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Original Article

Serum renalase and cerebellin levels in acute central serous chorioretinopathy

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ABSTRACT

Aim: To compare the blood renalase and cerebellin-1 levels of acute central serous chorioretinopathy (CSC) patients with healthy subjects.

Method: A total of 33 eyes with acute naïve CSC (less than 2 months duration) and 31 healthy subjects were enrolled in this study. Idiopathic CSC was diagnosed based on the presence of a serous detachment of the neurosensory retina involving the macula that was confirmed using optical coherence tomography and leakage at the retinal pigment epithelium level using fluorescein angiography. Blood samples were collected and centrifuged at 4000 g for 10 minutes. The serum samples were collected and stored at -80 °C until required for analysis. Serum renalase and cerebellin-1 levels were measured using an ELISA kit.

Results: In CSC group 11 patients were female and 22 patients were male. In control group 10 participants were female and 20 were male. The sex was similar between groups. Mean age in CSC group was 41, 04±5, 94, in control group was 40, 67±6, 53. Mean ages were similar between groups. Mean renalase levels in CSC group was 27, 19±14, 01 ng/ml and in control group was 19, 12±15, 57 ng/mL. Mean renalase level was higher in CSC group. Mean cerebellin levels were 57, 76±29, 72 pg/mL and 52, 50±29, 25 pg/mL in CSC and in control groups, respectively. Mean cerebellin levels were similar in groups.

Conclusion: Comparing with healthy subjects serum renalase levels were higher and cerebellin-1 levels were similar in CSC patients.

Keywords: Cerebellin, central serous chorioretinopathy, optical coherence tomography, renalase.

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Introduction

Central serous chorioretinopathy (CSC) is a common retinal disease characterized by a localized serous detachment of retina at macular region and has a relative high recurrence rate. Etiology and pathogenesis of CSC still remains ambiguous [1]. Focal retinal

pigment epithelium (RPE) barrier defects are likely related with CSC progression. Mechanical stress caused by intrachoroidal pressure elevation or dilated vessels in choroid, reduced RPE adhesion and RPE atrophy secondary to choriocapillaris hypoperfusion, high cortisol and catecholamine levels are some proposed mechanisms [2,3].

Cerebellin is derived from precerebellin and has neuromodulatory functions such as maintaining of synaptic structures and modulating of their functions. Today four cerebellin subtypes (Cbln1-4) are known [4]. It was previously thought that cerebellin genes were expressed only in the brain. However, it has been determined that cerebellin is secreted from adrenal gland, neuroendocrine system and pancreas [4-6]. Cerebellin mRNA was shown to be expressed in the tumour tissues of phaeochromocytoma, cortisol-producing adrenocortical adenoma, ganglioneuroblastoma and neuroblastoma [7]. Cerebellin has a stimulating effect on the secretion of aldosterone, cortisol and catecholamine from the adrenal glands [8]. Renalase is monoamine oxidase enzyme originating mainly from renal tissues directly degrades circulating catecholamines, (noradrenaline, adrenaline and dopamine) [9].

With the lights of aforementioned information, renalase and cerebellin speculatively may have role in the pathogenesis of CSC. In this study we aimed to compare the blood renalase and cerebellin-1 levels of acute CSC patients with healthy subjects.

Materials and Methods

The study followed the tenets of the Declaration of Helsinki and was approved by the institutional clinical researches ethics committee (Approval Date: 22th Nov 2017). Informed consent was obtained from all of the participants. A total of 33 eyes of 33 patients with acute naive CSC and 30 eyes of 30 healthy subjects were enrolled in this study. The inclusion criterion for the study group was to have CSC less than 2 months duration. Inclusion criterion for the control group was to be healthy without any systemic disorder. The exclusion criteria included the following; history of systemic disorders such as hypertension, diabetes mellitus etc, systemic or topical use of vaso-active drugs which may affect blood pressure (such as pseudoephedrine, timolol etc). Patients underwent complete ophthalmologic examination including best corrected visual acuity, ocular tonometry, biomicroscopy, detailed fundus examination, fundus fluorescein angiography and optical coherence tomography (OCT). All patients were referred to the internal medicine outpatient clinic for systemic evaluation, especially systemic hypertension. All OCT scans and measurements were acquired through a dilated pupil with using the RTVue XR Avanti with AngioVue (Optovue Inc., Fremont, CA, USA).

Idiopathic CSC was diagnosed based on the presence of a serous detachment of the neurosensory retina involving the macula that was confirmed using optical coherence tomography and leakage at the retinal pigment epithelium level using fluorescein angiography. Blood samples were collected from patients and centrifuged at 4000 g for 10 minutes. The serum samples were collected and stored at -80 $^{\circ}$C until required for analysis.

Enzyme-Linked Immunosorbent Assay

Serum renalase [Human Renalase ELISA kit catalog number: 201-12-3148 Shanghai Sunred Biological Technology Co., Ltd, Shanghai, China] and cerebellin-1 [Human Cerebellin-1 ELISA kit; catalog number: 201-12-3438

Shanghai Sunred Biological Technology Co. Ltd, Shanghai, China] levels were measured using enzyme-linked immunosorbent assay method according to the manufacturer's protocol. Specimen absorbance values were determined on Multiskan FC microplate reader (Scanlt for Multiscan FC 2.5.1, Thermo Fisher Scientific, and Finland) at a wavelenght of 450 nm. Values were expressed as nanogram/mL for renalase and picogram/mL for cerebellin. The intra-assay coefficient of variance (CV), inter-assay CV, detection range and sensitivity of the renalase kit were reported as <10%, <12%, and 3-700 ng/mL and 2.156 ng/mL, respectively. The intra-assay CV, inter-assay CV, detection range and sensitivity of the cerebellin-1 kit were reported as <10%, <12%, and 5-1500 pg/mL and 4.385 pg/mL, respectively.

Statistical Analysis

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS ver. 20) and p value <0.05 was considered statistically significant. Previously normality was evaluated by using the Kolmogorov Simirnov and Shapiro-Wilk tests. Independent samples t test was used for comparison of normally distributed data and Mann-Whitney U test was used for comparison of non-normally distributed data.

Results

In CSC group 11 patients were female and 22 patients were male. In control group 10 participants were female and 20 were male. The sex was similar between groups *(p=1.00)*. Mean age was 41.04±5.94 (32-55) years in CSC group and 40.67±6.53 (30-53) years in the control group, respectively. Mean ages were similar between groups *(p=0.829)*. Mean renalase level was 27.19±14.01 (4.36-61.60) ng/ml in CSC group and 19.12±15.57 (2.30-56.13) ng/mL in the control group. Mean renalase level was higher in CSC group *(p=0.039)*. Mean cerebellin-1 levels were 57.76±29.72 (15.18-138.76) pg/mL and 52.50±29.25 (12.18-162.94) pg/mL in CSC and in control groups, respectively. Mean cerebellin-1 levels were similar in groups *(p=0.776)*.

Discussion

Central serous chorioretinopathy is more frequently seen with personality prone to stress and these people were reported to have higher levels of serum and urinary cortisol and catecholamines than healthy subjects [10]. Corticosteroids have been hypothesized to inhibit collagen synthesis (a main component of Bruch's membrane), and to increase choriocapillaris permeability by altering ion transport across RPE [11]. Cortisol may also directly damage the RPE cells or their tight junctions [11]. Several studies investigated the association between cortisol levels and CSC. Zakir et al found statistically significant higher mean serum cortisol levels in CSC cases than controls [12]. Endogenous hypercortisolism named Cushing's syndrome is found to be associated with CSC [11]. Central serous chorioretinopathy is also reported to be related with use of both systemic and local glucocorticoids [13]. Exposure to elevated levels of epinephrine induces apoptosis to RPE cells in vitro. Epinephrine metabolites, 3,4-dihydroxyphenylglycolaldehyde and hydrogen peroxide that originated by monoamine oxidase can induce apoptosis in RPE cells. Evidence suggests that monoamine oxidase is present in RPE cells [14]. Norepinephrine can increase choroidal blood flow that may contribute the development or exacerbation of CSC [15]. Michael et al reported CSC patients with

excessive use of sympathomimetic drugs. Visual symptoms of the patients alleviated after discontinuation of the sympathomimetics [15].

As previously mentioned, renelase and cerebellin exert opposite effects. Cerebellin has a stimulating effect on the secretion of aldosterone, cortisol and catecholamines while renalase directly degrades circulating catecholamines. Cerebellin-1 levels were similar in CSC and control patients in this study. Corticosteroids have alternative metabolisms that occurs primarily in the liver. Presumably circulating corticosteroid levels might be regulated by alternative metabolic pathways without affecting serum cerebellin-1 levels in CSC patients. Recent studies suggest that renalase plays an important role in blood pressure regulation via decreasing the levels of circulating catecholamines [16]. Increased renalase levels might be due to increased catecholamine levels in our CSC patients. On the other hand, all of our patients were normotensive. Increased renalase levels may have contributed to the prevention of hypertension in CSC patients. Since the literature information is limited, this mentioned association is speculative and we cannot make any precise scientific conclusion about the increment of serum renalase levels in CSC patients. One limitation of our study is we did not measure the aforementioned molecules aldosterone, cortisol and catecholamines that contribute to the pathogenesis of CSC. If we measured the levels of these molecules we could make more detailed discussion about biochemical relationships.

To the best of our knowledge it is the first study about the serum renalase and cerebellin-1 levels in CSC patients according to medline search. Comparing with healthy subjects serum renalase levels were higher and cerebellin-1 levels were similar in CSC patients. More detailed studies with higher number of participants are needed to prove the exact pathophysiologic roles of these proteins in CSC.

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References

[1]Liu B, Deng T, Zhang J. Risk factors for central serous chorioretinopathy: a systematic review and meta-analysis. Retina. 2016;36(1):9-19.

[2]Daruich A, Matet A, Dirani A, et al. Central serous chorioretinopathy: Recent findings and new physiopathology hypothesis. Prog Retin Eye Res. 2015;48:82-118.

[3]Seigneur E, Südhof TC. Cerebellins are differentially expressed in selective subsets of neurons throughout the brain. J Comp Neurol. 2017;525(15):3286-11.

[4]Mazzocchi G, Andreis PG, De Caro R, et al. Cerebellin enhances in vitro secretory activity of human adrenal gland. J Clin Endocrinol Metab. 1999;84(2):632-35.

[5]Rucinski M, Malendowicz LK. Precerebellin-related genes and precerebellin 1 peptide in endocrine glands of the rat-pattern of their expression. Int J Mol Med. 2009;23(1):113-19.

[6]Strowski MZ, Kaczmarek P, Mergler S, et al. Insulinostatic activity of cerebellin-evidence

from in vivo and in vitro studies in rats. Regul Pept. 2009;157(1-3):19-24.

[7] Satoh F, Takahashi K, Murakami O, et al. Cerebellin and cerebellin mRNA in the human brain, adrenal glands and the tumour tissues of adrenal tumour, ganglioneuroblastoma and neuroblastoma. J Endocrinol. 1997;154(1):27-34.

[8] Aydın S. Can cerebellin and renalase measurements contribute to the elimination of false positive results in pheochromocytoma and paraganglioma diagnoses? Med Hypotheses. 2017;107:64.

[9] Wang F, Xing T, Li J, et al. Renalase's expression and distribution in renal tissue and cells. PLoS One. 2012;7(10):e46442.

[10] Scarinci F, Ghiciuc CM, Patacchioli FR, et al. Investigating the hypothesis of stress system dysregulation as a risk factor for central serous chorioretinopathy: a literature mini-review. Curr Eye Res. 2019;44(6):583-89.

[11] Iannetti L, Spinucci G, Pesci FR, et al. Central serous chorioretinopathy as a presenting symptom of endogenous Cushing syndrome: a case report. Eur J Ophthalmol. 2011;21(5):661-64.

[12] Zakir SM, Shukla M, Simi ZU, et al. Serum cortisol and testosterone levels in idiopathic central serous chorioretinopathy. Indian J Ophthalmol. 2009;57(6):419-22.

[13] Ricketti PA, Unkle DW, Cleri DJ, et al. Central serous chorioretinopathy secondary to corticosteroids in patients with atopic disease. Allergy Asthma Proc. 2015;36(2):123-29.

[14] Sibayan SA, Kobuch K, Spiegel D, et al. Epinephrine, but not dexamethasone, induces apoptosis in retinal pigment epithelium cells in vitro: possible implications on the pathogenesis of central serous chorioretinopathy. Graefes Arch Clin Exp Ophthalmol. 2000;238(6):515-19.

[15] Michael JC, Pak J, Pulido J, et al. Central serous chorioretinopathy associated with administration of sympathomimetic agents. Am J Ophthalmol. 2003;136(1):182-85.

[16] Maciorkowska D, Zbroch E, Malyszko J. Circulating renalase, catecholamines, and vascular adhesion protein 1 in hypertensive patients. J Am Soc Hypertens. 2015;9(11):855-64.

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Original Article

Effects of evening primrose oil and 5-fluorouracil on the healing of colonic anastomoses in rats

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ABSTRACT

Aim: This study was designed to evaluate the efficacy of evening primrose oil (EPO) on colonic anastomosis.

Methods: Sixty rats with colonic anastomosis were randomly divided into six groups. EPO and 5-Fluorouracil (5-FU) were administered at doses of 5 g/kg/day and 20 mg/kg/day, respectively. Group 1 served as sham control. The rats in Group 2 (EPO) received EPO (14 days preoperatively), in Group 3 (Extended EPO) received EPO (14 days preoperatively and 7 days postoperatively), in Group 4 (5-FU) received intraperitoneally 5-FU (5 days preoperatively), in Group 5 (5-FU+EPO) received EPO (14 days preoperatively), and 5-FU (5 days preoperatively), in Group 6 (5-FU+ extended EPO) received EPO (14 days preoperatively and 7 days postoperatively) and 5-FU (5 days preoperatively). Histopathological examination, bursting pressure, and hydroxyproline content were used for evaluation.

Results: Significant differences were found between the Groups 1, 2, and 3 and Groups 4, 5, and 6 in bursting pressures. Polymorphonuclear leukocyte (PMNL) and lymphocyte infiltration was significantly less in group 3, compared to the control and group 2. The least PMNL infiltration was in group 6 compared to groups 4 and 5. The hydroxyproline level was different in group 3 compared to the control and group 2. Furthermore, groups 5 and 6 were different compared to group 4.

Conclusion: EPO had favorable effects on colonic anastomosis even in groups where 5-FU was used.

Keywords: Anastomotic healing, evening primrose oil, 5-fluorouracil, bursting pressure.

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Introduction

Anastomotic leakage is a major problem that significantly increases mortality and morbidity in colorectal surgery. The incidence is 0.5-30% in the literature [1,2]. A mortality rate of 25-37% has been reported due to anastomotic leakage in a large series [3]. Anastomotic failure increases the duration of hospitalization 2-fold and perioperative mortality 3-fold [4]. Therefore, research is focused on a variety of systemic or local interventions with the hope of favorable effects on anastomosis healing [1,2,4-6].

5-fluorouracil (5-FU) application has been regarded as the standard chemotherapy agent for colorectal cancer for a long time [7]. The postoperative intraperitoneal application of 5-FU is known to clear disseminated cancer cells and eradicate micrometastases [4,8]. However, 5-FU has also been shown to negatively affect healing in colonic anastomosis in several studies [9-11]. It was shown that 5-FU increases inflammatory reaction, reduces the fibroblast and collagen formation and neovascularization at the anastomotic site [11].

Evening primrose oil (EPO) is rich in omega-six and essential fatty acids such as linoleic acid (LA) and gamma linoleic acid (GLA). These fatty acids are prostaglandin precursors and used in the production of prostaglandin-E1 (PGE1) exogenously [12]. PG-E1 has been demonstrated to favorably affect anastomotic healing in various studies [13-15]. PG-E1 exerts its effect by promoting the collagen synthesis and decreasing the inflammatory cell infiltration that negatively affect the process of anastomotic healing [14,15].

EPO, as a rich PG-E1 source, has vasodilator, anti-oxidative and anti-inflammatory effects when used in high doses [12]. It has been the subject of many studies due to its effects and found a place in the treatment of many diseases [16-18]. However, to the best of our knowledge, its effects on anastomotic healing has yet to be elucidated. The aim of this study is to evaluate the effects of EPO on colonic anastomosis in a rat experiment.

Materials and Methods

This study was approved by the Ethics Board of 19 Mayıs University, School of Medicine (Dated 29.01.2013 and issue number 2013/07). In addition to standard groups, rat groups who were subjected to the effects of 5-FU were created. Therefore, the efficacy of EPO was evaluated in the presence of an agent that is known to have unfavorable effects on anastomotic healing.

Sixty male Wistar albino rats, weighing 250-300 g, were used in the study. The subjects were fed with tap water, standard animal feed, and EPO and were kept in a laboratory environment. A cocktail with ketamine (Ketalar®, Parke-Davis, Eczacıbaşı), 80 mg/kg and xylazine (Rompun®, Bayer), 8 mg/kg was used intraperitoneally as anesthetic agent. Following appropriate field cleansing and placing sterile sheets, a midline incision, 3-3.5 cm in length was performed. A colon resection, 1 cm in thickness, was carried out in the middle part of the transverse colon and an end-to-end anastomosis was created using a 5/0 polyprolene suture. The abdominal incision was closed using a 3/0 silk suture.

The rats were randomly divided into six groups (Figure 1). Group 1 (control) received standard feed for 14 days preoperatively and 7 days postoperatively. The rats in Group 2 (EPO) received EPO, 5 g/kg/day through gavage for 14 days preoperatively in addition to standard feed. Rats were fed with standard feed for 7 days postoperatively. The rats in Group 3 (Extended EPO) received EPO, 5 g/kg/day through gavage for 14 days preoperatively and

7 days postoperatively in addition to standard feed. The rats in Group 4 (5-FU) received intraperitoneally 20 mg/kg 5-FU (Biosyn ®, Orna) diluted in 3 ml saline solution for 5 days preoperatively and resection and anastomosis was performed after an interval of 1 day. Subjects were fed with standard feed for 7 days postoperatively. The rats in Group 5 (5-FU+EPO) received EPO, 5 g/kg/day for 14 days and also intraperitoneally 20 mg/kg 5-FU diluted in 3 ml saline solution for 5 days preoperatively. Subsequently one day interval was given and resection and anastomosis were performed. Subjects were fed with standard feed for 7 days postoperatively. The rats in Group 6 (5-FU+ extended EPO) received EPO, 5 g/kg/day for 14 days and also intraperitoneally 20 mg/kg 5-FU diluted in 3 ml saline solution for 5 days preoperatively. Subsequently, a one-day interval was given and resection and anastomosis were performed. Subjects were fed with EPO, 5 g/kg/day through gavage in addition to standard feed for 7 days postoperatively. Rats were sacrificed after seven days postoperatively and after 12 hours of fasting, relaparotomies were performed.

Bursting pressure

Following relaparotomy, a segment of the colon, measuring 5 cm in length, 2.5 from each direction from the anastomosis, was resected carefully. This segment of the transverse colon was cleaned of feces and the distal end was tied with a 3/0 silk suture. A catheter connected to a sphygmomanometer was inserted to the proximal end and colon was carefully tied around the tube. A solution of 0.9% NaCl was infused at a constant speed of 1ml/min through the catheter. The level of pressure seen when the leakage appeared at the anastomosis was recorded. All the bursting pressures were evaluated immediately after the rats were sacrificed.

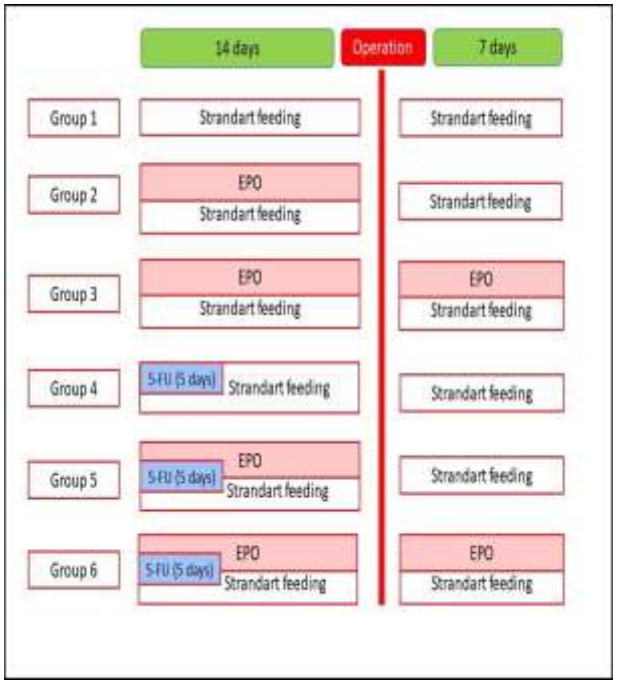

Figure 1. Diagram of experimental design.

Histopathological examination

Following the recording of the bursting pressure, the anastomosis line was resected, including approximately 0.5 cm proximal and distal colonic segments. The resected segment of colon was divided into two parts in equal lengths and one of the pieces was placed in a 10% buffered neutral formalin solution for histopathological examination. Evaluation was performed after staining with Hematoxylin Eosin (HE) using a semi-quantitative scale including many parameters related to wound healing, as defined by Biert et al. [19] (Table 1). According to this scale, the amount of necrosis was expressed as none (0 points), only small patches (1 point), some patches (2 points), or massive (3 points). In the anastomotic area, accumulation of polymorphonuclear cells (PMNs), macrophages, and lymphocytes was also assessed in terms of none or normal (0 points), slightly increased (1 point), marked infiltration (2 points), and massive infiltration

(3 points). Edema, expressed as the ratio of maximum thickness of the wall at the anastomosis and the thickness of the normal colon wall at the end of the section, was graded as none (0 points), some (1–1.5× normal thickness; 1 point), marked (1.5–2× normal thickness; 2 points) and severe (> 2x normal thickness; 3 points). Healing of the mucosa was expressed as normal, i.e. mucosa with restored glandular epithelium (0 points), intact mucosa with cubic epithelium but without glands (1 point), mucosa only partially covered by cubic epithelium (2 points), and mucosa completely devoid of epithelial coverage (3 points). Submucosal-muscular repair was assessed in terms of good (0 points), average (1 point), poor (2 points), or no (3 points) fibroblast stretching and bridging the anastomotic wound. This way, in each anastomosis, two observations from the mesenteric and two from the anti-mesenteric side were performed.

Hydroxyproline

The second piece taken from the anastomotic line was manually homogenized applying liquid nitrogen in a porcelain mortar and placed in an appropriately buffered phosphate solution (Phosphate Buffer Solution, 10 mM, pH 7.2). Following the sonication of the tissue samples for 1 minute in +4°C at 220V (Fisher, Sonic Dismembrator; Mosel 300), they were stored in a freezer at -80 °C. Homogenates were centrifuged at +4°C at 14000 rpm for 5 minutes and the obtained supernatants were used for analysis. Hydroxyproline levels were determined using a BioVision commercial kit (BioVision, CA, USA, Cat.No.#K555-100) based on reading at 560 nm of chromogen, which is formed as a result of a spectrophotometric reaction. The protein content of each sample in tissue homogenates was determined using the Lowry method [20] and levels of hydroxyproline were expressed in ng per mg protein (ng/mg.prot).

Statistical analysis

When Groups I, II, and III and Groups IV, V, and VI were compared within groups, and the characteristics of hydroxyproline and bursting pressure were evaluated one-way analysis variance (ANOVA) was used. Levene's homogeneity test was used to detect the homogeneity of the variances.

The Kruskal-Wallis test was used in the analysis of other features. The analysis of hydroxyproline levels and bursting pressure characteristics were performed using independent sample t-test in inter-group comparisons. The Mann-Whitney U-test was used in the analysis of other

Table 1. Scores are used to analyze semi-quantitatively the healing of anastomosis line in colon.

Score	Necrosis	PMN	Lymphocytes	Macrophages	Edema	Mucosal Epithelium	Submucosal -Muscular Layer
0	None	Normal pumber	Normal number	Normal Number	None	Normal glandular	Good bridging
1	Small patches	Slight increase	Slight increase	Slight increase	Some	Normal cubic	Average bridging
2	Some patches	Marked infiltration	Marked infiltration	Marked infiltration	Marked	Incomplete cubic	Poor bridging
3	Massive	Massive infiltration	Massive infiltration	Massive infiltration	Severe	Absent	No bridging

characteristics. P values <0.05 were accepted as statistically significant.

Results

Bursting pressure

The highest bursting pressure values were measured in the extended EPO group (198.0 ± 7.48). The lowest bursting pressures were in the 5-FU group (119.89 ± 2.88). Statistically significant differences were found between the intergroup analysis of Groups I, II, and III and between Groups IV, V, and VI ($p<0.001$) (Table 2).

Differences were reported between the control group and 5-FU group and between the EPO group and 5-FU+EPO group, as well ($p<0.001$). In addition, statistically significant differences were found between the extended EPO and 5-FU+ extended EPO groups ($p=0.002$) (Table 3).

Table 2. Hydroxyproline content levels and bursting pressure values according to group a≠b≠c.

Groups	Hydroxyproline (ng/mL)	Bursting Pressure (mmHg)
Control	1113..41 ± 62..32b	144.6 ± 3..81c
EPO	1380..98 ± 59..39b	177.4 ± 7.66b
Extended EPO	1915..08 ± 318..89a	198.0 ± 7.48a
P value	*0.014*	*0.001*
5- FU	789.23 ± 129.73b	119.89 ± 2.88c
5-FU+EPO	1183.48 ± 52.71a	136.40 ± 4.51b
5-FU+Extended EPO	1249.83 ± 58.54a	158.33 ± 7.26a
P value	*0.002*	*0.001*

Values: SEM, EPO: evening primrose oil, 5-FU: 5- fluorouracil.

Table 3. Hydroxyproline content levels and bursting pressure values according to groups a≠b.

Groups	Hydroxyproline (ng/mL)	Bursting Pressure (mmHg)
Control	1113.41 ± 62.32a	144.6 ± 3.81a
5- FU	789.23 ± 129.73b	119.89 ± 2.88b
P value	*0.045*	*<0.001*
EPO	1380.98 ± 59.39a	177.4 ± 7.66a
5-FU+EPO	1183.48 ± 52.71b	136.40 ± 4.51b
P value	*0.023*	*<0.001*
Extended EPO	1915.08 ± 318.89	198.0 ± 7.48a
5-FU+Extended EPO	1249.83 ± 58.54	158.33 ± 7.26b
P value	*0.057*	*0.002*

Values: SEM, EPO: evening primrose oil, 5-FU: 5- fluorouracil.

Histopathological examination

When the control, EPO and extended EPO groups were compared, it was observed that PMNL and lymphocyte infiltration was significantly lower in the extended EPO group compared to the other two groups *(p=0.003, and p=0.020)*. No differences were found in other histopathological examination criteria (Table 4). When the groups with 5-FU were compared, a significant difference between all groups was found in only the PMNL infiltration. The lowest PMNL infiltration was in the 5-FU+extended EPO group and was different from the other two groups *(p=0.029)*. Other criteria of histopathological examination were found to be similar in the groups (Table 4). Figures 2-4 show histopathological changes and inflammatory findings in different treatment groups.

When the groups with and without chemotherapeutic agents were comparatively analyzed, significant differences were found in macrophage infiltration, edema formation, and mucosal epithelial structure between the control and 5-FU groups *(p=0.005, p=0.025, and p=0.008, respectively)*. Macrophage infiltration and mucosal epithelial damage was significantly higher in the 5-FU group. In addition, more edema development was seen in this group. Mucosal epithelial structure was different in the EPO and 5-FU+EPO groups. The mucosal epithelial damage was more severe in the 5-FU+EPO group *(p=0.030)*. When the extended EPO and 5-FU+extended EPO groups were compared, edema development and mucosal epithelial damage was significantly greater in the 5-FU+extended EPO group *(p=0.005 and p=0.048, respectively)*. Other histopathological evaluation criteria were similar in all groups (Table 5).

Table 4. The results of histopathological examination according to groups a≠b.

Groups	Necrosis	PMNL	Lymphocytes	Macrophages	Edema	Submucosal-ML	Mucosal Epithelium
Control	0 (0 – 0)	2(0– 3)a	1 (0 – 3)a	0 (0 – 1)	1(0–2)	0 (0 – 0)	0 (0 – 0)
EPO	0 (0 – 3)	1(0–2)a	1 (0 – 2)a	1 (0 – 1)	1(0–2)	0 (0 – 3)	0 (0 – 0)
Extended EPO	0 (0 – 0)	0(0– 1)b	0 (0 – 1)b	0 (0 – 1)	0(0–1)	0 (0 – 0)	0 (0 – 2)
P value	*0.387*	*0.003*	*0.020*	*0.483*	*0.215*	*0.387*	*0.329*
5- FU	0 (0 – 3)	1(1–3)a	1(1 – 2)	1 (1 – 2)	1(1–3)	0 (0 – 0)	2 (0 – 3)
5-FU+EPO	0 (0 – 3)	1(0-2)ab	0.5(0 – 2)	0.5 (0 – 2)	1(0–3)	0 (0 – 0)	0 (0 – 3)
5FU+Extended EPO	0 (0 – 3)	0(0– 2)b	0 (0 – 2)	0 (0 – 2)	1(1–3)	0 (0 – 2)	2 (0 – 3)
P value	*0.814*	*0.029*	*0.072*	*0.072*	*0.068*	*0.348*	*0.851*

Values: Median (Min – Max), EPO: evening primrose oil, 5-FU: 5- fluorouracil, ML: Muscular Layer, PMNL: polymorphonuclear leukocyte.

Table 5. The results of histopathological examination according to groups a≠b.

Groups	Necrosis	PMN	Lymphocytes	Macrophages	Edema	Submucosal-ML	Mucosal epithelium
Control	0(0–0)	2(0–3)	1(0–3)	0(0 – 1)b	1(0–2)b	0(0–0)	0(0–0)b
5- FU	0(0–3)	1(1–3)	1(1–2)	1(1–2)a	1(1–3)a	0(0–0)	2(0–3)a
P value	*0.125*	*0.428*	*0.882*	*0.005*	*0.025*	*1.000*	*0.008*
EPO	0(0–3)	1(0–2)	1 (0 – 2)	1(0 – 1)	1(0 – 2)	0(0 – 3)	0(0–0)b
5-FU+EPO	0(0–3)	1(0–2)	0.5(0–2)	0.5(0 – 2)	1(0 – 3)	0(0 – 0)	0(0–3)a
p value	0.542	0.314	0.188	0.993	0.720	0.739	0.030
Extended EPO	0(0–0)	0(0–1)	0(0–1)	0(0–1)	0(0–1)b	0(0–0)	0(0–2)b
5FU+Extended EPO	0(0–3)	0(0–2)	0(0–2)	0(0–2)	1(1–3)a	0(0–2)	2(0–3)a
P value	*0.313*	*0.873*	*0.873*	*0.873*	*0.005*	*0.73*	*0.048*

Values: Median (Min – Max), EPO: evening primrose oil, 5-FU: 5- fluorouracil, ML: Muscular Layer, PMNL: polymorphonuclear leukocyte.

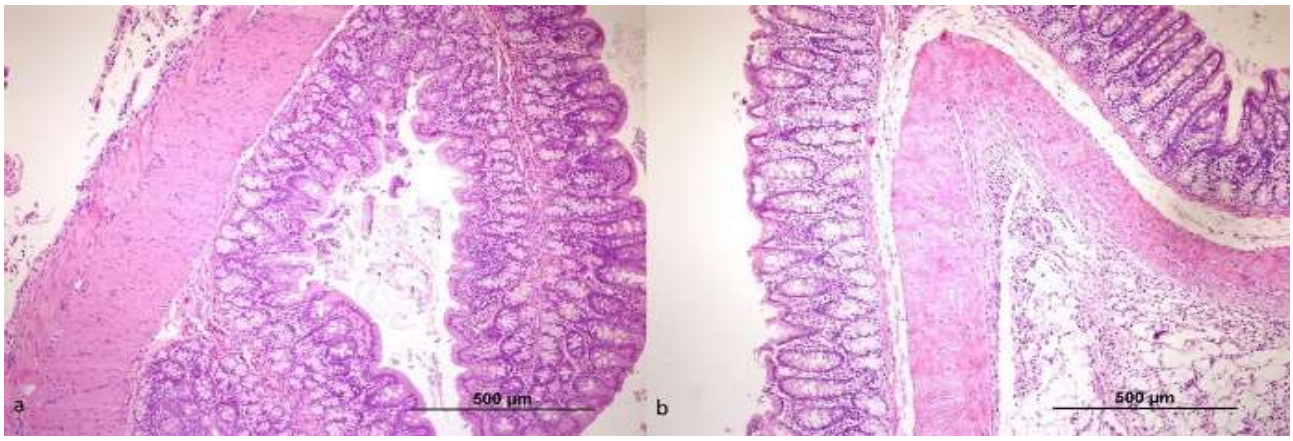

Figure 2. a) Mucosa is intact in the extended EPO group; acute and chronic inflammation are minimal (x100HE). b) Mucosa is in atrophic appearance in the control group, chronic active inflammation which is evident especially in serosa (x100 HE).

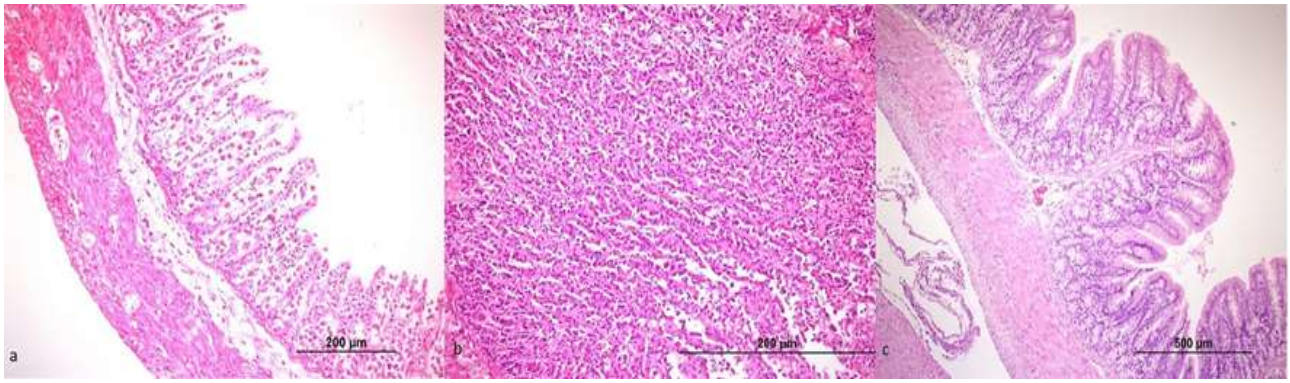

Figure 3. a) Extended necrosis and active inflammation present in mucosa and submucosa in the 5-FU group (x200HE). b) Extensive macrophage infiltration in lamina propria in the 5-FU group (x400 HE). c) Intact mucosa, minimal inflammation, no macrophage infiltration in the control group (x100HE).

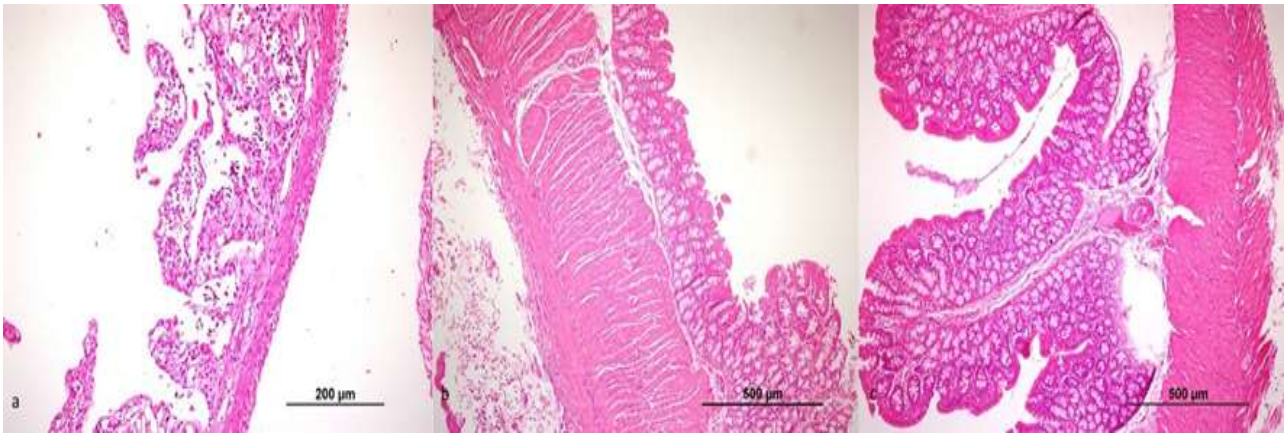

Figure 4. a) Extended ischemic damage is present in mucosa and submucosa in the 5-FU group. Intensive chronic and active inflammatory cells are seen (x200 HE). b) Mucosa with atrophic morphology and mild inflammatory cells are seen in 5-FU + EPO group (x100 HE). c) Intact mucosal and submucosal layers and minimal inflammatory cells are seen in the 5-FU + extended EPO group (x100 HE).

Hydroxyproline

Hydroxyproline levels in the extended EPO group was different when compared to the control and EPO groups *(p=0.014)*. The 5-FU+EPO and 5-FU+extended EPO groups were also significantly different from the 5-FU groups, as well *(p=0.002)* (Table 2).

When the groups with and without chemotherapeutic agents were comparatively evaluated, differences were found between the control and 5-FU groups and between EPO and 5-FU+EPO *(p=0.045 and p=0.023, respectively)* (Table 3).

Discussion

The salient finding of the present study was that EPO demonstrated beneficial therapeutic effects of on colonic anastomosis in rats. These favorable effects were still prominent in the presence of 5-FU that has deleterious effects on the anastomosis.

Anastomotic leakage after colorectal surgery is reported to be seen in up to 30% of the cases. [1,2,4,9]. In spite of the advances in the operative techniques and patient preparation procedures it is still a significant cause of postoperative morbidity and mortality [3].

Anastomotic healing is a complex process including a series of biological events. Coordination of cellular activity and humoral factors is necessary during this process [21]. Anastomotic healing is affected by the degree of the inflammatory response and the rate of mucosal re-epithelization, thus the power, quantity, and maturation rate of the collagen. Durability of the anastomosis line is basically dependent on the collagen fibrils and their degree of maturation in the submucosa [22].

We found that administration of EPO had a clear beneficial effect on both bursting pressures and hydroxyproline content. Hydroxyproline content was significantly higher in the extended EPO group compared to the other groups on the seventh day when we expected the collagen synthesis to be at its highest level. We also observed that 5-FU significantly decreased the hydroxyproline content. The adverse effect of 5-FU on fibroblast proliferation and collagen synthesis is well-known [7]. On the contrary, the observed higher hydroxyproline content in the 5-FU+EPO and 5-FU+extended EPO groups compared to the 5-FU group demonstrated that EPO reverses the deleterious effects of 5-FU.

Experimental studies have demonstrated that the hydroxyproline concentrations decrease in the anastomotic lines after colonic resections [15]. Hawley et al. [23] emphasized that the collagenase enzyme was responsible for the observed decrease in the level of hydroxyproline. Hawley et al. demonstrated that the production of this enzyme was increased both in the area of anastomosis and also in other parts of gastrointestinal system. This is a primary factor causing weakness of the anastomosis by increasing collagen breakdown. Therefore, the production and breakdown of collagen during the healing phase of the anastomosis should be well balanced.

The authors have stressed that it is necessary to prevent low suture tension, good local blood flow, weak local inflammation, and to reduce feces by a lavage of colon preoperatively for a good anastomotic healing after colonic surgery. These factors may have an effect on collagen synthesis by inhibiting collagenase. As a result, collagen content in the intestinal wall plays a major role in anastomotic healing and the prevention of complications such as leakage [15].

Since fibroblast proliferation and collagen synthesis occur in the submucosal layer, anastomotic strength primarily depends on the collagen fibrils in the submucosa, as stated above [21]. Maximal collagen synthesis occurs in the 5-7th days during the proliferation of local fibroblasts which produce collagen [22]. Therefore, this study was terminated on the 7th day, taking into account the experimental models in the literature [22,24].

EPO is a product that has been used for years as a dietary supplement and an alternative treatment in various diseases [12,16]. It is most commonly used in the treatment of diseases with chronic inflammation. Atopic dermatitis, rheumatoid arthritis, mastalgia, menopausal and premenstrual symptoms, cervical ripening, and birth induction are some of the conditions for which it is frequently used [16-18]. It is rich from omega-6 essential fatty acids such as LA and GLA, which are used in the production of PGE1 in the body [12]. Orally administered EPO has been proven to demonstrate vasodilator, anti-oxidative, and anti-inflammatory efficacy in various experimental studies [12,25]. Abo-Gresha et al. [12] demonstrated the healing effect of EPO in rats with experimentally-induced myocardial infarction. In that study, in addition to the antithrombotic activity of PGE1, which is synthesized from the fatty acids in EPO, it is reported to cause smooth muscle relaxation and vasodilation. Furthermore, PGE1 was reported to decrease oxidative stress and inflammation in the ischemic heart. El-Sayed et al. [25] on the other hand, in their experimental study, in which they evaluated the progression of rheumatoid arthritis, reported that EPO has a marked anti-inflammatory effect. In addition, they recorded that GLA, which increases the production of PGE1 in the body had a role in the anti-inflammatory and anti-oxidative effects. In experimental studies, the predominant vasodilator, anti-inflammatory and wound healing effects of PGE1 have been emphasized. Goertz et al. [26] evaluated the effects of various agents such as acetylsalicylic acid, isosorbide dinitrate, sodium chloride, and PGE1, which were considered to have treatment effect in rats with experimentally produced burns. The most successful results were obtained in the PGE1 group. PGE1 was proven to increase angiogenesis and blood flow and at the same time to demonstrate anti-inflammatory effects by decreasing rolling leukocytes in the circulation. Its effects on augmentation of wound healing by increasing perfusion through inducing vasodilation,

inhibiting thrombocyte aggregation, and thus delaying thrombus formation have also been reported. Kobayashi et al. [27] also demonstrated that PGE1, in addition to its vasodilator and anti-aggregating effects on thrombocytes, proliferates the production of epidermal keratinocytes and dermal fibroblasts. In addition, they reported that PGE1 was an anti-oxidative agent and all of its characteristic features have a positive effect on wound healing.

The limitation of the current work deserves to be stated. We applied 5-FU and EPO in a single dose in all groups. We determined the dose of EPO as 5 g/kg/day based on the results of previous studies in the literature [25,28,29], in which the dose of EPO varied between 1.25-10 g/kg/day and for an average of 14-28 days. We also determined the dose and duration of 5-FU in the appropriate groups according to the previous studies [7,10]. To limit the number of scarified animals and not to increase complexity of the experimental model, we opted for using a single EPO and 5-FU dose in the study.

For the first time in the literature, we evaluated the effects of EPO administered through the gavage method on colonic anastomosis in an experimental study. We administered the agent for 14 days preoperatively and 7 days postoperatively in the respective groups.

Conclusions

In conclusion, we demonstrated beneficial therapeutic effects of EPO on colonic anastomosis in rats. This was likely due to the essential fatty acid content of EPO because these fatty acids are used in the production of PGE1 in the body. The anti-inflammatory, vasodilator and anti-oxidative effects of EPO might favorably influence the anastomosis. The maintenance of these favorable effects in spite of the presence of an agent that has deleterious effects on the anastomosis such as 5-FU suggests that EPO use might be employed to decrease the rate of anastomotic leakage in patients who receive neoadjuvant chemotherapy and are scheduled to undergo surgical treatment.

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References

[1] Caglayan K, Erdogan N, Avci B, et al. Effect of Beta-glucan on intestinal anastomoses in a rat model. Adv Clin Exp Med. 2013; 22(2):157-63.

[2] Yaman I, Kara C, Derici H, et al. The effect of a special amino acid mixture on healing of left colonic anastomosis: an experimental study. Turkiye Klinikleri J Med Sci. 2013;33(3):678-84.

[3] Kanellos I, Blouhos K, Demetriades H, et al. The failed intraperitoneal colon anastomosis after colon resection. Tech Coloproctol. 2004; 8 :53-5.

[4] Zacharakis E, Demetriades H, Pramateftakis MG, et al. Effect of IGF-I on healing of colonic anastomoses in rats under

5-FU treatment. J Surg Res. 2008;144(1):138-44.

[5] Uludag M, Citgez B, Ozkaya O, et al. Effects of amniotic membrane on the healing of normal and high-risk colonic anastomoses in rats. Int J Colorectal Dis. 2009; 24(7):809-17.

[6] Symeonidis N, Papakonstantinou E, Psarras K, et al. The effect of celecoxib administration on the healing and neovascularization of colonic anastomosis in rats. J Invest Surg. 2014; 27(3):139-46.

[7] Ozel L, Ozel MS, Toros AB, et al. Effect of earlypreoperative5-fluorouracil on the integrity of colonic anastomoses in rats. World J Gastroenterol. 2009; 15(33):4156-62.

[8] Vaillant JC, Nordlinger B, Deuffic S, et al. Adjuvantintraperitoneal5-fluorouracil in high-risk colon cancer: A multicenter phase III trial. Ann Surg. 2000; 231(4):449-56.

[9] Kanellos D, Pramateftakis MG, Demetriades H, et al. Healing of colonic anastomoses after immediate postoperative intraperitoneal administration of oxaliplatin. Int J Colorectal Dis. 2008;23(12):1185-91.

[10] Kanellos D, Pramateftakis MG, Mantzoros I, et al. The effects of the intraperitoneal administration of oxaliplatin and 5-FU on the healing of colonic anastomoses: an experimental study. Tech Coloproctol. 2011; 15 :111-15.

[11] Pramateftakis MG, Kanellos D, Mantzoros I, et al. Intraperitoneally administered irinotecan with 5-fluorouracil impair wound healing of colonic anastomoses in a rat model: an experimental study. Tech Coloproctol. 2011; 15 :121-25.

[12] Abo-Gresha NM, Abel-Aziz EZ, Greish SM. Evening primrose oil ameliorates platelet aggregation and improves cardiac recovery in myocardial-infarct hypercholesterolemic rats. Int J Physiol Pathophysiol Pharmacol. 2014; 6(1):23-36.

[13] de Oliveira PG, Soares EG, Aprilli F. Influence of misoprostol, a syntheticprostaglandinE1analog, on the healing of colonic anastomoses in rats. Dis Colon Rectum. 1994; 37(7):660-63.

[14] Cali RL, Smyrk TC, Blatchford GJ, et al. Effect of prostaglandinE1 and steroid on healing colonic anastomoses. Dis Colon Rectum. 1993; 36(12):1148-51.

[15] Terzioglu T, Sonmez YE, Eldegez U. The effect of prostaglandin E1 on colonic anastomotic healing. A comparison study. Dis Colon Rectum. 1990; 33(1):44-8.

[16] Bayles B, Usatine R. Evening primrose oil. Am Fam Physician. 2009; 80(12):1405-8.

[17] Farzaneh F, Fatehi S, Sohrabi MR, et al. The effect of oral evening primrose oil on menopausal hot flashes: a randomized clinical trial. Arch Gynecol Obstet. 2013;288(5):1075-79.

[18] Senapati S, Banerjee S, Gangopadhyay DN. Evening primrose oil is effective in atopic dermatitis: a randomized placebo-controlled trial. Indian J Dermatol Venereol Leprol. 2008; 74(5):447-52.

[19] Biert J, Seifert WF, Verhofstad AA, et al. A semiquantitative histological analysis of repair of anastomoses in the rat colon after combined preoperative irradiation and local hyperthermia. Radiat Res. 1998; 149(4):372-77.

[20] Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265-75.

[21] Inan A, Koca C, Sen M. Effects of diclofenac sodium on bursting pressures of anastomoses and hydroxyproline contents of perianastomotic tissues in a laboratory study. Int J Surg. 2006;4(4):222-27.

[22] Aslan A, Temiz M, Hakverdi S, et al. Effect of mesalamine on healing in experimental colon anastomosis: a randomised experimental study. Int J Surg. 2008;6(1):40-4.

[23] Hawley PR, Faulk WP, Hunt TK, et al. Collagenase activity in the gastro-intestinal tract. Br J Surg. 1970; 57(12):896-900.

[24] Kosmidis C, Efthimiadis C, Anthimidis G, et al. Myofibroblasts and colonic anastomosis healing in Wistar rats. BMC Surg. 2011; 11:6.

[25] El-Sayed RM, Moustafa YM, El-Azab MF. Evening primrose oil and celecoxib inhibited pathological angiogenesis, inflammation, and oxidative stress in adjuvant-induced arthritis: novel role of angiopoietin-1. Inflammopharmacology. 2014 Mar 25. [Epub ahead of print]

[26] Goertz O, Ring A, Buschhaus B, et al. Influence of anti-inflammatory and vasoactive drugs on microcirculation and angiogenesis after burn in mice. Burns. 2011; 37(4):656-64.

[27] Kobayashi H, Katakura O, Morimoto N, et al. Effects of cholesterol-bearing pullulan (CHP)-nano gels in combination with prostaglandin E1 on wound healing. J Biomed Mater Res B Appl Biomater. 2009;91(1):55-60.

[28] Omran OM. Histopathological study of evening primrose oil effects on experimental diabetic neuropathy. Ultrastruct Pathol. 2012; 36(4):222-27.

[29] Ford I, Cotter MA, Cameron NE, et al. The effects of treatment with alpha-lipoic acid or evening primrose oil on vascular hemostatic and lipid risk factors, blood flow, and peripheral nerve conduction in the streptozotocin-diabetic rat. Metabolism. 2001; 50(8):868-75.

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Original Article

Influence of articular arthroscopy-like washout on fracture healing of intra-articular fractures; animal experiment

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ABSTRACT

Aim: To examine whether the application of intra-articular lavage during arthroscopic joint fracture surgery can disturb fracture union and cartilage healing.

Metods: Twenty New Zealand rabbits were then randomly divided into 3 groups; these groups consisted of 2 surgical groups including eight rabbits and a control group consisting of 4 rabbits. After both rear limbs exposed with a medial parapatellar incision, medial femoral condyle was fractured. Four groups were created by doing anatomic reduction or non-anatomic reduction and making irrigation or no irrigation. (Group 1: Fixed by creating a gap and no Irrigation; Group 2: Fixed by creating a gap and irrigation; Group 3: Fixed with complete reduction and no irrigation; Group 4: Fixed with complete reduction and irrigation) X-rays of both knees of all rabbits were taken at the end of the second week and at the end of the eighth week. The operated knees were collected for histopathological analysis.

Results: Radiological data show a significant difference in the level of ossification between the groups in the 2nd week; however, this difference was lost in the 8th week. Histopathologically, at the end of week 8, it was observed that the subchondral bone tissue was incompletely renewed in all the groups. The cartilage tissue of the joint surface was not fully formed and renewed and that it did not completely coalesce with the old cartilage tissue in all of the groups. Compared with the other groups, the group that fracture was anatomically reducted with no irrigation (Group 1), the cartilaginous tissue layer formed was thicker while the surface of the tissue was flatter.

Conclusion: There were no adverse effects of intra-articular lavage on fracture union and cartilage healing in an in vivo environment. Nonetheless, the findings of this study should be confirmed with a larger sample size.

Keywords: Intra-articular fracture, arthroscopy, irrigation, fracture union, cartilage healing.

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Introduction

In recent years the number of arthroscopic joint surgeries has increased dramatically, becoming an important part of orthopedic surgeries. The use of arthroscopy in fracture treatment is considered to be more advantageous compared to open surgery for determining the fracture type and associated soft tissue injury as it is less harmful to surrounding tissues and provides better reduction [1]. Fractures in the tibia plateau [2-5] and eminence [6-8]; ankle [9-11]; femoral head [12,13]; shoulder glenoid [14]; tuberculum majus [15]; distal clavicle [16]; elbow radial head [17], coronoid [18], capitellum [19]; wrist distal radius [20-22] and scaphoid [23-25] were reported to be successfully treated by arthroscopy assisted surgical techniques.

During arthroscopy, the joint surfaces and fracture zone are irrigated with excess of 0.9 % isotonic NaCl or Ringer's Lactate solutions for a few hours. It has been reported that irrigation with pressure lavage can break the healed fractures in the metaphyseal area but there is no evidence that application of saline solution into the joint without pressure for a long time and in high volume is harmful for the unification of fractures [26]. In addition, application of NaCl and Ringer Lactate to the solid cartilage during arthroscopy does not have any harmful effects but their effect on the broken cartilage is still unknown [27,28]. In this study we have examined whether the application of intra-articular lavage during arthroscopic joint fracture surgery can disturb fracture union and cartilage healing.

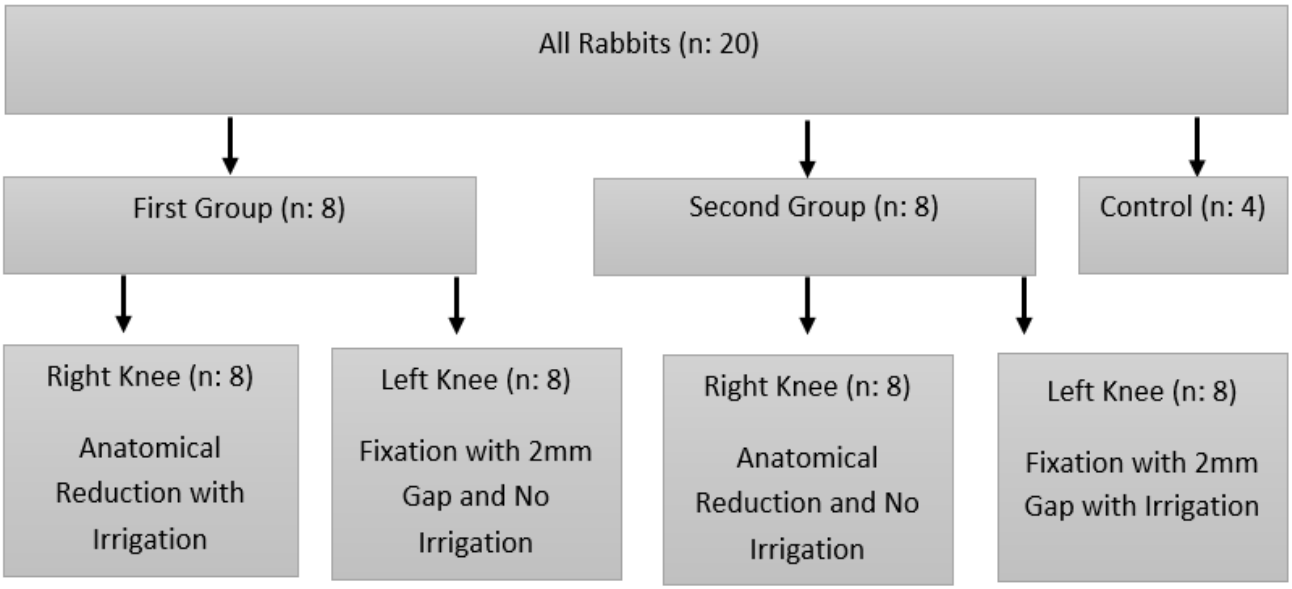
Materials and Methods

The study was approved by the Bezmialem Vakif University Animal Experiments Local Ethics Committee (Decision no: 2015/220). Twenty New Zealand rabbits were used which weighed between 2400 g - 2800 g and were 9 - 15 months of age. Prior to the surgery, the rabbits were acclimatized for three days and fed on a regular diet. The rabbits were then randomly divided into 3 groups; these groups consisted of 2 surgical groups including eight rabbits and a control group consisting of 4 rabbits (Graphic 1). Both knees of all rabbits were used in the study in order to reduce the number of subjects.

Surgical protocol

Thirty minutes before the operation, the antibiotic Cefazolin Na (50 mg IM) was applied prophylactically. Meloxicam (10 mg / kg) was administered subcutaneously on the day before the surgery and for 3 days postoperatively. The operations were conducted under general anesthesia using Ketamine (35-40 mg/kg) IM following sedation with Xylazine (3-5 mg/kg). After both rear limbs were shaved, they were fixed with a clip, covered with sterilized dressing and the knee joint was exposed with a medial parapatellar incision. The medial femur condyle was fractured at 45 degrees oblique and osteotomy was initiated from the middle of the joint. For the first group of rabbits, after the fractures created in the right knee underwent anatomical reduction without leaving a gap, they were fixed with one 2.7 mm cortical screw and 2 cannulas, which transversed the joint, were inserted. The wound was tightly stitched and made waterproof. The fracture of the left knee was fixed with a 2.7 mm screw, leaving a

Graphic 1. Grouping of subjects.



2 mm gap between the fracture lines and they were closed without any cannula placement. For the second group of rabbits, after the fractures created in the right knees underwent complete anatomical reduction, they were fixed with a 2.7 mm cortical screw and closed without placing cannulas. The fractures of the left knee were fixed with a 2.7 mm screw with a 2 mm gap and 2 cannulas, which transversed the joints, were placed. The wounds were tightly closed without leaving a gap and made waterproof.

Thirty minutes after finishing the surgery and before ending the anesthetic procedure, the cannulated knees were irrigated by infusing 1 liter of normal saline solution for 30 minutes and the cannulas were pulled out (Figure 1, 2). The rabbits were followed up on a regular diet for 8 weeks. During the follow up, rabbits that exhibited the presence of distal localization of the fracture, loss of fixation, development of infection, significant reduction lost and a weight loss of more than 20% [29,30] were excluded from the study.

X-rays of both knees of all rabbits were taken at the end of the second week and at the end of the eighth week followed by euthanasia with high dose Xylazain and Ketamine. The operated knees were collected for histological (Giemsa and fluorescent) analysis.

Radiological Analysis

Four dials around the bone containing the mineralized external callus were evaluated in anteroposterior and lateral direct radiographs taken at the end of week 2 and week 8 (Table 1) [29] .

Histopathological analysis

The collected tissues were fixed in 10% neutral buffered formalin and decalcified in a 10% EDTA (pH 7.4) decalcification solution. After decalcification, the tissues were rinsed with distilled water and an alcohol series (70%, 90%, 96% and 100%) followed by incubation with xylene and embedding in paraffin. 5mm thick sections prepared in a microtome were placed on positively charged slides. The sections were stained with hematoxylin & eosin and histopathologically analyzed using a light microscope (Nikon Eclipse i5, Tokyo, Japan). Histological findings were scored as previously

described by Wakitani et al [31]. The sections were rated for: 1) Cell morphology (maximum 4 points), 2) Matrix Staining Intensity (maximum 3 points), 3) Surface Regularity (maximum 3 points), 4) Thickness of Cartilage (maximum 2 points) and 5) Integration of donor with host adjacent cartilage (maximum 2 points). The maximum score was calculated as 14 points.

Statistical analysis

The non-parametric Kruskal Wallis and post-hoc Dunn Multiple tests were used for statistical comparison of the four surgical groups with each other. If any significant differences were seen in Kruskal Wallis Test, then post-hoc Dunn test was applied at the second stage in order to confirm the difference.

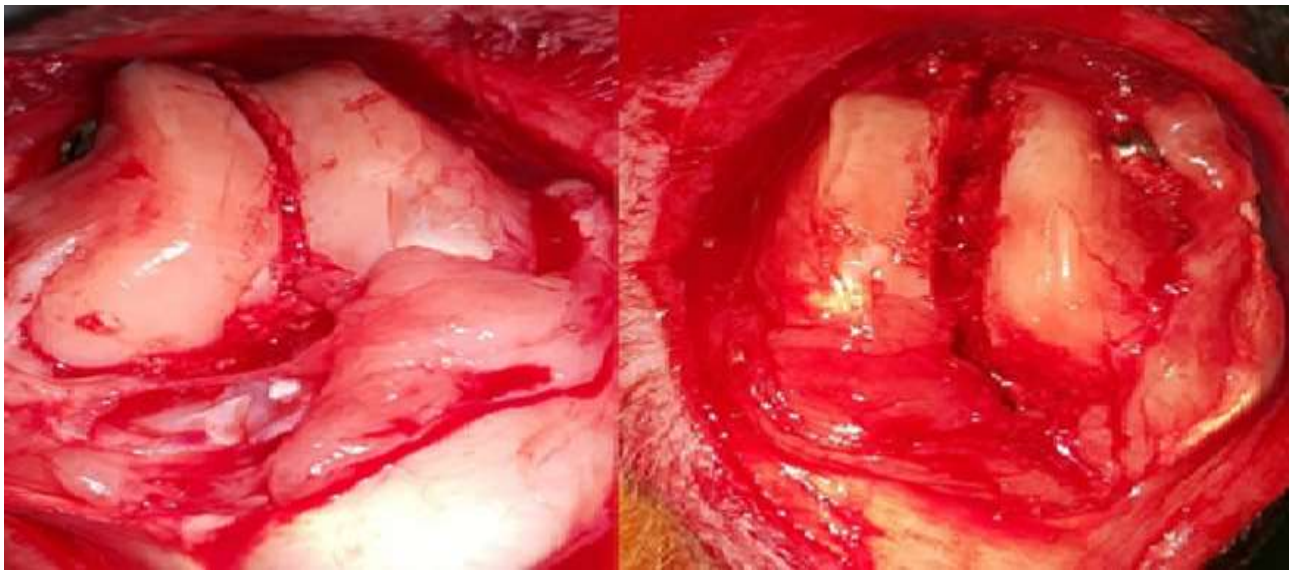


Figure 1. Intra-operative images, a) Right knee fixed without leaving a gap. b) Left knee fixed with 2mm gap.

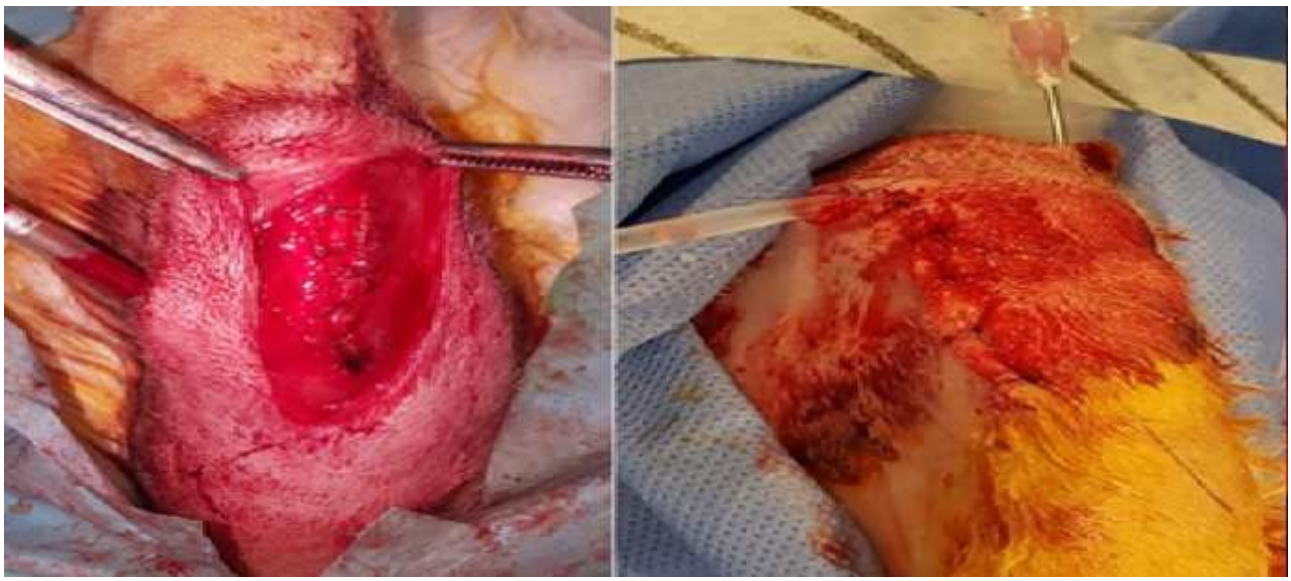


Figure 2. a) The joint capsule tightly closed with a cannula placement. b) In-knee irrigating; with 18 gauge injector, the liquid is drained from the plastic cannula while 0.9% saline solution is injected into the joint.

Table 1. Radiological evaluation scale.

0	No bridged mineralized callus appearing on any scale
1	A brilliant mineralized callus seen on a scale
2	Bridged mineralized callus seen on a scale
3	Triple bridged mineralized callus seen on a scale
4	Four bridged mineralized callus seen on a scale

Results

The study was initiated with 20 rabbits with the aim to assign eight subjects to each group. Bilateral femur diaphysis fracture developed in 1 subject, unilateral femur diaphysis fractures developed in 3 subjects; infection occurred on one side of the femur in 2 subjects; while one rabbit died during the study. The 7 subjects mentioned above were replaced with new subjects. The study was completed at the end of week 8 when all of the rabbits were sacrificed and histological specimens were obtained from them. After histological examination, those subjects with pseudoarthrosis and loss of implant position were excluded as well. Subject populations that were evaluated for each group are shown in Table 2.

The level of ossification was evaluated from the radiographic data obtained in the second and the eighth weeks and graded according to the method outlined in Table 2; the mean values of ossification for the different groups are given in Table 3. A significant difference (Kruskal-Wallis test) was found in the level of ossification between the groups in the 2nd week; however, this difference was lost in the 8th week. Additionally, the ossification data from the groups at Week 2 were subjected to a post-hoc Dunn's test. We observed significant differences between the first (G-NI) and fourth (C-I) groups; the fourth group exhibited less ossification in early time point. No significant differences were found in other comparisons (Table 4).

At the end of week 8, it was observed that the subchondral bone tissue was incompletely renewed in all the groups. A comparison of the extent of subchondral bone tissue renewal indicated an increase in Groups C-NI and C-I when compared to Groups G-NI and G-I.

Unlike the incomplete formation of new bone tissue in the subchondral area, an increase in connective tissue and vascularization were observed in all of the groups. When the

Table 2. Group names, abbreviations and number of samples.

Group	Process	Abbreviation	Number
1	Fixed by creating a gap and no irrigation	G-NI	7
2	Fixed by creating a gap and irrigation	G-I	6
3	Fixed with complete reduction and no Irrigation	C-NI	5
4	Fixed with complete reduction and Irrigation	C-I	7
5	Control		8

Table 3. Radiographic median values of the groups.

Groups		Median	Min	Max
Group 1 G-NI	2nd Week	1.00	0.00	1.00
	8th Week	3.00	2.00	4.00
Group 2 G-I	2nd Week	1.00	0.00	1.00
	8th Week	3.00	2.00	4.00
Group 3 C-NI	2nd Week	1.00	0.00	1.00
	8th Week	3.00	1.00	4.00
Grup 4 C-I	2nd Week	2.00	1.00	2.00
	8th Week	3.00	2.00	4.00

cartilage tissue of the joint surface was examined, it was observed that the cartilage tissue was not fully formed and renewed and that it did not completely coalesce with the old cartilage tissue in all of the groups. In Group C-NI, the new tissue, which was formed on the joint surface, was generally fibro-cartilage in nature and in some areas hyaline cartilage tissue was observed. In other groups, hyaline cartilage tissue formation was not observed but there was the formation of extensive connective tissue. Compared with the other groups, the cartilaginous tissue layer formed in Group C-NI was thicker while the surface of the tissue was

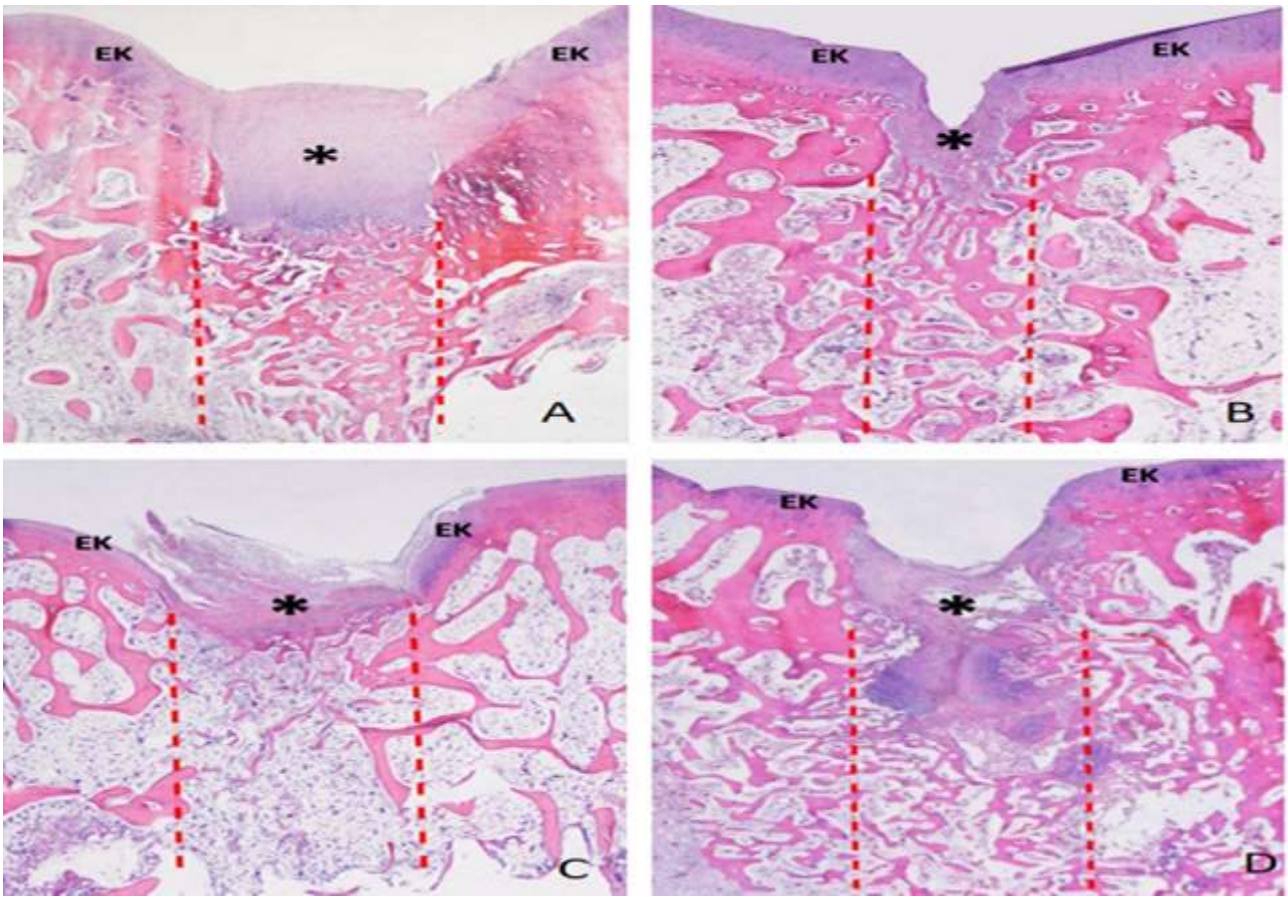


Figure 3. Histologic analysis of sections taken from the knee joints by staining with hematoxylin & eosin. In groups C-I **(B),** G-NI **(C)** and Group G-I **(D),** the area between the old cartilage tissue (EK) on the joint surface has a fibrous connective tissue feature (*) with a new developing density and a subchondral ossification area (red striated area) which is below this part. In group C-NI (A), fibrocartilage tissue (*) containing both connective tissue and newly formed hyaline cartilaginous tissue (*) and newly developed subchondral ossification area (red striated area) has been observed. (C-NI: Time reduction, no irrigation, C-I: Complete reduction, irrigation available, G-NI: Intermittent fix, no irrigation, G-I Reduction with gap with irrigation. Graphic 1. Grouping of subjects.

flatter. Additionally, the newly formed tissue was better fused with cartilaginous tissues adjacent to it. Statistical evaluation of the data and the Wakitani scoring indicated there was incomplete improvement in all groups; nonetheless, the Group C-NI demonstrated the best histological parameters for healing (Tables 5-8, Figure 3).

Discussion

Intra-articular fractures are compulsive fractures because they require both anatomic fracture reduction and rigid fixation; moreover it is often difficult to reach the fracture area. Even minor problems may affect the clinical results negatively. A better understanding and experience of arthroscopic methods may lead to

Table 4. Statistical comparison of the week 2 and week 8 data in different the groups.

	Kruskal Wallis Chi-square Test		Post-hoc Dunn Test					
	K-W Value	*P* Value	Group 1 - 2	Group 1 - 3	Group 1 - 4	Group 2 - 3	Group 2 - 4	Group 3 - 4
2 nd Week	10.336	0.016	1.000	1.000	0.024	1.000	0.272	0.065
8th Week	1.155	0.764	Not suitable for further evaluation.					

p<0.05, K-W: Kruskal Wallis

Table 5. Median values of ossification percentages at week 8.

Groups	Median	Minimum	Maximum
Group 1 (G-NI)	12.70	9.22	19.92
Group 2 (G-I)	11.81	2.03	33.68
Group 3 (C-NI)	24.44	21.46	29.33
Group 4 (C-I)	20.43	16.69	32.29

Table 6. Comparisons of ossification percentages at week 8 in different the groups

	Kruskal Wallis Chi-Square Test		Post-hoc Dunn Test					
	K-W Value	*P* Value	Group 1 - 2	Group 1 - 3	Group 1 - 4	Group 2 - 3	Group 2 - 4	Group 3 - 4
Percent of Ossification	10.053	0.018	1.000	0.065	1.000	0.106	0.353	0.231

K-W: Kruskal Wallis Value.

Table 7. Mean values according to Wakitani 31, 32 histological grading scale at eighth week.

		Cell Morphology	Matrix Staining	Surface consistency	Cartilage Thickness	Combining of the donor tissue with recipient neighboring cartilage.	Total
Group 1 (G-NI)	Median	4.00	3.00	3.00	2.00	2.00	2.00
	Maximum	4.00	3.00	3.00	2.00	2.00	14.00
	Minimum	2.00	2.00	1.00	1.00	1.00	7.00
Group 2 (G-I)	Median	3.50	3.00	3.00	2.00	2.00	13.50
	Maximum	4.00	3.00	3.00	2.00	2.00	14.00
	Minimum	2.00	2.00	1.00	2.00	1.00	8.00
Group 3 (C-NI)	Median	2.00	1.00	2.00	2.00	1.00	9.00
	Maximum	2.00	2.00	3.00	2.00	2.00	10.00
	Minimum	1.00	1.00	1.00	0.00	0.00	3.00
Group 4 (C-I)	Median	3.00	2.00	3.00	2.00	2.00	12.00
	Maximum	4.00	3.00	3.00	2.00	2.00	14.00
	Minimum	2.00	2.00	1.00	1.00	1.00	8.00

Table 8. Statistical comparison of cell morphology and matrix staining in the groups.

	Kruskal Wallis Chi-Square Test		**Post-hoc Dunn Test**					
	K-W Value	*P*	Group 1- 2	Group 1-3	Group 1-4	Group 2-3	Group 2-4	Group 3-4
Cell Morphology	10.710	**0.013**	1.000	**0.016**	1.000	**0.038**	1.000	0.349
Matrix Staining	12.873	**0.005**	1.000	**0.016**	1.000	**0.007**	0.632	0.432
Surface Consistency	4.832	0.185	Not suitable for further evaluation.					
Cartilage Thickness	3.470	0.325	Not suitable for further evaluation.					
Combining of the donor tissue with recipient neighboring cartilage.	7.435	0.059	Not suitable for further evaluation.					
Total	9.371	**0.025**	1.000	0.054	1.000	**0.035**	1.000	0.552

more extensive use of arthroscopy in intra-articular fracture surgery. Arthroscopically assisted fixation of intra-articular fractures has the following advantages over traditional fixation methods: a) much better visualization and full reduction despite minimal invasiveness, b) increased clinical improvement due to diagnosis and c) repair of other injuries accompanying the fracture [1]. Nevertheless, there are inherent limitations of arthroscopy-assisted intra-articular fracture treatment the most important of which are the long learning curve and the material for fixation. Our study was designed to examine another possible limitation, the effect of lavage during arthroscopy on the healing of fractures and cartilage tissue.

There are four separate mechanisms responsible for the healing of fractures: Enchondral ossification in which the fracture hematoma plays a role, intramembranous ossification in which periostea is responsible, appositional ossification and ossification through the direct Haversian system. These mechanisms contribute to fracture healing at various rates and may be affected by variables such as fracture shape, location, stability, and fixation type. Usually, in fractures that undergo anatomical reduction and rigid fixation, the healing is primarily through a more direct Haversian system (primary ossification) rather than hematomas. On the other hand, fractures that undergo healing without anatomic reduction and rigid fixation heal with hematoma by (secondary ossification) enchondral ossification [33]. Joint cartilage defects, on the other hand, heal with fibrous tissue formation. We tried to prevent secondary ossification at the osteotomy sites by removing the hematoma from the environment via arthroscopy like closed irrigation. In preliminary studies, some cases were created to remove the fracture hematoma. Park et al reported that after osteotomy in the rabbit tibia diaphysis, union was delayed or never developed with open irrigation in the first and the second day [34]. Dirschle et al observed a delay in union by 20 – 30% at early time points when pressureless irrigation with syringes and high pressure irrigation system were used after femur medial condyle osteotomy [35]. Cartilage tissue healing constitutes the subject of many animal experiments; Mitchell et al. [36] reported that medial femoral condylar fractures of the rabbit femur healed with hyaline cartilage when compression was done; however, without compression they healed with fibrous cartilage.

Although the effect of lavage solutions on natural cartilage tissue has been investigated previously, to our knowledge, no study in the published literature has examined the effects of arthroscopic lavage on fractured cartilage and bone tissue [27,28]. Rabbits were considered as appropriate subjects for this study since the Haversian system of rabbits are similar to human bones and because the cost and care of rabbits are more reasonable compared to larger animals. Our experimental setup was based on the femur medial epicondyle osteotomy and fixation method described by Mitchell et al. [36] Unlike other published studies; lavage after osteotomy was carried out by providing a closed lavage environment in order to simulate the arthroscopic environment [26,34,35]. In the rabbit knees that were fixated with gap and underwent irrigation (group 2), we expected to observe a decrease in fracture union tissue quantity and deterioration of the quality of cartilage tissue that were likely related to the removal of the hematoma; however, in the histological examinations, there was no significant difference between the groups in the percentage of ossification. Also when we

examined the cartilaginous tissue, we observed that the Wakitani score was lower in the second group compared to the third group where complete reduction was carried out without any irrigation. We also observed that cell morphology and matrix staining scores were lower in the second group. However, we also observed similar data in the first group of animals where a gap was created and the wound was not irrigated. For this reason, we think that the healing disorder of the cartilaginous tissue was caused by the created gap, and not by washing. We did not observe any harmful effect of washing on fracture and cartilage healing in our study.

The major weaknesses of our study are the low number of subjects and the use of both knees of the subjects. In addition to this, radiologic studies were performed with direct X-ray because of the lack of access to micro-computerized tomography, which would show the rabbit bone structure better.

Conclusion

There were no adverse effects of intra-articular lavage on fracture union and cartilage healing in an *in vivo* environment. Nonetheless, the findings of this study should be confirmed with a larger sample size.

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References

[1]Atesok K, Doral MN, Gideon TW, et al. Arthroscopy-assisted fracture fixation. Knee Surg Sports Traumatol Arthrosc. 2011;19(29:320–29).

[2]Caspari RB, Hutton PM, Whipple TL, et al. The role of arthroscopy in the management of tibial plateau fractures. Arthroscopy. 1985;1(2):76–82.

[3]Chan YS, Chiu CH, Lo YP, Chen AC, et al. Arthroscopy-assisted surgery for tibial plateau fractures: 2 to 10 year follow-up results. Arthroscopy. 2008;24(7):760–68.

[4]Kayali C, Ozturk H, Altay T, et al. Arthroscopically assisted percutaneous osteosynthesis of lateral tibial plateau fractures. Can J Surg. 2008;51(5):378-82.

[5]Rousseau MA, Biette G, Jouve F, et al. Long term outcomes after arthroscopic management of tibial plateau fractures. Eur J Trauma Emerg Surg. 2008;34(1):49-54.

[6]Hirschmann MT, Mayer RR, Kentsch A, et al. Physeal sparing arthroscopic fixation of displaced tibial eminence fractures: a new surgical technique. Knee Surg Sports Traumatol Arthrosc. 2007;17(7):741–47.

[7]Hunter RE, Willis JA. Arthroscopic fixation of avulsion fractures of the tibial eminence: Technique and outcome. Arthroscopy. 2004;20(2):113-21.

[8]Vega JR, Irribarra LA, Baar AK, et al. Arthroscopic fixation of displaced tibial eminence fractures: a new growth plate-sparing method. Arthroscopy. 2008;24(11):1239-43.

[9]Leontaritis N, Hinojosa L, Panchbhavi VK. Arthroscopically detected intra-articular lesions associated with acute ankle fractures. J Bone Joint Surg Am. 2009;91(2):333–39.

[10]Ono A, Nishikawa S, Nagao A, et al. Arthroscopically assisted treatment of ankle

fractures: arthroscopic findings and surgical outcomes. Arthroscopy. 2004;20(6):627–31.

[11] Thordarson DB, Bains R, Shepherd LE. The role of ankle arthroscopy on the surgical management of ankle fractures. Foot Ankle Int. 2001;22(2):123-25.

[12] Mullis BH, Dahners LE. Hip arthroscopy to remove loose bodies after traumatic dislocation. J Orthop Trauma. 2006;20(1):22-26.

[13] Yamamoto Y, Ide T, Ono T, et al. Usefulness of arthroscopic surgery in hip trauma cases. Arthroscopy. 2003;19(3):269–73.

[14] Tauber M, Moursy M, Eppel M, et al. Arthroscopic screw fixation of large anterior glenoid fractures. Knee Surg Sports Traumatol Arthrosc. 2008;16(3):326–32.

[15] Kim KC, Rhee KJ, Shin HD, et al. Arthroscopic fixation for displaced greater tuberosity fracture using the suture bridge technique. Arthroscopy. 2008;24(1):120.e1–120.e3.

[16] Checchia SL, Doneux PS, Miyazaki AN, et al. Treatment of distal clavicle fractures using an arthroscopic technique. J Shoulder Elbow Surg. 2008;17(3):395–98.

[17] Michels F, Pouliart N, Handelberg F. Arthroscopic management of mason type 2 radial head fractures. Knee Surg Sports Traumatol Arthrosc. 2007;15(10):1244–50.

[18] Adams JE, Merten SM, Steinmann SP. Arthroscopic assisted treatment of coronoid fractures. Arthroscopy. 2007;23(10):1060–65.

[19] Hardy P, Menguy F, Guillot S. Arthroscopic treatment of capitellum fracture of the humerus. Arthroscopy. 2002;18(4):422–26.

[20] Wolfe SW, Easterling KJ, Yoo HH. Arthroscopic-assisted reduction of distal radius fractures. Arthroscopy. 1995;11(6):706-14.

[21] Adolfsson L, Jorgsholm P. Arthroscopically-assisted reduction of intra-articular fractures of the distal radius. J Hand Surg Br. 1998;23(3):391–95.

[22] Ruch DS, Vallee J, Poehling GG, et al. Arthroscopic reduction versus fluoroscopic reduction in the management of intra-articular distal radius fractures. Arthroscopy. 2004;20(3):225–30.

[23] Shih JT, Lee HM, Hou YT, et al. Results of arthroscopic reduction and percutaneous fixation for acute displaced scaphoid fractures. Arthroscopy. 2005;21(5):620–26.

[24] Slade JF, Lozano-Caldero´n S, Merrell G, et al. Arthroscopic-assisted percutaneous reduction and screw fixation of displaced scaphoid fractures. J Hand Surg Eur. 2008;33(3):350–54.

[25] Caloia MF, Gallino RN, Caloia H, et al. Incidence of ligamentous and other injuries associated with scaphoid fractures during arthroscopically assisted reduction and percutaneous fixation. Arthroscopy. 2008;24(7):754-59.

[26] Polzin B, Ellis T, Dirschl DR. Effects of varying pulsatile lavage pressure on cancellous bone structure and fracture healing. J Orthop Trauma. 2006;20(4):261-66.

[27] Arciero, RA, Little JS, Liebenberg SP, et al. Irrigating solutions used in arthroscopy and their effect on articular cartilage. An in vivo study. Orthopedics. 1986;9(11):1511-15.

[28] Bulstra SK, Kuijer R, Eerdmans P, van der Linden AJ. The effect in vitro of irrigating solutions on intact rat articular cartilage. J Bone Joint Surg Br. 1994;76(3):468-70.

[29] An YH, Friedmen RJ. Animal models in orthopaedic research. CRC press, New York. 1999: pp155-32.

[30] Öztuna V. Ortopedi ve Travmatolojide Deneysel Hayvan Modelleri. BAYT Yayınları, Ankara. 2015.

[31] Wakitani S, Goto T, Pineda SJ. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am. 1994;76(4),579-92.

[32] Nagura I, Fujioka H, Kokubu T, et al. Repair of osteochondral defects with a new porous sythetic polymer scafold. J. Bone Joint Surg Br. 2007;89(2),258-64.

[33] Whittle AP. General Principles of Fracture Treatment. In: Azar FM, Beaty JH, Canale ST. Campbell's Operative Orthopaedics; 13th edn. Chapter 53, 2017. pp 2655-2711.

[34] Park SH, Silva M, Bahk WJ, et al. Effect of repeated irrigation and debridement on fracture healing in an animal model. J Orthop Res. 2002;20(6)1197-204.

[35] Dirschl DR, Duff GP, Dahners LE, et al. High pressure pulsatile lavage irrigation of intraarticular fractures: effects on fracture healing. J Orthop Trauma. 1998;12(7):460-63.

[36] Mitchell N, Shepard N. Healing of articular cartilage in intra-articular fractures in rabbits. J Bone Joint Surg Am. 1980;62(4):628-34.

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Original Article

Role of N-terminal pro b-type natriuretic peptide (NT-pro-BNP) in compensated chronic kidney disease

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ABSTRACT

Aim: To evaluate the role of N-terminal pro b-type natriuretic peptide (NT-pro-BNP) in the evaluation of hypervolemia in chronic kidney disease (CKD) and its relationship with CKD.

Methods: Sixty compensated chronic kidney disease patients enrolled in this study. NT-pro BNP levels and other routine biochemical laboratory parameters are studied. The associations between results were analyzed.

Results: NT-pro BNP levels were correlated with urea *($r = 0.66$, $p < 0.01$)*, creatinine *($r = 0.69$, $p < 0.01$)* and phosphorus *($r = 0.36$, $p < 0.01$)* values and were negative correlated with hemoglobin *($r = -0.32$, $p = 0.01$)*, hematocrit *($r = -0.36$, $p < 0.01$)*, albumin *($r = -0.29$, $p = 0.02$)* and glomerular filtration rate (GFR) values *($r = -0.35$, $p < 0.01$)*.

Conclusion: The positive correlation between NT-pro BNP levels and urea and creatinine values in our study and the negative correlation with GFR support that the severity of hypervolemia increases as the CKD stage progresses. BNP and NT-pro BNP are strong predictors of all-cause cardiovascular mortality in asymptomatic CKD patients. In the light of all these data, it is possible to suggest that NT-pro BNP is associated with hypervolemia and therefore increased cardiovascular mortality in subjects with CKD.

Keywords: Chronic kidney disease, NT-pro BNP, hypervolemia.

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Introduction

Chronic kidney disease (CKD) is a disease that causes significant morbidity and mortality and associated cardiovascular diseases are common [1]. Left ventricular hypertrophy (LVH), one of the cardiovascular problems, is a strong predictor of mortality in CKD [2,3]. Therefore, early detection of LVH in this population is of great importance in preventing mortality and in determining CVD risk. LVH is caused by prolonged exposure to increased volume load in CKD patients and can be detected by echocardiography. However, some serum markers have been shown to play a role in

determining LVH. For example, the N-terminal pro-brain natriuretic peptide (NT-pro BNP) has been reported in many studies as an important predictor of LVH in CKD patients [4-7].

Increased extracellular volume, myocardial strain and increased left ventricular pressure are among the main causes of elevation of NT-pro BNP levels in CKD patients [8]. This hormone is a member of the family of natriuretic peptides, known as vasoactive hormones, and when activated via neurohumoral pathways, plays a role in regulating blood pressure and maintaining volume balance by acting directly on the kidneys and systemic vessels [9,10].

In the light of current literature, in this study, we aimed to evaluate the role of NT-pro BNP in the evaluation of hypervolemia in CKD and its relationship with CKD.

Materials and Methods

This prospective study was conducted after approval by the local ethics committee (Decision no: B.30.2. ABÜ.0.20.05.04-050.01.04-08). Patients who were referred to our Internal Medicine-Nephrology clinic and diagnosed as compensated chronic renal failure were included in the study on a voluntary basis. Laboratory parameters were evaluated with blood samples taken at least eight hours after fasting were taken according to routine procedures in patients with compensated chronic renal failure. Complete blood count parameters were recorded on Mindrey BC-5380 analyzer; Glucose, urea, creatinine, sodium, potassium, total protein, albumin, calcium, phosphorus, parathormone, C-reactive protein (CRP) parameters were studied in Abbott C-8000 clinical chemistry autoanalyser. Plasma NT-pro BNP measurements; Electrochemiluminescence immunoassay method was used with Elecsys 2010 analyzer. Cockcroft-Gault formula, which is the most commonly used glomerular filtration rate (GFR) in clinical practice for the determination of CKD stage [Creatinine clearance = (140-Age) x (Ideal weight) (ml / min) / Serum creatinine (mg / dl) X 72 (female x 0.85)] was used [11].

Exclusion criteria were follows: Patients with congestive heart failure, heart valve diseases, coronary artery disease, cardiac operation history or cardiac pacemaker, cardiomyopathy, sick sinus syndrome, chronic obstructive pulmonary disease, morbidly obese and overweight, hypo / hyperthyroidism and chronic liver disease. Cases without consent to participate in the study were also excluded.

Statistical analysis

All statistical analyzes of the data were performed by SPSS (15.0 IBM Co., Chicago, Illinois, USA) program. The Pearson test was used to analyze the correlation coefficients and statistical significance between the clinical parameters whose normal distribution was demonstrated by analytical methods (using Kolmogorov Smirnov and Shapiro-Wilk tests). Spearman's correlation test was used for the relationship between at least one of the variables which did not show normal distribution or ordinal variables. The statistical difference between the independent parameters, whose normal distribution was shown by the tests mentioned above, was investigated by independent groups t test (Student's t test) and expressed as mean ± standard deviation. Kruskal Wallis test was used to compare the data with abnormal distribution and the results were expressed as median (min-max). Statistical significance level was accepted as $p < 0.05$.

Results

The study population consisted of 31 (51%) male and 29 (48%) female patients. The age of

Table 1. The clinical and laboratory findings of the study.

Parameter		Mean ± SD
Age (year)		60.8 ± 12.4
Gender	Female	29 (% 48)
	Man	31(% 51)
Weight (kilogram)		72.2 ± 11.1
Size (meter)		1.63 ± 0.1
BMI(weight/size²)		26.8 ± 3.6
DM time (year)		12 ± 5.7 *
Smoking cigarette	Yes	9 (% 15)
	No	51 (% 85)
Using Anti-HT	Yes	53 (% 88)
	No	7 (% 12)
Systolic blood pressure (mmHg)		136 ± 21
Diastolic blood pressure (mmHg)		79 ± 12
Heart rate (beat/min)		70 ± 7
Leukocyte (/mm³)		7163 ± 1955
Hemoglobin (g/dl)		11.7 ± 2.3
Hematocrit (%)		35.6 ± 6.9
Thrombocyte (/mm³x1000)		246 ± 71
Glucose (mg/dl)		122 ± 57
Urea (mg/dl)		84 ± 37
Creatinine (mg/dl)		2.36 ± 1.2
Sodium (mmol/l)		137 ± 4
Potassium (mmol/l)		4.6 ± 1.0
Total protein (g/dl)		6.8 ± 1.0
Albumin (g/dl)		3.8 ± 1.0
Calcium (mg/dl)		8.8 ± 1.0
Phosphorus (mg/dl)		3.9 ± 1.2
Parathyroid hormone (pg/ml)		130 ± 85.1
C-reactive protein (mg/l)		13.7 ± 19.1*
NT-pro BNP (pg/ml)		1181. 1 ± 2198.6
		473.7 * (min:37,5-max:14541)
GFR (Cocroft-Gault) (ml/min.)		36.3 ± 14.5
		33.6 * (min:12,1-max:73)

*Median value.

the patients was 60.8 ± 12.4 years. The mean GFR value of the CKD patients included in the study was 36.3 ± 14.5 ml / min. According to CKD stage, 5 (9%) patients were classified as stage 2, 31 (51%) patients as stage 3 and 24 (40%) patients as stage 4 CKD. Twenty-six (43%) patients were diagnosed with diabetes mellitus (DM) and the mean duration of diabetes was 12.0 ± 5.7 years. Nine (85%) people were smoking cigarette and 88% (53 cases) of the patients were using anti-hypertensive (anti-HT) treatment due to hypertension. The clinical and laboratory findings of the cases included in the study are presented in Table 1.

The mean NT-pro BNP values of the patients included in the study were 1181.1 ± 2198.6 pg / ml and the median value was 473.7 (min:37,55pg/ml-max: 14541pg / ml).

There was a positive correlation between NT-pro BNP levels and urea *(r = 0.66, p <0.01*), creatinine *(r = 0.69, p <0.01)* and phosphorus *(r = 0.36, p <0.01)* values and then negative correlation with hemoglobin *(r = -0.32, p = 0.01)*, hematocrit *(r = -0.36, p <0.01)*, albumin *(r = -0.29, p = 0.02)* and GFR values *(r = -0.35, p <0.01*). There was no correlation between age, height, weight, body mass index (BMI), smoking cigarette, use of anti-HT agents, DM time, blood pressures, heart rate and other laboratory findings and NT-pro BNP levels. The results of the analysis showing the correlation between NT-pro BNP level and clinical and laboratory parameters are shown in Table 2.

Discussion

The most important results of this study were that NT Pro BNP had a positive correlation with serum creatinine and phosphorus levels, which are important markers of renal function, and a

negative correlation with hemoglobin, hematocrit, albumin and GFR levels.

Table 2. The correlation between NT-pro BNP level and clinical and laboratory parameters.

Parameter	*p*	*r*
Age (year)	0.95	0.01
Size (meter)	0.20	0.16
Weight (kg)	0.27	0.14
BMI (weight/size²)	0.64	0.06
DM time (year)	0.19	0.27
Systolic blood pressure (mm Hg)	0.20	0.11
Diastolic blood pressure (mm Hg)	0.17	-0.17
Heart rate (Beat/min)	0.92	-0.01
Leukocyte (/mm³)	0.17	0.17
Hemoglobin (g/dl)	0.01	-0.32
Hematocrit (%)	<0.01	-0.36
Thrombocyte (mm³ x 1000)	0.15	-0.18
Glucose (mg/dl)	0.69	-0.05
Urea (mg/dl)	<0.01	0.66
Creatinine (mg/dl)	<0.01	0.69
Sodium (mmol/l)	0.43	-0.1
Potassium (mmol/l)	0.75	-0.41
Albumin (g/dl)	0.02	-0.29
Total protein (g/dl)	0.16	-0.18
Calcium (mg/dl)	0.58	-0.73
Phosphorus (mg/dl)	<0.01	0.36
Parathyroid hormone (pg/ml)	0.67	0.05
C-Reactive Protein (mg/dl)	0.09	0.25
GFR (ml/min.)	<0.01	-0.35

CKD is an important public health problem in the world and in our country (1). According to the data of the CREDIT study the prevalence of CKD from all causes in Turkey was estimated at 15.7% [12]. The main problem with this large group of patients is the inability to effectively control the complications as well as the disease. Cardiovascular diseases, commonly seen in patients with chronic renal failure, are a major cause of morbidity and mortality [1]. Cardiovascular diseases seen in CKD have a broad spectrum and these are mainly left ventricular hypertrophy, ischemic heart disease, heart failure, peripheral vascular diseases, cardiac arrhythmias and sudden death [13].

LVH is a strong predictor of mortality in CKD [13]. There are many studies in the literature that NT-pro BNP is a strong predictor of left ventricular hypertrophy. In a study by Satyan et al. [4] in asymptomatic HD patients, a significant relationship was found between NT-pro BNP and left ventricular mass index. In another study, it was shown that there is a direct relationship between NT-pro BNP and left ventricular hypertrophy in patients with end-stage renal disease (ESRD) [5]. Wang et al. [6] found that NT-pro BNP levels were significantly higher in patients with left ventricular dysfunction and severe left ventricular hypertrophy in a CKD cohort undergoing dialysis treatment. In another study performed by the same team, the association of NT-pro BNP with cardiovascular congestion, mortality and cardiac events was examined in a group of 230 patients with chronic peritoneal dialysis [7]. In this study, echocardiographic measurements were performed concurrently with basal NT-pro BNP value and all patients were followed up to 3 years. As a result of the study, a strong relationship was found between left ventricular EF, left ventricular mass index

and residual GFR and NT-pro BNP. NT-pro BNP has been shown to be an important predictor of cardiovascular congestion, mortality and cardiovascular side effects.

Studies have shown that left ventricular hypertrophy was detected at the beginning of dialysis treatment in more than 70% of CKD patients and that the frequency of left ventricular hypertrophy increased in this patient group over time [14,15]. The positive correlation between NT-pro BNP levels and urea and creatinine values in our study and the negative correlation with GFR support that the severity of hypervolemia increases as the CKD stage progresses. Deflippe et al. [16] found positive and significant correlation between NT pro BNP and LVH in asymptomatic compensated CKD patients and was consistent with GFR. In another study performed on the diagnostic values of natriuretic peptides in CKD, a negative correlation was found between BNP and GFR and it was reported that the increase in phosphorus values and anemia-related values were related to the progression of CKD stage [17]. In the evaluation of this group, hypoalbuminemia in patients was associated with CKD-induced urinary protein loss and malnutrition, and its association with natriuretic peptides was attributed to extracellular volume increase and hypervolemia. In our study, a positive correlation was found between the NT-pro BNP and phosphorus, and a negative correlation between NT-pro BNP and hemoglobin, hematocrit, albumin values. This suggests that as the degree of renal failure progresses in compensated CKD, it may show increased anemia and metabolic imbalance disturbances as well as a reflection of increased hypervolemia. In addition, hypoalbuminemia is an important indicator of inflammation and CRP elevation and hypoalbuminemia in CKD are two independent and strong predictors of mortality [18, 19]. In a study performed in patients with ESRD, it was shown that there was a 4.6-fold increase in deaths related to all diseases and 5.5-fold increase in CVD-related deaths due to the increase in CRP level [20]. Studies have shown that inflammation markers are significantly reduced in patients with euvolemia, where hypervolemia causes inflammation and cardiovascular morbidity and mortality in CKD patients [18,19]. In our study, there was a negative correlation between NT-pro BNP and albumin, which is consistent with the above-mentioned literature. In addition, we found a significant relationship between NT-pro BNP and CRP *(p=0.09)*. However, this value was above the level of statistical significance. We thought that this might be related to the scarcity of our patients.

Limitations of present study are lack of echocardiographic evaluation and relatively small study population. However, our results showing significant association between NT pro BNP and compensated CKD.

Conclusion

Among the main causes of BNP and NT-pro BNP increase in CKD patients are factors such as increased extracellular volume, myocardial strain and increased left ventricular pressure (9). In addition, causes such as left ventricular hypertrophy, endothelial dysfunction in advanced CKD, systolic and diastolic left ventricular insufficiency, and ischemic cardiac disease also contribute to this increase. In conclusion, BNP and NT-pro BNP are strong predictors of all-cause cardiovascular mortality in asymptomatic CKD patients. In the light of all these data, it is possible to say that NT-pro BNP is associated with hypervolemia and therefore increased cardiovascular mortality in CKD.

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References

[1]Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Hypertension. 2003; 42(5): 1050–65.

[2]Amann K, Rychlík I, Miltenberger-Milteny G, et al. Left ventricular hypertrophy in renal failure. Kidney Int Suppl.1998; 68:78-85.

[3]Krane V, Heinrich F, Meesmann M, et al. Electrocardiography and outcome in patients with diabetes mellitus on maintenance hemodialysis. Clin J Am Soc Nephrol. 2009; 4(2): 394-400.

[4]Satyan S, Light RP, Agarwal R. Relationships of N-terminal pro-B-natriuretic peptide and cardiac troponin T to left ventricular mass and function and mortality in asymptomatic hemodialysis patients. Am J Kidney Dis. 2007; 50(6): 1009–19.

[5]Madsen LH, Ladefoged S, Corell P, et al. N-terminal pro brain natriuretic peptide predicts mortality in patients with end-stage renal disease in hemodialysis. Kidney Int. 2007; 71(6): 548–54.

[6]Wang AY, Lam CW, Wang M, et al. Diagnostic potential of serum biomarkers for left ventricular abnormalities in chronic peritoneal dialysis patients. Nephrol Dial Transplant. 2009; 24(6):1962– 69.

[7]Wang AY, Lam CW, Yu CM, et al. N-terminal probrain natriuretic peptide: an independent risk predictor of cardiovascular congestion, mortality, and adverse cardiovascular outcomes in chronic peritoneal dialysis patients. J Am Soc Nephrol. 2007; 18: 321–30.

[8]Wang AY. Clinical utility of natriuretic peptides in dialysis patients. Semin Dial. 2012;25(3):326–333.

[9]Baughman KL. B-type natriuretic peptide -- a window to the heart. N Engl J Med. 2002;347(3):158–59.

[10]de Lemos JA, McGuire DK, Drazner MH: B-type natriuretic peptide in cardiovascular disease. Lancet. 2003; 362 (9380): 316–22.

[11]Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31–41.

[12]Süleymanlar G, Utaş C, Arinsoy T, et al. A population-based survey of Chronic REnal Disease In Turkey--the CREDIT study. Nephrol Dial Transplant. 2011;26(6):1862–71.

[13]Ausma J, Litjens N, Lenders MH, et al. Time course of atrial fibrillation-induced cellular structural remodeling in atria of the goat. J Mol Cell Cardiol. 2001; 33(12): 2083–94.

[14]Foley RN, Parfrey PS, Harnett JD, Kent GM, Martin CJ, Murray DC, et al. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. Kidney Int. 1995; 47(1):186-92.

[15]Wang AY, Wang M, Woo J, et al. Inflammation, residual kidney function, and cardiac hypertrophy are interrelated and combine adversely to enhance mortality and cardiovascular death risk of peritoneal

dialysis patients. J Am Soc Nephrol. 2004; 15(8): 2186–94.

[16] De Filippi CR, Fink JC, Nass CM, et al. N-terminal pro- B-type natriuretic peptide for predicting coronary disease and left ventricular hypertrophy in asymptomatic CKD not requiring dialysis. Am J Kidney Dis. 2005; 46(1): 35–44.

[17] Patrick B. Mark, Graham A. Stewart, et al. Jardin. Diagnostic potential of circulating natriuretic peptides in chronic kidney disease. Nephrol Dial Transplant. 2006; 21(2): 402–10.

[18] Ortega O, Gallar P, Muñoz M, et al. Association between C-reactive protein levels and N-terminal pro-B-type natriuretic peptide in pre-dialysis patients. Nephron Clin Pract. 2004; 97 (4):125-30.

[19] Yılmaz R. Ultrafiltrasyon ve hemodiyaliz sıklığının BNP ve CRP üzerine etkisi: Kardiyak fonksiyon bozukluğu, inflamasyon ve hipervolemi ilişkili mi? 22. Ulusal Nefroloji, Hipertansiyon, Diyaliz ve Transplantasyon Kongresi Özet Kitabı, 2005; s: 8.

[20] Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int. 1999; 55 (2): 648-58.

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Original Article

The role of diffusion weighted imaging in magnetic resonance to evaluate breast masses

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ABSTRACT

Aim: To investigate the role of diffusion weighted magnetic resonance imaging (MRI) in differentiation of benign and malignant breast lesions using apparent diffusion coefficient (ADC) values preoperatively.

Methods: A total of 30 women between the ages of 14-75 years (mean, 47, 6 years) with 30 histopathologically verified breast lesions were investigated in this study. The patients were examined by a 1, 5 T MRI device using bilateral phased array breast coil. Spin echo planar diffusion imaging was used to scan patients. Images were obtained by b values 0 and 500 seconds/mm². Mean ADC values of the benign and malignant lesions were measured and calculated. The comparison between the histopathological diagnoses and the mean ADCs were performed by Mann Whitney U test.

Results: The diagnosis of 30 patients with 30 breast lesions were as follows; malign lesions (n=13), benign lesions (n=17). The ADC values were as follows (in units of 10^{-3}mm² /sec): benign breast lesions (range: 1, 09-1, 98, mean: 1, 45) and malignant breast lesions (range: 0, 59-1, 08, mean: 0, 76). The mean ADC obtained from malignant breast lesions was statistically different from that observed in benign solid lesions *(p<0, 01)*.

Conclusion: Diffusion imaging can be used in differentiation of malign and benign breast lesions.

Keywords: Magnetic resonance imaging, diffusion, breast masses.

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Introduction

Breast cancer is the most common type of cancer in women. Despite significant improvements in treatment, it is still one of the major causes of cancer-related mortality worldwide with an estimated 500,000 deaths per year [1]. Accurate diagnosis is of critical importance because early diagnosis and treatment increases quality of life and survey [2].

Mammography is still the main imaging method for screening and detecting breast

lesions with a sensitivity of 69–90% [3-5]. In the presence of dens parenchyma or breast implant, where mammography is insufficient, ultrasonography (USG) is complementary to mammography in patients evaluated postoperatively or after radiotherapy, and it has a very significant place in breast imaging. However, USG alone is inadequate to detect microcalcifications and ductal carcinoma in situ cases [6].

MRI is increasingly used as a problem-solving method in the diagnosis of breast cancer [2]. Conventional dynamic breast MRI has a sensitivity of 94-99% in the diagnosis of invasive breast cancer, while its specificity varies between 37-86% [7-8]. In addition, it may not be possible to differentiate benign lesions from malignant lesions by conventional MRI sequences because sometimes morphological features and contrast enhancement patterns of benign and malignant lesions may be similar [9]. Therefore, more specific imaging techniques are needed to characterize breast lesions. DWI is an MRI technique based on different diffusion rates of water molecules in normal and pathological tissues. Compared with conventional MRI, this technique has been shown to have a higher specificity in differentiating benign and malignant lesions from 84% to 37% [10].

In addition, ADC maps are generated automatically by high-capacity computers over DWI, and measurements can be made on these maps. In many studies in the literature, it was found out that the average ADC values correlated with the cell density of breast tumors [10-11]. Studies have suggested that DWI and ADC measurements have high accuracy rate in differentiating malignant-benign breast lesions [11-14].

In this study, it was aimed to determine the contribution of signal abnormalities detected in DWI to diagnosis in breast MRI and, additionally, DWI planned in selected cases for different reasons, and to compare the ADC values measured with histopathological results for the lesions.

Materials and Methods

Thirty female patients whose lesion differentiation between malignant and benign could not be assessed by mammography or USG, and who had BI-RADS 4 and 5 lesions with a mass size of 1 cm or over were included into our study. MRI examinations of premenopausal patients were performed at the 2nd and 3rd weeks of the cycle to avoid possible effects of menstrual cycle on ADC values. After getting approval of the ethics committee (MoH Dışkapı Yıldırım Beyazıt Training and Research Hospital Ethics Evaluation Commission, 26.08.2010, Decision No: 03), all participants were informed and their written consent was obtained.

Breast MRI examinations of all patients were performed by 1, 5 Tesla MRI (Signa HDxt, GE, USA). 34 cm imaging area was obtained by using a standard breast coil in the prone position, TSE T1 and T2 weighted fat saturated axial, gradient echo T1 weighted axial, and contrast gradient eco T1 weighted dynamic and late phase axial sequences as conventional sequences. For pre-contrast FSE T1-weighted images TR: 650msec, TE: 9.6 msec, matrix: 512X512, sections with a cross-sectional thickness of 3 mm and a cross-section of 0.5 mm; For FSE T2-weighted images, TR: 10550 msec, TE: 83.8 msec, matrix: 256x256, cross-sectional thickness 3 mm and 0.5 mm cross-sectional range were obtained. 8 images for each section in the axial plane for dynamic operation TR: 6.6 msec, TE: 3.2 msec, angle of roll: 20 °, matrix: 512X512, cross-sectional thickness: 3 mm and cross-sectional range: 0.5

mm with 30 sec intervals in the T1-weighted FSE sequence were obtained. Gadolinium-containing contrast agent 0.1 to 0.2 mmol / kg dose was given intravenously within 20 seconds. Subtracted series were obtained by using the subtraction program as standard in the MRI console, and time signal intensity curves of the lesions were plotted.

Diffusion-weighted MRI images were obtained in the axial plane prior to contrast agent injection, through breath-hold command and single-shot echoplanar spin echo sequence by using the following parameters: TR / TE: 2500 / 74.4 msec ; matrix: 256X256; imaging area: 34 cm; section thickness: 3mm; gap between sections: 0.5mm. Two different b-values were used for each section, with b = 0 and b = 500 sec / mm². Both breasts were examined in 22 sections. In the console of the MRI device, ADC values were automatically measured and ADC map images were prepared. ADC measurements were made using a standard measuring area (ROI) of 25.0 mm². During ADC measurement, necrotic and cystic components of the tumors were excluded from the measurement area. ADC measurements were taken three times in each case in the lesion, and normal breast parenchyma, and a numerical value was determined by taking arithmetic mean for each localization. ADC value was measured by standard deviation in ROI.

Statically analysis

Statistical package for social sciences (SPSS) computer program was used for statistical evaluations. Descriptive statistics (mean, standard deviation, minimum maximum interval), were used in statistical evaluation on computer, and Mann Whitney U test and Chi-square test were used to compare continuous variables with each other. It was accepted that "p" value should be less than 0.05 ($p < 0.05$) as the statistical significance limit.

Results

The age of the patients ranged from 14 to 75 years, and the mean age was determined as 47.6 years. Lesions were detected in the right breast of 13 patients (43.3%), in the left breast of 17 patients (56.6%), in the upper outer quadrant of 17 patients (56.6%), in the upper internal quadrant of 7 patients (23,3 %), in the lower outer quadrant of one patient(3.3%) and in the lower inner quadrant of 5 patients (16.6%) by conventional breast imaging methods. None of the patients had BI-RADS (Breast Imaging-Reporting and Data System) 4-5 lesions in the contralateral breast. The size of the lesions detected were between 1.5 and 4 cm (mean: 2.3 cm). 30 lesions in total were detected on routine MRI. All patients had only one lesion. The smallest lesion detected on MRI was 1 cm and the largest lesion was 4 cm (mean: 2.06 cm). It was seen that the lesions covered a single quadrant correlated with conventional breast imaging methods. Of the 30 lesions sampled histopathologically 13 were malignant and 17 were benign. All patients underwent diffusion weighted breast MRI. The smallest lesion detected in DWI was 1 cm and the largest lesion was 4 cm in size, and signal abnormalities were detected in all of the lesions. There was, visually, diffusion limitation in 14 lesions (46.6%) and diffusion increase in 16 lesions (53.3%) in DWI. On ADC maps, the mean ADC value of 13 lesions with malignant histopathologic diagnosis was calculated as $0.76x10^{-3}$ mm² / sec ± 0.14 (highest value $1.08x10^{-3}$ mm² / sec, lowest value $0.59x10^{-3}$ mm² / sec) (Figure 1).

The mean ADC value for measurements from seventeen benign lesions was $1.45x10^{-3}$ mm² / sec. ± 0.26 (highest value 1, $98x10^{-3}$ mm² / sec,

lowest value 1, 09x10^{-3} mm² / sec). However, each lesion group was evaluated within itself, and ADC values of lesion subgroups were calculated (Table 1).

sensitivity rate in detecting breast cancer compared to mammography and USG [16]. Conventional breast MRI and dynamic phase contrast MRI, which is a part of this

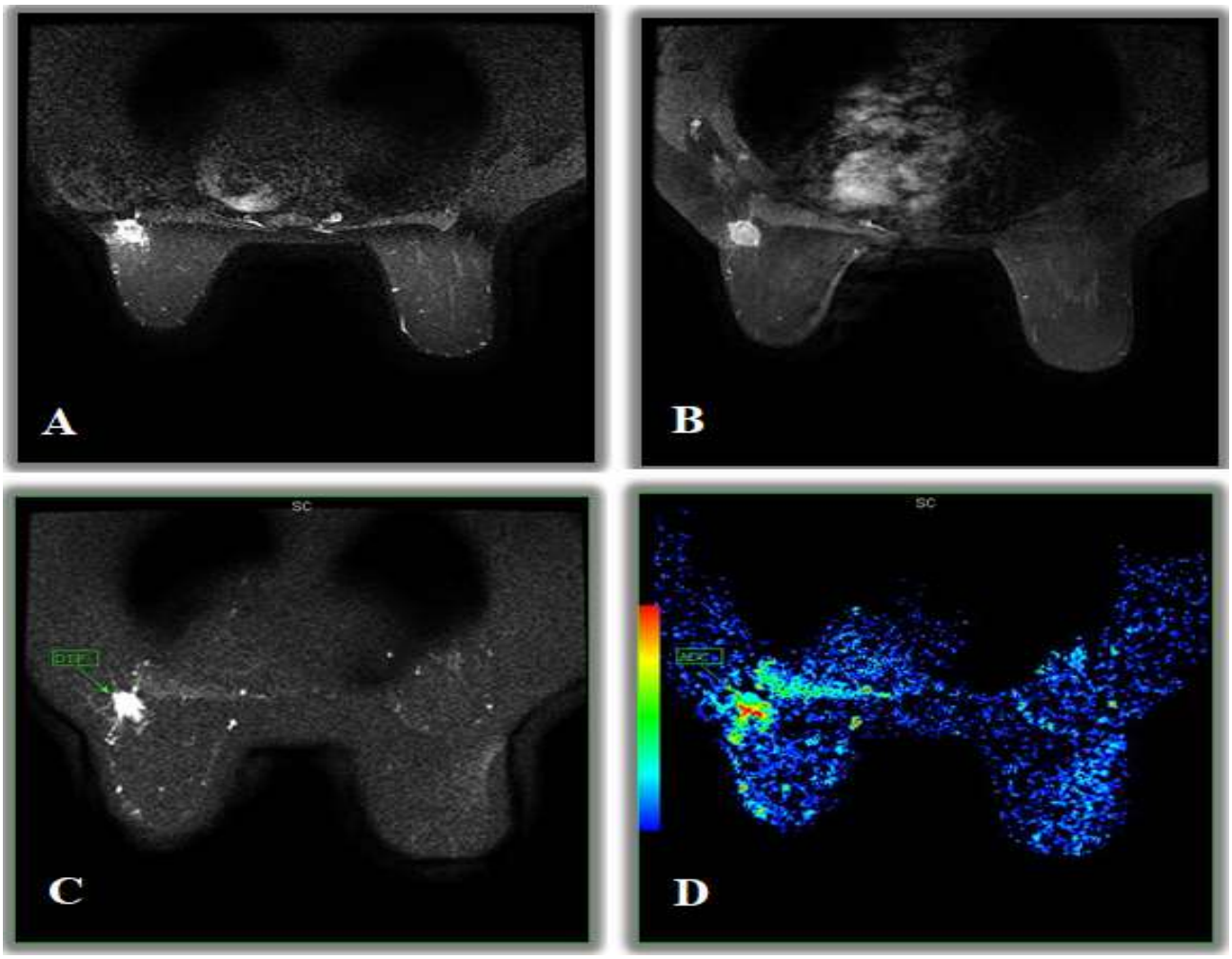

Figure 1 A-D. Conventional dynamic and diffusion weighted MR images from a patient with histologically proved invasive ductal carsinoma in the left breast. **A.** Fat saturated T2-weighted image shows a mass with high signal intensity compared with normal fibroglandular tissue **B.** Contrast-enhanced T1-weighted image demonstrates peripherally enhancement of the mass **C.** Diffusion-weighted image shows restricted diffusion compared to normal breast tissue in the left side and **D.** On ADC maps calculated mean ADC values of the mass was low (0,755x10^{-3} mm² /sec)

Discussion

Breast cancer, affecting 2.1 million women each year, is becoming an increasing health problem in both developed and developing countries. It is the primary cause of cancer-related deaths in women [15]. MRI has a higher

examination, are now widely used in breast evaluation in many centers. However, it may cause unnecessary biopsies with a high false-positive rate [17]. Dynamic examination reflects tissue vascularity, vascular

Table 1. Distribution of ADC values in lesion subgroups (ADC value x 10^{-3} mm^2/sec).

Pathological Diagnosis of Lesions	Number of Lesions	ADC Values (10^{-3} mm^2/sec)			
		The Lowest ADC	The Highest ADC	Mean ADC	Standard Deviation
Invasive ductal carcinoma	6	0,60	0,97	0,75	0,13
Infiltrative ductal carcinoma	5	0,59	0,85	0,73	0,11
Invasive lobular+ductal carcinoma	1	–	–	0,65	–
Invasive lobular carcinoma	1	–	–	0,88	–
Fibroadenoma	6	1,15	1,57	1,35	0,17
Fibrocystic changes of breast	4	1,32	1,58	1,47	0,1
Atypical hyperplasia	3	1,45	1,92	1,75	0,14
Tubular adenoma	1	–	–	1,05	–
Fat necrosis	1	–	–	1,43	–
Intraductal papilloma	1	–	–	1,15	–
Stromal fibrosis of the breast	1	–	–	1,98	–

permeability, changes in interstitial pressure, and changes in extracellular space content. This technique is directly related to the vascularity of the lesions, but there is no direct correlation between tumor cellularity and contrast enhancement pattern [18]. The only imaging method that reflects cellular cellularity in recent conditions is DWI [12]. The term diffusion is used for the randomized microscopic movement of molecules known as Brownian motion and measured by ADC values [19]. DWI and ADC maps obtained from them reduce the false positivity rates caused by conventional MRI in breast imaging by examining the biophysical properties of tissues [17]. Magnetic susceptibility and chemical shift

artifact are evident in the images obtained by echo planar imaging (EPI) technique, which is the most widely used diffusion sequence in clinical practice [13,20]. Especially in breast imaging, the sensitivity of the EPI sequence to the indicated artifacts increases because the breast tissue is surrounded by very dense adipose tissue and the air-tissue interface is high due to the anatomical localization of the breast. The resulting artifacts both create image distortion and cause the edges of the lesion to fade in isotropic diffusion images. In today's technological conditions, especially in breast imaging, it is recommended that the spatial resolution of DWI should be low, and the lesion to be examined should be larger than 2x2 pixels. The resulting image distortion does not significantly change the functional sensitivity of the diffusion up to a certain limit. Because the ADC map where measurements are made is created by making measurements per pixel [11]. Studies have shown that these artifacts and the disadvantages of the EPI sequence are eliminated by the DWI technique obtained by the HASTE (Half Fourier Single Shot Turbo Spin Echo) sequence [21,22]. In our study, in 3 of the DWI obtained by the EPI sequence, the artifacts which we think were caused by the above mentioned features and the dense fat tissue found in the breast tissue in the BI-RADS type 1 breast pattern were observed. The basis of the use of DWI in malignant lesions of the breast is constituted by its histological properties due to the microstructure of tumors. There is a direct correlation between tumor cellularity and ADC values. In a study on brain tumors carried out for the first time, ADC values of low-grade gliomas were shown to be higher than high-grade gliomas [23].

In many studies on breast lesions, it was shown that ADC values in malignant breast lesions with high cellularity were significantly lower compared to benign lesions, and it increased sensitivity in the differentiation of benign and malignant lesions. In the study performed by Kinoshita et al. [21], it has been reported that the average ADC value in 10 masses diagnosed with invasive ductal carcinoma in DWI obtained by HASTE sequence: 1.216 ± 189.10^{-3} mm² /sec, and the average ADC value in 6 lesions diagnosed with fibroadenoma: $1.495 \pm 0.181.10^{-3}$ mm² / sec. In this study, "b" value is taken as 0 and 700 sec / mm². In our study, the mean ADC value in 11 masses with pathologic diagnosis of invasive ductal carcinoma was obtained as : $0,74 \pm 0.12 .10^{-3}$ mm²/sec while the mean ADC value of the lesion from 6 pathological diagnosis of fibroadenoma was found out to be : $1,35 \pm 0.17 .10^{-3}$ mm²/sec.

The ADC values determined in some studies obtained with the EPI sequence were as follows; the average ADC value of 17 malignant lesions: $1,60 \pm 0,36 .10^{-3}$ mm²/sec, the mean ADC value of 6 benign lesions : $2,01 \pm 0,46.10^{-3}$ mm²/sec in the study in which b value was taken as 400 sec/ mm² by Sinha et al [11], the average ADC value of 31 malignant lesions: : $0,97 \pm 0,20 .10^{-3}$ mm²/sec , the mean ADC value of 24 benign lesions: $1,57 \pm 0,23 . 10^{-3}$ mm²/sec in the study in which b value was taken as 1000 sec/ mm² by Guo et al [22], again in another study in which b value was taken as 1000 sec/ mm² by Marini et al [24] it was found out that the average ADC value of 42 malignant lesions: $0,95 \pm 0,18. 10^{-3}$ mm²/sec and the mean ADC value of 21 benign lesions: $1, 48 \pm 0, 37. 10^{-3}$ mm²/sec. In another study in which Partridge et al [25] took b value as 600 sec / mm², the values were calculated as follows : the mean ADC value of 27 malignant lesions : $1,32 \pm 0,23. 10^{-3}$ mm²/sec and the mean ADC value of 91 benign lesions: $1, 71 \pm 0, 43. 10^{-3}$ mm²/sec.

Table 2. Comparison of previous studies with current study about diffusion imaging of breast lesions and ADC values (ADC value x 10^{-3} mm^2/sec).

Previous Studies	Methods of Study		ADC Value (10^{-3} mm^2/sec)	
	DWI Sequence	"b" Value (mm^2/sec)	Malignant Lesions	Benign Lesions
Kinoshita et al.	HASTE	0-700	1,21 ± 0,18	1,49 ± 0,18
Sinha et al.	EPI	0-400	1,60 ± 0,36	2,01 ± 0,46
Guo et al.	EPI	0-1000	0,97 ± 0,20	1,57 ± 0,23
Marini et al.	EPI	0-1000	0,95 ± 0,18	1,48 ± 0,37
Partridge et al.	EPI	0-600	1,32± 0,23	1,71 ± 0,43
Current study	EPI	0-500	0,76 ± 0,14	1,45 ± 0,26

The ADC values obtained in our study and in the literature show numerical differences for lesion groups (Table 2). This difference is thought to be caused by the differences in the value of "b". The gradient intensity applied in diffusion measurement is expressed with the value of "b". The unit is a parameter, sn/mm^2, which shows the power and time of the gradient. As the gradient intensity increases, the phase distribution in the moving protons increases, and thus the signal loss increases. Therefore, in the selected studies with high "b" values diffusion weights are high while their ADC values are low. The value of "b" is of great importance while obtaining DWI. When the value "b" is chosen as 400 sec / mm^2 and lower than this value, the image is not only affected by the molecular diffusion of water, but also by the microcirculation of the blood in the capillary bed of tissues and thus by the perfusion [12]. As predicted in malignant tumors, an increase in the number and size of these capillary vessels is observed [26,27]. Therefore, when the low "b" value is selected, the perfusion effects that will occur in the ADC value will be higher for malignant tumors rather than benign tumors.

In our study, diffusion-weighted images were used to determine and compare the mean ADC values of malignant and benign lesions. In the images, "b" value is selected as 0-500 sec / mm^2. The common result obtained from all studies in this field in the literature is that the ADC values of malignant and high-grade tumors are significantly lower compared to benign tumors. In the results of our study, a statistically significant difference was observed between the malignant lesions and benign breast lesions ($p < 0.01$) in the mean ADC values in accordance with the literature data. This difference is due to the fact that malignant tumors have low ADC values due to the reasons mentioned above. The ADC results of benign and malignant lesion groups obtained by Padridge et al. [25] (0-600 sec/mm^2) and Sinha et al. [11] (0-400 sec/mm^2), who took "b"

values close to the one in our study, and our findings showed numerical differences. In addition to this, it was found out that malignant and benign lesions could be differentiated with 100% sensitivity and 100% specificity with a cut-off value of $1.08.10^{-3}$ mm^2 / sec in our patient group. However, we think that these high sensitivity and specificity rates and the numerical differences observed in the closest "b" values stems from our limited patient group of thirty. It is estimated that sensitivity and specificity rates and ADC values may change if the patient group is expanded and diversified because the ADC values of the lesions are associated with tumor cellularity, the sensitivity and specificity values of the threshold values specified in the literature are lower [22].In fact, in some studies done using DWI, it has been shown that ADC values in invasive breast carcinomas are lower than noninvasive breast carcinomas and that ADC values may be useful for histopathological differentiation [28,29].

ADC measurement values vary depending on the structure of the tissues. Changes in ADC values of normal breast fibroglandular tissue during the menstrual cycle have been demonstrated by studies [20]. During the first and second weeks of the cycle, ADC values showed a steady decrease, but increased in the third and fourth weeks. These changes were not statistically significant. It was established that histopathologically indicated ADC changes were due to the varying water and epithelial tissue content of the breast parenchyma at different stages of the menstrual cycle, and in this study it was shown that fibroglandular tissue density of the breast also affected ADC values [30]. ADC values of adipose tissue located close to breast tissue are very low. While there is no fat tissue between the voxels measured in the breast tissue in the dense sclerosed pattern, the liposklerosis and lipomatous breast pattern inevitably have fat tissue in the voxels within the scope of measurement, which leads to a decrease in the measured ADC values. It is reported that, in order to remove the signal of adipose tissue, signal spectral-spatial RF pulses were inserted in single-shot turbo spin echo diffusion weighted sequences thanks to new technical advances [29]. Furthermore, free diffusion of water is limited in very dense fibrosis and it increases in liquids compared to solid lesions. In line with this information, it should be kept in mind that in a benign lesion such as fibrous fibroadenoma, ADC values may decrease depending on significant fibrous content and increase in malignant tumors showing intense central necrosis. In our study, DWIs were performed in premenopausal patients in the second and third weeks of menstrual phase as recommended in the literature.

Conclusion

DWI is a special MRI sequence, and when used together with conventional MRI sequences, it increases the diagnostic usefulness for differentiating malignant and benign lesions. One of the important advantages of DWI is the ability to obtain numerical data by measuring ADC. Thus, with the help of ADC measurements from breast lesions of sufficient size, a more accurate prediction can be made about the malignancy potential of the lesions before histopathological sampling. When DWI is being evaluated, ADC maps should be evaluated together with it. In this way, the T2 shine-through effect imitating limited diffusion can be eliminated.

There are some limitations to our study. The first is that we have conducted studies with limited number of patients, and if the patient group is expanded and diversified, the sensitivity and specificity rates will change. The second is the formation of artifacts due to

the use of EPI sequence with low spatial resolution. Finally, ADC measurements were performed by a single observer and there was no intra-observer correlation.

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References

[1]Ferlay J, Soerjomataram I, Dikshit R. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):359–86.

[2]Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893-917.

[3]Kopans DB. The positive predictive value of mammography. AJR Am J Roentgenol. 1992;158(3):521-26.

[4]Aberle DR, Chiles C, Gatsonis C, et al. Imaging and cancer: research strategy of the American College of Radiology Imaging Network. Radiology. 2005;235(3):741-51.

[5]Leung JW. Screening mammography reduces morbidity of breast cancer treatment. AJR Am J Roentgenol. 2005;184(5):1508-09.

[6]Mahesh M. AAPM/RSNA physics tutorial for residents: digital mammography: an overview. Radiographics. 2004;24(6):1747-60.

[7]Orel SG, Schnall MD. MR imaging of the breast for the detection, diagnosis, and staging of breast cancer. Radiology. 2001, 220(1): 13-30.

[8]Kuhl CK, Mielcareck P, Klaschik S, et al. Dynamic breast MR imaging: are signal intensity time course data useful for differential diagnosis of enhancing lesions?. Radiology. 1999;211(1): 101-10.

[9]Partridge SC, DeMartini WB, Kurland BF, et al. Quantitative diffusion-weighted imaging as an adjunct to conventional breast MRI for improved positive predictive value. AJR Am J Roentgenol. 2009;193(6):1716-22.

[10]Hetta W. Role of diffusion weighted images combined with breast MRI in improving the detection and differentiation of breast lesions. Egypt J Radiol Nucl Med. 2015; 46 (1): 259-70.

[11]Sinha S, Lucas-Quesada FA, Sinha U, et al. In vivo diffusion-weighted MRI of the breast: potential for lesion characterization. J Magn Reson Imaging. 2002;15(6):693–704.

[12]Provenzale JM, Sorensen AG. Diffusion-weighted MR imaging in acute stroke: theoretic considerations and clinical applications. AJR 1999; 173(6): 1459– 67.

[13]Le Bihan D, Breton E, Lallemand D, et al. Separation of diffusion and perfusion in intra-voxel incoherent motion imaging. Radiology. 1988;168(2): 497–505.

[14]Guo Y, Cai ZI, Cai YQ, et al. Elementary Clinical Estimation of DWI and ADC Value in Differentiating Benign and Malignant Breast Lesions [abstract]. In: Proceedings of the Eighth Annual Meeting of ISRMRM, Denver, 2000:2158. Proc Intl Sot Mag Reson Med. 2000; 8:2158.

[15]Van Bodegraven EA, van Raaij JC, Van Goethem M, et al. Guidelines and recommendations for MRI in breast cancer

follow-up: A review. Eur J Obstet Gynecol Reprod Biol. 2017;218:5–11.

[16] Mehnati P, Tirtash MJ. Comparative efficacy of four imaging instruments for breast cancer screening. Asian Pac J Cancer Prev. 2015;16(15):6177–86.

[17] Robinson IA, McKee G, Nicholson A, et al. Prognostic value of cytological grading of fine-needle aspirates from breast carcinomas. Lancet. 1999; 343(8903):947-49.

[18] Ducatman BS, Emery ST, Wang HH. Correlation of histologic grade of breast carcinoma with cytologic features on fine-needle aspiration of the breast. Mod Pathol. 1993;6(5):539–43.

[19] Le Bihan D, Breton E, Lallemand D, et al. MR Imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. Radiology. 1986;161(2): 401-7.

[20] Yamashita Y, Tang Y, Takahashi M. Ultrafast MR imaging of the abdomen: echo planar imaging and diffusion-weighted imaging. J Magn Reson Imaging. 1998;8(2):367–74.

[21] Kinoshita T, Yashiro N, Ihara N, et al. Diffusion weighted half-Fourier single-shot turbo spin echo imaging in breast tumors: differentiation of invasive ductal carcinoma from fibroadenoma. J Comput Assist Tomogr. 2002;26(6):1042-46.

[22] Guo Y, Cai YQ, Cai ZL, et al. Differentiation of clinically benign and malignant breast lesions using diffusion weighted imaging. J Magn Reson Imaging. 2002;16(2):172-78.

[23] Sugahara T, Korogi Y, Kochi M, et al. Usefulness of diffusion-weighted MRI with echo-planar technique in the evaluation of cellularity in gliomas. J Magn Reson Imaging. 1999; 9(1): 53–60.

[24] Marini C, Facconi C, Giannelli M, et al. Quantitative diffusion-weighted MR imaging in the differantial diagnosis of breast lesion. Eur Radiol. 2007; 17(10):2646-55.

[25] Partridge SC, Demartini WB, Kurland BF, et al. Differantial diagnosis of mammographically and clinically occult breast lesions on diffusion-weighted MRI. J Magn Reson Imaging. 2010;31(3);562-70.

[26] Buadu LD, Murakami J, Murayama S, et al. Breast lesions: correlation of contrast medium enhancement patterns on MR images with histopathologic findings and tumor angiogenesis. Radiology. 1996; 200(3):639–49.

[27] Von Dijke CF, Brasch RC, Roberts TP, et al. Mammary carcinoma model: correlation of macromolecular contrast-enhanced MR imaging characterizations of tumor microvasculature and histologic capillary density. Radiology. 1996;198(3):813–18.

[28] Kuroki Y, Nasu K, Kuroki S, Murakami K, et al. Diffusion-weighted imaging of breast cancer with the sensitivity encoding technique: analysis of the apparent diffusion coefficient value. Magn Reson Med Sci. 2004;3(2):79-85.

[29] Woodhams R, Matsunaga K, Iwabuchi K, et al. Diffusion-weighted imaging of malignant breast tumors: the usefulness of apparent diffusion coefficient (ADC) value and ADC map for the detection of malignant breast tumors and evaluation of cancer extension. J Comput Assist Tomogr. 2005; 29(5): 644–49.

[30] Partridge SC, McKinnon GC, Henry RG, etal. Menstrual Cycle Variation of Apparent Diffusion Coefficients Measured in the Normal Breast Using MRI. J Magn Reson Imaging. 2001;14(4):433-38.

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Original Article

Evaluation of symptomatological and hematological aspects of patients with dizziness in a sample of 744 subjects

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ABSTRACT

Aim: To evaluate the hematologic parameters and subgroups that may be useful in the treatment approach of patients presenting with dizziness, together with their symptomatological features.
Methods: 744 participants between the ages of 20-80 were included in this study. The participants were randomly divided into six groups. Participants' gender, educational levels, ages, comorbidities, symptomatological evaluations, complete blood count values, levels of the main electrolytes, kidney function tests, liver function tests, B12 vitamin level and ferritin and thyroid gland function values were investigated.
Results: There were statistically significant differences between some age groups in terms of white blood cell, hemoglobin, hematocrit, neutrophil, glucose, sT4, Na, K, aspartate aminotransferase, and creatinine levels. Study participants described their current dizziness as a feeling of shaking in their heads (5.4%), as if their surroundings are spinning around them (31.2%), imbalance and feeling dizzy (42%), blackening of eyes (1.1%), and as if they were turning and turning around them (20.4%). One hundred and four of 744 patients were diagnosed with benign paroxysmal positional vertigo (BPPV). At least one additional disease was detected in 392 (52.7%) of the participants.
Conclusion: Dizziness is one of the most common symptoms that should be considered more frequently because it causes a decrease in labor force and impacts significantly on quality of life in the affected individuals. Symptomatological and hematological evaluations are very effective on the treatment approach as well as supporting the diagnosis.
Keywords: Dizziness, vertigo, vestibular disease, vestibulopathy.

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Introduction

Dizziness, is one of the most common complaints in many branches of medical practice including otolaryngology, neurology and emergency medicine clinics. Dizziness affects about 30 % of the general population [1]. About 3 % of the patients aged 25 and over admit to the family physician or emergency services with dizziness [2,3]. Dizziness is more frequently seen in elderly people. Its incidence has been found 30 % in patients over 65 years old. This rate was found to be 50 % in the age

of 85 years and above [4,5]. Different studies have been carried out in the emergency services and in neurologic and otorhinolaryngological units, and peripheral dizziness was detected in 40 %, central pathologies in 10 %, psychiatric pathologies in 15 % and hematologic and systemic causes in 25 % of the patients. Etiological factors also vary according to age. Psychiatric disorders and presyncope are more common in younger ages, whereas central pathologies may be seen more frequently in the elderly [6].

It is also important to evaluate hematological parameters in the differential diagnosis of patients presenting with dizziness. Differential diagnosis can be made by evaluating hemoglobin, white blood cell, leukocyte, neutrophil, lymphocyte, platelet counts, liver function tests, renal function tests, vitamin levels and thyroid function tests [7-9].

Studies on etiology of the dizziness are important because dizziness is very common in the community and as a result it affects a very large part of the society and sometimes it is a symptom of life-threatening diseases. In the present study, etiological and symptomatological features, subgroups and hematological evaluations were performed with the intention to be useful in the management of the patients presenting with dizziness.

Materials and Methods

Study design

The study has been conducted in accordance with the principles of the Helsinki Declaration and approved by the local Institutional Review Board (2018-275/08). In the present study, the data of patients who applied to Ear Nose Throat outpatient clinics with dizziness in the previous year were retrospectively reviewed. Our study was performed with 744 participants between the ages of 20-80. Six age groups were formed in the study. Group 1; (20-30), Group 2; (31-40), Group 3; (41-50), Group 4; (51-60), Group 5 (61-70) and Group 6; (71-80).

Outcome parameters

Participants' gender, educational levels, ages, symptomatologic evaluations, comorbidities, complete blood counts [white blood cell (WBC), hemoglobin (Hb), hematocrit (Htc), neutrophil (Neu) Platelets (Plt), lymphocytes (Lym)], body electrolyte values (Na, K, Ca), kidney function tests (urea, creatinine), hepatic functional tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin], B12 vitamin level, ferritin and thyroid gland function values [thyroid-stimulating hormone (TSH) and free T4] were examined. Besides Dix-Hallpike test scores related to dizziness were also evaluated.

Statistical analysis

In the statistical analysis of the results, the scores were expressed as mean ± standard deviation (SD). Gender, age, educational level, hematological parameters were compared using chi-square test and t-test. $p < 0.05$ value was accepted as statistically significant.

Results

Five hundred and sixty women (75.3 %) and 184 men (24.7 %) were included in the study. The mean age of the participants was 45.10 ± 14.372 (range, 20 to 80) years. The mean age of the women was 46.03 ± 14.001 (range, 20 to 75) years. The mean age of males was 42.26 ± 15.256 (range, 20 to 80) years. When age groups were evaluated, 136 participants (18.3 %) in Group 1, 160 (21.5 %) in Group 2, 192 (25.8 %) in Group 3, 152 (20.4 %) in Group 4, 64 (8.6 %) in Group 5, and 40 in Group 6 (5.4 %) were detected.

When the educational levels of the participants were evaluated, 40 (5.4 %) participants were illiterate. 408 (54.8 %) participants were identified as primary school, 184 (24.7 %) as high school, and 112 (15.1 %) as university graduates. No additional disease was detected in 352 of the participants (47.3%) when the existing comorbidities of the patients who presented to our outpatient clinics with dizziness were evaluated. However, the participants had anemia (n=8; 1.1 %), Behçet's disease (n=8; 1.1 %), brain surgery (n=16; 2.2 %), neck hernia (n=192; 13 %), diabetes mellitus (n=40; 5.5 %), epilepsy (n= 16; 2.2 %), Familial Mediterranean Fever (FMF) (n=8; 1.1 %), thyroid dysfunction (n=24; 3.3 %), hypercholesterolemia (n=16; 2.2 %), hypertension (n=104; 14.1 %), migraine headache (n=8; 1.1 %), chronic otitis surgery(n=16; 2.2 %), osteoporosis (n=16; 2.2 %), tachycardia (n=8; 1.1 %) history of head trauma (n=4; 1 %) (Table 1).

Table 1. The existing comorbidities of the patients.

Comorbidities	N	%
Anemia	8	1,1
Behcet 's disease	8	1,1
Brain operation	16	2,2
Neck hernia	96	13
diabetes mellitus	40	5,5
Epilepsy	16	2,2
Familial Mediterranean Fever	8	1,1
Thyroid dysfunction	24	3,3
Hypercholesterolemia	16	2,2
Hypertension	104	14,1
Migraine	8	1,1
Chronic otitis surgery	16	2,2
Osteoporosis	16	2,2
Tachycardia	8	1,1
Head injury	8	1,1
No additional illness	352	47,3
Total	744	100,0

Study participants described their current dizziness as a feeling of shaking in their heads (n=40; 5.4 %), as if their surroundings are spinning around them (n=232; 31.2 %), imbalance and feeling dizzy (n: 312; 42 %), blackening of eyes (n=8; 1.1 %), and as if they were turning and turning around them (n=152; 20.4%) (Table 2).

Table 2. How do participants define dizziness?

How do participants define dizziness?	N	%
Feeling of shaking in their heads	40	5,4
Surroundings are spinning around them	232	31,2
Imbalance and feeling dizzy	312	42
Blackening of eyes	8	1,1
Turning around them	152	20,4
Total	744	100,0

When the Dix-Hallpike test results were evaluated according to the educational level of the participants; 16 (2.2 %) of the participants who had positive findings in the Dix-Hallpike test were illiterate, and the remaining participants were primary school (n=64; 8.6 %) high school (n=8; 1.1 %), and university graduates (n=16; 2.2 %). In 640 (85.9 %) participants, negative findings were found in the Dix-Hallpike test. In addition, the Head Shake test showed positive findings in 8 (1.1 %) high school and 16 (2.2 %). university graduates, while in 720 participants (96.7 %) we did not find any abnormal findings.

Two hundred and sixteen participants (29 %) had tinnitus, while 528 (71 %) participants had not any complaint of tinnitus. In addition, 96 (13 %) participants had hearing loss at certain levels, and 648 (87 %) participants did not have any hearing loss.

Any statistically significant difference was not found in the statistical evaluation of hematological parameters between the groups

in terms of Ca, ALT, total bilirubin, direct bilirubin, B12 vitamin level, ferritin and urea levels *(p > 0.05)*.

Table 3. Ferritin, B12 level, sT4 (free T4) and TSH levels in age groups.

Parameters	Groups*	N	Mean ±SD
TSH	1	136	1,67±0,61
	2	160	1,39±0,76
	3	192	2,14±1,68
	4	152	1,42±0,60
	5	64	2,14±1,57
	6	40	1,79±0,92
sT4	1	136	0,98±0,14
	2	160	0,98±0,22
	3	192	0,95±0,14
	4	152	1,04±0,15
	5	64	0,92±0,24
	6	40	0,91±0,05
B12 vitamin level	1	136	322±167,59
	2	160	276±115,44
	3	192	283±154,78
	4	152	297±165,58
	5	64	272±81,24
	6	40	387±195,62
Ferritin	1	136	20,38±32,49
	2	160	14,08±13,36
	3	192	16,18±16,64
	4	152	15,97±16,73
	5	64	11,20±7,11
	6	40	37,12±54,93

Values: Mean±SD, TSH: Thyroid stimulation hormone, *Group 1; (20-30), Group 2; (31-40), Group 3; (41-50), Group 4; (51-60), Group 5 (61-70) and Group 6; (71-80).

In comparisons between groups in terms of WBC values (Group 1 *vs* Group 2), (Group 1*vs* Group 4), (Group 2*vs* Group 6), (Group 3 *vs* Group 6), (Group 4*vs* Group 6) ($p < 0.05$) statistically significant intergroup differences were detected. In terms of hemoglobin values, statistically significant intergroup differences were observed; (Group 1*vs* Group 3), (Group 3*vs* Group 4), (Groups 3 *vs* Group 6) ($p < 0.05$). In terms of hematocrit values, statistically significant intergroup differences were observed (Group 3 *vs* Group 4), (Group 3 *vs* Group 5), (Group 3 *vs* Group 6) *(p < 0.05)*. In terms of neutrophil counts, statistically significant intergroup differences were observed (Group 1 *vs* Group 4), (Group 1 *vs* Group 6), (Group 2 *vs* Group 6), (Group 3 *vs* Group 4), (Group 4 *vs* Group 6) *(p < 0.05)*. Sodium (Na) values were significantly different between some groups; (Group 2 *vs* Group 5), (Group 3 *vs* Group 5) *(p < 0.05)*. Potassium (K) values were statistically significantly different between some groups; (Group 4 *vs* Group 5), (Group 4 *vs* Group 6) *(p < 0.05)*. AST values were statistically significantly different between some groups (Group 1 *vs* Group 4), (Group 1*vs* Group 5), (Group 3 *vs* Group 4) *(p <0.05)*. Creatinine (cre) values were statistically significantly different between some groups; (Group 1 *vs* Group 2), (Group 1 *vs* Group 3), (Group 1 *vs* Group 4), (Group 1 *vs* Group 5), (Group 1 *vs* Group 6) ($p < 0.05$). In terms of glucose (fasting) values, statistically significant difference was observed between Groups 1, and 4, *(p = 0.021, p < 0.05*). In terms of TSH values statistically significant differences were observed between Groups 3 and 4 *(p < 0.05)*. In terms of sT4 values statistically significant differences were observed between some groups; (Group 1 *vs* Group 6), (Group 3 *vs* Group 4), (Group 4 *vs* Group 5), (Group 4 *vs* Group 6) *(p < 0.05)*.

WBC, hemoglobin, hematocrit, platelet, neutrophil, ferritin, B12 level, fasting glucose value, s4 and TSH levels, serum electrolyte values (Na, K, Ca), liver function tests (AST, ALT, total bilirubin and direct bilirubin) and the mean ± standard deviation values of kidney function tests (urea, creatinine) were also evaluated within the scope of our study (Table 3 - 6).

Table 4. WBC, Hgb, Htc, Plt, Neutrophil, fasting glucose levels in age groups.

Parameters	Groups*	N	Mean ± SD	Parameters	Groups*	Mean ± SD
WBC	1	136	7,12±1,99	**Neutrophil**	1	4,20±1,52
	2	160	8,68±2,74		2	4,87±2,06
	3	192	7,70±2,32		3	4,19±1,48
	4	152	8,33±2,28		4	5,13±1,77
	5	64	8,01±3,09		5	4,62±2,03
	6	40	6,55±0,82		6	3,57±0,67
Hgb	1	136	13,42±1,65	**Platelet**	1	245±39,83
	2	160	13,23±2,15		2	276±50,63
	3	192	12,69±1,45		3	288±61,64
	4	152	13,60±1,28		4	273±53,14
	5	64	13,66±1,75		5	264±52,97
	6	40	13,50±0,73		6	215±47,51
Hematocrit	1	136	38,93±3,86	**Fasting glucose value**	1	99±22,40
	2	160	38,55±4,79		2	105±22,02
	3	192	37,68±2,76		3	105±20,38
	4	152	39,49±3,06		4	112±26,95
	5	64	40,03±4,13		5	102±18,52
	6	40	40,44±2,25		6	109±19,64

Values: Mean±SD, WBC: White bloodcell, Hgb: Hemoglobin, Htc: Hematocrit, Plt: Platelet, *Group 1; (20-30), Group 2; (31-40), Group 3; (41-50), Group 4; (51-60), Group 5 (61-70) and Group 6; (71-80).

Discussion

Dizziness is one of the most common complaints in the geriatric age group. The prevalence of dizziness in the general population is about 20 % to 30 %. Generally, symptoms and signs are indeterminate, nonspecific, and difficult to identify [10]. Nevertheless, a robust systematic approach can often lead to diagnosis. It may be difficult to establish a satisfactory diagnosis of the cause of vertigo for many physicians. In most cases, laboratory tests and radiological examinations may not be helpful in making a diagnosis. In fact, a detailed history with a systematic approach is the most important component in the evaluation of patients with dizziness. Although the causes of vertigo are mostly due to otologic reasons, it may be related to central, somatosensory, and visual etiologies [11]. In this study, patients who were admitted to our clinic with central dizziness were excluded from the study. Sociodemographic, hematological and symptomatologic evaluation of 744 patients with dizziness were performed. Five hundred and sixty women (75.3 %) and 184 men (24.7 %) were included in the study. The median age of the study participants was 46 years. The median ages of the women, and men who participated in the study were 46 and 42 years, respectively. In a study conducted with 907 participants, the mean age of the participants was 59 years. Female participants constituted 59 % of the study population [12]. In a different study, 62.8 % of 1194 patients above 70 years of age were female [13]. In the literature, it was determined that patients presenting with dizziness described the

Table 5. Serum electrolyte (Na, K and Ca) levels in age groups.

Parameters	Group*	N	Mean ± SD
Na	1	136	139,12±3,56
	2	160	138,75±2,16
	3	192	138,79±2,84
	4	152	139,68±2,48
	5	64	140,25±2,29
	6	40	140,20±2,61
K	1	136	4,23±0,36
	2	160	4,27±0,33
	3	192	4,25±0,34
	4	152	4,15±0,39
	5	64	4,37±0,22
	6	40	4,44±0,27
Ca	1	136	9,22±0,55
	2	160	9,40±0,60
	3	192	9,38±0,38
	4	152	9,27±0,41
	5	64	9,13±0,46
	6	40	9,62±0,64

**Group 1; (20-30), Group 2; (31-40), Group 3; (41-50), Group 4; (51-60), Group 5 (61-70) and Group 6; (71-80).*

dizziness as imbalance (16 %), feeling as if they were going to faint (14 %), and drowsiness (10 %) [3]. In another study with 185 patients, 33 % of the patients described dizziness as a drowsy feeling [14]. In this study, we determined that the patients described dizziness as a feeling of drowsiness in 42 %, feeling of everything rotating around them in 31.2 %, and feeling himself rotating in 20.4 % of the patients. Although the changes in the rates of symptoms vary in the literature and when we look at the study, patients generally describe dizziness with similar terms

When the presence of additional diseases with dizziness in patients was evaluated, in a multicenter study of 1092 participants, the effects of comorbidities on the recurrence rates of benign paroxysmal positional vertigo were compared and significant results were obtained. It has been shown that the increase in the number of comorbid diseases also increases the frequency of recurrence [15]. A different study reported that recurrence was observed in 29.3

Table 6. Kidney function tests and hepatic functional tests levels in age groups.

Parameters	Group*	N	Mean ± SD	Parameters	Group*	Mean ± SD
AST	1	136	22,94±4,91	Direct Bilirubin	1	0,11±0,05
	2	160	20,70±9,66		2	0,13±0,06
	3	192	22,42±9,58		3	0,11±0,05
	4	152	19,11±2,92		4	0,11±0,06
	5	64	19,56±5,62		5	0,10±0,05
	6	40	23,40±6,46		6	0,15±0,07
ALT	1	136	16,53±5,44	Blood urea nitrogen (BUN)	1	13,33±3,86
	2	160	20,13±12,41		2	12,43±4,91
	3	192	18,00±9,90		3	12,94±3,69
	4	152	17,11±6,15		4	13,33±3,52
	5	64	18,38±10,78		5	14,18±5,34
	6	40	18,00±5,85		6	15,52±4,83
Total bilirubin	1	136	0,51±0,25	Creatinine	1	0,64±0,09
	2	160	0,54±0,23		2	0,69±0,12
	3	192	0,54±0,26		3	0,69±0,11
	4	152	0,51±0,15		4	0,71±0,18
	5	64	0,55±0,33		5	0,71±0,08
	6	40	0,56±0,22		6	0,74±0,11

% of 475 patients. In patients with recurrence, comorbidities were detected in 72.6 % of the patients. Higher recurrence rates have been reported in patients with HT, DM, cardiovascular system disorders, rhythm disorders, migraine, endocrine disorders and psychiatric disorders [16]. In this study, we evaluated the comorbidity rates of patients presenting with dizziness. In our study, no additional disease was found in 352 (47.3 %) of the participants, while 392 (52.7 %) participants had different additional diseases. This suggests that the incidence of dizziness in patients may be higher in the presence of additional diseases.

In a different study concerning the effect of different levels of education on dizziness and the frequency of referrals to clinics, the educational level of patients admitted with complaints of dizziness was evaluated and patients were found to be university (15 %), primary school (38 %) and high school (11 %) graduates [17]. In this study, we determined that the participants were illiterate (n=40; 5.4 %), primary school (n=408; 54.8 %), high school (n=184; 24.7 %), and university (n=112; 15.1 %) graduates. This phenomenon shows us that the prevalence of dizziness is higher in individuals with primary education level.

When we look at the relationship between differences in educational level and benign paroxysmal positional vertigo (BPPV), in a study conducted with 105 patients diagnosed as BPPV, 21 % of the BPPV patients, and 16 % of 297 BPPV negative patients had higher education levels. However, there were no statistical differences between the two groups [18]. In this study, we identified 64 (62 %) of 104 patients diagnosed with BPPV as primary school and 16 (15 %) of them as university graduates. These results were found to be consistent with the literature. However, we concluded that there was no relationship between education level and BPPV positivity.

Additional pathologies such as tinnitus and hearing loss can also be detected in patients with dizziness [19]. In a study conducted with 400 patients, it was shown that dizziness may be accompanied by tinnitus and hearing loss in 46.9 %, and 38.2 % of the cases, respectively [20]. In our study, tinnitus and hearing loss accompanied dizziness in 29 %, and 13 % of the cases, respectively.

Inflammation and trauma in the neck or head area in BPPV were thought to be possible etiologic factors [21]. In addition, stress-related inflammation may be seen in cases of vertigo-related anxiety. Therefore, the relationship between the diagnosis of BPPV and inflammatory biomarkers in general can be shown [22]. In a study, 114 patients presented with dizziness were diagnosed as peripheral positional vertigo. We found significant differences between patients as for hemogram, biochemistry, vitamin B12 levels, thyroid function tests, urea, creatinine, total bilirubin and direct bilirubin levels [23]. In this study, we did not detect significant differences in terms of Ca, ALT, total bilirubin, direct bilirubin, B12 vitamin level, ferritin and urea levels. However, we found statistically significant differences among various age groups in terms of WBC, hemoglobin, hematocrit, neutrophil counts, fasting glucose value, free T4, Na, K, AST, and creatinine levels.In addition, in this study, 6 different groups were formed for each decade between 20 and 80 years of age. Differences in hematological values among these groups were revealed. When we look at the literature, we did not find any studies evaluating the differences according to age groups.

When the limitations in our study were evaluated; increasing the number of patients in groups and multi-center studies may reveal

more different results. In addition, detailed vestibular tests were not performed in this study. Studies can also be done to compare the results of videonystagmography and caloric test results in age groups.

As a conclusion, dizziness is one of the symptoms that should be considered more frequently because it is often seen in the community and causes a decrease in the quality of work and quality of life in the affected individuals. Symptomatological and hematological evaluations are highly effective on the treatment approach, and they also aid in diagnosis.

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References

[1]Chu Y, Cheng L. Vertigo and dizziness. Acta Neurol Taiwanica. 2007; 16(1): 50-60.

[2]Kerber KA, Hofer TP, Meurer WJ, et al. Emergency department documentation templates: variability in template selection and association with physical examination and test ordering in dizziness presentations. BMC Health Serv Res. 2011; 11(1): 65-73.

[3]Labuguen RH. Initial evaluation of vertigo. Am Fam Physician. 2006; 73(2): 244-51.

[4]Maarsingh OR, Dros J, Schellevis FG, et al. Causes of persistent dizziness in elderly patients in primary care. Ann Fam Med. 2010; 8(3): 196-205.

[5]Iwasaki S, Yamasoba T. Dizziness and imbalance in the elderly: age-related decline in the vestibular system. Aging Dis. 2014; 6(1): 38-47.

[6]Newman-Toker DE. Symptoms and signs of neuro-otologic disorders. Continuum (MinneapMinn) 2012; 18(5): 1016-40.

[7]Gulacti U, Lok U, Hatipoglu S, et al. Assessment of Vitamin B12 And Folic Acid Deficiency In Emergency Department As a Cause of Acute Presentation of Dizziness. Acta Medica Mediterranea. 2014; 30(1): 771-74.

[8]Lok U, Hatipoglu S, Gulacti U, et al. The role of thyroid and parathyroid metabolism disorders in the etiology of sudden onset dizziness. Med Sci Monit. 2014; 20(3): 2689–94.

[9]Edlow JA, Newman-Toker DE. Medical and nonstroke neurologic causes of acute, continuous vestibular symptoms. Neurol Clin. 2015; 33(3): 699-716.

[10]Lee A. Diagnosing the cause of vertigo: a practical approach. Hong Kong Med J. 2012; 18(4): 327-32.

[11]Neuhauser HK, Radtke A, von Brevern M, et al. Burden of dizziness and vertigo in the community. Arch Intern Med. 2008; 168(19): 2118-24.

[12]Atzema CL, Grewal K, Lu H. Outcomes among patients discharged from the emergency department with a diagnosis of peripheral vertigo. Ann Neurol. 2016; 79(1): 32-41.

[13]Katsarkas A. Dizziness in aging: a retrospective study of 1194 cases. Otolaryngol Head Neck Surg. 1994; 110(3): 296-301.

[14]Kroenke K, Lucas CA, Rosenberg ML, et al. Causes of persistent dizziness: a prospective

study of 100 patients in ambulatory care. Ann Intern Med. 1992; 117(11): 898-904.

[15] De Stefano A, Dispenza F, Suarez H, et al. A multicenter observational study on the role of comorbidities in the recurrent episodes of benign paroxysmal positional vertigo. Auris nasus larynx. 2014; 41(1): 31-36.

[16] Picciotti PM, Lucidi D, De Corso E, et al. Comorbidities and recurrence of benign paroxysmal positional vertigo: personal experience. Int J Audiol. 2016; 55(5): 279-84.

[17] Grill E, Strupp M, Müller M, et al. Health services utilization of patients with vertigo in primary care: a retrospective cohort study. J Neurol. 2014; 261(8): 1492-98.

[18] Al-Asadi JN, Al-Lami Q. Prevalence and risk factors of benign paroxysmal positional vertigo among patients with dizziness in Basrah, Iraq. Br J Med Med Res. 2015; 7(9): 754-61.

[19] Whitman GT. Examination of the Patient with Dizziness or Imbalance. Med Clin North Am. 2019;103(2):191-201.

[20] Müjdeci B, Dere H. Evaluation of the accompanying symptoms and results of the Dizziness Handicap Inventory of patients presenting with complaint of vertigo. Medeniyet Med J. 2016; 31(2):94-97.

[21] Novotný M, Skutil J, Trnka A, et al. Our experience with benign paroxysmal positional vertigo. Int Tinnitus J. 2006; 12(1): 71-73.

[22] Güçlütürk MT, Ünal ZN, İsmi O, et al. The Role of Oxidative Stress and Inflammatory Mediators in Benign Paroxysmal Positional Vertigo. J Int Adv Otol. 2016; 12(1): 101-5.

[23] Tekeşin A, Tunç A. Inflammatory biomarkers in benign paroxysmal positional vertigo: a turkey case-control study. Ideggyogysz. 2018; 71(11): 411-16.

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Original Article

Unexpected risk of gynecological malignant and premalignant disease in women undergoing hysterectomy for pelvic organ prolapse

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ABSTRACT

Aim: To investigate the incidence of unexpected malignant and premalignant gynecological pathological findings among women who underwent hysterectomy due to pelvic organ prolapse (POP).

Methods: In this retrospective study, the medical reports of women who underwent hysterectomy for POP between 2007 and 2019 were investigated to reveal unexpected malignant and premalignant lesions. The possible relationship between pathological results and other variables was evaluated statistically.

Results: The hysterectomy was performed by abdominal (160, 30.53%), laparoscopic (62%, 11.83%) and vaginal approaches (302, 57.63%) in 524 patients with POP indication. Thirty five patients (6.67%) had unexpected premalignant or malignant pathological findings found on hysterectomy specimens. Simple hyperplasia was found in 18 patients (3.44%), complex hyperplasia in two patients (0.38%); CIN-1 (LSIL) low grade cervical intraepithelial dysplasia in nine patients (1.7%), CIN-II, moderate dysplasia in two patients (0.38%); CIN-III, severe dysplasia in one patient (0.19%); vaginal carcinoma in two patients (0.38%) and endometrial carcinoma in one patient (0.19%). In the vaginal hysterectomy group, the incidence of unsuspected gynecological malignancy was founded at the rate of 0.57% (3/524) and the percentage of the group was significantly higher than laparotomic and laparoscopic hysterectomy groups. Statistically significant difference was not found between the groups with respect to unexpected uterine malignancy.

Conclusion: Women without abnormal vaginal bleeding do not have high risk of premalignant or malignant pathological reporting after uterovaginal prolapse surgery, however it should not be neglected.

Keywords: Pelvic organ prolapse, uterovaginal prolapse, hysterectomy, malignancy.

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Introduction

Pelvic organ prolapse (POP) is a common health problem which has significant negative effects on women's life quality. POP is seen in approximately 30-40% of women who had given birth. The lifetime risk of surgery for POP in the general female population is approximately 19% [1]. Vaginal hysterectomy (VH) is the most common procedure for the surgical treatment of uterovaginal prolapse (UVP) [2,3]. Gynecologists usually encounter POP during uterine conservation surgery [4]. However, many surgeons are unwilling to preserve the uterus during POP surgery, since there may be lesions that develop later and require uterine evaluation and /or hysterectomy.

In the literature, risk of unsuspected gynecological malignancy after hysterectomy for POP ranges between 0.0 and 0.9 % [5,6]. Hence, this study aimed to investigate the incidence of malignant and/or premalignant pathological results of women with normal cervical cytology and transvaginal ultrasound who underwent hysterectomy for POP.

Materials and Methods

This retrospective cohort was conducted after getting approval from the ethics committee of Abant İzzet Baysal University (Decision no: 2019/325). All patients were selected from İzzet Baysal State Hospital and Abant İzzet Baysal University Hospital, who underwent hysterectomy for POP between Jan. 1, 2007 and November. 14, 2019. Patients were evaluated according to International Classification of Diseases, Revision Ten codes. Data were collected from a retrospectively maintained departmental billing database. The database access granted from International Classification of Disease codes, current procedural terminology. Medical records of the patients including clinical characteristics and past medical histories were collected from the hospital database. Pathology reports were reviewed for the final pathology.

Patients who underwent hysterectomy according to International Classification of Disease Revision Ten codes (ICD 10) N 81.4 (Uterovaginal prolapse, unspecified), N 81 (Female genital prolapse), N81.2 (Incomplete uterovaginal prolapse), N81.3 (Complete uterovaginal prolapse), N81.8 other (Female genital prolapse) and N81.9 (Female genital prolapse, unspecified) diagnoses were included in this study. In addition, patients enrolled to this study had a normal cervicovaginal smear test in the last one year or a negative cervicovaginal cytology test and human papilloma virus within three years before surgery. Women without abnormal uterine bleeding symptoms or abnormal endometrial findings do not routinely undergo endometrial biopsies in daily surgical practice. Patients with premalignant and / or malignant adnexal, uterine or cervical pathology were excluded from the study. Women whose last menstruation was retarded more than 1 year, who were postmenopausal and over 40 years old were also discarded. Menorrhagia, intermenstrual bleeding and postmenopausal bleeding were considered as abnormal uterine bleedings. Treatment approaches in this study were recorded as laparoscopic hysterectomy, vaginal hysterectomy, and abdominal hysterectomy.

Statistical analysis

Data analysis was performed using SPSS version 23.0 (SPSS Inc. USA). Student *t*-test was used to evaluate the possible relationship between pathologic results and other variables. Chi-square test was used to evaluate abnormal pathologic results between each hysterectomy type. The results were assessed within 95%

confidence interval. A p value <0.05 was considered as statistically significant.

Results

Five hundred and twenty four hysterectomies with the indication of POP were performed during the study period, including abdominal (160, 30.53%), laparoscopic (62, 11.83%) and vaginal approaches (302, 57.63%) (Table 1). The mean age of the women in this study was 51.34 ± 9.62 years (50.5-52.2, 95% CI). Table 2 shows preoperative diagnosis of patients.

Table 1. Surgical procedures for pelvic organ prolapse (POP) treatment.

Procedure	n (%)
Hysterectomy, all	524 (100)
Vaginal	302 (57.7)
Abdominal	160 (30.5)
Laparoscopic	62 (11.8)
Oophorectomy	253 (48.3)
Incontinence procedure	196 (37.4)
Transobturator tape (TOT) technique	157 (29.9)
Marshall-Marchetti-Krantz procedure (MMK)	39 (7.4)
Colporrhaphy	421 (80.3)
Anterior	155 (29.6)
Posterior	53 (10.1)
Anterior and posterior	209 (39.9)

Primary indications for hysterectomy were (N 81.4) Uterovaginal prolapse, unspecified (275, 52.48%), (N 81) Female genital prolapse (101, 19.27%), (N81.2) Incomplete uterovaginal prolapse (61, 11.64%), (N81.3) Complete uterovaginal prolapse (51, 9.73%), (N81.8) other Female genital prolapse (24, 4.58%) and (N81.9) Female genital prolapse unspecified (12, 2.29%). In preoperative screening,

Table 2. Distribution of preoperative diagnosis.

Preoperative diagnosis (ICD 10)	n (%)
Female genital prolapse (N 81)	101(19.27)
Incomplete uterovaginal prolapse (N 81.2)	61 (11.4)
Complete uterovaginal prolapse (N 81.3)	51 (9.73)
Uterovaginal prolapse, unspecified (N 81.4)	275 (52.48)
Other female genital prolapse (N 81.8)	24 (4.58)
Female genital prolapse, unspecified (N81.9)	12 (2.29)
Abnormal uterin bleeding (N 92)	18 (3.44)
Leiomyoma uteri (D 25)	160 (30.53)
Stres urinary incontinence (N39.3)	196 (37.4)

eighteen patients (3.44%) were detected to have abnormal uterine bleeding symptoms. None of them was postmenopausal.

Preoperative diagnostic evaluation with ultrasound scanning and/or endometrial biopsy was negative for malignant and premalignant disease. Women with no symptoms or abnormal gynecological examination do not routinely undergo endometrial sampling in daily surgical practice.

Table 3 shows pathological results after hysterectomy of the study subjects. Thirty five patients (6.67%; 95% CI, 5.7–7.2) have unsuspected premalignant or malignant gynecological pathological result found after hysterectomy. Simple hyperplasia without atypia were detected in 18 patients (3.44%; 95% CI, 2.34–4.56), complex hyperplasia without atypia in two patients (0.38%; 95% CI, 0.21–1.49), CIN-I (cervical intraepithelial neoplasia) in nine patients (1.7%; 95 CI, 1.5-1.9) , CIN-II in two patients (0.38%; 95% CI, 0.21–1.49), one CIN-III in one patient (0.19%; 95% CI, 0.11–0.27), vaginal carcinoma in two patients (0.38% ;95% ,0.21-1.49) and endometrial carcinoma in one patient 0.19%; 95% CI, 0.11–0.27) after pathology reporting.

Table 3. The pathology results after the hysterectomy.

Specimen	n (%)	95% CI
Uterus (n = 524)		
Leiomyoma(s)	213 (40.6)	36.8–44.5
Adenomyosis	126 (24.1)	21.2–27.2
Endometrial polyp	45 (8.6)	6.5–10.7
Endometrial hyperplasia		
Simple	18 (3.44)	2.34–4.56
Complex	2 (0.38)	0.21–0.49
Endometrial carcinoma	1 (0.19)	0.11–0.27
Cervix (n = 524)		
CIN1	9 (1.7)	1.5– 1.9
CIN2	2 (0.38)	0.32–0.41
CIN3	1 (0.19)	0.11–0.27
Vaginal cancer	2 (0.38)	0.32-0.41

These unexpected premalign gynecological pathologies (6.09%) were detected in premenopausal women. In total, women diagnosed with unexpected gynecological malignancies after a hysterectomy was three (0.57%). These included two vaginal squamous cell carcinomas (0.38%) and one endometrioid type of endometrium adenocarcinoma (0.19%).

Comparison of the numbers of Unexpected Uterine Malignancy (UUM) among abdominal, laparoscopic and vaginal hysterectomy are shown in table 4. Statistically significant difference was not found with respect to mean age of abdominal hysterectomy, laparoscopic and vaginal hysterectomy groups (51.75± 9.83, 51.32± 9.51 and 51.39± 10.04, respectively, *p=0.299*).

No significant difference was observed with respect to the incidence of patients diagnosed with UUM after hysterectomy in laparotomic, laparoscopic and vaginal hysterectomy groups (laparotomy, 0 [0.0 %]; laparoscopy, 0 [0.0 %]; vaginal, 1 [0.19%] *p=0.077*). The incidence of unsuspected gynecological malignancy after hysterectomy was 0.57 % (3/ 524) which was significantly higher in vaginal hysterectomy group *(p=0.01)*. The incidences of UUM, unsuspected endometrial malignancy and unexpected gynecological malignancy other than endometrial malignancy were 0.19% (1/524 patients), 0.19% (1/524 patients) and 0.38% (2/524 patients), respectively.

In the cohort of women found to have an unanticipated uterine malignancy after hysterectomy, the median age at the time of diagnosis was 58 years (range 51–65). The age of unsuspected endometrial malignancy case

Table 4. The distribution of unexpected gynecological malignancies after the hysterectomy.

Gynecological malignancies	TAH	TLH	VH	Total	P[a]
Mean age (Years)	51.7± 9.8	51.3± 9.5	51.3± 10	51.3 ± 9.6	0.299
Leiomyosarcoma	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Endometrioid carcinoma	0 (0.0)	0 (0.0)	1 (0.19)	1(0.19)	0.077
Squamous-cell carcinoma of the vagina	0 (0.0)	0 (0.0)	2 (0.38)	2 (0.38)	0.043
Total	0 (0.0)	0 (0.0)	3 (0.57)	3 (0.57)	0.01

Data are expressed as n (%) and Maen±SD. [a]Chi-Square test. TAH: total abdominal hysterectomy, TLH: total laparoscopic hysterectomy, VH: vaginal hysterectomy.

was 51 years, her preoperative endometrial thickness was 15mm, and preoperative endometrial sampling pathologic result was proliferative endometrium and postoperative hysterectomy pathologic result was stage 0 endometrioid type adenocarcinoma grade I. This patient did not receive any additional surgery or treatments at the time of observation. Vaginal squamous cell carcinoma cases were determined to vaginal pathologic specimen who underwent vaginal hysterectomy. One of these cases was 58 and the other was 66 years old. In these two patients, vaginal prolapse was present for more than 10 years and they underwent vaginal hysterectomy with a diagnosis of stage 4 uterovaginal prolapse. The result of postoperative pathology in which tissues were extracted from the posterior vaginal walls revealed Stage 2A squamous cell carcinoma well differentiated type. These patients received radiotherapy after diagnosis. No surgical treatment was performed again.

Discussion

The results of uterine protective surgery for POP treatment showed very low risk of unexpected premalignant or malignant gynecologic disease (6.67%). The unsuspected malignancy risk was 0% among premenopausal women in our patient group. Though, 6.09% of premenopausal women were diagnosed with endometrial hyperplasia or cervical intraepithelial neoplasia after hysterectomy and reconstructive surgery, only 0.57 % of the patients had detected gynecological malignancy. The unexpected uterine malignancy risk was 0.19% and unexpected gynecological malignancy other than uterine malignancy risk was 0.38% in our study.

Patients without abnormal uterine bleeding symptoms had minimal risk of unsuspected disease. On the other hand, postmenopausal women suspected of bleeding patterns have significant risk for unsuspected malign disease and are not good candidates for uterine conservation [5]. But, postmenopausal patients without abnormal uterine or vaginal bleeding have a low risk of abnormal pathology (0.57%). Incidence rates were reported to be 0–0.9 % in previous studies (Table 5). Frick et al. [6] reviewed 644 hysterectomy cases with POP and found two unsuspected malignancies (0.3%). They found cancer cases in patients with postmenopausal bleeding. Ram et al. found that very few women undergoing POP surgery had undiagnosed serious endometrial pathologic results (0.7%), with five of them incidentally diagnosed with uterine cancers (0.6%) [7]. These cancer cases were detected in postmenopausal patients with abnormal endometrial thickness without abnormal uterine bleeding. Similar to this study, we also detected unexpected endometrial cancer cases were detected in postmenopausal patients with higher endometrial thickness and without postmenopausal hemorrhage. In the present study, the rate of unexpected endometrial malignancy was 0.19% and vaginal squamous cell carcinoma was 0.38% in patients who underwent vaginal hysterectomy with the diagnosis of UVP. Similarly; unexpected endometrial malignancy incidence has been reported between 0-0.54 percent in hysterectomies performed with the diagnosis of pelvic floor dysfunction [8-10]. Previous studies did not report unexpected cases of vaginal cancer. It may be because the vaginal tissue was not removed or the extracted vaginal tissues were not taken into pathological examination.

Our results show that abnormal gynecological pathology risk is low after hysterectomy and does not show the risk of uterine, adnexal, vaginal or cervical disease for the rest of

Table 5. Incidence of premalignant and malignant uterine findings after hysterectomy for pelvic organ prolapse – summary of previous studies.

Study	n	Significant [a] premalignant uterine findings	Unexpected uterine malignancy	Total
Frick et al. [6] (2010)	644	7 (1.08%)	2 (0.3%)	9 (1.39%)
Ramm et al. [7] (2012)[b]	708	2 (0.28%)	5 (0.7%)	7 (0.98%)
Wan et al. [8] (2013)	640	2 (0.31%)	2 (0.31%)	4 (0.62%)
Andy et al. [9] (2014)	324	3 (0.92%)	0	3 (0.92%)
Ackenbom et al. [10] (2016)	1196	10 (0.8%)	3 (0.3%)	3 (1.1%)
Grigoriadis et al. [11] (2015)	333	13 (3.9%)	1 (0.3%)[b]	14 (4.2%)
Mizrachi et al. [12] (2017)	677	5 (0.73%)	1 (0.15%)	6 (0.88%)
Present study	524	5 (0.95%)	1 (0.19%)	6 (1.14%)

[a] Significant premalignant pathological findings were defined as either atypical endometrial hyperplasia or cervical intraepithelial neoplasia grade 2–3 [b] cervical cancer.

patient's life. Surgical procedure should not include removing of the ovarian tissue. So, incidentally abnormal ovarian pathology is expected to occur only in a few patients, even a little normal appearance of the ovaries is seen intraoperative.

Our study had some limitations. First, this is a retrospective study. Second, this study does not provide information about ovarian pathologies, third, preoperative examinations are not performed in the same clinic and there may be inadequate or incomplete evaluations resulting from this.

The strengths of our study are that the number of patients is high and that the patients with postmenopausal bleeding are not included.

Conclusion

The rate of incidentally found premalignant or malignant gynecological pathological findings in patients who underwent hysterectomy with the diagnoses of POP were not frequent (0,57%) in this study, but the risk should not be ignored. Therefore, patients who underwent surgery due to POP must be informed about the risk of unexpected gynecological malignancy.

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References

[1]Swift SE. The distribution of pelvik organ support in a population of female subjects seen for routine gynecologic healthcare. Am J Obstet Gynecol. 2000;183(2):277-85.

[2]Johnson N, Barlow D, Lethaby A, et al. Surgical approach to hysterectomy for

benign gynaecological disease. Cochrane Database Syst Rev. 2005;(1):CD003677.

[3] Renganathan A, Edwards R, Duckett JR. Uterus conserving prolapse surgery–what is the chance of missing a malignancy? Int Urogynecol J. 2010;21(7):819–21.

[4] Schulten SFM, Detollenaere RJ, Stekelenburg J, et al. Sacrospinous hysteropexy versus vaginal hysterectomy with uterosacral ligament suspension in women with uterine prolapse stage 2 or higher: observational follow-up of a multicentre randomised trial. BMJ. 2019; 366:l5149.

[5] vanHanegem N, Prins MMC, Bongers MY, et al. The accuracy of endometrial sampling in women with postmenapozal bleeding: asystematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2016;197:147-55.

[6] Frick AC, Walters MD, Larkin KS, et al. Risk of unanticipated abnormal gynecologic pathology at the time of hysterectomy for uterovaginal prolapse. Am J Obstet Gynecol. 2010;202(5):507.e1–507.e5074.

[7] Ramm O, Gleason JL, Segal S, et al. Utility of preoperative endometrial assessment in asymptomatic women undergoing hysterectomy for pelvic floor dysfunction. Int Urogynecol J. 2012;23(7):913–17.

[8] Wan OY, Cheung RY, Chan SS, et al. Risk of malignancy in women who underwent hysterectomy for uterine prolapse. Aust N Z J Obstet Gynaecol. 2013; 53(2):190–96.

[9] Andy UU, Nosti PA, Kane S, et al. Incidence of unanticipated uterine pathology at the time of minimally invasive abdominal sacrocolpopexy. J Minim Invasive Gynecol. 2014;21(1):97–100.

[10] Ackenbom MF, Giugale LE, Wang Y, et al. Incidence of occult uterine pathology in women undergoing hysterectomy with pelvic organ prolapse repair. Female Pelvic Med Reconstr Surg. 2016;22(5):332–35.

[11] Grigoriadis T, Valla A, Zacharakis D, et al. Vaginal hysterectomy for uterovaginal prolapse: what is the incidence of concurrent gynecological malignancy? Int Urogynecol J. 2015;26(3):421–25.

[12] Mizrachi Y, Tannus S, Bar J, et al. Unexpected significant uterine pathological findings at vaginal hysterectomy despite unremarkable preoperative workup. Isr Med Assoc J. 2017;19(10):631–34.