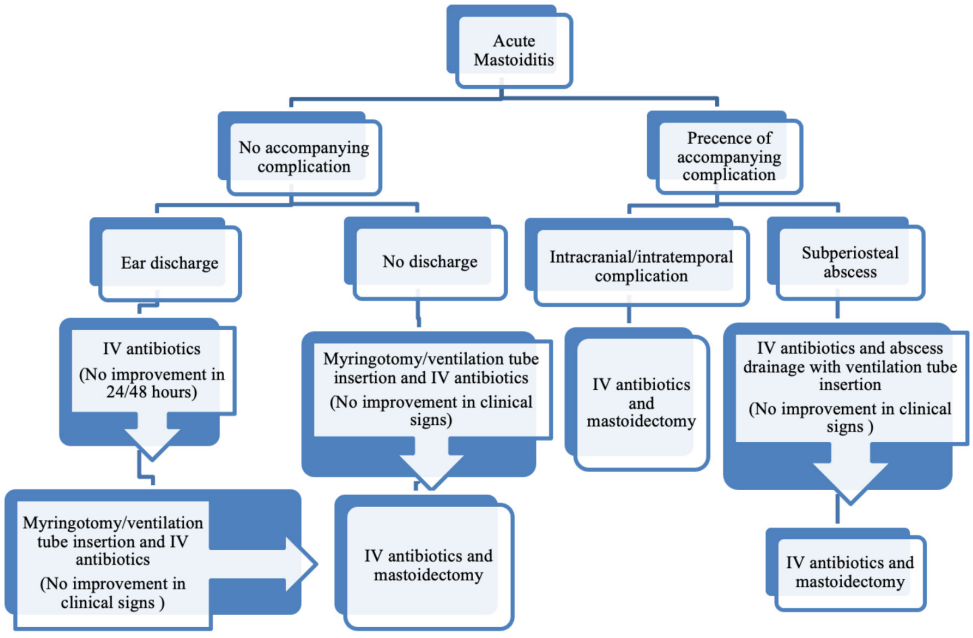

Figure 1. Management algorithm of acute mastoiditis in children

diagnosis and management, and the patient was transferred to the pediatrics department if needed.

Recurrent AM was defined as an episode of AM over 4 weeks after a previous episode and in the absence of symptoms or findings to suggest persistent infection between episodes (11). Records were surveyed for readmission or treatment for recurrent AM in the affected ear.

Statistical Analysis

NCSS (Number Cruncher Statistical System, Kaysvilee, UT) 2007 software was used for the statistical analysis. Descriptive statistical analyzes were performed. Mean values were compared with Mann-Whitney U test. The relationship between recurrence and age, surgery type, accompanying complications, and ICCs were analyzed with Fisher's Exact test. In all analyses, $p<0.05$ was considered to indicate statistical significance.

RESULTS

Over 6 years, 58 patients were hospitalized in our clinic due to AM. Of these 58 patients, 25 were female (43.1%) and 33 were male (56.9%). The mean age was 4.91±3.97 (0-16) years. There were 7 patients under one year of age (the youngest was 2 months old and the oldest was 9 months old). When calculating the mean age, the ages of these patients were accepted as 0 years. The demographic data and the age distribution of the patients are shown in Table 1. The average length of stay in the hospital was 11.5±8.86 (3-41) days. Most of the patient admissions were in the winter months.

When the complaints at the admission of the patients were evaluated, the most common symptoms were found to be otalgia, retro-auricular swelling-hyperemia, protruding of the auricle, and otorrhea. 34 (58.6%) out of 58 patients, had complications [SPA, SST, FP, petrositis, meningitis, and epidural abcess (EA)] accompanied by AM. There was more than one accompanying complication in 4 patients (SPA and petrositis in two patients, SPA and FP in one patient, SST and EA in one patient). SPA was the most common complication (n=24, 41.3%). 7 patients (12%) had ICCs, of whom one had multiple concurrent ICCs. The most common ICC was SST (n=5, 8.6%), followed by meningitis (n=2, 3.4%). Of the four patients with FP, one was Hause-Brackmann grade 4, two were grade 3, and one was grade 2.

Table 1. The demographic data and the age distribution of the patients

		n (%)
Gender	Male	33 (56.9%)
	Female	25 (43.1%)
Side	Right	32 (55.2%)
	Left	26 (44.8%)
Age (years)	<1	7 (12%)
	1-4	24 (41.4%)
	4-8	18 (31%)
	>8	9 (15.6%)

At admission, an empiric intravenous antibiotic treatment was administered to all patients after taking samples for culture if there was ear discharge. Samples for culture were taken during the surgical intervention from those who had no ear discharge at admission. According to the clinical condition of the patient, as a first-line antibiotic treatment, we used ceftriaxone (50-100 mg/kg) as monotherapy in 22 patients (37.9%), along with clindamycin (15-40 mg/kg) or metronidazole (20-40 mg/kg) in 24 patients (41.3%). Vancomycin (60 mg/kg) or meropenem (120 mg/kg) was preferred in certain patients depending on clinical symptoms or culture results in the line with pediatric infectious disease department recommendations. Intravenous corticosteroids (1 mg/kg prednisolone) were administered to patients with accompanying FP. All patients who initially presented with FP had fully recovered. For managing the five SST patients, one was placed on anticoagulation therapy for 45 days. During the follow-up, minor bruising was noted for this patient and no other bleeding concerns were reported. Of the four patients who were not anticoagulated, two had a nonocclusive thrombus and two had no progression of their thrombus on imaging studies during their hospital stay. No patient died during the hospital stay or follow-up.

When considering surgical management, a conservative

Table 2. Symptoms at admission, type, and management of accompanying complications and microbiological profile of patients with acute mastoiditis

		n (%)
Symptoms at admission	Otalgia	36 (62%)
	Retroauricular swelling-hyperemia	25 (43.1%)
	Protruding of the auricle	21 (36.2%)
	Otorrhea	21 (36.2%)
	Headache	19 (32.7%)
	Fever	17 (29.3%)
	Irritability	9 (15.5%)
	Vomiting	4 (6.8%)
	Facial paralysis	4 (6.8%)
	Neck stiffness	1 (1.7%)
Accompanying complications (n=34)	Subperiosteal abscess	21 (61.7%)
	Subperiosteal abscess + petrositis	2 (5.8%)
	Subperiosteal abscess + facial paralysis	1 (2.9%)
	Facial paralysis	3 (8.8%)
	Sigmoid sinüs thrombosis	4 (11.7%)
	Sigmoid sinüs thrombosis + epidural abscess	1 (2.9%)
	Meningitis	2 (5.8%)
Treatment protocol	Medical	16 (27.5%)
	Medical + VT	8 (13.7%)
	Medical + drainage + VT	16 (27.5%)
	Medical + CM + VT	18 (31%)
Microbiology	Negative	11 (26.8%)
	Streptococcus pneumoniae	17 (41.4%)
	Pseudomonas aeruginosa	5 (12.1%)
	Streptococcus pyogenes	5 (12.1%)
	Enterococcus species	2 (4.8%)
	Escherichia coli	1 (2.4%)

VT: Ventilation tube, CM: Cortical mastoidectomy

Table 3. The clinical characteristics of patients with recurrent AM

	Age at the first episode	Gender	Accompanying complications in the first episode	Culture results of the first episode	Treatment modalities of the first episode
Patient 1	5 month	F	SPA	*Streptococcus pneumoniae*	Medical + drainage + VT
Patient 2	6 years	M	SST	No growth	Medical + CM + VT
Patient 3	4 years	M	None	*Streptococcus pneumoniae*	Medical
Patient 4	7 years	M	SPA + petrositis	*Streptococcus pneumoniae*	Medical + CM + VT
Patient 5	3 years	F	SPA	No growth	Medical + drainage + VT
Patient 6	5 years	F	FP	No growth	Medical + CM + VT
Patient 7	9 years	F	None	No growth	Medical + VT
Patient 8	4 years	F	None	No growth	Medical + VT
Patient 9	6 years	M	None	No growth	Medical

SPA: Subperiosteal abcess, SST: Sigmoid sinus thrombosis, FP: Facial paralysis, VT: Ventilation tube, CM: Cortical mastoidectomy, AM: Acute mastoiditis

Table 4. The comparison of blood parameters in patients with and without accompanying complications and with and without ICCs

	Accompanying complication (n=34) (mean ± SD)	No accompanying complication (n=24) (mean ± SD)	p-value	ICCs (n=7) (mean ± SD)	No ICCs (n=51) (mean ± SD)	p-value
Hb (g/dL)	10.72±1.31	13.04±1.40	**0.000**	10.60±0.57	11.8±1.82	0.081
Hct (%)	33.09±4.03	39.31±3.74	**0.000**	33.05±2.23	36.02±5.14	0.090
WBC (10^3/IU)	15.44±6.16	10.79±4.29	**0.004**	12.02±4.02	13.72±6.11	0.527
NLR	2.65±2.11	3.34±3.21	0.528	4.47±3.14	2.72±2.50	0.185
CRP (mg/L)	37.7±37.0	24.1±28.6	0.114	66.4±60.6	25.4±25.8	**0.028**

n: Number, SD: Standard deviation, ICC: Intracranial complication, Hb: Hemoglobin, Hct: Hematocrit, WBC: White blood cell count, NLR: Neutrophil to lymphocyte ratio, CRP: C-reactive protein (Bold text indicates a statistically significant difference with a p-value less than 0.05)

approach was adopted in patients without ICCs. Sixteen patients (27.5%) were treated without surgical intervention. Eight patients (13.7%) underwent myringotomy + VT insertion. Twenty one (87.5%) of 24 patients with a SPA underwent incision and drainage of the abscess + VT insertion, and later 5 (23.8%) of these 21 patients underwent CM + VT insertion, during the same admission, due of lack of clinical improvement. This was performed on average 4 days after the original surgery. The remaining three patients with SPA underwent CM + VT insertion. Of these three patients, two had concomitant petrositis and one had FP. Ten of the 18 patients who underwent CM + VT insertion, three had FP, two had meningitis, four had SST, one had SST + EA.

Culture results were not available for 17 (29.3%) patients. Of the remaining 41 patients (70.6%) no growth was observed in 11 (26.8%), *Streptococcus pneumoniae* was isolated in 17 (41.4%), and the other bacteria in 13 (31.7%) patients. The symptoms at admission, type and management of accompanying complications, and microbiological profile of the patients are shown in Table 2.

Recurrent AM developed in 9 patients (15.5%) during follow-up. The time from the first AM to the second episode ranged from 6 weeks to two years. The clinical characteristics of patients with recurrent AM are shown in Table 3. There was no statistically significant difference in terms of recurrence between those with and without accompanying complications and those with and without ICCs (Fisher's Exact test, p=0.555, p=0.70). Also, there was no statistically significant relationship between the treatment protocol of the first episode and the development of recurrence (Fisher's Exact test, p=0.332).

Patients with and without complications accompanying AM were compared in terms of blood parameters, hemoglobin and Hct levels were found to be significantly lower (p values, respectively p=0.000, p=0.000), WBC levels were found to be significantly higher in the presence of accompanying complications (p=0.004). When patients with and without

ICCs were compared in terms of blood parameters, the CRP levels were found to be significantly higher in the presence of ICCs (p=0.028). The comparison of blood parameters in patients with and without accompanying complications and with and without ICCs is shown in Table 4.

DISCUSSION

In this study, we reported the clinical characteristics, management, and outcome of our patients with AM and compared blood parameters with and without accompanying complications and those with and without ICCs in children attending a large tertiary referral care center, covering a population of two million people.

Similar to the work of Ren et al. (11), we found, as expected, the highest rates of AM-related hospitalizations were highest in winter and lowest in summer, reflecting the seasonal variation of viral upper airway infections closely related to the development of AOM. In the line with previous studies, the most common presenting symptoms were otalgia, retro-auricular swelling-hyperemia, and protruding of the auricle (2,4). Only 17 patients (29.3%) had fever at admission. Although it is thought that this may have been due to the antibiotic treatment they may receive before, previous treatment data were not available for children in our study.

In our study, isolated AM was seen in 24 (41.3%) patients. The most common accompanying complication was SPA (n=24, 41.3%), followed by SST (n=5, 8.6%) and FP (n=4, 6.8%). ICCs were seen in 7 (12%) patients. Duygu and Şevik Eliçora (2) reported the rate of SPA as 28.6%, SST as 7.1%, and FP as 25% in complicated pediatric AM cases. Inconsistent with our study, Mattos et al. (4) reported the rate of SPA as 38%, SST as 8.3%, and FP as 16.7% in complicated pediatric AM cases. Mansour et al. (3) stated that although AM rates decreased with the introduction of antibiotics, the rate of ICCs due to AM remained high, ranging from 5% to 29%, and they reported the ICC development rate as 13% in their study. Similarly, we found the rate of ICC development due to AM to be 12%.

Although studies have described various treatment algorithms in the literature, no consensus has been reached yet in the treatment of AM and accompanying complications (2,5,12-14). Mierzwiński et al. (12) stated that incision and drainage of SPAs have disadvantages like poor cooperation and difficult daily wound toilet in children, it may require multiple episodes of general anesthesia and the inability to expose other existing complications of AM, such as EA which can be detected and immediately treated during mastoidectomy. They reported that they recommended mastoidectomy also in SPAs, performed mastoidectomy in 77% of the patients and a recurrence rate of 8% in their series of 73 cases. Psarommatis et al. (13) reported that nine out of 21 (42.9%) patients who had the first incision and drainage of a SPA needed mastoidectomy as they did not improve over time. Ghadersohi et al. (14) stated that favorable outcomes for children treated with otherwise uncomplicated AM with SPA who are managed with incision and drainage, myringotomy ± tympanostomy tube placement, and intravenous antibiotics. They reported that they reserved mastoidectomy to treat patients with ICCs and certain intratemporal complications, such as acute petrositis and labyrinthitis. However, recurrent AM rates were not available in their series of 48 cases. Duygu and Şevik Eliçora (2) stated that they performed a mastoidectomy if progression occurs despite a perforated tympanic membrane, or severe clinical presentation with multiple complications together with ICCs. In our practice, we performed a stepwise approach to AM and its accompanying complications. In isolated AM cases, if the tympanic membrane is perforated and pus drainage is sufficient, we only administered intravenous antibiotic therapy, if the tympanic membrane is not perforated or pus drainage is not sufficient from perforation, we performed myringotomy and VT insertion together with intravenous antibiotics. None of the patients with isolated AM required additional intervention. All the patients in our study with a SPA, without other complications, underwent myringotomy + VT insertion and incision and drainage of the abscess together with intravenous antibiotics. Due to the lack of clinical improvement, five (23.8%) of these patients required CM during the same admission. There were three patients with SPA who underwent CM + VT insertion in the first-line, because of the co-occurrence of a petrositis in two patients and FP in one patient. Also the patients with accompanying FP (n=3, 8.8%), meningitis (n=2, 5.8%), SST (n=4, 11.7%) and SST + EA (n=1, 2.9%) were managed with CM + VT insertion together with intravenous antibiotics in the first-line treatment. To the best of our knowledge, there are only a few studies in the literature that have focused on recurrent AM cases. Recurrent AM developed in 9 patients (15.5%) during the follow-up in our series of 58 cases. We performed the same stepwise management approach in the recurrent cases and we did not find a statistically significant relationship between the treatment protocol of the first episode and the development of recurrence. Based on these data and in the absence of formal national guidelines, we believe that our treatment protocol is sufficient for children with presenting AM.

Information on bacterial cultures was available for 41

(70.6%) patients and more than a quarter of these patients revealed 'no growth' samples. This may relate to previous treatments that they may have received and could reduce the probability of recovering causative bacteria. However, previous treatment data were not available in our study and the lack of this information represents one of our study's limitations. In the current opinion, *Streptococcus pneumoniae* is considered the predominant pathogen in children affected by AM (15). *Streptococcus pneumoniae* were the predominant pathogen in our study and this finding supports the current opinion.

Golz et al. (16) reported in their study that children with anemia had more otitis media attacks compared with healthy children with normal hemoglobin levels, and they also found a direct relationship between anemia and the number of AOM episodes. Although it varies according to gender and race in children, a hemoglobin level below 10.5 g/dL between 6 months and 2 years of age and below 11.5 g/dL between 2 years and 12 years of age is considered anemia (17). Based on this information, when we analyzed our data, we found statistically significant differences in laboratory findings between those with and without complications accompanying AM at the time of admission. As shown in Table 4, we found that mean Hb and Hct levels in the presence of accompanying complications were 10.72±1.31 g/dL and 33.09±4.03% and we found these values were significantly lower and WBC levels were significantly higher in the presence of accompanying complications. Mansour et al. (3) with the object of identifying those AM patients who are at a high risk of developing ICC, thus supporting their immediate brain imaging upon AM diagnosis, compared the clinical information available at the time of AM diagnosis in patients who developed and those who did not develop imaging-diagnosed ICCs and they reported that high CRP levels increased the risk of imaging-diagnosed ICCs. Similarly, Duygu and Şevik Eliçora (2) compared CRP values between the AM patients with and without ICCs and they reported that they found CRP values significantly higher in patients with accompanying ICCs. We also found CRP levels were significantly higher in the presence of ICCs.

The major limitation of our study is its retrospective nature. Due to its retrospective nature, we didn't have previous treatment and vaccination information data. Although pneumococcal vaccination was introduced in our country in 2009, the predominant pathogen was found to be *Streptococcus pneumoniae* in our study. To explain this predominance and influence of vaccination on accompanying complications, we think that large case series including previous vaccination data are needed.

CONCLUSION

AM is the most common complication associated with AOM in children. The most common symptoms in the children with AM were otalgia, retro-auricular swelling-hyperemia, protruding of the auricle, and otorrhea. Since no statistically significant relationship was found between the treatment protocol of the first episode and the development of recurrence, a conservative approach may be preferred for surgical treatment in patients without accompanying intratemporal or ICCs. Low hemoglobin and Hct levels with the symptoms and imaging of the patients may be a warning for developing accompanying complications, and high CRP values may be a warning for accompanying ICCs.

ETHICS

Ethics Committee Approval: The study protocol was approved by the Bakırköy Dr. Sadi Konuk Training and Research Hospital's local ethics board (decision no: 2020-18-07, date: 07.09.2020).

Informed Consent: Retrospective study.

Authorship Contributions

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

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. Balsamo C, Biagi C, Mancini M, Corsini I, Bergamaschi R, Lanari M. Acute mastoiditis in an Italian pediatric tertiary medical center: a 15 - year retrospective study. Ital J Pediatr 2018;44:71.
2. Duygu E, Şevik Eliçora S. Our experience on the management of acute mastoiditis in pediatric acute otitis media patients. Int J Pediatr Otorhinolaryngol 2020;138:110372.
3. Mansour T, Yehudai N, Tobia A, Shihada R, Brodsky A, Khnifies R, et al. Acute mastoiditis: 20 years of experience with a uniform management protocol. Int J Pediatr Otorhinolaryngol 2019;125:187-91.
4. Mattos JL, Colman KL, Casselbrant ML, Chi DH. Intratemporal and intracranial complications of acute otitis media in a pediatric population. Int J Pediatr Otorhinolaryngol 2014;78:2161-4.
5. Krishnan M, Walijee H, Jesurasa A, De S, Sinha A, Sharma R, et al. linical outcomes of intracranial complications secondary to acute mastoiditis: The Alder Hey experience. Int J Pediatr Otorhinolaryngol 2020;128:109675.

6. Pang LH, Barakate MS, Havas TE. Mastoiditis in a paediatric population: a review of 11 years experience in management. Int J Pediatr Otorhinolaryngol 2009;73:1520-4.
7. Zevallos JP, Vrabec JT, Williamson RA, Giannoni C, Larrier D, Sulek M, et al. Advanced pediatric mastoiditis with and without intracranial complications. Laryngoscope 2009;119:1610-5.
8. Thompson PL, Gilbert RE, Long PF, Saxena S, Sharland M, Wong IC. Effect of antibiotics for otitis media on mastoiditis in children: a retrospective cohort study using the United kingdom general practice research database. Pediatrics 2009;123:424-30.
9. Schwam ZG, Ferrandino R, Kaul VZ, Omorogbe A, Bu D, Faddoul DG, et al. The National Landscape of Acute Mastoiditis: Analysis of the Nationwide Readmissions Database. Otol Neurotol 2020;41:1084-93.
10. Stern Shavit S, Raveh E, Levi L, Sokolov M, Ulanovski D. Surgical intervention for acute mastoiditis: 10 years experience in a tertiary children hospital. Eur Arch Otorhinolaryngol 2019;276:3051-6.
11. Ren Y, Sethi RKV, Stankovic KM. Acute otitis media and associated complications in united states emergency departments. Otol Neurotol 2018;39:1005-11.
12. Mierzwiński J, Tyra J, Haber K, Drela M, Sinkiewicz A, Puricelli MD. Pediatric recurrent acute mastoiditis: Risk factors and insights into pathogenesis. Int J Pediatr Otorhinolaryngol 2018;111:142-8.
13. Psarommatis IM, Voudouris C, Douros K, Giannakopoulos P, Bairamis T, Carabinos C. Algorithmic management of pediatric acute mastoiditis. Int J Pediatr Otorhinolaryngol 2012;76:791-6.
14. Ghadersohi S, Young NM, Smith-Bronstein V, Hoff S, Billings KR. Management of acute complicated mastoiditis at an urban, tertiary care pediatric hospital. Laryngoscope 2017;127:2321-7.
15. Cassano P, Ciprandi G, Passali D. Acute mastoiditis in children. Acta Biomed 2020;91:54-9.
16. Golz A, Netzer A, Goldenberg D, Westerman ST, Westerman LM, Joachims HZ. The association between iron-deficiency anemia and recurrent acute otitis media. Am J Otolaryngol 2001;22:391-4.
17. Short MW, Domagalski JE. Iron deficiency anemia: evaluation and management. Am Fam Physician 2013;87:98-104.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:238-246
DOI: 10.4274/BMJ.galenos.2022.2021.12-15

Research

Comparison of Anemic and Non-anemic Iron-deficient Adolescents in Terms of Psychosocial Aspects and Quality of Life: A Case Control Study

Demir Eksikliği Olup Anemik Olan ve Olmayan Ergenlerin Psikososyal Durumlarının ve Yaşam Kalitelerinin Karşılaştırılması: Bir Olgu Kontrol Çalışması

Uğur Tekin[1], Sultan Aydın Köker[2], Halit Necmi Uçar[3]

[1]University of Health Sciences Turkey, Istanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Child and Adolescent Psychiatry, Istanbul, Turkey
[2]University of Health Sciences Turkey, Antalya Training and Research Hospital, Clinic of Child Hematology and Oncology, Antalya, Turkey
[3]Selçuk University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Konya, Turkey

ABSTRACT

Objective: In this study, anxiety levels, emotional and behavioral problems, self-esteem and quality of life (QoL) in adolescents with iron deficiency (ID) and iron deficiency anemia (IDA) were investigated.

Methods: The sample of this cross-sectional case-control study consisted of 115 adolescents (34 ID, 44 IDA and 37 healthy controls) aged 12–17 years. Strengths and Difficulties Questionnaire, Rosenberg Self-esteem Scale, The Pediatric QoL Inventory, Screen for Child Anxiety-Related Emotional Disorders were used for psychosocial assessment.

Results: The anxiety levels of patients with IDA were higher compared with healthy controls. The total, physical and psychosocial QoL scores of the adolescents with IDA was found to be lower than those with ID or healthy controls. The total iron binding capacity was correlated with total and psychosocial QoL, self-esteem, and anxiety scores.

Conclusion: Findings suggest that adolescents with IDA are affected in terms of anxiety and QoL. A psychosocial evaluation of adolescents with IDA would be appropriate.

Keywords: Quality of life, anxiety, adolescents, iron deficiency, anemia

ÖZ

Amaç: Bu çalışmada demir eksikliği (DE) ya da demir eksikliği anemisi (DEA) olan ergenlerin kaygı düzeyleri, duygusal ve davranışsal sorunlar, benlik saygısı ve yaşam kalitesi (YK) araştırıldı.

Gereç ve Yöntem: Bu kesitsel olgu-kontrol çalışmasının örneklemini 12-17 yaşları arasındaki 115 ergenden (34 DE, 44 DEA ve 37 sağlıklı kontrol) oluşturmuştur. Psikososyal değerlendirme için Güçler ve Güçlükler Anketi, Rosenberg Benlik Saygısı Ölçeği, Pediatrik Yaşam Kalitesi Envanteri, Çocukluk Çağı Anksiyete Tarama Ölçeği kullanıldı.

Bulgular: DEA olan hastaların anksiyete düzeyleri sağlıklı kontrollere göre daha yüksekti. DEA olan ergenlerin toplam, fiziksel ve psikososyal YK puanları, DE olanlara ve sağlıklı kontrollere göre daha düşük bulundu. Total demir bağlama kapasitesi, toplam ve psikososyal yaşam kalitesi, benlik saygısı ve kaygı puanları ile korele idi.

Sonuç: Bulgular, DEA olan ergenlerin kaygı ve yaşam kalitesi açısından etkilendiğini göstermektedir. DEA olan ergenlerin psikososyal yönden değerlendirilmesi uygun olacaktır.

Anahtar Kelimeler: Yaşam kalitesi, anksiyete, ergenler, demir eksikliği, anemi

Address for Correspondence: Uğur Tekin, University of Health Sciences Turkey, Istanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Child and Adolescent Psychiatry, Istanbul, Turkey
Phone: +90 543 450 52 30 E-mail: drutekin@hotmail.com ORCID ID: orcid.org/0000-0001-6720-4292

Cite as: Tekin U, Aydın Köker S, Uçar HN. Comparison of Anemic and Non-anemic Iron-deficient Adolescents in Terms of Psychosocial Aspects and Quality of Life: A Case Control Study. Med J Bakirkoy 2022;18:238-246

Received: 27.12.2021
Accepted: 23.05.2022

INTRODUCTION

Iron is an essential element that has many functions throughout the body and in the central nervous system, including myelination, cell development and neurotransmitter systems (1,2). Therefore, iron is of great importance in cognitive, behavioral and motor development and functions (3,4). Iron deficiency anemia (IDA) and iron deficiency (ID) are among the leading public health problems in children and adolescents in both developed and developing countries (5,6).

In clinical studies, it has been reported that ID causes social, academic and emotional problems (1). There is consistent evidence on the effects of IDA on neurocognitive development (7). However, there is limited information about psychosocial problems in iron-deficient adolescents with or without anemia. To date, studies have reported that children and adolescents with ID or IDA are at high risk of several psychiatric disorders such as major depression, bipolar disorder, anxiety disorder, autism spectrum disorder, and attention deficiency and hyperactivity disorder (8). But limited information reveals the difference between ID and IDA in adolescents in terms of these increased risks.

Quality of life (QoL) is defined by the World Health Organization as 'individuals' perception of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards and concerns. Health-related QoL can also be defined as the whole of a person's physical health, psychological state, beliefs, social relationships and relationships with the environment (9). Despite the increasing focus on QoL issues and outcomes in the past decades, and ID appearing so common, in this context, the consequences of ID and IDA have not been adequately studied. Moreover, there are limited data on the relationship between QoL and psychosocial status of adolescents with ID or IDA. In the studies conducted to date, there is a study stating that there is no relationship between serum iron levels and psychological distress unless anemia develops, while another study reported that regardless of anemia, no significant relationship was found between ID and QoL (10,11).

Consequently, ID related psychosocial problems, and their effects on life in adolescents are not fully understood yet, and many aspects of this issue still need to be investigated. It is also unclear whether ID and IDA differ from each other in mentioned aspects. So, in this study, we compared anemic and non-anemic iron-deficient adolescent, and healthy controls (HC) in terms of anxiety levels, emotional and behavioral problems, self-esteem and QoL. We hypothesized that there are differences between IDA, ID and HCs in these aspects. Moreover, we examined these psychosocial parameters' correlation with hematological parameters in ID regardless of the presence of anemia.

METHODS

The sample of this single-center cross-sectional case-control study consisted of 115 adolescents (34 ID, 44 IDA and 37 HCs) aged 12-17 years. The ID/IDA groups were collected from the pediatric hematology clinic, and HCs were collected from the adolescents who applied for a general health check-up through an announcement. Ethical consent and approval (Van Training and Research Hospital Clinical Research Ethics Committee- decision no: 2019/19, date: 17.10.2019) of the study, and written informed consent was obtained. A power analysis was performed using G*Power version 3.1 to detect a high effect size (0.40) when α=0.05 for a power of 0.95 using control for effects analysis (ANOVA). Based on these criteria, G*Power recommended a minimum sample size of 102, with 34 in each group.

The inclusion criteria were as follows: (1) to have ID (ferritin value below 30 mg/dL, and/or total iron binding capacity (TIBC) is above 350 ug/dL without anemia) or IDA (hemoglobin value below 11 g/dL for girls, 12 g/dL for boys due to ID); (2) to be between the ages of 12-17; (3) attending formal education and (4) not receiving treatment for ID or IDA. Adolescents with a history of psychiatric or neurological disorders, mental retardation or physical disability, or acute infection-like symptoms were excluded from the study. Adolescents with other hematologic conditions (e.g., thalassemia) were also excluded from the study.

The forms and scales used in the study were as follows:

Sociodemographic data form was used to collect information about age, gender, working and educational status of parents, family structure and monthly income.

Strengths and Difficulties Questionnaire (SDQ): This questionnaire is for screening behavioral and emotional problems in adolescents (12). The parent report form was used and cronbach's alpha score was 0.84 for the Turkish version. It contains 5 subscales and these subscales are as follows: attention deficit and hyperactivity, emotional problems, behavior problems, peer problems and prosocial behavior. The prosocial behaviors subscale measures positive social behavior, while the others measure problematic behavior and difficulties, and the total scores of each constitute a total difficulty score of 0-40.

Rosenberg Self-esteem Scale (RSS): To evaluate self-esteem in children and adolescents, the RSS (13). The 10 items are answered on a 4-point scale ranging from strongly agree to strongly disagree. The scale ranges from 10 to 40, with 40 indicating the highest possible score. Higher scores indicate higher levels of self-esteem. Cronbach's alpha score of this self-report scale was 0.75 for the Turkish version.

The Pediatric Quality of Life Inventory: This is an instrument that evaluates health-related QoL in 2-18-year-old children and adolescents. This questionnaire examines four distinct areas of health-related functioning: physical functioning, emotional functioning, social functioning and school functioning. The latter three scales are combined to determine a broad psychosocial summary score (14). The parent form of the scale was administered. Cronbach's alpha score was 0.84 for the Turkish version.

Screen for Child Anxiety-Related Emotional Disorders (SCARED): This instrument is designed to screen for DSM-IV anxiety disorders in childhood. The SCARED total score, derived by adding the responses of the 41 items, ranges from 0 to 82 (15). Cronbach's alpha score was 0.88 for the Turkish version. SCARED includes five distinct factors: somatic/panic, generalized anxiety, separation anxiety, social anxiety, and school refusal. The self-report form of the scale was used.

Statistical Analysis

The statistical data for the groups were expressed using the mean and standard deviation. For continuous normal distributed variables, the One-Way ANOVA test was used for comparison. Variables not normally distributed were compared using the Kruskal-Wallis Test. Comparison of non-parametric parameters between two groups was performed using the Mann-Whitney U test. Bonferroni-corrected Mann-Whitney U test was used for the Kruskal-Wallis test in multiple comparisons. The statistically significant value was $p<0.017$ in the Bonferroni correction. The Tukey honest significant difference test was used for post-hoc comparisons. Comparison between groups the categorical variables were performed using chi-squared tests. Correlations between variables were analyzed using the Pearson correlation coefficient and Spearman's rank correlation coefficient.

RESULTS

The ID, IDA and control groups were statistically similar in age and gender distribution.

There was no statistically significant difference between the groups in terms of monthly income, parental cohabitation, mother education, father education, mother working and father working status. The sociodemographic characteristics of all sample groups are shown in Table 1.

When the data were evaluated, it was observed that SCARED-Panic/Somatic, SDQ-Prosocial Behaviors scales did not fit into the normal distribution.

All anxiety scores were higher in the IDA group compared to HC, except for the social anxiety subscale. No significant difference was found between IDA and ID, or between ID and HC in the anxiety scores.

In terms of QoL, the IDA group's total, physical and psychosocial QoL scores were found to be statistically significantly lower than both the ID and HC. However, the difference between the ID and HC groups was not statistically significant.

In the SDQ, the SDQ-Emotional Problems score of the IDA group was higher than the HC ($p=0.013$). When self-esteem scores were examined, no statistically significant difference was found between the three groups (Table 2).

In the study, correlations of iron parameters with psychosocial measures, and correlations of QoL with other psychosocial measures were evaluated in combined IDA and ID groups.

Among hematologic parameters, solitarily TIBC showed a correlation with psychometric scores other than the QoL. TIBC showed mild positive correlation with the SCARED total, panic-somatic, separation anxiety, school phobia scores, and a mild negative correlation with self-esteem. TIBC showed a mild negative correlation with the total QoL and psychosocial health QoL. None of the SDQ scores showed a statistically significant correlation with any hematologic parameters.

Hemoglobin levels showed a mild positive correlation with all QoL scores. Serum iron levels and transferrin saturation showed mild positive correlation with physical health QoL scores. Ferritin levels showed a mild positive correlation with the total QoL and physical health QoL (Table 3).

All QoL scores showed moderate positive correlation with self-esteem, and mild, moderate, or strong negative correlations with all SCARED scores other than social anxiety. The social anxiety subscale was correlated just with psychosocial health QoL. Physical health QoL scores showed mild negative correlations with the SDQ total, emotional problems and attention deficit hyperactivity disorder (ADHD) problem scores (Table 4).

DISCUSSION

ID and IDA are common public health problems, and the relationship between ID/IDA and psychosocial and

behavioral problems in adolescence is less researched. This study aimed to evaluate the psychosocial and behavioral status, self-esteem and health-related QoL of adolescents with ID or IDA.

In our study, anxiety levels in the IDA group were higher in all areas except social anxiety compared to healthy controls. The total, physical and psychosocial QoL in the IDA group was found to be lower than that in the ID group and healthy controls. Also, emotional problems in the IDA group were found to be higher than in the healthy controls. The data of the ID and IDA combined group showed that ID parameters have a significant relationship with QoL. Furthermore, TIBC was correlated with self-esteem, and anxiety, in addition to the QoL correlation. There were significant relationships between QoL scores and psychosocial and behavioral scores such as anxiety, self-esteem.

There are few studies have examined the possible relationship between IDA/ID and anxiety. One study showed that severe and chronic ID in infancy increased the risk of anxiety, depression and attention problems (16). In a nationwide study in Taiwan about the relationship between IDA/ID and psychiatric morbidity in children and adolescents, it was found that patients with IDA were at higher risk of anxiety disorders (8). Consistent with the results of these studies, our study showed that anxiety levels of adolescents with IDA were higher than those of HCs. Although there was no statistically significant difference between the anxiety scores of ID and IDA or HCs, it was thought that there might be a possible relationship between the progression of ID to anemia and elevation of anxiety levels. Additionally, the correlation between QoL and anxiety scores in IDA/ID was considered as clinically important in terms of the possible effect of anxiety on patient's perceived health and functionality in ID. However, long-term follow-up studies are needed to establish a causal relationship between IDA/ID and anxiety.

Although ID is common, it is insidious and may not have a significant clinical symptoms, and it is difficult for patients to recognize themselves. IDA may lead to many nonspecific clinical symptoms because of disrupted hemoglobin synthesis due to ID. In our study, it was found that the QoL in adolescents with ID was similar to that of HCs,

Table 1. Sociodemographic variables of groups

		HC (n=37)	ID (n=34)	IDA (n=44)	p-value
		n (%)	n (%)	n (%)	
Gender	Female	29 (78.4)	25 (73.5)	41 (93.2)	0.054
	Male	8 (21.6)	9 (26.5)	3 (6.8)	
Age		15.1±1.72	15.05±1.73	14.79±1.65	0.569
Family income	<1500 TL	23 (62.2)	15 (44.1)	24 (54.5)	0.311
	>1500 TL	14 (37.8)	19 (55.9)	20 (45.5)	
Cohabitation of parents	Married	37 (100)	32 (94.1)	38 (86.4)	0.053
	Divorced	0 (0)	2 (5.9)	6 (13.6)	
Mother's educational level	Never went to school	11 (29.7)	5 (14.7)	9 (20.5)	0.211
	Primary school	16 (43.3)	16 (47.1)	23 (52.3)	
	Middle school	3 (8.1)	9 (26.6)	4 (9.1)	
	High school/university	7 (18.9)	4 (11.8)	8 (18.2)	
Mother's employment status	Unemployed	36 (97.3)	29 (85.3)	41 (93.2)	0.162
	Employed	1 (2.7)	5 (14.7)	3 (6.8)	
Father's educational level	Never went to school/primary school	17 (45.9)	19 (55.9)	30 (68.2)	0.345
	Middle school	7 (18.9)	6 (17.6)	4 (9.1)	
	High school/university	13 (35.1)	9 (26.5)	10 (22.7)	
Father's employment status	Unemployed	9 (24.3)	7 (20.6)	11 (25)	0.850
	Employed	28 (75.7)	27 (79.4)	33 (75)	

HC: Health controls, ID: Iron deficiency group, IDA: Iron deficiency anemia group, TL: Turkish Liras

Table 2. Comparison of SCARED, PedQoL, Self-Esteem and SDQ scores of the groups

	HC (n=37)	ID (n=34)	IDA (n=44)	F/Chi²	Comparisons
	M±SD	M±SD	M±SD	p-value	
SCARED-Total anxiety	15.02±8.91	20.67±11.59	25.34±15.71	F=6.684 p=0.002	IDA>HC (p<0.001)
					IDA=ID (p=0.109)
					ID=HC (p=0.063)
SCARED-Panic/somatic	2.24±1.93	3.97±3.03	5.52±5.52	Chi²=9.7 p=0.008	IDA>HC (p<0.001)
					IDA=ID (p=0.088)
					ID=HC (p=0.069)
SCARED-General anxiety	3.48±3.04	4.52±3.71	6.31±4.71	F=5.354 p=0.006	IDA>HC (p=0.002)
					IDA=ID (p=0.050)
					ID=HC (p=0.269)
SCARED-Separation anxiety	2.81±2.42	4.23±3.25	4.68±3.68	F=3.635 p=0.030	IDA>HC (p=0.010)
					IDA=ID (p=0.542)
					ID=HC (p=0.063)
SCARED-Social anxiety	5.43±3.59	6.32±3.49	6.90±4.05	F=1.564 p=0.214	IDA=HC (p=0.080)
					IDA=ID (p=0.496)
					ID=HC (p=0.320)
SCARED-School phobia	1.05±1.24	1.61±1.63	1.90±1.63	F=3.221 p=0.044	IDA>HC (p=0.013)
					IDA=ID (p=0.404)
					ID=HC (p=0.122)
Total PedQoL	82.10±13.9	75.28±14.10	62.25±21.40	F=13.977 p<0.001	IDA<HC (p<0.001)
					IDA<ID (p=0.001)
					ID=HC (p=0.098)
PedQoL-Physical health	81.67±17.6	71.96±21.43	53.19±26.52	F=16.917 p<0.001	IDA<HC (p<0.001)
					IDA<ID (p<0.001)
					ID=HC (p=0.072)
PedQoL-Psychosocial health	82.34±13.8	77.05±12.71	67.08±20.63	F=8.980 p<0.001	IDA<HC (p<0.001)
					IDA<ID (p=0.009)
					ID=HC (p=0.181)
RSS	27.89±5.79	30.47±5.20	29.34±5.18	F=2.044 p=0.134	IDA=HC (p=0.231)
					IDA=ID (p=0.362)
					ID=HC (p=0.047)*
SDQ-Total score	11.86±5.53	11.14±5.44	12.97±6.46	F=0.964 p=0.384	IDA=HC (p=0.398)
					IDA=ID (p=0.176)
					ID=HC (p=0.609)
SDQ-Emotional problems	2.35±2.21	3.0±2.32	3.75±2.77	F=3.240 p=0.043	IDA>HC (p=0.013)
					IDA=ID (p=0.187)
					ID=HC (p=0.272)

Table 2. Continued

	HC (n=37)	ID (n=34)	IDA (n=44)	F/Chi²	Comparisons
	M±SD	M±SD	M±SD	p-value	
SDQ-Behavioral problems	1.64±1.05	1.73±1.56	2.02±1.70	F=714 p=0.492	IDA=HC (p=0.260)
					IDA=ID (p=0.398)
					ID=HC (p=0.806)
SDQ-ADHD problems	3.67±1.90	3.47±2.20	3.97±2.09	F=594 p=0.554	IDA=HC (p=0.515)
					IDA=ID (p=0.286)
					ID=HC (p=0.677)
SDQ-Peer relationship problems	4.08±1.83	3.08±1.31	3.22±2.05	F=3.329 p=0.039	IDA=HC (p=0.035)*
					IDA=ID (p=0.734)
					ID=HC (p=0.021)*
SDQ-Pro-social behaviors	8.51±1.67	7.67±2.02	7.75±2.63	Chi²=3.16 p=0.164	IDA=HC (p=0.120)
					IDA=ID (p=0.883)
					ID=HC (p=0.110)

HC: Health controls, ID: Iron deficiency group, IDA: Iron deficiency anemia group, SCARED: Screen for Child Anxiety-Related Emotional Disorders, PedsQoL: The Pediatric Quality of Life Inventory, RSS: Rosenberg Self-esteem Scale, SDQ: Strengths and Difficulties Questionnaire, *p<0.017 in Bonferroni correction

Table 3. Correlations of iron parameters with psychosocial measures in anemic or non-anemic ID patients

	Hemoglobin	Serum iron	Ferritin	TIBC	Transferrin saturation
Total PedQoL	0.315**	0.218	0.253*	-0.224*	0.218
Physical Health-PedQoL	0.373**	0.248*	0.309**	-0.180	0.237*
Psychosocial Health-PedQoL	0.235*	0.169	0.155	-0.232*	0.179
SDQ-Total score	-0.080	-0.013	-0.074	0.134	-0.017
SDQ-Emotional problems	-0.091	-0.047	-0.028	0.081	-0.043
SDQ-Behavioral problems	-0.089	-0.109	-0.167	0.206	-0.123
SDQ-ADHD problems	-0.107	0.060	-0.101	0.075	0.058
SDQ-Peer relationship problems	0.096	0.064	0.080	0.066	0.058
SDQ-Pro-social behaviors	-0.086	-0.096	-0.001	0.042	-0.102
Total SCARED	-0.125	-0.084	-0.047	0.303**	-0.107
SCARED-Panic/somatic	-0.091	-0.073	-0.095	0.388**	-0.126
SCARED-General anxiety	-0.162	-0.150	-0.051	0.189	-0.159
SCARED-Separation anxiety	-0.055	0.030	-0.044	0.231*	0.017
SCARED-Social anxiety	-0.032	-0.099	0.071	0.189	-0.112
SCARED-School phobia	-0.044	0.074	-0.058	0.229*	0.044
Self-esteem	0.154	0.051	0.108	-0.261*	0.081

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

SCARED: Screen for Child Anxiety-Related Emotional Disorders, PedsQoL: The Pediatric Quality of Life Inventory, SDQ: Strengths and Difficulties Questionnaire, TIBC: Total iron binding capacity, ID: Iron deficiency

Table 4. Correlations of QoL with other psychosocial measures in anemic or non-anemic ID patients

	Total PedQoL	Physical Health-PedQoL	Psychosocial Health-PedQoL
SDQ-Total score	-0.202	-0.239*	-0.151
SDQ-Emotional problems	-0.211	-0.243*	-0.162
SDQ-Behavioral problems	-0.068	-0.108	-0.030
SDQ-ADHD problems	-0.179	-0.232*	-0.119
SDQ-Peer relationship problems	-0.085	-0.060	-0.095
SDQ-Pro-social behaviors	-0.124	-0.178	-0.054
Total SCARED	-0.586**	-0.430**	-0.639**
SCARED-Panic/somatic	-0.577**	-0.465**	-0.590**
SCARED-General anxiety	-0.518**	-0.364**	-0.575**
SCARED-Separation anxiety	-0.467**	-0.361**	-0.495**
SCARED-Social anxiety	-0.220	-0.106	-0.282*
SCARED-School phobia	-0.422**	-0.321**	-0.451**
Self-esteem	0.624**	0.492**	0.653**

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

SCARED: Screen for Child Anxiety-Related Emotional Disorders, QoL: Quality of life, PedsQoL: The Pediatric Quality of Life Inventory, SDQ: Strengths and Difficulties Questionnaire, ID: Iron deficiency

but it was found to be worse in all areas in IDA than in ID or controls. The relationship between IDA and QoL is an expected situation, but the results of the studies in this area are contradictory and the research investigating this issue in adolescents is rare in the literature. Furthermore, the relationship between the presence of anemia in ID and QoL has also been less studied. In a study using a general health questionnaire, it was reported that there was no relationship between serum iron levels and psychological distress in female students unless anemia developed (10). Similar to these results, another study found no significant relationship between ID and QoL (11). However, this study did not check all participants weather they had anemia or not. Contrary to these findings, Patterson et al. (17) reported a lower QoL for women with ID. However, in this study, patients with anemia were also included in the study. Similar to our results, a recent study of female university students in New Zealand reported no significant difference in QoL between patients with ID without anemia and HCs (18). A recent study conducted in Turkey revealed that several sub-dimensions of QoL, such as physical function, energy/fatigue and general health perception in IDA patients were affected (19). As seen, most of the research in this area was conducted in adults, and mostly in women. Our study is one of the few studies on this subject to examine the adolescent age group in this respect and aspect, and supports the findings that the QoL is not affected in ID however it will deteriorate as IDA develops.

The finding that there was no difference between the groups in terms of SDQ, was found to be consistent with the exclusion criteria for the presence of a psychiatric disease-treatment history, and it was considered significant and important in terms of reducing confounding factors in interpreting the results. The SDQ-emotional problems score in the IDA group was found to be higher than the HCs and it was interpreted that IDA may be a predisposing factor for emotional problems or cause conditions mimicking emotional pathologies. This finding is thought to be compatible with the literature on this subject (8,20). No previous study examining IDA/ID in terms of self-esteem has been found in the literature. In our study, results showed that self-esteem was not affected by IDA or ID in adolescents. Self-esteem may also be affected by chronic disease. In a recent study, it has been reported that hemophilia patients have a lower self-esteem score than their peers (21). However, this difference was not observed in adolescents with IDA in our study. Nevertheless, the relations between self-esteem, anxiety, and QoL in ID/IDA suggested that further studies are needed to increase our understanding of this issue.

Given the correlation of TIBC, it was interpreted that TIBC can be used as a sensitive and reliable parameter in future research because it is more specific and sensitive than serum iron and ferritin levels and is less affected by many other factors. The findings of the correlation of hemoglobin, serum iron, ferritin and transferrin saturation levels with the

QoL were interpreted as they will enrich the information and contribute to the literature about the relationships between hematological parameters and psychometric data in IDA/ID. In previous studies; one study reported the relationship between depression and ferritin levels in ID in adults (22); the other one reported the relationship between serum iron and hemoglobin level with physical function, as well as the relationship between hemoglobin level with mental health and general health perception (19); another study reported the relationship between ferritin levels and behavioral problems in patients with ADHD (21). Further studies are required to investigate the relationship between hematological and psychometric data in adolescents.

There are some limitations to our study. First, our study was conducted on a small sample. Secondly, in our study, no further psychiatric evaluation was performed in psychosocial and behavioral evaluations. Finally, our study is a cross-sectional study and it is thought that follow-up studies will provide clinically important information on the psychosocial and behavioral effects of ID/IDA treatments. Strengths of our study; this was one of the few studies conducted on adolescents, ID and IDA were taken as separate groups, psychosocial evaluation was made in several aspects, and their relationship between hematological data was examined.

CONCLUSION

In conclusion, our findings suggest that while iron-deficient adolescents without anemia are not affected, adolescents with IDA are affected in terms of anxiety and QoL. Adolescence is a period in which development accelerates and a period in which the first symptoms of many psychiatric disorders occur. Considering these; psychosocial evaluation of adolescents with IDA would be appropriate. Also, adolescents presenting with psychological problems should be evaluated for ID and IDA. We also recommend psychosocial support and intervention as a part of the treatment of adolescents with IDA in terms of adherence to treatment and improving their QoL.

ETHICS

Ethics Committee Approval: Approval for this study was obtained from the Clinical Research Ethics Committee of Van Training and Research Hospital (decision no: 2019/19, date: 17.10.2019).

Informed Consent: Written informed consent was obtained.

Authorship Contributions

Concept: U.T., S.A.K., H.N.U., Design: U.T., S.A.K., H.N.U., Data Collection or Processing: S.A.K., Analysis or Interpretation: U.T., S.A.K., H.N.U., Literature Search: U.T., S.A.K., Writing: U.T., S.A.K., H.N.U.

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. Beard J. Iron deficiency alters brain development and functioning. J Nutr 2003;133(5 Suppl 1):1468S-72S.
2. Ortiz E, Pasquini JM, Thompson K, Felt B, Butkus G, Beard J, et al. Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in three different animal models. J Neurosci Res 2004;77:681-9.
3. Hergüner S, Keleşoğlu FM, Tanıdır C, Cöpür M. Ferritin and iron levels in children with autistic disorder. Eur J Pediatr 2012;171:143-6.
4. Bilgiç A, Gürkan K, Türkoğlu S, Akça ÖF, Kılıç BG, Uslu R. Iron deficiency in preschool children with autistic spectrum disorders. Research in Autism Spectrum Disorders 2010;4:639-44.
5. WHO C. Worldwide prevalence of anaemia 1993–2005. WHO global database on anaemia. 2008.
6. Pasricha SR, Drakesmith H, Black J, Hipgrave D, Biggs BA. Control of iron deficiency anemia in low- and middle-income countries. Blood 2013;121:2607-17.
7. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 2001;131:649S-68S.
8. Chen MH, Su TP, Chen YS, Hsu JW, Huang KL, Chang WH, et al. Association between psychiatric disorders and iron deficiency anemia among children and adolescents: a nationwide population-based study. BMC Psychiatry 2013;13:161.
9. Harding L. Children's quality of life assessments: a review of generic and health related quality of life measures completed by children and adolescents. Clinical Psychology & Psychotherapy: An International Journal of Theory & Practice 2001;8:79-96.
10. Rangan AM, Blight GD, Binns CW. Iron status and non-specific symptoms of female students. J Am Coll Nutr 1998;17:351-5.
11. Grondin MA, Ruivard M, Perrève A, Derumeaux-Burel H, Perthus I, Roblin J, et al. Prevalence of iron deficiency and health-related quality of life among female students. J Am Coll Nutr 2008;27:337-41.
12. Güvenir T, Özbek A, Baykara B, Arkar H, Şentürk B, İncekaş S. Güçler ve güçlükler anketi'nin (gga) Türkçe uyarlamasinin psikometrik özellikleri. Turkish Journal of Child and Adolescent Mental Health 2008;15:65-74.
13. Saygısı ÇFAB. Yayımlanmamış Uzmanlık Tezi. Hacettepe Üniversitesi, Ankara. 1986.
14. Cakin Memik N, Ağaoğlu B, Coşkun A, Uneri OS, Karakaya I. Cocuklar Için Yaşam Kalitesi Olçeğinin 13-18 Yaş Ergen Formunun Geçerlik ve Güvenilirliği [The validity and reliability of the Turkish Pediatric Quality of Life Inventory for children 13-18 years old]. Turk Psikiyatri Derg 2007;18:353-63.
15. Çakmakçı F. Çocuklarda anksiyete bozukluklarını tarama ölçeği geçerlik ve güvenirlik çalışması. Çocuk ve Gençlik Ruh Sağlığı Dergisi 2004;11.
16. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics 2000;105:E51.

17. Patterson AJ, Brown WJ, Roberts DC. Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. J Am Coll Nutr 2001;20:337-42.
18. Beck KL, Conlon CA, Kruger R, Heath AL, Matthys C, Coad J, et al. Iron status and self-perceived health, well-being, and fatigue in female university students living in New Zealand. J Am Coll Nutr 2012;31:45-53.
19. Baş FY. Ruh Sağlığı ve Yaşam Kalitesine Demir Eksikliği Anemisinin Etkisi. SDÜ Sağlık Bilimleri Dergisi 2019;10:1-4.
20. Kim J, Wessling-Resnick M. Iron and mechanisms of emotional behavior. J Nutr Biochem 2014;25:1101-7.
21. Oner O, Alkar OY, Oner P. Relation of ferritin levels with symptom ratings and cognitive performance in children with attention deficit-hyperactivity disorder. Pediatr Int 2008;50:40-4.
22. Vahdat Shariatpanaahi M, Vahdat Shariatpanaahi Z, Moshtaaghi M, Shahbaazi SH, Abadi A. The relationship between depression and serum ferritin level. Eur J Clin Nutr 2007;61:532-5.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:247-251
DOI: 10.4274/BMJ.galenos.2022.2022.3-19

Research

Effects of Waterpipe Smoking on the Course of COVID-19 Infection

Nargile İçiminin COVID-19 Enfeksiyonu Sürecine Etkileri

Hayri Canbaz[1], Özgür Sirkeci[2], Emel Erkuş Sirkeci[3], Neslihan Ergün Süzer[4]

[1]Yıldırım Beyazıt University, Yenimahalle Training and Research Hospital, Clinic of Emergency Medicine, Ankara, Turkey
[2]Süleyman Demirel University Faculty of Medicine, Department of Gastroenterology, Isparta, Turkey
[3]Isparta City Hospital, Clinic of Emergency Medicine, Isparta, Turkey
[4]Gebze Fatih State Hospital, Clinic of Emergency Medicine, Kocaeli, Turkey

ABSTRACT

Objective: Smoking, and also water pipe smoking (hookah), is a common method of tobacco use in Southwest Asia and Middle East countries. Although the relationship between coronavirus disease-2019 (COVID-19) infection and smoking has been evaluated in many studies, no study has been conducted to evaluate the relationship between COVID-19 infection and water pipe smoking.

Methods: We enrolled 150 in-hospital patients. The severity of disease classified as mild, moderate, severe, and critically ill. The relationship between waterpipe smoker, smoker and non-smoker patients and severity of disease statistically evaluated.

Results: Patients with minimal involvement (1-25%) on thorax computed tomography were found to be higher in the smoker and cigarette-hookah smoking group compared to the non-smoking group, and the patients with moderate involvement (51-75%) were found to be less in the smoking-hookah group. in terms of disease degree; It was found that there were more mild and moderate smokers in the smoking and smoking-hookah group than the non-smoking group. The C-reactive protein and sedimentation values of cigarette-waterpipe tabocco smokers were found to be lower than non-smokers.

Conclusion: Waterpipe smoking does not aggravate the course of the disease in the young population, but new studies are needed for its effects on the elderly population.

Keywords: COVID-19, emergency department, pneumonia, waterpipe

ÖZ

Amaç: Sigara ve nargile içimi (Nargile), Güneybatı Asya ve Orta Doğu ülkelerinde yaygın bir tütün kullanım yöntemidir. Koronavirüs hastalığı-2019 (COVID-19) enfeksiyonu ile sigara kullanımı arasındaki ilişki birçok çalışmada değerlendirilmiş olsa da COVID-19 enfeksiyonu ile nargile içimi arasındaki ilişkiyi değerlendiren bir çalışma yapılmamıştır.

Gereç ve Yöntem: Hastanede yatan 150 hastayı kaydettik. Hastalığın şiddeti hafif, orta, şiddetli ve kritik olarak sınıflandırılır. Nargile içen, sigara içen ve içmeyen hastalar ile hastalık şiddeti arasındaki ilişki istatistiksel olarak değerlendirildi.

Bulgular: Toraks bilgisayarlı tomografide minimal tutulumu olan hastalar (%1-25), sigara içen ve sigara-nargile içen grupta sigara içmeyen gruba göre daha yüksek bulundu ve orta düzeyde tutulumu olan hastalar (%51-75) sigara-nargile grubunda daha az bulundu. Hastalık derecesi açısından; sigara ve nargile içen grupta, sigara içmeyen gruba göre daha hafif ve orta düzeyde sigara içenlerin olduğu bulundu. Sigara-nargile içenlerin C-reaktif protein ve sedimantasyon değerleri içmeyenlere göre daha düşük bulundu.

Sonuç: Nargile içimi genç popülasyonda hastalığın seyrini kötüleştirmez ancak yaşlı popülasyon üzerindeki etkileri için yeni çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: COVID-19, acil servis, pnömoni, nargile

Address for Correspondence: Hayri Canbaz, Yıldırım Beyazıt University, Yenimahalle Training and Research Hospital, Clinic of Emergency Medicine, Ankara, Turkey
Phone: +90 544 747 46 17 E-mail: dr_endorfin@yahoo.com ORCID ID: orcid.org/0000-0001-6989-7854

Cite as: Canbaz H, Sirkeci Ö, Erkuş Sirkeci E, Ergün Süzer N. Effects of Waterpipe Smoking on the course of COVID-19 Infection. Med J Bakirkoy 2022;18:247-251

Received: 30.03.2022
Accepted: 26.05.2022

INTRODUCTION

Coronavirus disease-2019 (COVID-19) infected over 140 million and caused more than 3 million deaths up to April 2021 (1). The main route of the transmission is respiratory droplets and patients usually present with respiratory symptoms like dry cough, fever, fatigue and sometimes atypical presentations also with loss of taste and smell.

Smoking, and water pipe smoking (Hookah), is a common method of tobacco use in Southwest Asia and Middle East countries. Recently, its popularity has increased and its usage has become widespread in Europe and America, carries the same risks as smoking (2,3). The prevalence of respiratory diseases, lung cancer, cardiovascular diseases and metabolic syndrome are increased in water pipe smoking. In addition, impairment in oral hygiene and a high incidence of periodontal disease are common outcomes of water pipe smoking (4). Although the relationship between COVID-19 infection and smoking has been evaluated in many studies, no study has been conducted to evaluate the relationship between COVID-19 infection and water pipe smoking. Therefore, we have designed this study whether water pipe smoking can is associated with the severity of COVID-19 infection or not.

METHODS

This is a retrospective single center study conducted at Gebze Government Hospital. We enrolled 150 in-hospital patients between 02 April 2020-03 May 2020. The study was approved by the Ankara City Hospital Clinical Research Ethics Committee (no: E2-22-1492, date: 02.03.2022) and the Ministry of Health COVID-19 Science Board.

COVID-19 was diagnosed according to World Health Organization guidance. Nasopharyngeal swap samples were used for real-time reverse transcriptase polymerase chain reaction (PCR) tests. Patients older than 18 years and confirmed of COVID-19 infection by PCR testing were enrolled in the study.

Those who use waterpipe tobacco at least twice a week was considered as a waterpipe tobacco smoker.

Data Collection

Patients demographic features, symptoms and signs, co morbidities, laboratory tests and chest computed tomography (CT) scans were evaluated. All data were collected from the electronic hospital information system. The medical records of the patients were reviewed by an expert investigator. Peripheral venous blood samples were measured at the biochemical laboratory of Gebze Government Hospital following standard operative procedures. The routine blood tests [including white blood cell count, leukocyte subtypes (neutrophil, lymphocyte, eosinophil, basophil), hemoglobin count, and platelet count] were measured with multi-function automatic blood analyzer. Biochemical parameters were measured using the ARCHITCT ci16200 automatic biochemistry analyzer (Abbott Laboratories, Illinois, United States) C-reactive protein (CRP) and serum ferritin were measured using latex-enhanced immunoturbidimetry (Cobas 8000; Roche).

Assessment of Disease Severity

Mild disease was defined as the absence of dyspnea and patients with fever, malaise, cough, upper respiratory symptoms, and/or less common features of COVID-19. Patients who developed dyspnea without hypoxia were defined as having moderate disease. Severe COVID-19 was defined as the existence of dyspnea, blood oxygen saturation ≤94% on air room and need for oxygenation or ventilatory support (4). Critical COVID-19 is defined as the occurrence of respiratory failure, septic shock and/or multiple organ functions (5).

Chest CT of the patients was assessed and classified for the degree of parenchyma involvement and noted as following: No involvement (0%), minimal involvement (1%-25%), mild involvement (26%-50%), moderate involvement (51%-75%) and severe involvement 76%-100%) (6).

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 24.0 (SPSS, Chicago, IL, USA). The one-sample Kolmogorov-Smirnov test was used to verify the normality of data distributions. Results are expressed as numbers, percentages; median, minimum, and maximum. Chi-square test was used for categorical variables. An independent sample t-test was used to analyze parametric numerical data, and the Mann-Whitney U test was used to analyze non-parametric data. One-Way ANOVA with a post hoc Bonferroni and Kruskal-Wallis tests were used in normally and non-normally distributed continuous data, respectively. Values of $p<0.05$ were considered statistically significant for all results.

RESULTS

Patients were divided into three groups; group one was a smoker, group two was cigarette and waterpipe smoker; and group three was non-smokers. The demographic, clinical and biochemical characteristics of all patients are summarized in Tables 1 and 2. The difference between thoracic CT involvement and severity index groups was

examined by chi-square and Bonferroni tests. No significant difference was found between smokers, cigarette and waterpipe smokers and non-smokers among patients thoracic CT with no involvement, mild involvement and severe involvement. Among the patients with minimal involvement on CT, there was no significant difference between cigarette smoker patients and cigarette and waterpipe smoker patients, the group with non-smoker

Table 1. Demographic characteristics of patients with COVID-19

	Non-smoker	Cigarette smoker	Cigarette + waterpipe smoker	Total
Age (year)				
18-35	1	9	16	26
36-50	0	21	16	37
51-65	11	28	0	39
>65	36	11	1	48
Male	17	51	27	95
Female	31	18	6	55
Co morbidity	1	18	18	37
Hypertension	3	10	4	17
Diabetes	7	11	3	21
Chronic kidney disease	2	4	0	6
Cardiovascular disease	24	12	3	39
Respiratory disease	6	10	5	21
Chronic liver disease	1	2	0	3
Solid tumour	2	2	0	4
Hematologic malignancy	1	0	0	1
Rheumatic disease	1	0	0	1
No co morbidity	1	18	18	37
Severity index				
Mild	1	7	7	15
Moderate	9	27	20	56
Severe	36	32	5	73
Critically ill	2	3	1	6
Bt involvement				
No involvement	1	2	4	7
<25%	4	19	14	37
25-50%	16	23	10	49
50-75%	20	20	4	44
>75%	7	5	1	13
Outpatient follow up	1	2	3	6
Ward follow up	14	38	23	75
Intensive care unit	25	22	7	54
Mechanic ventilation	7	6	0	13
Exitus	8	4	0	12

COVID-19: Coronavirus disease-2019, CT: Computed tomography

Table 2. Laboratory findings of patients with COVID-19

Laboratory	Non-smoker (n=15) mean/(STD)	Cigarette smoker (n=56)	Cigarette + waterpipe smoker
Leukocyte count, 10^3 cells/L	9.72 (4.76)	8.51 (4.68)	7.81 (3.54)
Neutrophil count, 10^3 cells/L	3.99 (2.4-9.07)	4.18(1.4312.12)	6.25 (0.00-78.0)
Lymphocyte count, 10^3 cells/L	2.04 (0.64-3.88)	1.78 (0.55-7.44)	1.49 (0.00-5.49)
Monocytes count, 10^3 cells/L	0.56 (0.36-1.46)	0.69 (0.18-5.00)	0.72 (0.00-5.20)
Hemoglobin level, g/L	12.7 (8.3-16.10)	13.8 (7.4-16.7)	12.0 (6.4-16.7)
Platelet count, 10^3 cells/L	288.0 (133.0-385.0)	250.0 (53.0-546.0)	223.0 (13.8-491.0)
Fibrinogen, g/L	245.0 (198.0-580.0)	356.0 (231-619)	455.0 (233.0-749.0)
D-dimer, mg/L	0.37 (0.14-0.58)	0.47 (0.08-6.29)	1.17 (0.08-25.06)
C-reactive protein level, mg/L	4.53 (1.0-9.0)	8.0 (2-17)	23.0 (9-54)
ESR, mm/60 min	12.0 (10.0-12.0)	14.0 (10.0-54.0)	23.0 (11.0-101.0
Serum ferritin ng/mL	90.6 (7.0-370.6)	63.5 (4.0-1086.0)	132.2 (6.9-1834.0)
Creatinine level, mg/dL	0.8 (0.58-1.38)	0.83 (0.38-2.64)	0.93 (0.55-3.2)
Urea level, mg/dL	12.0 (7.0-43.0)	12.0 (5.0-63.0)	17.0 (6.0-156.0)
Procalcitonin ng/mL	0.04 (0.01-0.41)	0.03 (0.00-1.93)	0.09 (0.0-60.96)
Neutrophil/lymphocyte	1.93 (0.89-4.07)	2.41(0.7-14.55)	4.05 (0.0-24.26)

ESR: Erythrocyte sedimentation rate, STD: Standard deviation, COVID-19: Coronavirus disease-2019

patients was found to be statistically significantly lower than the other two groups. Among the patients with moderate involvement, there was no significant difference between the smoker and non-smoker patient groups, while the group with cigarette and waterpipe smoker patients was found to be statistically significantly lower than the other two groups ($p<0.005$).

No significant difference was found between the smoker group and cigarette and waterpipe smoker groups among those with the COVID severity index-mild. Among those with COVID severity index-modarate, cigarette smoker, cigarette, and waterpipe smoker patients and non-smokers were all significantly different from each other. COVID severity index-severe patients, cigarette smokers, cigarette and waterpipe smokers and non-smokers were all significantly different from each other. There was no significant difference between patients with critical illness severity index ($p<0.001$). CRP values, respectively; cigarette smokers mean 14.8 (12.5-17.2), cigarette and waterpipe smoker patients mean 10.6 (8.0-13.3), non-smokers, mean 22.5 (18.9-26.1) detected. No statistically significant difference was found between group one and group two in terms of CRP values. A significant difference was found between the CRP values of only group three, group one and group two patients ($p<0.001$). Sedimentation values are respectively; average of 20.3 (17.6-23.1) in 67 smokers, 16.2 (13.9-18.4) in 31 cigarette-waterpipe smokers, 23.3 (19.2) in 47 non-smoking patients (27.4) and a statistically significant difference was found between the cigarette-smoking patient group and the non-smoking patient group. In other words, the sedimentation value was found to be higher in the non-smoker group ($p<0.005$).

DISCUSSION

Patients with minimal involvement (1%-25%) on thorax CT were found to be higher in the smoker and cigarette-hookah smoking groups compared to the non-smoking group, and the patients with moderate involvement (51%-75%) were found to be lower in the smoking-hookah group. In terms of disease degree; it was found that there were more mild and moderate smokers in the smoking and smoking-hookah groups than in the non-smoking group. The CRP and sedimentation values of cigarette and waterpipe smokers were found to be lower than non-smokers (5). In fact, our findings were unexpectedly confusing. In a study conducted in the UK, smokers were divided into two as current smokers and ex-smokers, and it was found that current smokers have an increased risk of hospitalization and death (6). Lippi and Henry (7) defined a relationship between smoking and COVID-19 in their study. It was emphasized that there was a significant

correlation between smoking and the need for ICU support and mechanical ventilation according to the metanalysis of Vardavas Nikitara (8). Cattaruzza et al. (9) stated that smoking is 'the most important preventable risk factor'. However, according to the results of our study, COVID-19 shows a milder course and inflammatory markers tend to be at a lower level in patients who smoke and hookah. In Turkey hookah smoking has become popular among the young population over the past decade. But it has not become popular among the middle and advanced age group. In our study, all those who smoked waterpipe were young patients. In our opinion, therefore the disease tended to have a mild course among cigarette and waterpipe smoker groups and as a result, inflammatory markers were not high.

Angiotensin converting enzyme (ACE-2) receptors play a great role in viral replication and modification of the immune response in cells infected with severe acute respiratory syndrome coronavirus 2. ACE-2 has anti-inflammatory effects and a decrease in activity may result in severe infection course. Tabocco usage negatively affects receptor activity and may predispose severe lung injury (10). Of course, this is not the only factor that affects the course of the disease. Age and comorbid diseases are one of the most important factors determining the disease course. In our study, we had no patient over fifty years. This was the most important factor in the mild course of disease.

Tobacco use is an important form of transmission for both active and passive smokers (11). With waterpipe use social distancing rule is often violated and the same equipment is used by other people, this creates a very suitable environment for disease transmission. It is obvious that although waterpipe smoking does not aggravate the severe course of the disease, it does cause an increase in the risk of transmission.

CONCLUSION

Waterpipe smoking does not aggravate the course of the disease in the young population, but new studies are needed on its effects on the elderly population.

ETHICS

Ethics Committee Approval: The study was approved by the Ankara City Hospital Clinical Research Ethics Committee (no: E2-22-1492, date: 02.03.2022) and the Ministry of Health COVID-19 Science Board.

Informed Consent: Permission from cases and/or their relatives was obtained with an informed consent form.

Authorship Contributions

Surgical and Medical Practices: N.E.S., Concept: H.C., Ö.S., Design: H.C., Ö.S., E.E.S., Data Collection or Processing: Ö.S., N.E.S., Analysis or Interpretation: H.C., Ö.S., E.E.S., Literature Search: H.C., E.E.S., N.E.S., Writing: H.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. World Health Organization. Coronavirus disease 2019 (COVID-19) Situation Report - 106, Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports (2020).
2. Cobb C, Ward KD, Maziak W, Shihadeh AL, Eissenberg T. Waterpipe tobacco smoking: an emerging health crisis in the United States. Am J Health Behav 2010;34:275-85.
3. Akl EA, Gaddam S, Gunukula SK, Honeine R, Jaoude PA, Irani J. The effects of waterpipe tobacco smoking on health outcomes: a systematic review. Int J Epidemiol 2010;39:834-57.
4. Shafique K, Mirza SS, Mughal MK, Arain ZI, Khan NA, Tareen MF, et al. Water-pipe smoking and metabolic syndrome: a population-based study. PLoS One 2012;7:e39734.
5. Grundy EJ, Suddek T, Filippidis FT, Majeed A, Coronini-Cronberg S. Smoking, SARS-CoV-2 and COVID-19: A review of reviews considering implications for public health policy and practice. Tob Induc Dis 2020;18:58.
6. Government of the United Kingdom - Cabinet Office. Staying alert and safe (social distancing). Available from: https://www.gov.uk/government/publications/staying-alert-and-safesocial-distancing. (Published May 11, 2020. Accessed May 21, 2020).
7. Lippi G, Henry BM. Active smoking is not associated with severity of coronavirus disease 2019 (COVID-19). Eur J Intern Med 2020;75:107-8.
8. Vardavas CI, Nikitara K. COVID-19 and smoking: A systematic review of the evidence. Tob Induc Dis 2020;18:20.
9. Cattaruzza MS, Zagà V, Gallus S, D'Argenio P, Gorini G. Tobacco smoking and COVID-19 pandemic: old and new issues. A summary of the evidence from the scientific literature. Acta Biomed 2020;91:106-12.
10. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 2005;11:875-9.
11. Ahmed N, Maqsood A, Abduljabbar T, Vohra F. Tobacco Smoking a Potential Risk Factor in Transmission of COVID-19 Infection. Pak J Med Sci 2020;36(COVID19-S4):S104-7.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:252-257
DOI: 10.4274/BMJ.galenos.2022.2022.2-16

Research

Protective Effects of Hesperetin Against Lipopolysaccharide-induced Acute Renal Injury in Rat

Hesperetinin Lipopolisakkarit-uyarımlı Sıçan Akut Böbrek Hasarına Karşı Koruyucu Etkileri

Serkan Kaya[1], İhsan Karaboğa[2]

[1]Tekirdağ Namık Kemal University Faculty of Medicine, Department of Thoracic Surgery, Tekirdağ, Turkey
[2]Tekirdağ Namık Kemal University School of Health, Department of Emergency and Disaster Management, Tekirdağ, Turkey

Presented in: Some of the data in this study were presented as an Oral Presentation at the International Scientific Research Congress (UBAK-2019).

ABSTRACT

Objective: In this study, it was aimed to investigate the effects of hesperetin on renal transforming growth factor-beta 1 (TGF-β1) expression and apoptosis in rat lipopolysaccharide (LPS)-induced acute renal injury model.

Methods: In the study, 18 adult male Wistar albino rats were used. Rats divided into three groups, respectively (n=6); control, LPS and LPS + hesperetin. Sepsis model was created with a singledose of LPS (*Escherichia coli*, O26: B6 serotype, Sigma-aldrich). LPS + hesperetin group was administered intragastrically with the aid of hesperetin oral gavage at a dose of 100 mg/kg, after LPS-induction. Twenty four hours after LPS administration, the rats were opened from the midline under ketamine-xylazine anesthesia and kidney tissue and cardiac blood were collected. Kidney tissue was examined with hematoxylin-eosin staining. TGF-β1 expression was determined by indirect immunohistochemical method. TUNEL method was used to determine renal apoptosis. Blood urea nitrogen (BUN) and creatinine levels in blood serum were determined using spectrophotometric methods.

Results: Decreased histopathological changes, TGF-β1 expression and apoptosis were determined in the LPS + hesperetin group compared to the LPS group ($p<0.05$). In addition, a significant decrease in BUN and creatinine levels was observed in the LPS + hesperetin group compared to the LPS group ($p<0.05$).

Conclusion: The data obtained show that in the LPS-induced rat sepsis model, hesperetin suppresses the expression of TGF-β1 in kidney tissue and provides a protective effect.

Keywords: Lipopolysaccharide, acute kidney injury, hesperetin, transforming growth factor-beta 1, renal apoptosis

ÖZ

Amaç: Bu çalışmada, sıçan lipopolisakkarit (LPS)-uyarımlı akut böbrek hasarı modelinde hesperetinin renal transforme edici büyüme faktör-beta 1 (TGF-β1) ifadesi ve apoptozis üzerine etkilerinin incelenmesi amaçlandı.

Gereç ve Yöntem: Çalışmada 18 adet erişkin erkek Wistar albino sıçan kullanıldı. Sıçanlar üç gruba ayrıldı (n=6); kontrol, LPS ve LPS + hesperetin. Sepsis modeli, tek doz LPS (*Escherichia coli*, O26: B6 serotipi, Sigma-aldrich) ile oluşturuldu. LPS + hesperetin grubuna, LPS uygulaması sonrası, 100 mg/kg dozda hesperetin oral gavaj yardımıyla intragastrik olarak uygulandı. LPS uygulamasını takiben 24 saat sonra, sıçanlar ketamin-ksilazin anestezisi altında orta hattan açılarak böbrek dokusu ve kardiyak kan alındı. Böbrek dokusu hematoksilen-eozin boyaması ile incelendi. Renal TGF-β1 ifadesi indirekt immünohistokimyasal yöntemle incelendi. Renal apoptozisin belirlenmesinde TUNEL metodu kullanıldı. Kan serumunda kan üre azotu (BUN) ve kreatinin seviyeleri spektrofotometrik yöntemle belirlendi.

Bulgular: LPS + hesperetin grubunda LPS grubuna kıyasla azalmış histopatolojik değişiklikler, TGF-β1 ekspresyonu ve apoptozis belirlendi ($p<0,05$). Ayrıca LPS + hesperetin grubunda LPS grubuna kıyasla BUN ve kreatinin seviyelerinde anlamlı derecede azalma gözlendi ($p<0,05$).

Address for Correspondence: Serkan Kaya, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Thoracic Surgery, Tekirdağ, Turkey
Phone: +90 543 834 39 39 E-mail: serkankaya@gmail.com ORCID ID: orcid.org/0000-0002-9817-744X

Cite as: Kaya S, Karaboğa İ. Protective Effects of Hesperetin Against Lipopolysaccharide-induced Acute Renal Injury in Rat. Med J Bakirkoy 2022;18:252-257

Received: 22.02.2022
Accepted: 26.05.2022

Sonuç: Elde edilen veriler LPS uyarımlı sıçan sepsis modelinde hesperetinin böbrek dokusunda TGF-β1 ekspresyonunu baskılayarak koruyucu etki sağladığını göstermektedir.

Anahtar Kelimeler: Lipopolisakkarit, akut böbrek hasarı, hesperetin, transforme edici büyüme faktör-beta 1, renal apoptozis

INTRODUCTION

Acute kidney injury (AKI) is characterized by acute tubular cell damage and renal dysfunction, and may develop due to septic, toxic or ischemic causes (1). One of the most important risk factors for the development of AKI is severe bacterial infection (2). AKI-associated Gram-negative bacterial infections continue to be a cause of high mortality in patients (3). While a specific method for the treatment of AKI has not been developed yet, the development of new agents in the treatment of septic AKI has a great potential to reduce mortality and morbidity in patients (4).

Lipopolysaccharide (LPS), which is an important pathogenic factor in the cell membrane of Gram-negative bacteria, consists of lipid and polysaccharide linked by covalent bond (5). Experimental studies have shown that it is an endotoxin that provides a strong immune response (6,7).

Various histopathological and biochemical changes have been reported in kidney tissue in LPS-induced experimental models. These are renal tubular damage, increase in blood urea nitrogen (BUN) and creatinine levels, inflammatory cell infiltration, pathological changes such as necrosis in tubular cells, and increased oxidative stress (8,9).

LPS stimulation induces the activation of nuclear factor-kappa beta and may activate the Toll-like receptor-4 pathway, which stimulates the release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β) and transforming growth factor-beta 1 (TGF-β1) (10,11). Excessive production of inflammatory cytokines may cause kidney damage by causing the migration of neutrophils and monocytes to the glomerulus (11,12). Therefore, suppression of inflammatory cytokine production is an important strategy in the prevention of kidney damage (13).

Some natural flavonoids and polyphenols have broader biological effects and targets than chemical-synthetic agents, which usually have a specific target, especially in diseases with multifactorial pathogenesis (12-14).

Hesperetin (3',5,7-trihydroxy-4-methoxyflavanone) is a natural flavonoid found abundantly in citrus fruits such as lemon, orange, grapefruit, and mandarin (15). Flavonoids are polyphenolic compounds with various biological activities, and the free hydroxyl groups in their molecular structures function as free radical scavengers (16). Previous studies have shown that hesperetin has potent anti-inflammatory and antioxidative properties, both *in vivo* and *in vitro* (17,18).

It has been reported that application of hesperetin in acrolein-induced experimental lung injury model suppresses oxidative stress and supports tissue structure by preventing apoptosis in cells (19). It has been reported that application of hesperetin in experimental rat ischemia/reperfusion-induced retinal injury model prevents retinal thinning and apoptosis in the retina (18).

These experimental studies show that hesperetin reduces damage by supporting cell and tissue structure in different tissues and organs.

When studies in which hesperetin was used as a treatment agent in different kidney damage models were examined, it was reported that it reduced lipid peroxidation, suppressed oxidative stress, decreased the release of inflammatory cytokines, and helped to preserve tissue structure by reducing histopathological changes (20,21).

The LPS-induced AKI model is the most commonly used in vivo experimental model to investigate the histopathological changes caused by sepsis in kidney tissue and the mechanisms underlying potential treatments (11). In the literature review, no study that examined the effects of hesperetin in the LPS-induced AKI model was found. In this study, it was aimed to examine the efficacy of hesperetin in the LPS-induced AKI model by histological, immunohistochemical and TUNEL methods.

METHODS

Experiment Model and Applications

The ethics committee approval required for this experimental study was obtained from the Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee (decision no: 4, date: 31.10.2018), and all applications were carried out in accordance with the Laboratory Animal Care and Guidelines. Our study was carried out at Tekirdağ Namık Kemal University Experimental Animals Application and Research Center (DHUAM) between March and May 2019. Eighteen healthy adult male Wistar albinos (3 months old, 250-300 g) were included in the study. The rats were fed ad libitum at 22±2 °C temperature, 12 hours light/12 hours dark cycle and 50-60% humidity during the experiment. Rats were divided into 3 groups, respectively (n=6), as

control, LPS, and LPS + hesperetin groups. *Escherichia coli* (O26:B6 serotype, Sigma-Aldrich) was administered intraperitoneally to the LPS group at a dose of 10 mg/kg (22). In the LPS + hesperetin group, hesperetin (SantaCruz, dissolved in 0.5 mL saline) was administered orally at a dose of 100 mg/kg, following endotoxin administration (14). At the end of 24 hours following LPS administration, the rats were opened from the midline under ketamine-xylazine (90-10 mg/kg) anesthesia. The kidney tissue was dissected and the animals were sacrificed after drawing blood from the heart. The kidney tissue was fixed in 10% formalin solution.

Biochemical Analysis

The blood samples were centrifuged at 4500 rpm for 5 minutes and serum samples were obtained. BUN and creatinine levels were determined from the obtained serum samples with the help of a biochemistry autoanalyzer (Cobas-501, RocheDiagnostic).

Histopathological Examination

Following the 24-hour fixation of the tissues in formalin, they were washed by keeping them under running tap water for a night. After washing, kidney tissues were exposed to alcohol series with increasing concentrations (60-70-80-90-96-100) and tissue blocks were obtained by embedding kidney tissues first in a soft paraffin-toluene mixture and then in hard paraffin (Slee, MPS). These blocks were used in histological, immunohistochemical and TUNEL examinations.

Hematoxylin-eosin (H&E) staining method was used for histopathological examination. Sections (5 µm) obtained using a semi-automatic microtome (Sleecut, 5061) were kept in an oven (37 °C) overnight to ensure complete adhesion to the slide surface. These sections were taken in toluene and dewaxed (5 min x 2 times). Afterwards, they were put into water after passing through alcohol series at decreasing concentrations (100-96-90-80-70-60%). After the hydration process, they were stained with hematoxylin for 3 minutes and after washing, with eosin for 5 minutes.

Afterwards, the slides were passed through a series of increasing concentrations of alcohol and placed in toluene. They were covered with enthallan following the transparentizing process.

Obtained H&E stained kidney tissue sections were examined using a light microscope (Olympus CX40) and image analysis program (Kameram, GEN III). The histopathological changes that occurred were detected and photographed.

TGF-β1 Immunohistochemical Staining

Avidin-biotin peroxidase complex technique was used for TGF-β1 immunohistochemical marking. Paraffin-free slides were passed through an alcohol series at decreasing concentrations and washed with phosphate buffer. Tissue areas on the slide were delimited with a hydrophobic pen. For antigen retrieval, sections were boiled in a microwave oven for 5 min in citrate buffer. The procedure of endogenous peroxide suppression was performed with hydrogen peroxide. In order to prevent non-specific binding, primary antibody application (TGF-β1, sc-52893) was carried out in a humid box for one hour at 37 °C, following a one-hour block serum step at laboratory temperature. After the application of secondary antibodies with biotin and streptavidin, which were applied for 20 minutes after the primary antibody application, the coloring process was carried out with chromogen (AEC; 3-amino-9-ethyl carbazole, Thermoscientific). Sections stained with Mayer's hematoxylin were examined under a light microscope. TGF-β1 staining intensity was graded semiquantitatively (0; no staining, 1; weak, 2; moderate, 3; strong).

Apoptotic Examination

TUNEL (Terminal deoxynucleotidyltransferase-mediated dUTP nickend-labelling) method was used to determine apoptosis in kidney tissue. The procedure for using the kit was applied to the deparaffinized slides. DAB (3,3'-diaminobenzidine tetrahydrochloride) was used as chromogen. After ground staining with Mayer's hematoxylin, the sections were examined under a light microscope. The apoptotic index was calculated by measuring the number of TUNEL-positive cells stained dark brown in the cell nucleus in photographs obtained from 10 different areas of each kidney section (TUNEL positive cell number/total number of cells counted x 100) (23).

Statistical Analysis

The obtained data were analyzed using SPSS (PASW, 18.0.0) statistical program. The data of the groups were evaluated using the non-parametric Kruskal-Wallis test. Differences between the groups were measured with the Mann-Whitney U test. When the difference between the groups was less than $p<0.05$, it was considered statistically significant.

RESULTS

In the histopathological evaluation, normal histological structure was observed in the kidney tissue of the control group. The structure of proximal and distal tubules, interstitial tissue and kidney bodies were found to be normal (Figure 1a). In the LPS group, shedding especially in

tubular epithelial cells and pycnotic nuclei in some tubular epithelial cells were observed (Figure 1b). Although the tubule epithelial shedding continued in the group treated with hesperetin, it was found to be less frequent compared to the LPS group (Figure 1c).

When immunohistochemical TGF-β1 staining was examined, it was observed that positive immune reactivity was intense in tubular epithelial cells (Figure 2a-c). TGF-β1 semiquantitative staining scores were 0.3±0.5, 2.5±0.54, and 1.66±0.51 in the control, LPS and LPS + hesperetin groups, respectively. It was determined that there was an increase in TGF-β1 immune reactivity in the LPS group compared to the control group ($p<0.05$). It was found that hesperetin administration provided a significant decrease in TGF-β1 immune reactivity ($p<0.05$).

In our study, apoptosis occurring in kidney tissue was determined by the TUNEL method based on the detection of apoptotic cells by marking broken DNA ends (Figure 3). In the control, LPS and LPS + hesperetin groups, the apoptotic index was calculated as 5.3±1.8, 29.5±5.2, and 15.8±5.3, respectively. While a significant increase was observed in the LPS group compared to the control group ($p<0.05$), a significant decrease was detected in the LPS + hesperetin group compared to the LPS group ($p<0.05$).

Serum BUN and creatinine levels are presented in Table 1. LPS administration caused a significant increase in serum BUN and creatinine levels compared to the control group ($p<0.05$). It was determined that hesperetin administration provided a significant decrease in kidney function tests BUN and creatinine levels compared to the LPS group ($p<0.05$).

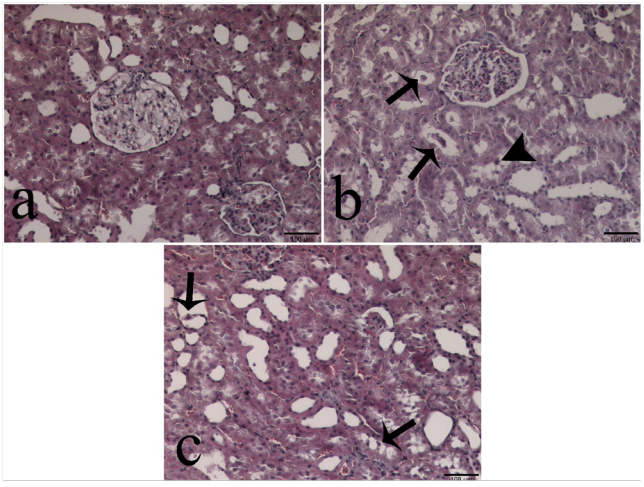

Figure 1. Histological structure of hematoxylin-eosin (H&E) stained kidney tissue (a; Control, b; LPS, c; LPS + hesperetin, bar; 100 µm, magnification; 200x, arrow; epithelial cell shedding, arrowhead; pycnotic cells, staining; H&E)
LPS: Lipopolysaccharide

DISCUSSION

Although the LPS-induced sepsis model does not represent all the features of sepsis encountered in the clinic, it is frequently used in the literature to create an AKI model because the findings of AKI caused by it are similar (24).

Although septic AKI is a complication that can be seen frequently in intensive care units and has a high mortality rate, there is no specific treatment method yet (25). In these patients, especially kidney transplantation is an effective treatment method (26). Today, studies examining the possible treatment efficacy of agents with known anti-inflammatory, anti-apoptotic and antioxidant properties against AKI damage are frequently encountered in the literature.

The findings obtained in our study demonstrated the renal protective effect of hesperetin in the rat LPS-induced AKI model with histological and biochemical data for the first time in the literature. Preservation of the renal histological structure by hesperetin administration may have been achieved by inhibiting TGF-β1 immunoreactivity and the activity of the inflammatory cytokine cascade and preventing

Table 1. Serum BUN and creatinine levels of the groups

	BUN (mg/dL)	Creatinine (mg/dL)
Control	12.1±2.3	0.7±0.1
LPS	42±5.8*	3.7±0.5*
LPS + hesperetin	27.1±5.7**	2.3±0.3**

BUN: Blood urea nitrogen, LPS: Lipopolysaccharide, *$p<0.05$ compared to the control group, **$p<0.05$ compared to the LPS group

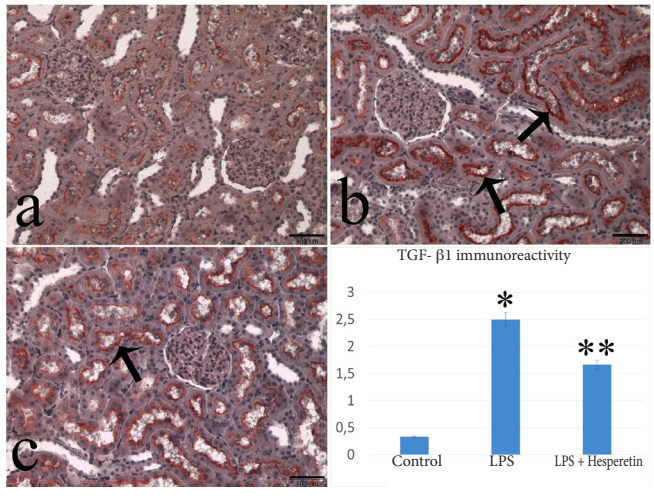

Figure 2. TGF-β1 immunohistochemistry findings (a; Control, b; LPS, c; LPS + hesperetin, bar; 100 µm, magnification; 200x, counterstaining; Mayer's hematoxylin, arrows; TGF- β1 immunoreactivity, *$p<0.05$ compared to control, **$p<0.05$ compared to LPS)
LPS: Lipopolysaccharide

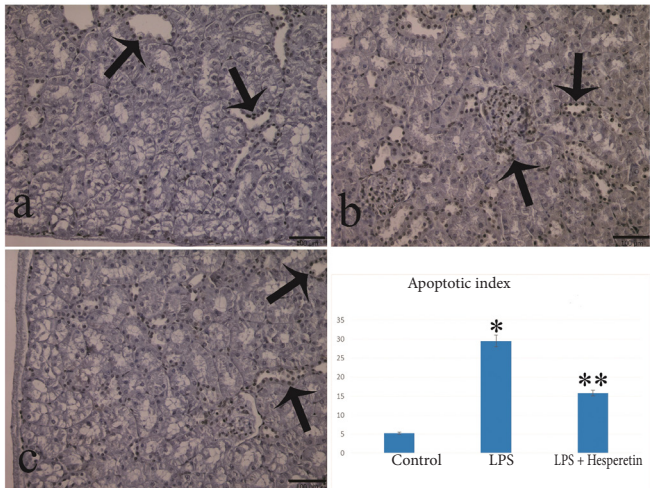

Figure 3. TUNEL staining findings of kidney tissue (a; Control, b; LPS, c; LPS + hesperetin, bar; 100 µm, magnification; 200x, counterstaining; Mayer's hematoxylin, arrows; TUNEL-positive apoptotic cells, *; p<0.05 compared to control, **; p<0.05 compared to LPS)
LPS: Lipopolysaccharide

apoptosis. There are many studies in the literature showing that hesperetin reduces/inhibits tissue damage in different experimental models due to its antioxidative, anti-inflammatory and anti-apoptotic properties (27,28).

Duran and Karaboğa (28) reported that administration of hesperetin prevented liver damage following blunt chest trauma. In the study of Trivedi et al. (29), it was revealed that heart damage caused by doxorubicin can be alleviated by the administration of hesperetin.

Kumar et al. (20) reported the therapeutic effect of hesperetin against kidney damage caused by cisplatin, a frequently used anticancer drug. In the aforementioned study, hesperetin treatment provided a significant decrease in histopathological changes caused by cisplatin application, serum BUN and creatinine levels, oxidative stress markers of superoxide dismutase, glutathione peroxidase, malondialdehyde, nitric oxide and inflammatory cytokines of TNF-α, IL-1β and IL-6 levels in kidney tissue.

It was determined that AKI-like findings occurred in the endotoxemia model created using LPS. Hesperetin administration can prevent the development of kidney damage by inhibiting the activation of the proinflammatory cytokine cascade and the expression of TGF-β1 and also by suppressing apoptosis in tubular epithelial cells.

There are some limitations in our study. Hesperetin treatment was applied following LPS administration and its effect was evaluated for a short time (24 h). This is not consistent with patient management in a clinical setting. In addition, hesperetin treatment was administered as a single dose. These limitations can be improved in new studies evaluating the effect of hesperetin application at different doses and longer than our study on kidney damage.

CONCLUSION

The results of our study show that hesperetin, a natural flavonoid, should be evaluated not only as a preventative but also for its therapeutic potential in diseases with kidney damage. More extensive *in vivo* and *in vitro* studies are needed to fully explain the treatment mechanism of hesperetin in the LPS-induced AKI model.

ETHICS

Ethics Committee Approval: The ethical aproval required to conduct this study was obtained from Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee (HADYEK- T2018-115) (decision no: 4, date: 31.10.2018).

Informed Consent: Animal experiment study.

Authorship Contributions

Surgical and Medical Practices: S.K., İ.K., Concept: S.K., İ.K., Design: S.K., İ.K., Data Collection or Processing: S.K., İ.K., Analysis or Interpretation: S.K., İ.K., Literature Search: S.K., İ.K., Writing: S.K., İ.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. Xin SB, Yan H, Ma J, Sun Q, Shen L. Protective Effects of Luteolin on Lipopolysaccharide-Induced Acute Renal Injury in Mice. Med Sci Monit 2016;22:5173-80.
2. Gursel G, Demir N. Incidence and risk factors for the development of acute renal failure in patients with ventilator-associated pneumonia. Nephrology (Carlton) 2006;11:159-64.
3. Zhang C, Walker LM, Mayeux PR. Role of nitric oxide in lipopolysaccharide-induced oxidant stress in the rat kidney. Biochem Pharmacol 2000;59:203-9.
4. Xu D, Chen M, Ren X, Ren X, Wu Y. Leonurine ameliorates LPS-induced acute kidney injury via suppressing ROS-mediated NF-κB signaling pathway. Fitoterapia 2014;97:148-55.
5. Bosshart H, Heinzelmann M. Targeting bacterial endotoxin: two sides of a coin. Ann N Y Acad Sci 2007;1096:1-17.
6. Abdelmageed ME, El-Awady MS, Suddek GM. Apocynin ameliorates endotoxin-induced acute lung injury in rats. Int Immunopharmacol 2016;30:163-70.
7. Xie X, Liu H, Wu J, Chen Y, Yu Z, De Isla N, et al. Rat BMSC infusion was unable to ameliorate inflammatory injuries in tissues of mice with LPS-induced endotoxemia. Biomed Mater Eng 2017;28(s1):S129-38.

8. Chunzhi G, Zunfeng L, Chengwei Q, Xiangmei B, Jingui Y. Hyperin protects against LPS-induced acute kidney injury by inhibiting TLR4 and NLRP3 signaling pathways. Oncotarget 2016;7:82602-8.
9. Tan F, Chen Y, Yuan D, Gong C, Li X, Zhou S. Dexmedetomidine protects against acute kidney injury through downregulating inflammatory reactions in endotoxemia rats. Biomed Rep 2015;3:365-70.
10. Nair AR, Masson GS, Ebenezer PJ, Del Piero F, Francis J. Role of TLR4 in lipopolysaccharide-induced acute kidney injury: protection by blueberry. Free Radic Biol Med 2014;71:16-25.
11. Ye HY, Jin J, Jin LW, Chen Y, Zhou ZH, Li ZY. Chlorogenic Acid Attenuates Lipopolysaccharide-Induced Acute Kidney Injury by Inhibiting TLR4/NF-κB Signal Pathway. Inflammation 2017;40:523-9.
12. Kiyonaga N, Moriyama T, Kanmura Y. Effects of landiolol in lipopolysaccharide-induced acute kidney injury in rats and in vitro. Shock 2019;52:117-23.
13. Zhao H, Zheng Q, Hu X, Shen H, Li F. Betulin attenuates kidney injury in septic rats through inhibiting TLR4/NF-κB signaling pathway. Life Sci 2016;144:185-93.
14. Polat FR, Karaboga I, Polat MS, Erboğa Z, Yılmaz A, Güzel S. Effect of hesperetin on inflammatory and oxidative status in trinitrobenzene sulfonic acid-induced experimental colitis model. Cell Mol Biol (Noisy-le-Grand) 2018;64:58-65.
15. Shirzad M, Heidarian E, Beshkar P, Gholami-Arjenaki M. Biological Effects of Hesperetin on Interleukin-6/Phosphorylated Signal Transducer and Activator of Transcription 3 Pathway Signaling in Prostate Cancer PC3 Cells. Pharmacognosy Res 2017;9:188-94.
16. Arul D, Subramanian P. Inhibitory effect of naringenin (citrus flavonone) on N-nitrosodiethylamine induced hepatocarcinogenesis in rats. Biochem Biophys Res Commun 2013;434:203-9.
17. Lee JH, Kim GH. Evaluation of antioxidant and inhibitory activities for different subclasses flavonoids on enzymes for rheumatoid arthritis. J Food Sci 2010;75:H212-7.
18. Kara S, Gencer B, Karaca T, Tufan HA, Arikan S, Ersan I, et al. Protective effect of hesperetin and naringenin against apoptosis in ischemia/reperfusion-induced retinal injury in rats. ScientificWorldJournal 2014;2014:797824.
19. Park JH, Ku HJ, Park JW. Hesperetin mitigates acrolein-induced apoptosis in lung cells in vitro and in vivo. Redox Rep 2018;23:188-93.
20. Kumar M, Dahiya V, Kasala ER, Bodduluru LN, Lahkar M. The renoprotective activity of hesperetin in cisplatin induced nephrotoxicity in rats: Molecular and biochemical evidence. Biomed Pharmacother 2017;89:1207-15.
21. Wang J, Zhu H, Yang Z, Liu Z. Antioxidative effects of hesperetin against lead acetate-induced oxidative stress in rats. Indian J Pharmacol 2013;45:395-8.
22. Kaya S, Albayrak Kaya S, Polat E, Fidanol Erboğa Z, Duran Y, Polat FR, et al. Protective effects of hesperetin on lipopolysaccharide-induced acute lung injury in a rat model. Turk Gogus Kalp Damar Cerrahisi Derg 2020;28:359-68.
23. Aksu F, Aksu B, Unlu N, Karaca T, Ayvaz S, Erman H, et al. Antioxidant and renoprotective effects of sphingosylphosphorylcholine on contrast-induced nephropathy in rats. Ren Fail 2016;38:1089-98.
24. Han M, Li Y, Liu M, Li Y, Cong B. Renal neutrophil gelatinase associated lipocalin expression in lipopolysaccharide-induced acute kidney injury in the rat. BMC Nephrol 2012;13:25.
25. Verbrugge FH, Dupont M, Shao Z, Shrestha K, Singh D, Finucan M, et al. Novel urinary biomarkers in detecting acute kidney injury, persistent renal impairment, and all-cause mortality following decongestive therapy in acute decompensated heart failure. J Card Fail 2013;19:621-8.
26. Lattanzio MR, Kopyt NP. Acute kidney injury: new concepts in definition, diagnosis, pathophysiology, and treatment. J Am Osteopath Assoc 2009;109:13-9.
27. Kumar B, Gupta SK, Srinivasan BP, Nag TC, Srivastava S, Saxena R, et al. Hesperetin rescues retinal oxidative stress, neuroinflammation and apoptosis in diabetic rats. Microvasc Res 2013;87:65-74.
28. Duran Y, Karaboğa İ. Effect of hesperetin on systemic inflammation and hepatic injury after blunt chest trauma in rats. Biotech Histochem 2020;95:297-304.
29. Trivedi PP, Kushwaha S, Tripathi DN, Jena GB. Cardioprotective effects of hesperetin against doxorubicin-induced oxidative stress and DNA damage in rat. Cardiovasc Toxicol 2011;11:215-25.

Medical Journal of Bakırköy

Med J Bakirkoy 2022;18:258-265
DOI: 10.4274/BMJ.galenos.2022.2022.4-7

Research

Impact of Malnutrition on Prognosis in Patients with HER2-negative Metastatic Gastric Cancer

Metastatik HER2-negatif Mide Kanseri Hastalarında Malnütrisyonun Prognoz Üzerine Etkisi

Atakan Topçu, Ayşe İrem Yasin

Bezmialem Vakif University Faculty of Medicine, Department of Medical Oncology, Istanbul, Turkey

ABSTRACT

Objective: Malnutrition is common in patients with gastric cancer and may adversely affect their prognosis. This study investigated the impact of malnutrition on overall survival (OS) in patients with metastatic gastric cancer by computing the Malnutrition Universal Screening Tool (MUST), one of the most used nutritional screening tools.

Methods: Seventy-seven patients diagnosed with HER2-negative metastatic gastric cancer were included in this retrospective study. All patients had pathologically metastatic disease at diagnosis for gastric adenocarcinoma and received standard-based chemotherapy as first-line treatment. The MUST was used to evaluate the malnutrition risk. It was considered the participants with a MUST score ≥1 (moderate and high risk) as malnourished patients and those with a MUST score of 0 (low risk) as not malnourished patients. We analyzed the patient characteristics, the MUST score and the OS outcomes.

Results: The mean age was 58.7±13.6, and 68.8% were male. The most common metastatic sites were the peritoneum (64.9%) and liver (44.2%). The median MUST score was 2 (0-4). According to the MUST, 50 patients (64.9%) had moderate-high risk in our study. The median OS was 11.2 months in this study. Patients with moderate and high risk had a shorter median OS than patients with low risk (8.8 months vs. 14.0 months, p=0.034). In the univariate Cox regression analysis for death risk, >10% of weight loss [hazard ratio (HR): 1.60], MUST score ≥1 (HR: 1.69), and albumin <3.5 g/dL (HR: 1.64) were found to be an increased risk factor for death. But, statistically significant results were not obtained in the multivariate analysis.

Conclusion: The median OS was significantly lower in malnourished patients than in non-malnourished patients. However, the effects of moderate-high risk MUST, low serum albumin, and >10% of weight loss on the death risk may not be evaluated independently. The high prevalence of malnutrition and its relation to poor survival highlights the significance of routine screening for malnutrition with MUST in patients with gastric cancer.

Keywords: Albumin, gastric cancer, malnutrition, Malnutrition Universal Screening Tool, overall survival, weight loss

ÖZ

Amaç: Mide kanseri hastalarında malnütrisyon yaygındır ve hastaların prognozlarını olumsuz etkileyebilir. Bu çalışma, en çok kullanılan malnütrisyon tarama araçlarından biri olan Malnutrition Universal Screening Tool (MUST) skorunu hesaplayarak metastatik mide kanserli hastalarda malnütrisyonun genel sağkalım (GSK) üzerine olan etkisini araştırmayı amaçlamıştır.

Gereç ve Yöntem: Bu retrospektif çalışmaya HER2-negatif metastatik mide kanseri tanılı 77 hasta dahil edildi. Tüm hastalar mide adenokarsinom tanısı sırasında patolojik olarak metastatik hastalığa sahipti ve birinci basamak tedavi olarak standart bazlı kemoterapi almışlardı. MUST, malnütrisyon riskini değerlendirmek için kullanıldı. MUST puanı ≥1 (orta ve yüksek riskli) olan katılımcılar malnütrise, MUST puanı 0 (düşük riskli) olanlar malnütrisyonu olmayan hasta olarak kabul edildi. Hasta özelliklerini, MUST skorunu ve GSK sonuçlarını analiz ettik.

Bulgular: Yaş ortalaması 58,7±13,6 idi ve %68,8'i erkekti. En sık metastatik bölgeler periton (%64,9) ve karaciğer (%44,2) idi. Medyan MUST skoru 2 (0-4) idi. MUST'ye göre çalışmamızda 50 hastada (%64,9) orta-yüksek risk vardı. Medyan GSK çalışmamızda 11,2 aydı. Orta ve yüksek riskli hastalar düşük riskli hastalara göre daha kısa medyan GSK'ye sahiplerdi (8,8 ay ve 14,0 ay, p=0,034). Ölüm riski için tek değişkenli Cox regresyon analizinde >%10 kilo kaybı [risk oranı (HR): 1,60], MUST skor ≥1 (HR: 1,69) ve albümin <3,5 g/dL (HR: 1,64) ölüm için artmış birer risk faktörleri olarak bulunmuştur. Ancak çok değişkenli analizde istatistiksel olarak anlamlı sonuçlar elde edilememiştir.

Address for Correspondence: Atakan Topçu, Bezmialem Vakif University Faculty of Medicine, Department of Medical Oncology, Istanbul, Turkey
E-mail: atakantopcu@hotmail.com ORCID ID: orcid.org/0000-0002-3634-4820

Cite as: Topçu A, Yasin Aİ. Impact of Malnutrition on Prognosis in Patients with HER2-negative Metastatic Gastric Cancer. Med J Bakirkoy 2022;18:258-265

Received: 08.04.2022
Accepted: 26.05.2022

Sonuç: Medyan GSK malnütrise hastalarda, malnütrisyonu olmayan hastalardan önemli ölçüde daha kısaydı. Ancak orta-yüksek risk MUST, düşük serum albümini ve >%10 kilo kaybının ölüm riski üzerindeki etkileri birbirlerinden bağımsız olarak değerlendirilemeyebilir. Malnütrisyonun yüksek prevalansı ve daha kötü sağkalım ile ilişkisi, mide kanserli hastalarda MUST ile rutin malnütrisyon taramasının önemini vurgulamaktadır.

Anahtar Kelimeler: Albümin, mide kanseri, malnütrisyon, Malnutrition Universal Screening Tool, genel sağkalım, kilo kaybı

INTRODUCTION

Gastric cancer is a highly lethal and frequently incurable malignancy. Most patients have pathological human epidermal growth factor receptor-2 (HER2)-negative disease at an advanced stage. While systemic chemotherapy is the most preferred treatment option, it often requires nutritional, pain, and other supportive management (1,2).

Malnutrition is a deficiency in energy and nutrients and skeletal muscle loss, associated with functional and physical impairment, increased adverse effects of chemotherapy, poor tumor response to chemotherapy, quality of life, and overall survival (OS). Malnutrition is present in 40%-80% of cancer patients and is significantly related to morbidity and mortality in patients with metastatic cancer (3-6). Malnutrition is also more common and severe in gastrointestinal tract cancer than in other malignancies (7,8). Thus, malnutrition might affect prognosis in gastric cancer patients; hence, screening for malnutrition might be crucial for providing appropriate nutritional management, clinical benefit, and the survival advantage in gastric cancer.

Several screening tools have been improved to evaluate the risk of malnutrition. Among them, the Malnutrition Universal Screening Tool (MUST) is a validated simple screening tool frequently used in patients with cancer (7,9-11). Additionally, the European Society for Clinical Nutrition and Metabolism (ESPEN) suggests routine screening in gastrointestinal cancer patients using validated scales to notice and treat malnutrition (12).

This study analyzed the impact of malnutrition on OS in patients with metastatic gastric cancer by computing the MUST score, one of the most used nutritional screening tools.

METHODS

Study Design and Patients

A total of 77 patients diagnosed with metastatic gastric cancer between January 2013 and March 2020 were included in this retrospective study. The inclusion criteria comprised [1] those who had pathological metastatic disease at diagnosis for gastric adenocarcinoma and [2] those who received standard-based chemotherapy as first-line treatment. The exclusion criteria were [1] those who did not receive chemotherapy; [2] those aged <18 years; [3] those who had Eastern Cooperative Oncology Group Performance Status (ECOG PS) >3; [4] those who could not be assessed by MUST because of lack of patient data; [5] and those who had HER2-positive disease.

Patient Evaluation

Patients' general clinical characteristics were noted. Disease evaluations were assessed with computed tomography as a standard. Treatment response was determined by Response Evaluation Criteria in Solid Tumors version 1.1 criteria. OS was defined as the time from the date of metastatic disease diagnosis until the last date the patient was alive or dead. Malnutrition risk status was screened for using MUST at the diagnosis of metastatic disease. The Bezmialem Vakif University Non-Interventional Research Ethics Committee approved the study with the reference number 2021/395 (date: 30.11.2021).

Malnutrition Screening Tool

The MUST was used to evaluate the malnutrition risk. The MUST scale is the sum of scores of 3 factors: the body mass index (BMI) at presentation (>20.0=0, BMI 18.5-20.0=1, BMI<18.5=2), the percentage of total body weight loss over the last 3-6 months (weight loss <5%=0, weight loss 5%-10%=1, weight loss >10%=2), and acute disease effect score (adding a score of 2 if there is no nutritional intake for >5 days). The overall risk of malnutrition was defined as low risk if MUST score=0, moderate risk if the MUST score=1, and high risk if MUST score≥2. Finally, we considered the participants with a MUST score≥1 as malnourished patients and those with a MUST score of 0 as not malnourished patients. MUST scores were calculated at diagnosis of metastatic disease.

Statistical Analysis

Statistical data were provided using the Statistical Package for the Social Sciences version 24.0 (SPSS Inc., Chicago, IL, USA). Qualitative variables were detailed by frequencies and percentages, and continuous and ordinal variables were detailed by mean, standard deviation, median and range. Kolmogorov-Smirnov test was performed to determine the normal distribution range. The Pearson $\chi 2$ test was used to compare qualitative variables. Patients' characteristics were assessed with descriptive analysis. The median cut-off value of the prognostic nutrition index (PNI) and neutrophil-to-lymphocyte ratio (NLR) was detected by performing the receiver operating characteristic curve analysis. OS analysis

was assessed using Kaplan-Meier survival curves and the log-rank test. Univariate and multivariate Cox proportional hazard models were used to identify predictors of death risk. Statistical significance was set at a p-value of <0.05.

RESULTS

Patients with HER2-negative *de novo* metastatic gastric cancer (n=77) were included in the study. The mean age was 58.7±13.6, and 68.8% were male. The mean BMI was 24.5±5.2. The ECOG PS of 76.6% of the patients was 0-1. All patients had metastatic disease at diagnosis. Standard chemotherapies were initiated for all patients as first-line treatment. The rate of patients who started second-line chemotherapy was 36.4%, the rate of patients who started third-line chemotherapy was 15.6%, and the rate of patients who began fourth-line chemotherapy was 2.6%. The most common metastatic sites were the peritoneum (64.9%) and liver (44.2%). Thirty-one (40.3%) patients had single-organ metastasis. The MUST was used to evaluate the malnutrition risk as a nutritional screening tool. The median MUST score was 2 (0-4). According to the MUST, 27 patients (35.1%) had a low risk, 8 patients (10.4%) had a moderate risk, and 42 patients (54.5%) had a high risk. Thus, 50 patients (64.9%) had moderate-high risk in our study. The participants with moderate and high risk were considered malnourished patients. While the number of patients with weight loss of >10% and loss of appetite was significantly higher in patients with moderate and high risk than in low-risk patients, the mean BMI and the number of patients with obesity was substantially lower. The baseline characteristics of the 77 patients are shown in Table 1. The comparison of the biochemical variables was also evaluated according to the MUST in our study, but no significant difference was found between the groups (Table 2).

The median follow-up time was 11.2 months (1.1-30.3) in our study. The median OS was 11.2 months [9.1-13.3, 95% confidence interval (CI)]. The rate of patients who reached median OS was 88% at 6 months, 46% at 1 year, and 9% at 2 years. Patients with moderate and high risk had a shorter median OS than patients with low risk [8.8 months (6.6-11.1, 95% CI) vs. 14.0 months (11.7-16.4, 95% CI), p=0.034; Figure 1].

In the univariate Cox regression analysis for death risk, >10% of weight loss [hazard ratio (HR): 1.60], MUST score≥1 (moderate-high risk) (HR: 1.69), and albumin <3.5 g/dL (HR: 1.64) were found to be an increased risk factor for death. Statistically significant results were not obtained in the multivariate analysis, but the MUST score reached an almost statistically significant result (p=0.051). The results of univariate and multivariate analyses for death risk are shown in Table 3.

DISCUSSION

Malnutrition is common in patients with gastric cancer and may adversely affect their prognosis (7,13,14). Therefore, the ESPEN recommends performing a nutritional assessment for patients with gastric cancer at diagnosis using validated scales to determine and treat malnutrition (12). Because metastatic gastric cancer is not curable, the aim is an OS advantage and best supportive care. These patients are thought to have a higher risk of malnutrition; hence, they should be evaluated from this perspective (1,7). Moreover, the malnutrition might reduce the OS in patients with gastric cancer.

Our study analyzed the risk of malnutrition by computing the MUST in 77 patients with HER2-negative metastatic gastric cancer and evaluated the effect of their malnutrition risk on OS. The HER2-positive patients were excluded from our study because of their different prognostic features and treatment. All patients were treated with standard chemotherapies. In our study, the prevalence of malnourished patients, according to the MUST, was 64.9%. They were considered moderate- and high-risk patients. We found that the malnourished patients with metastatic gastric cancer had a shorter median OS than non-malnourished patients (8.8 months vs. 14.0 months, p=0.034). Additionally, we found that albumin of <3.5 g/dL (HR: 1.64), weight loss of >10% (HR: 1.60), and the moderate-high risk by the MUST (HR: 1.69) were significant risk factors for death by the univariate analysis. However, the multivariate analysis found no statistically independent factors when we incorporated other variables on death risk. Nevertheless, the moderate-high risk MUST status showed a trend toward statistical significance (p=0.051). Therefore, the MUST score should be evaluated with albumin and weight loss status to predict the prognosis. We thought that these factors might be related to each other regarding the death risk.

The MUST is cited as one of the best scales to identify malnourished patients with gastrointestinal cancer (10,11,15). According to MUST, moderate and high risk are generally considered equal status for malnourished patients, as in our study (16-18). In a study, the prevalence of cancer patients receiving chemotherapy with moderate-high risk of malnutrition, according to the MUST, was 42% (19). In another study in Spain, the authors detected that 69.9% of the patients with cancer were at nutritional risk using the MUST, and malnutrition was associated with the length of hospital stay (20). In another study, the prevalence of elderly

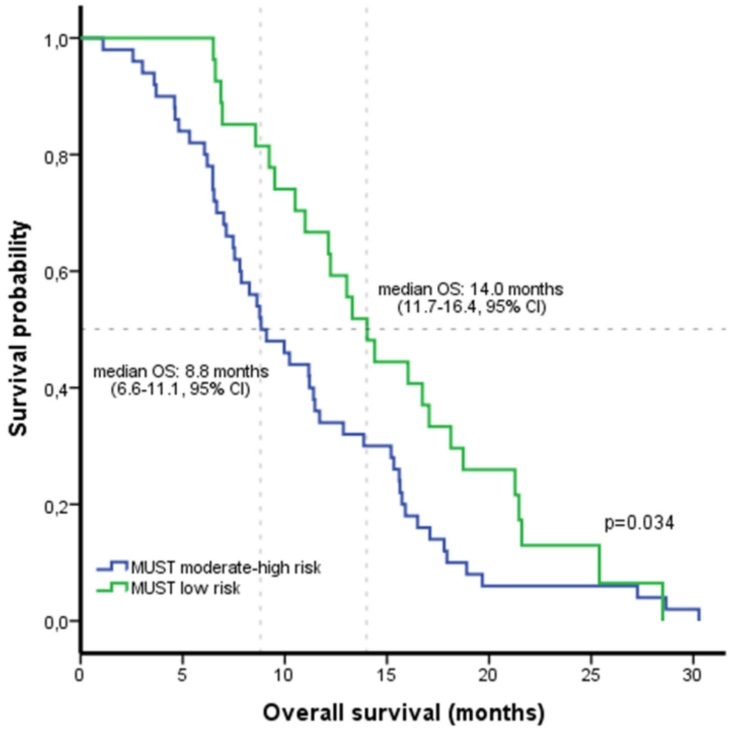

Figure 1. Kaplan-Meier curves of overall survival in patients with low risk and moderate-high risk according to the MUST
MUST: Malnutrition Universal Screening Tool, OS: Overall survival, CI: Confidence interval

cancer patients at risk of malnutrition was 64.8%. However, the authors evaluated the patients for malnutrition risk with the Global Leadership Initiative on the Malnutrition scale. This study also found that the patients at risk of malnutrition showed worse OS than those without risk of malnutrition (21). Moreover, one study that evaluated the importance of malnutrition in patients with esophageal achalasia found that 70% of the patients were at moderate and high risk of malnutrition, according to the MUST. Additionally, the moderate and high risks for malnutrition were associated with the severity of symptoms (17). In a French study that evaluated the malnutrition risk on prognosis in metastatic colorectal cancer patients receiving chemotherapy, malnourished patients had shorter median OS than non-malnourished patients. Malnutrition was evaluated with the nutritional risk index, and it was diagnosed in 65% of patients (5). Moreover, a critical study on MUST identified increased mortality for colorectal cancer patients at moderate and high risk of malnutrition. This relationship was found to be independent (22). According to MUST, those with moderate-high risk had a lower median OS than those with low risk in our research. Although when looking at the risk of death, this risk was not independent. Compared to this study, the relatively small sample size or different primary cancer types might have caused this phenomenon. Nevertheless, one study demonstrated that nutritional screening tools, including MUST, did not strongly influence the prognosis of gastric cancer, where only sarcopenia had predictive value for the prognosis (23). In a Malaysian study, more than half of the cancer patients were at risk of malnutrition with the MUST, and weight loss was identified as an important risk factor for malnutrition. It was also observed that weight loss was associated with poor outcomes (24). In our study, the malnourished patients were significantly associated with poorer median OS, and weight loss was also significantly higher in malnourished patients. Additionally, both malnutrition and weight loss status were associated with an increased risk of death in the univariate analysis. However, no independent effects were found on the risk of death. It might be assumed that these factors cannot be considered independently. We think that these findings highlight the importance of routine malnutrition screening with the MUST in patients with cancer.

Systemic inflammation in cancer patients is an important factor for nutrition and prognosis (25-27). It also contributes to the development of cachexia. Therefore, it might be challenging to manage and reverse these patients' malnutrition and weight loss (25). Cancer patients receiving chemotherapy with weight loss before chemotherapy have a poorer prognosis than those who remain weight-

Table 1. Comparison of the baseline characteristics according to MUST (n=77)

Characteristics	All n (%)	MUST Low risk n (%)	MUST Moderate-high risk n (%)	p-value
Age, years (mean ± SD)	58.7± 3.6	59.6±10.7	58.2±15	0.675
BMI (kg/m^2)	24.5±5.2	27.4±5.5	22.9±4.3	<0.001
Age				
<65 years	50 (64.9%)	19 (38.0%)	31 (62.0%)	0.463
≥65 years	27 (35.1%)	8 (29.6%)	19 (70.4%)	
Gender				
Female	24 (31.2%)	8 (33.3%)	16 (66.7%)	0.830
Male	53 (68.8%)	19 (35.8%)	34 (64.2%)	
ECOG PS				
0-1	59 (76.6%)	20 (33.9%)	39 (66.1%)	0.698
2-3	18 (23.4%)	7 (38.9%)	11 (61.1%)	
Smoking				
Yes	26 (33.8%)	9 (34.6%)	17 (65.4%)	0.953
No	51 (66.2%)	18 (35.3%)	33 (64.7%)	
Obesity (BMI≥30 kg/m^2)				
Yes	14 (18.2%)	9 (64.3%)	5 (35.7%)	0.011
No	63 (81.8%)	18 (28.6%)	45 (71.4%)	
Weight loss (>10%)				
Yes	35 (45.5%)	1 (2.9%)	34 (97.1%)	<0.001
No	42 (54.5%)	26 (61.9%)	16 (38.1%)	
Loss of appetite				
Yes	55 (71.4%)	6 (10.9%)	49 (89.1%)	<0.001
No	22 (28.6%)	21 (95.5%)	1 (4.5%)	
At least ≥1 comorbidity				
Yes	43 (55.8%)	14 (32.6%)	29 (67.4%)	0.604
No	34 (44.2%)	13 (38.2%)	21 (61.8%)	
Localization				
Gastric	57 (74.0%)	18 (31.6%)	39 (68.4%)	0.279
GEJ	20 (26.0%)	9 (45.0%)	11 (55.0%)	
Histologic classification				
Adenocarcinoma	57 (74.0%)	21 (36.8%)	36 (63.2%)	0.581
Signet ring cell carcinoma	20 (26.0%)	6 (30.0%)	14 (70.0%)	
Grade				
Grade 1&2	18 (28.6%)	6 (33.3%)	12 (66.7%)	0.728
Grade 3	45 (71.4%)	13 (28.9%)	32 (71.1%)	

Table 1. Continued

Characteristics	All n (%)	MUST Low risk n (%)	MUST Moderate-high risk n (%)	p-value
Metastatic organs				
1	31 (40.3%)	11 (35.5%)	20 (64.5%)	0.950
>1	46 (59.7%)	16 (34.8%)	30 (65.2%)	
Liver metastasis				
Yes	34 (44.2%)	11 (32.4%)	23 (67.6%)	0.657
No	43 (55.8%)	16 (37.2%)	27 (62.8%)	
Peritoneum metastasis				
Yes	50 (64.9%)	15 (30.0%)	35 (70.0%)	0.205
No	27 (35.1%)	12 (44.4%)	15 (55.6%)	
Lung metastasis				
Yes	19 (24.7%)	6 (31.6%)	13 (68.4%)	0.714
No	58 (75.3%)	21 (36.2%)	37 (63.8%)	

BMI: Body mass index, ECOG PS: Eastern Cooperative Oncology Group Performance Status, GEJ: Gastroesophageal junction, MUST: Malnutrition Universal Screening Tool, SD: Standard deviation

Table 2. Comparison of the biochemical variables according to MUST

Variables	All n (%)	MUST Low risk n (%)	MUST Moderate-high risk n (%)	p-value
PNI				
≤45.5	44 (57.9%)	14 (31.8%)	30 (68.2%)	0.606
>45.5	32 (42.1%)	12 (37.5%)	20 (62.5%)	
NLR				
≤3.08	30 (39.5%)	12 (40.0%)	18 (60.0%)	0.390
>3.08	46 (60.5%)	14 (30.4%)	32 (69.6%)	
Albumin				
<3.5 g/dL	35 (46.1%)	13 (37.1%)	22 (62.9%)	0.619
≥3.5 g/dL	41 (53.9%)	13 (31.7%)	28 (68.3%)	
Hemoglobin				
≤10 g/dL	24 (31.6%)	9 (37.5%)	15 (62.5%)	0.681
>10 g/dL	52 (68.4%)	17 (32.7%)	35 (67.3%)	

PNI: Prognostic nutrition index, NLR: Neutrophil to lymphocyte ratio, MUST: Malnutrition Universal Screening Tool

*It was considered the participants with moderate-high risk for MUST score ≥1 as malnourished patients

stable (25). The relationship between weight loss, systemic inflammation, and poor prognosis in patients with advanced gastrointestinal cancer had been previously demonstrated (25,28,29). However, the effect of weight loss on prognosis is still unclear in cancer patients. A comprehensive study showed that weight loss alone does not determine the full impact of cachexia and is not a prognostic factor. Weight loss, systemic inflammation, and loss of appetite affect patients' prognosis (30). Low serum albumin might reflect malnutrition, and recent studies showed that low serum albumin might be associated with poorer survival in patients with gastric cancer (31,32). One study showed that low serum albumin reflected both malnutrition risk and systemic inflammatory response and it was independently associated with poorer survival in patients with colorectal cancer (33). In our study, we found that low serum albumin increased the risk of death in the univariate analysis. However, it was not significant in the multivariate analysis. NLR and PNI, which were systemic inflammation markers in this study, did not significantly affect death risk.

The nature of gastric cancer and using chemotherapy can also be considered additional risk factors for malnutrition. Chemotherapy has also been demonstrated to be a risk factor for malnutrition (14). All patients received chemotherapy in our study. Thus, it might be essential to evaluate the patients for malnutrition risk from the beginning.

Our study had some limitations. Firstly, this was a retrospective study. Thus, prospective studies with a

Table 3. Results of univariate and multivariate analyses of overall survival

	Univariate analysis		Multivariate analysis	
Variables	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Age ≥65 years	1.02 (0.63-1.64)	0.953	-	-
Male gender	1.12 (0.68-1.85)	0.665	-	-
ECOG PS 2-3	1.15 (0.66-1.97)	0.623	-	-
Smoking	0.96 (0.59-1.56)	0.864	-	-
Obesity (BMI≥30 kg/m^2)	1.07 (0.59-1.92)	0.825	-	-
Weight loss (>10%)	1.60 (1.01-2.55)	**0.046**	1.21 (0.68-2.17)	0.516
Localization (non-gastric)	0.70 (0.41-1.19)	0.189	-	-
Signet ring cell carcinoma	1.03 (0.60-1.77)	0.902	-	-
Grade 3	1.42 (0.82-2.48)	0.215	-	-
≥1 Comorbidity	0.96 (0.61-1.53)	0.867	-	-
>1 Metastatic organs	1.03 (0.65-1.63)	0.909	-	-
Liver metastasis	0.99 (0.63-1.57)	0.972	-	-
Peritoneum metastasis	1.46 (0.90-2.36)	0.130	-	-
Lung metastasis	1.11 (0.65-1.90)	0.696	-	-
MUST score≥1 (moderate-high risk)	1.69 (1.03-2.75)	**0.036**	1.64 (0.99-2.70)	0.051
PNI≤45.5	1.50 (0.93-2.40)	0.095	-	-
NLR>3.08	1.34 (0.83-2.18)	0.229	-	-
Albumin<3.5 g/dL	1.64 (1.03-2.63)	**0.038**	1.57 (0.98-2.51)	0.060
Hemoglobin≤10 g/dL	1.10 (0.66-1.87)	0.729	-	-

BMI: Body mass index, ECOG PS: Eastern Cooperative Oncology Group Performance Status, HR: hazard ratio, CI: Confidence interval, NLR: Neutrophil to lymphocyte ratio, PNI: Prognostic nutrition index, MUST: Malnutrition Universal Screening Tool

larger number of patients can enable us to offer stronger recommendations. Second, the relationship of MUST with other clinical findings, such as quality of life, physical function, muscle strength measurement, and sarcopenia, should also be investigated. Additionally, the MUST was not compared with other malnutrition screening tools in this study.

CONCLUSION

In this study, the median OS was significantly lower in malnourished patients than in non-malnourished patients. It was found that moderate-high risk MUST, low serum albumin, and >10% of weight loss increased the death risk. However, these factors had no independent effects on the risk of death in the multivariate analysis. The high prevalence of malnutrition and its relation to poorer survival, as noted in many studies, highlights the significance of routine screening for malnutrition with MUST in patients with gastric cancer, as early intervention results in improved outcomes.

Acknowledgments: The authors thank Dr. Mehmet Beşiroğlu for editing assistance during the study period.

ETHICS

Ethics Committee Approval: The Bezmialem Vakif University Non-Interventional Research Ethics Committee approved the study with the reference number 2021/395 (date: 30.11.2021).

Informed Consent: Retrospective study.

Authorship Contributions

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

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. Digklia A, Wagner AD. Advanced gastric cancer: Current treatment landscape and future perspectives. World J Gastroenterol 2016;22:2403-14.
2. Rinninella E, Cintoni M, Raoul P, Pozzo C, Strippoli A, Bria E, et al. Effects of nutritional interventions on nutritional status in patients

with gastric cancer: A systematic review and meta-analysis of randomized controlled trials. Clin Nutr ESPEN 2020;38:28-42.

3. Nitenberg G, Raynard B. Nutritional support of the cancer patient: issues and dilemmas. Crit Rev Oncol Hematol 2000;34:137-68.
4. Senesse P, Assenat E, Schneider S, Chargari C, Magné N, Azria D, et al. Nutritional support during oncologic treatment of patients with gastrointestinal cancer: who could benefit? Cancer Treat Rev 2008;34:568-75.
5. Barret M, Malka D, Aparicio T, Dalban C, Locher C, Sabate JM, et al. Nutritional status affects treatment tolerability and survival in metastatic colorectal cancer patients: results of an AGEO prospective multicenter study. Oncology 2011;81:395-402.
6. Zhang X, Edwards BJ. Malnutrition in Older Adults with Cancer. Curr Oncol Rep 2019;21:80.
7. Deftereos I, Djordjevic A, Carter VM, McNamara J, Yeung JM, Kiss N. Malnutrition screening tools in gastrointestinal cancer: A systematic review of concurrent validity. Surg Oncol 2021;38:101627.
8. Chen XY, Zhang XZ, Ma BW, Li B, Zhou DL, Liu ZC, et al. A comparison of four common malnutrition risk screening tools for detecting cachexia in patients with curable gastric cancer. Nutrition 2020;70:110498.
9. Mueller C, Compher C, Ellen DM; American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) Board of Directors. A.S.P.E.N. clinical guidelines: Nutrition screening, assessment, and intervention in adults. JPEN J Parenter Enteral Nutr 2011;35:16-24.
10. Ye XJ, Ji YB, Ma BW, Huang DD, Chen WZ, Pan ZY, et al. Comparison of three common nutritional screening tools with the new European Society for Clinical Nutrition and Metabolism (ESPEN) criteria for malnutrition among patients with geriatric gastrointestinal cancer: a prospective study in China. BMJ Open 2018;8:e019750.
11. Boléo-Tomé C, Monteiro-Grillo I, Camilo M, Ravasco P. Validation of the Malnutrition Universal Screening Tool (MUST) in cancer. Br J Nutr 2012;108:343-8.
12. Muscaritoli M, Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, et al. ESPEN practical guideline: Clinical Nutrition in cancer. Clin Nutr 2021;40:2898-913.
13. Li Q, Zhang X, Tang M, Song M, Zhang Q, Zhang K, et al. Different muscle mass indices of the Global Leadership Initiative on Malnutrition in diagnosing malnutrition and predicting survival of patients with gastric cancer. Nutrition 2021;89:111286.
14. Fujiya K, Kawamura T, Omae K, Makuuchi R, Irino T, Tokunaga M, et al. Impact of Malnutrition After Gastrectomy for Gastric Cancer on Long-Term Survival. Ann Surg Oncol 2018;25:974-83.
15. Chao PC, Chuang HJ, Tsao LY, Chen PY, Hsu CF, Lin HC, et al. The Malnutrition Universal Screening Tool (MUST) and a nutrition education program for high risk cancer patients: strategies to improve dietary intake in cancer patients. Biomedicine (Taipei) 2015;5:17.
16. Zhang Z, Wan Z, Zhu Y, Zhang L, Zhang L, Wan H. Prevalence of malnutrition comparing NRS2002, MUST, and PG-SGA with the GLIM criteria in adults with cancer: A multi-center study. Nutrition 2021;83:111072.
17. Milito P, Aquilino K, Lazzari V, Boveri S, Munizio N, Ogliari C, et al. The Malnutrition Universal Screening Tool can predict malnutrition in patients with esophageal achalasia. Eur J Gastroenterol Hepatol 2020;32:1135-40.
18. Stratton RJ, Hackston A, Longmore D, Dixon R, Price S, Stroud M, et al. Malnutrition in hospital outpatients and inpatients: prevalence, concurrent validity and ease of use of the 'malnutrition universal screening tool' ('MUST') for adults. Br J Nutr 2004;92:799-808.
19. Hettiarachchi J, Madubhashini P, Miller M. Agreement between the Malnutrition Universal Screening Tool and the Patient-Generated Subjective Global Assessment for Cancer Outpatients Receiving Chemotherapy: A Cross-Sectional Study. Nutr Cancer 2018;70:1275-82.
20. Vidal-Casariego A, Amigo-Otero E, Pita-Gutiérrez F, Lugo-Rodríguez G, Almeida-Seoane C, Martínez-Ramonde T. Comparison of MUST and Nutriscore for the Screening of Malnutrition in Hospitalized Oncology Patients. Nutr Cancer 2021;73:1941-6.
21. Zhang X, Tang M, Zhang Q, Zhang KP, Guo ZQ, Xu HX, et al. The GLIM criteria as an effective tool for nutrition assessment and survival prediction in older adult cancer patients. Clin Nutr 2021;40:1224-32.
22. Almasaudi AS, McSorley ST, Dolan RD, Edwards CA, McMillan DC. The relation between Malnutrition Universal Screening Tool (MUST), computed tomography-derived body composition, systemic inflammation, and clinical outcomes in patients undergoing surgery for colorectal cancer. Am J Clin Nutr 2019;110:1327-34.
23. Huang DD, Cai HY, Chen XY, Dong WX, Wangchuk D, Yan JY, et al. Value of Sarcopenia defined by the new EWGSOP2 consensus for the prediction of Postoperative Complications and Long-term Survival after Radical Gastrectomy for Gastric Cancer: A comparison with four common nutritional screening tools. J Cancer 2020;11:5852-60.
24. Loh KW, Vriens MR, Gerritsen A, Borel Rinkes IH, van Hillegersberg R, Schippers C, et al. Unintentional weight loss is the most important indicator of malnutrition among surgical cancer patients. Neth J Med 2012;70:365-9.
25. McMillan DC. An inflammation-based prognostic score and its role in the nutrition-based management of patients with cancer. Proc Nutr Soc 2008;67:257-62.
26. Shimada H, Takiguchi N, Kainuma O, Soda H, Ikeda A, Cho A, et al. High preoperative neutrophil-lymphocyte ratio predicts poor survival in patients with gastric cancer. Gastric Cancer 2010;13:170-6.
27. Sakurai K, Ohira M, Tamura T, Toyokawa T, Amano R, Kubo N, et al. Predictive Potential of Preoperative Nutritional Status in Long-Term Outcome Projections for Patients with Gastric Cancer. Ann Surg Oncol 2016;23:525-33.
28. O'Gorman P, McMillan DC, McArdle CS. Impact of weight loss, appetite, and the inflammatory response on quality of life in gastrointestinal cancer patients. Nutr Cancer 1998;32:76-80.
29. O'Gorman P, McMillan DC, McArdle CS. Prognostic factors in advanced gastrointestinal cancer patients with weight loss. Nutr Cancer 2000;37:36-40.
30. Fearon KC, Voss AC, Hustead DS; Cancer Cachexia Study Group. Definition of cancer cachexia: effect of weight loss, reduced food intake, and systemic inflammation on functional status and prognosis. Am J Clin Nutr 2006;83:1345-50.
31. Ouyang X, Dang Y, Zhang F, Huang Q. Low Serum Albumin Correlates with Poor Survival in Gastric Cancer Patients. Clin Lab 2018;64:239-45.
32. Liu C, Li X. Stage-Dependent Changes in Albumin, NLR, PLR, and AFR are Correlated with Shorter Survival in Patients with Gastric Cancer. Clin Lab 2019;65.
33. Almasaudi AS, Dolan RD, Edwards CA, McMillan DC. Hypoalbuminemia Reflects Nutritional Risk, Body Composition and Systemic Inflammation and Is Independently Associated with Survival in Patients with Colorectal Cancer. Cancers (Basel) 2020;12:1986.