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Effect of α-lipoic acid and N-acetylcysteine on liver oxidative stress, preneoplastic lesions induced by diethylnitrosamine plus high-fat diet

α-Lipoik asit ve N-asetilsisteinin sıçan karaciğerinde dietilnitrozamin ve yüksek yağlı diyetin neden olduğu oksidatif stres ve preneoplastik lezyonlar üzerine etkisi

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Abstract

Aim: Oxidative stress and inflammation are important for development of nonalcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (HCC). High fat diet (HFD) acts as promoter and induces cancer formation by diethylnitrosamine (DEN)-initiated carcinogenesis. DEN+HFD experimental model may be suitable to investigate the relationship between diet, cirrhosis, and cancer.

Methods: Rats were injected with DEN (50 mg/kg/once a week; i.p.) for 4 weeks. After 15 days, rats received HFD with/without supplementations of α-lipoic acid (ALA; 2 g/kg chow), N-acetylcysteine (NAC; 1% w/v drinking water) and their combination for 12 weeks.

Results: DEN+HFD-treatment resulted in increase of serum hepatic damage markers, hepatic oxidative stress parameters (lipid/protein oxidation products) and fibrotic changes. However, no HCC nodule was detected. Hepatic GST-pi and Ki-67 expressions also increased. Accordingly, DEN+HFD-treatment resulted in precancerous lesions and high rate of proliferation in the liver. NAC supplementation decreased hepatic oxidative stress and formation of fibrotic and preneoplastic lesions of DEN+HFD-treated rats. However, ALA supplementation did not have a curative effect on these lesions. No synergistic effect was seen with co-administration of ALA and NAC.

Conclusion: According to present results NAC, acting as an antioxidant, has ameliorating effect on DEN+HFD-induced oxidative stress and the formation of preneoplastic lesions in liver.

Keywords: High fat diet, diethylnitrosamine, oxidative stress, α-lipoic acid, N-acetylcysteine, liver injury.

Öz

Amaç: Oksidatif stres ve inflamasyon steatohepatit (NASH), siroz ve hepatoselüler karsinom (HCC) gelişimi için önemlidir. Yüksek yağlı diyet (HFD) promotör görevi görür ve dietilnitrosamin (DEN) ile başlatılan karsinogenez modelinde kanser oluşumunu indükler. DEN + HFD uygulaması diyet, siroz ve kanser arasındaki ilişkiyi araştırmak için uygun bir deneysel model olabilir.

Yöntemler: Sıçanlara 4 hafta boyunca DEN (haftada bir kez 50 mg / kg / i.p.) enjekte edildi. 15 gün sonra, sıçanlara HFD tek başına veya α -lipoik asit (ALA; 2 g / kg yem), N-asetilsistein (NAC; içme suyunda % 1 w/v) takviyeleri 12 hafta süreyle verildi.

Bulgular DEN + HFD uygulaması serum hepatik hasar belirteçleri ve hepatik oksidatif stres parametrelerinin (lipit/protein oksidasyon ürünleri) armasına ve fibrotik değişikliklere neden oldu. Ancak, HCC nodülü tespit edilmedi. Hepatik GST-pi ve Ki-67 ekspresyonları de artmış olarak bulunduğundan dolayı, DEN + HFD uygulaması, prekanseröz lezyonlara ve karaciğerde yüksek proliferasyon oranlarına neden oldu. NAC takviyesi, DEN + HFD ile muamele edilen sıçanların karaciğerinde oksidatif stresi ve fibrotik ve preneoplastik lezyonların oluşumunu azalttı. Bununla birlikte, ALA takviyesinin bu lezyonlar üzerinde iyileştirici bir etkisi olmamıştır. ALA ve NAC'nin birlikte uygulanmasıyla hiçbir sinerjistik etki görülmedi.

Sonuç: Sonuçlarımıza göre, bir antioksidan olan NAC, karaciğerde DEN + HFD'nin neden olduğu artmış oksidatif stresi azaltmada ve preneoplastik lezyonların oluşumu üzerine iyileştirici etkiye sahiptir.

Anahtar Kelimeler: yüksek yağlı diyet, dietilnitrozamin, oksidatif stres, α-lipoik asid, N-asetilsistein, karaciğer hasarı

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is frequent disorder linked with variety of conditions including simple steatosis (non-alcoholic steatohepatitis, NASH), fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Several hypotheses have recommended to elucidate the pathogenesis of advancement of steatosis to serious liver pathologies. The ''two hit'' hypothesis is the most common one. According to this, the ''first hit'' is simple steatosis (initiation phase) which sensitize liver to secondary injuries to promote its advancement to NASH. Oxidative stress, following lipid peroxidation and varios cytokines are recommended to be the ''second hit'' and conduce to the progress of NASH to several serious liver pathologies [1, 2].

Diethylnitrosamine (DEN) is an environmental hepatocarcinogen and is frequently used to produce experimental liver cancer [3]. In fequently applied models, initiation and promotion phases are essential in HCC generation. DEN initiation is mostly followed by phenobarbital, carbon tetrachloride (CCl_4), 2-acetylaminofluorine and partial hepatectomy promotion [3,4]. High fat diet (HFD) may also have a promoter effect and induce cancer formation by DEN-initiated carcinogenesis in rats [5]. HFD is an animal model of NAFLD/NASH [6] and causes steatosis, changes in prooxidant-antioxidant balance and inflammation in the liver [7, 8]. Indeed, HFD-induced NASH was found to promote DEN-initiated hepatocarcinogenesis by increasing oxidative stress and inflammation [9-11]. So that, this animal model was used to investigate the pathophysiological mechanisms in NASH-promoted liver cirrhosis and carcinogenesis and to test reducing or preventing potential of some antioxidants against these changes in the liver [9-11].

α-Lipoic acid (ALA) and N-acetylcysteine (NAC) are sulfur-containing antioxidants. As an antioxidant ALA is competent to scavenge reactive oxygen species (ROS), regenerate various antioxidants such as glutathione (GSH), and vitamins E, C, and possess metal chelating activity [12]. Additionally, ALA treatment was noticed to be protective in oxidative stress-induced pathologies including liver damage [12-14]. On the other hand, ALA was found to suppress tumor growth in mice treated with Ehrlich carcinoma cells and improve prooxidant-antioxidant balance in the liver [15]. It has also been reported that ALA shows an anti-carcinogenic effect by suppressing HCC that develops because of DEN and thioacetamide administration [16]. Contrarily, ALA administration was detected to aggravate liver damage and stimulate the development of preneoplastic lesions in DEN-initiated and choline-methionine-deficient (MCD) diet promoted HCC model [17].

Like ALA, NAC is an antioxidant compound with free radical scavenging properties and activating enzymes responsible for the regeneration of GSH, leads to increased intracellular GSH levels [18]. NAC was found to be an alleviating compound in oxidative stress-induced conditions including liver disorders [19, 20]. In addition, there are a few studies demonstrating the antineoplastic effect of NAC [21-23].

In the light of this information, we wanted to investigate whether the supplementation of ALA and NAC and their combination have a protective role against the development of liver lesions in DEN plus HFD-treated rats.

Material and methods

DEN, ALA, NAC used chemicals were purchased from Sigma-Aldrich (USA).

Experimental design

Approval for the experimental procedures used in this study was obtained from Bezmialem Vakif University Animal Experiments Local Ethics Committee (No: 2014/170). Male Sprague Dawley rats weighing 200-220 g were haused in stainless cages at 22°C on daily 12/12-hour light/darkness cycles and supplied with food (standard diet) and water ad libitum. Standard and HFD were obtained from Barbaros Denizeri A.Ş. (Kocaeli-Turkey). HFD included 34.3% fat (31% bovine oil, 3.4% corn oil), 27.3% carbohydrate, 23.5% protein, salt mixture and vitamins.

Rats were fed with standard diet and injected with DEN (50 mg/kg, once a week for a total of 4 doses) intraperitoneally. 15 days after the fourth injection of DEN, rats were divided into four groups as follows and started to receive HFD with and without antioxidants for 12 weeks. In addition, a control group was performed as the fifth group. No food and water restriction were performed in experimental period and total food and water intake were recorded.

1) DEN+HFD group (n=7): Rats were fed with HFD for 12 weeks. This group was used as model group.
2) DEN+HFD+ALA group (n=7): Rats were fed with HFD containing ALA (2 g/kg) for 12 weeks. The consumption of ALA was roughly equivalent to 100 mg/kg/day.
3) DEN+HFD+NAC group (n=6): Rats were fed with HFD and received NAC (1%; w/v) in drinking water for 12 weeks. The consumption of NAC was rougly equivalent to 500 mg/kg/day.
4) DEN+HFD+ALA+NAC group (n =7): Rats were fed with HFD containing ALA (2 g/kg) and received NAC (1%; w/v) in drinking water for 12 weeks.
5) Control group (n=6): Rats were injected with % 0.9 NaCl (once a week for a total of 4 doses) and fed on normal commercial chow during experimental period.

In our study, HFD composition [24] and the used doses of DEN [25], ALA [13,14] and NAC [19,20] were selected with respect to previous studies.

Blood and tissue samples

Following an overnight fast, rats were sacrificed by cardiac puncture taking their blood under sodium pentobarbital (50 mg/ kg, i.p.) anesthesia into dry tubes. Blood samples were centrifuged at 1500 x g for 10 min to obtain serum samples. Liver tissues were removed, washed in ice-cold saline and homogenized in 0.15 M KCl. A portion of homogenates was centrifuged (600xg/10 min/4°C) to remove unhomogenized tissue residues and nuclear portion. Postnuclear supernatants (PNS) were used for biochemical analyses. From another portion of supernatants (centrifuged at 10000g/20 min) postmitochondrial fractions (PMF) were obtained in which superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were measured. The materials were stored at -80 °C until analyses.

Determinations in serum

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)

activities and glucose, cholesterol, triglyceride and albumin levels were measured with autoanalyzer (Cobas Integra 800, Roche Diagnostics, Mannheim, Germany).

Determinations in the liver

Reactive oxygen species (ROS) levels (relative fluorescence units, RFU)

Modified fluorometric assay was used to test ROS generation [26]. After incubation of PNS samples with 2',7'-dichlorodihydrofluorescein diacetate (37°C/30 min) the fluorescence of the formed profuct was assayed by fluorometer (Fluoroskan Ascent FL, Thermo Scientific Inc, USA) ($\lambda_{excitation}$: 485 nm and $\lambda_{emission}$: 538 nm).

Malondialdehyde (MDA) levels (pmol/mg protein)

Lipid peroxidation in PNS was determined by measuring the levels of MDA [27]. The formed product 1,1,3,3-tetraethoxypropane was used as a standard.

Advanced oxidation protein products (AOPP) levels (nmol chloramine-T /mg protein)

Measurements of AOPP were detected spectophotometrically and calibrated with chloramine-T [28].

Advanced glycation end products (AGEs, relative fluorescence units, RFU)

Serum samples were diluted (1:50) with phosphate-buffered saline (PBS, pH 7.4), and fluorescence intensity was measured ($\lambda_{emission}$: 440 nm; $\lambda_{excitation}$: 350 nm) [29].

Ferric reducing antioxidant power (FRAP, nmol/mg protein)

FRAP assay [30] was used to determine the antioxidant power in PNS samples. At low pH, the formation of ferrous-tripyridyltriazine complex was monitored at 593 nm.

Glutathione (GSH) levels (nmol/mg protein)

GSH levels were detected spectophotometrically with 5,5'-dithiobis-2-nitrobenzoate (DTNB) at 412 nm [31].

Superoxide dismutase (SOD) activity (U/mg protein)

The SOD activity was determined by its ability to increase the riboflavin-sensitized photooxidation of o-dianisidine [32].

Catalase (CAT) activity (μmol/min/mg protein)

CAT activity was determined spectrophotometrically at 240 nm using hydrogen peroxide (H_2O_2) as substrate [33]. One unit of CAT was considered the activity of enzyme needed to degrade 1 μmol H_2O_2 per min at 25 °C.

Glutathione peroxidase (GSH-Px) activity (nmol/min/mg protein)

GSH-Px activity was assessed using cumene hydroperoxide as a substrate [34]. Extinction coefficient of NADPH ($6.22x10^3\ M^{-1}cm^{-1}$) was used for calculaton of results.

Glutathione transferase (GST) activity (nmol/min/mg protein)

GST activity was tested using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate [35].

Extinction coefficient ($9600\ M^{-1}cm^{-1}$) of the formed conjugation product (CDNB+GSH) was used for calculaton of results.

Protein levels

Protein levels were detected by bicinchoninic acid [36].

Immunohistochemical analysis for GST-Pi and Ki-67

GST-Pi and Ki-67 expressions were analyzed as markers of preneoplastic lesions and proliferation, respectively. All specimens were prefixed in 10 % buffered formalin.

Slides were rehydrated by graded ethyl alcohol, immersed in citrate buffer (Citrate Buffer, Thermo Scientific, Germany) and put in a microwave oven (20 min). Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Primary antibodies of GST-Pi (#311-H; Anti-GST-P rabbit polyclonal antibody, MBL, Nagoya, Japan) and Ki-67 (#PRM-325AA, rat monoclonal antibody, Biocare, USA) were done and incubated for 60 min. Then, biotinylated second antibody (goat anti-rabbit IgG; Santa Cruz Biotechnology, Heidelberg, Germany), streptavidin peroxidase, and substrate-chromogen (AEC) solution were done, respectively. Hematoxylin was used for nuclear staining. Staining intensity for GST-Pi was assigned as a percentage and given a score in the range from (+) to (+++): 5–30% (+), 30–60% (++), and >60% (+++). Nuclear brown staining was considered as positive for Ki-67 and staining intensity was defined as a percentage. Negative and positive cells numbers were counted on Leica DM 6000 Digital microscope. For each sample, "percent expression" was calculated formula: positive cells/ total number of counted cells x100.

Histopathological examination

Liver tissues were prefixed in 10% buffered formalin intombed in paraffin, splited, and stained with hematoxylin and eosin (H&E). Fibrosis was evaluated by using H&E staining considering Ishak's stage [37].

Statistical analysis

The results were expressed as mean ± SD. One-way analysis of variance (ANOVA) with Tukey's honestly significant differece post-hoc test, Kruskal–Wallis test with post-hoc Mann–Whitney U test were used. Difference was considered significant when $p < 0.05$.

Results

Effects of ALA and NAC and their combination (ALA+NAC) on body and liver weights and liver index in DEN+HFD-treated rats

Body and liver weights and liver index at the end of experiment remained unchanged in DEN+HFD-treated rats compared to control values. Similarly, NAC and ALA+NAC treatments did not affect these parameters in DEN+HFD rats. Only, decreased final body weight and increased liver index were detected in DEN+HFD-treated rats due to ALA supplementation (Table 1).

Effects of ALA and NAC and their combination (ALA+NAC) on biochemical parameters in serum of DEN+HFD-treated rats

Serum glucose, triglyceride and albumin levels did not alter in DEN+HFD group as compared to controls, but serum cholesterol levels increased (Table 1).

Serum ALT, AST and LDH activities increased significantly in DEN+HFD group as compared to controls. There were no changes in serum ALT and AST activities due to ALA, NAC and ALA+NAC supplementations in DEN+HFD-rats. However, these supplementations were detected to significantly decrease serum LDH activities in DEN+HFD-rats (Figure 1).

Effects of ALA, NAC and their combination (ALA+NAC) on hepatic histopathology of DEN+HFD-treated rats

Extensive nodular disarrangement surrounded with thin fibrotic tissue was observed in DEN+HFD group. Few inflammatory cell infiltrations were also seen in some areas (Figure 2A). ALA supplementation caused decreases in nodular structures as compared to DEN+HFD group. These structures showed the tendency to interconnect with each other and there

were thin fibrous bands around them (Figure 2B). It was seen that the number of nodular structures in the liver decreased with NAC application and they occupied less area. Fibrous bands and inflammatory response were not observed (Figure 2C). ALA+NAC administration showed similar changes to NAC administration in the liver, but nodules were higher than the NAC

Table 1. The effect of α-lipoic acid (ALA), N-acetylcysteine (NAC) and their combination (ALA+NAC) on final body weight, liver weight, liver index as well as some biochemical parameters in serum of diethylnitrosamine plus high fat diet (DEN+HFD)-treated rats (Mean±SD).

Variable	Control (n=6)	DEN+HFD (n=7)	DEN+HFD +ALA (n=7)	DEN+HFD +NAC (n=6)	DEN+HFD +ALA+NAC (n=7)
Final body weight (g)	310.8±24.4	262.8±60.0	247.1±34.3 [a]	286.3±35.2	283.7±21.5
Liver weight (g)	7.40±0.83	6.41±1.15	7.40±1.40	7.08±0.65	7.95±0.92
Liver index* (%)	2.37±0.14	2.48±0.33	2.97±0.28 [a,b]	2.48±0.22	2.79±0.16
Glucose (mmol/L)	8.83±1.66	11.1±2.36	9.71±1.32	11.3±1.38[a]	10.5±1.98
Cholesterol (mmol/L)	2.45±0.37	3.31±0.39[a]	2.93±0.55	2.87±0.21[a]	3.22±0.45[a]
Triglyceride (mmol/L)	0.72±0.11	0.89±0.27	0.77±0.33	0.99±0.36	0.98±0.30
Albumin (g/dL)	3.96±0.20	3.74±0.63	3.85±0.36	3.77±0.13	3.94±0.21

[a] $p < 0.05$ as compared to controls; [b] $p<0.05$ as compared to DEN+HFD.
*Liver index= Liver weight x 100 / body weight.

group and few fibrous bands and inflammatory changes were observed (Figure 2D).

Effects of ALA, NAC and their combination (ALA+NAC) on hepatic GST-pi expression of DEN+HFD-treated rats

GST-Pi expression was found to be (+++) in DEN+HFD group. This expression did not alter due to ALA supplementation. However, NAC and NAC+ALA supplementations caused (+) and (++) staining, respectively, in DEN+HFD-rats (Figure 3 A-D).

Effects of ALA and NAC and their combination (ALA+NAC) on hepatic Ki-67 expression in DEN+HFD-treated rats

Ki-67 expression in DEN + HFD group was 4%. This expression was determined as 4%, 2% and 2-3%, respectively, due to ALA, NAC and ALA + NAC supplementations in DEN+HFD rats (Figure 4 A-D).

Effects of ALA and NAC and their combination (ALA+NAC) on hepatic oxidative stress parameters in DEN+HFD-treated rats

Hepatic MDA and AOPP (27.3% and 43.7%, respectively) levels increased significantly in DEN+HFD rats as compared to controls. ROS (21.2%) and AGE (17.7%) levels were also increased, but these increases were not statistically significant. ALA, NAC and ALA+NAC supplementations decreased high levels of MDA and AOPP in DEN+HFD group. However, these supplementations did not alter ROS and AGE levels in the liver of DEN+HFD rats (Figure 5).

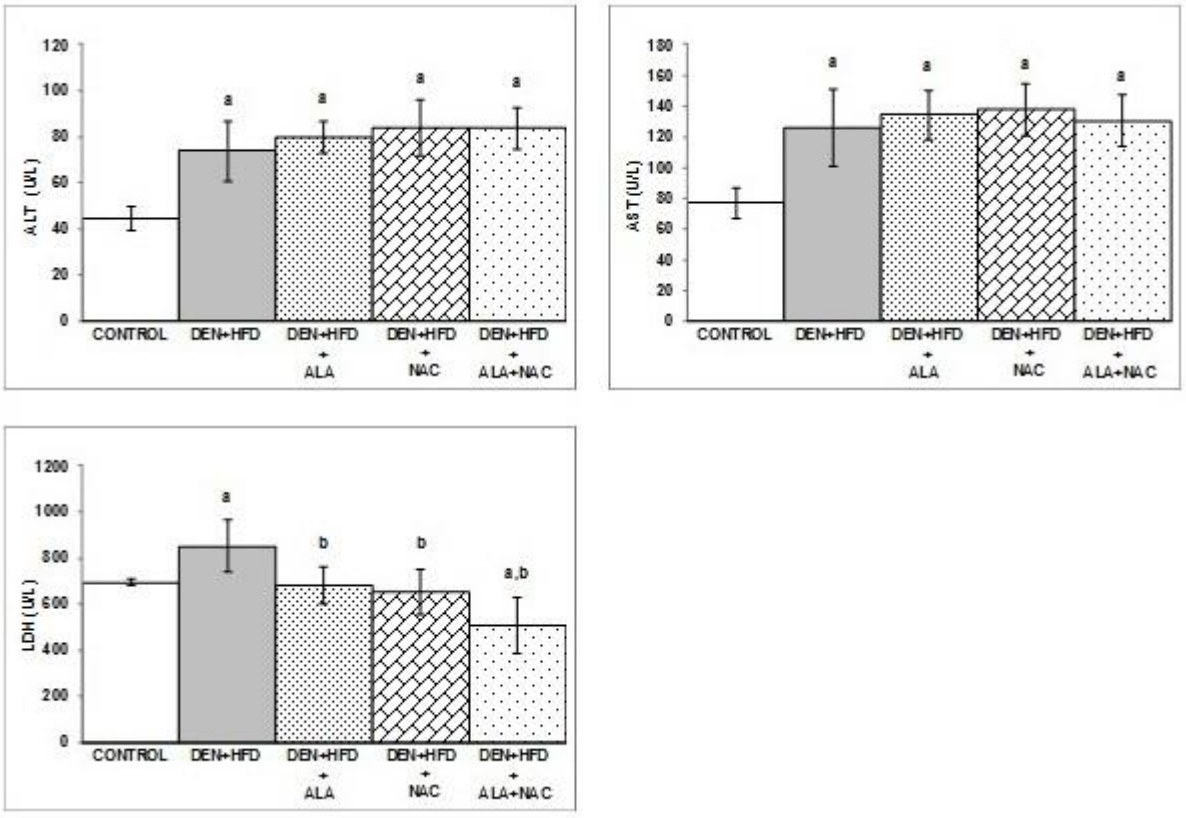

Figure 1. The effect of α-lipoic acid (ALA), N-acetylcysteine (NAC) and their combination (ALA+NAC) on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities of diethylnitrosamine plus high fat diet (DEN+HFD)-treated rats (Mean±SD). [a] $p < 0.05$ as compared to controls. [b] $p<0.05$ as compared to DEN+HFD.

Table 2. The effect of α-lipoic acid (ALA), N-acetylcysteine (NAC) and their combination (ALA+NAC) on ferric reducing antioxidant power (FRAP) and glutathione (GSH) levels, and superoxide dismutase (SOD), catalase (CA), glutathione peroxidase (GSH-Px) and glutathione transferase (GST) activities in the liver of diethylnitrosamine plus high fat diet (DEN+HFD)-treated rats (Mean±SD).

Variable	Control (n=6)	DEN+HFD (n=7)	DEN+HFD +ALA (n= 7)	DEN+HFD +NAC (n=6)	DEN+HFD+ALA +NAC (n=7)
FRAP (nmol/mg protein)	54.0±14.1	52.2±6.72	47.9±7.34	48.2±5.86	45.6±3.37
GSH (nmol/mg protein)	27.1±3.57	38.2±9.68	48.0±13.0[a]	35.1±9.50	38.1±8.13
SOD (U/mg protein)	13.6±2.05	17.9±4.35	15.1±1.53	11.9±2.2[b]	11.4±1.75[b]
CAT (µmol/min/mg protein)	277.5±48.1	256.5±100.1	330.4±47.2	301.2±72.4	321.5±59.6
GSH-Px (nmol/min/ mg/protein)	564.1±87.0	665.2±172.5	816.3±94.1[a,b]	719.3±77.4[a]	671.0±75.3[a]
GST (nmol/min/ mg/protein)	531.9±121.0	734.1±147.9[a]	756.1±74.8[a]	617.0±68.0	554.2±86.6[b]

[a] $p< 0.05$ as compared to controls.
[b] $p<0.05$ as compared to DEN+HFD.

Hepatic GSH (31.3%) levels, and SOD (22.6%) and GSH-Px (17.9%) activities were found to increase in DEN+HFD rats in comparation with controls, but these increases were not significantly. FRAP levels and CAT activities remained unchanged, but GST activity (38.0%) increased significantly in DEN+HFD group. Although ALA treatment did not alter antioxidant parameters in DEN+HFD group, only GSH-Px (22.7%) activity increased significantly. Similarly, no changes in antioxidant parameters were observed, excluding SOD activity, in DEN+HFD rats due to NAC supplementation. ALA+NAC supplementation significantly decreased SOD (36.3%) and GST (24.5%) activities in DEN+HFD rats (Table 2).

Discussion

NASH is the progressive form of NAFLD and features of NASH on liver biopsy include steatosis, inflammation and varying degrees of fibrosis. Persistent fibrosis may lead to cirrhosis and HCC. Although fibrosis is reversible, advanced stages of cirrhosis are very difficult to reverse [1, 2, 6]. The molecular basis of HCC formation in fibrotic/cirrhotic livers is not elucidated yet. Oxidative stress and inflammation has important roles in pathogenesis of NASH [1, 2]. Since dietary animal models of NAFLD such as HFD or MCD-diet require a relatively long period to produce cirrhosis/ HCC [6], new models are needed to investigate the development of NASH-induced cirrhosis/ HCC.

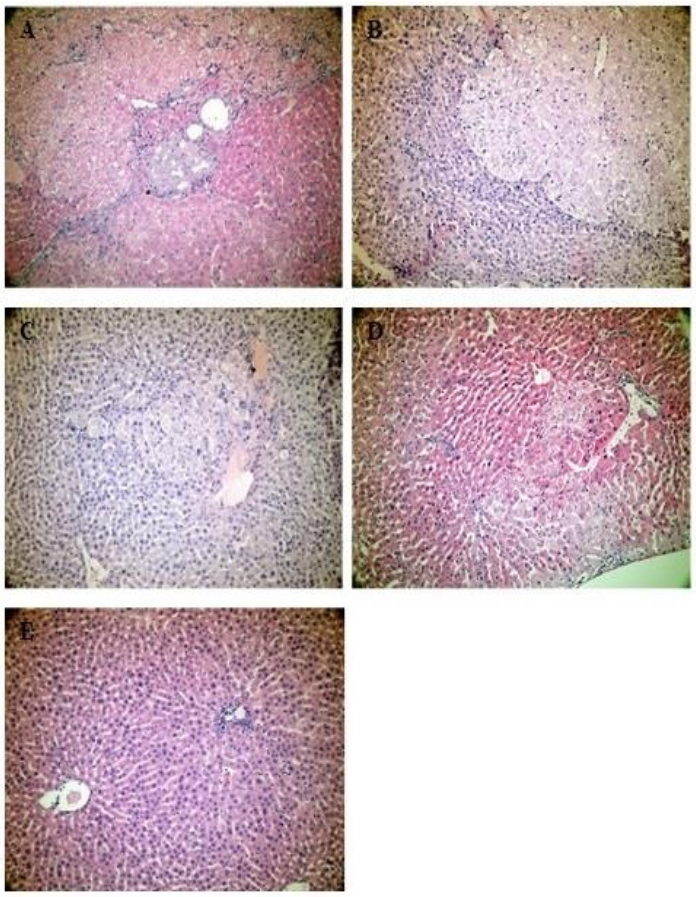

Figure 2. Histopathological appearance of liver in groups (H&Ex200). (A) Diethylnitrosamine plus high fat diet (DEN+HFD)-treated group. (B) α-lipoic acid (ALA)- supplemented DEN+HFD-treated rats. (C) N-Acetylcysteine (NAC)-supplemented DEN+HFD-treated rats. (D) ALA+NAC-supplemented DEN+HFD-treated rats. (E) Control group.

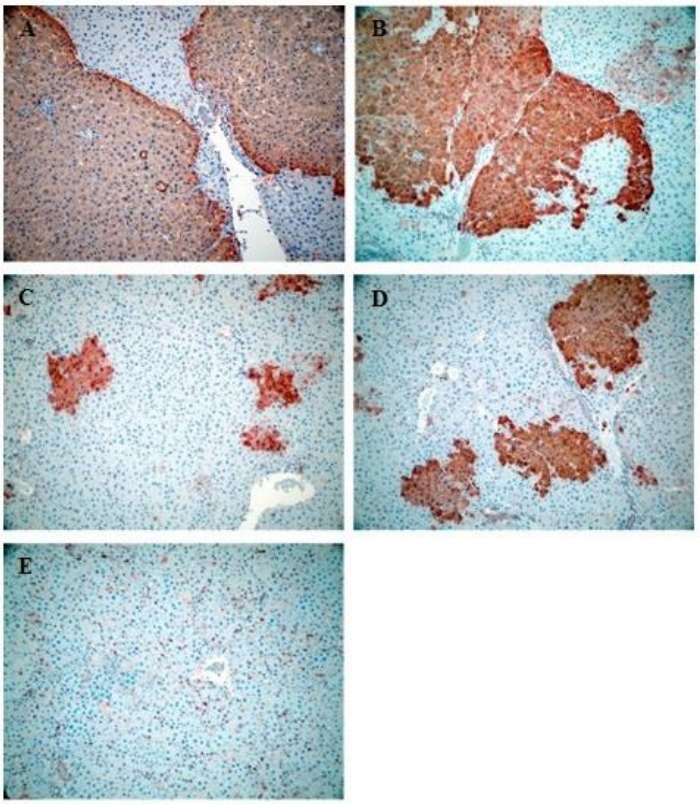

Figure 3. Immunohistochemical detection of hepatic glutathione transferase-Pi (GST-Pi) expression in groups (x200). (A) Diethylnitrosamine plus high fat diet (DEN+HFD)-treated group. (B) α-lipoic acid (ALA)- supplemented DEN+HFD-treated rats. (C) N-Acetylcysteine (NAC)-supplemented DEN+HFD-treated rats. (D) ALA+NAC-supplemented DEN+HFD-treated rats. (E) Control group.

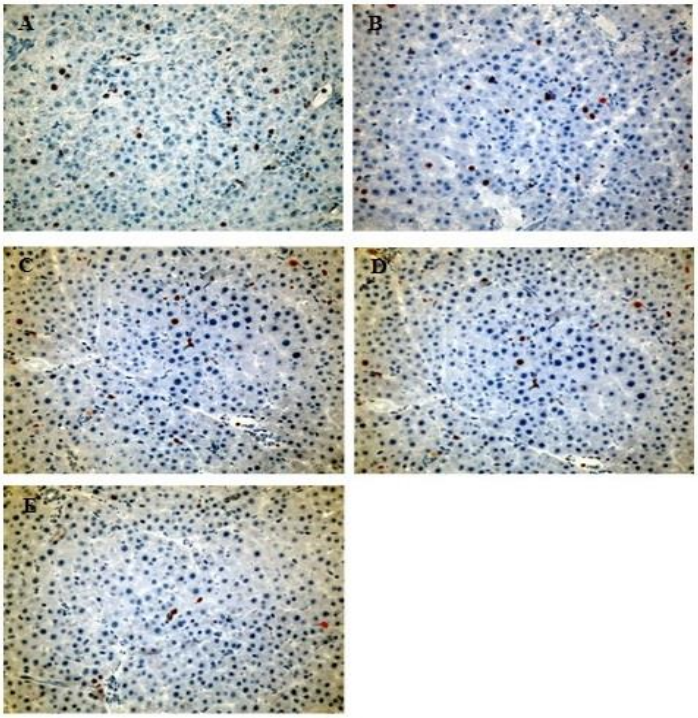

Figure 4. Immunohistochemical detection of hepatic Ki-67 expression in groups (x400). (A) Diethylnitrosamine plus high fat diet (DEN+HFD)-treated group. (B) α-lipoic acid (ALA)- supplemented DEN+HFD-treated rats. (C) N-Acetylcysteine (NAC)-supplemented DEN+HFD-treated rats. (D) ALA+NAC-supplemented DEN+HFD-treated rats. (E) Control group.

DEN is important hepatotoxin and hepatocarcinogen. DEN leads to hepatocellular necrosis and increased cell proliferation [3, 4, 38]. DEN-induced hepatic injury was observed to be related to increased ROS generation. DEN following ROS generation is related to biotransformation in cytochrome P450 system (CYPs), especially CYP2E1 [3, 39]. Meanwhyle, DEN changes the DNA structure and forms alkyl DNA adducts in the rat liver [3, 4, 38].

DEN has been used to initiate the liver cancer either alone or in combination with promoters. The initiation can be brought either by administering a single necrogenic dose of DEN or by administering a lower dose of DEN in combination with promoters [3, 4, 38]. On the other hand, previous studies in experimental animals have shown that repeated subnecrogenic doses of DEN without promoters cause progressive liver fibrosis and cirrhosis followed by HCC [40, 41].

HFD has been reported to have a promoter effect on DEN-induced hepatocarcinogenesis [5]. Bioactivation of DEN is controlled by CYP2E1. Increase in CYP2E1 activity is a probable risk factor in the progression of hepatofibrosis to hepatocarcinogenesis [39]. Since HFD elevates hepatic CYP2E1 activity [42], it may increase NAFLD progression and carcinogenesis [39]. According to this, HFD combined DEN exposure may be a suitable model to investigate the relationship between diet and liver cirrhosis and cancer formation.

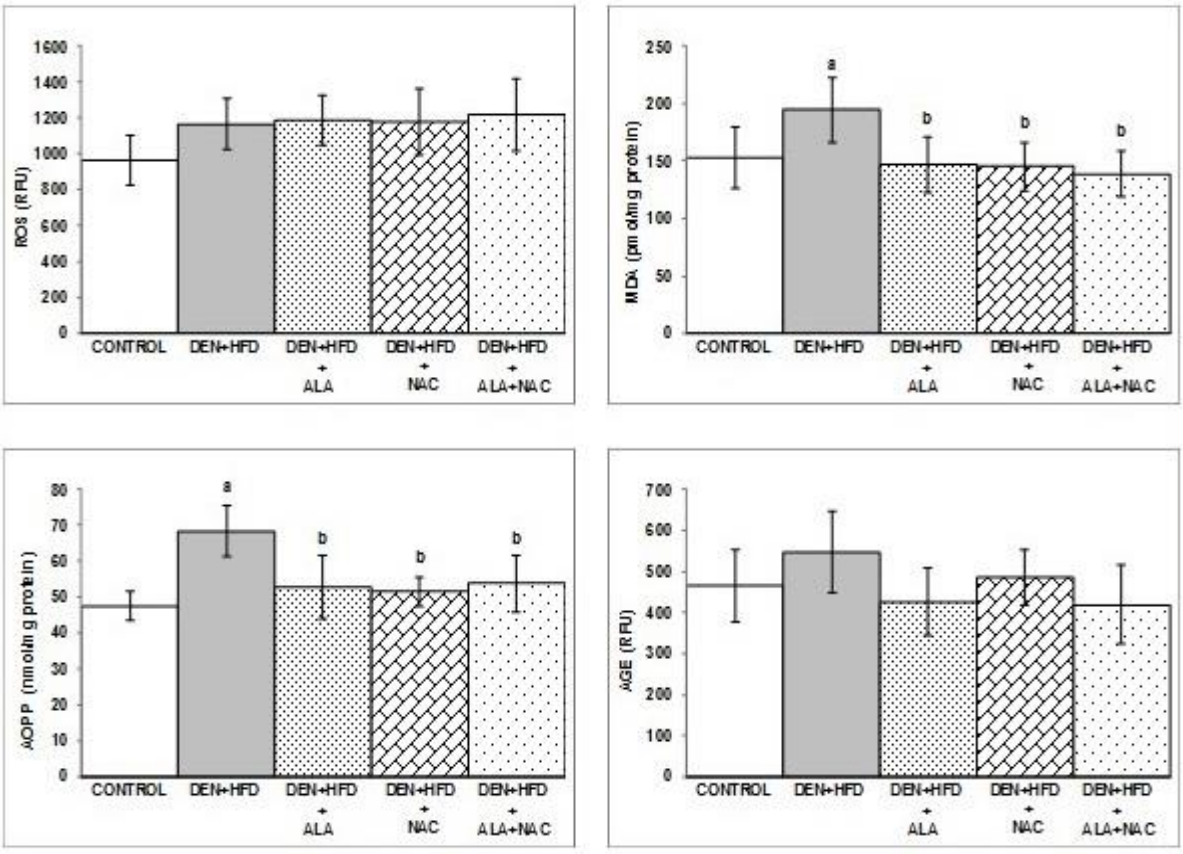

Figure 5. The effect of α-lipoic acid (ALA), N-acetylcysteine (NAC) and their combination (ALA+NAC) on reactive oxygen species (ROS) formation, malondialdehyde (MDA), advanced oxidation protein products (AOPP) and advanced glycation end products (AGE) levels in the liver of diethylnitrosamine plus high fat diet (DEN+HFD)-treated rats (Mean±SD). [a] $p< 0.05$ as compared to controls. [b] $p<0.05$ as compared to DEN+HFD.

In studies using DEN+HFD model, the subnecrogenic dose of DEN was followed by HFD application with different composition and duration. However, liver damage resulting in fibrosis and finaly to HCC was found to be different depending on some factors such as DEN dosage, examination time, HFD composition and application period [8-10]. In the present study, rats were injected with DEN (50 mg/kg; i.p.) 4 times with an interval of one week, and then fed a HFD for 12 weeks. This DEN+HFD protocol elevated serum ALT, AST and LDH activities and caused fibrotic changes in the liver. Serum aminotransferases such as ALT and AST are sensitive markers of hepatocellular injury. Increases in ALT and AST activities are also well described in hepatic fibrosis progression. However, no HCC nodule was detected macroscopically and histopathologically. Moreover, hepatic GST-pi and Ki-67 expressions were observed to increase immunohistochemically as compared to controls. GST-pi is used as a marker of preneoplastic lesions [5]. However, Ki-67 is an indicator of cell proliferation [11]. Accordingly, our findings indicate that DEN+HFD treatment caused mild fibrosis together with precancerous lesions and a high rate of proliferation in hepatocytes of rats.

Oxidative stress is very important in development and progression of liver cirrhosis and HCC [5, 21, 23, 40]. In the present study, DEN+HFD treatment increased MDA and AOPP levels. No significant changes in liver antioxidant parameters were found, but there was a tendency towards increases in GSH levels and SOD, GSH-Px activities of DEN+HFD rats. This situation may prevent further increases in prooxidant mileu in the liver. Our results agree with previous studies showing that DEN+HFD treatment produces a prooxidant state in the liver of rats [5, 11].

In our study, we aimed to investigate the effects of ALA and NAC and their combination on DEN-initiated HFD-promoted hepatic lesions. ALA is an antioxidant with protective effects against various hepatic injuries [13, 14]. ALA supplementation was reported to reduce lipid accumulation, inflammation, and oxidative stress in the liver of MCD- and HFD-fed mice [13]. Moreover, ALA administration alleviated CCl_4-induced liver cirrhosis in rats [14]. Although ALA administration was reported to have an anti-carcinogenic effect in DEN-initiated and thioacetamide-promoted HCC [16], it showed a pro-carcinogenic effect in DEN-initiated MCD-promoted HCC [17]. However, in the present study, ALA supplementation decreased nodular structures in the liver, but this treatment did not affect the formation of preneoplastic lesions in DEN+HFD rats.

NAC is also an antioxidant and antiinflammatory compound [18]. There are some studies showing that NAC attenuates oxidative stress and liver pathology in rats with NASH promoted by HFD [19, 20]. NAC treatment was also reported to supress hepatic GST-pi expression and oxidative stress in DEN-initiated and indole-3-carbinol-promoted hepatocarcinogenesis model [21]. Moreover, it was found that NAC supplementation improved DEN-induced HCC in toll-like receptor 2 [TLR2]-deficient mice by suppressing oxidative/endoplasmic stress [22]. In addition, NAC treatment improved liver function, hepatic ROS levels and DNA damage in mice with DEN-initiated and high cholesterol-promoted hepatocarcinogenesis [23]. In the current study, according to histopathological observations, decreases in number of nodular structures was detected in the liver due to NAC supplementation in DEN+HFD-treated rats. However, fibrous bands and inflammatory response were not seen. Moreover, NAC supplementation decreased GST-pi and Ki-67 expresions were in DEN+HFD treated rats. In contrast, the results of liver histology and GST-pi and Ki-67 expressions in ALA+NAC-treated group were worse than NAC- and better than ALA-supplemented rats.

On the other hand, there were no changes in serum ALT and AST activities in DEN+HFD-treated rats due to ALA, NAC and ALA+NAC supplementations. However, these antioxidants significantly decreased serum LDH activity in DEN+HFD-treated rats. In addition, the effects of these antioxidants on hepatic prooxidant-antioxidant balance were observed to be similar and decrease prooxidant status in the liver.

In conclusion, NAC treatment decreased hepatic oxidative stress and formation of fibrosis and preneoplastic lesions in DEN+HFD-treated rats. However, ALA supplementation was found not to have a curative effect on these lesions and the co-administration of NAC and ALA does not produce a synergistic effect in the liver of DEN+HFD-treated rats.

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The Effect of BCG vaccine on COVID-19 severity and mortality

BCG aşısının COVID-19 ağırlığına ve mortalitesine etkisi

Tayfun Çalışkan [1], Bengü Saylan [1]

Abstract

Aim: To investigate the effect of Bacille Calmette-Guérin (BCG) vaccine on the severity and mortality of the disease in hospitalized patients with COVID-19 in this study.

Methods: This study was conducted as a retrospective observational study in a tertiary reference hospital in Turkey. The patients were divided into two groups as BCG scar positive and negative according to the presence of BCG scar. BCG scar positive and negative groups were compared in terms of radiological involvement, oxygen desaturation, need for intensive care unit (ICU) and intubation, and mortality.

Results: BCG scar negative patients required more inpatient treatment (p = 0.001). The radiological involvement was more severe in BCG scar negative group (p = 0.016). Forty-one (22.7%) patients in the BCG scar positive group and twenty-two (42.3%) patients in the BCG scar negative group were treated in the ICU, and the difference was statistically significant (p = 0.007). More patients in the BCG scar negative group had oxygen desaturation than the BCG scar positive group (59.6% vs. 40.9%, p = 0.018). The BCG scar negative group needed more intubation (21.2% and 8.8%, p = 0.024). Nine (17.3%) patients in the BCG scar negative group and 12 (6.6%) patients in the BCG scar positive group died, and the difference was statistically significant (p = 0.027).

Conclusion: The length of hospital stay was longer, radiological involvement was more severe, the need for ICU and intubation, oxygen desaturation and mortality were higher in BCG-unvaccinated patients with COVID-19.

Keywords: BCG vaccine, COVID-19, SARS-CoV-2, pandemics, prognosis

Öz

Amaç: Hastanede yatan COVID-19 hastalarında Bacille Calmette-Guérin (BCG) aşısı yapılmış olmanın hastalığın ciddiyeti ve mortalitesi üzerine etkisini araştırmaktır.

Yöntemler: Bu çalışma, Türkiye'deki üçüncü basamak bir referans hastanesinde geriye dönük gözlemsel bir çalışma olarak gerçekleştirildi. Hastalar BCG skarının varlığına göre BCG skarı pozitif ve negatif olarak iki gruba ayrıldı. BCG skarı pozitif ve negatif gruplar radyolojik tutulum, oksijen desatürasyonu, yoğun bakım ünitesi (YBÜ) ihtiyacı ve entübasyon ve mortalite açısından karşılaştırıldı.

Bulgular: BCG skarı negatif hastaların daha uzun süre yatarak tedavi ihtiyacı oldu (p = 0,001). Radyolojik tutulum, BCG skarı negatif grupta daha ağırdı (p = 0,016). BCG skarı pozitif grupta 41 (% 22,7) hasta ve BCG skarı negatif grupta 22 (% 42,3) hasta YBÜ'de tedavi edildi ve aradaki fark istatistiksel olarak anlamlıydı (p = 0,007). BCG skarı negatif grupta BCG skarı pozitif gruba göre daha fazla hastada oksijen desatürasyonu vardı (% 59,6 ve % 40,9, p = 0,018). BCG skarı negatif grup daha fazla entübasyona ihtiyaç duydu (% 21,2 ve% 8,8, p = 0,024). BCG skarı negatif grupta 9 (% 17,3) hasta ve BCG skarı pozitif grupta 12 (% 6,6) hasta öldü ve aradaki fark istatistiksel olarak anlamlıydı (p = 0,027).

Sonuç: BCG ile aşılanmamış COVID-19 hastalarında hastanede kalış süresi daha uzun, radyolojik tutulum daha şiddetli, yoğun bakım ve entübasyon ihtiyacı, oksijen desatürasyonu ve mortalite daha yüksekti.

Anahtar Kelimeler: BCG aşısı, COVID-19, SARS-CoV-2, pandemi, prognoz

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Introduction

Bacille Calmette-Guérin (BCG) vaccine is a live attenuated vaccine that has been used widely to prevent tuberculosis disease for about 100 years. BCG's protection against pulmonary tuberculosis varies between 0 and 80%, and it primarily protects against severe forms of tuberculosis such as disseminated and meningitis tuberculosis in children [1]. World Health Organization (WHO) recommends BCG vaccination for countries with a high incidence of tuberculosis and therefore it is not included in the routine vaccination program in countries with low tuberculosis incidence [2]. Turkey has a universal BCG vaccination policy.

The coronavirus disease 2019 (COVID-19) pandemic still continues. Although there are a few vaccines that have been developed against COVID-19, these vaccines have not been approved yet. After the approval process of the vaccines, it is estimated that the production and distribution of the vaccines worldwide and the vaccination process can be completed in about approximately two years. BCG vaccine is protective against tuberculosis and the vaccine is also protective against different respiratory diseases, especially those of viral origin [3]. BCG induces trained immunity and has been proposed to be protective against COVID-19 [4]. There are studies reporting that the severity and mortality of COVID-19 disease was lower in countries with national BCG vaccination policy [5]. However, there are also studies concluding that BCG was not protective against COVID-19 [6,7]. WHO has stated that BCG vaccination for COVID-19 protection is not recommended due to the insufficient level of evidence yet [8]. Randomized controlled clinical studies are ongoing on this subject.

This study was conducted to investigate the effect of BCG vaccination in hospitalized patients with COVID-19 on the severity and mortality of the disease.

Material and methods

Study design, setting, participants and ethics

This study was a single center, retrospective observational study. Adult patients (> 18 years old) with COVID-19 hospitalized in Sultan 2. Abdulhamit Han Training and Research Hospital which is a tertiary center for COVID-19 in Istanbul, Turkey between April 15 and June 15, 2020 were included in the study. Ethics committee approval was obtained for the study. Patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR) positive in nasopharyngeal swap and those with SARS-CoV-2 PCR negative tests who were diagnosed with clinical, radiological, and laboratory findings were enrolled in the study. The data of the patients were analyzed retrospectively. The patients were divided into two groups as BCG scar positive and negative according to the presence of BCG scar. Oxygen desaturation was defined as the oxygen saturation (SpO2) in room air measured by pulse oximetry was <93%. Chest computed tomography scans were examined to evaluate the extent of lung involvement associated with COVID-19. According to radiological involvement scale, each of the 5 lobes was scored as; none (0%) 0 point, minimal (1-25%) 1 point, mild (26-50%) 2 points, moderate (51-75%) 3 points and severe (76-100%) 4 points [9]. Total radiological involvement score was obtained by summing the scores of 5 lobes (0-20 point). Those with a radiology score of 0 point were in stage 0, 1-5 points were in stage 1, 6-10 points were in stage 2, 11-15 points were in stage 3, and 16-20 points were in stage 4. BCG positive and negative groups were compared in terms of radiological involvement, presence of oxygen desaturation, need for intensive care unit (ICU) and intubation, and mortality.

Statistical analysis

The patient data collected in the study were analyzed with the IBM SPSS version 21.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) package program. Frequency and percentage were used for discrete data and mean ± standard deviation was given as descriptive values for continuous data. "Independent Sample t-test" was used to compare two groups between groups. Results were considered statistically significant when p value was less than 0.05.

Results

A total of 233 hospitalized patients, 133 males and 100 females, were included in the study (Table 1).

Table 1. Demographic characteristics of the patients.

		N (%) or mean ± SD
Gender	Male	133 (57.1)
	Female	100 (42.9)
Age (year)		51±19.3
Hospital stay (day)		9±7.0
BCG scar	Negative	52 (22.3)
	Positive	181 (77.7)
Radiological stage	0	28 (12.0)
	1	48 (20.6)
	2	94 (40.3)
	3	52 (22.3)
	4	11 (4.7)
Need for ICU	No	170 (73.0)
	Yes	63 (27.0)
Oxygen desaturation	No	128 (54.9)
	Yes	105 (45.1)
PCR	Negative	23 (9.9)
	Positive	210 (90.1)
Need for intubation	No	206 (88.4)
	Yes	27 (11.6)
Mortality	Survivor	212 (91.0)
	Non-survivor	21 (9.0)

SD: Standard deviation, BCG: Bacillus Calmette Guerin, ICU: Intensive care unit, PCR: Polymerase chain reaction.

The mean age of the patients was 51 ± 19.3 years. The average length of stay in the hospital was 9 ± 7.0 days. Sixty-three of the patients have been treated in ICU and 105 patients had oxygen desaturation. Twenty-seven patients were intubated and needed mechanical ventilator support, and 21 patients died. BCG scar was positive in 181 and negative in 52 of the patients. There was no statistically significant difference between the BCG scar positive and negative patient groups in terms of gender and PCR test results (Table 2, p = 0.341 and p = 0.305). The mean age of BCG scar positive patients was 47 ± 17.6 years and the mean age of BCG scar negative patients was 64 ± 19.2 years, and the difference between the two groups was statistically significant (p <0.0001). When BCG scar positive and negative patients were compared in terms of length of hospitalization, it was found that BCG scar negative patients required statistically significantly more inpatient treatment (12 ± 6.7 and 8 ± 6.9 days, p = 0.001). The radiological involvement due to COVID-19 was

more severe in BCG scar negative patients (p = 0.016). Forty-one (22.7%)

Table 2. The comparison between patients with and without BCG vaccine.

Variable	BCG scar Negative (N:52) N (%) or mean ± SD	BCG scar Positive (N:181) N (%) or mean ± SD	p
Gender			
Male	33 (63.5)	100 (55.2)	0.341
Female	19 (36.5)	81 (44.8)	0.317
Age (year)	64±19.2	47±17.6	<0.001
Hospital stay (day)	12±6.7	8±6.9	0.001
Radiological Stage			
0	2 (3.8)	26 (14.4)	
1	8 (15.4)	40 (22.1)	
2	19 (36.5)	75 (41.4)	0.016
3	19 (36.5)	33 (18.2)	
4	4 (7.7)	7 (3.9)	
Treated in ICU	22 (42.3)	41 (22.7)	0.007
Patients with oxygen desaturation	31 (59.6)	74 (40.9)	0.018
PCR positive	45 (86.5)	165 (91.2)	0.305
Intubated patients	11 (21.2)	16 (8.8)	0.024
Mortality			
Survivor	43 (82.7)	169 (93.4)	
Non-survivor	9 (17.3)	12 (6.6)	0.027

BCG: Bacillus Calmette Guerin, SD: Standard deviation, ICU: Intensive care unit, PCR: Polymerase chain reaction.

patients in the BCG scar positive group and twenty-two (42.3%) patients in the BCG scar negative group were treated in the ICU, and the difference in need for intensive care between the two groups was statistically significant (p = 0.007). More patients in the BCG scar negative group than the BCG scar positive group had oxygen desaturation (59.6% vs. 40.9%, p = 0.018). The number of intubated patients was 11 (21.2%) in the BCG scar negative group and 16 (8.8%) in the BCG scar positive group, and the difference was statistically significant (p = 0.024). Nine (17.3%) patients in the BCG scar negative group and 12 (6.6%) patients in the BCG scar positive group died, and the difference was statistically significant (p = 0.027)

Discussion

In this study, patients who were hospitalized with COVID-19 were divided into two groups according to BCG vaccine status, and the effect of BCG on the length of hospital stay, the severity and the mortality of the disease was investigated. Patients in BCG scar negative group had longer length of hospital stay, more severe radiological involvement and oxygen desaturation. The need for ICU and intubation and mortality were also higher for non-vaccinated patients.

BCG vaccine has been shown to be protective against novel viruses by affecting the maturation and production of native T cells that cause accelerated and long-acting innate immune responses [10]. There is a negative correlation between reported COVID-19 cases and BCG vaccination status [11]. Miller et al. investigated the relationship between COVID-19 mortality and BCG vaccination programme in different countries. COVID-19 mortality was higher in countries such as Italy, the Netherlands and the USA where no national BCG vaccination program was implemented, than in countries with universal BCG vaccination policy, and having a policy of universal BCG vaccination correlated with a reduction in mortality associated with COVID-19 [12]. COVID-19 mortality was 5.8 times lower in countries where BCG vaccine is currently applied than in countries not applied (95% CI, 1.8-19.0) [13]. Escobar et al. compared countries for deaths related to COVID-19 by dividing them into 3 different groups (no BCG vaccination, discontinued and currently applying) according to BCG vaccination status. It was determined that for every 10% increase in BCG index, there was a 10.4% decrease in deaths [14]. In studies investigating the relationship between BCG and COVID-19, its effect on the spread of the disease as well as its effect on mortality was investigated.

The mandatory BCG vaccination policy, after controlling for other confounding factors, had a significant impact on the rate of increase in both the number of COVID-19 cases and deaths, and a significant advantage of the universal BCG policy in reducing the spread of COVID-19 [15]. A study conducted in Turkey, compared COVID-19 cases and deaths related with COVID-19 in different countries according to the BCG vaccination status and countries with national BCG vaccination programme had lower case numbers and mortality rates (p <0.0001 and p <0.0058) [16]. A study from Israel showed a strong negative association between the ages of BCG administration and the spread and severity of COVID-19. This relationship was most prominent especially in younger (<25 years) population, and especially in recent vaccination [17]. According to a cohort study, COVID-19 patients with BCG vaccine needed less hospitalization during the course of the disease, and it has been suggested that BCG vaccination can prevent more severe COVID-19 [18]. The relationship between BCG vaccination and COVID-19 spread, severity and mortality has been investigated with mathematical modeling, and it has been observed that the rate of COVID-19 spread was lower, the hospital stay was shorter and the recovery rate was higher in those with BCG vaccination. The incidence of COVID-19, disease severity, and mortality were lower in countries where BCG has been used for a long time [19]. In this study, similar results to those in previous studies were obtained. In COVID-19 patients with BCG vaccination, length of hospital stay was shorter, disease severity was lower, oxygen desaturation, need for ICU and intubation, and mortality was lower.

There are also studies reporting that BCG is not protective against COVID-19. A study conducted with regression discontinuity method in Switzerland, which has stopped BCG vaccination in newborns since 1975, reported that BCG vaccination at birth did not have a protective effect against COVID-19 [20]. In another study, there was no relationship between universal BCG vaccination and COVID-19 mortality and the difficulties of obtaining reliable epidemiological results based on available data on the COVID-19 pandemic all over the world have been revealed [21]. There was no difference between the two groups (p = 0.9) in the study conducted in Israel comparing the rates of COVID-19 in BCG-vaccinated and non-vaccinated young people. BCG vaccine applied in childhood did not have a protective effect against COVID-19 in adulthood [22]. The relationship between BCG vaccination and COVID-19 pneumonia severity pneumonia was evaluated in a retrospective study in Turkey. 30 patients with BCG positive had mild disease and 61 patients had severe disease, and the difference was statistically significant (p = 0.026) in the study. Severe disease was more common in those who did not have BCG vaccines.

However, in binominal logistic regression analysis, BCG vaccination was not found to be associated with the severity of COVID-19 pneumonia [6]. Wassenaar et al. also concluded that there was no available evidence that BCG vaccination has a beneficial effect on the number or fatality of COVID-19 reported cases [7].

Ecological studies have suggested that countries where BCG vaccination is mandatory have fewer COVID-19 cases, lower disease severity and mortality. However, the results of these studies should be evaluated carefully. Variables such as demographic features, genetic variabilities, differences in the treatment of COVID-19, the number of diagnostic tests, and the epidemiological period of the disease may affect the results of these studies. BRACE (Phase III, Australia), BCG-CORONA (Phase III, Netherlands) and BADAS (Phase IV, USA) randomized controlled studies on the protection of BCG against COVID-19 are continuing. The results of these studies can provide the necessary evidence in this regard [23].

The most important limitation of this study was that it was designed retrospectively. In addition, the patients included in the study were not randomized. The efficacy of the BCG vaccine varies on populations due to different BCG strains applied in different countries, genetic and geographic characteristics and exposure to non-mycobacterial infections.

In conclusion, the length of hospital stay was longer, radiological involvement was more severe, the need for ICU and intubation, oxygen desaturation and mortality were higher in BCG-unvaccinated patients with COVID-19.

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Is aspartate aminotransferase to alanine aminotransferase ratio (De Ritis ratio) helpful in predicting breast cancer?

Aspartat aminotransferaz/alanin aminotransferaz oranı (De Ritis oranı) meme kanserini tahmin etmede yardımcı olur mu?

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Abstract

Aim: De Ritis ratio (aspartate transaminase/alanine transaminase) may be a useful prognostic biomarker for certain malignant tumors. However, the predictive value of the De Ritis ratio before treatment in preoperative staging in patients with breast cancer is unknown. This study aimed to evaluate the De Ritis ratio in benign and malignant breast diseases and investigate the predictive value of it for breast cancer.

Methods: Retrospective analysis was made of the clinicopathological data of 301 patients with benign breast disease and breast cancer treated between April 2017 and April 2020 in a single center. 64 Patients were excluded from the study due to chronic illness or incomplete data. The relationship between the De Ritis ratio and clinicopathological findings before treatment was evaluated in patients. The Mann Whitney U test and Kruskal Wallis test were used in the comparisons between groups.

Results: Of the total 237 patients, the number of patients with benign breast disease was 96 and the number of the patients with breast cancer was 141. No statistically significant results were determined between the benign breast disease and breast cancer groups, in respect of pre-treatment evaluation of the De Ritis ratio and as a predictive factor for preoperative staging in molecular subtyping, tumor diameter, lymph node metastasis, and Ki 67 index.

Conclusion: It was concluded that the De Ritis ratio examined before treatment was not an independent predictive factor in breast cancer diagnosis and staging.

Keywords: De ritis ratio, aspartate transaminase, alanine transaminase, breast cancer, predictive factor

Öz

Amaç: De Ritis oranı (aspartat transaminaz/alanin transaminaz) belirli malign tümörler için yararlı bir prognostik biyobelirteç olabilir. Bununla birlikte, meme kanseri olan hastalarda tedavi öncesi incelenen De Ritis oranının preop evrelemede prediktif değeri büyük ölçüde bilinmemektedir. Bu çalışmanın amacı, benign ve malign meme hastalıklarında De Ritis oranını değerlendirmek ve bunun meme kanseri için prediktif değerini araştırmaktır.

Yöntemler: Nisan 2017- Nisan 2020 yılları arasında benign meme hastalığı ve meme kanserli 301 hastanın klinikopatolojik verileri tek bir merkezde retrospektif olarak analiz edildi. Kronik hastalığı olan veya veri eksiği olan 64 hasta çalışmadan çıkarıldı. De Ritis oranının tedavi öncesi değeri ile, hastaların klinikopatolojik bulguları arasındaki ilişki değerlendirildi. Gruplar arası karşılaştırmalarda Mann Whitney U testi ve Kruskal Wallis testi kullanıldı.

Bulgular: 237 hastada, benign meme hastalığı olan 96, meme kanseri olan hasta sayısı ise 141 idi. De ritis oranın tedavi öncesi değeri ile, benign meme hastalığı ve meme kanseri arasında, ayrıca meme kanserinde prognostik faktör olan moleküler subtiplendirme, tümör çapı, lenf nodu metastazı ve Ki 67 indeksinde preoperatif evrelemede prediktif faktör olarak istatisksel anlamlı sonuçlara ulaşılamadı.

Sonuç: Tedavi öncesi incelenen De ritis oranın meme kanseri evrelemesinde bağımsız prediktif faktör olmadığı sonucuna ulaşıldı.

Anahtar kelimeler: De ritis oranı, aspartat transaminaz, alanin transaminaz, meme kanseri, prediktif faktör

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Introduction

Breast cancer is the most common malignancy in women and the second most frequent cause of cancer-related death [1]. Since most of the patients are diagnosed at an advanced stage, the response and prognosis of the disease are negatively affected. The effective treatment of breast cancer is mostly based on early diagnosis and a definitive treatment plan. Mammography is the most effective screening method for breast cancer diagnosis, but there are some limitations to this method, such as the high cost, radiation exposure, and the low number of experienced breast radiologists. Alternatively, many tumor markers have been developed, such as serum carcinoembryonic antigen (CEA) and carbohydrate antigen (CA15-3). Unfortunately, the use of these markers in the diagnosis of breast cancer is limited due to inadequate sensitivity and specificity [2, 3]. The development of cancer screening tests and cancer biomarkers with clinical utility would overcome these problems.

Aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) are liver enzymes commonly used in clinical laboratory tests. These enzymes released from liver cells into the blood are produced by both malignant and non-malignant cells. AST also acts in the brain, liver, gastric mucosa, adipose tissue, skeletal muscle, and kidneys, especially the liver and heart muscle. ALT is predominantly found in the liver, whereas in other tissues, a small amount can be detected [4, 5]. AST and ALT values are stable in normal healthy individuals but may be affected by many other non-tumor-related factors, including chronic hepatitis, coronary heart disease, impaired kidney function, and medications. As the numerical values are unstable when they act as single predictors, the combination of AST and ALT is more suitable as a composite parameter. The AST-ALT ratio, also called the De Ritis ratio, was originally defined by De Ritis in 1957 [6]. Although the De Ritis ratio was first used to identify viral hepatitis and some chronic liver diseases, it has been identified in recent studies as an independent prognostic factor for the prediction of disease stage and patient survival in some malignancies [7-9].

In the preoperative period, many laboratories and radiological tests are performed to determine the stage of cancer. Therefore, surgical planning and the neoadjuvant or adjuvant systemic treatment plan is decided with a multidisciplinary approach before the operation according to these test results. The De Ritis ratio is an inexpensive, simple and non-invasive test, which can be useful as a predictive factor in preoperative staging.

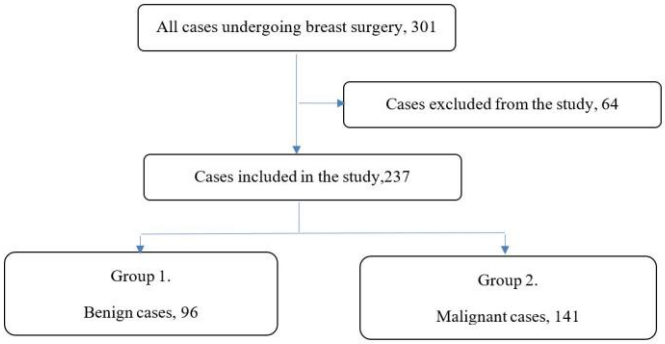
This study aimed to evaluate the De Ritis ratio in benign and malignant breast diseases and investigate the predictive value of it for breast cancer.

Material and methods

Clinical Research Ethics Committee approved the study. Approval date and number: 27.08.2020, 2020/20:360. Informed consent was not taken due to the retrospective nature of the study.

301 women who underwent surgery with a diagnosis of benign breast disease or breast cancer in the General Surgery Department between April 2017 and April 2020 were retrospectively evaluated. A total of 64 patients were excluded from the study for various reasons; 1) known chronic liver disease (eg, chronic viral hepatitis, non-alcoholic fatty liver disease, cirrhosis), 2) receiving neoadjuvant chemotherapy or stage 4 breast cancer 3), recurrent tumor, 4) other known malignancy, 5) incomplete or unavailable data (Figure 1). The remaining 237 women were divided into two groups as benign breast disease (Group 1) and breast cancer (Group 2) according to the histopathological results.

Figure 1: Patient selection.



Data related to age, liver function tests (AST, ALT) before surgery, and in Group 2 molecular subtypes (luminal A, luminal B, triple-negative, and HER2 +), diameter of the tumor, lymph node involvement, and Ki 67 index were retrieved from the hospital database. The De Ritis ratio was calculated by the simple proportioning method in the electronic system. Tumor staging was applied according to the American Joint Committee on Cancer (AJCC) 8th edition tumor, lymph grade metastasis, distant metastasis (TNM) staging system.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS vn 21 software. The conformity of the data to normal distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed parametric data were presented as mean ± standard deviation (SD) values and the significance of intergroup variance was analyzed using the Student's t-test. The Mann Whitney U and Kruskal Wallis tests were used in the analysis between the groups. A value of $p<0.05$ was considered statistically significant.

Results

Of the total 237 patients, Group 1 included 96 (40.50%) patients with a mean age of 52.07±10.83 years, and Group 2 included 141 (59.49%) patients with a mean age of 54.50 ±12.15 years. There was no statistical significance between groups according to the ages ($p = 0.095$). The AST, ALT values and De Ritis ratio were not statistically significant in Group 1 and Group 2 ($p = 0.058$, $p = 0.148$, $p = 0.908$, respectively) (Table 1).

Table 1. Comparison of the Impact of the De Ritis ratio in Benign Breast Disease and Breast Cancer.

	Group 1 (benign)	Group 2 (malignant)	
	Mean±sd	Mean±sd	p value
Age	52.07±10.83	54.50±12.15	0.095
AST (U/L)	18.17 ±5.32	20.13±10.44	0.058
ALT (U/L)	17.42±8.49	19.85±14.84	0.148
AST/ALT ratio	1.19 ±0.49	1.17±0.41	0.908

In the molecular subtype staging of the Group 2 patients, 72 (51.06%) were luminal A, 54 (38.29%) were luminal B, 4 (2.83%) were triple-negative and 11 (7.80%) were HER2 +. No statistically significant difference was determined between

the molecular subtypes in respect of the De Ritis ratio (p = 0.850) (Table 2).

Table 2. Comparison of the Impact of the De Ritis Ratio on Prognosis in Breast Cancer Subgroups

Group 2 (malignant)	Number of Cases (n=141)	Percentage (%)	AST/ALT mean ± SD	p value
Molecular subtype				0.850
Luminal A	72	51.06	1.15 ±0.35	
Luminal B	54	38.29	1.18 ±0.46	
Triple negative	4	2.83	1.07 ±0.39	
HER2+	11	7.80	1.24 ±0.51	
Tumor size				0.722
T1 (≤2cm)	70	49.64	1.17 ±0.37	
T2 (<2cm, ≤5cm)	57	40.42	1.19 ±0.49	
T3 (>5cm)	14	9.92	1.09 ±0.28	
Lymph node involvement				0.154
N0	93	65.95	1.21 ±0.42	
N1, N2	48	34.04	1.10 ±0.37	
Ki 67				0.198
<20	92	65.24	1.20 ±0.43	
≥20	49	34.75	1.10 ±0.37	

AST: Aspartate transaminase, ALT: Alanine transaminase, SD: Standard deviation, HER2: Human epidermal growth factor receptor 2

According to tumor size, 70 (49.64%) patients were T1, 57 (40.42%) were T2 and 14 (9.92%) were T3. There was no statistically significant difference in the De Ritis ratio between tumor sizes (p = 0.722) (Table 2).

There was no lymph node metastasis in 93 (65.95%) of the patients, and 48 (34.04%) had lymph node metastasis. No statistically significant difference was found in the De Ritis ratio according to the presence of lymph node metastasis (p = 0.154) (Table 2).

In 92 (65.24%) patients, the Ki-67 index was <20, and in 49 (34.75%) it was >20. There was no statistically significant difference in the De Ritis ratio according to the Ki 67 index (p = 0.154) (Table 2).

Discussion

The De Ritis ratio is frequently used as a predictive index in various diseases including acute stroke, arteriosclerosis, kidney, and respiratory dysfunction, and critical limb ischemia [10-13]. The effect of the De Ritis ratio on cancer prognosis has also been reported in various studies [14-16]. Wu et al. found in a meta-analysis involving 9400 patients that high De Ritis ratio before treatment was a prognostic factor for overall survival, cancer-specific survival, and disease-free survival in solid tumors (renal cell cancer, urinary tract urethral carcinoma, bladder cancer, and liver cancer) [17]. In a retrospective study of 109 patients by Nishikawa et al, it was found that the De Ritis ratio in urinary tract urothelial carcinoma was an independent predictive factor in TNM staging [18]. Katzke et al. conducted a prospective randomized cohort study and demonstrated that a high ratio of De Ritis before treatment in all four cancers (lung, prostate, breast, and colorectum) increased mortality but it was seen to only increase the risk of prostate cancer and not the risk of breast cancer [19]. In contrast, Thriveni et al. reported that the De Ritis ratio before treatment statistically significantly increased TNM staging according to stages in breast cancer and could be an independent predictive factor [4]. However, among these studies, there are very few studies on breast cancer, and very different results have been found in terms of the De Ritis ratio being a predictive and prognostic factor in breast cancer. Therefore, the value of the De Ritis ratio on breast cancer prognosis remains uncertain. The current study was conducted to investigate the predictive factor effect of the De Ritis ratio in breast cancer preoperative staging. The study results showed that the De Ritis ratio did not increase the risk of breast cancer, which was consistent with the findings of Katzke et al. [18]. Contrary to the study by Thriveni et al. [4], the results of the current study demonstrated that the De Ritis ratio does not increase statistically significantly according to stages and tumor diameter in the TNM staging system. In addition to these studies, among the molecular subtypes (luminal A, luminal B, triple-negative, and HER2 +), which are important prognostic indicators in breast cancer, the De Ritis ratio was not statistically significant in the Ki 67 index. It may be because of the smaller sample size of the T3 group and exclusion of the patients at stage 4, as the De Ritis ratio has been found to be more significant in the advanced stage.

Hypotheses related to the high De Ritis ratio in cancer patients have stated that AST is predominantly found in the mitochondria of cells and ALT is found only in hepatocyte cytoplasm. Therefore, in cases involving tissue damage, mitochondrial DNA is damaged due to excessive release of reactive oxygen species, which can cause serious tissue damage and leads to the release of more mitochondrial enzymes [5]. Oxidative stress parameters and inflammatory markers increase cancer development and increase the De Ritis ratio. A second hypothesis states that cancer cells support more aerobic glycolysis than normal cells, and AST is known to play a vital role in aerobic glycolysis. This pathological condition leads to the activation of more AST than ALT in rapidly growing cancer tissues [20]. A third hypothesis is that oxidative stress and inflammation can cause liver damage. Therefore, the high De Ritis ratio may be a prognostic factor associated with the overall survival (OS) of patients with different types of cancer [21].

Limitations of this study can be said to be the retrospective design, the limited number of patients, the exclusion of stage 4 patients, and the unequal distribution of patients among the subgroups.

In conclusion, the results of this study showed that the pre-treatment De Ritis ratio was not an independent predictive factor differentiating between benign breast disease and breast cancer, in addition to molecular subtyping, tumor diameter, lymph node metastasis, and Ki 67 index preoperative staging in breast cancer. There are few studies in the literature on the fact that the De Ritis ratio is an independent predictive factor in breast cancer staging and different results have been obtained in those studies. Therefore, to overcome the above-mentioned limitations, there is a need for more comprehensive clinical trials to be conducted to confirm the prognostic role of the De Ritis ratio before treatment in preoperative staging in patients with malignancies.

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Comparison of proximal femoral nail and hemiarthroplasty outcomes in elderly (over 80 years old) patients with intertrochanteric fractures

İntertrokanterik kırığı olan ileri yaş (seksen yaş ve üstü) hastalarda, proksimal femoral çivi ve hemiartroplasti sonuçlarının karşılaştırılması

Ali Şişman [1], Şevki Öner Şavk [2], Serdar Kamil Çepni [3]

Abstract

Aim: Proximal femoral nail (PFNA) and hemiarthroplasty are the two most frequently used methods in the surgical treatment of intertrochanteric femur fractures. The study aimed to determine the priority choice for surgical treatment in elderly patients (over 80 years old).

Methods: Patients treated for intertrochanteric femur fractures between 2012 and 2017 were retrospectively analyzed. Patients aged 80 years and over who were treated with hemiarthroplasty or PFNA were included in the study. The length of the operation, the need for postoperative intensive care, Harris Hip Score, postoperative complications, and mortality rates in the first year were compared.

Results: A total of 120 patients with intertrochanteric femur fractures older than 80 years were evaluated. There were 43 patients (35.8) in the hemiarthroplasty group and 77 (64.2) in the PFNA group. No significant differences were found between the two groups in terms of mortality, need for postoperative intensive care, Harris Hip Score, and postoperative complications in the first year. While the median operation time was 45 minutes (IQR 40-50) in the PFNA group, it was 80 minutes (IQR 75-85) in the hemiarthroplasty group ($p < 0.001$). The length of the operation was shorter in the PFNA group.

Conclusion: In elderly patients, there were no significant differences between the surgical treatment modalities as hemiarthroplasty and PFNA in treating intertrochanteric fractures considering the postoperative outcomes and mortality rates within the first year. However, shorter operation time might be an advantage of PFNA.

Keywords: Femoral intertrochanteric fracture, hemiarthroplasty, proximal femoral nail.

Öz

Amaç: İntertrokanterik femur kırığı olgularının cerrahi tedavisinde en sık kullanılan iki yöntem proksimal femoral çivi (PFNA) ve hemiartroplastidir. Çalışmanın amacı ileri yaştaki (80 yaş üzeri) olgularda öncelikli tedavi seçimini belirlemektir.

Yöntemler: 2012 -2017 yılları arasında yaşı 80 ve üzerinde olan intertrokanterik femur kırığı nedeni ile hemiartroplasti ya da PFNA uygulanan hastalar retrospektif olarak incelendi. Ameliyat süreleri, ameliyat sonrası yoğun bakım ihtiyacı, Harris Kalça Skoru, ameliyat sonrası komplikasyonlar ve ilk bir yıl içindeki ölüm oranları karşılaştırıldı.

Bulgular: İntertrokanterik femur kırığı olan ve 80 yaşın üzerinde toplam 120 hasta değerlendirildi. Hastaların 43'ü (35,8) hemiartroplasti grubunda, 77'si (64,2) PFNA grubunda yer aldı. Her iki grup arasında ilk bir yıl içinde ölüm oranları, ameliyat sonrası yoğun bakım ihtiyacı, Harris Kalça Skoru ve ameliyat sonrası komplikasyonlar açısından anlamlı fark tespit edilmedi. Operasyon süresi PFNA grubunda medyan 45 dakika (çeyrekler arası açıklık 40-50) iken, hemiartroplasti grubunda medyan 80 dakika (çeyrekler arası açıklık 75-85) ($p<0.001$). Operasyon süresinin PFNA grubunda daha kısa olduğu görüldü.

Sonuç: İleri yaş olgularda, intertrokanterik kırık tedavisinde hemiartroplasti ve PFNA arasında ameliyat sonrası sonuçlarda ve ilk bir yıl içindeki ölüm oranları arasında anlamlı fark bulunamamıştır. Buna rağmen ameliyat süresinin daha kısa olması PFNA'nın bir avantajı olarak değerlendirilebilir.

Anahtar Kelimeler: Femur intertrokanterik kırık, hemiartroplasti, proksimal femoral çivi.

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Introduction

Intertrochanteric fractures are the common clinical situations in the elderly population and are mainly associated with osteoporosis. These fractures cause severe morbidity and mortality [1]. In the treatment of trochanteric fractures, there is a consensus on surgical treatment choices that allow early mobilization and restore function to the extremity to prevent complications and reduce the mortality rates [2, 3].

Although there are different treatment options, intramedullary nails are among the most frequently used methods due to the high blood supply potential of the region, their biomechanical advantage, and their easy application with minimally invasive techniques [4, 5]. However, complications and poor functional results, especially in osteoporotic patients due to intramedullary fixation methods, have recently increased the popularity of hemiarthroplasty applications. The advantage of hemiarthroplasty has been reported as enabling mobilization with full load early and eliminating healing problems due to fracture [6]. Also, complications due to the longer duration of the operation with hemiarthroplasty and increased postoperative mortality in elderly patients appeared as disadvantages [6, 7].

For these reasons, there is no consensus on which method is superior in the surgical treatment of intertrochanteric fractures, especially in elderly patients with poor bone quality. Our study's hypothesis was to show the proximal femoral nail (PFNA) choice is superior considering the mortality in patients with intertrochanteric femur fractures in the advanced age. Accordingly, we aimed to compare the literature by comparing which of the hemiarthroplasty and PFNA choices is superior in patients with intertrochanteric femur fractures over 80 years old.

Material and methods

Study design and study criteria

At the beginning of the study, we took approval from Adnan Menderes University, Faculty of Medicine, Clinical Research Ethics Committee (Date: January 4th, 2018, Number: 2018/1294). The study was performed in accordance with the Declaration of Helsinki. Written consent could not be taken due to the retrospective design of the study and unanimity of data.

The cases treated in our clinic due to intertrochanteric femur fracture between 2012 and 2017 were retrospectively analyzed using the hospital information system and the picture archiving systems. Patients over 80 years of age who underwent hemiarthroplasty or PFNA due to intertrochanteric fractures were included in the study. Patients who underwent conservative treatment, fixed with plate screws in surgical treatment, lost follow-up or whose follow-up period was less than one year, and patients whose archive records could not reach sufficient information were excluded from the study. Following the inclusion and exclusion criteria, the patients were divided into two groups: those who underwent hemiarthroplasty (Zimmer, Inc, Warsaw, IN) and PFNA (Synthes® Oberdorf, Switzerland). Surgery was performed using the same brand of implant in accordance with the group in which all patients were included.

A total of 143 patients with advanced age and intertrochanteric fractures were evaluated. Of them, 23 of the cases were not included in the study. Of the 120 patients included in the study, 43 (35.8) were in the hemiarthroplasty group, and 77 (64,2) were in the PFNA group.

Standard anteroposterior hip radiographs and pelvic radiographs were obtained for all patients included in the study. Unless contraindicated, all patients received thromboembolism prophylaxis with subcutaneous anticoagulant therapy from the first day of hospitalization to the first month after the surgery. Mechanical prophylaxis was applied with embolism stockings. All patients were administered a single dose of 1 gram cephalosporin 30 minutes before the surgery. In the case of prolonged surgery, an additional dose of cephalosporin was administered after the second hour.

In-bed exercise was started for the patients in both groups the day after the surgery. The patient group, who was applied PFNA, was raised with a walker in the early period without any load. At the earliest first month after the radiography control, the callus was seen, and mobilization with full load was allowed. Hemiarthroplasty patients were raised with full load bearing in the first period when their general condition was available.

Surgical techniques

All cases were operated by the same surgeon, in the same operating room, and using the same fluoroscopy device. Fractures were evaluated according to the Evans-Jensen classification. None of the fractures included in the study were reverse oblique according to the Evans-Jensen classification. The fractures included in the study were not subclassified. Regardless of the fracture classification, firstly, closed reduction was tried in all cases on the traction table. After being taken to the traction table, abduction, external rotation, adduction, and internal rotation maneuvers were performed on all fractures for reduction purposes. A suitable position was provided for reduction, and anteroposterior and lateral fluoroscopy images were obtained. While evaluating the reduction, the continuity of the posteromedial cortex was checked. The patient was stained and covered with the reduction appreciated, and PFNA was applied (Figure 1). Patients with successful reduction formed the PFNA group. In cases where reduction was not appropriate, the cases were placed in the lateral decubitus position, and hemiarthroplasty was performed with a posterior approach (Figure 2). The patients whose reduction failed constituted the hemiarthroplasty group. Hemiarthroplasty was applied to all patients for whom arthroplasty was preferred, and total hip arthroplasty was not applied to any patient.

Operation duration, postoperative intensive care need, postoperative complications, mortality rates within the first year, and Harris Hip Score at the 12 months after the surgery were compared.

Statistical analysis

SPSS 21.0 statistics program was used in our study. Whether the quantitative variables were suitable for normal distribution was examined by the Kolmogorov-Smirnov test. Groups were compared using the independent-samples t-test for variables with normal distribution and the Mann Whitney U test for variables that did not show normal distribution. Descriptive statistics of normally distributed quantitative variables were shown as mean ± standard deviation, and descriptive statistics of non-normally distributed quantitative variables were shown as median (interquartile range (IQR) 25-75th percentile). The dependence between qualitative variables was examined using chi-square analysis. Descriptive statistics of these variables were

expressed as frequency (%). A p <0.05 value was considered statistically significant.

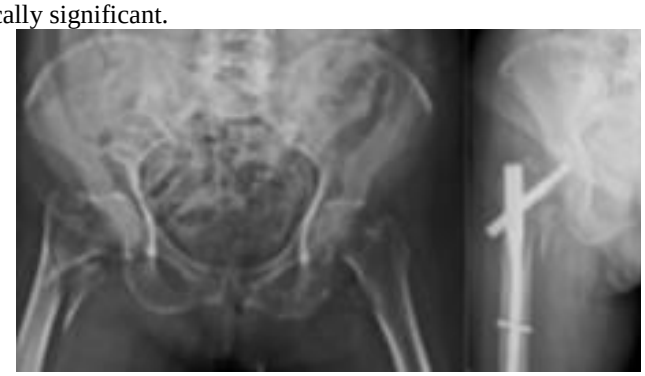

Figure 1. Pre-operative and postoperative first-year radiographs of an 83-year-old patient who underwent PFNA.

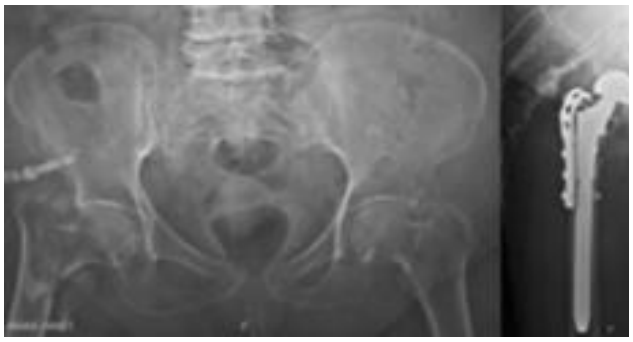

Figure 2. Pre-operative and postoperative radiographs of an 83-year-old patient who underwent hemiarthroplasty.

Results

The median age of those who underwent hemiarthroplasty was 85 years (range 81-86), and the median age of those who received PFNA was 83 years (IQR 81-86). The gender distribution between the groups was homogeneous. 48 (62.3%) of 77 patients who were applied PFNA were female, and 29 (37.7%) were male. Of the 43 patients who underwent hemiarthroplasty, 29 (67.4%) were female and 14 (32.6%) were male (p=0.718). While the median operation time was 45 minutes (IQR 40-50) in the PFNA group, it was 80 minutes (IQR 75-85) in the hemiarthroplasty group (p<0.001). Postoperative intensive care need was 58.4% (45 cases) in the PFNA group and 79.1% (34 cases) in the hemiarthroplasty group (p=0.037). The Harris Hip Score was evaluated at 12 months postoperatively. The mean score was 60.5±18.9 in the PFNA group, and the mean score was 53.52±16.11 in the hemiarthroplasty group. The median duration of the follow-up was 48 months (IQR 25-65) in the PFNA group and 36 months (IQR 25-61) in the hemiarthroplasty group (p=0.186). While the mortality rate in the first year was 35.1% (27 cases) in the PFNA group, it was found to be 41.9% (18 cases) in the hemiarthroplasty group (p=0.589) (Table 1).

In our study, heterotopic ossification in three (7%), wound infection in two (4.7%), prosthesis infection in one (2.3%), and dislocation in one (2.3%) occurred in patients who underwent hemiarthroplasty. Wound infection in three (3.9%), implant failure in two (2.6%), cut-out in two (2.6%), nonunion in one (1.3%), and periprosthetic fracture in one (1.3%) occurred in the PFNA group (Table 2).

Discussion

In our study, PFNA and hemiarthroplasty outcomes were compared in elderly patients with intertrochanteric femur fractures, and similar results were obtained. The significantly shorter operative time was regarded as an advantage of PFNA.

The ideal treatment method for intertrochanteric fractures continues to be debated. In treating intramedullary nails, which is the most common treatment method, failures, especially in elderly patients, have recently made the hemiarthroplasty option popular [8]. Problems such as loss of fixation, loss of reduction, and malunion increase the concerns of failure, especially in patients over 80 years of age with poor bone quality [5, 9]. Postoperative complications decrease with the early mobilization of elderly patients. It has made hemiarthroplasty an alternative in the treatment of intertrochanteric fractures with its early full-load mobilization advantage [10,11].

Table 1. Comparison of demographic information, general results, surgical data and postoperative results between groups.

Variables	PFNA Group (n=77)	Hemiarthroplasty Group (n=43)	p
Age (year) †	83 (81-86)	85 (83-88)	0.034
Gender ‡			0.718
Male	29 (37.7)	14 (32.6)	
Female	48 (62.3)	29 (67.4)	
The median duration of follow-up (day) †	48 (25-65)	36 (25-61)	0.186
Operation duration (minute) †	45 (40-50)	80 (75-85)	<0.001
Need for intensive care unit ‡	45 (58.4)	34 (79.1)	0.037
Mean death time (days) †	88 (35-291)	41.5 (18-142)	0.123
Mortality in the first year ‡	27 (35.1)	18 (41.9)	0.589
Harris Hip Score ¥	60.5±18.96	53.52±16.11	0.118

†: median (IQR), ‡: n (%), ¥: mean ± standard deviation

The most important advantage of PFNA is that it reduces surgery-related complications due to its short operation duration. In the study of Parker et al. [12], PFNA operation duration was 36 minutes, while hemiarthroplasty operation duration was 57 minutes. Also, the operation duration of PFNA was found to be shorter than hemiarthroplasty in many other studies [13-15]. Similar to the literature, the operation time of the PFNA group was shorter in our study. The average operation duration in patients with PFNA was 45 minutes, while it was 80 minutes in the hemiarthroplasty group. Intramedullary nailing significantly shortened the operation duration that is regarded as an advantage.

Studies are claiming higher mortality rates after hemiarthroplasty compared to PFNA in the surgical treatment of intertrochanteric fractures. Nie et al. [13] reported that the first year's mortality rate in patients who underwent hemiarthroplasty was higher than those with PFNA. Some studies did not find a significant difference in mortality rates within the first year [12, 16]. In the study of Kumar et al. [17] and other similar studies [18, 19], patients with advanced age were evaluated as in our study, and no significant difference was found between the two groups in terms of mortality in the first year. In our study, the mean day of death in the PFNA group was 88 days, whereas it was 41.5 days in the hemiarthroplasty group (not given in the text). Although there was an earlier death in the hemiarthroplasty group, no statistically significant difference was found.

Table 2. Comparison of postoperative complications between groups.

Complications ‡	PFNA Group (n=77)	Hemiarthroplasty Group (n=43)	p
Heterotopic ossification	-	3 (7)	
Wound infection	3 (3.9)	2 (4.7)	
Prosthesis infection	-	1 (2.3)	0.076
Dislocation	-	1 (2.3)	
İmplant failure	2 (2.6)	-	
Cut-out	2 (2.6)	-	
Non-union	1 (1.3)	-	
Periprosthetic fracture	1 (1.3)	-	

‡: n (%)

The prolongation of the operation duration and the deterioration of hemodynamics due to bleeding cause an increased need for postoperative intensive care. According to the studies performed, blood loss and blood transfusion need are higher with hemiarthroplasty [17, 19]. In our study, when the ratio of patients who needed postoperative intensive care was examined, no significant difference was found between the two groups.

Görmeli et al. [20] found the Harris Hip Score of 74.7 in the hemiarthroplasty group and 79.7 in the PFNA group. Li et al. [21] reported a Harris Hip Score of 85.6 in patients with intertrochanteric fractures to which they applied PFNA. In their study published in 2017, Hari Prasad et al. [22] found that the Harris Hip Score was higher in patients who underwent hemiarthroplasty. They recommended hemiarthroplasty for the treatment of intertrochanteric fractures. There was no significant difference between the two groups in the 12-month evaluation of Harris Hip Score in our study.

This study has several limitations. The main limitations were retrospective study, small sample size, and not including subgroups in the classification. Besides, since the follow-up periods in elderly patients were short, long-term analyzes could not be included. Besides, performing all operations by the same surgeon, using the same implants, performing operations with the same operating room and fluoroscopy device were the advantages of this study.

In conclusion, there was no significant difference between the two groups in terms of mortality in the first year, need for postoperative intensive care, 12-month Harris Hip scores, and postoperative complications. Due to the shorter operation time in the PFNA group, it is seen as an advantage in patients' surgical treatment with intertrochanteric fractures over 80 years old.

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Evaluation of the relationship between HbA1c values of pregnant women with gestational diabetes and anthropometric measurements of their newborn

Gestasyonel diyabetli gebelerin HbA1c değerleri ile yenidoğanlarının antropometrik ölçümleri arasındaki ilişkinin değerlendirilmesi

Birgül Deniz Doğan [1], Emin Pala [2], Süleyman Ersoy [2], Murat Doğan [3]

Abstract

Aim: In our study, we aimed to evaluate the relationship between the HbA1c values of pregnant women who were diagnosed with gestational diabetes mellitus (GDM) and the anthropometric measurements of their newborns.

Methods: 200 pregnant women with GDM between the ages of 18 and 43 with sufficient data were accepted to our study. Also, 200 healthy pregnant women formed the control group. The data of both groups were statistically compared.

Results: Gravidity of pregnant women was 1-8 and parity 1-7 and all between 27-42 gestational weeks. HbA1c values ranged from 4.3 to 8.3. The height of the babies was between 30 and 57 cm, birth weight between 800 and 4760 grams, and head circumference between 22 and 65 cm. While 1st minute apgar values varied between 2 and 9, 5th minute apgar values varied between 4 and 10. While 55.3% of the pregnant women had a normal birth, 44.7% of them had cesarean section. 95.5% of HbA1c values were less than 6.5 and 4.5% of them were at least 6.5. 45% of the newborns were girls and 55% were boys. 7% were low birth weight, 87% normal weight and 6% macrosomic. While 21.5% of newborns had complications, 78.5% had no complications.

Conclusion: The complication rate was higher in pregnant women with GDM and cesarean rate was higher due to increasing birth weight in our study. Further, multi-center and prospective studies may provide new perspectives for prevention of GDM and its complications.

Keywords: Gestational Diabetes Mellitus, HbA1c, Newborns

Öz

Amaç: Çalışmamızda gestasyonel diyabetes mellitus (GDM) tanısı alan gebelerin HbA1c değerleri ile bebeklerinin antropometrik ölçümleri arasındaki ilişkiyi değerlendirmeyi amaçladık.

Yöntemler: Çalışmamıza yaşları 18 ile 43 arasında değişen, yeterli veriye sahip 200 GDM'li gebe kabul edildi. Rastgele seçilen 200 sağlıklı gebe de kontrol grubunu oluşturdu. Her iki grubun verileri istatistiksel olarak karşılaştırıldı.

Bulgular: 1 ile 8 arasında değişen gravide ve 0 ile 7 arasında değişen parite değerlerine sahip gebeler 27 ile 42 hafta arasındaydılar. HbA1c değerleri 4,3 ile 8,3 arasında değişmekteydi. Bebeklerin boyları 30 ile 57 cm, doğum ağırlıkları 800 ile 4760 gram, baş çevreleri ise 22 ile 65 cm arasındaydı. 1. dk apgar değerlerinin 2 ile 9 arasında değişmesine karşılık, 5. dk apgar değerleri 4 ile 10 arasında değişmekteydi. Gebelerin %55,3' ü normal doğum yaparken, %44,7' sine sezeryan uygulanmıştı. HbA1c değerlerinin %95,5' i 6.5' ten küçük, %4,5' inin ise en az 6,5 idi. Gebelerin %53,5' ine diyet yanında insülin verilirken, %46,5' ine sadece diyet önerilmişti. Bebeklerin %45' i kız, %55' i erkekti. %7' si düşük doğum ağırlıklı, %87' si normal ağırlıklı ve %6'sı makrozomikti. Yenidoğanların %21,5' inde komplikasyon görülürken, %78,5' inde komplikasyon tespit edilememiştir.

Sonuç: Çalışmamızda GDM'li gebelerde komlikasyon ve artan doğum kilosuna bağlı olarak sezaryan oranı daha yüksek bulunmuştur. Maternal ve fetal morbidite ve mortalite üzerindeki olumsuz etkilerinin daha iyi anlaşılabilmesi ve önlenebilmesi için çok merkezli ve prospektif çalışmaların yapılmasına ihtiyaç vardır.

Anahtar Kelimeler: Gestasyonel Diyabetes Mellitus, HbA1c, Yenidoğan

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Introduction

GDM, is an intolerance to carbohydrates at second or third trimester of pregnancy in women without a history of diabetes mellitus, arising due to the hyperinsulinemia and insulin resistance caused by several hormones released from the placenta [1,2]. It occurs resulting from failure of pancreas to overcome insulin resistance [3].

Although the incidence is higher in countries where diabetes is more prevalent, usually it is seen in 2-4% of pregnant women. In our country the prevalence of diabetes is 13.7% in the population over 20 years old, thus GDM risk is medium. It is known that GDM may lead to many complications both in the mother and the fetus such as pre-eclampsia, hydramnios, macrosomia etc. [4]

One stage or two stage oral glucose tolerance test is the golden standard for diagnosis [5] and insulin is the golden standard in pharmacological therapy [6].

The objective of this study is, to evaluate the relationship between HbA1C values of pregnant women with GDM and anthropometric measurements of their newborn and compare them with normoglycemic pregnant women.

Material and methods

This was a retrospective case control study, and it was carried out by evaluation of the data that belong to pregnant women referring to University of Health Sciences, Umraniye Training and Research Hospital between December 2013 and December 2018.

Case group consists of pregnant woman who referred to our hospital for routine follow up and who met any of the following GDM diagnostic criteria:

50 g glucose challenge test 1-hr Plasma Glucose (PG) level ≥ 180 mg/dl,

50 g glucose challenge test 1-hr PG 140- 180 mg/dl and exceeding 2 of the 4 cut-off values after 100 g glucose challenge for OGTT to reach definitive diagnosis of GDM or in 75 g OGTT without any challenge test exceeded one of the fasting, 1-hr or 2-hr cut-off values.

The subjects who had Type I or Type II DM, any other endocrine disease or history of drug use, those with multiple pregnancy and under 18 years old and those with missing medical records were excluded from the study.

Case group was 200 pregnant women who met inclusion criteria regarding GDM diagnosis. A control group was selected randomly among pregnant women who do not have any comorbid disease or history of drug use.

The data of pregnant women with GDM and their newborns are compared with the control group.

Ethics approval

University of Health Sciences, Umraniye Training & Research Hospital Clinical Research Ethics Committee has approved the study on 22 May 2019 with B. 10. 1. TKH. 4. 34. H. GP. 0. 01/ 105 decision no and the study had clearance regarding ethical and scientific integrity of the study.

Statistical analysis

IBM SPSS Statistics 22 (IBM SPSS, Turkey) program is used for statistical analysis in assessment of data obtained from the study. Conformity of the parameters to normal distribution was tested by Shapiro Wilks test. For assessment of study data, in addition to descriptive statistical methods (mean, standard deviation, frequency) comparison of quantitative data and intergroup comparison of parameters with normal distribution were done by using one-way Anova test. Parameters with normal distribution were compared between two groups by Student t test, parameters with non-normal distribution were compared between two groups by Mann Whitney U test. Qualitative data were compared by using Chi-square test, Fisher's Exact test, and Continuity (Yates) Correction. The correlation between parameters that are in conformity with normal distribution was studied by using Pearson correlation analysis. $p < 0.05$ is considered significant.

Results

Our study included 200 pregnant women with GDM diagnosis and 200 pregnant women who served as control group in an age range of 18-43 years and their newborns.

Time span was 27-42 gestational weeks. Gravidity was 1-8 and parity was 0-7 and HbA1c values ranged between 4,3 - 8,3. and in 82 pregnant women the values ranged between 5,7-6,4. Complications were observed in 21.5% of the newborns and the remaining newborns were free from complications.

Table 1. Distribution of study parameters.

	Min-Max	Mean±SD
Age at pregnancy	18-43	29.14±5.79
Gestational week $_{(median)}$	27-42	38.08±2.26(38)
Gravidity$_{(median)}$	1-8	2.81±2.32 (3)
Parity$_{(median)}$	0-7	1.33±1.14 (1)
HbA1c (n=200)	4.3-8.3	5.61±0.54
Height at birth (cm)	30-57	50.22±2.89
Weight at birth (g)	800-4760	3300.68±540.95
Head circumference (cm)	22-65	34.63±2.79
Apgar 1 $_{(median)}$	2-9	8.73±0.8 (9)
Apgar 5 $_{(median)}$	4-10	9.8±0.65 (10)
	n	%
Gestational week group		
Preterm (under 37 weeks)	44	11
Term (37-42 weeks)	334	83.5
Post-term (over 42 weeks)	22	5.5
Type of birth		
Normal	221	55.3
Caesarian	179	44.8
HbA1c group (n=200)		
<6.5	191	95.5
≥ 6.5	9	4.5
Treatment (n=200)		
Insulin +Diet	107	53.5
Diet	93	46.5
Sex		
Female	180	45
Male	220	55
Birth weight (g) group		
Low (under 2500)	28	7
Normal (2500- 4000)	348	87
Macrosomia (above 4000)	24	6
Presence of complication		
Yes	314	78.5
No	86	21.5

Table 2. Intergroup assessment of study parameters.

	GDM group (Min-Max)-(Mean±SD)	Control group (Min-Max)-(Mean±SD)	p
Age at pregnancy (y)	(20-42)-(30.93±5.21)	(18-43)-(27.36±5.81)	[1]0.000*
Gestational week (median)	(27-42)-(37.62±2.22 (38))	(19-42)-(38.54±2.21 (39))	[2]0.000*
Gravidity (median)	(1-40)-(3.2±2.92 (3))	(1-8)-(2.43±1.41 (2))	[2]0.000*
Parity (median)	(0-6)-(1.49±1.03 (1))	(0-7)-(1.18±1.23 (1))	[2]0.000*
HbA1c	(4.3-8.3)-(5.61±0.54)	-	-
Height at birth	(30-57)-(49.79±3.17)	(36-56)-(50.66±2.51)	[1]0.002*
Birth weight	(800-4760)-(3315.72±599.04)	(1265-4310)-(3285.65±476.88)	[1]0.579
Head circumference	(22-65)-(34.87±3.55)	(29.5-46)-(34.4±1.72)	[1]0.092
Apgar 1 (median)	(2-9)-(8.63±0.94 (9))	(5-9)-(8.82±0.62 (9))	[2]0.002*
Apgar 5 (median)	(4-10)-(9.74±0.74 (10))	(6-10)-(9.86±0.53 (10))	[2]0.014*
	n (%)	n (%)	
Gestational week			
Preterm	30 (15)	14 (7)	[3]0.005*
Term	164 (82)	170 (85)	
Postterm	6 (3)	16 (8)	
Type of birth			
Normal	87 (43.5)	134 (67)	[3]0.000*
Caesarian	113 (56.5)	66 (33)	
HbA1c			
≤6.5	193 (96.5)	-	-
>6.5	7 (3.5)	-	
Treatment			
Insülin+Diet	107 (53.5)	-	-
Diet	93 (46.5)	-	
Sex			
Female	98 (49)	82 (41)	[3]0.108
Male	102 (51)	118 (59)	
Birth weight			
Low	15 (7.5)	13 (6.5)	[3]0.094
Normal	168 (84)	180 (90)	
Macrosomia	17 (8.5)	7 (3.5)	
Presence of complication			
Yes	141 (70.5)	173 (86.5)	[3]0.000*
No	59 (29.5)	27 (13.5)	

[1] Student t Test, [2] Mann Whitney U Test, [3] Ki-Kare Test, * p<0.05

The height of the newborns was between 30 and 57 cm, birth weight between 800 and 4760 grams, and head circumference between 22 and 65 cm. While 1st minute apgar values varied between 2 and 9, 5th minute apgar values varied between 4 and 10. Pregnancies have ended at preterm in 11%, term in 83.5% and post-term in 5.5% of the pregnant women. 55.3% of the pregnant women had a normal birth and 44.7% of them had cesarean section. Only diet was recommended as treatment in 46.5% of the subjects diagnosed as GDM and the remaining subjects had additional insulin injections. 45% of the babies were girls and 55% were boys. 7% were low birth weight, 87% normal weight and 6% had macrosomia (Table 1).

The most common complications were hyperbilirubinemia (36%) and transient tachypnea of the newborn (30,2%) followed by ASD (8.1%), prematurity (8.1%), sepsis (7.0%), congenital pneumonia (5.8%) and VSD (4.7%).

Mean age, gravidity values, parity values and birth by cesarean section were statistically significantly higher in pregnant women with GDM compared to the control group (p<0.001). However, gestational week values and height of the newborns at birth were significantly lower than the control group (p<0.001) (Table 2).

In the newborns of the group with GDM transient tachypnea of the newborn and early neonatal sepsis rates were statistically significantly lower compared to the control group regarding complications. (p<0.05) However, rate of hyperbilirubinemia was statistically significantly higher compared to the control group (p<0.05) (Table 3).

Table 3. Intergroup complication rates.

Complications	GDM group	Control group	p
	n (%)	n (%)	
Transient tachypnea of newborn	13 (22)	13 (48.1)	[1]0.028*
Atrial septal defect	5 (8.5)	2 (7.4)	[2]1.000
Hypoglycemia	6 (10.2)	0 (0)	[2]0.170
Small for gestational age	1 (1.7)	0 (0)	-
Large for gestational age	2 (3.4)	0 (0)	-
ventricular septal defect	3 (5.1)	1 (3.7)	[2]1.000
Hyperbilirubinemia	28 (47.5)	3 (11.1)	[1]0.003*
Pulmonary hypoplasia	1 (1.7)	0 (0)	-
Congenital cardiac defects	1 (1.7)	0 (0)	-
Intrauterine growth retardation	3 (5.1)	1 (3.7)	[2]1.000
Patent ductus arteriosus	4 (6.8)	2 (7.4)	[2]1.000
Sepsis	2 (3.4)	1 (3.7)	[2]1.000
Tricuspid insufficiency	1 (1.7)	1 (3.7)	-
Inguinal hernia	1 (1.7)	0 (0)	-
Prematurity	5 (8.5)	2 (7.4)	[2]1.000
Brachial plexus injury	1 (1.7)	0 (0)	-
Low birth weight	2 (3.4)	1 (3.7)	[2]1.000
Intubation	1 (1.7)	0 (0)	-
Pneumothorax: decreased aeration of lungs	3 (5.1)	0 (0)	[2]0.549
Asphyxia	1 (1.7)	0 (0)	-
Congenital pneumonia	3 (5.1)	2 (7.4)	[2]0.647
Exitus	2 (3.4)	0 (0)	-
Respiratory distress syndrome	2 (3.4)	1 (3.7)	[2]1.000
Early neonatal sepsis	1 (1.7)	5 (18.5)	[2]0.011*
Urinary tract infection	1 (1.7)	0 (0)	-
Thrombocytopenia	1 (1.7)	1 (3.7)	-
Clavicular fracture	1 (1.7)	0 (0)	-
Aorta coarctation	0 (0)	1 (3.7)	-
Down syndrome	0 (0)	1 (3.7)	-
Spina bifida: nerve damage in spinal cord	0 (0)	1 (3.7)	-
Other complications	0 (0)	3 (11.1)	[2]0.029*

[1] Continuity (Yates) Correction, [2] Fischer's Exact Test, *: p<0,05, Chi Square

Presence of complications, type of birth and birth weight did not affect HbA1c values statistically in both groups (Table 4).

In subjects with GDM, there was no statistical correlation between HbA1c values and age, gravidity, parity of the subjects and birth weight of the newborns (p>0.05).

Mean age of the mothers of newborns with complications was higher than the mothers of newborns without complication; birth weight of newborns with complications was lower than newborns without any complications (p<0.001).

Insulin treatment in subjects with HbA1c <5.7 (45%) was statistically significantly lower than subjects with HbA1c 5,7 - 6,4(59,8%) and HbA1c >6,5(100%) (p=0.024).

There was no statistically significant difference regarding the rate of complications and birth weights of the newborns in mothers with GDM who are treated with insulin or not (Table 5).

Table 4. Assessment of HbA1c values in GDM group according to the presence of complication, type of birth and birth weight.

GDM group	HbA1c		p
	Min-Max	Mean±SD	
Presence of complication			0.459[1]
No	4.5-7.2	5.59±0.49	
Yes	4.3-8.3	5.65±0.65	
Type of birth			0.523[1]
Normal	4.3-8.3	5.58±0.56	
Caesarian	4.5-7.4	5.63±0.53	
Birth weight group			0.167[2]
Low	5.1-8.3	5.77±0.78	
Normal	4.3-7.2	5.58±0.5	
Macrosomia	4.6-7.4	5.78±0.68	

[1] Student t test, [2] Oneway Anova Test

There is no significant correlation between HbA1c and height and head circumference of the newborns (r=0.118, p=0.096 and r=0.020, p=0.774 respectively).

Table 5. Assessment of presence of complications according to insulin treatment in subjects with GDM.

	Insulin treatment		
	Yes (n%))	No (n%))	p
Presence of complication			
No	70 (65.4)	71 (76.3)	[1]0.091
Yes	37 (34.6)	22 (23.7)	
Birth weight. *Mean±SD*	3303.74±659.94	3329.49±523.51	[2]0.763

[1] Ki-kare Test, [2] Student t test

Discussion

In the literature it is reported that advanced age is a risk factor for GDM in pregnancy. In a Canadian study by Denice et al. 1,109,605 pregnant women were assessed and GDM prevalence was found as 10% in women older than 30 [7]. In Finland, Lamminpää et al. studied 230,003 pregnant women over 35 years old with GDM and 5532 non-diabetic pregnant women younger than 35 years old and have found that GDM risk increases by increasing age [8]. In our study, mean age, gravidity, and parity values of women with GDM were found to be statistically significantly higher than the control group.

GDM is a significant health issue since it substantially impacts maternal and perinatal morbidity. In a study by Carolan M et al. it is observed that maternal hypertension, pre-eclampsia rates and interventions such as caesarian have increased in women with GDM [9] Also in our study C/S ratio (56.5%) of pregnant women with GDM was significantly higher than the control group (33%).

Measuring HbA1c in GDM patients is a controversial issue. The International Experts Committee on diabetes has reported that individuals with a HbA1c value 5.7-6.4% (39-46 mmol/mol) are at high risk for diabetes and should be included into the prevention programs [3]. In our study, HbA1c was 5.7-6.4% in 82 (41%) pregnant women and ≥ 6.5 in 9 (4.5%) of them. Mean value of HbA1c was 5.61 in pregnant women with GDM.

Macrosomia is a common complication observed in newborns of diabetic mothers and described as birth weight over 4000 g or more than 90th percentile in population-based growth charts. In the study of Figueroa et al., it is reported that in newborns of patients with borderline gestational diabetes LGA and macrosomia incidence have increased 2 and 1.6 times, respectively. In the study by Bonomo et al. it is reported that even slight alterations in glucose intolerance [10] may lead to excessive growth of the fetus [11]. In our study there was no significant difference between newborns regarding birth weight.

In the literature, correlation between fetal birth weight and maternal is a debated issue. In the study by Coen et al., it is concluded that there is no correlation between maternal HbA1c and birth weight [12]. In a comprehensive HAPO study, it is stated that plasma glucose level is more relevant in determining birth weight compared to HbA1c values [13]. In another study carried out in Turkey, it is reported that there is a positive and independent correlation between 2nd trimester maternal HbA1c values and birth weight [14]. In our study, we could not find any significant relationship between HbA1c values and birthweight, height at birth and head circumference of the newborns. This may be associated with strict antenatal follow up, proper insulin therapy, diet and exercise.

In some research it is observed that in pregnant women with previous diabetes diagnosis or with high HbA1c values during first trimester, risk of hazardous outcomes such as miscarriage, premature birth, neonatal mortality, fetal anomaly is higher compared to normal pregnancy. And it is reported that risk increases proportionally by increasing HbA1c values [15,16]. In different studies, the incidence of congenital anomalies was found higher in pregnant women with higher HbA1c values [17,18]. In our study as well, HbA1c values were significantly higher in diabetic pregnants who had complications compared to those without complications.

It is known that GDM may cause a lot of complications. In a study carried out in Poland the most common complications observed in newborns of the pregnant women with GDM were hyperbilirubinemia (17.3%), hypoglycemia (15.6%), congenital defects (4.3% - most commonly cardiac anomalies) [19]. In our study the most common complications among those with GDM were hyperbilirubinemia (47.5%), transient tachypnea of the newborn (22%), hypoglycemia (10.2%)

Advanced maternal age is among risk factors for GDM. In the study of Lamminpää et al.; it was observed that the advanced age of the mother increased the risk of prematurity, fetal mortality, and admission of the newborn to neonatal intensive care unit [9]. Based on this information we have compared the mean age of the pregnant woman with GDM who had complications (32.27 years) and those who did not have any complications (30.36 years). Mean age or the mothers with GDM was significantly higher in those who had complications compared to the ones without complications.

Preterm birth is defined as births occurring before 37th gestational age and GDM is a risk factor for preterm birth. In a study by Beigelman et al. incidence of preterm birth was found as 10% among 3841 pregnant women with GDM [20]. Moreover, in a study carried out by Heddeson et al. glucose intolerance is found to be related with spontaneous preterm births. In our study preterm birth rate was significantly higher in pregnant women with GDM (15%) compared to the control group (7%).

Due to various metabolic disorders occurring in newborns because of maternal diabetes the newborns of diabetic mothers may have lower apgar scores at birth. There are studies also on the contrary. In Israel Bental et al. investigated newborns of 120 pregestational diabetic and 825 gestational diabetic mothers prospectively and have found that APGAR score was higher in newborns of diabetic mothers compared to the newborns of non-diabetic mothers [21]. In our study 1st minute (8.63) and 5th minute (9.74) mean APGAR scores of the newborns of mother with GDM were found to be statistically lower than the mean APGAR scores of the newborns of non-diabetic mother group (8.82-9.86).

In a study carried out in Turkey it is suggested that HbA1c value >5,4 in pregnant women with GDM may be a predictor for starting insulin treatment [22]. In our study rate of insulin use in those with HbA1c <5.7 was 45%, it was 58.8% when the value is between 5.7-6.4 and 100% when it is ≥6.5.

According to these results it is observed that need for insulin increase as HbA1c value increases.

In our study there was no significant difference in terms of occurrence of complications between pregnant women with GDM who use insulin and who do not. However, in a randomized, multi-center study performed in the USA two groups with slight GDM were compared and in one of the groups normal gestational follow up was sustained and the other group was treated (diet, blood glucose monitoring and insulin when needed). In the treatment group complications such as macrosomia, shoulder dystocia and incidence of sectio was lower [23].

Anthropometric measurements of the newborns were compared in terms of treatment applied to the mothers with GDM diagnosis in Italy by Mello et al.; LGA rate was 18.8% in mothers treated only by diet; 9.9% in mothers treated with both diet and insulin and 8.3% in the control group [24]. Fetal macrosomia is a common adverse infant outcome of GDM if unrecognized and untreated in time. For the infant, macrosomia increases the risk of shoulder dystocia, clavicle fractures and brachial plexus injury and increases the rate of admissions to the neonatal intensive care unit [25] It may be suggested that proper follow up and treatment may decrease macrosomia. In our study, we could not find any significant difference between the birth weights of newborns who were born from pregnant women with GDM who were treated with insulin or not.

Consequently, the complication rate was higher in pregnant women with GDM and cesarean rate was higher due to increasing birth weight in our study. The negative impact of GDM on maternal and fetal morbidity and mortality may be mitigated by diet, insulin treatment and strict antenatal follow up. Further, multi-center and prospective studies may provide new perspectives for prevention of GDM and its complications.

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Evaluation of perianal fistulas with magnetic resonance imaging: Significance of T2-weighted BLADE sequence in disease diagnosis

Perianal fistüllerin manyetik rezonans görüntüleme ile değerlendirilmesi: T2 ağırlıklı BLADE sekansının hastalık tanısında önemi

Safiye Sanem Dereli Bulut[1], Zakir Sakcı [1]

Abstract

Aim: Anatomical details of perianal fistulas can be revealed more clearly by T2-weighted (T2-W) periodically rotated overlapping parallel lines with enhanced reconstruction (BLADE) sequence during magnetic resonance imaging (MRI). This study aimed to compare the efficacy of the T2-W BLADE sequence and the T2-W turbo spin echo (TSE) sequence in diagnosing perianal fistula, as well as to compare the findings with the results obtained using the dynamic contrast-enhanced (DCE) T1-weighted (T1-W) sequence during routine pelvic MRI examination.

Methods: Ninety patients (male/female: 67/23) who had undergone pelvic MRI examination (1.5Tesla) for the prediagnosis of perianal fistula were included in this prospective study.

In addition to our routine pelvic MRI protocol, T2-W BLADE sequence imaging and T2-W TSE sequence imaging in the axial, sagittal, and coronal planes were performed. Using a three-stage scoring system, two radiologists independently compared the T2-W BLADE and T2-W TSE sequences with each other and then with the DCE T1-W sequence in terms of perianal fistula imaging. Statistical analysis was performed using a sample t-test and the Cronbach's α test.

Results: Compared with the T2-W TSE sequence, the T2-W BLADE sequence was associated with fewer ghosting artifacts, with higher overall image quality, and with clearer visualization of the anatomical details of perianal fistula ($p < 0.05$). Images with high anatomic details and contrast resolution were obtained using the T2-W BLADE sequence similar to those obtained using the DCE T1-W sequence ($p < 0.05$).

Conclusion: On the basis of the reduced imaging time and on the higher image resolution, T2-W images can be obtained with the BLADE technique for the diagnosis of perianal fistula.

Keywords: Magnetic resonance imaging, perianal fistula, artifacts, T2-weighted sequence, BLADE technique

Öz

Amaç: Manyetik Rezonans Görüntüleme (MRG) ile perianal fistül teşhisinde, T2-ağırlıklı (A) BLADE sekansın etkinliğini, T2-A Turbo Spin Eko (TSE) sekans ile karşılaştırmak ve bulguları, rutin pelvik MRG incelemede Dinamik Kontrastlı (DCE) T1-A sekans görüntüleri ile karşılaştırmak.

Yöntemler: Perianal fistül ön tanısı ile pelvik MRG incelemesi (1,5 Tesla) yapılan toplam 90 hasta prospektif çalışmamıza dahil edildi. Rutin pelvik MRG protokolümüze, üç planda T2A BLADE sekansı ve T2A TSE sekansları da dahil edildi. Perianal fistül tanısında T2A BLADE sekans ve T2A TSE sekansların üstünlüğünü birbiriyle ve ardından üç aşamalı skorlama ile DCE T1A sekans ile karşılaştırıldı. Değerlendirme iki radyolog tarafından, farklı zamanlarda ve birbirinden bağımsız olarak yapıldı. İstatistiksel analiz için Simple t-test ve Cronbach's Alpha testi kullanıldı.

Bulgular: Perianal fistül tanısında, T2A BLADE sekans ile T2A TSE sekansa göre daha kısa sürede ve artefaktlara daha az duyarlı, yüksek çözünürlüklü görüntüler elde edildi ($p <0,05$). T2A BLADE sekans ile DCE T1A sekansına benzer yüksek kontrast çözünürlüğü olan görüntüler elde edildi ($p <0,05$).

Sonuç: Daha kısa sürede ve yüksek çözünürlüklü görüntüler elde edebilmesi nedeniyle, perianal fistül tanısında T2A görüntüler BLADE tekniği ile elde edilebilir.

Anahtar kelimeler: Manyetik rezonans görüntüleme, perianal fistül, artefaktlar, T2 ağırlıklı sekans, BLADE tekniği

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Introduction

A perianal fistula is characterized by an inflammatory process; once it has formed, a perianal fistula adversely affects the surrounding areas in the anal canal [1-3]. Perianal fistulas can cause considerable morbidity, and the majority of patients unfortunately undergo multiple medical and surgical treatments, as perianal fistulas demonstrate treatment resistance and exhibit a high recurrence rate [3].

The type and extent of fistulas and the co-existing pathologies, along with their radiological classification based on magnetic resonance imaging (MRI) findings, are important. Therefore a treating physician could plan the appropriate medical and surgical interventions that promote complete healing and ultimately prevent recurrence [3].

While multiple imaging techniques, including fistulography and endocavitary ultrasonography, have been used to evaluate perianal fistulas, MRI remains to be the preferred imaging modality. T2-weighted (T2-W) MRI sequences have been recently found to be the best techniques in acquiring a detailed anatomical view of the pelvis when evaluating perianal fistulas, and they also allow for the assessment of the anal canal [4, 5]. However, the susceptibility of T2-W MRI to motion artifacts related to respiratory, intestinal, or vascular pulsatility can obscure findings [5]. Different sequences have been developed to quickly acquire images and thereby reduce artifacts; these techniques include the HASTE and steady-state precession techniques [6]. In addition, a promising sequence called the PROPELLER (periodically rotated overlapping parallel lines with enhanced reconstruction) sequence was developed by Pipe in 1999 [7-11]. Meanwhile, the BLADE sequence is relatively insensitive to respiratory movements, enabling the correction of in-plane motion, rotation, and translation [7, 8].

This study aimed to compare the efficacy of the T2-W BLADE sequence with the T2-W TSE sequence in diagnosing perianal fistula, as well as to compare the findings with the results of DCE T1-W sequence in routine pelvic MRI. Our hypothesis was that the T2-W BLADE sequence could obtain images of higher quality within a reduced imaging duration.

Material and methods

Patients

Patients who had undergone pelvic MRI examination for perianal fistula pre-diagnosis from January 1, 2017 to August 1, 2017 were included in this prospective study.

Our inclusion criteria were as follows: presence of a discharge from the perianal fistula and antibiotherapy was not given within the last month. The exclusion criteria were as follows: a history of having perianal fistula operation in the last two months, having a contraindicated condition for MR examination (stent history, pacemaker history, and claustrophobia), and younger than 18 years old.

Ethical consideration

Approval from the concerned ethics committee was sought (No. 21194), and informed written consent was obtained from all the included patients. And all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

MRI Protocol

Pelvic MRI was performed in the supine position with a six-channel body coil in 1.5 Tesla MR (Magnetom Avanto; Siemens Healthcare, Erlangen, Germany). The pelvic MRI protocols used in our study are summarized in Table 1.

Since the anal canal is tilted forward, the field of view and the scan extent were defined using a sagittal T2-W single shot image with the center line running along the anal canal. Axial and coronal images were obtained relative to the long axis of the anal canal. No antiperistaltic agent was used in our study.

Table 1. Perianal fistula magnetic resonance (MR) sequence protocols for the study.

	Routine pelvic MR sequences				Added sequence
Parameters	T2-weighted TSE	T1-weighted TSE	DCE 3D T1 vibe	Diffusion-weighted imaging (b:0, 400, 800 s/mm^2)	T2-weighted BLADE
TR/TE (ms)	6200/117	557/18	4,7/2,3	6250/81	4000/83
FOV (mm)	260	320	220	220	260
matrix	256×256	352×352	256×256	96×96	256×256
ETL	-	-	-	-	27
FA (^{0})	-	-	-	10	-
Slice thickness/ spacing (mm)	4/1,2		4/0	4/1	4/1,2
Fat saturation	yes	no	yes	yes	yes
Acquisition time	3 min 52 s	3 min 50 s	5 min 18 s	2 min 13 s	2 min 24 s

The DCE T1 3D vibe sequence was performed before 30, 60, and 120 s after the peripheral administration of 0.1 mmol/kg gadobutrol at a rate of 2 mL/s. T2-W TSE and T2-W BLADE sequences were obtained using the fat sat technique in the axial, coronal, and sagittal planes. (TR: time of repetition; TE: time of echo; FOV: field of view; ETL: echo train length; and FA: flip angle).

Image Analysis

Image datasets were transferred to a Picture Archiving and Communication System (PACS) workstation for analysis (Centricity® PACS; GE Healthcare, Milwaukee, WI, USA). The images were independently evaluated by two radiologists with six and eight years of experience in abdominal radiology, respectively.

Image evaluation was performed in three phases (Figure 1). In the first phase, T2-W BLADE and T2-W TSE sequences were compared with each other in terms of image quality: presence of artifacts (bowel or respiratory motion, ghosting artifacts, aliasing, and radial artifact) and visibility of anatomic details in the perianal region.

The presence of artifacts was scored as follows: 1 = marked, 2 = moderate, 3 = mild, 4 = minimal, and 5 = absent.

Some structures were set as references in assessing the visibility of anatomical details. These anatomical structures were the sphincter complex, the levator ani–pelvic diaphragm, and the anatomical details of the ischioanal and ischiorectal fossa. The scoring was as follows: 1 = cannot be seen, 2 = blurry but visualized, 3 = acceptable, 4 = good, and 5 = excellent.

In the second phase, the T2-W TSE and T2-W BLADE sequences were compared in terms of their ability to distinguish the fistula type. In this evaluation, the DCE T1-W sequence was taken as a reference.

Finally, both observers were asked about their preferred T2-W sequence and the reasons behind such a preference. We categorized these reasons as follows: reduction of artifacts, higher image quality, clearer anatomical details of the perianal region, and shorter acquisition time.

Perianal fistula classification was made according to the St James's University Hospital classification [2, 3].

Statistical Analysis

The distribution of outcome categories was assessed using the Kolmogorov–Smirnov test. The Cronbach's α test was utilized to compare the performance of the T2-W sequences. P-values of <0.05 were considered statistically significant.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 22.0; SPSS Inc., Chicago, IL, USA).

Results

Patients

A total of 90 patients (23 females and 67 males) aged 22–75 years (median age: 46 years) were included in this study. Fourteen of these patients had undergone perianal fistula surgery more than two months prior. The remaining 76 patients were newly diagnosed to have perianal fistula. In seven patients, the etiology of this condition was an inflammatory bowel disease. Eighty patients had clinical chronic pain caused by fissure and by hemorrhoids in the perianal region, and three of them had fecal incontinence.

The distribution of patients according to perinal fistula types is summarized in Table 2.

Table 2. The distribution of patients according to perianal fistula types.

Types of perianal fistula	Distribution of Perianal fistula types
Type 1	62%
Type 2	7%
Type 3	14%
Type 4	8%
Type 5	9%

Presence of Artifacts

The amounts of aliasing and ghosting artifacts were significantly reduced in the T2-W BLADE sequence images relative to those in the T2-W TSE sequence images ($p < 0.05$; Cronbach's α: 0.87).

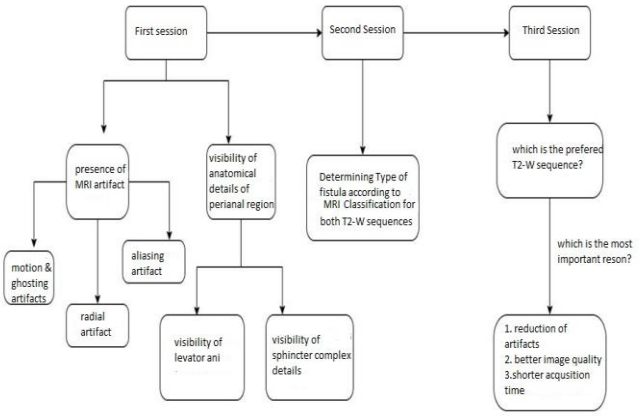

Figure 1. Flowchart showing the research methodology.

In terms of movement artifacts and radial artifacts, no significant difference was observed between the two sequences ($p = 0.07$; Cronbach's α: 0.65). The findings are detailed in Table 3.

Visibility of Anatomical Features

The sphincter anatomy and the levator ani were visualized more clearly with the T2-W BLADE sequence than with the T2-W TSE sequence ($p < 0.05$; Cronbach's α: 0.68) (Figures 2a and 2b).

No significant difference was observed between the two sequences in terms of the anatomical details of the ischioanal and ischiorectal fossa ($p > 0.05$).

Overall Image Quality

Both observers found that the T2-W BLADE imaging was superior to the T2-W TSE imaging in terms of image quality ($p < 0.01$; Cronbach's α: 0.73) (Figure 3).

Detection of Fistula Presence and Extension

In the evaluation performed with DCE T1-W vibe images, no significant difference was found between the T2-W BLADE and T2-W TSE images in terms of fistula presence and type ($p > 0.05$).

A complete consensus was reached between the two radiologists on the typing of perianal fistulas (Table 2).

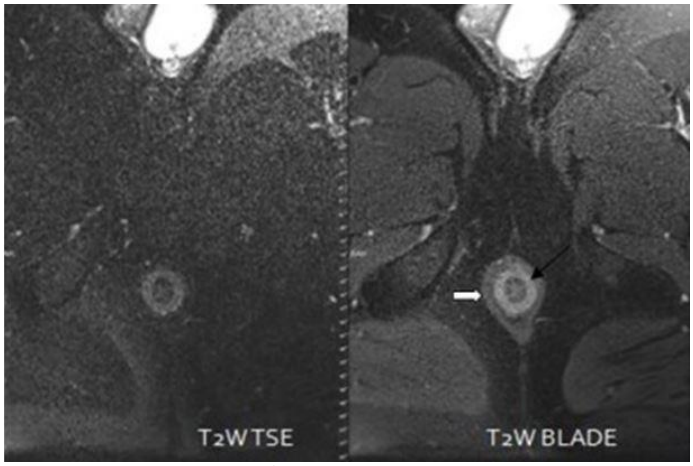

Figure 2a. Pelvic MRI image of a patient with chronic diarrhea by transverse plane fat-suppressed T2-weighted (T2-W) turbo spin echo (TSE) sequence and T2-W BLADE sequence.
A normal anatomy of the perianal region and the sphincter complex were observed. The perianal region found in the center of the image, as well as the internal sphincter (black arrow) and external sphincter (white arrow) forming the anal sphincter complex are clearly distinguishable in the T2W BLADE sequence.

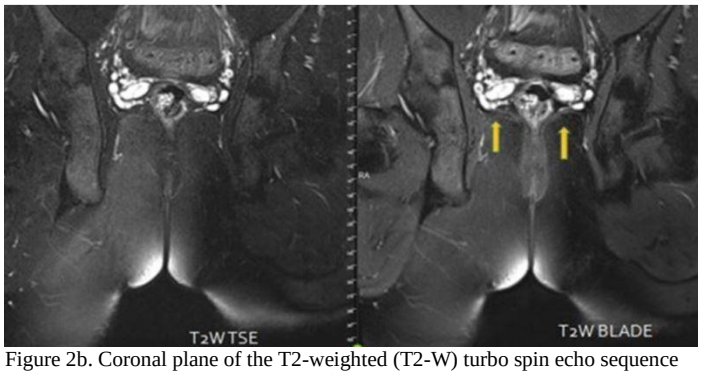

Figure 2b. Coronal plane of the T2-weighted (T2-W) turbo spin echo sequence and T2W BLADE sequence images of the same patient. Levator plates (thick yellow arrows), which are an essential landmark in fistula classification, are clearly distinguishable in the T2-W BLADE sequence image with a high-contrast resolution.
Levator plates (thick yellow arrows), which are an essential landmark in fistula classification, are clearly distinguishable in the T2-W BLADE sequence image with a high-contrast resolution.

Preferred sequence

Both observers preferred the T2-W BLADE sequence. The first reason was that the T2-W BLADE sequence required a shorter acquisition time than the T2-W TSE sequence (Cronbach's α: 0.74).

The second reason was the T2-W BLADE sequence's ability to reveal anatomical details of higher quality (Cronbach's α: 0.68).

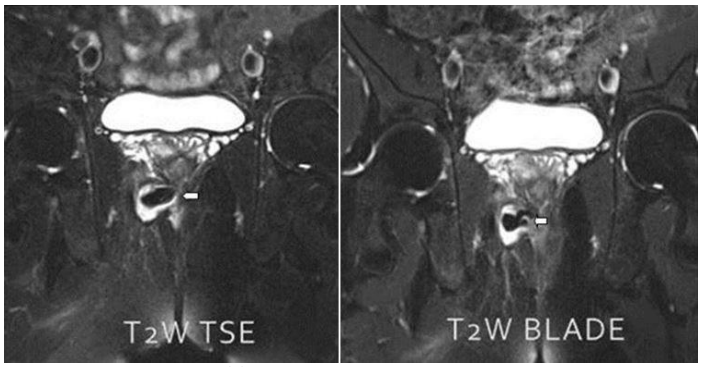

Figure 3: Coronal plane of fat-suppressed T2-weighted (T2W) BLADE sequence and T2W TSE images obtained from a 42-year-old man suffering from pain and discharge in the perianal region.
Grade 4 perianal fistula with abscess was noted according to the magnetic resonance classification of perianal fistulas. The correlation between abscess and fistula tract is more clearly seen in the T2W BLADE sequence than in the T2W TSE sequence. When looking at the free air in the fistula tract, it is noteworthy that two abscess-related fistulas were observed in the fistula tract.

Table 3. Average scores given by the observers.

	Average Scores			
	Observer 1		Observer 2	
Presence of artifacts	BLADE (mean ± SD)	TSE (mean ± SD)	BLADE (mean ± SD)	TSE (mean ± SD)
Motion	4.96 ± 0.18	4.66 ± 0.72	4.92 ± 0.26	4.67 ± 0.76
Aliasing	4.95 ± 0.20	4.69 ± 0.61	4.98 ± 0.10	4.83 ± 0.4
Radial	4.98 ± 0.10	4.73 ± 0.49	5	4.86 ± 0.34
Visibility of anatomical features	BLADE (mean ± SD)	TSE (mean ± SD)	BLADE (mean ± SD)	TSE (mean ± SD)
Sphincter complex anatomy	5	2.92 ± 0.27	5	2.85 ± 0.35
Pelvic diaphragm visibility	5	2.94 ± 0.27	5	3.79 ± 0.40
Details of ischioanal-ischiorectal fossa	4.94 ± 0.23	4	5	4.38 ± 0.12
Image quality	5	4.89 ± 0.30	4.97 ± 0.14	4.83 ± 0.45

The table shows the average scores for T2W BLADE and T2W TSE sequences. The presence of artifacts was scored for each of the following three categories: artifacts due to bowel or respiratory motion, artifacts due to aliasing, and presence radial artifact (1 = marked, 2 = moderate, 3 = mild, 4 = minimal, and 5 = absent). Some structures were used as references in assessing the visibility of anatomical details. These anatomical structures were the sphincter complex (the visualization of internal and external sphincter), the levator ani-pelvic diaphragm, and the anatomical details of ischioanal and ischiorectal fossa (1 = not visible, 2 = blurry but visualized, 3 = acceptable, 4 = good, and 5 = excellent). The overall image quality was also assessed visually and scored as follows: 1 = unacceptable, 2 = poor, 3 = average, 4 = good, and 5 = excellent.

Moreover, both observers preferred to employ the T2-W BLADE over the T2-W TSE in patients with a history of perianal fistula surgery and in patients who had grade 5 complex fistula.

In evaluating the co-existing pathologies in the ischioanal and ischiorectal fossa, both observers preferred the T2-W BLADE sequence in order to provide a more detailed assessment (Cronbach's α: 0.74).

Discussion

T2-W sequences are important in pelvic and abdominal MR imaging. However, because of their long acquisition time, they are susceptible to motion artifacts [5,6]. Compared with the other T2-W sequences, the T2-W BLADE sequence is promising for the reduction of motion artifacts that obscure important imaging findings as well as for the reduction of the time spent by patients in the scanner bed [7-11].

In this study, we compared the use of T2-W BLADE sequence and T2-W TSE sequence in patients with perianal fistulas. We found that the former produced images with fewer artifacts, with superior anatomical visualization, and with a higher image quality. Moreover, the fact that it shortens the imaging duration is beneficial in maintaining patient comfort. Sequence duration was approximately 1minute and 28 seconds shorter when performed with BLADE sequence.

Designed to compensate and correct for motion artifacts, the T2-W BLADE sequence is increasingly used as a respiratory-triggered sequence in non-compliant patients, including those who have difficulty performing breath-holding maneuvers [10, 11].

While the conventional T2-W TSE sequence techniques fill the k-field in a sequential fashion, the T2-W BLADE sequence fills the k-field with radially oriented blades wherein the center of the k-field is further sampled, resulting in oversampling of the center of the k-space. This oversampling corrects phase, rotation, translation, and weighting to reduce spatial inconsistencies. Furthermore, redundancy in sampling can increase the signal-to-noise ratio, yielding higher quality images [7-9].

In the literature, such an advanced method as radial k-space sampling has been shown to reduce motion artifacts in different body regions [8-12]. For example, Lane et al. compared pelvic imaging using T2-W BLADE and T2-W TSE sequences in 26 female patients; they found that the T2-W BLADE was superior in evaluating the anatomy of the uterine junctional zone, in determining the contours of ovaries as well as in depicting follicles, and in detecting fibroids with fewer motion artifacts [12]. Although the T2-W BLADE technique may reduce motion artifacts due to factors such as patient motion and breathing, it may lead to streak artifacts (i.e., wrap-around and other types of aliasing artifacts) [11, 12].

While we did not directly assess streak artifacts in our study, we did not experience any diagnostic issues related to streak artifacts given that our region of interest (i.e., the perianal region of the pelvis) was located in the center of the image rather than in the periphery where streak artifacts appear.

Another inherent issue in T2-W BLADE is the non-uniform weighting of k-space data in the phase-encoding direction, which may lead to artifacts associated with signal non-uniformity [8, 9]. However, we were not able to assess this issue in our study.

Sahnan et al. stated that the key to understanding the treatment options and the likelihood of their success is deciphering the exact morphology of the tract(s) and the amount of sphincter involved. They also used the T2-W BLADE sequence. They reported that they could easily reveal the anatomy of complex fistulas with the 3D images they created by marking the sphincter complex, the levator plate, and the fistula [13]. In our study, we evaluated the fistula anatomy in three planes. Similarly, we were able to obtain detailed anatomical information about the perianal fistula.

As reported in the literature, the use of endoanal coils to aid in the preoperative diagnosis and evaluation of perianal fistulas have been found inadequate compared with the use of phase sequence coils. In particular, supralevatoric and cutaneous extensions have not been clearly assessed. Moreover, it has been emphasized that endoanal coil application cannot be tolerated by some patients [14].

We obtained the BLADE sequence with the fat suppression technique. Thanks to the suppression of the surrounding fatty tissue in the perianal region, the required contrast for perianal fistula visibility has been increased [1-3, 13].

In our study, the T2-W BLADE sequence allowed for the easy visualization of the supralevator region, especially in coronal plane images. The fact that the imaging does not require any endocavitary application and that the imaging time required less than 25 s for each sequence would ensure patient comfort during the preoperative evaluation for perianal fistula. Another

advantage of this sequence is that antiperistaltic agent was not used.

Our study has some limitations. Firstly, no quantitative measurements were performed to evaluate the T2 contrast of the sequences; instead, we qualitatively evaluated the overall quality of the images. Secondly, diagnosis of active fistula was made with reference to the high-contrast T1-W DCE VIBE sequence images and without reference to pathology. Thirdly, our patient sample consisted of adult patients only. However, we speculate that the T2-W BLADE sequence can be applied in the pediatric age group and may be especially useful for this group, who may have more difficulty in staying still and in holding their breath. Finally, the observers' interpretation of the T2-W BLADE and T2-W TSE images might have been biased as both types of images presented unique image characteristics.

In conclusion, our findings support that the T2-W BLADE is clinically promising for the diagnosis of perianal fistulas and that it offers substantial benefits, including reduced imaging time and significantly reduced ghosting and aliasing artifacts in the images. T2-W BLADE is promising as an important tool for improving the confidence of radiologists in assessing perianal fistulas as well as in yielding important information that will help a treating physician to determine the best medical or surgical treatment for patients.

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Review of ovarian carcinoma with peritoneal metastasis: Rethinking of management

Peritoneal metastazlı over karsinomunun derlemesi: Yaklaşımın yeniden düşünülmesi

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Abstract

Epithelial ovarian carcinoma (EOC) is a type of cancer that is usually diagnosed in advanced stages. To date, the standard treatment of EOC is surgery with neoadjuvant or adjuvant platin and taxane-based systemic chemotherapies. Hyperthermic intraoperative intraperitoneal chemotherapy (HIPEC) is considered in cases of recurrence of EOC. HIPEC has been accepted as a gold standard for treating pseudomyxoma peritonei of ovarian and appendiceal origin. More recently, a randomized clinical trial supports that HIPEC is a promising treatment option for peritoneal metastasis (PM) of primary ovarian cancer (OC) in surgical series. HIPEC is also an effective treatment option in primary and recurrent cancer cases with PM of OC. As a result, the standardization and optimization of the HIPEC technique, determination of patient subgroups with PM of the OC responding to treatments, personalized evaluation, and the treatments currently carried out by the multidisciplinary team still need to be re-evaluated. This review aimed to update the standard treatment approach in PM of OC, along with the systemic treatments and HIPEC treatment approaches combined with surgery.

Keywords: Peritoneal metastasis, ovarian cancer, HIPEC, cytoreductive surgery, hyperthermic intraperitoneal intraoperative chemotherapy.

Öz

Epitelyal tipte over karsinomu (EOK), genellikle ileri evrelerde teşhis edilen bir kanser türüdür. Günümüzde, EOC'nin standart tedavisi neoadjuvan veya adjuvan platin ve taksan bazlı sistemik kemoterapi ile kombine edilen cerrahidir. EOC'nin rekürrensinde hipertermik intraoperatif intraperitoneal kemoterapi (HİPEK) düşünülmektedir. HİPEK, over ve apendiks kaynaklı psödomiksoma peritonei tedavisinde altın standart olarak kabul edilmiştir. Son zamanlarda, randomize bir klinik çalışma, HIPEK'in, cerrahi serilerde primer over kanserinin (OK) peritona metastazı (PM) için umut verici bir tedavi seçeneği olduğunu desteklemektedir. zamanlarda, randomize bir klinik çalışma, HIPEK'in, cerrahi serilerde primer over kanserinin (OK) peritona metastazı (PM) için umut verici bir tedavi seçeneği olduğunu desteklemektedir. HİPEK'in ayrıca primer ve rekürren OK'nin PM vakalarında da etkili bir tedavi seçeneği olduğu bildirilmektedir. Sonuç olarak, HİPEK tekniğinin standardizasyonu ve optimizasyonu, OK'nin PM olan hastalarda tedavilere yanıt veren alt gruplarının belirlenmesi, hali hazırda kişiselleştirilmiş değerlendirme ile multidisipliner ekip tarafından yürütülen tedavilerin yeniden değerlendirilmesi gerekmektedir. Bu derlemede OK'nin PM durumunda standart tedavi yaklaşımı ile birlikte uygulamadaki sistemik tedaviler ve cerrahi ile kombine edilmiş HİPEK tedavi yaklaşımlarının güncellenmesi amaçlanmıştır.

Anahtar Kelimeler: Periton metastazı, over kanseri, HİPEK, sitoredüktif cerrahi, hipertermik intraperitoneal intraoperatif kemoterapi.

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Introduction

Epithelial ovarian carcinoma (EOC) arises from the surface epithelium composed of peritoneal mesothelial cells [1]. Patients with EOC usually present at advanced stages of the disease due to the absence of symptoms in the early stages.

There are four main types of EOC: serous ovarian carcinoma (30% to 70%), mucinous ovarian carcinoma (5% to 20%), endometrioid ovarian carcinoma (10% to 20%), clear cell carcinoma (3% to 10%) as well as a small group of transition cells (Brenner tumor) (1%) and mixed epithelial carcinosarcoma (3%) [2]. EOC, fallopian tube carcinoma, and primary peritoneal carcinoma (PPC) are treated as a single disease entity according to NCCN protocol.

The latest evidence showed that the fallopian tube carcinomas might also be the source of some EOC subtypes [3]. EOC usually remains in the peritoneum, a barrier to all peritoneal surfaces in the abdominal cavity. Therefore, EOC is generally designated as a Peritoneal Surface Malignancy (PSM). The surgeons specializing in peritonectomy procedures and hyperthermic intraoperative chemotherapy believe that these patients can be treated or even cured with cytoreductive surgery, including peritonectomy and combinations of intraperitoneal and systemic chemotherapy.

Here, we would like to review the standard care of the EOC and evidence-based treatment success of intraperitoneal chemotherapy applications such as hyperthermic intraperitoneal chemotherapy with an ultraradical surgical approach.

Standard of care as a first-line therapy for EOC

The standard systemic chemotherapy regimen for EOC is the combination of carboplatin (CarboDDP) area under curve 5 or 6 and paclitaxel (PTX) 175 mg/m2 administered every three weeks [4]. The benefit of secondary surgery in patients who had a residual tumor size bigger than 1 cm after primary surgery as part of first-line therapy was evaluated in the EORTC-55865 trial [5]. This trial included FIGO stage IIb-IV patients with residual disease to complete ≥ X6 chemotherapy or 3Xchemotherapy followed by interval debulking surgery (IDS) and then additional adjuvant X3 chemotherapy. There are significant survival advantages in patients who underwent IDS, according to the result of this study. Unfortunately, this result has not been confirmed in the GOG-152 trial. When the chemotherapy regimen is replaced with a combination of paclitaxel (PTX) and cisplatin (CDDP) instead of cyclophosphamide and cisplatin, OS has shown no superiority to each other between IDS and primary DS groups. Accordingly, the IDS approach did not improve OS in these patients [6].

Intraperitoneal chemotherapy

EOC is confined into the peritoneal cavity as a natural feature of the disease. Therefore, locoregional treatment has been considered as a treatment option of EOC for almost three decades. Indeed, GOG-172 was designed for small volume residual disease [7] (Table 1). A significant increase in progression-free survival (PFS) and 15.9 months OS with intraperitoneal (IP) chemotherapy arm than systemic chemotherapy arm with this trial. Two more large phase III trials GOG 104 [8] and GOG 114 [9], have also demonstrated longer OS compared to systemic chemotherapy ($p<0.05$) (Table 1). IP chemotherapy's role in treating newly diagnosed advanced EOC has not been subjected to controversy for almost three decades. Also, IP chemotherapy is not widely used in US or European Centers due to the high rate of complication and low rate of completing six cycles of chemotherapy, even though both NCI accepted, and Phase III trials indicated that the IP treatment has a survival superiority systemic chemotherapy.

In EOC treatment, physicians do not prefer IP chemotherapy due to complications such as catheter-related complications that may develop due to IP chemotherapy. We demonstrated in our unpublished series that i.p. chemotherapy can be tolerated safely and effectively for up to four cycles in patients with peritoneal metastasis. Other experts also reported that i.p. chemotherapy could be well tolerated for up to 6 cycles for peritoneal metastases.

Besides these studies, the GOG-252 trial reported that IP chemotherapy did not have an OS advantage to the systemic chemotherapy when bevacizumab was added to both IP and systemic chemotherapy arms [10]. GOG 252 has another advancement toward identifying upfront treatment of advanced stages EOC using combinations of the systemic use of bevacizumab with IP platinum and paclitaxel.

Altogether, these trials unfortunately failed to define IP chemotherapy's exact role in advanced EOC at the time of initial diagnosis. GOG-104 did not assess progression-free survival with cyclophosphamide. GOG-114 was designed with high dose induction chemotherapy and a weekly and dose intense regimen in the IP arm that seems irrelevant for clinical practice. Finally, GOG 252 is the only trial that evaluated the effect of chemotherapy with IP route and a comparison of cisplatin with carboplatin. More recently, the advantage of IP chemotherapy was analyzed on OS. This study showed that IP chemotherapy has some advantages as OS beyond ten years, effective cytotoxicity in patients with macroscopic (gross) residual disease of <1 cm; and prolonged OS in association with the number of IP cycles was detected when the retrospective data of GOG 114 and 172 compared [11].

Dose dense therapy

Novel trial testing dose-dense weekly paclitaxel showed longer progression-free survival (28.1 vs. 17.5 mo) and better median OS (100.5 vs. 62.2 mo) in patients with OC [12]. However, this survival benefit could not be confirmed in the Western GOG-262 Trial (median OS 40.2 vs. 39.0 months) [13]. This trial also supported that bevacizumab alone was effective as much as weekly paclitaxel dose-dense therapy. According to the ICON-8 trial, weekly dose-dense therapy of paclitaxel as a part of first-line therapy did not prolong progression-free survival [14]. Progression-free survival also did not show a difference between carboplatin/paclitaxel regimen and weekly carboplatin plus paclitaxel regimen according to Multicenter Italian Trials [15].

Molecular therapy

Angiogenesis is the initiation stage of tumorigenesis for tumor proliferation and invasion. Therefore, Anti-angiogenic therapy and, more recently, BRCA mutation have also been selected as a targeted molecular therapy of EOC. Indeed, vascular endothelial growth factor (VEGF) has been reported as the most important progressive factor for EOC [16]. In this study, progression-free survival is significantly longer in the bevacizumab arm, whereas OS was not different in double-blind 3-arm with bevacizumab and paclitaxel carbo platinum in the GOG-18 study [17]. A similar result with no OS and no

progression-free survival benefit was also reported using bevacizumab to the standard systemic chemotherapy in 2 arm ICON-7 trial [18]. Besides this, progression-free survival and median OS were more remarkable in high-risk group patients with Grade III or clear cell histology in the group added bevacizumab to the Standard chemotherapy arm [18].

The patients were recently randomized for other anti-angiogenic agents, Pazopanib (oral tyrosine kinase inhibitor), after completing debulking surgery and standard first-line chemotherapy in the AGO OVAR-16 trial [19]. Again, progression-free survival was improved in the Pazopanib arm, whereas no difference was observed for OS in this study.

Table 1. Intraperitoneal chemotherapy trials for EOC; n: number of the patients; PTX: paclitaxel; CDDP: Cisplatin; iv: intravenous, ip: intraperitoneal; p value<0.05 is statistically significant; OAS: overall survival; PFS: progression free survival; Ns: non significant.

Study	Regimen	OAS advantages (months) ip *vs* iv	PFS advantages (months) ip *vs* iv
GOG 172 [7] (n=175)	iv PTX+CDDP *vs* iv PTX with ip PTX+CDDP	65.6 vs 49.7 (p=0.03)	23.8 *vs* 18.3 (p=0.05)
GOG 104 [8] (n=546)	ip CDDP/iv CDDP *vs* iv CDDP/iv cyclophosphamid	49 *vs* 41 (p=0.02)	Ns *vs* Ns
GOG 114 (n=462)	High-dose iv carboplatinx ip CDDP *vs* iv paclitaxel/iv CDDP	63 *vs* 52 (p=0.05)	28 *vs* 22 (p=0.01)
GOG 252 (n=1381)	ip carboplatin/iv PTX+bevacizumab *vs* ip CDDP/ivPTX/ip PTX day 8		28.7 (p=0.41) 27.8 (p=0.73)
ICON 7 (n=1528)	iv carboplatin/iv PTX/iv bevacizumab-placebo for 1 year *vs* iv carboplatin+iv PTX/iv bevacizumab by bevacizumab for 1 year	45.5 *vs* 44.6 (p=0.85)	24.1 *vs* 22.4 (p=0.04)

Trebananib is a recombinant peptide that blocking the binding of Angiopoietin-1 and -2 to Tie2 to inhibit angiogenesis. Trinova-3/ENGOT-ov2 Phase III trial did not support additional overall survival improvement compared to systemic carboplatin and paclitaxel treatment [20].

Nintedanib, which is a potent oral inhibitor of growth factor receptors (vascular growth factor receptors-1,-2,-3; platelet-derived growth factor receptors-α, -β and fibroblast growth factor receptors), has been investigated as first-line chemotherapy in addition to standard systemic chemotherapy in AGO-OVAR-12 as Phase III clinical trial [21]. Even though OS has not been reported, progression-free survival was improved with Nintedanib arm in addition to systemic chemotherapy in patients with EOC after primary complete surgical resection.

In addition to these achievements, the inhibitors of the enzyme poly-ADP ribose polymerase (PARP) promise new agents that report impressive survival improvement in recurrent EOC [22]. This result led to the design of the PAOLA, PRIMA, GOG-3005, and SOLO-trials to investigate the role of PARP inhibitors in the first-line maintenance therapy. SOLO-1 Phase III trial was designed in BRCA1/2 mutated EOC patients who primarily responded to platinum-based chemotherapy. These patients were received two years of Olaparib therapy. Progression-free survival was improved by 70%, with a lower risk of disease recurrence. Progression or death was found to be lower in Olaparib's arm.

Neoadjuvant chemotherapy

Neoadjuvant chemotherapy (NAC) followed by interval debulking surgery and adjuvant chemotherapy has also been considered in stage IIIC and Stage IV patients with EOC to achieve complete resection. NAC was investigated in EORTC trial 55971 [23] and Chorus Trial [24]. However, OS or progression-free survival was not improved with NAC whereas less complication and lower postoperative mortality were recorded in the NAC arm. Therefore, NAC seems to be very important in decreasing morbidity and mortality related to surgery. Two further studies, JCOG-602 [25] and SCORPION trial [26], also reported similar results for NAC, but also NAC did no have inferiority to surgery.

Surgery and HIPEC

Complete cytoreduction to remove all macroscopic residual disease is the most important independent prognostic factor to prolong overall survival and even to cure patients with EOC [23]. Indeed, when the surgical series were examined, some patients had a bulky residual tumor after surgery.

Therefore, surgery and experience in upper abdominal surgery with peritonectomy procedures seem to be essential to perform surgery to achieve maximum surgical success. Peritoneal Surface Oncology Group International (PSOGI) has been designated as the organization aiming to standardize the techniques and procedures for diseases characterized by peritoneal metastasis. The peritoneum is also considered as a potential metastasis site for EOC. We consider that peritoneum is the potential metastasis site of EOC due to the peritoneal metastasis being a part of this disease's natural history. Therefore, a combination of radical surgical resections with peritonectomy procedures and intracavitary application of heated chemotherapy was developed by peritonectomy surgeons.

The rationale of HIPEC is that locoregional disease could only be treated with the addition of regional therapies to the systemic therapy. Peritonectomy surgeons also perform complete surgical resections with total greater and lesser omentectomy. Then HIPEC is added to kill intraabdominal microscopic tumor cells that penetrated the intraperitoneal tissue after surgical resection. This relatively new therapeutic approach, which consists of the addition of HIPEC to cytoreductive surgery, has been widely used by surgeons dealing with peritoneal metastasis developed from appendiceal mucinous neoplasms and colorectal cancer.

The first report of HIPEC performed for EOC was presented in 1993 [27]. Then, 141 patients with EOC were treated with cytoreductive surgery and HIPEC either frontline,

interval debulking, for recurrence and consolidation [28]. Platinum sensitive disease, shorter hospital stays and completeness of cytoreduction were found to be associated with increased overall survival, and the median OS was 30.3 months.

A systematic review and meta-analysis demonstrated that the benefit of HIPEC in addition to cytoreductive surgery was up to 8 years compared to cytoreductive surgery alone. Cytoreductive surgery with HIPEC had significantly better 1-year survival than standard surgery and systemic chemotherapy group [29].

More recently, there are two phase III trials investigating the effects of HIPEC. The first multicenter randomized study was designed by adding HIPEC to frontline cytoreductive surgery that showed no benefit for both progression-free survival and OS with HIPEC. These results were presented at ASCO Annual Meeting 2017 [30]. However, Van Driel et al. [31] investigated the effects of HIPEC in addition to the interval debulking surgery in patients with stage III EOC patients. The median progression-free survival was 14.2 months in the cytoreductive surgery plus-HIPEC group, whereas it was 10.7 months in the cytoreductive surgery group alone. Median overall survival was 45.7 months in the cytoreductive surgery-plus-HIPEC group and 33.9 months in the cytoreductive surgery group. These results showed a 12-month improvement in OS with the addition of HIPEC to cytoreductive surgery in interval debulking surgery setting [32].

A phase III prospective randomized study on HIPEC in recurrent cases reported an improved overall survival up to 13.3 months [32]. However, this trial was reported to have limitations such as statistical analysis and randomization, and validity problems [33].

There are several limitations of the above studies. Firstly, all studies were reported by Gynecologic Oncology groups. Peritoneal metastasis of EOC can be considered as a gynecologic oncological disorder. However, Stage IIIC EOC is already metastatic disease and the disease spreads into the peritoneal surface in the entire abdomen. When the PM of OC develops the right diaphragmatic and the left diaphragmatic surface and subcapsular area of the liver with lesser and greater omentum and omental bursa are heavily affected with the disease as much as pelvic peritoneum and bowels and their mesentery. These areas are called as upper abdomen and can be resected by peritonectomy surgeons easily. Therefore, incomplete cytoreduction is highly likely performed during the first surgery due to non-resected greater and lesser omentum and diaphragmatic peritoneum in these cases. These patients are also admitted to gynecologic surgery departments when the ascites or Stage IIIC ovarian carcinoma was occurred. Oncological surgeons might involve the surgery if they were called by the patients or the gynecologic oncologists during surgery. Therefore, above studies were carried out by gynecological oncologists. Based on peritoneal spreading of the disease, PM of OC can be managed with both peritonectomy surgeons and gynecologic oncologists specialized for OC. Therefore, above studies have a limitation to achieve complete cytoreduction to leave no macroscopic disease either in upfront surgery arms or in neoadjuvant arms. Again, a collaborative effort between surgical oncology and gynecological oncology seems to be not possible even though both groups' efforts are the cure of these patients. Secondly, intraperitoneal port placement and intraperitoneal chemotherapy are challenging and invasive procedures. Therefore, the intraperitoneal chemotherapies perform in special trained centers in worldwide. Intraperitoneal port can cause intraabdominal infection and sepsis and perforation and obstruction and fibrosis. These serious complications prevent the widely use of intraperitoneal chemotherapy and as well as HIPEC in all centers.

In addition to peritonectomy procedures as surgical training, intraperitoneal chemotherapy applications and usage of intraperitoneal chemotherapy need to be optimized and to become standard protocol for these patients. Altogether, we still have failed to cure these patients with these standard treatment protocols stated previously.

In conclusion, the standard of care for peritoneal metastasis of EOC is debulking surgery and followed by a combination of paclitaxel and carboplatin as a frontline therapy. The addition of bevacizumab to standard chemotherapy is suggested for patients with a high risk of disease progression. To date, the effects of HIPEC were not determined except the last Phase III trial supporting the improvement in overall survival with HIPEC in primary cases of EOC.

To achieve the cure of this disease;

- Subgroups of EOC need to be classified on molecular bases and documented well with symptoms and pathological characteristics.

-Neoadjuvant chemotherapy approach needs to be improved by changing the route of application such as intracavitary applications of chemotherapy.

-The role of hyperthermia and pressurized application of intraperitoneal chemotherapy applications need to be well characterized with future studies.

- Intraoperative diagnosis of peritoneal metastasis needs to be improved.
- Multicenter randomized clinical trials with specialized surgeons and gynecological oncologists and medical oncologists and pathologists seem essential.
- Consensus statements need to be developed with the shared efforts of gynecological oncologists and surgeons and medical oncologists, and pathologists.
- The clinical significance of HIPEC and other techniques needs to be clarified, and the standard of care effort for the selection of the patients needs to be defined with international collaborative effort.

Therefore, new treatment strategies seem to be essential to cure these patients from their initial treatment stages.

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DOI:10.25000/acem.828037 Olgu Sunumu / Case Report

Simultaneous scar-less laparoscopic cholecystectomy with abdominoplasty

Abdominoplasti ile eşzamanlı skarsız laparoskopik kolesistektomi

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Abstract

With abdominoplasty, loosening and sagging problems caused by excessive weight loss are corrected. After bariatric surgery, especially Roux-en-Y gastric bypass (RYGB), the frequency of gallstone formation increases compared to the normal population due to rapid weight loss. Even if the patient is asymptomatic, it is important to perform an abdominal ultrasound examination of all patients who admit for abdominoplasty, especially following postoperative weight loss for morbid obesity. In this study, we tried to state that scarless laparoscopic cholecystectomy and abdominoplasty can be performed safely.

Keywords: bariatric surgery, cholecystectomy, abdominoplasty

Öz

Karın germe ameliyatı ile aşırı kilo vermeye bağlı oluşan gevşeme ve sarkma problemleri düzeltilir. Bariyatrik cerrahi, özellikle de Roux-en-Y gastrik bypass (RYGB) sonrasında, hızlı kilo kaybından dolayı safra taşı oluşum sıklığı normal populasyona göre artar. Hasta asemptomatik olsa bile, özellikle morbid obezite için ameliyat sonrası kilo kaybını takiben, abdominoplasti için gelen tüm hastaların abdominal ultrason muayenesi yapılması önem arzeder. Bu çalışmada, izsiz laporoskopik kolesistektomi ve abdominoplastinin güvenli bir şekilde uygulanabileceğini ifade etmeye çalıştık.

Anahtar Kelimeler: bariatrik cerrahi, kolesistektomi, abdominoplasti

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Introduction

Today, abdominoplasty is the most commonly used procedure among body shaping procedures [1]. With this surgical intervention, while loosening and sagging problems caused by excessive weight loss are corrected, the body image and quality of life of the patients also improve [2-4]. After bariatric surgery, especially Roux-en-Y gastric bypass, the frequency of gallstone formation increases compared to the normal population due to rapid weight loss [5, 6]. Supersaturation of hepatic bile with cholesterol, gallbladder stasis and increased concentration of mucin in the bile are the possible causes [7].

The objective of this study was to present the phenomenon of laparoscopic cholecystectomy and abdominoplasty which we diagnosed at the same period the abdominal laxation, diastases of muscle recti and cholelithiasis without leaving any trocar entrance mark.

Case Presentation

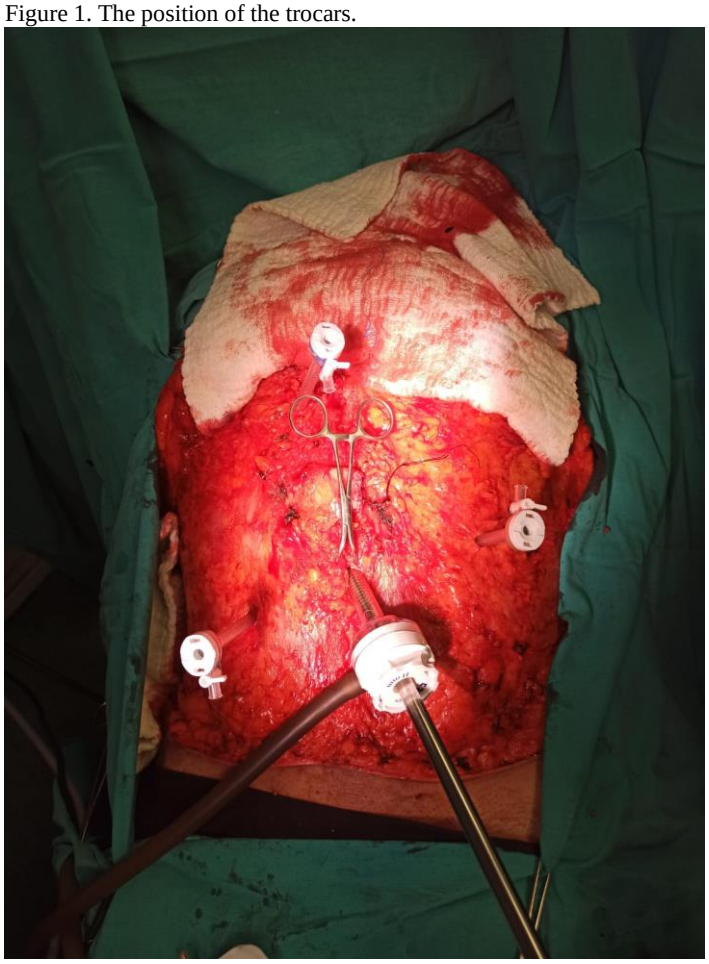
Our patient was 24 years old male patient presented to our hospital with abdominal wall laxity after massive weight loss of about 30 kilograms. He came seeking for a cosmetic procedure (abdominoplasty) to reduce the abdominal wall laxity and skin excess. During the clinical follow-up, he presented three recurrent biliary colic and recurrent attacks of abdominal pain in right upper quadrant. Abdominal ultrasound examination was performed and revealed chronic calcular cholecystitis with multiple gall bladder stones with non-dilated common bile duct and no manifestations of jaundice. The initial plan of management of the patient was to combine both the abdominoplasty procedure with laparoscopic cholecystectomy.

The surgery was started in supine position under general anesthesia. At the beginning of the intervention the plastic surgeon started marking the abdominal flap. An extended Pfannenstiel incision and the generation of the abdominal skin flap were made up to the xiphoid process. On the muscular wall a 10mm Hasson trocar was placed at the umbilical level to generate pneumoperitoneum and was used as a chamber port. Next, 5 mm trocars were introduced under direct view. The position of the trocars can be seen in Figure 1. Then the patient was placed in a reverse Trendelenburg position of 30° while rotating the table to left side by 15°. Laparoscopic cholecystectomy was performed. Cystic artery and cystic duct were identified, clamped, and divided. The gallbladder was removed through epigastric midline port using retrieval bag. Abdominal wall parts were closed with non-absorbable suture material. The patient was repositioned in supine position. Abdominoplasty, that is, rectus plication, neo-omphaloplasty, and dermolipectomy of infra-umbilical region was performed. Abdominoplasty wound was closed in layers with a suction drain in situ in the plane of dissection, that is, in between the rectus sheath and the fat layer and flap held with tight dressing. The procedure of combined abdominoplasty and cholecystectomy was completed without leaving any signs of cholecystectomy, and this led to a better cosmetic result from the patients' point of view. There was no complication from both procedures, and full ambulation was obtained post operatively. The patient's consent was obtained for this case study.

Discussion

Standard abdominoplasty is generally applied in patients with upper and sub-umbilical skin laxity in the area limited to the anterior aspect of the lower body. An incision is made in the lower abdominal area that will remain in a bikini or underwear, and a second incision must be made around the umbilicus. The skin and fat layer are separated from the abdominal wall up to the lower border of the ribs. The abdominal muscles are tightened by a surgical procedure called plication. Excess skin and adipose tissue are excised. The operation is terminated by placing drains on both sides of the abdomen [8, 9].

Figure 1. The position of the trocars.



The amount of cholesterol and mucin in bile increases after bariatric surgery. As fatty food intake will decrease after bariatric surgery, gallbladder movements decrease. Bile salt release decreases with weight loss. For these reasons, the possibility of stone formation in the gallbladder increases with rapid weight loss after bariatric surgery. Too high BMI (body mass index) of the patient before bariatric surgery and losing more than 25% of his weight before bariatric procedure are also risk factors for stone development [5-7].

The combined abdominoplasty with cholecystectomy was performed in 3 cases laparoscopically in 2012 with no complications and this led to a better cosmetic result from the patients' point of view [10]. Combined laparoscopic cholecystectomy with abdominoplasty were achieved with successful outcome without any complications [11]. Combining two or multiple abdominal procedures may reduce the potential risks of multiple anesthesia for each procedure, shorten total hospital stay and operating room time, and, perhaps most importantly for the patient, reduce costs and time off work [12].

Scarless cholecystectomy has been described in the past using the single port technique through the umbilicus and natural orifice translumenal endoscopic surgery (NOTES) from the vagina [13-15]. Our technique is a modification of the routine 'French technique' [16, 17] used for laparoscopic cholecystectomy, where all ports were placed directly on the fascia and muscle wall during abdominoplasty, thus avoiding visible scars of cholecystectomy. Good surgical teams can safely and effectively combine abdominoplasty with intra-abdominal procedures [18]. The combined surgery in the same surgical time allows to avoid skin incisions and reduce the potential risks of multiple anesthesia for each procedure, and it also reduces costs and postoperative recovery [19].

Conclusion

In the present study, even if the patient is asymptomatic, we recommend abdominal ultrasound examination of all patients who admit for abdominoplasty following postoperative weight loss especially for morbid obesity.

The present study also suggests the technique we use for the port placement because it is convenient, easy, and has no side effects on patients compared to other techniques.

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An interesting foreign body in the urinary system: Metal clips

Üriner sistemde sıradışı bir yabancı cisim: Metal klips

Cem Şenol [1], Coşkun Kaya [2]

Abstract

Migration of surgical materials into the urinary system is a rare situation. Forgotten and/or fragmented ureteral stents were defined more frequently as foreign bodies in urinary system in the literature. But surgical clips migration is an interesting case. In this paper, we aim to present a 31-year-old man with a medical history of multiple urinary operations. We extracted two metal clips, one of them was from the ureter and the other from the renal pelvis of this patient. The case is discussed in the light of the literature.
Keywords: urinary system, metal clips, foreign body

Öz

Cerrahi materyallerin üriner sisteme taşınması nadir bir durumdur. Literatürde daha sık olarak unutulmuş ve/veya parçalanmış üreteral stentler üriner sistemdeki yabancı cisimler olarak tanımlanmıştır. Ancak cerrahi klipslerin yer değiştirmesi ilginç bir durumdur. Bu yazıda, çoklu üriner operasyon öyküsü olan 31 yaşındaki bir erkeği sunmayı hedefliyoruz. Bu hastanın biri üreterden, diğeri renal pelvisten olmak üzere iki metal klips çıkardık. Olgu literatür ışığında tartışılmıştır.
Anahtar kelimeler: metal klips, üriner sistem, yabancı cisim

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Introduction

Surgical material migration into the urinary system does not occur frequently. Foreign bodies detected in the urinary system in urological surgeries can usually be together with stones. Although the most common foreign bodies are urinary stents and stent residues, metallic clips used for bleeding control can also be encountered in the urinary tract.

We present a case of migration of a metal clip into the right ureter 12 years after an operation for right kidney calculi.

Case report

A 31-year-old man was referred to our clinic with symptoms of right flank pain, hematuria, and dysuria for two months leading up to our consultation. In his medical history he had undergone urinary operations seven times, he was unaware what these were for, he only knew that they were performed for stones in his urinary system. We received the information that no other abdominal organ surgery was performed. In a physical examination, we found he had four laparoscopic port entry scars on the right side of the abdomen and a 20 cm median abdominal incision. His renal functions were normal, and he had microscopic hematuria in a urine analysis. We performed a plain abdominal radiograph and a non-contrast computerized tomography (CT) after the urinary ultrasonography showed bilateral hydroureteronephrosis (Figure 1, 2). The CT showed a 7 mm long right ureteral stone in the distal ureter, another stone in the right kidney, and grade 4 hydroureteronephrosis in proximity to the stone in the distal ureter (Figure 2).

We decided to perform a ureterorenoscopy of the right ureter and reached the stone, and holmium laser lithotripsy was performed. Inside the stone we saw a metal fragment. After all the stone was cleared, we removed the metal fragment from the ureter with forceps and we saw that it was a metal clip, as used in laparoscopic procedures to control bleeding (Figure 3). We inserted a double j catheter into the right ureter. In the following month we performed a second ureterorenoscopy for the kidney stone. We removed the double j catheter out of the ureter and in the renal pelvis we saw another metal clip with a stone covering it, which was lodged into the renal pelvis wall. Holmium laser lithotripsy was performed, the stone was cleared, and the metal clip was extracted from the renal pelvis. A double j catheter was again inserted into the right ureter. Post-operative first day plain abdominal radiography was performed and there was no evidence of any residual stone or foreign body.

Written informed consent was obtained from patient for publication..

Discussion

A foreign body can reach the urinary tract by retrograde migration from the bladder, during the operation, from the intestinal tract, or after renal trauma [1]. Forgotten and fragmented ureteral stents have been defined more frequently as foreign bodies in the urinary system in the literature. Stone encrustation on such forgotten ureteral stents may cause complications and further medical approaches would then be needed. [2]. Similar cases, such as ours, have been reported by Miller et al. [3], Massud W [4], Brusky et al. [5]. In our case, we think that our patient had initially undergone a laparoscopic pyelolithotomy, but, surgical clips were applied and one of the metal clips fell in the collecting system because of bleeding during the surgery. There have been case reports of clips migrating to the urinary system after the partial nephrectomy [6,7]. So, the migrating of the clips should be kept in mind and the patients should be informed after the laparoscopic pyelolithotomy like the partial nephrectomy.

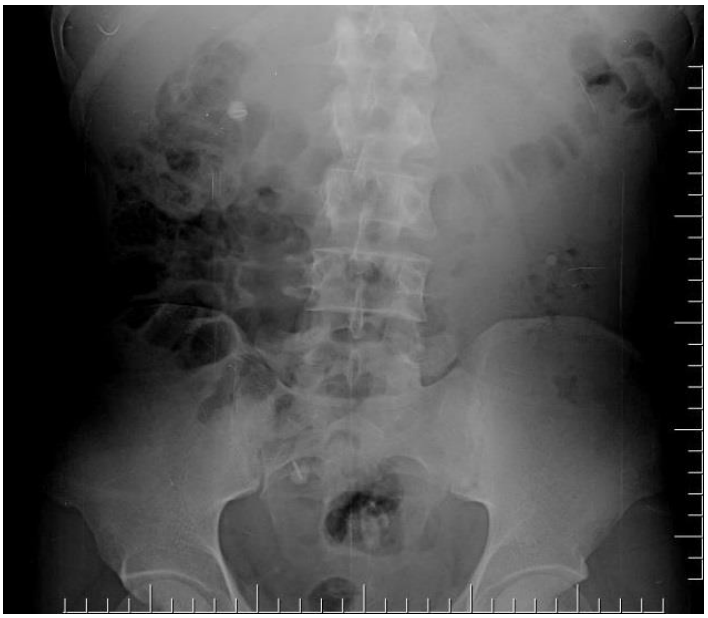

Figure 1: Plain abdominal radiograph of the patient.

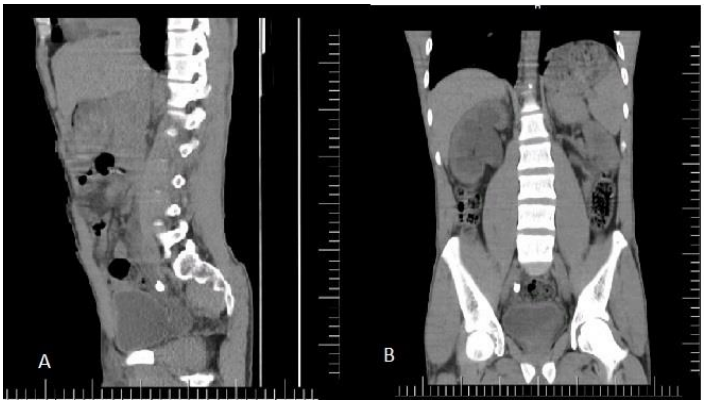

Figure 2: a. Sagittal plane image of the stone and the metal clips, b. Coronal plane image of the metal clip in the stone settled in the ureter.

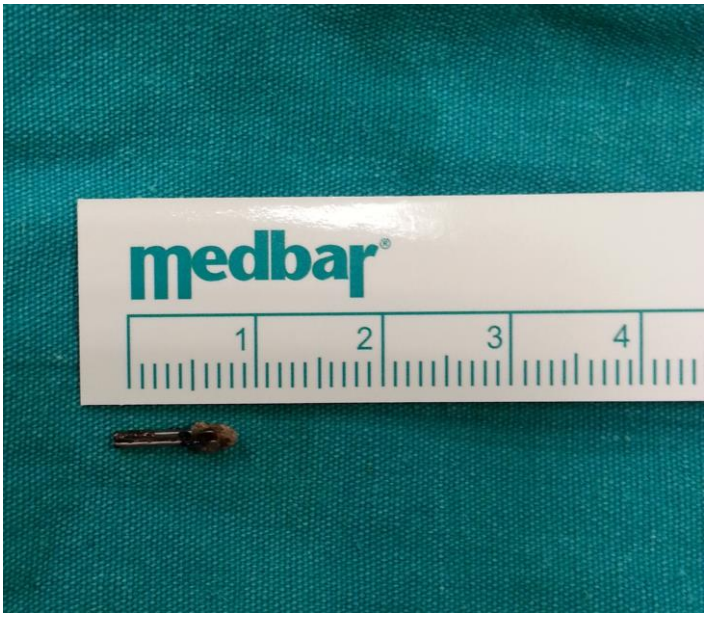

Figure 3: The metal clip which had been extracted from the ureter.

In conclusion, surgical clips are useful tools in laparoscopic cases, but we should keep in mind that over time they may migrate other than the anatomic area where they were

used, and we should consider the possibility of clip displacement in patients with follow-up complaints, otherwise it may cause serious problems in the future.

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