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Evaluation of the protective effect of Red Ginseng on lipid profile, endothelial and oxidative damage after splenectomy in rats

Ratlarda splenektomi sonrası Red Ginseng'in lipit profili, endotel ve oksidatif hasar üzerinde koruyucu etkisinin değerlendirilmesi

Koray Koşmaz[1], Abdullah Durhan[1], Abdullah Şenlikçi[1], Marlen Süleyman[1], Mustafa Taner Bostancı[2], Mevlüt Recep Pekçici[1], Mehmet Şeneş[3], İlknur Alkan Kuşabbi[3], Mehmet Alparslan Gönültaş[4], Sema Hücümenoğlu[4], Aziz Mutlu Barlas[1], Yusuf Murat Bağ[5], Can Ersak[1], Ender Ergüder[1]

Abstract

Aim: It was aimed to examine the effects of Red Ginseng (RG), which has anti-inflammatory and antioxidant properties, on the histopathological alterations and lipid metabolism following splenectomy.

Methods: Rats were randomly divided into 3 groups of 10 rats each. Only laparotomy was conducted in group 1 (sham). In group 2 (control), splenectomy was performed but no therapy was offered. Splenectomy was performed and RG extract was supplied orogastrically at a dosage of 100mg/kg/day for 28 days in group 3 (RG group). At the end of the 28-day period, tissue samples were obtained for the assessment of histopathological alterations in the vascular, intestinal, and lung tissues, and blood samples were obtained for biochemical analysis.

Results: The RG group had reduced significantly total cholesterol (TC), triglyceride (TG), and very low density lipoprotein (VLDL) levels than the control group ($p<0.05$). When mononuclear cell infiltration in colon and lung tissues was evaluated, it was shown to be considerably lower in the RG group ($p<0.05$). For aortic tissues, there was no difference between the groups. Malondialdehyde (MDA) levels were observed to be considerably lower in the RG group compared to the control group ($p<0.05$). The total sulfhydryl (t-SH) level increased considerably in the RG group as compared to the control group. ($p<0.05$)

Conclusions: After splenectomy, RG usage decreases oxidative stress in mice. It has also been demonstrated to have a regulating impact on lipid metabolism. Furthermore, the administration of RG following splenectomy was found to have a poor likelihood of improving vascular, lung, and colon epithelial regeneration.

Keywords: Splenectomy, Red ginseng, lipid profile, antioxidant.

Öz

Amaç: Anti-inflamatuar ve antioksidan etkili red ginsegin splenektomi sonrası görülen histopatolojik değişiklikler ve lipid profili üzerindeki etkisi araştırıldı.

Yöntemler: Sıçanlar rastgele her biri 10 sıçandan oluşan 3 gruba ayrıldı. Grup 1'de (sham), sadece laparotomi yapıldı. Grup 2'de (kontrol), splenektomi uygulandı fakat ek hiçbir tedavi uygulanmadı. Grup 3'te (Red ginseng grubu), splenektomi yapıldı ve Red Ginseng (RG) ekstresi orogastrik yoldan 100 mg / kg / gün doz olarak 28 gün süreyle uygulandı. 28 günün sonunda vasküler, intestinal ve akciğer dokularındaki histopatolojik değişiklikler incelemesi için doku örnekleri alındı ve biyokimyasal analiz için kan örnekleri alındı.

Bulgular: Total kolestrol (TC), trigliserit (TG) ve çok düşük yoğunluklu lipoprotein (VLDL) düzeyleri kontrol grubuna göre RG grubunda anlamlı olarak azalmıştır ($p<0,05$). Kolon ve akciğer dokusundaki mononükleer hücre infiltrasyonunun RG grubunda, kontrol grubuna göre anlamlı derecede azaldığı görüldü ($p<0,05$). Aort dokusunda hiç bir grup arasında fark saptanmadı. Malondialdehit (MDA) seviyelerinin de RG grubunda kontrol grubuna göre anlamlı derecede azaldığı görüldü ($p<0,05$). Total sülfhidril (t-SH) seviyesinin ise RG grubunda kontrol grubuna göre anlamlı derecede artış gösterdiği saptandı ($p<0,05$).

Sonuç: RG kullanımı splenektomi yapılan ratlarda oksidatif stresi azaltmakta ve lipid metabolizması üzerinde düzenleyici etki göstermektedir. Bununla birlikte RG kullanımının splenektomi sonrası ortaya çıkan vasküler, akciğer ve kolon epitelindeki rejenerasyonu artırıcı etkisi gözlenmemiştir.

Anahtar Kelimeler: Splenektomi, Red ginseng, lipid profili, antioksidan.

KK: 0000-0003-2111-3162
AD: 0000-0002-5622-9678
AŞ: 0000-0002-4321-4004
MS: 0000-0001-6979-4150
MTB: 0000-0003-2876-2683
MRP: 0000-0002-5566-8134
MŞ: 0000-0002-7924-5478
İAK: 0000-0003-0000-1196
MAG: 0000-0002-4024-585X
SH: 0000-0002-6898-4101
AMB: 0000-0002-4941-3186
YMB: 0000-0002-0159-9356
CE: 0000-0002-9658-6063
EE: 0000-0001-5289-3718

[1] University of Health Sciences, Ankara Education and Research Hospital, Department of General Surgery, Ankara, Turkey.
[2] University of Health Sciences, Dış Kapı Yıldırım Beyazıt Education and Research Hospital, Department of General Surgery, Ankara, Turkey.
[3] University of Health Sciences, Ankara Education and Research Hospital, Department of Biochemist, Ankara, Turkey.
[4] University of Health Sciences, Ankara Education and Research Hospital, Department of Medical Pathology, Ankara, Turkey.
[5] University of Health Sciences, Van Education and Research Hospital, Department of General Surgery, Ankara, Turkey.

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Sorumlu yazar / Corresponding author:
Koray Koşmaz
Adres/Address: Ankara Eğitim ve Araştırma Hastanesi Genel Cerrahi Kliniği A blok 1 kat Hacettepe Mah, Ulucanlar Cad, No:87, Altındağ, Ankara, Turkey.

e-mail: koraykosmaz@hotmail.com
Tel/Phone: 00905065362371

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Introduction

Today, the most common reason for splenectomy is trauma. Furthermore, splenectomy is performed in response to a variety of etiological reasons, including hematological and primary spleen disorders. Hematological consequences of splenectomy include leukocytosis and thrombocytosis [1, 2]. The spleen also plays a significant role in lipid metabolism and works as a lipid reservoir, according to research conducted in humans and rats, and as a result, hyperlipidemia is seen in many cases following splenectomy [3, 4]. The filtration of aberrant and aged erythrocytes by activated macrophages is another crucial function of the spleen [5]. Because the phagocytosis function is lost in splenectomy patients, the number of injured cells in the bloodstream rises, vascular homeostasis is disrupted, and the coagulation system is activated, resulting in vascular problems [2]. Pulmonary hypertension is one of the most common diseases linked with vascular problems after splenectomy [1].

In today's world, Panax ginseng is one of the most often utilized herbal remedies in both Asian and Western countries. Ginseng is a succulent perennial herb that grows slowly. Red ginseng (RG) is the most important sub-component of this plant root, which is made up of ginsoids. RG; It has been used in traditional medicine for many years, particularly in far eastern countries like Korea and Japan, and its effects are still being investigated in many studies. It is used in many fields of medicine, including liver, cardiovascular disease, kidney disease, autoimmune systemic diseases, various malignancies, and immune system strengthening [6-8]. According to research, RG regulates the activity of inflammatory signaling pathways, which have anti-inflammatory properties in inflammatory responses [9]. Although several animal research has demonstrated the possible influence of RG on blood lipid profiles, there is not enough research to evaluate the lipid profile after splenectomy [10, 11].

In this study, we examined the histopathological changes in vascular, intestinal, and respiratory tissues after splenectomy and the possible effects of RG on these changes.

Material and methods

The experimental procedures and technique of this study met the requirements of the National Guidelines for the Use and Care of Laboratory Animals, and the Animal Ethics Committee approved this study (approval date: 16.04.2021, issue 0065:661). All transactions were carried out by adhering to ethical rules and principles.

Animals and experimental-surgical procedures

The study sample consisted of 30 female adult Wistar albino rats, each weighing 230 ± 22 g. Rats have housed in wire mesh cages at a sustained temperature of 21 ± 2 °C with a 12-hour light/dark cycle. The animals had a diet consisting of standard laboratory food and as much water as they wanted. For rats, access to food was stopped 12 hours before anesthesia and access to water 2 hours before anesthesia. The same team performed anesthesia and surgical intervention under sterile conditions. Before intervention procedures, 50 mg/kg ketamine hydrochloride (Ketalar; Parke-Davis, Detroit, MI, USA) and 5 mg/kg Xylazine (Rompun; Bayer AG, Leverkusen, Germany) were anesthetized via intramuscular injection. Rats were randomly divided into 3 groups of 10 rats: sham group, control group, and treatment group. In group 1 (sham), the only laparotomy was performed. In group 2 (control), the spleen peduncle was ligated with 4/0 silk and splenectomy was performed In group 3 (RG group), spleen peduncle was ligated with 4/0 silk, splenectomy was performed, then RG [Korean 6-Year Root Red Ginseng Extract (Samsung, Korea)] was administered with the help of an orogastric tube at a dose of 100 mg/kg/day for 28 days. After the aforementioned procedures were completed, the abdominal incisions were closed in two continuous layers with 3/0 silk sutures in all three groups. The rats were allowed to feed after surgery. All animals were euthanized after 28 days using an overdose of ketamine. After laparotomy and sternotomy, 4-5 cc blood samples were taken for biochemical analysis of lipid profile, platelet, leukocytes, antioxidant parameters, and oxidative stress markers. Aortic segment, 8 cm distal segments of the total colectomy specimen, and lung tissue samples were taken for histopathological examination.

In the Ankara Training and Research Hospital Medical Biochemistry Department, white blood cell (WBC), hemogram, lipid profiles, malondialdehyde (MDA), and total sulfhydryl (t-SH) in serum were assessed. On the day the samples were obtained, lipid profiles and hemograms were taken. Serum samples were stored at -80 ° C until analysis day for the determination of MDA and t-SH. Hemogram measurements were performed on the Sysmex XN-Series instrument. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) measurements in serum were made using Roche Cobas 8000 biochemistry analyzer using Roche Cobas commercial kits for clinical purposes. Very low density lipoprotein (VLDL) values were calculated by total cholesterol-(HDL-C) + LDL-C) formula. The levels of MDA were determined using the fluorometric approach, as described by Wasowicz et al. [12]. The reaction product of thiobarbituric acid (TBA) and MDA was separated in butanol following the reaction. Then, at a wavelength of 547 nm for emission and 525 nm for excitation, a spectrofluorometrical measurement was performed. The standard was a solution of 1,1',3,3' tetra ethoxy propane ranging from 0 to 5 mol/L. To assess MDA levels in tissue, 50 mL serum was placed in 10 ml glass tubes, each with 1 ml distilled water. The acetic acid was then combined with a 1 mL solution containing 29 mmol/L TBA. Following that, the samples were placed in a water bath and exposed to heat between 95° and 100°C for 1 hour. After cooling the heated samples, they were mixed with 25 liters of 5 mol/L hydrochloric acids (HCL) and agitated for 5 minutes to extract the reaction mixture using 3.5 mL n-butanol. A fluorometer (HITACHI F-2500) was used to measure the fluorescence in the butanol extract at 547 nm and 525 nm wavelengths for emission and excitation, respectively, after the butanol phase was separated by centrifugation for 10 minutes at 1500 g. MDA concentrations were expressed in milligrams per liter (mol/L). The principle described by Taylan et al. was used to perform the t-SH measurement by adapting the Ellman reaction to the microplate method, they were able to use this method [13]. The study utilized a methanolic 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) solution that reacts with sulfhydryl groups. A multi-measurement mode microplate reader with a monochromator was used to measure the reaction products. The measurement was carried out at 412 nm. 200 µL of Tris buffer (0.25 M Tris-HCl, pH 8.2 containing 20 mM EDTA) was pipetted and 25 µL of homogenate was added. 10 µL of DTNB reagent (4 mg / mL in methanol) was added to each well and incubated for 15 minutes at room temperature, then the absorbance of yellow 5-thio-2-nitrobenzoic acid (TNB) at 412 nm (A2) was measured against blank (A1). The same settings were used to test glutathione standards, and the curve was created using linear regression analysis. The standard curve developed using reduced

glutathione (GSH) (between 0 and 450 M) was used to calculate the quantity of the t-SH group in the samples according to the net absorbance (A2-A1) mol/L is the unit of measurement for t-SH levels.

Histopathological analysis

Samples were obtained after all tissues were preserved with 10% neutral formalin. Tissues were traced using an automatic tissue tracking system, and subsequently, paraffin-embedded blocks were obtained. Hematoxylin and eosin were used to stain the blocks, which were sliced into 6 mm thick slices (H&E). The histopathologist assessed all samples without definition under a light microscope (Nikon Optiphat-2). Oculometrically, the wall thickness of the aorta tissue was assessed. The presence and absence of calcification in the artery wall, mononuclear cell infiltration, foamy macrophages, and fatty streaks findings were also assessed. Saverymuttu et al. developed a technique for evaluating intestine biopsy samples, which was followed [14]. Changes in enterocytes, crypts, and lamina propria were scored in this table, and a total value between 0 and 12 was assigned to each sample. The presence and absence of lymphoid nodules and alveolar degeneration were investigated in lung tissue, and the presence of mononuclear cells and polymorphonuclear leukocytes were graded independently between 0 and 3 values. It was given a score of 0 (none), 1 (mild), 2 (moderate), and 3 (severe) based on previous research (severe) (Table 1).

Table 1. Histopathological scoring of the intestinal tissue samples.

Cells/Regions	Findings	Grade
Enterocytes	Normal	0
	Loss of single cells	1
	Loss of groups of cells	2
	Frank ulceration	3
Mononuclear cells in LP	Normal	0
	Slight increase	1
	Moderate increase	2
	Marked increase	3
Neutrophils in LP	Normal	0
	Slight increase	1
	Moderate increase	2
	Marked increase	3
Crypts	Normal	0
	Single inflammatory cells	1
	Cryptitis	2
	Cryptic abscesses	3
Total		**12**

LP: Lamina propria

Statistical Analysis

The Shapiro-Wilk test was used to assess the normality of the distribution of continuous variables. Normally distributed continuous variables were given as mean ± standard deviation and were compared using the one-way analysis of variance (ANOVA) test followed by Tukey's honestly significant difference (HSD) post hoc test. Non-normally distributed continuous variables were described as median (minimum-maximum) and were compared using the Kruskal-Wallis test followed by Mann-Whitney U multiple comparison test. A P value < 0.05 was considered significant. Statistical analyses were performed using the IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA).

Results

Throughout the trial, one rat from the Sham group, two rats from the control group, and two rats from the RG group died after surgery, and no further issues were identified. Table 2 shows the results and comparisons of serum lipid profile and hematological parameters. The control group had the highest mean TC level, with significant disparities between it and the other groups. Furthermore, there was no significant difference in TC levels between the sham and RG groups.

The levels of HDL and LDL were not substantially different between the groups. The control group had much greater TG levels than the other groups, and the difference between the control and RG groups was substantial. When the TG levels of the Sham and RG groups were examined, there was no significant difference. VLDL levels differed significantly between the control and other groups, with the control group having the highest levels. When the VLDL levels of the Sham and RG groups were examined, there was no significant difference. PTL and WBC levels were found to be significantly higher in the control group compared to the sham group. When the control group and the RG group were compared, there was a decrease in WBC and PTL counts, but there was no statistically significant difference between these two groups. Table 3 summarizes MDA and total SH levels. The control group showed the highest median MDA level. There were significant differences in median MDA values between the control group and the other groups (p=0.009, and p=0.039). A sham group measured the highest median total SH level. The differences in median total SH levels between the control group and the other groups were likewise significant (p=0.002 and p=0.012, respectively), similar to the differences in median MDA levels.

Table 4 shows the histopathological scores of the tissue samples. A large difference was seen in the mononuclear cell infiltration score in the colon samples. The highest score was seen in the control group, and the lowest score was in the sham group. The differences were significant in all comparisons. The cryptid was significantly higher in the control group than in the sham group, but there was no significant difference when compared with the RG group. Enterocyte damage was seen at a higher rate in the control group than in the sham and RG groups, but it was not statistically significant (Figure 1).

Table 2. Results and comparisons of serum lipid profile and hematological parameters.

	Sham group (Group 1)	Control group (Group 2)	RG group (Group 3)	P value (2-3)	P value (1-2)	P value (1-3)
TC (mg/dL)	47.33 ± 6.61	58.75 ±12.24	48.87 ± 13.72	0.006	0.010	0.994
HDL (mg/dL)	31.55 ± 5.14	30.85 ± 9.89	31.30 ± 10.86	0.868	0.985	0.860
LDL (mg/dL)	5.84 ± 1.75	7.62 ± 2.64	6.57 ± 1.92	0.165	0.220	0.238
TG (mg/dL)	36 (16-96)	79 (30-155)	40.5 (26-80)	0.021	0.014	0.834
VLDL (mg/dL)	9.5 (2.5-15.8)	19.5 (7-54.4)	10.25(0.9-25.6)	0.005	0.003	0.962
WBC ($x10^3$/uL)	6.32 ± 1.15	9.80 ± 3.25	8.81 ± 2.57	0.006	0.020	0.010
PLT ($x10^3$/uL)	357.62 ±196.43	753.12±207.39	688.44±146.25	0.001	0.001	0.021

RG: Red ginseng TC: Total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglycerides, VLDL: Very-low-density lipoprotein, WBC: White blood cell, PLT: Platelet

Table 3. Results and comparisons of malondialdehyde and total sulfhydryl levels in blood.

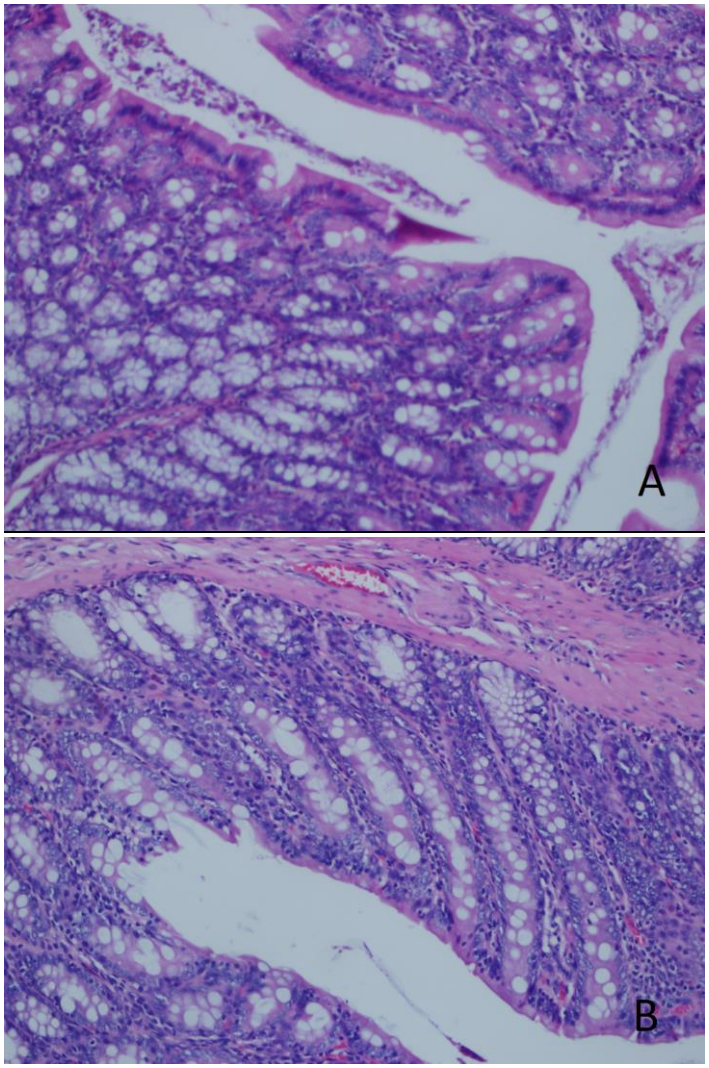
	Sham group (Group 1)	Control group (Group 2)	RG group (Group 3)	P value (2-3)	P value (1-2)	P value (1-3)
MDA (micromol/L)	0.15 (0.14-0.23)	0.21 (0.18-0.4)	0.17 (0.15-0.26)	0.010	0.009	0.086
Total SH (micromol/L)	280.50 ± 22.48	221.40 ± 37.33	271.49 ± 34.05	0.002	0.002	0.828

RG: Red ginseng, MDA: Malondialdehyde, SH: Sulfhydryl

Table 4. Results and comparisons of histopathological scores of tissue samples

	Sham group (Group 1)	Control group (Group 2)	RG group (Group 3)	P value (2-3)	P value (1-2)	P value (1-3)
			Colon			
Enterocyte damage	0 (0-0)	0 (0-2)	0 (0-1)	0.555	0.289	0.289
Mononuclear cell infiltration	0 (0-0)	1 (1-2)	1 (0-1)	<0.001	<0.001	0.002
Neutrophils infiltration	0 (0-1)	0.5 (0-1)	0.5 (0-1)	0.163	0.088	0.088
Cryptitis	0 (0-0)	0.5 (0-2)	0 (0-1)	0.056	0.020	0.050
			Lung			
Lymphoid follicle	0 (0-1)	1.5 (1-3)	1 (1-2)	0.003	0.004	0.009
Alveolar degeneration	2 (0-2)	2.5 (2-3)	2 (1-3)	0.027	0.012	0.300
Mononuclear cell infiltration	1 (1-2)	2.5(2-3)	2 (1-2)	0.012	0.012	0.514
Neutrophils infiltration	0 (0-1)	1 (0-2)	1 (0-1)	0.039	0.020	0.102
			Aorta			
Aortic diameter	190 (100-200)	205.5 (150-280)	200 (100-201)	0.246	0.119	0.414
Neutrophils infiltration	0 (0-0)	0 (0-0)	0 (0-0)	-	-	-
Macrophage infiltration	0 (0-0)	0 (0-0)	0 (0-0)	-	-	-
Fatty streak	0 (0-0)	0 (0-0)	0 (0-0)	-	-	-

RG: Red ginseng



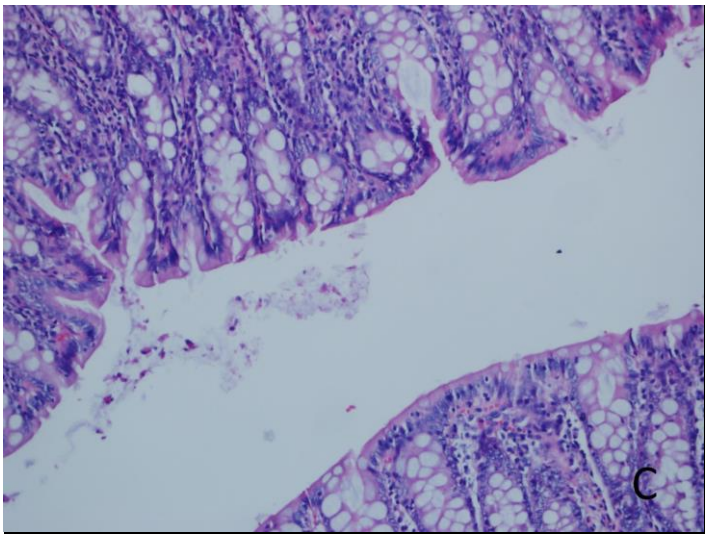
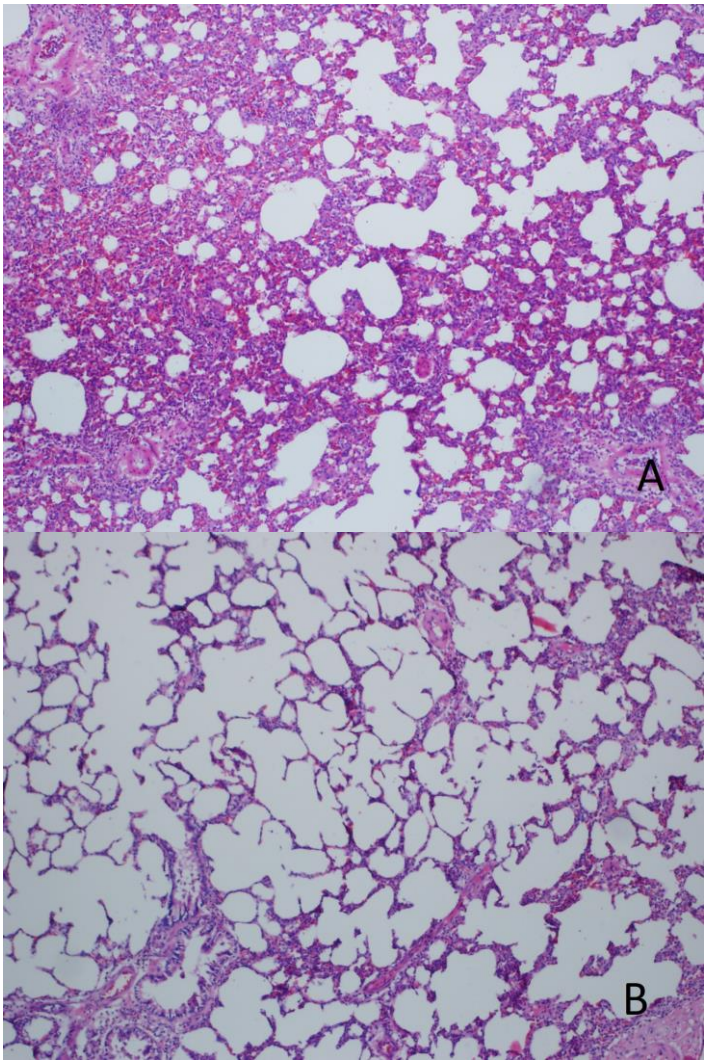


Figure 1: Histological images of intestines from A. Sham group B. Control group C. Red Ginseng group.



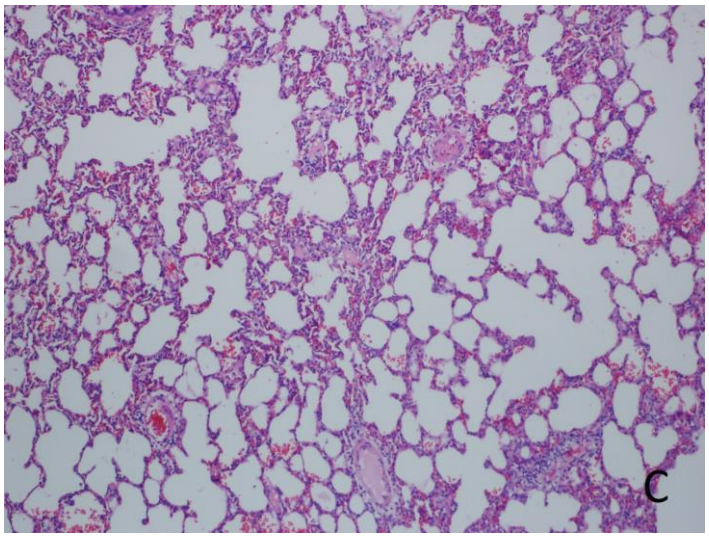
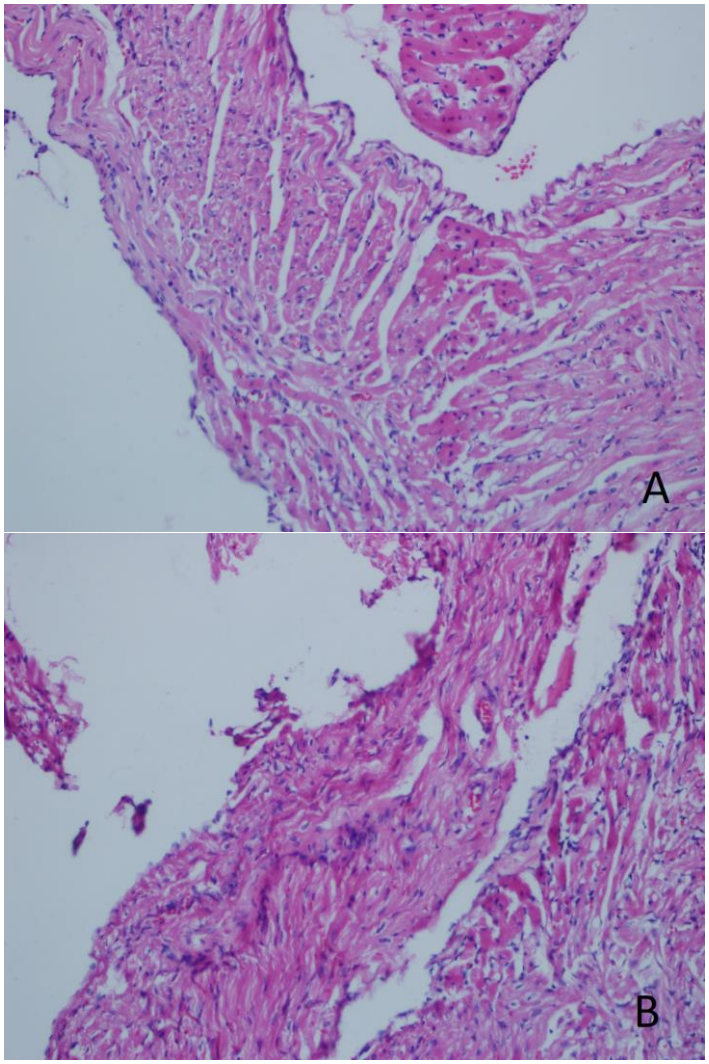


Figure 2: Histological images of lung tissues from A. Sham group B. Control group C. Red Ginseng group.



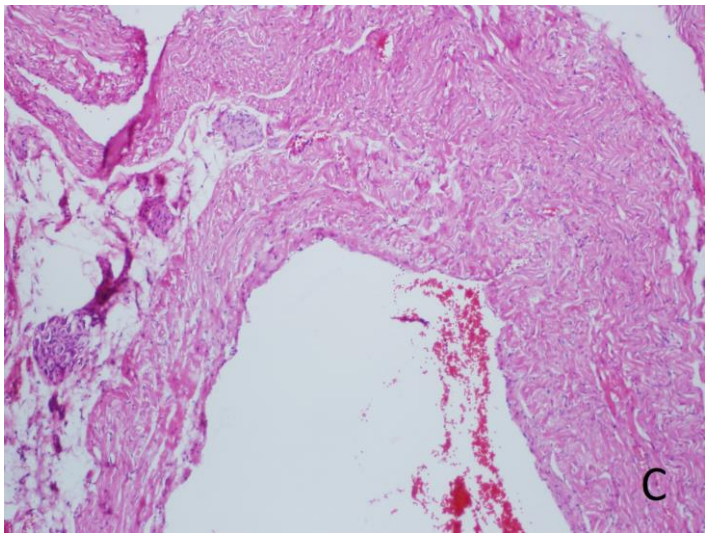


Figure 3: Histological images of aorts of rats from A. Sham group B. Control group C. Red Ginseng group.

The control group and the other groups had significantly different mononuclear cell infiltration scores in lung tissue samples, but there was no significant difference between the RG group and the sham group. There were significant differences in the lymphoid follicle, alveolar degeneration, and neutrophil infiltration ratings between the control and sham groups, but none between the control and the RG group (Figure 2). The aortic diameter measurements were not significantly different between the groups, and there was no evidence of neutrophil or macrophage infiltration or fatty streak in any of the samples (Figure 3).

Discussion

This is the first study to examine the effect of RG use on histopathological changes in vascular, lung, and colon tissue after differential splenectomy. Because of its immunological function, much recent information has been distributed on the need of attempting to protect the spleen during elective or emergency surgical procedures. Many researchers have prompted them to work toward total splenectomy in cases where it is unavoidable in order to avoid problems and metabolic alterations [15]. The relevance of splenic tissue as a key regulator of immunological and hematopoietic activities cannot be overstated today [16]. Well-designed studies have revealed that the spleen has a significant influence on lipid metabolism [17] Many theories have been presented to explain the possible mechanism, the majority of which are based on strong innate immune activity, specifically the mononuclear phagocytic system, however, the exact processes underlying this mysterious phenomena remain unknown. safeguards While the incidence of atherosclerosis (AS) was higher in individuals who had a splenectomy after a splenic injury, the incidence of AS did not change in instances with conservative follow-up [18]. After splenectomy, vascular and systematic hemostasis due to damaged erythrocytes or thrombocytosis is damaged and causes serious systemic complications such as pulmonary hypertension [1].

In this study, we first evaluated the histopathological changes in endothelial, alveolar, and vascular tissues after splenectomy in rats, as well as their effects on lipid profile and antioxidant mechanisms. Second, we focused on the therapeutic effects of RG, a well-known antioxidant and anti-inflammatory agent, on the lipid profile, respiratory, gastrointestinal, and vascular systems. Panax ginseng is one of the most widely used herbal remedies in Asian and Western countries. Ginseng is a slow-growing, succulent perennial herb, the root of which is

composed of ginsenosides and the most important subcomponent is red ginseng (RG). Panax ginseng has many bioactive elements such as triterpene saponins, polysaccharides, sesquiterpenes, polyacetylenes, amino acids, fatty acids, carbohydrates, alkaloids, peptidoglycans, minor elements, vitamins, and phenolic compounds [19-20]. Numerous research has demonstrated the effects of RG, including immune system activation, pharmacological effects on the endocrine and cardiovascular systems, anti-cancer, antioxidant, cognitive performance enhancement, and aging deferral. Obesity, diabetes, and hyperlipidemia are just a few of the metabolic disorders linked with aging that have historically been treated with RG [21-24]. Furthermore, the administration of RG dramatically reduces visceral adipocyte hypertrophy, which is linked to metabolic disorders such as insulin resistance and hepatic steatosis. In the study of Lee et al., RG has shown that diet-induced and ovariectomized obese mice reduce adipose tissue mass and prevent obesity [25-26]. After total splenectomy in rats, they noticed a rise in plasma cholesterol levels, indicating the spleen's role in lipid metabolism [27]. In another rat study, TG, HDL-C, and VLDL levels increased, although LDL-C levels decreased when compared to rats that underwent total splenectomy and were all fed with standard feed and underwent only laparotomy [28]. The spleen may also influence lipid metabolism in the gut by triggering inflammatory processes and oxidative stress, leading to lower lipid peroxidation after splenectomy [29]. Increased TC, TG, and VLDL plasma levels in splenectomy rats considerably lowered TC, TG, and VLDL levels compared to the control group in our investigation, indicating that RG has a therapeutic effect on lipid metabolism. In addition, in the biochemical analysis of serum lipid profile in our study, there was no difference between the groups in HDL and LDL levels. This may be a result of impaired macrophage functions and changes in lipoprotein metabolism after splenectomy [30]. The number of mononuclear cells in the intestinal tissue of the splenectomy rats was considerably larger than in the sham and RG groups, according to our histological analyses. When the RG group was compared to the control group, however, there was decreased mononuclear cell infiltration in the RG group, which was statistically significant. This discovery shows that RG has an anti-inflammatory effect on splenectomy rats' intestinal tissue. Erythrocyte damage was found to be higher in splenectomy rats and enterocyte damage was observed to be less compared to the RG group, but there was no statistically significant difference. Cryptitis was found to be significantly higher in the splenectomy group compared to the sham group, but although cryptitis was less common in the RG group than in the control group, it was not statistically significant.

Vascular complications and related pulmonary hypertension may occur after splenectomy [1]. In addition, Andres-Hernando et al. reported that splenectomy exacerbated lung damage and inflammatory reactions in their study on rats. In the study of Cho et al., they showed that the use of RG reduced lung damage due to the use of ventilators [32]. Therefore, we examined the vascular and respiratory changes and possible effects of RG after splenectomy in rats. Lymphoid follicle, alveolar degeneration, mononuclear cell infiltration, and neutrophil infiltration scores were found to be significantly higher in splenectomized rats compared to the sham group. In the RG group, compared to the control group, there was a statistically significant decrease in mononuclear cell infiltration. Although there was a decrease in the lymphoid follicle, alveolar degeneration, and neutrophil infiltration scores in the RG group compared to the control group, it was not statistically significant. When the aortic diameters of the sham and control groups were compared, an increase in aortic diameters was observed in the control group, but it was not statistically significant. In addition, when the RG group was compared with the control group, aortic diameters were decreased, but it was not statistically significant. Neutrophil infiltration, macrophage infiltration, and fatty streaking were not observed in any of the groups. Durhan et al. created a mechanical icterus model in rats and demonstrated the anti-inflammatory and antioxidant effects of RG. In their study, they showed that RG significantly decreased MDA values, which indicates intracellular oxidative stress, and significantly increased the level of catalase, which is an important antioxidant [31]. Another study showing the antioxidant effect of RG is the study of Cho et al. They showed that the use of RG in lung injury caused by mechanical ventilation in rats significantly decreased MDA values [32]. In our study, the MDA level, which indicates intracellular oxidative stress, increased significantly in splenectomy rats compared to the sham group. When the RG group and the control group were compared, MDA values were found to be significantly decreased. Total SH values, which is an antioxidant parameter, decreased in the control group and increased significantly in the RG group. Especially platelet and leukocyte values of 113 patients who underwent splenectomy were found to be significantly higher when compared with healthy individuals without a specific disease [33]. In another study on rats, leukocyte and thrombocyte levels were found to be significantly higher in the splenectomy group compared to the control group [28]. In our study, a significant increase was observed in platelet and WBC counts after splenectomy compared to the sham group, in line with many studies in the literature. However, although there was a decrease in platelet and WBC counts in the RG group, this decrease was not statistically significant.

In conclusion, the use of RG after splenectomy in rats has been shown to have a significant anti-inflammatory effect by reducing mononuclear cell infiltration within the histopathological changes occurring in the lungs and colon tissue. However, the probability of improving epithelial regeneration was low. It was observed that the use of RG was significantly effective on rats in the regulation of the antioxidant system and lipid profile, which deteriorated after splenectomy. While promising beneficial effects of RG have been demonstrated, more clinical studies are needed to apply these positive results in clinical settings.

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Arch Clin Exp Med 2021;6(2):50-55. e-ISSN: 2564-6567

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The change in the frequency of community acquired acute respiratory tract infections in weight loss period after bariatric surgery: An observational case-crossover study. Are those who have had bariatric surgery at risk?

Bariatrik cerrahi sonrası kilo kaybı döneminde toplum kökenli akut solunum yolu enfeksiyonlarının sıklığındaki değişim: bir gözlemsel vaka-çapraz çalışma. Bariatrik cerrahi geçirenler risk altında mı?

Serdar Coşkun [1], Cüneyt Kırkıl [1], Gamze Kırkıl [1]

Abstract

Aim: It is unknown whether there is an increase in the frequency of acquired respiratory tract infections (ARTIs) while the weight loss process continues after bariatric surgery (BS).

Methods: An observational case-crossover study of 366 patients underwent BS between September 2018 and September 2019 was performed. Prospectively recorded data of ARTI events in the first pre- and post-operative ARTI seasons were compared.

Results: Mean age was 36.4 ± 10.9 years and mean body mass index (BMI) was 44.1 ± 6.2 kg/m2. Mean change in BMI in an ARTI season was 13.2 ± 6.1 kg/m2. Among 366 patients, 180 (49.2%) were diagnosed with ARTI and the average numbers of ARTIs was 0.69 ± 0.85 in preoperative season. During the postoperative season, 134 (36.6%) patients were diagnosed with ARTI and the average numbers of ARTIs was 0.49 ± 0.76. Both period prevalence and average number of ARTIs in preoperative ARTI season was significantly higher than postoperative season (P = 0.001 for both). The change in BMI in an ARTI season was correlated with the postoperative period prevalence of ARTIs (r = 0.119, p = 0.022).

Conclusion: These findings showed that the period prevalence and incidence of ARTIs reduced after BS. However, the frequency of ARTIs was higher in patients with greater weight loss.

Keywords: Bariatric surgery, weight loss, acute respiratory tract infection

Öz

Amaç: Bariatrik cerrahi (BC) sonrası kilo verme süreci devam ederken, edinilmiş solunum yolu enfeksiyonlarının (ASYE) sıklığında bir artış olup olmadığı bilinmemektedir.

Yöntem: Eylül 2018-Eylül 2019 tarihleri arasında BC uygulanan 366 hastanın gözlemsel vaka-çapraz çalışması yapıldı. Ameliyat öncesi ve sonrası ilk ASYE mevsimlerinde ASYE olaylarının prospektif olarak kaydedilen verileri karşılaştırıldı.

Bulgular: Ortalama yaş 36,4 ± 10,9 yıl, ortalama vücut kitle indeksi (VKİ) 44,1 ± 6,2 kg/m2 idi. Bir ASYE mevsiminde VKİ'ndeki ortalama değişim 13,2 ± 6,1 kg / m2 idi. 366 hasta arasında 180 (%49,2) ASYE tanısı kondu ve preoperatif sezonda ortalama ASYE sayısı 0,69 ± 0,85 idi. Postoperatif dönemde 134 (%36,6) hastaya ASYE tanısı kondu ve ortalama ASYE sayısı 0,49 ± 0,76 idi. Preoperatif ASYE sezonunda hem dönem prevalansı hem de ortalama ASYE sayısı postoperatif sezona göre anlamlı derecede yüksekti (her ikisi için de p = 0,001). Bir ASYE mevsiminde VKİ'deki değişim, ASYE'nun postoperatif dönem prevalansı ile korele idi (r = 0,119, p = 0,022).

Sonuç: Bu bulgular, BC sonrası ASYE prevalansının ve insidansının azaldığını göstermiştir. Bununla birlikte, daha fazla kilo kaybı olan hastalarda ASYE sıklığı daha yüksekti.

Anahtar kelimeler: Bariatrik cerrahi, kilo kaybı, akut solunum yolu enfeksiyonu

[1] Gazi State Hospital, Department of General Surgery, Samsun, Turkey.
[2] Fırat University, Faculty of Medicine, Department of General Surgery, Elazığ, Turkey.
[3] Fırat University, Faculty of Medicine, Department of Chest Diseases, Elazığ, Turkey.

SC: 0000-0002-7238-6032
CK: 0000-0001-8756-4850
GK: 0000-0003-4799-5589

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Sorumlu yazar / Corresponding author:
Cüneyt Kırkıl
Adres/Address: Fırat Üniversitesi Hastanesi, Genel Cerrahi Kliniği, 23119 Elazığ, Turkey.

e-mail: ckirkil@yahoo.com
Tel/Phone: 00905065009933

Introduction

It is conceivable that in excess of a half million patients globally undergo bariatric procedures each year considering total number of bariatric surgery (BS) is approximately 252,000 in 2018 in USA [1]. Bariatric surgeons and candidates for BS discussed whether individuals who had undergone BS had a higher COVID-19 infection risk due to suppression of immune response in the first weeks of the global outbreak of coronavirus disease 2019 (COVID-19). It was a reasonable question because various forms of stress, trauma, infections, malignancies, and nutritional status could modulate the immune system. It can be thought that the answer of the question should be probably yes, at least during perioperative period. Even if the perioperative immunological response, secondary to tissue injury, anesthesia, and blood transfusion, is not immunosuppressive all the way, it is extensively so [2]. Previous studies that quantify the postoperative immune response have consistently demonstrated that levels of interleukin-6 and interleukin-10 increased during postoperative period [3, 4]. Early postoperative levels of these cytokines correlate with an increase in the frequency of nosocomial infections.

It is known that adipose tissue takes part in various immune processes due to secretion of pro- and anti-inflammatory factors and causes to obesity-related complications [5]. Bariatric surgery does not only reduce inflammatory state both in adipose tissue and blood by reducing adiposity [6] but also improves co-morbidities as T2DM, which is associated with a substantial increase in morbidity and mortality risks associated with acute respiratory tract infections (ARTI) like seasonal influenza [7]. Thus, one can expect that patients who will undergo BS may have metabolic and immune benefits that will be protective in terms of getting ARTI. But could the same thing be told for patients who were still in the process of weight loss? They had still some extra fat tissue which could produce pro- and anti-inflammatory factors. Moreover, they could be at a higher risk because of their nutritional status.

Until now, there is no data in literature about rates of community acquired ARTIs during weight loss period following BS. There are some seasonal differences in the epidemiological data of ARTIs around countries. In Turkey, ARTIs occur mostly in the period between late September and April [8]. And weight loss rate is not constant following BS. The vast majority of patients lose weight faster in early period. Hypothetically, there might be a difference for getting ARTIs among patients related to the month of surgery. Non-seasonal ARTIs could have an effect on the results.

In the present study, it was aimed to detect whether there was any change in the frequency of ARTIs in a common ARTIs season while weight loss process was still going on following a BS.

Material and methods

Patients

Prospectively recorded data of 423 patients who underwent BS by a single surgeon between September 2018 and September 2019 were analyzed retrospectively. Compliance with the validated international criteria for BS as having body mass index (BMI) $\geq$ 40 kg/m2 or $\geq$ 35 kg/m2 in the presence of obesity-related medical co-morbidities was the inclusion criteria for the study. Exclusion criteria were noncompliance with BS criteria and follow-up protocols, and flu vaccination history. Informed consent was obtained from all individuals included in the study. All smokers were advised to quit smoking after surgery.

Follow-up

Patients followed by a team composed of two clinical secretaries, a nurse, and a medical doctor. The postoperative follow-up protocol for first year was as follows. Body weight was recorded monthly. Complete blood count, liver function tests, blood levels of glucose, glycated hemoglobin, urea, creatinine, sodium, potassium, calcium, albumin, ferritin, thiamine, folic acid, cyanocobalamin, and 25-hydroxyvitamin D levels were measured in the first postoperative month, and were repeated by 3-months intervals for the first postoperative year by their own primary care physicians. Doses of supplements were adjusted according to the test results by the medical doctor in bariatric team. Complete remission of T2DM defined as a level of glycated hemoglobin less than 6.0% without any anti-diabetic medication in the postoperative period.

Data acquisition

After obtaining approval from the Scientific Research Committee of Turkish Ministry of Health (2020-06-06T15_18_09), data for ARTI of all patients were obtained in June 2020 from the electronic database of Turkish Ministry of Health (e-nabiz). The database gets data from the Social Security Institution (SSI), the only organization that provides public health insurance in Turkey. Every physician work in primary care clinics, public hospitals, and private hospitals contracted with SSI have to enter the data of pre- and definitive diagnosis into the database of SSI using International classification of diseases (ICD) version 10. ARTI-related diagnoses in the first September-April period before and after operation date were evaluated. Data of the postoperative 30-days was not included for the probability of hospital-acquired infection. In June 2020, the clinical secretaries called the patients for getting information about flu vaccine (within one year before or after surgery), and smoking status.

Statistical analysis

The results were presented as mean, ratio, per cent or dichotomy. Distributions of parameters were evaluated by Kolmogorov-Smirnov test. Student's T-test was used for the comparisons of parametric data, and chi-square test was used for the comparisons of non-parametric data. The bivariate analysis was used to evaluate the correlations between age, T2DM, chronic pulmonary diseases, smoking, BMI, delta (Δ) BMI (change in BMI) in a season of ARTI, percentage excess weight loss (% EWL = 100% $\times$ [baseline weight - last weight] / [baseline weight - ideal weight]) and ARTIs. A simple linear regression analysis was used for correlation between BMI and Δ BMI in season. All calculations were performed using the IBM SPSS version 22 (IBM Corporation, Chicago, IL, USA). A 2-tailed P value less than 0.05 was considered statistically significant. Power analysis was performed by G Power 3.1.9.2 (written by Franz Faul, Universität Kiel, Germany).

Results

The flow chart of patient enrollment is shown in Figure 1. Gastroesophageal reflux was sole indication for revisional surgery for three patients with a BMI less than 31 kg/m2. They were excluded because they would not be expected to lose too much weight. Others met the validated international criteria for bariatric surgery. Because of noncompliance with follow-up protocols, additional 49 patients were excluded. Because of flu vaccination history within one year, 5 patients were also excluded. The remaining 366 patients received multivitamin and iron supplements at least 9 months during postoperative period were enrolled into the study. Table 1 summarizes preoperative characteristics and the changes in demographics of patients

during postoperative follow-up. Mean postoperative follow-up was 13.2 ± 3.4 months (range, 8 to 19), but a mean of 11.0 ± 1.4 months (range, 8 to 12) was in the ARTI season. Mean %EWL was 90.1% ± 19.4% (range, 31% to 141%) during follow-up. Mean of Δ BMI in a season was 13.2 ± 6.1 kg/m2 (range, 0.8 to 32.2). There was a significant correlation between baseline BMI and Δ BMI in a season (Beta = 0.563, 95% CI, –0.50 to 0.78, P = 0.000). Since the patients who had surgery towards the end of ARTI season lost the majority of their excess weight in summer when ARTIs were not common, they had a lower Δ BMI in the next ARTI season. Among 70 patients with T2DM, 61 (87.1%) had complete remission in the postoperative 3rd month. All but one of the remaining patients stopped using insulin treatment. Indeed, a reduction in the insulin doses and glycated hemoglobin levels were recorded for this patient. Although 10 smokers (16.1%) quit smoking in postoperative period, smoking rates were not statistically different in both periods (16.9% in preoperative period vs. 14.2% in postoperative period, p=0.359).

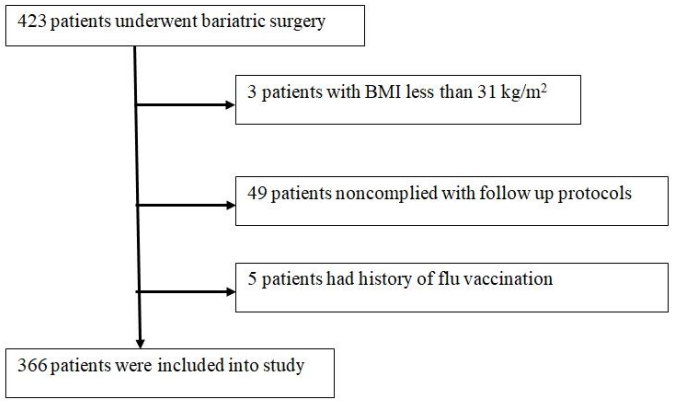

Figure 1. The flow chart illustrating patient enrollment.

Table 1. The preoperative characteristics and the changes in demographics of patients during postoperative follow-up.

	Preoperative (n = 366)	Postoperative (n = 366)	P value
Age (years) (mean ± SD)	36.4 ± 10.9	37.3 ± 10.9	0.258
Sex (F / M)	268 / 98		NA
BMI (kg / m^2) (mean ± SD)	44.1 ± 6.2	27.8 ± 4.5	<0.001
Type 2 Diabetes Mellitus (yes / no)	70 / 296	9 / 357	< 0.001
Chronic Pulmonary Disease (yes / no)	16 / 350	16 / 350	1.000
Smoking (yes / no)	62 / 304	52 / 314	0.359
ARTIs (yes / no)	180 / 186	134 / 232	0.001
Average number of ARTIs (mean ± SD)	0.69 ± 0.85	0.49 ± 0.76	0.001
Operations			
SG (%)	270 (73.8)		
OAGB (%)	40 (10.9)		
RYGB (%)	20 (5.5)		
TB (%)	18 (4.9)		
Revisional RYGB (%)	16 (4.4)		
BPD-DS (%)	2 (0.5)		

BMI: Body mass index, ARTI: Acute respiratory tract infection, SG: Sleeve gastrectomy, OAGB: One anastomosis gastric bypass, RYGB: Roux-en-Y gastric bypass, TB: Transit bipartition with sleeve gastrectomy, BPD-DS: Biliopancreatic diversion with duodenal switch.

Among 366 patients, 180 (49.2%) were diagnosed with ARTI at least one, at most, five times in the last preoperative ARTI season. During the first ARTI season after surgery, 134 (36.6%) patients were diagnosed with ARTI (maximum three times in a season). All ICD-10 codes used to diagnose both preoperative and postoperative ARTI seasons were listed in table 2. The most common ICD-10 codes were J06 and J20 in both periods. The period prevalence (number of persons with an episode of ARTI over a defined period of time / number of persons in the population over the same period) in preoperative ARTI season was significantly higher than postoperative season (49.2% vs. 36.6%, respectively, P = 0.001). The postoperative period prevalence of patients operated during ARTI season (between September and April) was similar to those operated in post-ARTI season (between May and August) (86 / 228, 37.7% vs. 48 / 138, 34.8%, respectively, P = 0.654). The average numbers of ARTIs per cohort were also significantly reduced. It was 0.49 ± 0.76 in postoperative season, while it was 0.69 ± 0.85 in preoperative season (P = 0.001). So, incidence proportions for ARTIs were 69% and 49% per an ARTI season in preoperative and postoperative periods, respectively. Considering the average numbers of ARTIs, the effect size was 0.25 and power of the study was 99.9 percent.

Table 3 summarizes the correlations between ARTIs and risk factors. Bivariate analysis for preoperative period showed that the period prevalence of ARTIs was negatively correlated with age and T2DM (r = -0.122, P = 0.02 and r = -0.173, P = 0.001, respectively). Sex, BMI, having chronic pulmonary diseases, and smoking habit were not correlated with the period prevalence of ARTIs. While the preoperative frequency of ARTIs was negatively correlated with age (r = -0.133, P = 0.011), it was positively correlated with BMI (r = 0.104, P = 0.048). There was no correlation between the preoperative frequency of ARTIs and sex, T2DM or chronic pulmonary diseases, and smoking habit. In the postoperative ARTI season, there was no correlation between postoperative ARTIs frequency and sex, T2DM, smoking habit, baseline BMI, and operation type. Both the period prevalence and incidence of ARTIs was negatively correlated with age and chronic pulmonary diseases as a comorbidity (P < 0.05 for all). There was a positive correlation between Δ BMI in ARTI season and the period prevalence of ARTIs (r = .119, P = 0.022). There were also strong correlations between preoperative and postoperative periods in both the period prevalence and incidence proportions (r = 0.364, P = 0.000, for prevalence, and r = 0.334, P = 0.000, for incidence). Among 134 patients who had ARTIs in postoperative period, 98 (73.1%) had also ARTIs in preoperative period. Only one-fifth of the patients (19.4%) who had not any ARTIs in the preoperative period experienced ARTIs in the first postoperative ARTI season.

Table 2. The distribution of acute respiratory tract infections in preoperative and postoperative periods.

ICD-10 codes	Diagnosis	Preoperative cases (n)	Postoperative cases (n)
J00	Acute nasopharyngitis (common cold)	18	8
J01	Acute sinusitis	32	25
J02	Acute pharyngitis	29	24
J03	Acute tonsillitis	8	4
J06	Acute upper respiratory infections of multiple and unspecified sites	72	61
J11	Influenza	11	6
J18	Pneumonia	16	6
J20	Acute bronchitis	57	40
J22	Unspecified acute lower respiratory infection	11	6
Total		254	180

ICD-10: International Classification of Diseases version 10

Discussion

In the present study, it was observed that the period prevalence and incidence of ARTIs were significantly reduced in weight loss period following BS. BMI was positively correlated with the incidence of ARTIs in preoperative season. Δ BMI in ARTI season was also positively correlated with the period prevalence of ARTIs postoperatively. It was associated with higher baseline BMI in these patients.

It is well known that being overweight (BMI = 25.0 – 29.9 kg/m2) and in particular obesity (BMI ≥ 30 kg/m2) have a role in predisposition to RTIs [9,10]. Moreover, two recent Danish population-based studies reported an excess of a large spectrum of RTIs including pneumonia among obese people [11,12]. Some obesity-related mechanisms can alter immune system, and lead to predisposition to infections. Obesity is characterized by excessive accumulation of fat tissue. Adipokines produced by adipocytes primarily composing adipose tissue can significantly alter immune function. Adiponectin is a member of adipokines and reduces macrophage activity and proinflammatory cytokine production. Its production decreases in obese patients. Whereas, leptin that is also a member of adipokines is considered to be proinflammatory, and is elevated in obese individuals. The anti-inflammatory action of adiponectin is partly attributed to the induction of interleukin 10 and the suppression of nuclear factor kB in macrophages. Thus, it suppresses of T cell–mediated responses and innate immune responses. When they were dealt as a whole, low plasma levels of adiponectin causes overresponse of the innate immune system to pathogens [13]. It results in more pronounced immune response against to viruses in obese persons than that of lean persons, and can explain why the courses of some ARTIs like COVID-19 and H1N1 are severe in obese patients. One explanation for obesity and ARTIs relationship is increased vulnerability to infections in obese individuals. The vast majority of evidence is found regarding nosocomial infections, except for the findings in influenza pandemics. It is caused by lack of retrieving record, because BMI is not usually recorded in health care providers in the case of community acquired infections [14]. But it has shown that obesity affected the influenza course and increased mortality rate during the influenza H1N1 pandemic [15]. In the present study, there was no mortality caused by ARTIs. Both the period prevalence and incidence of ARTIs decreased in obese patients treated with BS.

Table 3. The correlations between the frequency of ARTIs and risk factors in both preoperative and postoperative seasons.

		Preoperative		Postoperative	
		Prevalence	Incidence	Prevalence	Incidence
Age	r	- 0.122	- 0.133	- 0.103	- 0.124
	p	0.020 *	0.011 *	0.049 *	0.018 *
Sex	r	- 0.052	0.029	0.027	0.047
	p	0.323	0.582	0.605	0.369
Type 2 diabetes mellitus	r	- 0.173	- 0.086	- 0.084	- 0.056
	p	0.001 †	0.099	0.108	0.283
Chronic pulmonary diseases	r	- 0.050	- 0.017	- 0.107	- 0.103
	p	0.341	0.741	0.041 *	0.049 *
Smoking	r	0.051	0.094	0.048	0.066
	p	0.329	0.073	0.359	0.207
BMI baseline	r	0.091	0.104	0.073	- 0.007
	p	0.081	0.048 *	0.164	0.894
Type of operation	r	NA	NA	- 0.067	0.012
	p			0.366	0.869
EWL percent	r	NA	NA	0.083	0.130
	p			0.111	0.013 *
Δ BMI in season	r	NA	NA	0.119	0.032
	p			0.022 *	0.537
BMI last	r	NA	NA	- 0.052	0.005
	p			0.317	0.931
Preoperative prevalence	r	NA	NA	0.364	0.269
	p			0.000 ‡	0.000 ‡
Preoperative incidence	r	NA	NA	0.340	0.334
	p			0.000 ‡	0.000 ‡

BMI: Body mass index, EWL: Excess weight loss, Δ BMI: Change in BMI, NA: Not applicable, *p value less than 0.05, † p value equals to 0.001, ‡p value less than 0.001

In this study, it was observed that most of the patients who had ARTI in the preoperative period also experienced ARTI in the postoperative period. This observation in the weight-losing obese population suggests that there are some risk factors other than obesity that predispose to ARTIs. The risk factors as living in crowded conditions, malnutrition, lack of immunization, and exposure to tobacco or indoor smoke may contribute the development of ARTIs [16]. The patients included into the study had not immunization history for influenza that was a common cause of ARTIs. Cigarette smoking has been reported as a major environmental risk factor for recurrent and severe RTIs [17,18]. Although there were patients who quit smoking in this cohort, the decrease in the frequency of smoking was not statistically significant. Smoking habit was not correlated with the period prevalence and incidence of ARTIs. It is known that individuals with diabetes are also at risk of infections as influenza and pneumonia [19]. Nevertheless, there was also no positive correlation between T2DM and the period prevalence and incidence of ARTIs in the study. A reduction in the frequency or severity of acute respiratory infections might be expected in weight losing obese patients for the reasons mentioned earlier. However, weight loss process following BS is actually a period when the energy balance is negative. Malnutrition is considered the most common cause of immunodeficiency throughout the World. It can facilitate pathogen invasion and propagation by compromising host defense [20]. The nutritional status of the host has critical importance for the outcome of infection. Because initiation of both innate and adaptive immune responses requires additional anabolic energy and it has been shown that acute protein-energy malnutrition can affect immune response at various stages [21]. The positive correlation between change in BMI in a season and postoperative ARTIs that was observed in this study might be due to energy deficit following BS. However, the malnutrition-infection relationship is not only based on energy deficit. Both the adaptive and innate arms of the immune system are adversely affected by different types of nutritional deficiency state. It has been shown that vitamin A or vitamin D deficient children are at risk of developing more frequent and more severe respiratory tract infections [22,23]. Vitamin C also reduces severity of cold by perhaps improving natural killer cells and lymphocyte activities [24]. Similarly, deficiencies of fatty acids, amino acids, iron, and trace elements also increases susceptibility to major human infectious diseases [21]. It can be said that the patients having energy deficit in the present cohort were protected from ARTIs by the protective effect of regularly used multivitamin supplements in the postoperative period. The decrease in the incidence of ARTIs in smokers and patients with T2DM, although not statistically significant, can also be attributed to multivitamin and mineral supplements. But it is impossible to place a final judgement, because postoperative levels of vitamins and minerals were analyzed in different laboratories using various methods in primary care clinics.

Having a chronic pulmonary disease is another important risk factor for ARTIs [25]. Chronic diseases, foremost asthma and chronic obstructive pulmonary diseases, are associated with both an increased risk of RTIs and obesity [26-29]. Interestingly, it was negatively correlated with both the period prevalence and incidence of ARTIs in postoperative period. This observation may be due to the protective effect of vitamin supplements, or may be due to changes in patients' awareness of health or self-protection efforts from diseases in the postoperative period. But also, it may be due to inappropriate using of ICD-10 codes by health care providers, such as using of acute exacerbation of chronic pulmonary disease diagnosis code (J44) instead of any ARTIs code. The overlapping clinical syndromes caused by the etiologic agents of acute respiratory infections make it difficult to assign a specific etiology based on the clinical presentations [30]. Physicians dealing with ARTIs do not usually make an effort to identify the microorganism causing infection. They endeavor to identify pathogens only for pandemics or epidemiological studies, though public databases

are important for determining the variables that affect public health and making decisions about public health. Moreover, a lot of people prefer to use some over-the-counter medications instead of consulting a doctor in flu-like conditions. Nonspecific clinical presentation, and a lack of medical attention or recordkeeping cause to underestimation of ARTIs. For these reasons, it does not seem possible to reliably determine the prevalence and incidence of acute respiratory tract infections.

This study has some limitations, firstly it has a retrospective design. The possibility of the presence of patients who do not apply a health care provider in the case of any ARTIs, and the possibility of misused ICD-10 codes by healthcare professionals are other limitations. However, data of the cohort includes all applications to family doctors, state hospitals, university hospitals, and private hospitals contracted with SSI. Another limitation is that the prevalence of influenza like illness in the population varies between years due to epidemiological reasons. Indeed, considering Turkish Ministry of Health data, the frequency of influenza-like illness doubled between 2017-2018 (2017/week 40th to 2018/week 20th) and 2018-2019 (2018/week 40th to 2019/week 20th) seasons (Figure 2) [31]. The data for 2019-2020 season has not yet been reported by Turkish Ministry of Health. Despite this increase between two seasons, the decrease in prevalence and incidence of ARTIs in patients who lost weight following BS is remarkable. This is the first study focused on the effect of weight losing following BS on the seasonal ARTIs, and it may enable physicians to do comment whether BS reduces in the frequencies of ARTIs like H1N1 or COVID-19.

As a conclusion, the frequency of ARTIs decreased while weight loss continued after BS in this retrospective cohort of adults receiving vitamin and mineral supplements. Further studies are needed to determine whether ARTIs decrease in patients with vitamin or mineral deficiencies in postoperative period. Sub-group analysis should be included in future studies for the risk factors predisposing to ARTIs such as socioeconomic status, living and working conditions, and nutritional parameters of patients. Based on these findings, it can be said that patients with a history of BS are expected to be less likely to get COVID-19 than obese patients, unless they have vitamin or mineral insufficiency.

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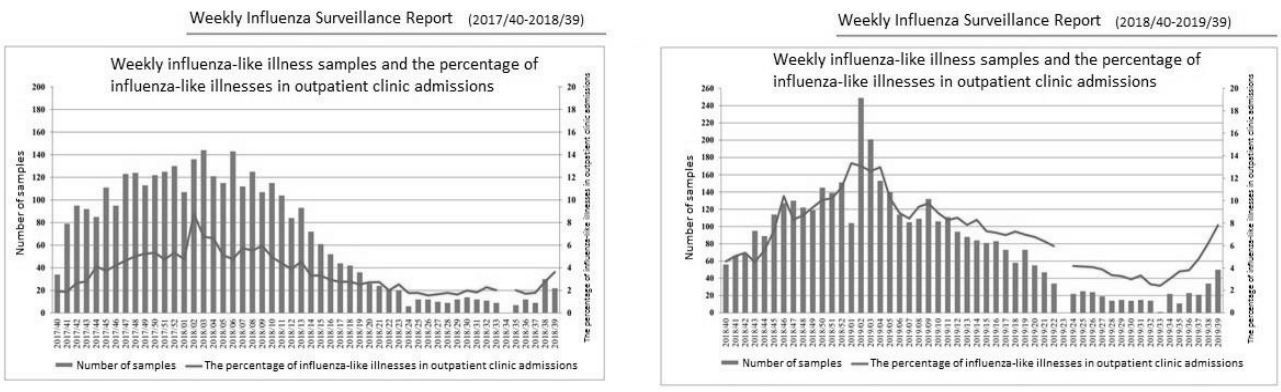

Figure 2. The change in frequency of influenza-like illness in Turkey between 2017-2018 and 2018-2019 influenza seasons (https://www.grip.gov.tr/tr/2017-2018-haftalik-influenza-raporlari.html)

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Correlation of residual subjective cognitive and depressive symptoms with social functioning in patients with remitted major depressive disorder

Remisyonda major depresif bozukluk tanılı hastalarda rezidüel subjektif bilişsel ve depresif belirtilerin sosyal işlevsellik ile ilişkisi

Ender Kaya[1], Fatma Barlas[2]

Abstract

Aim**:** In Major Depressive Disorder (MDD) residual symptoms cause disability in the remission phase even if they are mild. This study investigated the correlation of residual depressive and subjective cognitive symptoms with social functioning in remitted MDD patients.
Methods: In the study, 51 patients who had been diagnosed with MDD before, were followed regularly and had been in remission for at least 6 months, were included. The socio-demographic data form, Beck Depression Inventory, Perceived Deficits Questionnaire-Depression, and Social Adaptation Self-evaluation Scale were applied to all participants. In the statistical analysis; descriptive analyses, Pearson's Correlation Analysis and linear regression analysis were used.
Results: Residual depressive symptom severity (r=-0.357, p<0.05) and residual subjective cognitive symptom severity (r=-0.356, p<0.05) were negatively correlated with social functioning level. In the linear regression analysis, it was determined that residual depressive and subjective cognitive symptom scores were a predictor of social functioning (p<0.05).
Conclusion: In the study, it was determined that residual depressive and subjective cognitive symptoms encountered in MDD remission phase might affect social functioning negatively. Rapid and practical subjective cognitive tests can be preferred in identifying cognitive symptoms in MDD remission phase.

Keywords: Depression, remission, residual subjective cognitive symptoms, residual depressive symptoms, psychosocial functioning.

Öz

Amaç: Major Depresif Bozuklukta (MDB) remisyon döneminde rezidüel belirtiler hafif şiddette olsa bile yeti yitimine neden olurlar. Bu çalışmada remisyonda olan MDB hastalarında, rezidüel depresif ve subjektif bilişsel belirtilerin sosyal işlevsellikle ilişkisi araştırılmıştır.
Yöntemler: Çalışmaya öncesinde MDB tanısı almış, düzenli takip edilen ve en az 6 ay süre ile remisyonda olan 51 hasta dahil edilmiştir. Tüm katılımcılara sosyodemografik veri formu, Beck Depresyon Ölçeği, Algılanan Bilişsel Kusur Anketi-Depresyon, Sosyal Uyum Kendini Değerlendirme Ölçeği uygulanmıştır. İstatiksel analizde tanımlayıcı analizler, Pearson Korelasyon Analizi ve lineer regresyon analizi kullanılmıştır.
Bulgular: Rezidüel depresif belirti (r=-0,357, p<0,05) ve subjektif bilişsel belirti şiddeti (r=-0,356, p<0,05) ile sosyal işlevsellik düzeyi arasında negatif korelasyon saptandı. Lineer regresyon analizi ile rezidüel depresif ve subjektif bilişsel belirti puanlarının sosyal işlevselliğin yordayıcıları olduğu tespit edildi (p<0,05).
Sonuç: Bu çalışmada MDB remisyon döneminde görülen rezidüel depresif ve subjektif bilişsel belirtilerin sosyal işlevselliği olumsuz etkileyebileceği tespit edilmiştir. MDB da remisyon döneminde bilişsel belirtilerin saptanmasında hızlı ve pratik kullanımı olan subjektif bilişsel testler tercih edilebilir.

Anahtar Kelimeler: Depresyon, remisyon, rezidüel subjektif bilişsel belirtiler, rezidüel depresif belirtiler, psikososyal işlevsellik.

[1] University of Health Sciences, Prof. Dr. Cemil Tascioglu City Hospital, Clinic of Psychiatry, Istanbul, Turkey.
[2] University of Health Sciences, Prof. Dr. Cemil Tascioglu City Hospital, Clinic of Psychiatry, Istanbul, Turkey.

EK: 0000-0002-8767-3798
FB: 0000-0001-7893-5437

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Sorumlu yazar / Corresponding author:
Ender Kaya

Adres/Address: University of Health Sciences, Prof. Dr. Cemil Tascioglu City Hospital, Clinic of Psychiatry, Istanbul, Turkey.

e-mail: enderkaya76@yahoo.com

Tel/Phone: 0212 314 55 55
Fax: 0212 221 78 00

Introduction

Depression is a mental disorder which is commonly encountered and usually progresses with relapses. It is known that 29-46% of patients with depression partly or never respond to antidepressant treatment. Residual symptoms are observed not only in patients in partial remission, but also in patients meeting the remission criteria. Even if residual symptoms are mild, they lead to disability [1]. In addition, residual symptoms may increase the depression relapse risk by extending the depressive syndrome and aggravating the clinical picture [2]. The two most commonly encountered residual symptoms are cognitive and psychosocial dysfunctions in MDD in remission phase [3].

It has been reported that cognitive functional disorder in MDD can be healed with the treatment of depressive symptoms; however, residual cognitive symptoms might even be encountered in the remission period. In full or partial remission, residual cognitive symptoms might be seen at the level of 44% [4]. MDD patients in remission have residual cognitive symptoms in the areas of attention, memory, data processing speed and executive cognitive function [5, 6]. In depression, the presence of cognitive impairment is an important factor affecting social and occupational functioning in illness and remission phases and affects the course of disease negatively [7].

Treatments in MDD make improvement in psychosocial functioning and quality of life. However, functional disorders often continue in depressive patients even if improvement is made in symptom severity [8]. Moreover, these patients cannot reach the functional levels of non-depressive individuals [9]. Also it is known that residual cognitive functional symptoms in MDD patients in remission phase may impair psychosocial functioning [10], which arises a curiosity about the correlation of residual cognitive symptoms of depression encountered in the remission phase with dysfunctions.

Neuropsychological tests are usually accepted as 'gold standard' in evaluating cognitive dysfunction. However, as neuropsychological evaluation requires a specific expertise and is hard to access for patients and clinicians, it is not practical for routine clinical practice [11]. Cognitive evaluation scales which are rated by the patient may provide a more detailed evaluation beyond the standard clinical evaluation of cognition. In addition it has been reported that subjective cognitive changes are strongly correlated with changes in neuropsychological tests [12]. Within this framework, the correlation of subjective cognitive changes with social functioning in major depressive patients has aroused curiosity. Our study can contribute to the elimination of the deficiency in the literature.

The aim of this study was to investigate the correlation of subjective residual depressive and cognitive symptoms with social functioning in MDD patients in remission. Also the study investigated the correlation between subjective residual cognitive symptoms, depressive symptoms and disease process. In accordance with this purpose, our primary hypothesis is that subjective residual cognitive and depressive symptoms in remitted MDD patients may affect social functioning negatively.

Material and methods

The study included 51 patients who were previously diagnosed with MDD in the psychiatry outpatient clinic and were followed up regularly (55 patients were invited to the study. 4 patients did not want to participate in the study). This study was conducted between November 2020 and March 2021. In the study the patients who were voluntary to take part in the study, were aged 18 to 65 years, were previously diagnosed with MDD and were remitted for at least 6 months, were included (BDI <17) [13]. Presence of a neurological or general medical disease that might impair cognitive functions, mental retardation, psychotic disorder, bipolar disorder and psychoactive substance use/abuse were determined as exclusion criteria. The patients meeting the study criteria were given detailed information about the study. Informed consent form was received from the patients who agreed to take part in the study. Following the diagnostic interview (SCID-1) conducted with the patients, the socio-demographic information form, Perceived Deficits Questionnaire-Depression (PDQ-D), Social Adaptation Self-evaluation Scale (SASS), and Beck Depression Inventory (BDI) were applied to the patients.

The study was conducted in compliance with Good Clinical Practice requirements and the Declaration of Helsinki.

Data Collection Tools

Socio-demographic information form: Questions the demographic characteristics of patients (such as age, gender, number of depressive episodes (NDE), number of admissions to hospital with depression diagnosis (NHA), number of suicide attempts (NSA)).

Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I): Developed as a structured clinical diagnosis tool, the SCID-I enables investigating whether there was any axis I diagnosis in the past and/or it has been present within the past month or not according to the DSM diagnostic criteria via mutual interviews. SCID-I was developed by First et al., and its Turkish adaptation and reliability study was conducted by Özkürkçügil et al [14, 15].

Table 1. Socio-demographic and clinical characteristics and psychometric test scores of the patients.

	Mean/Number	Sd/%
Gender (female)	42	82
Age (year)	41.8	8.5
NHA	0.2	0.5
NSA	0.6	0.8
NDE	2.5	1.2
BDI	9.7	3.4
SASS	34.5	9.4
PDQ-D		
Attention/Concentration	6.7	3.7
Prospective Memory	5.8	2.8
Retrospective Memory	6.7	4.0
Planning/Organization	5.8	2.5
Total	25.6	11.8

NHA: Number of hospital admissions, NSA: Number of suicide attempts, NDE: Number of depressive episodes, BDI: Beck Depression Inventory, SASS: Social Adaptation Self-evaluation Scale, PDQ-D: Perceived Deficits Questionnaire-Depression.

Perceived Deficits Questionnaire-Depression (PDQ-D): The Perceived Deficits Questionnaire [16] is a self-report questionnaire which was initially developed to measure cognitive dysfunction as part of the Quality of Life Battery in multiple sclerosis patients [17]. The questionnaire has four subscales each of which comprises five items, making 20 items in total: attention/concentration, retrospective memory, prospective memory and planning/organization. Scoring is made according to the last four weeks. Each item is rated in a five-point scale: 0=never, 1=seldomly, 2=sometimes, 3=frequently, 4=almost always. Total score ranges from 0 to 80. Higher scores indicate

that the perceived deficit is higher. The questionnaire has been made appropriate for use in Major Depressive Disorder patients. Scoring for the PDQ-Depression (PDQ-D) is made according to the last seven days and some questions have been altered in such a way to comprise patients with depression more [18]. Validity and reliability study of its Turkish version was conducted by Aydemir et al. in 2017 [19].

Social Adaptation Self-evaluation Scale (SASS): The scale was developed by Bosc et al. [20]. The Turkish validity and reliability study of the scale was conducted by Akkaya et al [21]. It is a 21-item self-evaluation scale developed to determine the 'social functioning' levels of patients with depression. As only one of the first and second items is completed according to the professional status, each person responds to 20 items which are rated in the range of 0-3 in total. In the scale, which is evaluated in the range of 0-60 points, it is reported that the individual must get at least 35 points in order to have normal social functionality, and if he/she gets a score below 25, there is a problem in social functioning.

Beck Depression Inventory (BDI): Measures physical, emotional and cognitive symptoms in depression. It is a self-assessment inventory containing 21 symptom categories. The highest score is 63. Highness of the total score indicates the severity of depression [22]. Its Turkish validity and reliability study was conducted by Hisli and the limit value for the inventory was determined to be 17 [13].

Statistical analysis

In the assessment of the findings, the IBM SPSS Statistics 22 (IBM SPSS, Turkey) program for statistical analyses was used. When evaluating the study data, whether the parameters were normally distributed or not was evaluated via the Shapiro-Wilk test. As the parameters were normally distributed, the Pearson's correlation analysis was used in examining the correlation. The linear regression analysis was used in evaluating the effect of the BDI and PDQ-D total scores on SASS. The statistical significance value was accepted as $p<0.05$.

Results

Table 1 shows the socio-demographic and clinical characteristics and psychometric test scores of the patients.

Correlation Analysis

It was determined that there was a positive significant correlation between BDI scores and PDQ-D total scores ($r=0.360$, $p<0.01$). There was a significant correlation between BDI scores and the attention/concentration ($r=0.459$, $p<0.01$), retrospective memory ($r=0.335$, $p<0.05$), and planning/organization ($r=0.399$, $p<0.05$) subscales of PDQ-D. There was a negative significant correlation between BDI scores and SASS scores ($r=-0.357$, $p<0.05$). A negative significant correlation was found between PDQ-D total scores and SASS scores ($r=-0.356$, $p<0.05$). There was a positive significant correlation between PDQ-D total scores and number of depressive episodes (NDE) ($r=289$, $p<0.05$).

Table 2: Correlations between BDI, PDQ-D and SASS and points of remitted MDD patients with disease characteristics.

		1	2	3	4	5	6	7	8	9	10
1.BDI	r	1									
	p										
2.Attention/ Concentration	r	0.459**	1								
	p	*0.001*									
3.Prospective Memory	r	0.145	0.277	1							
	p	*0.311*	*0.049*								
4. Retrospective Memory	r	0.335*	0.819**	0.543**	1						
	p	*0.016*	*0.000*	*0.000*							
5.Planning/ Organization	r	0.399**	0.624**	0.608**	0.586**	1					
	p	*0.004*	*0.000*	*0.000*	*0.000*						
6. PDQ-D Total	r	0.360**	0.794**	0.726**	0.891**	0.782	1				
	p	*0.009*	*0.000*	*0.000*	*0.000*	*0.000*					
7. SASS	r	-0.357*	-0.044	-0.528**	-0.168	-0.432**	-0.356*	*1*			
	p	*0.010*	*0.761*	*0.000*	*0.239*	*0.000*	*0.010*				
8. NDE	r	0.358**	0.021	0.376**	0.234	0.259	0.289*	*-0.531***	*1*		
	p	*0.010*	*0.885*	*0.006*	*0.098*	*0.066*	*0.040*	*0.000*			
9. NHA	r	-0.162	-0.223	0.038	-0.089	0.153	-0.035	*-0.163*	*0.238*	*1*	
	p	*0.257*	*0.116*	*0.792*	*0.535*	*0.283*	*0.807*	*0.254*	*0.092*		
10. NSA	r	-0.057	-0.188	0.536**	0.144	0.134	0.220	*-0.495***	*0.360***	*0.376***	*1*
	p	*0.689*	*0.186*	*0.000*	*0.315*	*0.347*	*0.120*	*0.000*	*0.009*	*0.007*	

* Statistical significance was defined as $p< 0.05$, ** $P \leq 0.01$. BDI: Beck Depression Inventory, PDQ-D: Perceived Deficits Questionnaire-Depression, SASS: Social Adaptation Self-evaluation Scale, NDE: Number of depressive episodes, NHA: Number of hospital admissions, NSA: Number of suicide attempts.

A negative significant correlation was determined between SASS scores and prospective memory (r=-0.528, p<0.01), planning/organization (r=-0.432, p<0.01) and number of suicide attempts (r=-495, p<0.01) (Table 2).

Regression Analysis

While SASS was used as dependent variable, BDI and PDQ-D total scores were assigned as independent variable. The linear regression analysis results indicated that BDI (R2=0.128) and PDQ-D total (R2=0.127) scores were a predictor of SASS (p< 0.05) (Table 3).

Table 3. Linear regression analysis results for variables predicting SASS total points.

Dependent Variable	Predictors	B	Std E	Beta	T	p
SASS	BDI	-0.993	0.371	-0.357	-2.679	0.01
	PDQ-D Total	-0.283	0.106	-0.356	-2.668	0.01

Statistical significance was defined as p< 0.05, BDI: Beck Depression Inventory, SASS: Social Adaptation Self-evaluation Scale, PDQ-D: Perceived Deficits Questionnaire-Depression.

Discussion

The study investigated the correlation of residual depressive and subjective cognitive symptoms with social functioning in remitted MDD patients. In the present study, it was determined that there was a significant correlation between subjective cognitive and depressive symptoms and social functioning.

In the present study it was found that there was a significant correlation between the number of past episodes and subjective cognitive impairment in MDD patients in remission phase. Similarly there are studies emphasizing the correlation between the number of depressive episodes and cognitive impairment in patients with depression in remission phase [23]. On the other hand, there are studies finding no evidence to this correlation at all [24, 25].

In the present study it was determined that there was a significant correlation between residual depressive symptoms and residual subjective cognitive symptoms and social dysfunction. In addition, residual depressive symptoms were a predictor of social dysfunction. Residual depressive symptoms may impair psychosocial functioning [26]. However, residual depressive symptoms alone do not explain the social dysfunction. Moreover it has been reported that as long as residual depressive impact is controlled, cognitive symptoms play a key role in functional improvement [7].

In the present study it was determined that there was a significant correlation between residual subjective cognitive symptoms and social dysfunction. Also residual subjective cognitive symptoms were a predictor of social dysfunction. 60% of MDD patients have neurocognitive deficits six months after the treatment [7]. In previous studies, it was reported that residual cognitive symptoms might be encountered in MDD patients in remission [27] and these symptoms might play a role in social dysfunction [5]. In addition, Nierenberg et al., suggested that residual symptoms in the remission phase might be associated with impairment in psychosocial functioning in 80% of cases [28]. The presence of these residual symptoms may increase dysfunction and destroy the quality of life of patients [4]. As a consequence, psychotherapeutic interventions aiming to heal residual cognitive symptoms in the remission period, may contribute to the improvement of social functioning.

In the present study, it was determined that there was a negative significant correlation between the prospective memory and planning/organization subscales of PDQ-D and social dysfunction. In the remission phase of major depression, impairment is observed in attention, memory and executive functions [10, 29]. Decrease in the speed of mental processes associated with cognitive functions may restrict the daily life functions of patients and reduce their interaction with other people. Thus, social functioning of patients may be affected negatively.

Cognitive tests are used in identifying the depression risk in the early period. By this way, evaluating cognitive symptoms in individuals under risk, following the changes in these symptoms and initiating appropriate treatment interventions in the early period may contribute to the course of disease positively [30]. In the evaluation process of these patients, rapid and practical subjective cognitive tests can be preferred as an alternative to objective neurocognitive batteries.

The present study had some limitations. First of all, as the study was cross-sectional, it was hard to make an inference about the cause and effect relationship. Secondly the depression patients who suffered from depression with psychotic characteristics in the past could not be distinguished. The third limitation was the small number of cases. Finally the patients took psychotropic drugs (antidepressants, antipsychotics, benzodiazepines, mood stabilizers). As it would not be ethical to terminate the medications causing residual cognitive symptoms, the study was conducted with the patients who were under medication.

As a consequence, residual depressive and subjective cognitive symptoms are associated with social dysfunction in remitted MDD patients. Residual depressive and subjective cognitive symptoms in these patients may be a determinant of social functioning. Thus it is crucial to identify cognitive symptoms early and intervene in these symptoms in order to enhance social functioning in the remission phase in MDD. Also rapid and practical subjective cognitive scales in clinics can be used as an alternative to neuropsychological tests in evaluating cognitive changes.

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Retrospective donor hepatectomy results in living donor liver transplant– A single-center experience

Canlı vericili karaciğer naklinde retrospektif donör hepatektomi sonuçları- Tek merkez deneyimi

Ramazan Dönmez [1], Oya Andaçoğlu [2]

Abstract

Objectives: We aimed to describe our technique and donor selection for donor hepatectomy, review our case series and report our complication rates and outcomes.
Materials and Methods: We retrospectively reviewed 41 consecutive donor hepatectomy cases between October 2019 and November 2020 at Yeditepe University, Istanbul, Turkey. Complications were graded according to Clavien-Dindo classification. All cases were performed via laparotomy.
Results: Out of 41 donor cases, 38 (92.6%) were right lobe, 2 (4.8%) were left lobe and 1 (2.4%) was left lateral segment donor hepatectomy. Follow up was 9 ±2,2 months (4-16 months). There were 8 (19.5%) complications and all were minor (grade 1 or 2). There were no grade 3 or higher complications. Three (7.3%) of our donors are heterozygous for Factor Leiden mutation and 4 (9.7%) of our donors had heterozygous prothrombin mutation. Length of stay was average 6.4±1.4 days (range=5-12). Ten donors lost weight with a supervised diet and exercise program. There was one wound complication in this subset of patients.
Conclusions: We present our single center donor hepatectomy series with excellent results. We also describe successful weight loss for donors with Body Mass Index >30 kg/m^2. Donor safety is the most important component of living donor liver transplantation. As donor results continue to improve, living donor liver transplant will continue expand worldwide.

Keywords: Donor complication, donor hepatectomy, liver transplantation, weight loss

Öz

Amaç: Donör hepatektomi tekniğimizi ve donör seçimimizi tanımlamayı, vaka serimizi gözden geçirmeyi, komplikasyon oranlarımızı ve sonuçlarımızı bildirmeyi amaçladık.
Gereç ve Yöntemler: Yeditepe Üniversite Hastanesi'nde Ekim 2019- Kasım 2020 tarihleri arasında 41 ardışık donör hepatektomi olgusunu retrospektif olarak inceledik. Komplikasyonlar Clavien-Dindo sınıflamasına göre derecelendirildi. Tüm olgular laparotomi ile yapıldı.
Bulgular: 41 donör olgusunun 38'i (%92,6) sağ lob, 2'si (%4,8) sol lob ve 1'i (%2,4) sol lateral segment donör hepatektomisi idi. Takip süresi 9 ± 2,2 aydı (4-16 ay). Sekiz (%19,5) komplikasyon vardı ve hepsi minördü (derece 1 veya 2). Derece 3 veya daha yüksek komplikasyon görülmedi. Üç donörde heterozigot Faktör 5 Leiden mutasyonu ve 4 tanesinde heterozigot protrombin mutasyonu vardı. Kalış süresi ortalama 6,4±1,4 gündü (aralık = 5-12). Kontrollü diyet ve egzersiz programı ile 10 donör kilo verdi. Bu hasta alt grubunda bir yara komplikasyonu vardı.
Sonuç: Tek merkezli donör hepatektomi serimizi çok iyi sonuçlarla sunuyoruz. Ayrıca Vücut Kitle İndeksi > 30 kg/m^2 olan donörler için başarılı kilo vermeyi de tanımlıyoruz. Donör güvenliği, canlı donör karaciğer naklinin en önemli bileşenidir. Donör sonuçları iyi oldukça, canlı vericili karaciğer nakli dünya çapında yaygınlaşmaya devam edecektir.

Anahtar Kelimeler: Donör hepatektomi, donör komplikasyonu, karaciğer nakli, kilo kaybı

[1] Fenerbahce University, Vocational School of Health Services, Istanbul, Turkey.
[2] Akdeniz University, Organ Transplantation Research and Application Center, Antalya, Turkey.

RD: 0000-0003-2455-6380
OA: 0000-0001-5777-0266

Ethics Committee Approval: This study was approved by Yeditepe University Clinical Research Ethics Committee with an approval number of 18.02.2021, 1393.
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Sorumlu yazar / Corresponding author:
Ramazan Dönmez

Adres/Address: Atatürk Mah. Ataşehir Bulvarı, Metropol İstanbul, 34758, Ataşehir – İstanbul, Turkey.

E-mail: donmez3570@gmail.com
Tel/Phone: +90 216 910 1907

Introduction

First living donor liver transplant (LDLT) in Turkey was performed by Dr. Haberal and his team in 1990. As of 2020, there are 48 liver transplant centers in Turkey according to the Ministry of Health web page [1]. Although not a part of the publicly available report, approximately 15-20 of these centers perform LDLT at least 10 or more cases annually. Donor outcomes mainly based on single center studies. Common complications after donor hepatectomy are wound infection, bile leak, and incisional hernia. According to a recent meta-analysis by Brown at al. they reported that any complication rate could up to 60% including the minor ones after donor hepatectomy [2].

Herein, we report our own center experience with donor hepatectomy, including weight loss for the potential donor, our technique, complication rates and outcomes.

Material and Methods

We retrospectively reviewed all of our donor hepatectomy medical records between October 2019 and November 2020. It is allowed up to 4th degree relatives to donate an organ to the recipient in Turkey. We had ethics committee approval for all donor candidates if the donor was unrelated to the recipients. Institutional Review Board (IRB) permission was obtained (KAEK#1393) for this study. Statistical analyzes were performed using SPSS v22.0 (IBM, Armonk, NY, USA). Quantitative variables were expressed as mean ± SD, median, min-max, and range. Qualitative variables were reported as numbers and percentages (%). Mean and standard deviations are used for homogeneous distributions, while median and range values are given for heterogeneous distributions.

Preoperative preparation of the donor

Comprehensive laboratory tests are performed for the donor candidate including complete blood count, full chemistry, C-reactive protein, coagulation mutations and routine coagulation labs, urinary analysis, viral serology and lipid panel. Triphasic computed tomography (CT) was obtained for the vascular anatomy and liver steatosis was determined via Hounsfield unit (HU) difference between liver and spleen and absolute HU values of the liver on non-contrast phase. If the liver average HU was ≥50 HU in addition to HU difference Liver-spleen was ≥5, it was deemed that there was no significant steatosis. In addition, ratio of liver/spleen HU> 1.1 was also considered as no significant steatosis. None of the donors had liver biopsy. Magnetic resonance cholangiopancreatography (MRCP) was performed in order to delineate the biliary anatomy. We utilized graft to the recipients' weight ratio (GRWR) and used ≥ 0.8 cut off in addition to future liver remnant (FLR) volume of minimum 30%. If liver/spleen HU ratio was <1.1 and/or absolute liver HU average was <50, this was determined as significant steatosis. Donors with BMI>30 or donors with significant steatosis on CT scan as described above, were counseled to our dedicated nutritionist and were provided a specific diet and encouraged to lose weight before donation if there were no alternative donors. Liver cutting area and volume calculations were made in Myrian XP-Liver program. The measurements were made together by a ten-year experienced radiologist and donor surgeon. Weight loss program included the following: Uptempo walk at least twice daily, for 45 minutes minimum, black coffee twice daily, metformin 850 mg daily, lean protein rich and low fat and low carbohydrates diet (tailored based on the needs of the individual donor by the dietician). These donors had a repeat CT scan in order to ensure weight loss and to quantify the decease in steatosis. All donors and recipients underwent an evaluation process through a multi disciplinary discussion conference which included gastroenterology, anesthesia, transplant surgery and psychiatry.

Donor Hepatectomy Operation

All donor operations were performed by a dedicated donor surgeon (RD). The donor surgeon who performed the operations had an experience of 10 years and approximately 1000 cases. All cases were open. All recipients had central venous catheter, nasogastric catheter, Foley catheter and ampicillin prophylaxis. Central venous pressure was targeted for 0-2 mmHg. Thompson automatic retractor was used in operations. Patient was on 30° reverse Trendelenburg position. J-Shaped incision was used to enter the abdomen. Triangular ligaments were released. Short hepatic veins were ligated. After encircling hepatic vein, liver hanging maneuver was performed and attention was turned to the porta hepatis. After identifying the hepatic artery and portal vein, demarcation line was obtained by gentle clamping of the inflow with bulldog clamps (Figure 1). Intended resection point of the bile duct was marked with clips and routine intraoperative cholangiogram was performed through cystic duct (Figure 2).

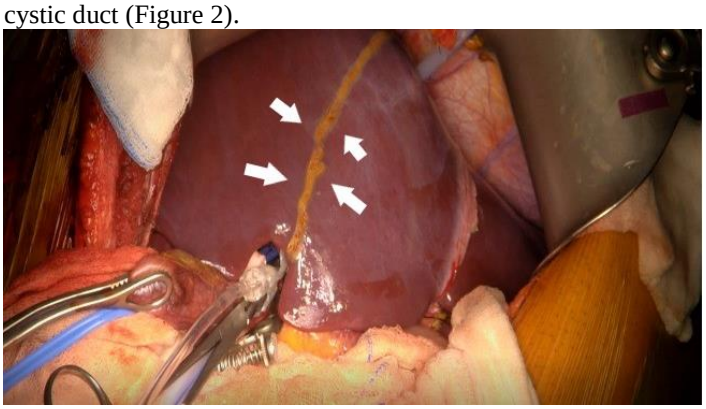

Figure 1. Demarked liver, liver hanging maneuver

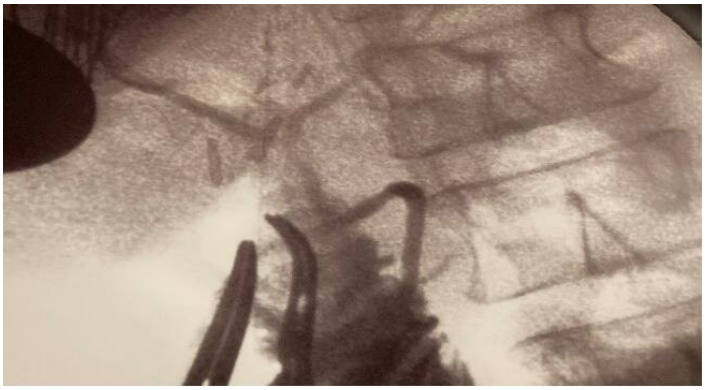

Figure 2. Intraoperative cholangiogram

Parenchyma resection was performed using Cavitron ultrasonographic aspirator (CUSA, Excel 2016-01 Version) (Figure 3-4).

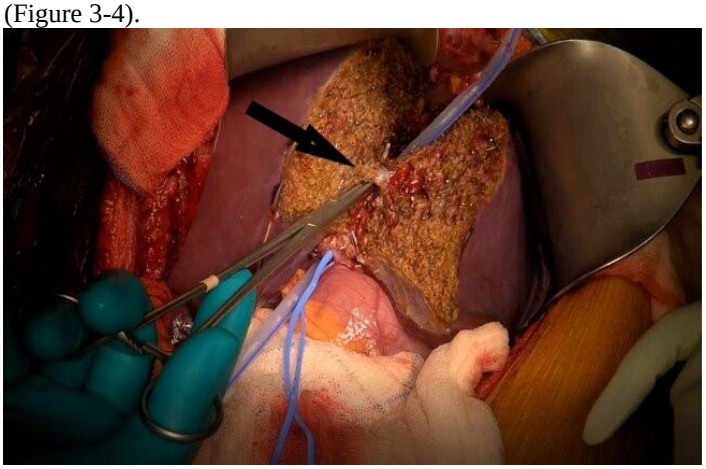

Figure 3. Paranchymal resection and Segment V vein.

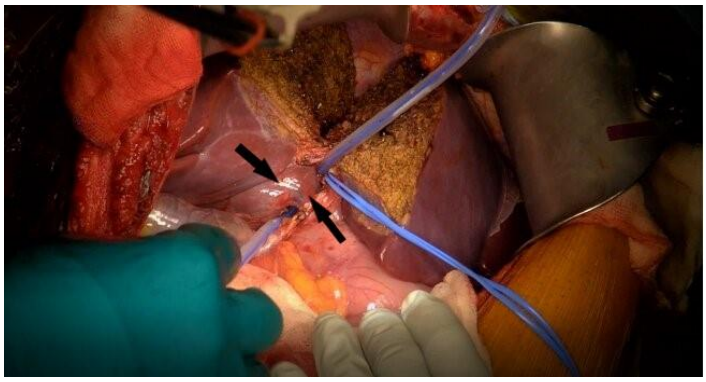
Figure 4. Paranchymal resection of the caudate lobe.

We also utilized bipolar cautery and metal clips. Middle hepatic vein was preserved for all right lobe donors. Segment V and VIII veins were re-constructed if they were ≥5 mm. Extra caution was exercised to divide the biliary plate in order avoid too much dissection and to avoid ischemia of both the remnant and the graft bile duct. The bile ducts, hepatic artery, and right portal vein were cut, respectively, and dissection was completed (Figure 5-8).

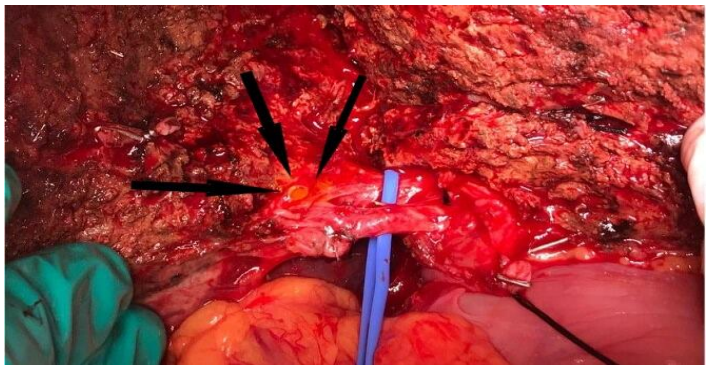
Figure 5. Transected bile duct.

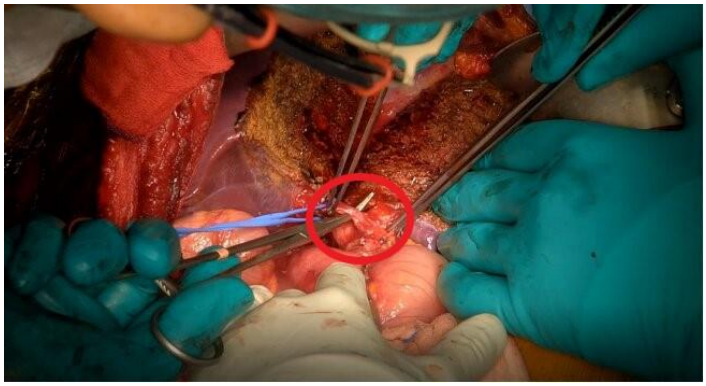
Figure 6. The right hepatic artery.

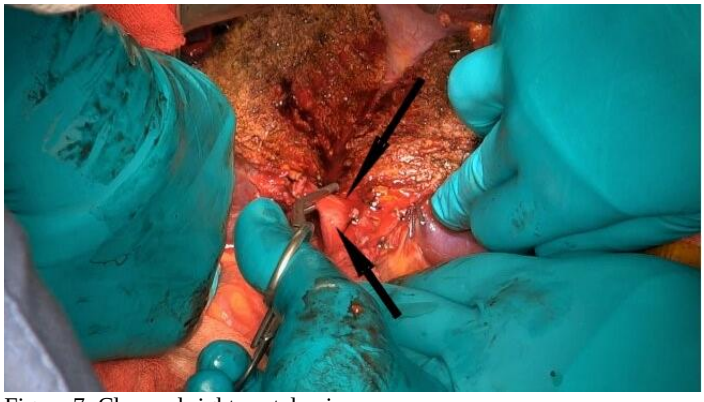
Figure 7. Clamped right portal vein.


Figure 8. Clamped right hepatic vein.

In left and left lateral donor hepatectomies, the left triangular ligament was released from the top and bottom to the level of the vena cava. Parenchyma transection was performed over the line drawn from the left hepatic vein to the portal bifurcation, leaving the liver 0.5 cm to the right of the falciform ligament in the left lateral segmentectomy. Once graft was removed, hepatic vein stump was sutured with 4-0 prolene, portal vein and hepatic artery stumps were sutured with 6-0 prolene, and bile duct openings were sutured with 6-0 PDS. Remnant left lobe was fixed to the abdominal wall by suturing falciform ligament to the abdominal wall after right hepatectomy cases. One silastic drain was left in the surgical site for all cases (Figure 9). Facia was closed in single layer with 1-0 PDS. Skin was closed with absorbable sutures. We utilized N-acetyl cysteine (NAC) IV infusion intra-operatively for all cases. We also utilized 1000 units IV heparin before hepatic artery division. All patients were extubated at the end of the operation, and they were observed in the intensive care unit for the initial 24 hours.

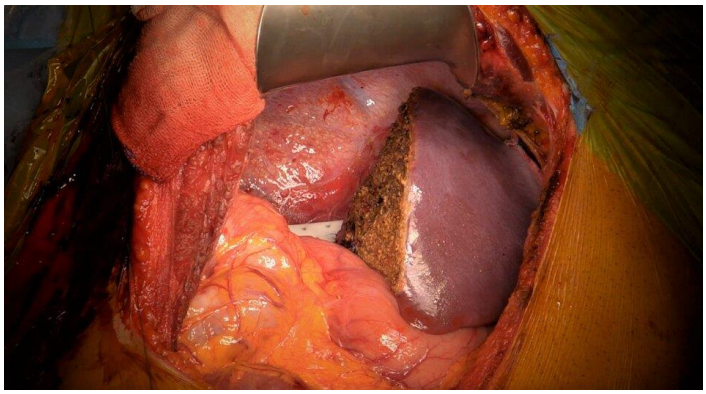
Figure 9. Remnant liver image.

Post-operative Care

All patients remained in the intensive care unit for the initial 24 hours with laboratory tests and routine Doppler ultrasound was performed on post-operative day 1. They were moved to the regular floor on postoperative day one. Early mobilization was encouraged. Incentives spirometer was used vigorously on a daily basis. Routine antibiotics were continued for 24 hours and was continued if wound infection was suspected. We continued the parenteral NAC for 5 days. Once INR was below 1.8, low molecular weight heparin was initiated as a prophylaxis. All the donors continued prophylactic anticoagulants for two weeks after discharge and for a month if they had any prothrombotic mutations such as factor five Leiden or prothrombin gene mutation at preoperative work up. Heterozygous Factor V Leiden mutation or Prothrombin gene mutation are not an exclusion criteria from donation at our center. Drain was removed on post-operative day 6 or 7 or when the output was less than 100 mL/day and serous.

Results

Demographic features are summarized in table 1.

Table 1. Baseline characteristics of donors.

Features	n	range or %
Age	33 ±8.1	20-47
Gender Male/female	32/9	
BMI	25.14 ±3.9	17.4-33.9
FLR	36.18 ±5.7	30-46
Blood loss (mL)	108 ±33	100-300
RL	38	92.68
LL	2	4.8
LLS	1	2.4
Operative time (min)	4.4 ±0.5	3.3-6.3
LOS (days)	6.4 ±1.4	5-12
Weight loss donor	10	24.4
Factor V Leiden mutation(Heterozygous)	3	7.3
Prothrombin gene mutation(Heterozygous)	4	9.7
Follow up (mo)	9 ±2.2	3-16

BMI: Body mass index. FLR: Future liver remnant. RL: Right lobe, LL: Left lobe, LLS: Left lateral segment, LOS: Length of stay.

We had total of 41 donor hepatectomy cases Our average age was 33, with a mean BMI of 25. We had 10 donors with BMI above 30 or steatosis greater than 30% on CT scan who underweight weight loss and exercise program under supervision. Follow up time was 9 months. Blood loss was minimal (108 ±33 mL, range=100-300). There was no blood transfusion. Mean length of stay was 6.4 days (5-12 days). There were no re-operations due to complications. We did have total of 8 donors with complications (19.5%) and all were minor (grade 1 or 2). There were no grade 3 or higher complications (Table 2).

Table 2. Complications of the donors.

Complications	n	Grade	Management
Wound infection	3	2	Prolonged antibiotic
Prolonged dyspepsia	2	1	Symptomatic treatment
Ascites	2	2	Diuretics, albumin, prolonged surgical drain
Bile leak	1	2	Prolonged surgical drain

Two donors had prolonged dyspepsia, nausea and vomiting, requiring prolonged parenteral anti-emetics and proton pump inhibitors. Wound complications were treated with prolonged perioperative antibiotics. None of the wounds were opened. There was one bile leak that was managed by keeping the surgical drain for total of 8 weeks. Surgical drain was removed once the output was less than 50 mL/week. Repeat ultrasound at 10 weeks showed no collection. Ascites was defined as greater than 1000 mL/day serous output. Albumin and diuretics were used for these patients. We also had 10 donors who lost weight before they were accepted as liver donor. Median BMI before weight loss was 33.2 (range=28.4-37, SD=2.48) and median BMI at operation was 29.7 (range=25.7-33.4, SD=2.29). Three (7.3%) of our donors are heterozygous for Factor 5 Leiden mutation and 4 (9.7%) of our donors had heterozygous prothrombin mutation. Median LOS was 6 days for the weight loss donors. One weight loss donor had wound seroma, others had no complication. Regarding the recipients who received liver from the donors after the weight loss program, there was one bile leak and one rejection.

Discussion

The safety of the donor is the most important aspect of any living donor transplant. As this safety becomes the norm, living donor liver transplant rates would continue to increase worldwide. Therefore safe surgical techniques remain the key of successful outcomes. Herein we describe our safe technique with excellent donor outcomes.

Majority of the literature states that surgeon and center experience is closely related with lower complication rates [2-5]. Kim et al. reported that the experience of the center was important in order to decrease the complications rates of donor hepatectomy with their series of over 500 donors [3]. They divided patients into 3 periods: period A (n = 100), period B (n = 200), and period C (n = 200). They found that over time, the operative time, the amount of transfusions during surgery, hospital stay, and the incidence of biliary complications decreased. There was no mortality. Even though the total complication rate was high (21.6 %, n = 108) including 10.6 % (n = 53) of biliary complications, the grade 3 complication rate was 9.4 % (n = 47). In most patients with grade 3 complication, interventional therapies via radiologic or endoscopic approaches corrected these complications, and reoperation was required for ten patients (2 %). They also reported that biliary complications were related with operation period and operative time. They concluded that optimization of donor selection as well as institutional experience is imperative to improve the surgical outcome [3]. Even though donor hepatectomy was associated with relatively higher complication rate, most complications showed low-grade severity which could be corrected by interventional therapies. Shaji Matthew et al. reported 1 mortality due to biliary sepsis [6]. There are other series reporting biliary and overall complications after donor hepatectomy [2-10]. A recent systematic review by Braun et al. they reviewed 33 studies, reporting outcomes from 12,653 donors (right lobe: 8231, left lobe: 4422) [2]. Of 33 studies, 12 reported outcomes from right lobe donors, 1 from left lobe donors, 14 compared left and right, and 6 focused specifically on biliary complications. A total of 830 biliary complications (6.6%) were reported, with 75 donors requiring re-operation for biliary complications and 1 donor death attributed to biliary complications [2]. They concluded that although bile leaks and strictures are still relatively common following living donor hepatectomy, the majority of complications are minor and resolve with conservative measures. Approximately 6% of living donors will experience a biliary complication and, of these 6%, approximately 9% (total of 0.6% of donors) will require operative management of the biliary complication [2]. In our series, overall complication rate was 19.5% and these were all minor complications. There was one bile leak case and this resolved without the need of any intervention. However due to out small sample size, we were not able to make any comparison.

Erdogan et al. reported complications after 1521 donor hepatectomy between June 2010 and January 2018, (1291 right lobe grafts, 230 left lobe grafts) of patients who underwent LDLT [7]. Of these, 63 donors underwent endoscopic retrograde cholangiography (ERC) due to biliary complication. Biliary stricture was found in 1.6% (25/1521), biliary leakage in 2.1% (33/1521), and stricture and leakage together in 0.3% (5/1521) donors. Their endoscopic success rates in patients with biliary leakage, biliary stricture, and stricture and leakage were 85% (28/33), 92% (23/25), and 80% (4/5), respectively. Surgical treatment was performed on 12.6% (8/63) donors who failed ERC. They concluded that ERC is a successful treatment for post-LDLT donors who have biliary complications [7].

There also some reports reporting higher complication

rates based on graft type (higher complication rates for right lobe donors as opposed to left lobe), however there are contradicting reports as well. [2,8-10]. In our series none of the left lobe donors had complications however we cannot make any conclusions regarding graft type and complication due to our small sample size.

Future remnant liver (FLR) is also evaluated as a risk factor for complications of the donors. Hsu et al. reported that when the future of remnant liver volume was less than 35% there was higher rate of complications. Also post operative AST, ALT, bilirubin, length of intensive care and stay and length of overall hospital stay was higher [11]. In our series among the donors with any complication, only 1 donor had less than 35% future remnant liver, all others had greater than 35% FLR.

Lastly, there are other reports about weight loss for steatosis for living liver donors [12-16]. While most of these focus on steatosis for the potential graft, most of our cohort had high BMI in addition to the steatosis. We also report a safe and supervised weight loss program approach so that the donor pool could be further expanded. This is in concordance with the literature.

In the literature, it is reported that donors with deficiencies in Factor V Leiden, prothrombin mutation and anticoagulant proteins (protein C, protein S and antithrombin) or coagulation factors should be rejected [17,18]. We also excluded donors who are homozygous. Heterozygous Factor V Leiden mutation or Prothrombin gene mutation is not a donor exclusion criterion at our center. We did not observe any complications in these donors.

The effect of standardized donor hepatectomy technique and surgical experience on the results has been demonstrated in studies in the literature [19,20].

Weaknesses of our report includes the very limited sample size, short follow up, and the inability to perform statistical analysis due to aforementioned issue.

Standardization of the technique of the donor hepatectomy is the key to minimize complications. We also believe separate donor and recipient surgeons help to minimize complications. Most complications after donor surgery could be dealt with minimal invasive interventions. We also describe safe weight loss program for donors with BMI greater than 30 or donors with steatosis. These donors can donate liver safely if they comply with a strict exercise and diet program. It should be always kept in mind that donors are completely healthy individuals and the priority should always be the minimization of the donor complications.

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Evaluation of the nutritional status changes of resident physicians before and during the COVID-19 Pandemic

Asistan hekimlerde Covid-19 öncesi ve sırasında çalışma ortamında beslenme durumu değişikliğinin saptanması

Nazlı Batar [1], Ceren Pak [1], R.Nükra Tüfekçi [1], Betül Koçak [2] , Rümeysa Özçalkap [1], Pelin Pak [3] , Seçil Arıca [3]

Abstract

Aim: This study was conducted to determine nutritional changes in the working environment of resident physicians before and during the coronavirus 2019 (COVID-19) pandemic.

Methods: Resident physicians working at Prof. Dr. Cemil Taşçıoğlu City Hospital and who agreed to participate were included in the study. Data were collected using an online questionnaire method developed by the researchers for determining demographic characteristics and nutritional changes in the study participants. Results were analyzed with the SPSS statistical program.

Results: The COVID-19 outbreak affected the nutritional status of 82.2% of the resident physicians. The rate of physicians trying to eat a healthy diet during the pandemic was 79.1%. However, the number of physicians who increased their consumption of fruits and vegetables during the epidemic was 58.2%. During the pandemic, water consumption of the resident physicians was evaluated, and a 51.1% decrease in water consumption was found. While 78.3% of the physicians slept 6–8 hours per night before the pandemic, this rate decreased to 56.6% during the pandemic while the number of physicians who slept for 1 to 5 hours increased to 38% during the Covid-19 outbreak. A significant relationship between sleep duration before and during the pandemic was found ($p < 0.05$).

Conclusion: The Covid-19 outbreak has greatly and continues to affect the living standards of resident physicians. During the study period, it was observed that the diet and sleep patterns of resident physicians who worked in an intense environment had been disrupted.

Keywords: Covid-19, Resident physician, Healthy diet, Sleep

Öz

Amaç: Bu çalışmada, koronavirüs (COVİD-19) salgını öncesi ve sırasında asistan hekimlerin çalışma ortamında beslenme durumu değişikliklerinin saptanması amacıyla yapılmıştır.

Materyal ve Metod: Prof. Dr. Cemil Taşçıoğlu Şehir Hastanesinde görev yapan ve çalışmaya katılmayı kabul eden asistan hekimler çalışmaya dahil edilmiştir. Araştırmaya katılan bireylerin demografik özelliklerini ve beslenme durumu değişikliklerini belirlemek için araştırmacılar tarafından geliştirilen online anket yöntemi ile veriler toplanmıştır. Sonuçlar SPSS istatistik programı ile analiz edilmiştir.

Bulgular: Covid-19 salgını asistan hekimlerin %82,2'lik kısmının beslenme durumunu etkilemiştir. Salgın sırasında sağlıklı beslenmeye çalışan hekim oranı %79,1 olarak saptanmıştır. Bununla beraber meyve ve sebze tüketimini salgın sırasında arttıran hekim sayısı ise %58,2'dir. Salgın sırasında asistan hekimlerin su tüketimleri sorgulanmış ve %51,1 oranında azalma saptanmıştır. Salgın öncesi hekimlerin %78,3'ü 6-8 saat oranında uyurken, salgın sırasında bu oran %56,6'ya düşmüş ve 1-5 saat uyuyan hekim sayısı %38'e çıkmıştır. Salgın öncesi ve salgın sırasında uyku süresi arasında anlamlı bir ilişki olduğu görülmüştür ($p<0.05$).

Sonuç: Covid-19 salgını asistan hekimlerin yaşam standartlarını büyük ölçüde etkilemiştir. Bu dönemde yoğun çalışma temposu içerisinde olan asistan hekimlerin beslenme ve uyku düzenlerinin bozulduğu görülmüştür.

Anahtar kelimeler: Covid-19, Asistan hekim, Sağlıklı beslenme, Uyku

[1] Istanbul Kültür University, Health Sciences Faculty, Department of Nutrition and Dietetics, Istanbul, Turkey.
[2] Atlas University, Health Sciences Faculty, Department of Nutrition and Dietetics, Istanbul, Turkey.
[3] Prof.Dr.Cemil Taşcıoğlu City Hospital, Department of Family Physician, Istanbul, Turkey.

NB: 0000-0001-9527-5709
CP: 0000-0002-9418-6795
RNT: 0000-0002-8294-3197
BK: 0000-0003-3664-9066
RÖ: 0000-0002-8006-0411
PP: 0000-0002-0831-355X
SA: 0000-0003-0135-6909

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Sorumlu yazar / Corresponding author:
Nazlı Batar
Adres/Address: İstanbul Kültür University, Health Sciences Faculty, Department of Nutrition and Dietetics, E5 Karayolu Üzeri, No: 22 Bahçelievler 34191 Istanbul, Turkey.
e-mail: n.batar@iku.edu.tr
Tel/Phone: 0533-413-3717

Introduction

In 2019, the World Health Organization's (WHO) China Country Office announced cases of pneumonia with unknown etiology and unknown mode of transmission had spread in Wuhan located in Hubei province. On January 7, 2020, this disease was defined as a new type of coronavirus (2019-nCoV/COVID-19) that had not previously been seen in humans. WHO described the COVID-19 outbreak as an "international public health emergency" on January 30 after which COVID-19 cases were found in 113 countries outside of China and had spread to six continents except Antarctica. Immediately after the first positive case was seen in Turkey on March 11, 2020 and due to the rapid spread and severity of the virus, COVID-19 was defined as a global epidemic/pandemic [1].

During this pandemic, healthcare workers who work at a very intense pace had to adapt to working at this newer pace [2]. It is the responsibility of the hospital management to protect and care for healthcare professionals during this process. In one study, it was determined that a patient who came to the hospital during the pandemic could transmit the COVID-19 virus to 10 physicians in the same environment [3]. Resident physicians, who are at the forefront of the pandemic process, are at risk during this period, both physically and psychologically [4]. Multiple factors, such as the fact that the hospital in which they work is a pandemic hospital, the increase in working hours, the fact that their colleagues could be or become infected, the equipment is not sufficient, and the treatment of the disease is not certain, increase the negative psychological and physical effects of handling this crisis for these healthcare workers [5].

Healthy nutrition is defined as adequate and balanced nutrition. It has been found that individuals who are healthy and well-nourished have an increase in their quality of life and have stronger immune systems when combatting diseases. It is very important to keep the immune system as strong as possible as a precaution against COVID-19 [6]. It is recommended that employees obtain good nutrition and when necessary, take nutritional supplements, such as vitamin–mineral supplements, and prebiotics and probiotics to allow them to maintain their hectic working schedules. Although no food or beverage that prevents or treats the transmission of COVID-19 exists, healthy nutrition, physical activity, and regular sleep have proven to be supportive when combatting this virus. In this process, an accepted precaution against this virus is that people will quarantine at a distance from each other. Mood changes, such as quarantine-associated stress and depression, can increase the tendency to eat foods with high carbohydrate density. As a result, unwanted weight gain may occur with the high sugar content found in these carbohydrate foods [7]. Published by the Turkish Dietetic Association, Covid-19 nutritional guidelines were created according to the suggested Healthy Eating Plate. In each main meal of this plate, one quarter of the meal consists of vegetables, the other quarter is whole grain products, and the remaining half consists of three equal pieces of fruit, high-protein foods (such as legumes, meat, eggs, fish, chicken, oilseeds) and dairy products (such as milk, yogurt, buttermilk, cheese). In addition, COVID-19 nutritional recommendations, such as ensuring sufficient daily water consumption and adding olive oil to meals, are found in these guidelines [9].

This study was conducted to determine the nutritional changes in resident physicians before and during the COVID-19 pandemic.

Material and methods

This study is a cross-sectional study, and all 200 resident physicians working in the Prof. Dr. Cemil Taşçıoğlu City Hospital constitute the study population while 129 residents who agreed to participate in this study formed the study sample. The data were collected by researchers using a questionnaire created by compiling information from the literature. The questionnaire included questions about anthropometric and demographic characteristics of individuals and nutritional status changes before and during the pandemic. Due to the pandemic, the questionnaire was not applied face-to-face but was converted into an online questionnaire form and delivered to the resident physicians. Before starting this study, the institution's approval was obtained from the hospital's chief physician, and study approval was obtained from the Ministry of Health. Resident physicians were required to read and approve the informed consent form before participating in this study. This study was based on voluntary participation, and no reward was given for participating.

Ethics committee approval for this study was obtained from Istanbul Kültür University Ethics Committee (Decision number: 2021.12).

Statistical analysis

The SPSS 20 program was used for statistical analyses. In the analysis of the data, frequencies and percentages were used to describe the group according to gender, marital status, branch, and people who lived together. Average and standard deviation values were given for body mass index (BMI) in women, men, and the whole group. The effects of working during the COVID-19 pandemic on diet, efforts to eat healthy, increases in probiotic use and vitamin C intake, water consumption, number of meals, fruit and vegetable consumption, sleep time before the pandemic, locations for meals before and during the pandemic, and percentage analysis were evaluated. Relationships between gender and Covid-19 diagnosis, pandemic-associated effects on diet, weight changes during the pandemic, changes in water consumption, changes in the number of meals during the pandemic, efforts to eat healthy, changes in fruit and vegetable consumption, and intake of vitamins, probiotics, and vitamin C was examined by using the chi-square independence test. In addition, the chi-square test of independence was used for the relationship between the location for meals before and during the pandemic. A p value < 0.05 was considered statistically significant.

Results

The study group consisted of 74 women and 55 men (57.4% and 42.6%, respectively) and a total of 129 doctors. Ninety-six (74.4%) of the doctors were not married and 33 (25.6%) were married. Branches consisted of emergency medicine (8), primary care (57), internal medicine (23), dermatology (3), obstetrics (4), otolaryngologist (3), neurology (1), orthopedics and traumatology (4), pediatrics (25), and plastic surgery (1). Lifestyle status of the participants included living alone (49), with their spouse/friend (29), with their spouse and children (18), with their parents (30), and with a large family (3). The mean BMI was 22.40 ± 8.29 kg/m2 for women, 26.01 ± 5.24 kg/m2 for men, and 23.94 ± 7.35 kg/m2 for the whole group.

The frequency and percentages according to the variables are shown in Table 1. Accordingly, 33.3% of the doctors were diagnosed with COVID-19. Most (82.2%) of the participants stated that the COVID-19 pandemic affected their diet. It can be seen that 79.1% of the participants made an effort at healthy nutrition during the pandemic. The rate of those who

stated that they increased the use of probiotics was 63.6%, and the rate of those who stated that they increased their vitamin C intake was 75.2%.

Table 1. Frequency and percentages of variables

Variables	Group n(%)	Women	Men	Total
COVID-19 diagnosis	Yes	30 (40.5)	13 (23.6)	43 (33.3)
	No	44 (59.5)	42 (76.4)	86 (66.7)
Affects diet	Yes	66 (89.2)	40 (72.7)	106(82.2)
	No	8 (10.8)	15 (23.3)	23 (17.8)
Efforts to eat healthy	Yes	61 (82.4)	41 (17.6)	102(79.1)
	No	13 (74.5)	14 (25.5)	27 (20.9)
Increase in probiotic intake	Yes	47 (63.5)	35 (63.6)	82 (63.6)
	No	27 (36.5)	20 (36.4)	47 (36.4)
Increase in vitamin C intake	Yes	59 (79.7)	15 (69.1)	97 (75.2)
	No	15 (20.3)	17 (30.9)	32 (24.8)

COVID-19: Coronavirus 2019

Nutritional behavior during the epidemic is given in Table 2. Participants stated that water consumption decreased by 55.1% during the pandemic. Similarly, 59.7% of the study doctors stated that their number of meals decreased. However, 58.2% of the participants increased their consumption of fruits and vegetables. In addition, vitamin supplement use was checked during the pandemic. The rate of doctors who took vitamin supplements during the study period was 53.3% (70), while the rate of those who did not take was 30.2% (39), and the rate of those who used vitamin supplements independent of the pandemic was 15.5% (20).

Table 2. Nutritional behavior during the pandemic

Variables n (%)	Water Consumption	Women	Men	Total
Water consumption	Increased	12 (16.2)	13 (23.6)	25 (19.4)
	Decreased	46 (62.2)	20 (36,4)	66 (51.1)
	Unchanged	16 (21.6)	22 (40)	38 (29.5)
Number of meals	Increased	5 (6.8)	5 (9.1)	10 (7.8)
	Decreased	44 (59.4)	33 (60)	77 (59.7)
	Unchanged	25 (38.8)	17 (30.9)	42 (32.5)
Fruit and vegetable consumption	Increased	48 (64.9)	27 (49)	75 (58.2)
	Decreased	4 (5.4)	3 (5.5)	7 (5.4)
	Unchanged	22 (29.7)	25 (29.5)	47 (36.4)

Information on sleep status before and after the epidemic is shown in Table 3. While 78.3% of the participants slept between 6 and 8 hours before the epidemic, the rate of those who slept 6–8 hours during the pandemic was 56.6%, and the rate of those who slept 1–5 hours was 38.0%.

Places to eat in the working environment before and during the pandemic are shown in Table 3. While 67.2% of the participants ate at a restaurant or cafe before the epidemic, it was observed that 55% ate their meals at home during the study period. In addition, no significant difference between eating places in the work environment before and during the pandemic was found ($p > 0.05$).

In Table 4, it can be seen that significant relationships between gender, COVID-19 diagnosis, the effect of the pandemic on the diet, weight change during epidemic, and the change in water consumption during pandemic study period ($p < 0.05$). The diets of female doctors changed at a higher rate than male doctors. The fact that the epidemic affected the diet of female doctors more than males may have contributed to the relationship that emerged in two variables. Less than half (41.3%) of the female doctors in the study experienced weight loss during the pandemic. Weight loss of female doctors during the epidemic may have affected the relationship of weight loss based on gender. During the pandemic, 62.2% of female doctors reduced their water consumption. The reduction in women's water consumption may have an impact on the water consumption relationship based on gender. Although not included in the table, no relationship between gender and change in the number of meals during the pandemic, efforts to eat healthy, change in fruit and vegetable consumption, and vitamin supplement use, probiotic, and vitamin C consumption was found ($p > 0.05$).

Table 3. Sleep duration and places to eat from in the working environment before and during the pandemic

Variables	Groups n (%)	Women	Men	Total
Sleep duration before the pandemic	1-5 Hours	7 (9.4)	12 (21.8)	19 (14.7)
	6-8 Hours	62 (83.8)	39 (70.9)	101(78.3)
	9 Hours and more	5 (6.8)	4 (7.3)	9 (7)
Sleep duration during the pandemic	1-5 Hours	31 (41.9)	18 (32.7)	49 (38)
	6-8 Hours	42 (56.7)	31 (56.4)	73 (56.6)
	9 Hours and more	1 (1.4)	6 (10.9)	7 (5.4)
Place to eat from before the pandemic	Dining Hall	24 (32.9)	9 (16.4)	33 (25.8)
	Restaurant / Cafe	45 (61.6)	41 (74.5)	86 (67.2)
	From Home	4 (5.5)	5 (9.1)	9 (7)
Place to eat from during the pandemic	Dining Hall	13 (17.6)	15 (27.3)	28 (21.7)
	Restaurant / Cafe	16 (21.6)	14 (25.5)	30 (23.3)
	From Home	45 (60.8)	26 (47.2)	71 (55)

Table 4. Relationships of variables according to gender

Variables	Category	Gender n (%) Women	Men	Total	X^2	df	p
COVID-19 diagnosis	Yes	30 (40.50)	13(23.60)	43(33.30)	3.33	1	0.033
	No	44 (59.50)	42(76.30)	86(66.70)			
Pandemic's affect on diet	Affected	66 (89.20)	40(72.70)	106(82.20)			
	Not affected	8 (10.80)	15(27.30)	23(17.80)	4.77	1	0.015
		10.80%	27.30%	17.80%			
Weight during pandemic	Increased	23 (31.10)	17(30.90)	40(31.00)			
	Decreased	31 (41.30)	13(23.60)	44(34.10)	6.15	2	0.046
	Not changed	20 (27.00)	25(45.50)	45(34.90)			
Water consumption during pandemic	Increased	12 (16.20)	13(23.60)	25(19.40)			
	Decreased	46 (62.20)	20(36.40)	66(51.20)	8.62	2	0.013
	Not changed	16 (21.60)	22(40.00)	38(29.50)			

Table 5 shows the relationship between sleeping hours before and after the pandemic started. It is observed that 78.3% of the participants slept between 6 and 8 hours daily before the pandemic. In addition, a significant relationship between pre- and pandemic period sleep time was found ($p < 0.05$). The fact that 33 people (67.3%) who slept for 6 to 8 hours before the epidemic only slept for 1 to 5 hours during the pandemic led to a significant change in sleep time.

Table 5. Relationship between sleep duration before and after the pandemic.

Variables	Category	Sleeping duration during the pandemic n (%) 1–5 Hours	6–8 Hours	≥ 9 Hours	Total	X^2	df	p
Sleeping duration before the pandemic	1-5 Hours	14(28.60)	3 (4.10)	2 (28.60)	19 (14.7)			
	6-8 Hours	33(67.30)	66 (90.40)	2 (28.60)	101(78.3)	30.94	4	0.000
	≥ 9 Hours	2 (4.10)	4 (5.50)	3 (42.80)	9 (7.00)			

Discussion

The aim of this study was to question and evaluate the nutritional changes of resident physicians in their work environments before and after the start of the COVID-19 pandemic. Only a limited number of studies concerning this issue can be found in the literature, and most of them are focused on the general population rather than healthcare professionals/students studying in health-related departments. For this reason, this study about healthcare professionals is important because it is one of the only studies to address this important issue. The mean BMI was 22.40 ± 8.29 kg/m² (normal) for female resident physicians, 26.01 ± 5.24 kg/m² (overweight) for male resident physicians, and 23.94 ± 7.35 kg/m2 for the whole group. It has been found based on the total body surface area (TBSA) of a population that the frequency of being obese and overweight in women was 29.7% and 41.0%, respectively; in males, it was found to be 39.1% and 20.5%, respectively [10]. In a study conducted by Yücel et al., 41.7% of male physicians compared to 22.2% of female physician were overweight. On the contrary, in whole society studies conducted throughout the country, it was found that female physicians were heavier than male physicians. It has been found that the proportion of overweight and obese health workers tends to be lower than in the general population. The reason for this tendency was thought to be related to the level of consciousness of healthcare workers about diet and nutrition [11]. In our study, the average BMI value of male physicians was 26.01%, and that of female physicians was found to be 22.40%. These results are consistent with those from other studies.

Our physicians have played and continue to play a major role in battling this pandemic [4]. Nutrition also has been shown to have an important role in epidemic-associated protection methods. In the intense work atmosphere of physicians, disruption of their nutrition can have a negative effect on their immune systems and increase their risks of contracting Covid-19. This process can have negative results on the pace of work in this health-related battle [8]. One-hundred six (82.2%) of the physicians participating in this study stated that the COVID-19 pandemic affected their diet. It is thought that vitamin C, known as an immune system enhancer, may be particularly protective against COVID-19 [12]. It is known that this protection is due to the antioxidant properties of vitamin C [6]. It has been reported that vitamin C supplementation is protective against viral infections as it strengthens a person's immunity. In a study conducted in China, in addition to the treatment of patients with moderate and severe COVID-19, high doses of vitamin C (10–20g/day, 8–10 hours) were administered to 50 patients and found to be successful in treating the virus[13]. In our study, the increase in vitamin C consumption by resident physicians during the pandemic was found to be 75.2%. In addition, those who started using additional vitamin supplements during the epidemic was found to be 53.3%; 58.2% of the participants were attentive to increasing fruit and vegetable consumption. The reason for the increase in vegetable/fruit consumption and vitamin supplement during the pandemic is thought to be due to immune system enhancing effects of fruits and vegetables and the COVID relationship [14]. Fruit and vegetable consumption is also very important for strengthening the immune system and ensure adequate fiber intake for the body [15]. In some studies, fruit and vegetable consumption was found to have decreased during the pandemic. Among the reasons for this decrease, a deterioration of individuals' healthy eating habits occurred with the decrease in the number of meals. According to a study by Macit, it was determined that the use of nutritional supplements increased during the COVID pandemic. In the study, it was emphasized that individuals should be informed about adequate balanced nutrition, physical activity, and nutritional supplements [16]. In the article by Coelho-Ravagnani et al., the effects of vitamins and minerals, such as zinc, selenium, and vitamins C, A, and D during the COVID-19 pandemic were described, and it was emphasized that these nutritional supplements should be used in case of food insufficiencies; however, when possible, fresh vegetables and fruits and whole grain foods should be included in the daily diet [17]. In our study, when fruit and vegetable consumption was analyzed based on gender, it was found that consumption increased by 64.9% for female physicians and 49.0% for male physicians during the pandemic study period. However, no statistically significant relationship between the increase of fruit and vegetable consumption and gender was found ($p > 0.05$). In the study by Celorio-Sardà et al., food consumption of individuals before and during the quarantine was examined, and a decrease in alcohol consumption with a concurrent increase in fresh vegetable and fruit consumption was found [18].

Physicians working in an intense, fast-paced environment usually cannot consume the necessary amount fruit and vegetables due to working both shifts and the in-hospital environment conditions [15]. In another study, it was determined that as the quarantine period increased, emotional eating and BMI increased, while healthy eating attitudes decreased [19]. In this study, the nutritional status of physicians before and during the pandemic was examined. It has been determined that 79.1% of the physicians tried to eat healthier during the pandemic study period than before the pandemic. It is thought that physicians see this process as an opportunity to develop healthy eating habits and after considering the possible infection risks under pandemic conditions, they might make an effort to eat healthy in order to strengthen their immune systems.

In this study, it was also observed that the healthy diets of 82% physicians were affected. In a study, the diets of healthcare workers working different shifts before the pandemic was investigated in the work environment, and it was found that they mostly preferred biscuits, crackers, and chips as snacks [20]. In the study conducted by Kesgin et al. involving nurses, it was found that more than half of the individuals generally preferred easily accessible foods and beverages such as hamburgers, toast, bagels, biscuits, chocolates, colas, and ready-made fruit juices [21]. In our study, it is thought that the reason for dietary deterioration of most of the physicians during the pandemic was that they could not find the time to eat a healthy diet due to the busy work pace, rapid and easy access of unhealthy foods, and the pandemic-related effects they experienced in their daily lives. Several other studies have shown that women placed more importance on healthy eating than men [22]. In this study, a statistically significant relationship was found that the diet of female physicians was affected more than male physicians ($p < 0.05$).

Probiotics, which have a positive effect on the immune system and are frequently used during this pandemic period, have also been proven to have positive effects on our health [14]. It has been found that especially garlic and kefir may exert antiviral effects against viruses affecting the respiratory tract and lungs [23]. Addition of probiotic foods (Yogurt, kefir, pickle, vinegar) to our meals is among one protective measure against Covid-19. However, in some sources, it is stated that more studies are needed because there is no clear information about the protective effect of probiotics [16]. In our study, in line with this information, 63.6% increase in the use of probiotics by resident physicians was observed. It is thought that physicians consciously add probiotics to their meals as a protective measure

against the virus, knowing the positive effects of probiotics on the immune system.

About one-third (33.3%) of the resident physicians who participated in our study had been diagnosed with COVID-19, 40.5% of the female resident physicians and 23.6% of the male resident physicians. The reason for this difference is thought to related to the diet of women, which has been more affected by pandemic conditions than the diets of the male participants. A statistically significant relationship was found between the status of diagnosis, the diet, water consumption, and body weight change of the participants ($p < 0.05$). It is thought that sensory loss, such as taste and smell, may have been experienced by the resident physicians who have been diagnosed, and therefore has led to a decrease in the number of meals and weight loss. In another study, it was found that regardless of being diagnosed with COVID-19, individuals may skip their main meals, especially lunch, more than before the pandemic period [24]. The effect of the epidemic on the number of meals was found to have caused significant changes not only for working individuals but also in the number of meals for all individuals. In this study, the way in which the number of meals changed in resident physicians before and during the pandemic was investigated. Over half (59.7%) of the physicians stated that the number of meals decreased among resident physicians who were involved in an intense working environment during the pandemic period. While the rate of the number of physicians who said that the number of meals increased was 7.8%, those who said that they did not change their number of meals was only 32.5%. The pandemic period caused a significant decrease in the number of meals of resident physicians. Although studies involving resident physicians are inadequate, when looking at other studies examining the pandemic and the number of meals, it can be seen that a great majority of the society experienced a serious decrease in the number of meals during this period. It was determined that the individuals whose number of meals increased were actually in a constant state of snacking. Considering the number of meals according to gender in this study, it was observed that the number of meals for female physicians decreased by 59%, and the number of meals by male physicians decreased by 60%. In another study, it was observed that the decrease in the number of meals of female physicians was greater than that of male physicians [25]. As a result of this study, a statistically significant decrease was observed in the number of meals of resident physicians regardless of gender in this intense work environment ($p < 0.05$).

In a study conducted before the pandemic with healthcare professionals, water consumption was examined and found to be 7.43 ± 3.51 glasses per day [11]. In our study, it was found that water consumption during the pandemic decreased significantly (62.2%), especially in female residents. The reason for this decrease is thought to be the mask used as protective equipment against the virus in the working environment in addition to the fear and anxiety that the virus may be transmitted through the working environment. In a study conducted with doctors and nurses, it was found that stress and anxiety increased in participants during the pandemic period. In addition, stress levels and sleep times were also examined, and it was found that sleep times and stress levels were inversely proportional; as the stress level increased, sleep duration decreased [26]. In a study by Celorio-Sardà et al. involving food science students and professionals, it was determined that individuals' sleep quality decreased and their working and staying awake time increased during the COVID-19 quarantine. [18]. In a study conducted with medical faculty students, it was found that the level of anxiety and stress increased during the pandemic period [27, 28]. The results of our study show that intense physical and mental stress have negatively affected the frequency of water consumption and nutritional status in the work environment during the pandemic period.

Sleep is one of the most important health factors that increases the living standards of individuals and ensures continuity of social life. During the pandemic period, changes in living conditions have caused serious changes in the sleep patterns of individuals. This study investigated how sleep patterns have been affected in resident physicians. While 78.3% of the physicians slept between 6 and 8 hours before the start of the pandemic, the rate of those who slept 6–8 hours during the study period dropped to 56.6%, and the rate of those who slept 1–5 hours was 38.0%. When compared to the pre-epidemic rates, it can be seen that the pandemic causes sleep problems in physicians who are in a stressful working environment. Studies show that individuals who do not work have an increase in sleep times, which is due to the prolonged period of being at home during the pandemic. In another study conducted with physicians, it was observed that the sleep times of individuals exposed to intense working conditions during the pandemic had significantly reduced and shifts in the pandemic clinic were the main reason for this decrease [29]. In this study, a significant relationship between pre- and epidemic sleep duration was found ($p < 0.05$). We think that 67.3% of the physicians who slept for 6 to 8 hours before the epidemic slept for only 1 to 5 hours during the epidemic, which may have caused the statistically significant difference in the data. In the study of Macit and colleagues, it was proven that the shortage of sleep time causes a tendency toward malnutrition [15]. In this study, it is felt that the pandemic has led to a reduction in sleep duration of resident physicians and caused changes in nutrition and lifestyle.

In these times, eating already prepared food is quite common among working individuals. In a study conducted involving healthcare professionals, it was found that 64.2% of them ate five or more times a week outside the home [30]. In our study, the rate of eating at restaurants and cafes while working before the epidemic was 67.2%, while this rate decreased to 23% during the pandemic, and the rate of resident physicians bringing meals from home increased to 55%. Although the European Food Safety Authority (EFSA) has proven that the virus is not transmitted from food, the surfaces used for food preparation are not disinfected, so people are afraid and worried that the surfaces can act as a tool for transmitting viruses from other fomites (the object carrying the infectious disease). That is why the resident physicians started bring their own food from their homes to their working environments after paying attention to hygiene rules [30].

First, even though COVID-19 was declared a pandemic by WHO in March 2020, not enough information on this subject is available at this point. Studies are limited, and even fewer of these limited studies involve healthcare professionals. As the pandemic continues, study results may not be conclusive. Despite these limitations, our study is the first study conducted on resident physicians, and we feel that it will pave the way for future studies.

The COVID-19 outbreak is a global pandemic that is still ongoing. As in the rest of the world, very significant healthcare measures are being taken by the health committee in Turkey. Although the health measures taken are not always sufficient within the scope of combatting the pandemic, it has been observed that the biggest measure involves the hygiene rules. This global pandemic is causing more and more infections every day; and the number of confirmed cases, the number of patients requiring intensive care treatment, and the number of deaths are still increasing. Healthcare workers are the most affected by this increase. The COVID-19 outbreak has greatly

affected and continues to affect the living standards of resident physicians. During this study period, it was observed that the diet and sleep patterns of physicians who are in an intense work environment were significantly disrupted.

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Can irisin be used in the follow-up of osteoporosis treatment?

İrisin osteoporoz tedavisi takibinde kullanılabilir mi?

Esra Demirel [1], Kadri Yıldız [2], Kenan Çadırcı [3]

Abstract

Aim: This study aims to investigate the possible roles of irisin as a biomarker in the diagnosis and follow-up of osteoporosis.
Methods: A total of 32 postmenopausal osteoporotic and 23 healthy postmenopausal women were received in this study. Bone mineral densitometry (BMD) measurements were done for all patients and control groups. Clinical follow-ups were performed every 3 months. To elicit post-treatment values, at the end of the 12-month follow-up period, all patients underwent BMD and biochemical parameters. Serum irisin concentrations were measured by competitive Enzyme-Linked Immunosorbent Assay (ELISA). The detection range of the used kit was 0.5-30 ng/ml.
Results: T-scores were determined as -3.28±0.6 in the BT group (Before treatment) and -2.49±0.7 in the AT group (After-Treatment), and -0.7±0.4 in the control group (C). Significant differences were observed in T scores between BT and AT ($p<0.001$), BT and C ($p<0.001$), and AT and C ($p<0.001$) statistically. Significant differences were observed between BT and C ($p<0.001$) and AT and C ($p=0.002$) statistically. There was no significant difference between BT and AT values ($p=0.327$) statistically. At the correlation analysis, irisin was positively correlated with T score ($p=0.01$, $r=0.25$) and 25-OH-D ($p=0.02$, $r=0.23$), and negatively correlated with development of osteoporosis ($p=0.02$, $r=-0.23$). According to the ROC analysis, irisin levels of 4.1 ng/ml or less can predict pre-treatment osteoporosis with 65.6% specificity and 60% sensitivity (AUC: 65.8%, $p=0.014$).
Conclusion: We concluded that irisin was a protective factor against osteoporosis. It may be used as a biomarker in the diagnosis of osteoporosis.

Keywords: Irisin, osteoporosis, zoledronic acid, biomarker.

Öz

Amaç: Bu çalışma, osteoporozun tanı ve takibinde bir biyobelirteç olarak irisinin olası rollerini araştırmayı amaçlamaktadır.
Yöntemler: Bu çalışmaya toplam 32 postmenopozal osteoporotik ve 23 sağlıklı postmenopozal kadın alındı. Tüm hasta ve kontrol gruplarında kemik mineral dansitometri (KMY) ölçümleri yapıldı. Klinik takipler 3 ayda bir yapıldı. Tedavi sonrası değerleri ortaya çıkarmak için 12 aylık takip süresinin sonunda tüm hastalara BMD ve biyokimyasal parametreler uygulandı. Serum irisin konsantrasyonları, rekabetçi Enzyme-Linked Immunosorbent Assay (ELISA) ile ölçüldü. Kullanılan kitin algılama aralığı 0,5-30 ng/ml idi.
Bulgular: T-skorları BT grubunda (Tedavi öncesi) -3,28±0,6, AT grubunda (Tedavi Sonrası) -2,49±0,7 ve kontrol grubunda (C) -0,7±0,4 olarak belirlendi. BT ve AT ($p<0,001$), BT ve K ($p<0,001$) ve AT ve K ($p<0,001$) arasında T skorlarında istatistiksel olarak anlamlı farklılıklar gözlendi. BT ile K ($p<0,001$) ve AT ile K ($p=0,002$) arasında istatistiksel olarak anlamlı farklar gözlendi. BT ve AT değerleri arasında istatistiksel olarak anlamlı fark yoktu ($p=0,327$). Korelasyon analizinde irisin, T skoru ($p=0,01$, $r=0,25$) ve 25-OH-D ($p=0,02$, $r=0,23$) ile pozitif, osteoporoz gelişimi ile negatif korelasyon gösterdi ($p=0,02$, $r=-0,23$). ROC analizine göre, 4,1 ng/ml veya daha düşük irisin seviyeleri, %65,6 özgüllük ve %60 duyarlılıkla tedavi öncesi osteoporozu öngörebilir (EAA: %65,8, $p=0,014$).
Sonuç: İrisinin osteoporoza karşı koruyucu bir faktör olduğu sonucuna vardık. Osteoporoz tanısında biyobelirteç olarak kullanılabilir.

Anahtar kelimeler: İrisin, osteoporoz, zoledronik asit, biyobelirteç.

[1] University of Health Sciences, Erzurum Regional Training and Research Hospital, Department of Orthopedics and Traumatology, Erzurum, Turkey.
[2] Kafkas University Faculty of Medicine, Department of Orthopedics and Traumatology, Kars, Turkey
[3] University of Health Sciences, Erzurum Regional Training and Research Hospital, Department of Internal Medicine, Erzurum, Turkey.

ED: 0000-0002-8468-6286
KY: 0000-0002-8164-7687
KÇ: 0000-0002-2765-4288

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Sorumlu yazar / Corresponding author:
Kadri Yıldız
Adres/Address: Kafkas University Faculty of Medicine, Department of Orthopedics and Traumatology, Kars, Turkey.
e-mail: dr_2880@hotmail.com
Tel/Phone: +90 474 225 21 05

Introduction

Irisin is a thermogenic protein that exhibits its effect by inducing the conversion of white fat cells into brown fat cells [1]. It is a product of the Pep protein and Fibronectin Type-III Domain 5 (FNDC5) and has been determined to be an important factor in metabolic homeostasis [2]. It also exhibits autogenic, paracrine, and endocrine effects.

Fat serves as a thermogenic and energy storage tissue in the mammalian body, and it has significant effects on the endocrine system [3]. Lipo-blast cells are converted into two different adipose tissues, white and brown (thermogenesis) through diversification into adipocytes. Brown adipose tissue is thought to play a protective role against a decrease in body temperature in infants and affects energy storage, metabolism, and the immune system. This brown adipose tissue has not been in infants only, a small amount of brown adipose tissue has also been in adults. On the other hand, white adipose tissue may be regarded as the largest endocrine tissue in adults. Leptin, ghrelin, glucocorticoids, plasminogen activator inhibitor (PAI-1), TNF-α, IL-6, angiotensin, visfatin, resistin, irisin, and many other cytokines are secreted from adipose tissue and are basic compounds affecting metabolism [4]. These molecules, with important roles in communication between organs in the maintenance of metabolic homeostasis and that lead to various metabolic diseases in case of dysfunction, are known as adipocytokines [5].

Osteoporosis, one of the metabolic disorders that threaten the human body, is a common metabolic bone disease characterized by impaired bone mass and bone micro-architecture and eventually an increased risk of fracture. It seems like irisin is a potential junction molecule on the metabolism of obesity and osteoporosis. Recently, Morgan et al concluded that irisin treatment in the ovariectomized rats protected the bone architecture. They declared irisin as a possible target in the prohibition of postmenopausal osteoporosis [6].

The research questions of the study were as follows: i) irisin has a potential bio-marker features to follow up for osteoporosis, ii) in the metabolic pathway, there may be a protective role of irisin against to bone fractures, iii) irisin may be accepted as both diagnostic biomarker and therapeutic agent, and iv) irisin is a junctional molecule between osteoporosis and obesity metabolism.

The purpose of this study was to investigate whether irisin is capable of use as a marker of the follow-up osteoporosis treatment. The main aim was to search for the role of irisin as a biomarker in the diagnosis of osteoporosis.

Material and methods

This study was planned as a prospective case-control study on the follow-up of the irisin treatment for osteoporosis. The study was approved by the Local Ethics Committee (Erzurum Regional Research and Education Hospital, 2016/5-18) and was by the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice. Written informed consent was obtained from all patients.

For preventing potential sources of bias that might affect the study, all bone mineral densitometry (BMD) measurements, biochemical tests, and statistical analyses were made blinded. The patient group included postmenopausal women between the ages of fifty-five and seventy who applied to the internal medicine and orthopedics outpatient clinics between April and December 2016 and were diagnosed with osteoporosis (T score <-2.5).

Postmenopausal women between the ages of fifty-five and seventy who were not diagnosed with osteoporosis (T score ≥ -1) were recruited into the control group. All participant diagnoses were based on their BMD measurements. Three groups were created as Before Treatment (BT), After Treatment (AT), and Control (C) groups.

Inclusion criteria for the patient group were diagnosis of osteoporosis, no previous receipt of treatment for osteoporosis, and absence of any systemic or localized disease. Exclusion criteria were receipt of any surgical procedure during the study, the detection at follow-up during the study of any systemic disease requiring medication use, or the impossibility of clinical follow-up. Twenty-three postmenopausal women with no metabolic or systemic disease, including osteoporosis and osteopenia at BMD measurements, with no recent history of major surgery/disease, were included as the control group.

All patients included in the study and providing blood specimens received a single intravenous 5 mg/100 ml dose of Zoledronic Acid (Novartis Pharma Stein AG, Stein, Switzerland). These patients underwent three-monthly clinical follow-ups. The 32 patients remaining at the end of the 12-month follow-up period underwent BMD to elicit post-treatment values. Various biochemical values were again investigated post-treatment.

For pre-treatment irisin measurements, 10 cc venous blood specimens were collected between 07:30 and 09:30 am following 12-h overnight fasting. These were centrifuged for 10 min at 4000 rpm using an Electromag M4808 P Centrifuge device and stored at -80°C. The same procedure was performed to determine post-treatment irisin values, and blood specimens collected from the 32 patients were centrifuged and stored at -80°C.

Serum irisin concentrations were measured by competitive ELISA (Enzyme-Linked ImmunoSorbent Assay) (Eastbiopharm Company, Shangai) according to the manufacturer's instructions. The detection range of the kit was 0.5-30 ng/ml. Patients' BMD measurements were performed using a D.M.S STRATOS bone densitometry device (France) with dual X-ray absorptiometry (DXA). Routine biochemical parameters, vitamin, parathormone (PTH), and TSH were measured on an Abbott Architect i2000 SR device using the Chemiluminescent Microparticle Immunoassay (CMIA) method.

Statistical analysis

Statistical analyses were performed on SPSS 19.0 (Windows) software. The variables were normally distributed according to the presentation with mean ±SD. The student's t-test and the Mann-Whitney U test were applied for comparisons. Kruskal Wallis H Test was used for correlation analysis between categorical and numerical values. Pearson's correlation test and the Spearman correlation test were applied to determine relations between variables. ROC analysis was performed to determine a cut-off value for irisin. P values less than 0.05 were regarded as statistically significant.

Results

A total of 32 postmenopausal osteoporotic and 23 healthy postmenopausal women were received. The mean age of the groups was 62.5±7.5 years in the patient group and 60.0±4.5 years in the control group (p=0.054). Mean BMI values were

29.0±3.5 kg/m^2 in the patient group and 31.1±3.9 kg/m^2 in the control group (p=0.240).

Table 1. Clinical and biochemical characteristics of the patient and control groups.

	Patients (n=32)		Controls (n=23)		p	
Age (year) †	62.5±7.5		0.0±4.5		0.054	
BMI (kg/m^2) †	29.2±3.5		1.1±3.9		>0.05	
	BT	**AT**	**C**	**p_1**	**p_2**	**p_3**
T score†	-3.2±0.6	-2.4±0.7	0.7±0.4	<0.001	<0.001	<0.001
Irisin (ng/ml) †	5.23±6.08	6.03±5.19	6.70±3.34	<0.001	0.002	0.002
25-Hydroxy vitamin D (ng/ml) †	12.9±11.5	17.4±15.0	3.6±21.0	0.010	>0.05	>0.05
PTH (pg/ml) †	108.1±87.7	89.7±64.4	63.5±26.4	0.008	>0.05	>0.05
TSH (µIU/mL) †	1.3±0.9	1.2±0.7	0.0±1.8	>0.05	>0.05	>0.05
Creatinin (mg/dl) †	0.7±0.1	0.8±0.1	0.7±0.1	>0.05	>0.05	>0.05
Calcium (mg/dl) †	9.1±0.7	9.0±0.7	9.4±0.4	>0.05	>0.05	>0.05
Phosphorus (mg/dl) †	3.45±0.5	3.3±0.5	3.4±0.8	>0.05	>0.05	>0.05
ALP (U/L) †	97.7±51.2	80.7±32.4	84.8±28.4	>0.05	>0.05	>0.05
CK (U/L) †	96.4±85.5	71.4±20.6	92.9±37.4	>0.05	>0.05	>0.05

†: mean standard ± deviation

BT: before treatment, AT: after treatment, C: controls, BMI: Body Mass Index, PTH: Parathormone, TSH: Thyroid –Stimulating Hormone, ALP: Alkaline phosphatase, CK: Creatinine kinase.

p_1: statistically relationship between before treatment and controls

p_2: statistically relationship between after treatment and controls.

p_3: statistically relationship between before treatment and after treatment.

Pre-treatment and post-treatment biochemical data, T scores, and irisin levels in the patient group were examined and subjected to statistical analysis as BT, AT, and C groups. T scores were -3.28±0.6, -2.49±0.7, and -0.7±0.4 in the groups of BT, AT, and C, respectively. Irisin levels were 5.23±6.08 ng/ml, 6.03±5.19 ng/ml, and 6.70±3.34 ng/ml in the groups of BT, AT, and C, respectively. Clinical and biochemical values in the study groups and the Group C are shown in Table 1.

Statistically significant differences were observed in T scores between the groups BT and C (p<0.001). Statistically significant differences were observed between the groups of BT and C (p<0.001).

Statistically significant differences were observed in T scores between BT and AT groups (p<0.001), and AT and C groups (p<0.001). Statistically significant differences were observed between the groups of between AT and C (p=0.002) groups, and BT and AT (p=0.002) groups.

At correlation analysis, irisin was positively correlated with T score (p=0.01, r=0.25) and 25-OH-D (p=0.02, r=0.23), and negatively correlated with development of osteoporosis (p=0.02, r=-0.23). Correlation analysis data are shown in Table 2. Development of osteoporosis was positively correlated with age (p=0.003, r=0.312) and PTH (p= 0.001, r= 0.352), and negatively correlated with T score (p=0.000, r=- 0.868) and 25-OH-D (p=0.01, r=-0.26). There was no correlation between other parameters.

Table 2. Correlation analysis between the irisin and other parameters.

	p	**r**
T score	0.01	0.25
25-Hydroxy vitamin D	0.02	0.23
Osteoporosis development	0.02	-0.23

ROC analysis showed that irisin is a useful marker for predicting the diagnosis of osteoporosis and follow-up treatment. According to the ROC analysis, irisin levels of 4.1 ng/ml or less can predict osteoporosis with 65.6% specificity and 60% sensitivity (AUC: 65.8%, p=0.014) (Figure 1).

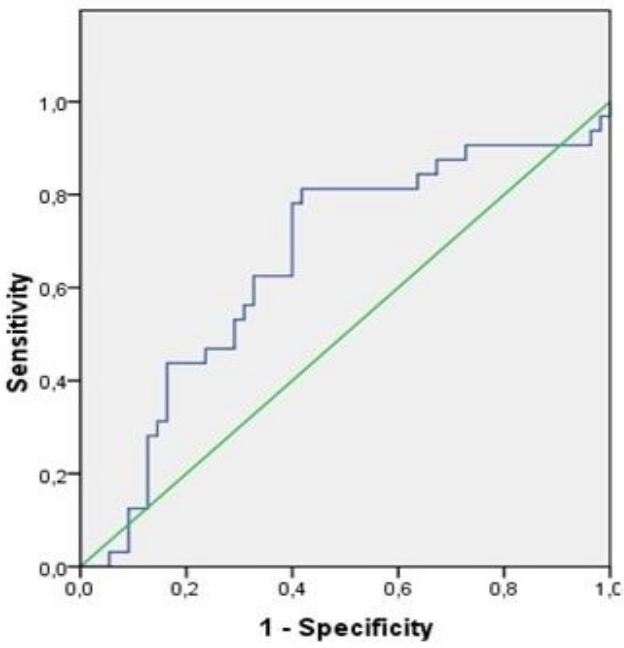

Figure 1. The irisin levels for predicting osteoporosis before the treatment.

Discussion

According to our results, the mean age was 6th decade. There was no significant difference between groups. The mean BMI values were similar in groups. In BT, AT, and C groups, irisin levels were 5.23±6.08 ng/ml, 6.03±5.19 ng/ml, and 6.70±3.34 ng/ml, respectively. There was significant difference statistically between BT, AT and C groups, in pairwise comparisons between groups. We found that irisin was positively correlated with T scores, and and negatively correlated with development of osteoporosis. Development of osteoporosis was positively correlated with age, and negatively correlated with T score. According to our ROC analysis, the specificity was 65.6% and the sensitivity was 60%, when irisin levels of 4.1 ng/ml or less.

Recent findings have shown that irisin affects both bone and glucose metabolism in humans. Irisin is an adipokine directly associated with obesity and glucolipid metabolism and increases cortical bone mass by affecting the osteoblast metabolic pathways [7, 8]. In vitro data from one study showed that the myokine irisin can affect bone metabolism by encouraging osteoblast differentiation [9].

One study examined the relation between irisin and bone metabolism in a Chinese population (n=6,308). Irisin levels were significantly higher in this population. A positive relation was determined between plasma irisin levels and BMD in elderly individuals [10]. Another study examined the relationship between BMD values and irisin levels in 160 Chinese women who were 70-90 years old. In addition, the irisin values were detected significantly lower in osteoporotic and osteopenic women compared to the control group, the low irisin levels in women were associated with an increased rate of femoral neck fracture and the lumbar spine fracture [11].

The basic aim of Palermo A et al.'s study [12] was to investigate the association between irisin and body composition in postmenopausal women with osteoporosis and to examine the effect of irisin on brittleness and vertebra fractures. Their databases confirmed an inverse correlation between irisin levels and vertebral osteoporotic fractures, but no significant relation was determined with BMD or non-fat mass. In another study, irisin levels in circulation were associated with previous osteoporotic fractures, but not with bone mass, and were not affected by 3-month denosumab (Dmab) or teriparatide (TPTD) therapy. That study was intended to investigate irisin levels in the circulation in postmenopausal women with low bone mass and the potential effects of three-month Dmab or TPTD therapy. No difference was determined in serum irisin levels between women with or without low bone mass, and these were unaffected by three-month Dmab or TPTD therapy. Irisin levels in circulation were found to be associated with previous osteoporotic fractures. However, the study was unable to conclude whether this relation was independent of whether or not it potentially derived from low bone mass reflected by low creatinine levels [13].

Physical activities such as sports are known to have a positive effect on BMD. A study from Italy, including members of the Bari soccer team, determined a positive correlation between BMD and irisin. The irisin levels of circulation were related to the improving Z-scores. From that perspective, irisin which is a myokine could be described as a 'sports hormone' [14]. One experimental mouse study reported that irisin has a positive effect on cortical bone mass, bone strength, and periosteal circulation. This anabolic effect, stimulation of bone formation, is independent of osteoclast activity. As anticipated, a low dose of r-irisin modulated the skeleton genes, Opn and Sost, rather than Ucp1 or Ppar gamma expression in white adipose tissues. Although the irisin precursor FNDC5 is abundantly expressed in skeletal muscle, other regions such as bone and brain have also been found to express FNDC5 at low levels. In addition, increased FNDC5 positivity and increased expression of FNDC5 mRNA were shown in muscle fibers in mice that were injected r-irisin [15].

It was known that irisin is a molecule linked to Type 2 Diabetes Mellitus, lipid metabolism disorders, cardiovascular diseases, non-alcoholic fatty liver, polycystic ovarian syndrome, and metabolic bone diseases. In addition, in terms of metabolic bone diseases, irisin is directly related to BMD and athletic performance. Irisin may be in the future a therapeutic target in metabolic bone diseases [16, 17].

Irisin has a pathophysiologic corner point in metabolic diseases. Also, ıt has therapeutic targets for metabolic pathways [18]. Briganti SI et al declared the regulator role of irisin in both bone and glucose metabolism [19]. Sarcopenia has a pioneering role before osteopenia. Li G et al emphasized the roles of various factors affecting muscle bone crosstalk and potential therapeutic approaches. They decided that irisin has an important role in the muscle-bone crosstalk and potential therapies for sarco-osteoporosis [20]. In another study, Xu L et al. [21] searched the effects of irisin on osteoblast apoptosis and osteoporosis in postmenopausal osteoporosis rats. They found that irisin inhibited the incidence of apoptosis, but also treated postmenopausal OP through upregulating Nrf2 and inhibiting NLRP3 expression. Notable for her work on irisin, Colaianni G emphasized that irisin has a key player in bone metabolism and its role is emerging as a possible therapeutic option to treat bone diseases [22]. In the growing rats, HFD has negative effects on BMD, bone microstructure, and bone metabolism. Swimming exercise decreased body weight, body fat, and pro-inflammatory cytokines. The swimming exercises increased serum irisin levels and expression of PGC-1/FNDC5 in the bone. This study advocated that swimming can improve bone microstructure, even if a high-fat diet [23].

The limitations of this study may be listed as follow: i) the number of patients in the group we used in the study could have been higher, ii) the study could be a comprehensive meta-analysis involving multiple medical centers with a larger number of patients, iii) since irisin is a junction molecule in human metabolism, other bio-regulatory compounds associated with metabolism and irisin could also be investigated in the blood material taken for our study.

Irisin level may be used as a marker in the diagnosis of osteoporosis. A significant difference was determined in irisin levels between before and after the treatment we think that further studies are now needed concerning the potential use of irisin as a parameter in the follow-up of osteoporosis treatment and maybe treatment agent.

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Myeloprotective and hematoprotective role of kefir on cyclophosphamide toxicity in rats

Kefirin Sıçanlarda Oluşturulmuş Siklofosfamid Toksisitesi Üzerine Myeloprotektif Ve Hematoprotektif Etkileri

Gülay Gözüoğlu[1], Songül Çetik Yıldız[1]

Abstract

Aim: Kefir is a probiotic and prebiotic beverage produced from milk and kefir grains containing a mixture of bacteria and yeast. Drugs like cyclophosphamide (CPx) that are used for cancer chemotherapy are generally limited due to numerous unwanted side-effects such as multiple organ toxicity. For this purpose, the cell-protective effects of kefir, a natural probiotic known for its antitumor and antioxidant properties, on CPx-induced hemotoxicity and myelotoxicity were investigated in this study.

Methods: Group 1 (control, 0.5 ml SF). Group 2 were administered a single dose of 150mg/kg CPx. Group 3 and 5 were given 5 and 10mg/kg kefir. Group 4 and 6 were given 5 and 10mg/kg kefir+150mg/kg CPx. While kefir was administered to the rats by gavage method for 12 days, CPx was administered as single-dose on the 12th day.

Results: The DPPH results showed that kefir possesses high antioxidant activity. It was observed that the leukocytes, thrombocytes, erythrocytes, hemoglobin, hematocrit and bone marrow nucleated cell levels decreased in the group that was administered only CPx, and increased relatively in the groups that were administered CPx+kefir, drawing close to the control.

Conclusion: The results of the present study showed that kefir had antioxidant and cytoprotective activity, protecting blood and bone marrow cells against CPx-induced damage.

Keywords: Kefir, CPx, myelotoxicity, hemotoxicity, rat

Öz

Amaç: Kefir, bakteri ve maya karışımı içeren kefir taneleri ile sütten üretilen probiyotik ve prebiyotik bir içecektir. Siklofosfamid (CPx) gibi kanser kemoterapisi için kullanılan ilaçların kullanımı, genellikle, istenmeyen çoklu organ toksisitesi gibi yan etkiler nedeniyle sınırlıdır. Bu amaçla yapılan çalışmamızda antitümör ve antioksidan özellikleri iyi bilinen ve doğal bir probiyotik olan kefirin CPx nedenli hemotoksisite ve myelotoksisite üzerine koruyucu etkileri araştırılmıştır.

Gereç ve Yöntem: Grup 1 (kontrol, 0,5 ml SF), grup 2; tek doz 150 mg/kg/b.w/i.p CPx, Grup 3; 5 mg /kg / b.w kefir, grup 4; 5 mg/kg/b.w+150 mg/kg/b.w/i.p CPx, grup 5; 10 mg/kg/b.w kefir, Grup 6; 10 mg /kg/b.w kefır+150 mg/kg/b.w/i.p CPx. Kefir, hayvanlara gavaj yöntemiyle 12 gün boyunca verildi. CPx ise 12. gün tek doz olarak verildi. Deney sonunda tüm sıçanlardan kan ve kemikiliği örnekleri anestezi altında alındı.

Bulgular: DPPH sonuçları kefirin yüksek antioksidan aktiviteye sahip olduğunu göstermiştir. Sadece CPx uygulanan grupta lökosit, trombosit, eritrosit, hemoglobin, hematokrit ve kemik iliği çekirdekli hücre düzeylerinin azaldığı, CPx+kefir verilen gruplarda ise göreceli olarak arttığı ve kontrole yaklaştığı gözlendi.

Sonuç: Sonuçlarımız antitümör, antioksidan ve sitoprotektif özellikleri olan kefirin kan ve kemik iliği hücrelerini CPx hasarına karşı koruduğunu göstermiştir.

Anahtar sözcükler: Kefir, Siklofosfamid, Myelotoksisite, Hemotoksisite, rat

[1] **Mardin Artuklu University, The Institute for Graduate Educational Studies, Department of Biology, Mardin, Turkey.**

GG: 0000-0001-9903-9754

SÇY: 0000-0002-7855-5343

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Sorumlu yazar / Corresponding author:

Songül Çeltik Yıldız

Adres/Address: Department of Biology, The Institute for Graduate Educational Studies, Mardin Artuklu University, 47200, Mardin, Turkey.

e-mail: songulcetik@gmail.com

Tel/Phone: +90-482-2134002 / 7270

Introduction

Todays, the demand for probiotic, prebiotic and functional food products in particular has been gradually increasing [1]. Probiotic microorganisms can improve intestinal barrier integrity and immune response while preventing the negative effects of pathogens [2]. Prebiotics, on the other hand, are short-chain carbohydrates that selectively reinforce the activity of certain beneficial bacteria groups that cannot be digested by human digestive enzymes [3]. Kefir contains high amounts of probiotics and prebiotics [4]. Kefir grains contain a rich microbial community consisting of a microflora of yeast and bacteria responsible for the fermentation of kefir [5, 6]. Lactobacilli constitute 65-80% of the microbial structure in kefir while streptococci account for 20% and yeast make up 5% [7]. As a result of the synthesis of certain vitamins, the development of bioactive substances and the partial fragmentation of lactose and proteins with the effect of the fermentation induced by the microorganisms in its formation, the nutritional value of kefir increases further [8]. Kefir also contains certain fatty acids and essential amino acids that must be obtained through nutrition [9], Vitamins B1, B2, B6 and B12, folic acid, Vitamin K, biotin (vit H), macrominerals such as calcium, phosphorus, potassium, magnesium, and microminerals such as zinc, copper, manganese, iron and cobalt [4, 10]. It has been determined in many studies that kefir consumption had positive and regulatory effects on the immune system [10-12]. A previous experimental study has shown that the microorganisms in kefir could significantly affect the immunoregulatory properties in rats [13]. It has been shown that kefir reduces lipid peroxidation and positively affects the antioxidant parameters in carbon tetrachloride-induced toxicity in rats [14]. Kefir possesses antioxidative, antimicrobial, anticarcinogenic properties and demonstrates anti-apoptotic activity [10, 15, 16].

Leukopenia, thrombocytopenia and lymphopenia develop due to the immunosuppressive effects of alkylating drugs. Therefore, high doses and/or more frequent use of such drugs prevent stronger therapeutic efficacy [17]. High-dose alkylating agents such as Cyclophosphamide (CPx) are needed to be used in effective doses in order to be efficient [18]. However, the use of high-dose cytotoxic drugs and the prolongation of patients' survival time also increase the side-effects of the drugs [19]. CPx is a cytotoxic drug that can repress both humoral and cellular immunity [20]. CPx, an alkylating chemotherapeutic prodrug, is metabolized by cytochrome P450 liver enzymes, i.e. CYP3A4 and CYP2B6, which show active therapeutic and cytotoxic metabolites and spread from hepatocytes to plasma [21]. CPx spreads to the body and produces two active metabolites: phosphoramide mustard (PAM) and acrolein (ACR). While the immunosuppressive and antineoplastic effects of CPx are related to PAM, ACR is responsible for its unwanted toxicity [22]. ACR produces oxidative stress in hepatocytes and highly-reactive oxygen species [23]. Therefore, it damages the antioxidant defense mechanism of tissue as it causes changes in the structure and functions of enzymes by interacting with proteins [24]. CPx, which has been known to be effective in the treatment of cancer and non-malignant diseases since 1958 and has a wide area of use in many neoplastic diseases as a stand-alone drug or in combination with other chemotherapeutics, also includes side-effects [25]. The fact that CPx causes multi-organ damage limits the effective high-dose use of the drug [26]. Hematopoietic depression is among the main side-effects of CPx. CPx includes considerable toxic side-effects such as hematoxicity, bone marrow suppression, carcinogenicity and mutagenicity [11]. In CPx chemotherapy, these toxic effects must be eliminated using certain antioxidant agents in order to avoid the toxic side-effects of acrolein [27]. For this purpose, in the present experimental study, the potential antioxidant and cytoprotective effects of kefir on peripheral blood and bone marrow nucleated cells in CPx-induced toxicity in rats were investigated.

Material and methods

Kefir fermentation

In the present study, commercially available freeze-dried kefir grains and 1 liter of cow's milk were used. For the kefir fermentation, the method adopted by Marshall et al., (28) was used. For the fermentation process, three groups of kefir classified as 24, 36 and 48 hours were created at a temperature of 24-26°C and preserved for use at +4°C. Kefir groups fermented on different days were used due to the formation of different microbial flora around the kefir grains.

Chemical Substances and Injections

CPx (Sigma-Aldrich) was obtained commercially. 500 mg of CPx was dissolved in 25 ml of bidistilled water and 150 mg/kg of CPx was made ready for injection. The injection was administered intraperitoneally as a single-dose using sterile disposable syringes on the 12th day of the experiment.

Also DPPH solution (in 60 micromolar in methanol) was obtained commercially.

Determination of antioxidant capacity (Scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical)

The milk (using as control) used for kefir fermentation and waited for 24, 36, 48 hours were weighed as 2 g samples. 20 ml of methanol (75%) was added on it. Stirred with magnetic stirrer in the dark for 4 hours. It was centrifuged (3500 rpm, 30 minutes, room temperature). The supernatant was put into a tube. The final volume was adjusted to 22 ml with 75% methanol. 100 µl extract was put into a tube. 3.9 ml DPPH solution was added on it and the absorbance was read at 517 nm by leaving it to incubate for 30 minutes. In blind reading (blank), the solvent was used instead of the sample (solvent only). Only DPPH solution + solvent were used for control purposes. The antioxidant activity was given as percentage (%) DPPH scavenging, calculated as [(control absorbance-extract absorbance)/(control absorbance)□100]. The extract concentration providing 50% inhibition (IC50) was calculated from the graph of scavenging effect percentage against extract concentration.

Experimental Groups

In the present experimental study; healthy male Wistar albino rats that were approximately 3 months old and weighing 200±20 grams were used. Throughout the experiment, the animals were kept in rooms with 12;12 bright/dark lighting, automatically adjusted to 22±2 C° temperature and 45-50% humidity. Standard pellet feed and tap water were provided to the rats. The 42 rats used in the present study were divided into 6 groups with 7 in each group. Group 1 used as control. Group 2 were administered a single dose of 150 mg/kg/b.w. CPx while Group 3 was given 5 mg/kg/b.w. kefir, Group 4 was given 5 mg/kg/b.w. kefir+150 mg/kg/b.w. CPx, Group 5 was given l0 mg/kg/b.w. kefir and Group 6 was given l0 mg/kg/b.w. kefir+150 mg/kg/b.w. CPx (Table 1). Kefir was administered to the groups for 12 days using the gavage method. CPx was administered as single-dose and intraperitoneal (i.p.) at the 12th and final day of the experiment.

Table 1: Experimental groups, kefir and injection protocol.

Groups	Treatment	The number of rats
Control	0.5 ml SF	7
Cyclophosphamide (CPx)	150 mg/kg /b.w CPx	7
Kefir	5 and 10 mg/kg/b.w kefir	7
Kefir +CPx	5 and 10 mg/kg b.w kefir + 150 mg/kg/b.w CPx	7

Peripheral blood and bone marrow nucleated cell count

Following the conclusion of the experiment, all of the rats were dissected under ketamine/xylazine anesthesia on the 13th day. Approximately 5 ml of blood was taken intracardially into tubes containing 3.8% sodium citrate (1:9, sodium citrate: blood). The blood taken from the rats in each experimental group was immediately counted in the rat calibration of the Hemavet 850 hematology analyzer. Afterwards, the statistical analyzes of the leukocyte, thrombocyte, erythrocyte, hemoglobin and hematocrit values obtained were performed using the SPSS 22.0 statistical package program. Following this process, a femur of the euthanized animals was exposed by thoroughly scraping off the muscles. The bone marrow was cut at both ends, held with pliers and placed entirely into the tube. To ensure the homogeneous distribution of the cells in the graduated tube containing 5 ml of physiological saline solution and bone marrow, the cellular fluid in the tube was drawn and emptied several times using the same injector. During this process, it was ensured that the fluid did not boil over. The tubes containing bone marrow were centrifuged at 3000 rpm for 5 minutes and the supernatant was drawn using the injector. The mixture was homogenized, and a bone marrow count was performed using the hematology analyzer.

Statistical analysis

The analysis of the present study was performed in a computer environment using the SPSS 11.0 statistical program. In the analysis, the One-Way ANOVA test was used to determine the importance of intergroup differentiation and the arithmetic mean ± standard deviation of the measurement data. If significant differentiation was detected in the analysis of variance, paired comparisons were performed using the Post Hoc Tukey test. The significance level was regarded as $p<0.001$. Differences between the means obtained from DPPH, acidity and pH analyzes were analyzed by Duncan Multiple Comparison Test ($p<0.05$)..

Results

The kefir samples, which were fermented for 24, 36 and 48 hours, were quantitatively tested for free radical scavenging activity using a DPPH. The samples were also tested for the pH and acidity. Our results showed that kefir that had been stored for 24, 36, and 48 h had strong antioxidant activity compared to milk. However, there was not found any significant difference between the 24, 36 and 48 h. While the acidity gradually increased on the 24, 36 and 48 h kefir, the pH gradually decreased (Table 2).

In the present study, the changes in blood and bone marrow nucleated cell levels were analyzed to determine the toxic effect of CPx and the changes that occur based on the antioxidant feature of kefir. In the group that was administered CPx, the bone marrow nucleated cell level demonstrated a statistically significant decrease compared to the control values ($p<0.001$). In comparison with the group that was administered only CPx, the bone marrow nucleated cell level of Groups 4 and 6, which were administered kefir in combination with CPx, demonstrated a statistically significant increase (particularly Group 6) and this level drew even closer to the control value with the increase in Group 6 ($p<0.001$). In Groups 3 and 5, which were administered 5 and 10 mg/kg kefir, the bone marrow nucleated cell level was found to be higher than the control value, albeit not statistically significant (Table 3, Figure 1). Compared to the control value, the leukocyte level demonstrated a statistically significant decrease in the group that was administered CPx ($p<0.001$). When Group 2, which was administered CPx, was compared to Groups 4 and 6, which were administered CPx+kefir, a statistically significant increase was observed in the leukocyte level ($p<0.001$). In Groups 3 and 5, which were administered 5 and 10 mg/kg kefir, the leukocyte level was found to be higher than the control value, albeit not statistically significant (Table 3, Figure 2). Regarding the thrombocyte level, there was a statistically significant decrease in the CPx group compared to the control value ($p<0.001$). In Groups 4 and 6, which were administered kefir in combination with CPx, this level increased significantly and drew near the control value ($p<0.001$). In Groups 3 and 5, which were administered 5 and 10 mg/kg kefir, the thrombocyte level was found to be higher than the control value, and this is statistically significant ($p <0.001$) (Table 3, Figure 3). In the CPx group, the erythrocyte level demonstrated a statistically significant decrease compared to the control value ($p<0.001$). In the groups that were administered kefir in combination with CPx, a significant increase was observed in the erythrocyte level (particularly in Group 6) ($p<0.001$). In Groups 3 and 5, which were administered 5 and 10 mg/kg kefir, this value was higher than the control value, and this is statistically significant ($p <0.001$) (Table 3, Figure 4). In Group 2, which was administered CPx, the hemoglobin level demonstrated a significant decrease compared to the control value ($p<0.001$). In Groups 4 and 6, which were administered CPx+kefir, a statistically significant increase was observed in the hemoglobin level and with this increase, the hemoglobin level drew near the control value particularly in Group 6 ($p<0.001$). In Groups 3 and 5, which were administered 5 and 10 mg/kg kefir, the hemoglobin level was found to be higher than the control value (particularly in Group 5) (Table 3, Figure 5). In Group 2, which was administered only CPx, the hematocrit value demonstrated a significant decrease compared to the control value ($p<0.001$). In Groups 4 and 6, which were administered kefir in combination with CPx, this level increased significantly and drew close to the control value to a large extent ($p<0.001$). This value was also high in Groups 3 and 5, which were administered 5 and 10 mg/kg kefir, albeit not statistically significant (Table 2, Figure 6).

Table 2: Antioxidant (inhibition of DPPH free radical), acidity and pH of 24, 36, 48 hours kefir groups and milk (used as control) stored at 4°C.

Samples	Antioxidant (%Inhibition of DPPH free radical)	Acidity (%)	pH
Milk	$^{*}15.93\pm0.9^{a**}$	0.09 ± 0.003^{a}	6.91 ± 0.01^{a}
Kefir – 24 h	30.01 ± 0.06^{b}	0.36 ± 0.005^{b}	5.81 ± 0.02^{b}
Kefir – 36 h	30.18 ± 0.10^{b}	0.930 ± 0.009^{c}	4.34 ± 0.01^{c}
Kefir – 48 h	30.29 ± 0.05^{b}	1.340 ± 0.009^{d}	4.28 ± 0.0^{d}

Table 3. The impact of 5 and 10 mg/kg kefir on bone marrow nucleated cell, erythrocyte, leukocyte, thrombocyte, hemoglobin and hematocrit count against 150 mg/kg CPx toxicity.

Groups	Bone marrow nucleated cells ($x10^3$/ml)	Leucocytes ($\times10^3$/ml)	Thrombocytes ($\times10^3$/ml)	Erythrocytes ($\times10^3$/ml)	Hemoglobin	Hematocrit
1- Control	3536.00±61.11[a]	**825.67±40.41[a*]	725.50±36.64[a]	9.70±0,42[a]	16.18±0.93[a]	46.90±1.50[a]
2- CPx (150 mg/kg)	617.71±21.52[b]	198.86±11.19[b]	574.14±24.97[b]	8.21±0.45[b]	14.37±0.56[b]	44.17±0.364[b]46.5
3- 5mg/kg Kefir	3615.00±88.83[a]	879.33±29.63[a]	694.33±22.78[c]	9.81±0.46[c]	16.17±0.68[a]	0±0.81[c]
4- 5+150 mg/kg Kefir+CPx	1347.71±75.01[c]	440.00±17.55[c]	629.71±35.54[c]	8.36±0.46[d]	14.40±0.77[b]	45.33±0.794[a]46.0
5- 10mg/kg Kefir	3727.86±60.49[a]	843.57±62.07[a]	703.71±21.68[c]	9.88±0.60[c]	15.80±1.04[b]	7±1.33[c]
6- 10+150 mg/kg Kefir+CPx	2673.86±56.96[d]	673.57±47.15[d]	697.71±9.34[c]	10.08±0.62[c]	16.17±0.76[a]	46.76±1.10[a]

*Values reported are means±standard deviation; means followed by different letters in same columns are significantly different ($p<0.001$: statistically significant differences). **Means are the averages of 3 replicates.

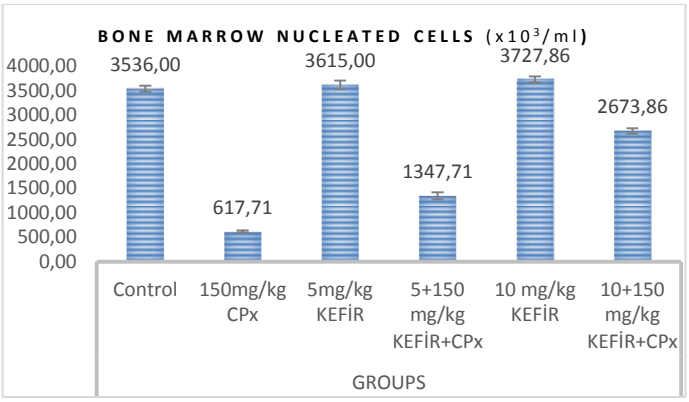

Figure 1: The effects of 5 and 10 mg/kg kefir on bone marrow nucleated cells under 150 mg/kg CPx toxicity.

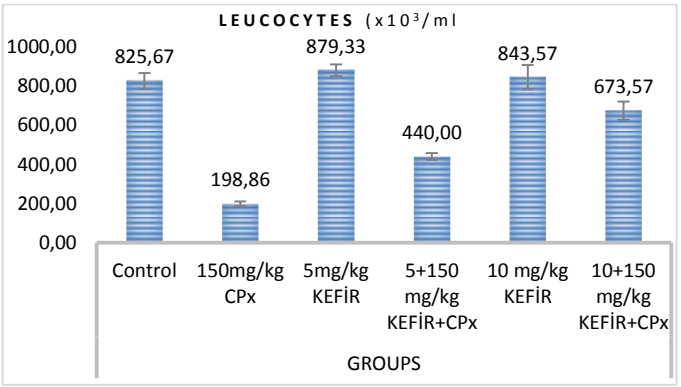

Figure 2: The effects of 5 and 10 mg/kg kefir on leukocyte count under 150 mg/kg CPx toxicity.

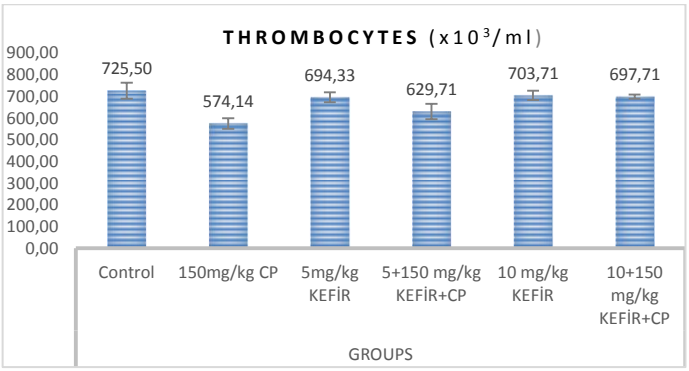

Figure 3: The effects of 5 and 10 mg/kg kefir on thrombocyte count under 150 mg/kg CPx toxicity.

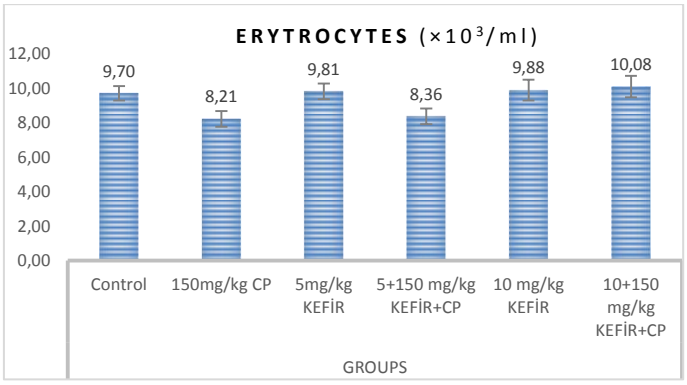

Figure 4: The effects of 5 and 10 mg/kg kefir on erythrocyte count under 150 mg/kg CPx toxicity.

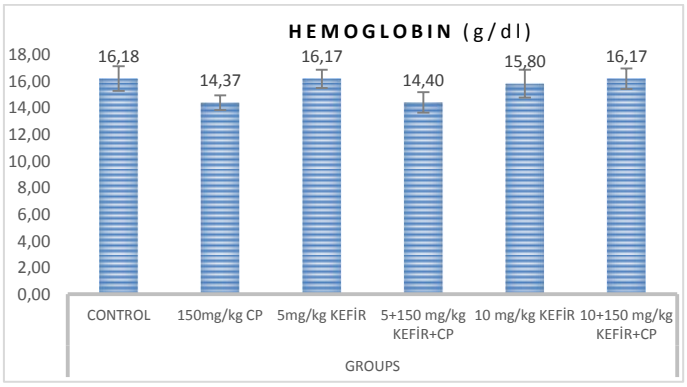

Figure 5: The effects of 5 and 10 mg/kg kefir on hemoglobin count under 150 mg/kg CPx toxicity.

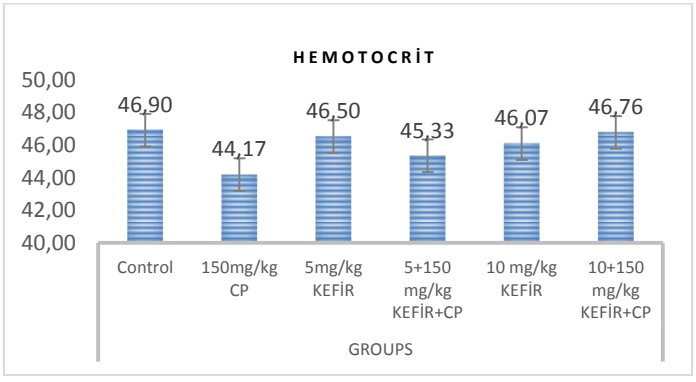

Figure 6: The protective effects of 5 and 10 mg/kg kefir on hematocrit count under 150 mg/kg CPx toxicity.

Discussion

The chemical compound of kefir may differ based on the properties of the milk used, production method, microbial flora of the kefir culture used, conditions of fermentation and the

conditions and time of storage. The reason why cow's milk was used in the present study is that most of the kefir sold in shops was fermented with cow's milk.

In a study conducted by Kubo et al. [29], it was stated that kefir, applied for 10 days, showed antitumor effect. In our study, we determined the duration of treatment with kefir as 12 days, as it was in the study of Matsuu et al. [30]. Due to the selenium, Vitamin E, catalase and superoxide dismutase enzymes in its content, kefir demonstrates an antioxidative effect and becomes an anticarcinogenic factor [31]. 24, 36, and 48 hours of kefir we used in our study showed strong antioxidant activity when compared to milk ($p < 0.05$). However, there was not found any significant difference between the 24, 36, and 48 hours (Table 2). The microbiota in kefir grains may differ, which may cause significant changes in the taste and nutritional properties of kefir [32]. Our results showed that there was a significant difference in acidity and pH of 24, 36 and 48 hours stored kefir ($p<0.05$) (Table 2). In many previous studies, it was reported that the pH value of kefir decreased in contrast with fermentation time [33-35].

The anticarcinogenic effects of kefir have been a point of emphasis for researchers in recent years [8,36,37]. Kefir demonstrates anticarcinogenic activity by decelerating the growth of cancer cells and accelerating apoptosis [38]. This anticarcinogenic effect is mostly attributed to amino acid groups that contain sulfur in their structure [39]. Although many studies were conducted on kefir and CPx, no study on the combination of CPx and kefir was found in the literature. In the present experimental study, the effects of kefir, which demonstrated antioxidant activity, on blood and bone marrow in CPx-induced hemotoxicity and myelotoxicity in rats were examined.

Chemotherapy is effective in active and rapidly growing cancer cells. Therefore, it also affects active and rapidly growing blood cells that are healthy. Myelosuppression is an unwanted side-effect of CPx chemotherapy. In the present study, the bone marrow nucleated cell level demonstrated a statistically significant decrease in the group that was administered CPx compared to the control value. In a similar study, it was reported that CPx reduced bone marrow nucleated cell levels and caused myelotoxicity [26,40]. It was reported in a previous studies that CPx-induced myelosuppression / immunotoxicity limited the use of CPx [41,42]. In the present study, when Groups 4 and 6 (which were administered kefir in combination with CPx) were compared with the groups that were administered only CPx, the bone marrow nucleated cell level demonstrated a statistically significant increase particularly in Group 6, where the levels drew near the control value ($p<0.001$) (Table 3, Figure 1). In parallel with this finding, it was reported in previous studies that regular kefir consumption had antioxidant, anticarcinogenic and immunoregulatory effects [7, 8, 35, 43].

It was observed that in Group 2, which was administered CPx, the leukocyte count decreased in comparison with the control value. In Groups 4 and 6, which were administered kefir in combination with CPx, a significant increase was observed in leukocyte count. Based on this finding, it can be said that kefir demonstrates a protective effect against CPx toxicity in leukocyte cells (Table 3, Figure 2). In the CPx group, it was observed that the thrombocyte level decreased compared to the control value. In Groups 4 and 6, which were administered CPx+kefir, it was observed that this value increased to a large extent and drew near the control value (Table 3, Figure 3). Based on this, it can be said that kefir protects thrombocytes in CPx-induced toxicity, and that an increase in thrombocyte count would serve as a precaution against potential bleeding. In the CPx group, it was observed that the erythrocyte level demonstrated a statistically significant decrease in comparison with the control value. In the groups that were administered CPx+kefir (particularly Group 6), it was determined that the erythrocyte level increased significantly (Table 3, Figure 4). As can be inferred from the results of the present study, CPx has considerable toxic side-effects on proliferating healthy cells such as bone marrow and blood. In many previous studies, it was reported that bone marrow was damaged, and that the damaged or dead bone marrow stem cells were unable to produce new blood cells, causing thrombocytopenia and leukopenia [26,44,45]. In another study, it was reported that CPx treatment significantly reduced erythrocyte, leukocyte, thrombocyte and bone marrow nucleated cell levels [46]. In a similar experimental study, it was reported that there was a decrease in the number of bone marrow nucleated cells and the erythrocytes and leukocytes produced in the bone marrow in rats treated with CPx [47]. In a research it is reported that CP is toxic to bone marrow, leukocytes and platelets parallel to the dose increase [48,49]. In the present study, CPx caused leukopenia, thrombocytopenia and erythrocytopenia while it was observed that this situation improved in the groups that kefir was administered (Table 3, Figures 2-4).

In the group that was administered CPx, it was observed that the hemoglobin level decreased significantly in comparison with the control value. In the groups that were administered kefir in combination with CPx (particularly Group 6), a significant increase was observed in the hemoglobin level compared to the group that was only administered CPx (Table 3, Figure 5). In 2 groups that were administered CPx, the hematocrit level demonstrated a significant decrease compared to the control value. In Groups 4 and 6, which were administered CPx+kefir, the hematocrit level demonstrated a significant increase and drew near the control value to a large extent (Table 3, Figure 6).

In conclusion, although CPx is commonly used as a chemotherapeutic agent, its high-dose use is limited by the toxic side-effects it possesses. For this reason, new effective agents are needed to protect normal tissue from toxicity associated with chemotherapy without preserving tumor and tumor growth stimulation properties. Therefore, combining treatment regimens with antioxidant and cytoprotective properties may be beneficial in protecting healthy cells and tissue against CPx-induced oxidative damage. As a result of the gravitation of consumer preferences towards natural probiotic/prebiotic food products in recent years, it was observed that fermented milk drinks had acquired a different position as products that were consumed in large quantities with an emphasis on human health and nutrition. For this purpose, in the present study, it was aimed to eliminate CPx-induced toxicity on blood and bone marrow using kefir, a highly valuable fermented milk product. As a result of the present study, it was observed that kefir was able to protect blood and bone marrow cells from the toxic effects of CPx to a significant extent. The findings of the present study reinforce the idea that kefir may serve as a potentially effective component in the prevention and treatment of CPx-induced damage.

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Impact of prediabetes on cardiovascular disease risk in patients with acute myocardial infarctions

Akut miyokard enfarktüslü hastalarda prediyabetin kardiyovasküler hastalık riskine etkisi

Özge Çakmak Karaaslan [1], Murat Oğuz Özilhan [1], Cem Çöteli [1], Orhan Maden [1]

Abstract

Aim: Prediabetes is known to be associated with increased cardiovascular diseases (CVD) risk and mortality. It has been reported that more than 70% of pre-diabetic patients develop Diabetes Mellitus (DM). In patients with acute myocardial infarction (AMI), diabetes increases a 2 to 4 fold risk of adverse cardiovascular events compared to non-diabetic patients. This morbidity-mortality relationship begins in the progression phase from normal glucose tolerance to diabetes. We evaluated the relationship between the presence of pre-diabetes by using hemoglobin A1c (HbA1c) values and CVD risk in patients presenting with newly diagnosed AMI.

Methods: This study was a retrospective, single-centre and we examined consecutive patients who underwent coronary angiography with a diagnosis of AMI at our hospital between March 2019 and September 2020. A total of 332 patients with AMI were enrolled; patients were divided into two groups according to their HbA1c levels: non-diabetic group (HbA1c <5.7%) and pre-diabetic group (5.7% ≤HbA1c <6.05%). The primary composite endpoints were cardiovascular death, myocardial infarction or stroke.

Results: Of the 332 patients, 204 (61%) patients had non-diabetic groups, and 128 (39%) patients were between the pre-diabetic groups. During the follow-up period, the primary composite endpoints amounted to 7.4% and 15.6% in the non-diabetes and prediabetes group (p=0.026). The incidences of cardiovascular death and myocardial infarction were significantly higher in the prediabetic group than the nondiabetic group (p= 0.021, p=0.004; respectively). Independent predictors for the primary composite endpoints following the multivariate analysis included SYNTAX score (odds ratio [OR]: 0.912; %95 confidence interval [CI]: 0.832- 0.999, p: 0.047), left ventricular ejection fraction (OR: 0.812; %95 CI: 0.753- 0.876, p< 0.001), systolic blood pressure (OR: 0.955; %95 CI: 0.927- 0.985, p: 0.003) and valuable categorical HbA1c (5.7% ≤HbA1c<6. 5%) (OR: 2.787; %95 CI: 1.091- 7.120, p: 0.032).

Conclusion: Prediabetes group has been shown to have a higher incidence of the primary composite endpoints than non-diabetes. SYNTAX score, left ventricular ejection fraction, systolic blood pressure, and HbA1c (categorical variable) were found as independent predictors for the primary composite endpoint. Preventive measures in the stage of pre-diabetes might help the prevention of developing CVD.

Keywords: Cardiovascular diseases, myocardial infarction, prediabetic state.

Öz

Amaç: Prediyabetin artmış kardiyovasküler hastalıklar (KVH) riski ve mortalite ile ilişkili olduğu bilinmektedir. Prediyabetik hastaların %70'inden fazlasında Diabetes Mellitus (DM) geliştiği bildirilmiştir. Akut miyokard enfarktüsü (AME) olan hastalarda diyabet, diyabetik olmayan hastalara kıyasla istenmeyen kardiyovasküler olay riskini 2-4 kat artırır. Bu morbidite-mortalite ilişkisi normal glukoz toleransından diyabete ilerleme aşamasında başlar. Yeni tanı almış AME ile başvuran hastalarda, hemoglobin A1c (HbA1c) değerleri kullanılarak prediyabet varlığı ile KVH riski arasındaki ilişkiyi değerlendirdik.

Yöntemler: Bu çalışma retrospektif, tek merkezli olup, Mart 2019-Eylül 2020 tarihleri arasında ardışık olarak, hastanemizde AME tanısı ile koroner anjiyografi yapılan hastalar incelendi. Toplam 332 AME hastası kaydedildi; hastalar HbA1c düzeylerine göre 2 gruba ayrıldı: diyabetik olmayan grup (HbA1c <%5,7, n = 204) ve prediyabetik grup (%5,7 ≤HbA1c <%6,5, n = 128). Birincil bileşik son noktalar, kardiyovasküler ölüm, miyokardiyal enfarktüs ve inme olarak belirlendi.

Bulgular: 332 hastanın 204'ü (%61) diyabetik olmayan gruba, 128 (%39) hasta prediyabetik grup arasındaydı. Takip süresi boyunca, birincil birleşik sonlanım noktaları diyabet olmayan ve prediyabet grubunda %7,4 ve %15,6 olarak bulundu (p = 0,026). Prediyabetik grupta kardiyovasküler ölüm ve miyokard infarktüsü insidansı, diyabetik olmayan gruba göre anlamlı olarak daha yüksekti (sırasıyla p = 0,021, p = 0,004). Çok değişkenli analizi takiben birincil birleşik sonlanım noktaları için bağımsız öngörücüler arasında SYNTAX skoru (OR: 0,912; %95 GA: 0,832- 0,999, p: 0,047) , sol ventriküler ejeksiyon fraksiyonu (OR: 0,812; %95 GA: 0,753- 0,876, p< 0,001), sistolik kan basıncı (OR: 0,955; %95 GA: 0,927- 0,985, p: 0,003) ve kategorik değerli HbA1c (%5,7 ≤HbA1c<%6,5) (OR: 2,787; %95 GA: 1,091- 7,120, p: 0,032) yer aldı.

Sonuç: Prediyabet grubunun diyabet olmayanlara kıyasla birincil bileşik sonlanım noktalarının daha yüksek insidansına sahip olduğu gösterilmiştir. Bileşik sonlanım noktası için bağımsız öngörücü olarak SYNTAX skoru, sol ventriküler ejeksiyon fraksiyonu, sistolik kan basıncı ve HbA1c (kategorik değişken) bulundu. Prediyabet aşamasında önleyici tedbirler, KVH gelişiminin önlenmesine yardımcı olabilir.

Anahtar kelimeler: Kardiyovasküler hastalıklar, miyokard enfarktüsü, prediyabetik durum.

[1] University of Health Sciences, Ankara City Hospital, Department of Cardiology, Ankara, Turkey.

ÖÇK: 0000-0003-0173-4017
MOÖ: 0000-0003-4801-966X
CÇ: 0000-0001-5520-7103
OM: 0000-0003-3170-1179

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Sorumlu yazar / Corresponding author:
Özge Çakmak Karaaslan
Adres/Address: Ankara City Hospital, Cardiology Clinic, Ankara, Turkey.
e-mail: ozgecakmak2323@gmail.com
Tel/Phone: +90 5377395670
Fax: +90 3123061000

Introduction

Diabetes mellitus (DM) is a significant risk factor for cardiovascular diseases (CVD) [1]. In patients with acute myocardial infarction (AMI), diabetes increases a 2- to 4-fold risk of adverse cardiovascular events compared to nondiabetic patients [2]. DM is one of the most critical risk factors in the development of coronary artery disease (CAD), and it alone is responsible for 9.9% of the risk of having the first AMI [3]. However, previous studies have shown that this morbidity-mortality relationship begins in the progression phase from normal glucose tolerance to diabetes [4] [5].

Prediabetes is defined as the period before developing symptomatic diabetes when fasting blood glucose concentration is below the limit values for the diagnosis of DM [6]. Prediabetes is known to be associated with increased cardiovascular risk and mortality [7]. It has been acknowledged that prediabetes status is diagnosed based on fasting blood glucose measures, 2-hours plasma glucose after an OGTT, or hemoglobin A1c (HbA1c) [8]. The OGTT in patients with AMI may lead to false evaluation due to acute stress hormones during the peri-infarction period. It isn't easy to perform in this period, so HbA1c is commonly preferred over the OGTT in clinical practice [9]. In addition, it has been shown that prediabetes defined by HbA1c was associated with a poor prognosis for CVD than prediabetes defined by fasting blood glucose or 2-hours plasma glucose [10].

It has been observed that there are significant inconsistencies between reports due to the use of different cutting points and reference groups [11]. It has been known that direct comparisons using HbA1c measurement with CVD development are rare in the same population [12].

Therefore, we evaluated the relationship between prediabetes status defined by HbA1c and CVD development in patients presenting with newly diagnosed AMI.

Material and methods

This study was a retrospective, single-centre and we examined consecutive patients who underwent coronary angiography with a diagnosis of AMI (ST-elevation and non-ST-elevation myocardial infarction) at our hospital between March 2019 and September 2020. We have retrospectively evaluated records of 1016 patients diagnosed with acute myocardial infarction between March 2019 and September 2020. This study was prepared by the Declaration of Helsinki and good clinical use guidelines. The local ethics committee approved this study (Date: 03.02.2021, Number: E1-21-1486).

A total of 608 patients who a history of known coronary artery disease, diabetes or newly diagnosed diabetes (HbA1c ≥ 6.5%) during hospital admission, and 76 patients with missing medical data were excluded from the study. The remaining 332 patients comprised the study cohort.

Patients were divided into 2 groups according to their HbA1c levels: nondiabetic group (HbA1c <5.7%, n=204) and prediabetic group (5.7% ≤HbA1c <6.5%, n=128) according to 2020 American Diabetes Association (ADA) definition [6].

The primary composite endpoints were cardiovascular death, myocardial infarction or stroke. Cardiovascular death was included sudden cardiac death, death due to acute myocardial infarction, death due to heart failure, death due to a cerebrovascular event and deaths for which there was no clearly documented non-cardiovascular cause (presumed cardiovascular death). Stroke was defined as an acute event of neurologic dysfunction attributed to a central nervous system vascular cause documented by imaging (e.g., computed tomography or magnetic resonance imaging). AMI is diagnosed based on the Universal Myocardial Infarction definition published by Thygesen et al. [13] in 2018.

The reason for death was evaluated by obtained death information from National Survival Registry and the hospital database's records. Patients were followed up for a median of 10 months (± 6 months). It was recorded the 1st, 3rd and 6th months follow-up visits after discharge from the hospital records. The primary composite endpoints were adjudicated by a researcher that was blinded to admission HbA1c level.

As a previous diagnosis of diabetes, DM was defined as usage of oral hypoglycaemic agents or insulin, or HbA1C levels ≥6.5% in the blood sample at admission hospital [14]. Pre-diabetes was defined as an admission HbA1c value of 5.7–6.4% in patients without previously diagnosed DM [15]. Dyslipidemia (total cholesterol level of ≥200 mg/dL in the fasting state or low-density lipoprotein (LDL) cholesterol ≥140 mg/dL or use of cholesterol-lowering agents) and hypertension (HT) (use of anti-hypertensive medications or a previous diagnosis of HT) evaluated as risk factors for CAD.

The diagnosis of AMI was established on the existence of an increase or fall of cardiac biochemical markers (creatinine kinase-MB and troponin I or T) except normal range and with at least one of the following: clinical symptoms of ischemia, novel or assumed new important ST-segment–T wave changes or novel left bundle branch block, the establishment of pathological Q waves in the electrocardiography, and imaging evidence of new regional wall motion abnormality [13].

Samples for creatinine, alanine aminotransferase (ALT), estimated glomerular filtration rate (eGFR), hemogram, HbA1c, and C-reactive protein (CRP), troponin I were taken at admission and fasting blood glucose, triglycerides (TG), total cholesterol (TC), high- density lipoprotein (HDL) cholesterol and LDL cholesterol were measured on the at admission. Data respecting baseline characteristic and clinical features, risk factors and laboratory parameters of all patients were obtained from follow-up visits, patients' records and the electronic hospital database.

Left ventricular ejection fraction (LVEF) was measured using the modified Simpson method.

Statistical analysis

Data were analysed using the IBM SPSS 22.0 Statistical Package Program for Windows (IBM SPSS, Inc., IL, USA). The Shapiro-Wilk test was utilized to test the normality of distribution. Continuous variables were expressed as mean ± standard deviation if normally distributed or median median (min-maks) if not distributed normally. Categorical variables were expressed as the number of patients and percentages. Student t-test was used to compare mean values and Chi-square or Fisher's Exact test was used to compare categorical variables as appropriate. The Mann-Whitney U test was utilized to compare continuous variables that were not distributed normally.

A univariate logistic regression analysis was used to evaluate each variable's association with the primary composite endpoint occurrence. Univariate logistic regression analysis was performed for the following parameters: SYNergy between PCI with TAXUS and Cardiac Surgery (SYNTAX) score, Thrombolysis in Myocardial Infarction (TIMI) thrombus grade, LVEF, age, ST-Elevation myocardial infarction (STEMI), heart rate, systolic blood pressure (SBP), eGFR, hemoglobin and HbA1c as a categorical parameter. The enter methods were used for determining variables with the association of the primary composite endpoint in the multivariate logistic regression analysis. Parameters smaller than p-value 0.05 are put into

multivariate logistic regression analysis was made. A p-value <0.05 was considered significant.

Results

Baseline clinical, demographic and laboratory characteristics are showed in Table 1. Of the 332 patients, 204 (61%) patients had HbA1c <5.7% (Nondiabetic Group) and 128 (39%) patients were between HbA1c 5.7-6.4% (Prediabetic Group). The prediabetic group has an older, higher heart rate and higher systolic blood pressure than the non-diabetic group (p= 0.014, p<0.001, p<0.001; respectively). The male gender ratio was similar between the two groups. There were no differences in terms of hypertension, dyslipidemia and known heart failure between groups. LVEF was similar in both groups. As expected, glucose values were higher in the prediabetic group (p<0.001). There was no statistically significant difference regarding, creatinine, ALT, TC, TG, LDL, HDL, eGFR, white blood cell count, hemoglobin, CRP, troponin levels, between non-diabetes and pre-diabetes groups. There was no difference in the use of the prescribed medications after discharge among the groups (Table 1). The rate of NSTEMI and STEMI were similar between groups. In prediabetes group had a higher prevalence of left main coronary artery disease (p<0.001). The prevalence of left anterior descending coronary artery, left circumflex coronary artery, and right coronary artery diseases were similar. There were no differences in terms of angiographic TIMI thrombus grade and SYNTAX scores between the groups. The Selection of revascularization was similar between groups (Table 2).
Table 1.

Table 1. Baseline demographical and clinical characteristics of the study population.

	Non-diabetic patients (n=204)	Prediabetic patients (n=128)	p
Age (year) †	55 ± 19	60.50 ± 17.75	0.014
Male ‡	164 (80%)	108 (84%)	0.359
Heart rate (beat/min)	69 ± 13	79.50 ± 21.75	<0.001
SBP mmHg †	130 ± 10	120 ± 20	<0.001
Hypertension ‡	52 (25.5)	36 (28.1)	0.611
Dyslipidemia ‡	16 (7.8)	12 (9.4)	0.686
Known heart failure	40 (19.6)	16 (12.5)	0.092
LVEF‡ (%) †	50 ± 10	50 ± 10.25	0.636
NSTEMI ‡	92 (45)	64 (50)	0.429
STEMI ‡	112 (55)	64 (50)	0.384
Admission			
Glucose (mg/dl)†	96.00 ± 20	106.50 ± 24	<0.001
Creatinine (mg/dl)	0.80 (0.4- 1.1)	0.80 (0.4- 1.5)	0.274
ALT (U/L)†	24.00 ± 18.00	25.00 ± 16.00	0.888
TC (mg/dl) †	180.00 ± 51.00	183.50 ± 53.25	0.398
Triglycerides (mg/dl) †	106.00 ± 77.00	113.00 ± 81.25	0.085
LDL-C (mg/dl) †	115.00 ±52.00	124.00 ± 58.50	0.247
HDL-C (mg/dl) †	35.00 ± 12.00	41.00 ± 12.75	0.124
eGFR (ml/min/1.73m²) †	100.00 ± 13.00	97.00 ± 18.00	0.100
WBC (x10^9/ L) †	11.05 ± 3.17	10.50 ± 3.41	0.836
Hemoglobin (g/dl) †	14.30 ± 2.00	14.00 ± 1.22	0.435
CRP (mg/L) β	4.41 (1.37- 112)	4.40 (1.0- 95)	0.992
Troponin (ng/L) †	6.20 ± 2.38	8.82 ± 2.35	0.456
Medications at discharge			
Aspirin ‡	201 (98.5)	124 (96.9)	0.307
B-blocker ‡	192 (94)	124 (96.9)	0.254
Statins ‡	192 (94)	124 (96.9)	0.302
ACEIs or ARBs ‡	200 (98)	124 (96.9)	0.491
Diuretics ‡	12 (5.9)	12 (9.4)	0.277
P2Y12 receptor inhibitors ‡	196 (96)	124 (96.9)	0.773

†: mean ± standard deviation, ‡: n (%), ‡: median (min-max).
SBP: Systolic Blood Pressure; LVEF: Left ventricular ejection fraction; ALT: Alanine aminotransferase; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate; WBC: White blood cell; CRP: C-reactive protein; ACEI: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin II receptor blockers

Table 3 demonstrates the primary composite endpoints according to HbA1c category. The primary composite endpoints amounted to 7.4% and 15.6% in the nondiabetic and prediabetic group (p=0.026). The incidence of cardiovascular death and myocardial infarction were higher in the prediabetic than the nondiabetic (p= 0.021, p= 0.004; respectively). The incidence of stroke was higher in the nondiabetic group (p= 0.046) (Table 3).

Table 1. Coronary artery disease, angiographic data and selection of revascularization among the study population.

	Non-diabetic patients (n=204)	Prediabetic patients (n=128)	p
Angiographic findings ‡			
Left main	0	8 (6.3)	<0.001
Left anterior descending	92 (45)	52 (40)	0.423
Left circumflex	68 (33)	32 (25)	0.112
Right coronary artery	72 (35)	56 (43)	0.123
TIMI thrombus grade	4.0 ± 3.0	4.0 ± 2.0	0.171
SYNTAX Score	6.0 ± 5.0	5.5 ± 8.2	0.968
Revascularization ‡			
Coronary artery bypass grafting	4 (2)	4 (3.1)	0.491
Percutaneous coronary intervention	176 (86.3)	108 (84.3)	0.632
Non-interventional treatment	24 (11.7)	16 (12.6)	0.464

‡: n (%).
NSTEMI: Non-ST elevation myocardial infarction; STEMI: ST elevation myocardial infarction; TIMI: Thrombolysis in Myocardial Infarction; Syntax: SYNergy between PCI with TAXUS and Cardiac Surgery.

Univariate logistic regression analyses showed that the SYNTAX score, TIMI grade, LVEF, STEMI, SBP, eGFR, hemoglobin and HbA1c (categorical variable) was significantly associated with the primary composite endpoints, as shown in Table 4. Independent predictors for the primary composite endpoints following the multivariate analysis included SYNTAX score, LVEF, SBP and HbA1c (categorical variable) (Table 4).

Table 3. Primary endpoints according to admission HbA1c.

	Non-diabetic patients (n=204)	Prediabetic patients (n=128)	p
Primary composite endpoints ‡	15 (7.4)	20 (15.6)	0.026
Cardiovascular death ‡	0	4 (3.1)	0.021
Myocardial infarction ‡	8 (3.9)	16 (12.5)	0.004
Stroke ‡	7 (3.4)	0	0.046

‡: n (%).

Discussion

The main findings were as follows: the prediabetes group has a higher incidence of primary compound endpoint than the nondiabetic group, despite similar medical history, angiographic findings, and revascularization selection. Incidence of cardiovascular death and myocardial infarction were higher in the prediabetic group. In addition, prediabetes status has been found predictive for the primary composite outcome. Contrary to expectations, the incidence of stroke was higher in the nondiabetic group.

It has been reported that 25-35% of patients who develop AMI are prediabetic [16, 17]. Ford et al. showed that the risk of CVD increased 0.65-2.5-fold in patients with fasting blood glucose of 110 mg/dl and above [18]. Satman et al. [19]reported that CVD risk increased 1.28-fold in patients with impaired fasting glucose 100-125 mg/dL and 1.20-fold in those with 110-125 mg/dL. It is known that the risk of developing CVD in prediabetic patients is significantly higher than those with normoglycemia [18]. In a study evaluating long-term follow-up results of the DECODE cohort, prediabetics with high OGTT (2nd hour) and high initial or fasting blood sugar values have reported an increased cardiovascular risk to the non-high group [20]. OGTT may cause misinterpretation in patients with AMI due to acute stress hormones during a peri-infarction period and may reflect incorrect plasma glucose levels [9].

Table 4. Univariate and multivariate logistic regression analyses for prediction of primary endpoints.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
SYNTAX Score	1.113 (1.067- 1.161)	<0.001	0.912 (0.832-0.999)	0.047
TIMI	1.308 (1.045- 1.637)	0.019	1.338 (0.942-1.900)	0.104
LVEF	0.865 (0.828- 0.905)	<0.001	0.812 (0.753-0.876)	<0.001
Age	1.012 (0.989- 1.036)	0.297		
STEMI	2.998 (1.462- 6.146)	0.003	1.571 (0.577- 4.278)	0.377
Heart Rate.	1.008 (0.984- 1.031)	0.532		
SBP	0.942 (0.922- 0.963)	<0.001	0.955 (0.927- 0.985)	0.003
eGFR	0.976 (0.961- 0.990)	0.001	0.985 (0.947- 1.025)	0.466
Hb	0.719 (0.613- 0.843)	<0.001	0.867 (0.590- 1.275)	0.469
HbA1c (5.7% ≤HbA1c<6. 5%)	2.333 (1.147- 4.746)	0.019	2.787 (1.091- 7.120)	0.032

OR: Odds ratio; CI: Confidence interval

Syntax: SYNergy between PCI with TAXUS and Cardiac Surgery; TIMI: Thrombolysis in Myocardial Infarction; LVEF: Left Ventricular Ejection Fraction; STEMI: ST elevation myocardial infarction; SBP: Systolic blood pressure; eGFR: estimated glomerular filtration rate; Hb: Hemoglobin.

In these previous studies, prediabetes status was evaluated with fasting blood glucose, impaired fasting glucose, or impaired glucose tolerance. In our study, prediabetes status was evaluated using HbA1c, considering the confounding effect of the catecholaminergic reaction in the AMI period. HbA1c has recommended as a diagnostic test for prediabetes and DM in addition to conventional criteria based on fasting plasma glucose or the OGTT by the ADA [6]. Prediabetes status is considered to HbA1c from 5.7 to 6.4% [20, 21]. The HbA1c has various benefits in the diagnosis of prediabetes in the status of AMI, for instance, fasting is not necessary, and as a marker of glycaemic control over the former 8–12 weeks average, it is less affected than admission plasma glucose by acute stress reaction caused by AMI [22]. Elevated HbA1c has been shown to connect to cardiovascular mortality and be important for the prognosis after AMI in nondiabetics patients [23]. Tomizawa et al. [24] reported that patients with between HbA1c levels 6.0-6.5% are potentially at risk of adverse cardiovascular events.

A significant proportion of dysglycemic patients develop vascular disorders during the prediabetes stage, although their glucometabolic impairment often remains undetected until the first cardiovascular event [25]. Free fatty acids, hyperglycemia, and insulin resistance in prediabetes status cause changes in blood vessels' function and structure [26]. Increased free fatty acid causes damage to the ischemic myocardium by increasing myocardial oxygen requirement, resulting in the occurrence of ventricular arrhythmias and depressed myocardial contractility [27]. The glucose dysregulation in prediabetes status has associated with the release of cytokines or cell death, inflammation, and consequent cardiomyopathy [28]. The vasoconstriction and inflammation increase and contribute to the progression of coronary atherosclerosis [29]. Prediabetes individuals have more advanced coronary artery disease such as diffuse coronary stenosis and impaired coronary collateral circulation compared to normoglycemia [16, 30]. Several mechanisms can explain the increased proportions of CVD in prediabetic individuals; the most widely accepted are all-day exposure of prediabetics to high blood sugar, high frequency of metabolic syndrome and vascular damage which is the cause of a diurnal cycle change and fluctuation of blood glucose levels in these patients [15, 31].

Previous studies reported that the mean age of the prediabetic acute coronary syndrome patients was higher than the nondiabetic acute coronary syndrome patients [32]. In this study, prediabetic patients were advanced age than nondiabetic patients. Although the risk of atherosclerotic coronary disease increases with age, nondiabetic patients who had AMI were younger age; it might be associated with the individual's other risk factors independent of high glucose levels.

It is known that prediabetes has a significant association with heart failure [33]. In our study, LVEF was found to be similar between groups, contrary to previous studies. The cardiovascular outcomes of prediabetes might be considered not yet reflected in the clinical findings. In addition, though the LVEF value was similar, the prediabetes group had higher cardiovascular mortality due to more AMI and complications rather than related heart failure.

In this study, the SYNTAX score showing coronary artery severity angiographically was similar between the groups, which were not surprising as no expected silent MI in prediabetes. Coronary atherosclerosis in diabetic patients is usually recognized at an advanced age, whereas the disease in its premature or asymptomatic stages regrettably frequently remains undetected or delayed [34]. Therefore, premature atherosclerosis in the prediabetic status might cause similar STYTAX and TIMI scores.

Several studies have evaluated the prevalence of prediabetes and DM type 2 in patients who had stroke and in the majority of those studies, the prevalence was assessed by OGTT and fasting plasma glucose [35]. Using impaired fasting glucose or impaired glucose tolerance level-based criteria, about 10% of the adult people were in prediabetes state, whereas HbA1c-based criteria identify a significantly lower ratio of the patients [36]. Contrary to the literature, stroke was less frequent in prediabetic patients in this study. This situation has been considered associated with the small size of our study population and the diagnosis of prediabetes based on HbA1c.

First, this study was limited by the cross-sectional nature of patients presenting with AMI, a single-centre retrospective study, including a limited number of participants and results, may not be generalized to the population as a whole. Second, the effect of factors that can change HbA1c levels regardless of glycaemia, such as anaemia or hemoglobinopathies, should be considered. The development of cardiovascular diseases and the occurrence of diabetes complications are a long process. Therefore, the short follow-up time of our study may lead to lower cardiovascular disease incidence. It might not be consistent compliance with treatment regimens, which could be difficult to assess in retrospective data analysis. We did not perform the OGTT for diagnosing diabetes mellitus. Therefore, according to the criteria described in the Methods section, patients with undiagnosed diabetes might have been misclassified as nondiabetic.

In this study, the prediabetes group has been shown to have higher incidences of the cardiovascular death and myocardial infarction than nondiabetics. Our data have been consistent with previous studies showing that prediabetes was a risk factor for CVD [17]. Otten et al. demonstrated that impaired fasting glucose was an independent predictor of in-hospital outcome, including death, reinfarction and the composite of major cardiovascular adverse events in the different nondiabetic categories [38]. As a result of multivariate analysis, it was determined that HbA1c categorical variable and reduced LVEF, SBP and SYNTAX score were associated with the primary composite endpoints. In agreement with our study, the study by Naito et al. showed that reduced LVEF was an independent predictor of poor prognosis in nondiabetic patients with AMI [39]. It has also been reported that the SYNTAX score has been used in the estimation of long-term mortality and major adverse cardiovascular events in prediabetics [39]. Thus, preventive measures in the stage of prediabetes might help the prevention of developing CVD.

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Arch Clin Exp Med 2021;6(2):88-91. e-ISSN: 2564-6567

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Evaluation of peripheral nerves in patients receiving anti-tumor necrosis factor-alpha drug therapy

Anti-tümör nekroz faktör-alfa ilaç tedavisi alan hastalarda periferik sinirlerin değerlendirilmesi

Bilge Ekinci [1], Hasan Yaşar [2], Yusuf Kemal Arslan [3]

Abstract

Aim: Anti-tumor necrosis factor-alpha (TNF-α) drug treatments are widely used in many inflammatory diseases. Neurological complications have rarely been reported in these treatments. Our aim in this study was to investigate the neurological findings that occurred in our patients receiving this treatment.
Methods: A case-control study conducted in (institutional information was blinded) between September 2018-September 2019. The study included 35 patients receiving tumor necrosis factor-alpha blocker drug, and 37 healthy control subjects with similar demographic characteristics. The disease activity scores of the patient group and physical function scores of the patient and control groups were questioned. All patients underwent a detailed physical and neurological examination. Afterward, peripheral nerves were evaluated neurophysiologically. According to distribution Mann-Whitney U test or independent samples t-test was used when comparing groups. The relationship between Short Form-36 and age or body mass index was determined by using Spearman's rank correlation coefficient.
Results: The results obtained in sensory and motor nerve conduction examinations were compared between groups. Patients using anti-tumor necrosis factor-alpha had peripheral sensory neuropathy. Examination of peripheral motor nerves was within normal limits.
Conclusions: Anti-tumor necrosis factor-alpha drugs have good effects in inflammatory diseases. These patients should be carefully monitored for neurological findings.

Keywords: Tumor necrosis factor-alpha, nerve conduction, electromyography

Öz

Amaç: Anti-tümör nekroz faktör-alfa (TNF-α) ilaç tedavileri, birçok enflamatuar hastalıkta yaygın olarak kullanılmaktadır. Bu tedavilerde nadiren nörolojik komplikasyonlar bildirilmiştir. Bu çalışmadaki amacımız, bu tedaviyi alan hastalarımızda ortaya çıkan nörolojik bulguları araştırmaktı.
Yöntemler: Eylül 2018-Eylül 2019 arasında (kurumsal bilgi körlendi) yürütülen bir vaka kontrol çalışmasıdır. Çalışmaya tümör nekroz faktör-alfa bloker ilaç alan 35 hasta ve benzer demografik özelliklere sahip 37 sağlıklı kontrol deneği dahil edildi. Hasta grubunun hastalık aktivite skorları ile hasta ve kontrol grubunun fiziksel fonksiyon skorları sorgulandı. Tüm hastalara detaylı fiziksel ve nörolojik muayene yapıldı. Daha sonra periferik sinirler nörofizyolojik olarak değerlendirildi. Dağılıma göre gruplar karşılaştırılırken Mann-Whitney U testi veya Independent samples t-testi kullanıldı. Kısa Form-36 ile yaş veya vücut kitle indeksi arasındaki ilişki, Spearman'ın sıra korelasyon katsayısı kullanılarak belirlendi.
Bulgular: Duyusal ve motor sinir ileti incelemelerinde elde edilen sonuçlar gruplar arasında karşılaştırıldı. Anti-tümör nekroz faktör-alfa ilacı kullanan hastalarda periferik duyusal nöropati tespit edildi. Periferik motor sinirlerin incelemesi normal sınırlardaydı.
Sonuç: Anti-tümör nekroz faktör-alfa ilaçları enflamatuar hastalıklarda oldukça etkilidir. Bu ilaçları kullanan hastalar nörolojik bulgular açısından dikkatle izlenmelidir.

Anahtar kelimeler: Tümör nekroz faktör-alfa, sinir iletimi, elektromiyografi

[1] Erzincan Binali Yıldırım University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Erzincan, Turkey.
[2] Erzincan Binali Yildirim University, Faculty of Medicine, Department of Neurology, Erzincan, Turkey.
[3] Erzincan Binali Yildirim University, Faculty of Medicine, Department of Biostatistics, Erzincan, Turkey.

BE: 0000-0002-7520-0205
HY: 0000-0001-8601-1277
YKA: 0000-0003-1308-8569

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Sorumlu yazar / Corresponding author:
Hasan Yaşar

Adres/Address: Erzincan Binali Yildirim University, Faculty of Medicine, Department of Neurology, Erzincan, 24100, Turkey.

e-mail: yasar_hsan@hotmail.com
Tel/Phone: +90 505 631 57 19
Fax: +904462261819

Introduction

Tumor necrosis factor (TNF) is a pleomorphic proinflammatory cytokine, which plays an important role in the pathogenesis of many chronic inflammatory diseases, mainly produced in monocytes, macrophages, and T-lymphocytes [1]. TNF has also been shown to be produced by natural killers, fibroblasts, granulocytes, keratinocytes, muscle cells, and neurons. It is the first cytokine to respond to tissue damage, bacteria, viruses, immune complex, tumor cells and is referred to as "fire alarm'' our body [2].

TNF released from macrophages has been shown to have endotoxic shock development, cachexia in the course of infections as well as suppressing replication of viruses and facilitating the elimination of pathogens by macrophages [3]. TNF is first synthesized as membrane TNF-bound transmembrane TNF (tmTNF), and TNF is transformed into soluble-TNF (sTNF) by TNF-alpha converting enzyme (TACE) and released from the cell. Both tmTNF and sTNF are biologically active and have important roles [4].

Recently, anti-tumor necrosis factor-alpha (anti-TNF-α) drugs have been widely used as immunosuppressive agents in chronic inflammatory diseases such as rheumatoid arthritis (RA), juvenile rheumatoid arthritis, ankylosing spondylitis (AS), psoriasis, psoriatic arthritis, and Crohn disease. The five anti-TNF-α agents currently in clinical use are etanercept (circulating receptor fusion protein), infliximab, adalimumab, golimumab (Ig G monoclonal antibodies), and certolizumab (PEGylated Fab 1 fragment of an Ig G1 monoclonal antibody) [5]. TNF blockers are known to stimulate phagocytosis, degranulation, cytokine release, and antibody-mediated cellular cytotoxicity through which the cell bind to Fc receptors through Fc portions [6]. Anti-TNF drugs with monoclonal antibody features show their effects by suppressing proinflammatory cytokines, inducing cellular apoptosis, and stimulating cytotoxicity through complement [7]. In Etanercept, this effect is weak, and unlike other anti-TNFs, it also blocks lymphotoxin α (LTα3) [8]. Anti-TNF-α drugs were found to be faster and more effective in controlling disease activity and preventing underlying structural tissue damage than traditional disease-modifying drugs (DMARD) treatments [7].

Anti-TNF-α has been associated with different adverse effects, including infections (especially tuberculosis reactivation), local site reactions, hemocytopenia, congestive heart failure, T-cell lymphomas, lupus-like syndromes and vasculitis, autoimmune and neurological events [9-11].

Additionally, with the widespread use of anti-TNF-α drugs, an increasing number of demyelinating pathologies have been reported, including central nervous system (optic neuritis, multiple sclerosis, acute transverse myelitis) and peripheral nervous system disorders (Guillain-Barre syndrome, Miller Fisher syndrome, mononeuropathy multiplex, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy with conduction block, and axonal sensorimotor polyneuropathies) [12].

Our aim in this study was to investigate the patients with the chronic inflammatory disease using TNF-α blockers in terms of peripheral nerve damage.

Material and methods

The study includes patients with RA and Spondyloarthropathy (SpA) using TNF-α blocker drug and who applied to (institutional information was blinded) Physical Medicine and Rehabilitation clinic between September 2018-September 2019. The study was approved by the Local Ethical Committee (date, meeting and decision no: 09.01.2018, 18/05). Patients with RA were previously diagnosed according to the American College of Rheumatology (ACR) 1987, patients with SpA, according to the Assessment of SpondyloArthritis İnternational Society (ASAS) diagnostic criteria. The study included 35 patients receiving TNF-α blocker drug (20 SpA, 15 RA) and 37 healthy control subjects with similar demographic characteristics. Of the patients, 35 in the patient group, 14 were receiving adalimumab, 9 were etanercept, 6 were infliximab, and 5 were golimumab.

Patients with diabetes mellitus, hypertension, dyslipidemia, heart disease, iron, and B12 deficiency, endocrinological, neurological disease, atherothrombotic attack, head and neck trauma, and neurological surgery were excluded from the study. The control group was consisted of healthy individuals aged between 18-65 years and demographically compatible with the patient group that without any acute, chronic diseases and vitamin deficiency, using no medication, non-smoking and no alcohol consumption and not pregnant. Disease activity scores of the patient group and physical function scores of the patient and control groups were questioned. All patients underwent a detailed physical and neurological examination. Afterward, peripheral nerves were evaluated neurophysiologically by electromyography (EMG). Neurological examination and neurological tests were performed by a specialist neurologist.

In the present study, nerve conduction studies were performed with a Medelec Synergy model device. Sensory nerve conduction was performed antidromically. Peak amplitude values, conduction velocities were measured and compared in the sensory nerve examinations between the patient and control groups. In sensory nerve examinations, distal latency was accepted as the time until the first positive peak of the potential generated by the stimulation artifact. The amplitude was evaluated as the amplitude measured between the first electronegative peak and the second electropositive peak. Compound muscle action potentials, distal motor latency, peak amplitude values, conduction velocities recorded by distal and proximal stimulation in motor nerve examinations were measured and compared between groups. Latency was evaluated as the time between the warning artifact and the point where the potential left the baseline in an electronegative direction. The amplitude was evaluated as the amplitude of the oscillation between the baseline and the electronegative peak. While calculating the motor nerve conduction velocity, the proximal latency of the compound muscle action potential obtained by proximal stimulation was obtained by subtracting the distal latency of the compound muscle action potential obtained by distal stimulation, and the conduction velocity was calculated by dividing the distance between the two stimulation points by this difference latency [13].

Statistical analysis

All statistical analyses were performed by using IBM SPSS 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). While reporting categorical variables number and percentage n (%) was used. Continuous variables were reported as mean±standard deviation (SD) and median (minimum-maximum). The normality assumption for variables was checked with the Kolmogorov-Smirnov test. When data were not normally distributed Mann-Whitney U test, otherwise independent

samples t-test, was used. The relationship between SF-36 and Age or BMI was determined by using Spearman's rank correlation coefficient. For correlation coefficient 0,0-0,19 was accepted as no correlation, 0,20-0,39 as weak correlation, 0,40-0,69 as moderate, 0,70-0,89 as strong and 0,90-1,00 as perfect correlation.

Results

The average age of the patient group who participated in our study was similar to the control group (p=0.683). Besides, BMI was similar between the patient group and the control group (p=0.123). In patients undergoing neurophysiological evaluation, the mean duration of disease was 124.3±80.3 (months), and mean duration of drug use was 35.6±19.9 (months). The demographic information is shown in Table 1.

Table 1. Demographic and Biochemical Findings of Study Groups.

	Case	Control	p
Age (years)	43.2±13.0	44.6±15.1	0.683†
BMI	27.4±4.4	26.1±2.8	0.123†
SF-36 (Physical)	5.0 (50.0-95.0)	90.0 (80.0-100.0)	<0.001
Duration of disease (months)	120.0 (24.0-360.0)	-	-
Duration of drug use (months)	36.0 (6.0-96.0)	-	-
RF (IU/mL)	8.8 (8.8-316.0)	-	-
CCP (U/mL)	4.7 (0.5-161.9)	-	-
CRP (mg/L)	3.2 (2.4-44.7)	3.0 (3.0-6.8)	0.002
Sedimentation (mg/dL)	10.0 (2.0-45.0)	8.0 (2.0-34.0)	0.623
AST (U/L)	21.0 (12.0-71.0)	20.0 (14.0-30.0)	0.709
ALT (U/L)	22.0 (9.0-91.0)	20.0 (11.0-42.0)	0.826
GGT (U/L)	28.3±12.7	22.5±6.3	0.018†
Urea (mg/dL)	28.0±6.2	28.5±6.9	0.731†
Creatinine (mg/dL)	0.9±0.1	0.9±0.2	0.154†
WBC (10^3/μL)	7.4 (4.2-12.2)	6.8 (5.2-10.2)	0.216
HGB (g/L)	13.4 (11.0-16.8)	14.1 (12.0-16.4)	0.263
HCT (%)	41.8±4.7	42.7±3.9	0.367†
PLT (10^3/mL)	274.1±67.2	271.9±59.1	0.888†

BMI: Body mass index, SF: Short form, RF: Rheumatoid factor, CCP: Cyclic citrullinated peptide, CRP: C- reactive protein, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, GGT: Gamma-glutamyl transferase, WBC: White blood cell, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelets. Results were presented as mean ± standard deviation value or median (minimum-maximum) value. †Independent samples t-test was performed; Otherwise Mann-Whitney U test was used. Statistically significant (p<0.05).

According to BASDAI, 11 (55%) of AS patients had low disease activity, and 9 (45%) had high disease activity. According to ASDAS, one out of 9 patients had very high disease activity. The functional level of those with low disease activity, according to BASFİ, is good; the functional level of those with high disease activity was moderate. According to the DAS 28 score, 3 (20%) of 15 RA patients had low, and 12 had moderate disease activity. The SF-36 physical function score was higher in the control group than the patient group for both genders.

A moderate negative relationship was found between age and SF-36 physical functional score (r =-0.54, p < 0.001). Similarly, a weak negative relationship was found between BMI and SF-36 physical functional score (r=-0.36, p=0.002). Five (33.3%) of the patients with RA were RF positive, and 10 (66.7%) were RF negative; Anti-CCP was positive in 7 (46.7%) of patients and negative in 8 (53.3%) of patients. HLA B27 was positive in 16 (80.0%) of patients with AS and negative in 4 (20.0%).

The EMG results of sensory nerve conduction between the patient and control groups for left median, right median, ulnar and sural nerves were presented in for Table 2.

Table 2. Sensory Nerve Conduction Values of Study Groups.

		Case	Control	p
Left Median Nerve	NP-amplitude	24.3 (5.9-54.7)	34.4 (9.4-76.1)	0.060
	Velocity	54.9 (38.1-90.3)	56.5 (31.3-73.7)	0.761
	Latency	2.5 (1.6-3.2)	2.3 (1.7-4.2)	0.020
Right Median Nerve	NP-amplitude	22.5 (3.0-68.7)	31.2 (9.3-65.2)	0.008
	Velocity	55.6±8.5	56.0±6.9	0.842†
	Latency	2.4 (2.0-4.5)	2.3 (1.8-3.7)	0.025
Ulnar Nerve	NP-amplitude	25.8 (10.1-52.9)	26.8 (9.0-49.8)	0.685
	Velocity	58.9±6.3	61.3±5.8	0.091†
	Latency	2.0 (1.6-2.6)	1.8 (1.5-2.5)	<0.001
Sural Nerve	NP-amplitude	12.8 (4.4-44.4)	19.5 (9.3-38.6)	0.001
	Velocity	48.8 (29.4-68.6)	60.6 (46.8-69.0)	<0.001
	Latency	2.1 (1.6-4.2)	1.6 (1.2-2.4)	<0.001

NP: Negative-peak. Results were presented as mean ± standard deviation value or median (minimum-maximum) value. †Independent samples t-test was performed; Otherwise Mann-Whitney U test was used. Statistically significant (p<0.05).

Similarly, EMG results of motor nerve conduction between the study groups for left median, right median, ulnar, common peroneal and tibial nerves were presented in for Table 3.

Table 3. Motor Nerve Conduction Values of Study Groups.

		Case	Control	p
Left Median Nerve	NP-amplitude	10.5 (4.2-16.6)	9.8 (7.7-15.5)	0.191
	Velocity	59.5 (53.4-70.3)	61.2 (51.9-76.2)	0.237
	Latency	3.0 (2.1-4.7)	3.0 (2.3-5.3)	0.883
Right Median Nerve	NP-amplitude	10.9±4.0	9.4±2.7	0.076†
	Velocity	57.4 (45.7-70.8)	59.2 (51.4-73.0)	0.289
	Latency	3.1±0.6	3.1±0.6	0.368†
Ulnar Nerve	NP-amplitude	10.4±2.2	10.0±2.0	0.380†
	Velocity	63.2±7.1	64.7±6.3	0.355†
	Latency	2.3±0.3	2.4±0.4	0.353†
CP Nerve	NP-amplitude	4.9 (2.2-13.2)	5.0 (2.4-8.1)	0.644
	Velocity	51.5±6.8	49.8±5.4	0.240†
	Latency	3.8±0.7	3.8±0.6	0.833†
TIB Nerve	NP-amplitude	9.2±4.3	8.0±2.6	0.140†
	Velocity	48.6 (24.0-67.3)	47.4 (42.6-54.8)	0.090
	Latency	4.3±1.0	4.5±0.7	0.179†

NP: Negative-peak, CP: Common peroneal nerve, TIB: Tibial nerve. Results were presented as mean ± standard deviation value or median (minimum-maximum) value. †Independent samples t-test was performed; Otherwise Mann-Whitney U test was used, Statistically significant (p<0.05).

Groups compared in terms of sensory nerves results. Right median nerve amplitude and sural nerve amplitude were considerably difference (p=0.008, p<0.001, respectively). Amplitudes were lower in the patient group. When the groups are compared in terms of conduction velocities, only the difference between the sural nerves were detected (p<0.001). Conduction velocity was lower in the patient group. When the groups were compared in terms of latency values, a statistical difference was found between the left median, right median, ulnar and sural nerves (p = 0.020, p = 0.025, p <0.001, p <0.001, respectively). Latency values were longer in the patient group. When the motor nerve findings were compared between the groups, no statistical difference was found in terms of amplitude values, nerve conduction velocities and latency.

Discussion

Nowadays, anti-TNF-α drugs are widely used in the treatment of autoimmune inflammatory diseases. It has been demonstrated that these anti-TNF-α drugs have a faster effect in reducing disease activity and the capacity to retard radiographic progression compared DMARD. Anti-TNF-α drugs in rheumatic diseases have rarely been reported to have neurological side effects. Peripheral neurological side effect is one of these and may cause drug discontinuation [14, 15]. Tektonidou et al., reported peripheral neuropathy in the form of mono neuritis multiplex or axonal sensorial polyneuropathy in two RA patients during infliximab therapy. Peripheral neuropathy has been described in RA either in vasculitis or as a side effect from medications and comorbid conditions. RA was on remission when peripheral neuropathy developed, and there were no risk factors associated with the development of rheumatoid vasculitis. Infliximab therapy, conduction block, and multifocal motor neuropathy, as well as the discontinuation of infliximab therapy, have been associated with axonal sensory polyneuropathy that returns with intravenous gamma globulin therapy [16]. In a French survey study, Seror et al., reported demyelinating findings in 33 patients receiving anti-TNF-α therapy. As a result of the study, they stated that peripheral neurological demyelinating complications might occur during the anti-TNF-α treatment [17]. Makol et al., presented a rheumatoid arthritis patient who received treatment with adalimumab and who had symptoms of mononeuritis multiplex as a case report and stated that mononeuritis multiplex and adalimumab therapy might be related [18].

In the present study, it was investigated whether there was any effect on the peripheral nervous system in patients using TNF-α inhibitors. TNF-α a has many effects on neurons [19]. It prevents the increase of Reactive Oxygen Species (ROS) in the cell. Thus, it prevents ROS from being toxic to neurons. Anti-TNF-α drugs can cause ROS increase and It has been reported that this situation may cause neuronal toxicity [20]. Studies have reported polyneuropathies in which both motor and sensory nerves are affected. Reports of only sensory polyneuropathy are limited in patients using anti-TNF-α [11]. In many studies, the results are controversial. In the presented study, it was observed that sensory nerves were affected in patients using anti-TNF- α drugs, but motor nerves were within normal limits. In the peripheral sensory nerve examination, in the patient group, longer latency values, lower amplitude values, and slower conduction velocity were measured. Motor nerve conduction velocity examination was evaluated within normal limits. It has been observed that sensory polyneuropathy occurs as a result of the use of anti-TNF-α drugs. In present study, pathological conditions in peripheral nerves were investigated primarily in patients using anti-TNF-α drug. Results suggesting that peripheral sensory nerves are affected.

This study has some limitations. A small sample size is the major limitation of the current research.

Taken together, although it is concluded that the only peripheral sensory involvement mentioned in the literature is rare and may occur under the influence of multiple factors [18], there is a need for a large number of multicenter studies involving a large number of patients.

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