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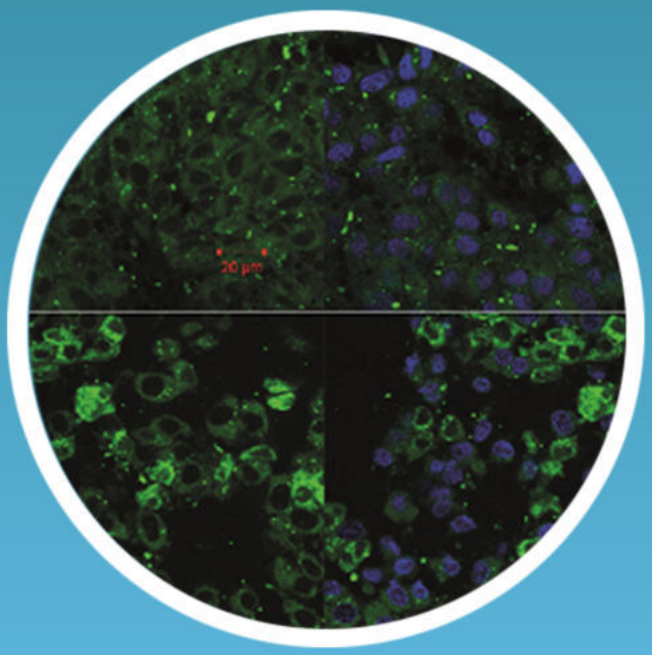

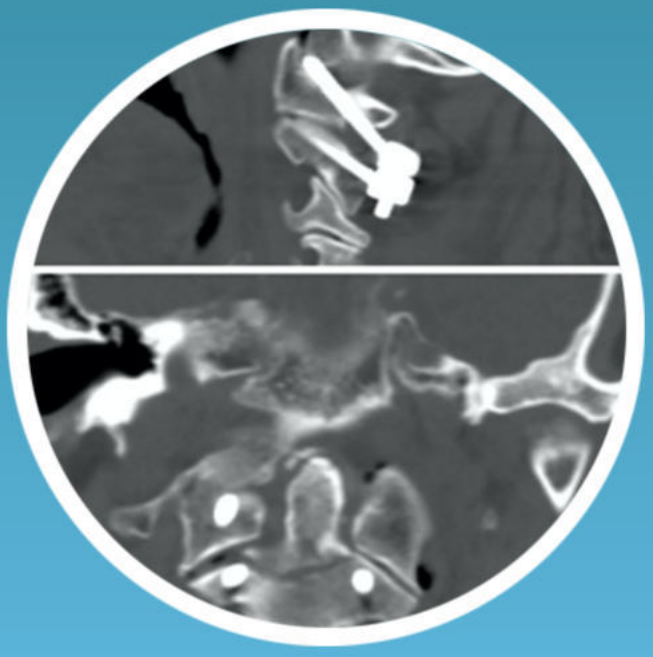

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The European Research Journal 2023;9(2):178-185 Original Article

DOI: 10.18621/eurj.1209679 *Histology and Embryology*

Ameliorative effects of apigenin on a rat model of endometriosis

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ABSTRACT

Objectives: Apigenin and parthenolide as natural products have potent antioxidant and anti-inflammatory outcomes that could make them a perfect option for endometriosis therapy. This study aimed to determine the effects of apigenin and parthenolide on created endometrial implants in a rat model of endometriosis.

Methods: Thirty-nine mature, female Sprague-Dawley rats were assigned randomly to six experimental groups four weeks after endometriosis induction. Group 1 (n = 5): Control (CTRL) that opened and closed the abdomen; Group 2 (n = 6): Peritoneal and ovarian endometriosis (POE) + drug-free; Group 3 (n = 7): POE+ Apigenin (APG) (50 mg/kg); Group 4 (n = 7): POE+ Parthenolide (PTL) (10 mg/kg); Group 5 (n = 7): POE+ Apigenin (APG) (50 mg/kg) + Parthenolide (PTL) (10 mg/kg); Group 6 (n = 7): POE+ DMSO. An endometriosis model was created, and histopathological analysis and biochemical evaluation were performed. Serum and peritoneal levels of pro-and-anti-inflammatory cytokine, and oxidative stress of implant tissue were measured.

Results: Serum IL-37 levels decreased significantly in the APG-treated group compared to the drug-free group ($p = 0.016$). The peritoneum and ovary endometriosis histopathologic scores were significantly lower in APG-treated ($p = 0.001$) and PTL-treated ($p = 0.001$) groups in comparison to the drug-free group. The oxidative stress index (OSI) values were increased statistically significantly in ovary endometriosis tissue in the drug-free group, ($p = 0.001$). However, compared to the drug-free group, OSI values decreased statistically significantly in the APG-treated group ($p = 0.003$)

Conclusions: The application of apigenin caused a decrease in oxidative stress and an improvement in histopathological grade. Apigenin may be a novel therapeutic agent for the treatment of endometriosis.

Keywords: Apigenin, parthenolide, oxidative stress, endometriosis, IL-6, IL-37

Endometriosis is an inherited condition that is affected by both genetic and environmental factors. It has been suggested that low progesterone levels and hormone imbalances in women with endometriosis

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may be genetic [1]. One of the most persuasive theories, the retrograde menstruation hypothesis indicates that endometrial fragments reach the pelvis via transtubal retrograde flow, implant onto the peritoneum and abdominal organs, and proliferate and cause endometriosis [2]. Due to the uncertain etiology of endometriosis, gonadotropin-releasing hormone (GnRH) agonists, progestin, and androgens are used for therapeutic purposes [3]. Therefore, new molecular studies that prevent or treat endometriosis are needed using therapeutic approaches for treatment.

Plant extracts that possess a broad range of biological activities have been investigated to prevent and treat various health issues, including gynecological disease, due to their anti-inflammatory and anti-oxidative properties [4]. Previous studies have also determined the effects of biological compounds on the restraint of endometriosis [4, 5]. For example, resveratrol and epigallocatechin-3-gallate have been shown to hinder a sharp increase in cell productivity and give rise to apoptosis in both in vitro and in vivo endometriosis models [5]. Rosaceae extracts have been determined to cause decreased cytokine levels and endometrial implants in surgically induced endometriosis [6].

Apigenin (4′, 5,7-trihydroxyflavone), found in many plants, belongs to the flavone class. Studies have shown that Apigenin has better pharmacokinetics and oral bioavailability than other flavonoids. Apigenin has recently attracted attention as a beneficial and health-promoting agent due to its striking effects, which are relatively non-toxic and non-mutagenic against normal cells compared to other flavonoids. It has been shown to have various biological properties such as antioxidant and anti-inflammatory [7]. While parthenolide is a natural product obtained from the shoots of Tanacetum parthenium, which has been shown to have immunomodulatory effects in several diseases [8]. In some studies, it has been suggested that parthenolide also shows anti-inflammatory and antioxidant activity [9].

There is an amount of evidence that inflammation and immune responses play a crucial role in the pathogenesis of endometriosis [10]. Especially, the cytokines – interleukin (IL)-1beta (IL-1β), 6, 10, and tumor necrosis factor-alpha (TNF-α), have been implicated in the pathogenesis of endometriosis [11]. Recent studies have proven that increased levels of IL-1β and TNF-α stimulate the production of IL-6 by peritoneal mesothelial cells, and as a result, it may cause local inflammation in endometriosis [12, 13]. In addition, it has been found that serum and peritoneal fluid IL-37 levels are significantly elevated in patients with endometriosis and are associated with the stage of endometriosis [14].

Furthermore, among the hypotheses put forward to explain the pathophysiology of endometriosis, oxidative stress is one of the leading theories. There is a body of evidence from animal and human studies that oxidative stress is one of the main processes in the pathogenesis of endometriosis [15].

Apigenin and Parthenolide are therapeutic agents with few side effects since they do not act directly hormonally. The effect of apigenin and parthenolide on pro-and anti-inflammatory cytokine secretion and oxidative stress in the pathogenesis of peritoneal and ovarian endometriosis has not been studied in-vivo yet.

In studies conducted to date, experimental peritoneal and ovarian endometriosis models have been created in rats, and many drugs' effects on endometriosis have been determined. The present study aimed to investigate the treatment effects of apigenin and parthenolide, which have not been used in endometriosis studies before and have proven potent anti-inflammatory and antioxidant activity, on endometriosis models in rats.

METHODS

The rats were obtained from the University of Health Sciences Animal Research Laboratory. In this study, 39 adult non-pregnant Wistar-Albino female rats weighing 145-208 g were used. Rats were housed under standard care and feeding conditions. All experimental procedures and protocols performed in this study were ethically approved by the Health Sciences University Hamidiye Animal Experiments Local Ethics Committee (Istanbul, Turkey) (No: 2020-01/01). All experimental animal procedures were performed following standard ethical guidelines.

Rats were randomly selected and divided into six groups (group 1 to group 6). All rats underwent laparotomy in the estrus phase. Only in the control group (n = 5) was the abdomen opened and closed. Experimen-

tal groups were listed below.

Group 1 (n = 5): Control (CTRL) group that opened and closed the abdomen. Group 2 (n = 6): Peritoneal and ovarian endometriosis (POE) + drug-free group. Group 3 (n = 7): POE+ Apigenin (APG) (50 mg/kg). Group 4 (n = 7): POE+ Parthenolide (PTL) (10 mg/kg). Group 5 (n = 7): POE+ Apigenin (APG) (50 mg/kg) + Parthenolide (PTL) (10 mg/kg). Group 6 (n = 7): POE+ Dimethyl sulfoxide (DMSO).

Establishment of the Endometriosis Model

The peritoneal area of the rats of the CTRL group was opened 3-4 cm in length after anesthesia and was sutured with 6/0 vicryl immediately afterward. In 34 rats other than the CTRL group, the right uterine horn was opened, turned inside out, and it was sutured in the right pelvic peritoneum of the rat uterus to create peritoneal endometriosis, and the left uterine horn was opened, turned inside out, and sutured to the left ovarian surface to form ovarian endometriosis.

In 34 rats (groups 2 to 6) with peritoneal and ovarian endometriosis, laparotomy was performed again 29 days later to detect the occurrence of endometriosis. Then, rats were rested for 3 days, and on the 3rd day (31st day of the experiment), APG (50 mg/kg) was given to POE + APG group, PTL (10 mg/kg) was given to POE + PTL group, and APG (50 mg/kg) + PTL (10 mg/kg) were given to POE + APG + PTL group. Intraperitoneal injection (IP) was applied five days a week for a month. POE + DMSO group was administered DMSO, IP in the concentration and volume in which we dissolved the apigenin and parthenolide herbal medicine five days a week for a month.
On 59 the day of the experiment, laparotomy was performed again on all rats. Rats were euthanized by cervical dislocation following intracardial blood collection (approximately 4 ml) under anesthesia. Peritoneal and ovarian endometriosis tissue was taken for clinical parameters and histopathological evaluation. In addition, the results of Hematoxylin and Eosin (H&E) staining were evaluated for gland-stroma structure in endometriosis foci and surface epithelial changes in the left ovary.

Tissue Procedures

Peritoneal and ovarian endometriosis tissue was divided into two, half of them for oxidative stress analysis, and another half of them for histopathological analysis. Tissues were weighed, homogenized with 1/5 (w/v) cold (1.15%) KCl solution in a homogenizer (Qiagen Tissue Layser LT) and centrifuged at 10,000 g for half an hour to separate the supernatants +4ºC. Protein measurements from tissues were analyzed colorimetrically by the Bradford method [16]. This method is based on the protein binding of Coomassie Brilliant Blue G250 dye (Sigma-Aldrich, Steinham, Germany). Bovine serum albumin (BSA) was used as a standard (Sigma-Aldrich, Steinham, Germany). 150 µL of Coomassie Brilliant Blue G250 were mixed with 10 µL of sample and then incubated for 10 minutes at room temperature with a shaker. The absorbance at 595 nm was measured (Synergy-HTX, Biotek, USA). The protein concentrations of the samples were calculated according to the standard protein curve.

Oxidative Stress Analyses

Peritoneal and ovarian endometriosis tissue samples were analyzed spectrophotometrically in the multi-plate reader (Synergy-HTX, Biotek, USA) for oxidative stress parameters. TAS and TOS were measured using commercially available kits. In agreement with the standard curve, TAS levels were presented as mmol ascorbic acid Eq/mL/mg protein and TOS levels were computed as µmol H2O2 Eq/mL mg protein. The oxidative stress index (OSI) was calculated as TOS/TAS.

Inflammation Biomarkers

During the last laparotomy surgery, 1 ml of PBS was injected into the abdominal cavity and allowed the PBS to spread all over the abdominal cavity. Then the PBS was withdrawn and used as the peritoneal fluid. IL-1β, IL-6, IL-10, TNF-α, and IL-37 cytokines, which are inflammation biomarkers were investigated in serum and peritoneal fluid. Cytokine levels were measured spectrophotometrically with ELISA kits (Abcam) in a multi-plate reader (Synergy-HTX, Biotek, USA) according to manufacturers' instructions.

Histopathological Investigation

Formalin-fixed, paraffin-embedded tissue samples were cut with 4 µm-thick and stained with H&E. A

semi-quantitative evaluation of all samples was performed in the ovary and peritoneum endometriosis tissue. Score 3: Well-protected endometrial glandular epithelium; Score 2: Moderately spilled endometrial glandular epithelium; Score 1: Highly spilled endometrial glandular epithelium; Score 0: No endometrial glandular epithelium left [17]. The histopathological analysis was conducted by a pathologist, who do not know each test group.

Statistical Analysis

IBM SPSS 21 statistical software was applied to analyze the data. The distribution of variables was evaluated using the Homogeneity of variance test. The difference between the groups was analyzed with the Kruskal-Wallis H test or the one-way ANOVA test. If there was a difference, compared furtherly in pairs using the Games-Howell test, or Tukey test, one of the post-hoc methods.

RESULTS

Oxidative stress-related biochemical parameters in peritoneal tissue and ovary

Oxidative stress biochemical parameters such as TAS, TOS, and OSI were measured. The Mean ± SD values achieved from the peritoneal and ovary endometriosis tissue are shown in Table 1.

Parameters of TAS, TOS, and OSI in peritoneum endometriosis tissue between groups

OSI values were increased in peritoneal endometriosis tissue in the drug-free group compared to the control group and were decreased compared to the other treatment groups. However, there were no significant differences identified between the groups (Table 1, Fig. 1).

Parameters of TAS, TOS, and OSI in ovary endometriosis tissue between groups

When compared with the control group, OSI values were increased statistically significantly in ovary endometriosis tissue in the drug-free group, (p = 0.001). Meanwhile, compared to the drug-free group, OSI values decreased statistically significantly in the APG-treated group (p = 0.003) (Table 2) (Fig. 1).

IL-1β, IL-6, IL-10, TNF-α, and IL-37 cytokines production in apigenin and parthenolide-treated endometriosis rat model

ELISA evaluated all serum samples to ascertain IL-1β, IL-6, IL-10, TNF-α, and IL-37 cytokines. IL-37 was quantified in all the analyzed samples and its serum concentration was significantly decreased in the POE+ APG group when compared to POE + drug-free (p = 0.016) group. However, no significant differences were identified in the production of IL-1β, IL-6, IL-10, and TNF-α between groups.

Peritoneal fluid samples for detecting IL-1β, IL-6, IL-10, TNF-α, and IL-37 cytokines were also evaluated. However, there were no significant differences

Table 1. TAS, TOS, and OSI biochemical parameters obtained from peritoneal endometriosis tissue

Groups	TAS (mmol Ascorbic acid Eq/L/mg protein)	TOS (μmol H_2O_2/L/mg protein)	OSI (AU)
CTRL	0.25 ± 0.04	0.38 ± 0.16	1.24 ± 0.60
POE + drug-free	0.15 ± 0.12	1.49 ± 1.11	6.49 ± 1.08
POE + APG	0.24 ± 0.05	0.98 ± 0.42	3.34 ± 1.89
POE + PTL	0.43 ± 0.18	0.73 ± 0.44	1.76 ± 1.48
POE + APG+ PTL	0.27 ± 0.02	1.69 ± 0.94	5.47 ± 3.35
POE + DMSO	0.26 ± 0.08	1.36 ± 0.36	4.40 ± 1.33

All values are expressed as mean ± SD. APG = apigenin, CTRL = control, DMSO = dimethyl sulfoxide, OSI = oxidative stress index, POE = peritoneal and ovarian endometriosis, PTL = parthenolide, TAS = total antioxidant status, TOS = total oxidant status

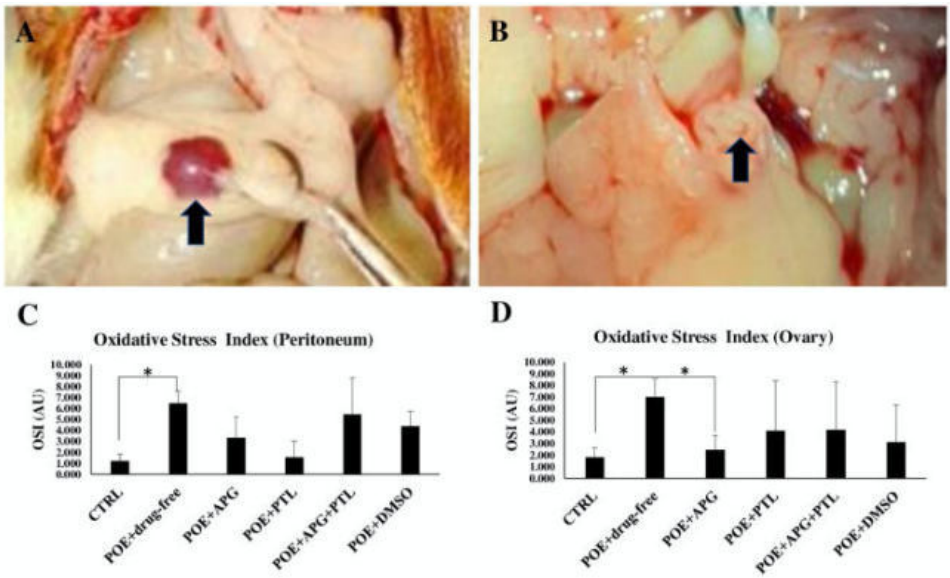

Fig. 1. The gross appearance of endometriotic lesion: pre-treatment enlarged cystic lesion (A), and the atrophic endometriotic lesion after treatment with APG (B). The oxidative stress index was evaluated by TAS, and TOS and analyzed spectrophotometrically. (OSI) for peritoneal endometriotic implants for all experimental groups (C), and ovarian endometriotic implants for all experimental groups (D).
Statistical comparisons were made using a Kruskal-Wallis H test with further Mann-Whitney U tests. A two-sided p-value of less than 0.05 was considered statistically significant. *$p < 0.05$. APG = apigenin, CTRL = control, DMSO = dimethyl sulfoxide, OSI = oxidative stress index, POE = peritoneal and ovarian endometriosis, PTL = parthenolide, TAS = total antioxidant status, TOS = total oxidant status

in the production of IL-1β, IL-6, IL-10, TNF-α, and IL-37 between groups.

Histopathological changes

We evaluated endometriosis foci in ovarian and peritoneal tissues with H&E staining. Histopathologically, grading evaluating the endometriosis that occurs in the peritoneum and ovary is given in Table 3. There was a significant difference between the groups in terms of preserved epithelium and inflammation in

Table 2. TAS, TOS, and OSI biochemical parameters obtained from the ovary endometriosis tissue

Groups	TAS (mmol Ascorbic acid Eq/L/mg protein)	TOS (μmol H_2O_2/L/mg protein)	OSI (AU)
CTRL	0.20 ± 0.03	0.41 ± 0.19	1.83 ± 0.81
POE+ drug-free	0.14 ± 0.03	1.22 ± 0.15	7.01 ± 1.58‡
POE+ APG	0.29 ± 0.07	0.96 ± 0.51	2.45 ± 1.25*
POE+ PTL	0.24 ± 0.05	1.33 ± 1.53	4.11 ± 4.30
POE+ APG+ PTL	0.20 ± 0.05	0.97 ± 0.38	4.17 ± 4.16
POE+ DMSO	0.21 ± 0.07	0.84 ± 0.19	3.13 ± 3.20

All values are expressed as mean ± SD. APG = apigenin, CTRL = control, DMSO = dimethyl sulfoxide, OSI = oxidative stress index, POE = peritoneal and ovarian endometriosis, PTL = parthenolide, TAS = total antioxidant status, TOS = total oxidant status

*$p = 0.003$ POE+APG versus POE+drug-free; ‡$p = 0.001$ POE+drug-free versus CTRL.

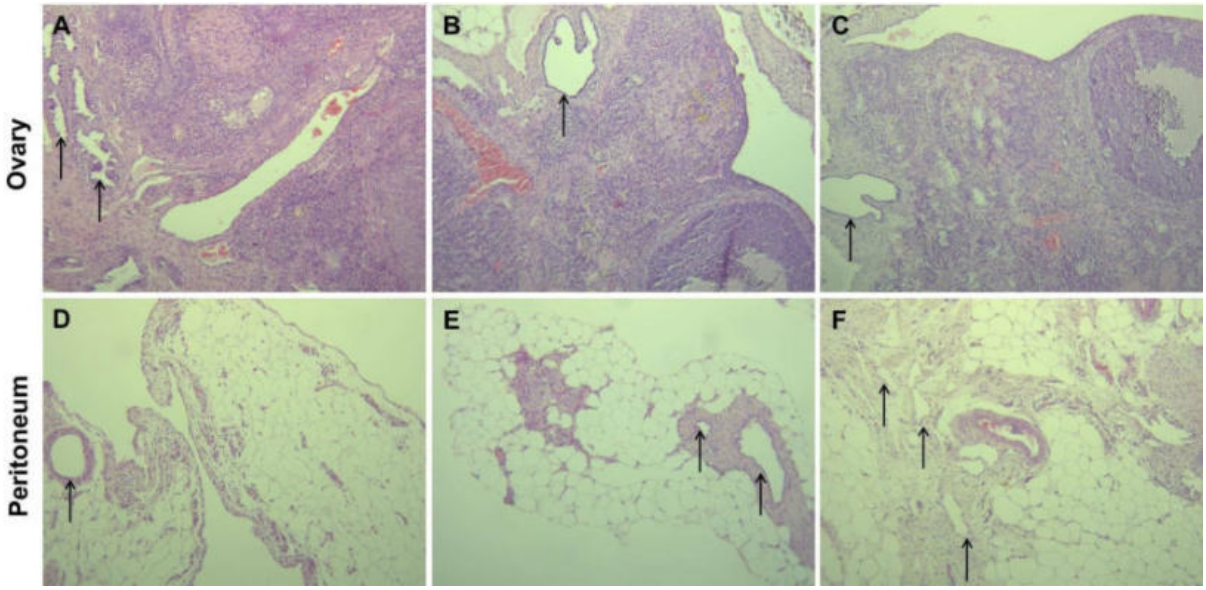

Fig. 2. Effect of APG or PTL treatment on the deterioration of the epithelial layer in endometriotic implants. The hematoxylin and eosin (H&E) images show representative histological sections of induced endometriotic implants in the ovary in the rat POE+drug-free group (A) and APG-treated rat POE+APG group (B), and PTL-treated rat POE+PTL group (C). In the peritoneum in rat POE+drug-free group (D) and APG-treated rat POE+APG group (E), and PTL-treated rat POE+PTL group (F). Arrows indicate endometrial glands. Magnification: 200×.

the peritoneum and ovary ($p < 0.001$) (Figs. 2 and 3).

DISCUSSION

Although many studies have been performed to better understand and explain the exact cause of endometriosis, the pathogenesis of endometriosis is still controversial and thus treatment options are not successful. In addition to modern treatment methods, some therapies have focused on oxidative stress in endometrial implants, and it has been proven that antioxidants can hamper endometriosis. In our rat models, apigenin and parthenolide, as strong antioxidant and anti-inflammatory herbal agents, were used to treat endometriosis.

Table 3. Endometriosis grading obtained from the peritoneum and ovary

Groups	Peritoneum	Ovary
POE+ drug-free	3.00 ± 0.00	3.00 ± 0.00
POE+ APG	0.33 ± 0.52*	0.50 ± 0.55*
POE+ PTL	0.33 ± 0.52‡	0.67 ± 0.52‡
POE+ APG+ PTL	2.20 ± 0.45	2.75 ± 0.50
POE+ DMSO	3.00 ± 0.00	3.00 ± 0.00

All values are expressed as mean ± SD. APG = apigenin, DMSO = dimethyl sulfoxide, POE = peritoneal and ovarian endometriosis, PTL = parthenolide

*p = 0.001 POE+APG versus POE+drug-free; ‡p = 0.001 POE+PTL versus POE+drug-free.

Iwabuchi *et al*. [18] has revealed that the inauguration of oxidative stress in endometriosis might be associated with the presence of degenerated endometrial tissue, and cell debris may have been transported to the abdominal cavity by menstrual reflux. In the present study, ovary endometrial implants showed significantly higher oxidative stress levels. These findings are in line with the results of studies showing that oxidative stress and inflammation have a pivotal function in the pathogenesis of endometriosis [17]. There may be a cause-result relationship between boosted oxidative stress and the pathogenesis of endometriosis [19]. It is well known that the disproportion between reactive oxygen species and antioxidants is associated with endometriosis [20]. We hypothesized in the current study that targeting oxidative stress probably results in a reduction in the histopathological grade of endometrial implants. In our study, the application of apigenin leads to the alleviation of lesional endometrial tissue in the rat ovary. This could be the mecha-

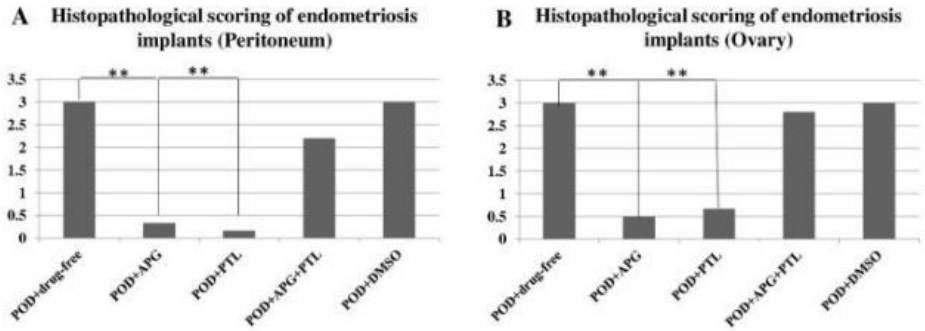

Fig. 3. Comparison of histopathological evaluations between groups. Histopathological scores were applied (stain: hematoxylin and eosin, H&E) Score 3: Well-protected endometrial glandular epithelium; Score 2: Moderately spilled endometrial glandular epithelium with inflammation; Score 1: Highly spilled endometrial glandular epithelium; Score 0: No endometrial glandular epithelium.
Statistical analysis was performed using a Kruskal-Wallis H test with further post-hoc Games-Howell tests. **$p = 0.001$. APG = apigenin, DMSO = dimethyl sulfoxide, POE = peritoneal and ovarian endometriosis, PTL = parthenolide

nism here that apigenin generates ROS-dependent apoptosis and endoplasmic reticulum stretching in endometriosis cells [21]. While Parthenolide inhibits cell proliferation in human endometriotic stromal cells, diminishes prostaglandin estradiol synthesis, and hinders the development of endometriosis in the murine model [22]. However, in the present study, histopathological grade and oxidative stress index could not match after the application of parthenolide treatment. Moreover, in the current study, there was no significant increase in serum levels of IL-1β, TNF-α, and IL-6 in the drug-free model group when compared to the CTRL group. Meanwhile, there was no remarkable decrease in cytokine IL-1β, TNF-α, and IL-6 in the APG and PTL-treated group after administration of apigenin and parthenolide. These findings are inconsistent with other studies which have reported that apigenin and parthenolide inhibit IL-1β, TNF-α, and IL-6 expression [23, 24]. This is probably due to the different degrees of inflammation in endometriosis foci, different doses of apigenin and/or parthenolide used, and the different types of experimental animals used in the studies. In our study, we found less inflammation in either ovary endometrial implants or peritoneal endometrial implants in contrast to the control. This is probably the reason why there are no significant differences identified between groups regarding pro-inflammatory cytokine production in our endometriosis model.

Differential expressions of anti-inflammatory cytokines and their roles in the pathogenesis of endometriosis are gradually emerging. Considering its immunological background, studies have shown that endometriosis is not only an inflammatory disorder but also a disease in which pro-and anti-inflammatory mechanisms work [25]. The current study analyzed the level of IL-10 and IL-37 in serum and peritoneal fluid with a rat model of endometriosis. We found that serum IL-37 levels were significantly lower than those measured in the drug-free group after treatment with APG; this suggests that APG may have inhibited immune responses. Based on present results, APG may play an anti-inflammatory role in endometriosis by regulating immune inflammation.

CONCLUSION

Apigenin application leads to a decreased histopathologic score of the induced endometrial implants and a depletion of oxidative stress in the rat model. Oxidative stress and inflammation are closely linked and have been suggested to play a role in the etiology of endometriosis. Apigenin can be a good target in the treatment of endometriosis.

Authors' Contribution

Study Conception: GH, SA, AD, KS, MT, NS, EMG, FM, HTC, HHP, TS, EK; Study Design: GH, SA, AD, KS, MT, NS, EMG, FM, HTC, HHP, TS, EK; Supervision: GH, SA, AD, KS, MT, NS, EMG, FM, HTC, HHP, TS, EK; Funding: GH, HTC, EK; Materials: GH, HTC, EK; Data Collection and/or Processing: GH, SA, AD, KS, MT, NS, EMG, FM, HTC, HHP,

TS; Statistical Analysis and/or Data Interpretation: GH, SA, AD, KS, MT, EK, NS, EMG, FM, HTC, HHP, TS; Literature Review: GH; Manuscript Preparation: GH and Critical Review: GH.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Fauser BC, Diedrich K, Bouchard P, Domínguez F, Matzuk M, Franks S, et al. Contemporary genetic technologies and female reproduction. Hum Reprod Update 2011;17:829-47.
2. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol 2014;10:261-75.
3. Kupker W, Felberbaum RE, Krapp M, Schill T, Malik E, Diedrich K. Use of GnRH antagonists in the treatment of endometriosis. Reprod Biomed Online 2002;5:12-6.
4. Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JP. Polyphenols and human health: prevention of disease and mechanisms of action. Nutrients 2010;2:1106-31.
5. Ricci AG, Olivares CN, Bilotas MA, Baston JI, Singla JJ, Meresman GF, et al. Natural therapies assessment for the treatment of endometriosis. Hum Reprod 2013;28:178-88.
6. Buwa CC, Mahajan UB, Patil CR, Goyal SN. Apigenin attenuates beta-receptor-stimulated myocardial injury by safeguarding cardiac functions and escalating the antioxidant defense system. Cardiovasc Toxicol 2016;16:286-97.
7. Tang D, Chen K, Huang L, Li J. Pharmacokinetic properties and drug interactions of apigenin, a natural flavone. Expert Opin Drug Metab Toxicol 2017;13:323-30.
8. Weihuan M, Zhu Z. Parthenolide inhibits hydrogen peroxide-induced osteoblast apoptosis. Mol Med Rep 2018;17:8369-76.
9. Ghantous A, Sinjab A, Herceg Z, Darwiche N. Parthenolide: from plant shoots to cancer roots. Drug Discov Today 2013;18:894-905.
10. Bulun SE. Endometriosis. N Engl J Med 2009;360:268-79.
11. Wu MY, Ho HN. The role of cytokines in endometriosis. Am J Reprod Immunol 2003; 49:285-96.
12. Urata Y, Koga K, Hirota Y, Akiyama I, Izumi G, Takamura M, et al. IL-1β increases expression of tryptophan 2,3-dioxygenase and stimulates tryptophan catabolism in endometrioma stromal cells. Am J Reprod Immunol 2014;72:496-503.
13. Mosbah A, Nabiel Y, Khashaba E. Interleukin-6, intracellular adhesion molecule-1, and glycodelin A levels in serum and peritoneal fluid as biomarkers for endometriosis. Int J Gynaecol Obstet 2016;134:247-51.
14. Jiang J, Jiang Z, Xue M. Serum and peritoneal fluid levels of interleukin-6 and interleukin-37 as biomarkers for endometriosis. Gynecol Endocrinol 2019;35:571-5.
15. Gupta S, Agarwal A, Krajcir N, Alvarez JG. Role of oxidative stress in endometriosis. Reprod Biomed Online 2006;13:126-34.
16. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
17. Cetinkaya N, Attar R, Yildirim G, Ficicioglu C, Ozkan F, Yilmaz B, et al. The effects of different doses of melatonin treatment on endometrial implants in an oophorectomized rat endometriosis model. Arch Gynecol Obstet 2015;291:591-8.
18. Iwabuchi T, Yoshimoto C, Shigetomi H, Kobayashi H. Oxidative stress and antioxidant defense in endometriosis and its malignant transformation. Oxid Med Cell Longev 2015;2015:848595.
19. Donnez J, Binda MM, Donnez O, Dolmans MM. Oxidative stress in the pelvic cavity and its role in the pathogenesis of endometriosis. Fertil Steril 2016;106:1011-7.
20. Harlev A, Gupta S, Agarwal A. Targeting oxidative stress to treat endometriosis. Expert Opin Ther Targets 2015;19:1447-64.
21. Park S, Lim W, Bazer FW, Song G. Apigenin induces ROS-dependent apoptosis and ER stress in human endometriosis cells. J Cell Physiol 2018;233:3055-65.
22. Takai E, Taniguchi F, Nakamura K, Uegaki T, Iwabe T, Harada T. Parthenolide reduces cell proliferation and prostaglandin estradiol synthesis in human endometriotic stromal cells and inhibits the development of endometriosis in the murine model. Fertil Steril 2013;100:1170-8.
23. Kowalski J, Samojedny A, Paul M, Pietsz G, Wilczok T. Effect of apigenin, kaempferol and resveratrol on the expression of interleukin-1beta and tumor necrosis factor-alpha genes in J774.2 macrophages. Pharmacol Rep 2005;57:390-4.
24. Zhang M, Liu RT, Zhang P, Zhang N, Yang CL, Yue LT, et al. Parthenolide inhibits the initiation of experimental autoimmune neuritis. J Neuroimmunol 2017;305:154-61.
25. Zhou WJ, Yang HL, Shao J, Mei J, Chang KK, Zhu R, et al. Anti-inflammatory cytokines in endometriosis. Cell Mol Life Sci 2019;76: 2111-32.

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Efficacy of topical mineralocorticoids in a rabbit model of ocular inflammation

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ABSTRACT

Objectives: The aim of this study was to determine the efficacy of mineralocorticoids in the treatment of ocular inflammation, whose potential use has not been assessed.

Methods: Thirty-five New Zealand albino rabbits were used in the study. Rabbits were divided into five groups. Only one eye was used for experimental purposes and the other eye was used as control. 11-deoxycortisol, deoxycorticosterone acetate, fludrocortisone acetate, aldosterone and 11-deoxycorticosterone were studied in a rabbit model of ocular inflammation. All animals in a group received the same corticosteroid. Paired t-tests and analysis of variance between subjects (ANOVA) were used to evaluate efficacy.

Results: The eyes treated with 11-deoxycortisol, deoxycorticosterone acetate, and fludrocortisone acetate had statistically significant lower fluorescence compared to control eyes. 11-deoxycortisol and deoxycorticosterone acetate provided a greater reduction in fluorescence compared to other corticosteroids.

Conclusions: Topical use of corticosteroids, especially those with mineralocorticoid activity can decrease ocular inflammation in a rabbit model. Clinical application of topical mineralocorticoids in human ocular inflammation needs to be performed.

Keywords: Topical mineralocorticoids, ocular inflammation, uveitis

Ocular inflammation is a common problem encountered in ophthalmology. Inflammatory responses in the eye are frequently seen in systemic diseases (e.g. rheumatoid arthritis), ocular diseases (e.g. infections), ocular surgeries (e.g. cataract extraction), or idiopathic conditions (e.g. anterior uveitis) [1-5].

The efficacy of glucocorticoids in treating ocular inflammation is well documented [6, 7]. In the treatment of ocular disease, this class of medications can be given topically. They can also be given through sub-tenon or intravitreal injections, with good ocular absorption [8-10]. Increased intraocular pressure is the primary complication of these medications [11].

The potential use of other classes of topical corticosteroids in the treatment of ocular inflammation has not been assessed, but they have theoretical utility. For example, mineralocorticoid receptors have been documented in rodentia ocular tissue [12] to maintain sodium-water homeostasis by regulating sodium channels [13, 14]. Through this role, the mineralocorticoids may impact eye function via modalities such as aqueous humor formation and corneal fibroblast formation [15, 16].

Given the anti-inflammatory properties of steroids and known intraocular receptors for mineralocorti-

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coids, it has been hypothesized that topical corticosteroids with primarily mineralocorticoid activity may also reduce intraocular inflammation. In this study, this hypothesis was tried to be tested.

This study had two specific aims: (1) to assess the reduction of ocular inflammation associated with topically administered mineralocorticoids in a rabbit model of prostaglandin-mediated uveitis, (2) to compare the efficacy of mineralocorticoids in reducing inflammation.

METHODS

Corticosteroid Solutions

We studied the following corticosteroids: 11-deoxycortisol, deoxycorticosterone acetate, fludrocortisone acetate, aldosterone, and 11-deoxycorticosterone. These compounds were obtained from Sigma Company (Sigma Chemical, St. Louis, MO).

Experimental Animals

Thirty-five New Zealand albino rabbits weighing between 2.5 and 3.5 kg were used in the study. Rabbits were treated in accordance with the principles of the Association for Research in Vision and Ophthalmology (ARVO) for the humane treatment of animals. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985), the Office for Protection from Research Risks (OPRR) Public Health Service Policy on the Humane Care and Use of Laboratory Animals (revised 1986), and the U.S. Animal Welfare Act were also followed. Only one eye was used for experimental purposes and the other eye was used as control. Slit-lamp and indirect fundoscopic examinations were performed on all eyes before and after euthanasia. No animals with pre-existing signs of ocular infection or inflammation were included in the study. The study was approved by Tulane University and conducted in the research laboratory of Tulane University Faculty of Medicine.

Rabbits were divided into five groups (Group 1:11-deoxycortisol; Group 2: Deoxycorticosterone acetate; Group 3: Fludrocortisone acetate; Group 4: Aldosterone; Group 5:11-deoxycorticosterone) with seven rabbits in each group (total of 35 rabbits, 70 eyes), based on corticosteroid use. All animals in a group received the same corticosteroid. All corticosteroids were administered topically as a 4 mg/ml solution.

Corticosteroid Administration

In this study, the experimental model described by Waterbury and Flach [17] was systematically followed. Accordingly, the rabbits were anesthetized with an intramuscular injection mixture of 1 ml of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Corticosteroid solutions were applied to the right eye and saline was applied to the left eye. Both corticosteroid and saline solutions were administered four times per hour. The first drop application represented the time zero of the experiment. Drugs and saline were administered to the eyes as a single 50µL drop.

Dextran-isothiocyanate-fluoroscein FITC-dextran, (Sigma Chemical, St. Louis, MO) was used as a systemic marker in an anterior chamber fluorophotometric model to evaluate the impact of corticosteroids on inflammation [17]. A 20 mg/ml dose was prepared and injected into the marginal ear vein of each rabbit at hour 1.5 of the experiment. Endotoxin (2.5 µg/kg) isolated from Salmonella Typhimurium (Sigma Chemical) was injected into the same vein at hour 2, coinciding with the administration of drop three to initiate an inflammatory response.

The concentration of dextran-FITC in the anterior chamber was evaluated using a prototype flurophometer (OcuMetrics, Mountain View, CA) 90 minutes after endotoxin administration (30 minutes after the final drop application). This time frame is best to observe leakage of the fluorescent material [17]. The excitation wavelengths of this instrument are approximately 410 to 490 nm, with emission wavelengths of approximately 520 to 630 nm. The scanning optic head of the device is computer controlled. The computer also stores and processes all data.

Rabbits were put in a plexiglas holder and then placed in front of the instrument. Blue excitation light was focused on the eye of interest. The resulting fluorescence emitted by FITC-dextran was identified by a photomultiplier tube. Both right and left eyes were scanned.

Statistical Analysis

Paired t-tests were used to evaluate the effects of each corticosteroid versus saline control. Therefore,

each animal served as its control. Analysis of variance between subjects (ANOVA) was used to evaluate the efficacy of the corticosteroids compared to each other. Post-hoc analyses were done with Tukey's LSD. A p value <.05 was considered statistically significant.

RESULTS

Table 1 shows the mean levels of fluorescence emitted by Dextran-FITC in treatment and control eyes for the various corticosteroids. Eyes treated with 11-deoxycortisol, deoxycorticosterone acetate, and fludrocortisone acetate had statistically significantly lower fluorescence compared to control eyes ($p = 0.021$, $p = 0.003$, and $p = 0.042$; respectively). This suggested lower levels of leakage of dextran-FITC, and thereby, lower levels of inflammatory response in treatment eyes.

Table 2 shows the mean reduction levels in fluorescence seen for each of the corticosteroids. The reduction in fluorescence for each study animal was calculated by subtracting fluorescence in the treated eye from fluorescence in the control eye. Means for this difference were then calculated for each corticosteroid. Therefore, larger means indicate a greater reduction in fluorescence and suggest a greater reduction in the inflammatory response.

ANOVA results showed a significant difference in the mean fluorescence reduction among the mineralocorticoids [$F(6, 27)=3.95$, $p < 0.01$]. Tukey's LSD results revealed that 11-deoxycortisol and deoxycorticosterone acetate provided greater reductions ($p < 0.05$) in fluorescence compared to aldosterone and 11-deoxycorticosterone. Other pairwise comparisons were not statistically significant.

DISCUSSION

Of the five corticosteroids studied, none showed mineralocorticoid activity and only two showed glucocorticoid activity (minimal activity only, defined as $1/1000^{th}$ of the mineralocorticoid effect [18]). Our results showed that topically administered corticosteroids with little or no glucocorticoid activity reduced ocular inflammation in a rabbit model. Three of the topical corticosteroids produced a significant decrease in ocular inflammation in the experimental animals. 11-deoxycortisol, deoxycorticosterone acetate, and fludrocortisone acetate resulted in decreased endotoxin-induced fluorescein leakage into the anterior chamber compared to control eyes. Of these, 11-deoxycortisol and deoxycorticosterone acetate had only

Table 1. Mean anterior chamber fluorescence emitted by dextran-FITC in treatment versus control rabbit eyes

Mineralocorticoid	Right Eye (Treatment)		Left Eye (Control)		$t(4)$*	p value
	Mean (nm)	SD (nm)	Mean (nm)	SD (nm)		
Group 1 (11-deoxycortisol)	330.80	283.42	519.00	233.78	-3.67	**0.021**
Group 2 (Deoxycorticosterone acetate)	200.80	150.04	379.00	123.34	-6.35	**0.003**
Group 3 (Fludrocortisone acetate)	165.80	185.02	251.60	240.38	-2.94	**0.042**
Group 4 (Aldosterone)	247.80	180.56	113.61	113.61	0.25	0.815
Group 5 (11-deoxycorticosterone)	233.40	277.08	215.80	197.13	0.39	0.716

*t-test with 4 degrees of freedom

Table 2. Mean reduction in fluorescence by mineralocorticoid usage in rabbit eyes

Mineralocorticoid	Mean Reduction (nm)	SD (nm)
Group 1 (11-deoxycortisol)	188.20	114.25
Group 2 (Deoxycorticosterone acetate)	178.20	62.78
Group 3 (Fludrocortisone acetate)	85.80	65.35
Group 4 (Aldosterone)	-13.60	122.48
Group 5 (11-deoxycorticosterone)	-17.60	100.11

mineralocorticoid activity. The ocular anti-inflammatory properties of these mineralocorticoids are unique. Fludrocortisone acetate has both mineralocorticoid and glucocorticoid activity. However, in a model of inhibition of corneal angiogenesis, fludrocortisone was effective at dosages as low as 5 µg/ml, which was a dose where glucocorticoid activity would be minimal (Peyman GA, unpublished observation).

In comparing the different corticosteroids, neither aldosterone nor 11-deoxycorticosterone had a statistically significant effect on inflammation. Interestingly, aldosterone actually increased vascular inflammation when presented systemically [19, 20].

The rabbit model used in this study was used in other studies of ocular inflammation similarly [17, 21, 22]. The model was considered an appropriate estimate of human disease. Therefore, the results showed that topical use of a variety of corticosteroids, especially those with mineralocorticoid activity, could decrease ocular inflammation in a rabbit model.

The mineralocorticoid receptor (MR) may be active in the anterior segment of the eye, in particular in the iris and ciliary body epithelium of rat and human eyes. MRs are found in all cells that make up the blood-aqueous barrier and raises the question of their role in acute inflammatory conditions, such as uveitis [23].

There are publications in the literature showing that stimulation of mineralocorticoid receptors increases inflammation and plays a role in many vascular and inflammatory processes.

Long-term inappropriate or excessive MR activation was found to promote inflammation and increase oxidative stress markers in kidney and cardiovascular pathologies in humans as well as in animal models [24].

Bousquet *et al*. [26] demonstrated that aldosterone reduced the intensity of clinical inflammation in a dose-dependent manner in an experimental model of uveitis. The clinical benefit of aldosterone may be attributed to its being an MR antagonist [26].

Topical administration of Spironolactone-Loaded Nanomicelles, an MR antagonist, inhibited glucocorticoid-induced corneal wound healing in rabbits [27].

The mineralocorticoid receptor (MR) has a role in regulating retinal fluid homeostasis and also manages water and ion channel expression in Müller glial cells. In addition, the MR significantly contributes to the pathological mechanism behind central serous chorioretinopathy (CSCR) and it is thought to play a role in choroidal vascular bed relaxation.

Zhao *et al*. investigated the role of MRs in the development of CSCR, showing that intravitreal injection of glucocorticoids or aldosterone in rats propagated CSCR [25]. This was identified by choroidal vessel dilation and increased leakage and expression via the K channel KCa2.3 in ECs. This demonstrated that EC-MRs significantly contributed to the observed effects. Furthermore, subsets of patients thought to have chronic CSCR who were given oral eplerenone exhibited a marked improvement in visual acuity, which was linked to the resolution of choroidal vasodilation effects and even retinal detachments [25, 28].

Considering the results of these studies showing that MR stimulation increases inflammation, suppression of inflammation seems contradictory in our study. However, mineralocorticoids are likely to suppress inflammation using pathways and mechanisms similar to glucocorticoids. This may be dose-dependent or related to other unexplained mechanisms.

Limitations

The major limitation of the study is the lack of different corticosteroids. Further studies in large groups need to be performed to generalize the results, as our study was conducted with specific corticosteroids.

CONCLUSION

The ocular anti-inflammatory properties of corticosteroids with mineralocorticoid activity have not yet been determined. Our current results indicate the need for more comprehensive research on this class of corticosteroids for the treatment of ocular inflammation, e.g. reducing inflammation without increasing intraocular pressure. In addition, the clinical application of topical mineralocorticoids in human ocular inflammation needs to be established and it is an important topic to be investigated in future studies. In addition, more comprehensive studies are needed to explain the reasons for the results related to the suppression of inflammation, which is inconsistent with the literature.

Authors' Contribution

Study Conception: MK; Study Design: MK; Supervision: MK; Funding: Gholam A. PEYMAN; Materials: MK; Data Collection and/or Processing: MK; Statistical Analysis and/or Data Interpretation: MK; Literature Review: MK; Manuscript Preparation: MK and Critical Review: MK.

Conflict of interest

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REFERENCES

1. Fraunfelder FW, Rosenbaum JT. Drug induced uveitis. Incidence, prevention and treatment. Drug Saf 1997;17:197-207.
2. Dunn JP, Nozik RA. Uveitis: Role of physician in treating systemic causes. Geriatrics 1994;49:27-32.
3. McCluskey P, Powell RJ. The eye in systemic inflammatory diseases. Lancet 2004;364:2125-33.
4. Martin TM, Smith JR, Rosenbaum JT. Anterior uveitis: current concepts of pathogenesis and interactions with the spondyloarthropathies. Curr Opin Rheumatol 2002;14:337-41.
5. Afshari NA, Afshari MA, Foster CS. Inflammatory conditions of the eye associated with rheumatic disease. Curr Rheumatol Rep 2001;3:453-8.
6. Gaudio PA. A review of evidence guiding the use of corticosteroids in the treatment of intraocular inflammation. Ocul Immunol Inflamm 2004;12:169-92.
7. Herman DC. Endogenous uveitis: Current concepts of treatment. Mayo Clin Proc 1990;65:671-83.
8. McGhee CN, Dean S, Danesh-Meyer H. Locally administered ocular corticosteroids: benefits and risks. Drug Saf 2002;25:33-55.
9. Weijtens O, Schoemaker RC, Romijn FP, Cohen AF, Lentjes EG, van Meurs JC. Intraocular penetration and systemic absorption after topical application of dexamethasone disodium phosphate. Ophthalmology 2002;109:1887-97.
10. Weijtens O, Feron EJ, Schoemaker RC, Cohen AF, Lentjes EG, Romijn FP, van Meurs JC. High concentration of dexamethasone in aqueous and vitreous after subconjunctival injection. Am J Ophthalmol 1999;128:192-7.
11. Kersey JP, Broadway DC. Corticosteroid-induced glaucoma: a review of the literature. Eye 2006;20:407-16.
12. Mirshahi M, Nicolas C, Mirshahi A, Hecquet C, d'Hermies F, Faure JP, Agarwal MK. The mineralocorticoid hormone receptor and action in the eye. Biochem Biophys Res Commun 1996;219:150-6.
13. Mirshahi M, Agerwal MK. Receptor-mediated adrenocorticoid hormone signaling in ocular tissue. Biochem Pharmacol 2003;65:1207-14.
14. Golestaneh N, Picaud S, Mirshahi M. The mineralocorticoid receptor in rodent retina: Ontogeny and molecular identity. Mol Vis 2002;8:221-5.
15. Suzuki T, Sasano H, Kaneko C, Ogawa S, Darnel AD, Krozowski ZS. Immunohistochemical distribution of libeta-hydroxysteroid dehydrogenase in human eye. Mol Cell Endocrinol 2001;173:121-5.
16. Mirshahi M, Mirshahi S, Golestaneh N, Nicolas C, Mishal Z, Lounes KC, et al. Mineralocorticoid hormone signaling regulates the 'epithelial sodium channel' in fibroblasts from human cornea. Ophthalmic Res 2001;33:7-19.
17. Waterbury LD, Flach AJ. Efficacy of low concentrations of

ketorlac tromethamine in animal models of ocular inflammation. J Ocular Pharm Ther 2004;20:345-52.
18. Dubois EF. Clinical potencies of glucocorticoids: what do we really measure? Curr Respir Med Rev 2005;1:103-8.
19. Joffe HV, Adler GK. Effect of aldosterone and mineralocorticoid receptor blockade on vascular inflammation. Heart Fail Rev 2005;10:31-7.
20. Kuster GM, Kotlyar E, Rude MK, Siwik DA, Liao R, Colucci WS, et al. Mineralocorticoid receptor inhibition ameliorates the transition to myocardial failure and decreases oxidative stress and inflammation in mice with chronic pressure overload. Circulation 2005;111:420-7.
21. Rooks WH, Malone PJ, Shott LD, Schuler ME, Sevelius H, Strosberg AM, et al. The analgesic and anti-inflammatory profile of ketorlac and its tromethamine salt. Drugs Exp Clin Res 1985;11:479-92.
22. Ohia OE, Mancino M, Kulkarni PS. Effects of steroids and immunosuppressive drugs on endotoxin-uveitis in rabbits. J Ocular Pharm 1992;8:295-307.
23. Stokes J, Noble J, Brett L, Phillips C, Seckl JR, Colm OB, et al. Distribution ofglucocorticoid and mineralocorticoid receptors and 11 beta-hydroxysteroiddehydrogenases in human and rat ocular tissues. Invest Ophthalmol Vis Sci 2000;41:1629-38.
24. Gilbert KC, Brown NJ. Aldosterone and inflammation. Curr Opin Endocrinol Diabetes Obes 2010;17:199-204.
25. Zhao M, Célérier I, Bousquet E, Jeanny JC, Jonet L, Savoldelli M, et al. Mineralocorticoid receptor is involved in rat and human ocular chorioretinopathy. J Clin Invest 2012;122:2672-9.
26. Bousquet E, Zhao M, Ly A, Leroux les Jardins G, Goldenberg B, Naud MC, et al. The Aldosterone-mineralocorticoid receptor pathway exerts anti-inflammatory effects in endotoxin-induced uveitis. PLoS One 2012;7:e49036.
27. Dahmana N, Gabriel D, Gurny R, Kalia YN. Development and validation of a fast and sensitive UHPLC-ESI-MS method for the simultaneous quantification of spironolactone and its metabolites in ocular tissues. Biomed Chromatogr 2018;32:e4287.
28. Barrera-Chimal J, Jaisser F. Vascular mineralocorticoid receptor activation and disease. Exp Eye Res 2019;188:107796.

The European Research Journal 2023;9(2):192-199 Original Article

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The relationship between sustainable nutrition and healthy food choice: a cross-sectional study

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ABSTRACT

Objectives: The aim of this study was to evaluate university students' attitudes toward sustainable nutrition, their level of knowledge in this area, and the associations between these variables and food preferences.

Methods: This study was conducted with university students between the ages of 19-45. The study data were collected between May 2022 and July. In the study, the Scale of sustainable nutrition knowledge was used for the sustainable nutrition knowledge level of individuals. The food choice scale was used to determine the food preference and the Sustainable and Healthy Eating Behaviors scale was used to determine the health food choice preference.

Results: The study involved 467 participants, and female made up 64.67% of the study's participants. The mean age of the group was 23.21 ± 6.13 years. Knowledge of sustainable nutrition increases by 1.365 for every 0.821 increase in educational level. The sustainable nutrition knowledge score increased by 1.529 points, the scale score for sustainable and healthy eating behaviors increased by 0.651 points, and the monthly income to the model increased by 1.611 times for every 1.109 unit ($p = 0.005$).

Conclusions: Our survey shows that most college students believe eating sustainably is important. As people's education and knowledge about sustainable nutrition rise, so do their healthy eating habits. Expand sustainable nutrition education, add it to the curriculum, and create nutrition guides to promote this novel concept in our country.

Keywords: Sustainable nutrition, university student, food preference, food-choice

More than 10 billion people are projected to live on the planet in 2050 [1]. In a world with limited resources, feeding a growing population is a significant challenge. The world's current food systems are unable to feed everyone, putting both the ability of the present generation and that of future generations to fend for themselves in danger [2, 3].This results in food insecurity, detrimental health effects, and environmental harm. The current food system is to blame for 20 to 30% of anthropogenic greenhouse gas emissions that contribute to climate change, in addition to biodiversity loss, deforestation, and altered land use [2]. Agriculture uses more than one-third of the world's arable land and 70% of its fresh water [4]. The entire food system uses a significant amount of natural resources. The environment is impacted by every step that food takes in the food system before it reaches your plate. Agriculture that produces both crops and

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livestock is more likely to use harmful practices. Livestock is a significant cause of climate change, contributing 14.5 percent of global greenhouse gas emissions, mostly from the production of meat, milk, and eggs [5]. The agricultural sector, which produces food for the population, is under pressure due to the need to find resources to feed a growing global population, and the problems brought on by climate change make this pressure even greater [2, 6]. The current food system needs to be changed for the benefit of the health of the planet and people. By 2050, there will likely be an increase in environmental pressure from the food system of 50-92% due to predictions that the world's population will reach 10 billion people and that the global income will triple [7]. If we want to avoid this environmental pressure, the global food system needs to be healthier and more environmentally friendly [3]. People are becoming more interested in sustainable nutrition because issues brought on by population growth and climate change may endanger our natural resources [8]. Our current diet contributes to an increase in disease burden. Obesity is on the rise on a global scale [9]. As a result, one in three people are obese or overweight [10]. Even though obesity is a serious health concern on the one hand, the fact that one in nine people worldwide experience hunger is a significant indicator of the injustice of the current food system and illustrates the importance of sustainable nutrition Because environmentally friendly nutrition is just, inexpensive, sufficiently nutritive, and has few unfavorable effects. A diet that is good for your health and the environment consists of eating more plants and less animal products [10, 119.

The aim of this study was to evaluate university students' attitudes toward sustainable nutrition, their level of knowledge in this area, and the associations between these variables and food preferences.

METHODS

Participant and Approval of the Ethics Committee

This study received approval from the Malatya Turgut Ozal University non-interventional studies Ethics Committee at its meeting on November 29, 2021, with meeting number 2021/16. The study's voluntary consent is included in the first section of the questionnaire that needs to be filled out. Volunteers who agreed to participate in the study were added to the list after they signed the consent form. Ethics dictate that no private information about people will be disclosed, that data will only be used for research, and that personal privacy will be respected when the results of the study are shared and published. The Declaration of Helsinki was followed when conducting the study.

Data Collection

General participant information (gender, age range, enrollment status (full- or part-time), program of enrollment, and level of the university courses) was divided into four sections of the questionnaire used to collect the data. Other sections covered a food selection test, behaviors related to sustainable nutrition, and a knowledge level data form for sustainable nutrition. The self-reported familiarity, perceived importance, and perspectives of university students on sustainability were also examined in this study for potential applications using a descriptive design. The following were rated on a Likert scale of 1 to 5: (1) Have you ever heard of the following terms? Environmental sustainability, social sustainability, and economic sustainability; sustainability (not at all familiar, very familiar); (2) How familiar are you with sustainability concepts? [12] cross-cutting issues, such as the use of food and agricultural policies, social development (for instance, social justice in the food system), environmental integrity (for instance, the use of synthetic chemicals and pesticides), economic resilience (for instance, resilience to economic risk); (3) How big of a problem do you generally think sustainability is? (4) The extent to which sustainability influences their daily decisions (not always); and, as a fifth person, the significance of sustainability for future practice (from very insignificant to very important). How would you characterize your attitude toward sustainability? was the final query, and there were five possible responses. (A) I have a strong advocacy. B) I think it's a good idea C) I don't care too much D) It's okay if other people want to do it.

Scale of Sustainable Nutrition Knowledge

A questionnaire consisting of 22 questions was created using a 5-point Likert scale to assess students' knowledge of sustainable nutrition (Strongly Disagree, Disagree, Undecided, Agree, Totally Agree). Students were asked to offer the most appropriate response to

each of these statements. Points were assigned in the following order: 1, 2, 3, and 4, from the most inappropriate criterion for sustainable nutrition knowledge (I strongly disagree) to the most appropriate criterion (I completely agree). For questions 18, 19, and 20, the results are flipped. The highest score possible for this section is 105. Since there is no scale for evaluating sustainable nutrition behavior and knowledge level, the researcher combed through the literature to create the questionnaire that will be used to evaluate this information.

Food-Choice Evaluation Scale

Health, mood, convenience, sensory appeal, natural content, price, weight control, familiarity, and ethical concern were the four dimensions that Steptoe *et al*. created for this study. The test involved choosing foods in a Likert-style manner. The construct validity of the Turkish validation study was determined using confirmatory factor analysis, and factor loadings were found to be similar to those of the original questionnaire. Test-retest reliability was examined using the infraclass correlation coefficient (ICC), which had values between 0.89 and 0.95. The results show that the Turkish FCQ is a valid and reliable research tool [12].

Sustainable and Healthy Eating Behaviors Scale

In order to evaluate sustainable and healthy eating behaviors for the idea of sustainable diet, Gazi University Nutrition and Dietetics students used the Turkish version of the "Sustainable and Healthy Eating Behaviors" scale. This scale was created in accordance with the FAO definition, the LiveWell approach, and the principles of sustainable and healthy eating habits [13]. This scale has eight factors and a total of 34 items. The eight factors mentioned above are: a healthy and balanced diet, quality marks (local and organic), less meat consumption, local foods, low fat, preventing food waste, animal health, and seasonal foods. A Likert-type scale was used to ask the participants to rate each of the scale's 34 items as either never, very rarely, rarely, sometimes, often, or very often, or always. Never = 1, Always = 7, and vice versa.

Statistical Analysis

All survey data were imported into IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Armonk, NY, USA) to prepare the data for statistical analysis. The analyzes used all available survey data. All analyzes had a significance level of 0.05. The summaries of all sociodemographic variables were proportions and frequencies and were either nominal or ordinal. Progress was made in a retrospective procedure in which variables were removed in order of importance until the model included only variables with $p < 0.05$ (for any level). Interaction terms between covariates were not considered in the model.

RESULTS

Table 1 displays the general characteristics of the people. 64.67% of the participants in the study were female. The group's mean age was 23.21 ± 6.13 years and 46.68% of the study participants had undergradu-

Table 1. General characteristics participants of study (n = 467)

	n	%
Gender		
Female	302	64.67
Male	165	35.33
Age(years) (mean ± SD)	23.21 ± 6.13	
Education level		
Associate degree	102	21.84
Licence	218	46.68
Masters	99	21.20
Doctorate	48	10.28
Department of study		
Health	145	31.05
Education	111	23.77
Liberal arts	108	23.13
Engineering	103	22.06
Monthly income		
1000 TL and below	215	46.04
1001-2000 TL	125	26.77
2001-3000 TL	78	16.70
3000 TL and above	49	10.49

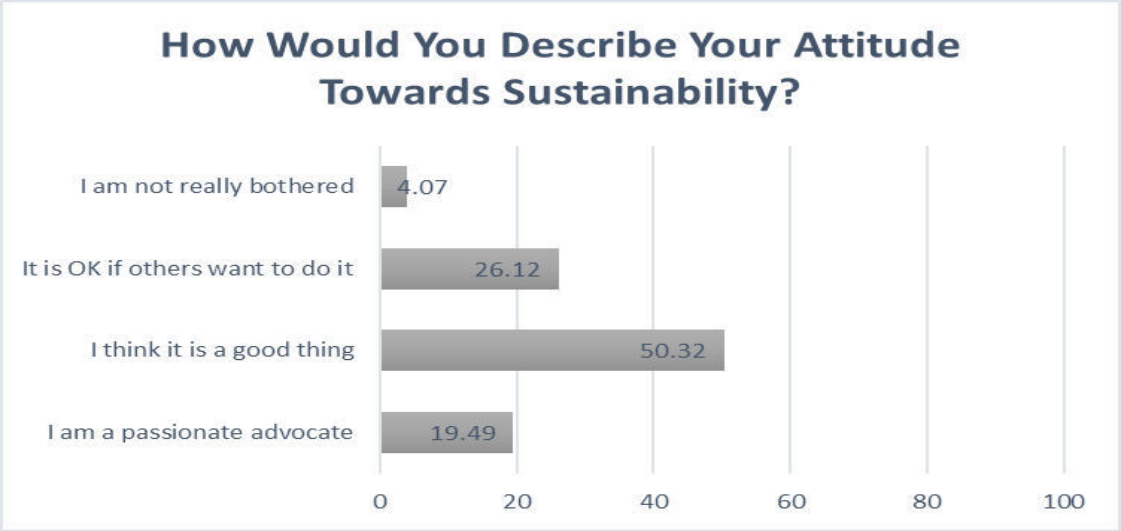

Fig. 1. Demonstrates 'how students feel about sustainability.

ate degrees, according to an analysis of the participants' educational backgrounds. However, it was discovered that 31.05% of the population had health-related education. It has been found that 46.04% of the study participants earn no more than 1000 TL per month.

One question was posed to respondents, and Fig. 1 displays their responses regarding sustainability. The percentages of responses provided by the respondents were looked at in relation to the four options for the question. The response "I think it is a good thing" received the highest response (50.23%).

The factors influencing people's knowledge of sustainable nutrition were looked at (Table 2). Individuals' food preferences, sustainable behavioral patterns, and knowledge of sustainable nutrition all showed a positive and significant correlation ($p < 0.05$).

It was found that using logistic regression models can assist in determining a person's level of familiarity with sustainable nutrition (Table 3). The following model was created: years of education, score on a scale measuring sustainable and healthy eating practices, and monthly income (TL). The analyses made use of the high-level LR model. Every 0.821 increase in education level results in a 1.365 increase in knowledge of sustainable nutrition. The sustainable and healthy eating behaviors scale score increased by 0.651, the sustainable nutrition knowledge score increased by 1.529, and the monthly income to the model increased by 1.611 times for every 1.109 unit ($p = 0.005$). The sustainable nutrition knowledge score is influenced by factors such as monthly income, educational attainment, and the Sustainable and Healthy Eating Behaviors scale score.

The relationship between the scale measuring sus-

Table 2. Analysis of the relationships between the food-choice evaluation scale, the sustainable and healthy eating behaviors scale, and an individual's level of knowledge about sustainable nutrition

	Food-choice evaluation scale	The sustainable and healthy eating behaviors scale
The sustainable nutrition knowledge level	r	r
	0.345*	0.571**

r = spearman correlation coefficients, $**p < 0.001$ $*p < 0.05$

Table 3. Regression analysis of factors that determine a person's knowledge of sustainable nutrition

		B	*p* value	OR	95% CI	
Model					**Lower**	**Upper**
Sustainable nutrition knowledge score	Education level (years)	0.821	0.064	1.365	1.000	1.790
	Sustainable and Healthy Eating Behaviors scale score	0.651	**0.002**	1.529	1.211	3.230
	Monthly income(TL)	1.109	**0.032**	1.611	1.003	3.102

CI = confidence interval, OR = Odds Ratio, TL = Turkish Lira.

tainable and healthy eating behaviors and the degree of sustainable nutrition knowledge was mediated by the food selection scale score. The major premise of the study is supported by this finding. Fig. 2 shows the statistical significance of the standardized regression coefficients ($p < 0.01$) between the food choice scale score and the level of sustainable nutrition knowledge as well as between the food choice scale score and the scale of sustainable and healthy eating behaviors. The sustainable nutrition knowledge level is influenced by the significant food selection scale score, and both are significantly influenced by the sustainable and healthy eating behaviors scale. The coefficient values between the food selection scale score and the sustainable nutrition knowledge level (B = .45, $p < 0.01$) and between the food selection scale score and the sustainable and healthy eating behaviors scale (B = .54, $p < 0.01$) show the positive significance of the relationships. Beta values and standard error results were obtained after standardized regression analyses were evaluated using the Sobel Test (Sobel, 1982). The Sobel Test results showed that the food selection

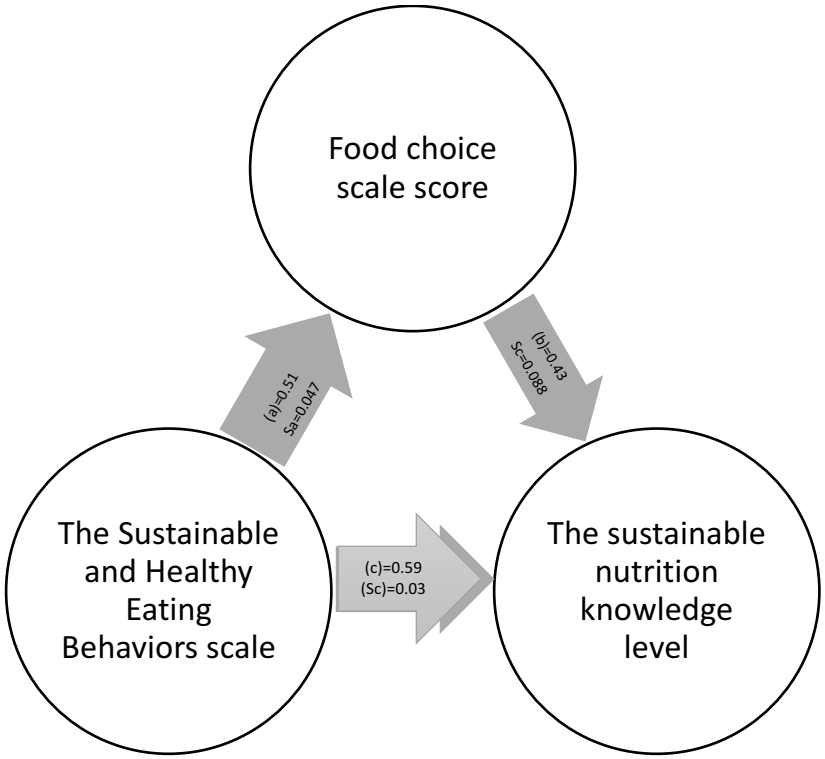

Fig. 2. Beta coefficients for analyzing the food selection scale score's mediating role in figuring out how much a person knows about nutrition and how to make sustainable and healthy eating choices. .

scale score ($p < 0.01$) was statistically significant. Here, a mediating factor is the score on the food choice scale.

DISCUSSION

Sustainable nutrition aims to maintain low negative environmental impacts while ensuring that all people have access to enough, safely, and basic nutrients for their health and the health of present and future generations. University education is a crucial phase in preparing students for adult life. It is crucial at this time for young people to become more environmentally conscious and to acquire life skills that will enhance their level of health.

Education is a potent force for personal and societal change. Education, which raises people's awareness, is crucial for promoting environmental awareness and developing sustainable eating practices. Studies on education and environmentally friendly consumption patterns also demonstrate the influence of education on environmental attitudes. According to Haanpää [14] there is a link between education level and environmental anxiety. According to the research by Barone *et al*. [15] people with higher education levels are more likely to practice sustainable nutrition and resource conservation. Rejman et. al [16] in contrast to other studies, found that education level had no bearing on sustainable eating habits and food preferences. In this study, it was discovered that a rise in education level of 0.821 units resulted in a rise in sustainable nutrition knowledge scale of 1.365 units. It has been found that knowledge of sustainable nutrition is influenced by education level.

Sustainable consumption behavior rises along with sustainability knowledge [17]. Sustainable behaviors are affected by knowledge levels. People will pay more attention to sustainable nutrition if their level of knowledge can be raised. In a study by Torabian-Riasati *et al*. [18] on undergraduate students, it was discovered that attitudes toward sustainable food practices and knowledge of food sustainability are positively and significantly related. According to a study, switching people to a sustainable diet will reduce their risk of developing obesity, one of the non-communicable diseases [19]. People who switch to a sustainable diet will consume fewer processed and packaged foods that contribute to obesity, consume less animal-based food, which will reduce their intake of saturated fat, and prefer more plant-based foods, which will result in a decrease in the amount of energy they obtain from food will lower the likelihood of obesity.

The food choice assessment scale, the sustainable healthy eating behaviors scale, and the level of sustainable nutrition knowledge were all found to be positively and significantly correlated in this study. It was found that a rise in the sustainable nutrition knowledge score of 1.529 units resulted in a rise in the sustainable healthy eating behavior scale score of 0.621 units. With the help of this study, it can be said that understanding sustainable nutrition has an impact on sustainable behaviors.

According to Burkhart *et al*. [20] 97.00% of nutrition and dietetics students had heard of the term "sustainability" before, and the majority of them believed that it was a significant concern in terms of dietary practices. According to Heidelberger *et al*. [21] 47.00% of dietitians address environmental concerns in their work. Only 38.00% of dietitians were found to consider diet to be a factor in climate change when Hawkins *et al*. [22] evaluated the attitudes and behaviors of dietitians regarding climate change. According to Truckner [23] 80% of medical professionals think that environmental degradation brought on by humans has a negative impact on human health, but 93% do not discuss this topic with their patients. According to a study by Sarfaty *et al*. [24] on physicians, 88% of participants believed that climate change had a direct impact on patient care, but 71% had no idea how to talk to patients about it. The majority of people in this study believe that sustainability is a good idea, but 122 people do not think that it is a problem that others want to implement it.

It is well known that students' eating habits and their available income have a connection. Personal disposable income and nutrition and food security are inversely correlated [25]. In this study, it was found that an increase in monthly income of 1.611 units was followed by an increase in sustainable nutrition knowledge scale of 1.109 units. The FAO defined sustainable diets in 2010; it is defined as diets that are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economical, and affordable, nutritionally adequate, and reliable. Sustainable diets use nature and human resources in

the best possible ways. From this perspective, it's crucial that sustainable nutrition is affordable [26].

CONCLUSION

Our findings indicate that the majority of the university students we surveyed think it's critical to eat sustainably. It has been found that as people's levels of education and knowledge about sustainable nutrition rise, so do their sustainable healthy eating behaviors and practices. Sustainable nutrition education should be expanded, added to the curriculum, and nutrition guides should be created in this regard in order to promote sustainable nutrition, which is a novel concept in our nation.

Authors' Contribution

Conception: HA, NA; Study Design: HA, NA; Supervision: HA, NA; Funding: HA, NA; Materials: NA; Data Collection and/or Processing: NA; Statistical Analysis and/or Data Interpretation: HA, NA; Literature Review: NA; Manuscript Preparation: NA and Critical Review: NA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. United Nations, Department of Economic and Social Affairs, Population Division (2017). World Population Prospects: The 2017 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP/248. 2017.
2. Fischer CG, Garnett T. Plates, pyramids, and planets: developments in national healthy and sustainable dietary guidelines: a state of play assessment: Food and Agriculture Organization of the United Nations; 2016.
3. Wiseman SA, Dötsch-Klerk M, Neufingerl N, de Oliveira Martins F. Future food: Sustainable diets for healthy people and a healthy planet. Int J Nutrology 2019;12:23-8.
4. Whitmee S, Haines A, Beyrer C, Boltz F, Capon AG, de Souza Dias BF, et al. Safeguarding human health in the Anthropocene epoch: report of The Rockefeller Foundation -Lancet Commission on planetary health. Lancet 2015;386:1973-2028.
5. Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, et al. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities: Food and Agriculture Organization of the United Nations (FAO); 2013.
6. Macdiarmid JI, Kyle J, Horgan GW, Loe J, Fyfe C, Johnstone A, et al. Sustainable diets for the future: can we contribute to reducing greenhouse gas emissions by eating a healthy diet? Am J Clin Nutr 2012;96:632-9.
7. Springmann M, Clark M, Mason-D'Croz D, Wiebe K, Bodirsky BL, Lassaletta L, et al. Options for keeping the food system within environmental limits. Nature 2018;562:519-25.
8. Auestad N, Fulgoni III VL. What current literature tells us about sustainable diets: emerging research linking dietary patterns, environmental sustainability, and economics. Adv Nutr 2015;6:19-36.
9. Scheelbeek P, Green R, Papier K, Knuppel A, Alae-Carew C, Balkwill A, et al. Health impacts and environmental footprints of diets that meet the Eatwell Guide recommendations: analyses of multiple UK studies. BMJ Open 2020;10:e037554.
10. Micha R, Mannar V, Afshin A, Allemandi L, Baker P, Battersby J, et al. 2020 global nutrition report: action on equity to end malnutrition. 2020.
11. Alexandratos N, Bruinsma J. World agriculture towards 2030/2050: the 2012 revision. 2012.
12. Dikmen D, İnan-Eroğlu E, Göktaş Z, Barut-Uyar B, Karabulut E. Validation of a Turkish version of the food choice questionnaire. Food Qual Prefer 2016;52:81-6.
13. Żakowska-Biemans S. Polish consumer food choices and beliefs about organic food. Br Food J 2011;113:122-37.
14. Haanpää L. Consumers' green commitment: indication of a postmodern lifestyle? Int J Consumer Studies 2007;31:478-86.
15. Barone B, Nogueira RM, Behrens JH. Sustainable diet from the urban Brazilian consumer perspective. Food Res Int 2019;124:206-12.
16. Rejman K, Kaczorowska J, Halicka E, Laskowski W. Do Europeans consider sustainability when making food choices? A survey of Polish city-dwellers. Public Health Nutr 2019;22:1330-9.
17. Seifi S, Zulkifli N, Yusuff R, Sullaiman S. Information requirements for sustainable consumption. Socl Responsibil J 2012;8:433-41.
18. Torabian-Riasati S, Lippman S, Nisnevich Y, Plunkett S. Food sustainability knowledge and its relationship with dietary habits of college student. Austin J Nutr Food Sci 2017;5:1089.
19. Hawkes C, Popkin BM. Can the sustainable development goals reduce the burden of nutrition-related non-communicable diseases without truly addressing major food system reforms? BMC Med 2015;13:143.
20. Burkhart S, Verdonck M, Ashford T, Maher J. Sustainability: Nutrition and dietetic students' perceptions. Sustainability

2020;12:1072.
21. Heidelberger L, Smith C, Robinson-O'Brien R, Earthman C, Robien K. Registered dietitian nutritionists' perspectives on integrating food and water system issues into professional practice. J Acad Nutr Diet 2017;117:271-7.
22. Hawkins IW, Balsam AL, Goldman R. A survey of registered dietitians' concern and actions regarding climate change in the United States. Front Nutr 2015;2:21.
23. Truckner RT. Health care provider beliefs concerning the adverse health effects of environmental and ecosystem degradation. Wilderness Environ Med 2009;20:199-211.
24. Sarfaty M, Mitchell M, Bloodhart B, Maibach EW. A survey of African American physicians on the health effects of climate change. Int J Environ Res Public Health 2014;11:12473-85.
25. Anderson PM, Butcher KF. The relationships among SNAP benefits, grocery spending, diet quality, and the adequacy of low-income families' resources. Center for Budget and Policy Priorities Policy Futures. 2016.
26. Alsaffar AA. Sustainable diets: the interaction between food industry, nutrition, health and the environment. Food Sci Technol Int 2016;22:102-11.

The European Research Journal 2023;9(2):200-206 Original Article

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The impact of radioactive iodine treatment on subclinical left ventricular dysfunction in patients treated for differentiated thyroid cancer: assessing the predictive value of presystolic wave

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ABSTRACT

Objectives: Despite strong evidence regarding the impact of radioactive iodine (RAI) on the hematopoietic, genitourinary, and gastrointestinal systems, its role in the cardiovascular system needs to be clarified. We hypothesized that the presence of a presystolic wave could be predictive for cardiovascular dysfunction in subjects receiving RAI. Thus, we investigated presystolic wave presence in differentiated thyroid cancer (DTC) patients with and without RAI therapy after undergoing total thyroidectomy.

Methods: Patients were included in the study within 1-5 years after thyroidectomy or thyroidectomy and RAI treatment. Participants were divided into three groups as follows: the first group included patients with DTC who received RAI following total thyroidectomy (Group 1; n = 33), the second group included patients with DTC who did not receive RAI following total thyroidectomy (Group 2; n = 34). The third group of age-matched subjects who underwent total thyroidectomy with suspicion of DTC and resulted in benign pathologies was also selected (Group 3; n = 37). All subjects underwent transthoracic echocardiography. The presence of a presystolic wave was assessed with Doppler imaging of the left ventricular outflow tract.

Results: Presystolic wave was more common in subjects receiving RAI (Group 1) compared to Group 2 (those without RAI) (90.9% vs. 61.8% respectively, $p = 0.003$), and its prevalence in Group 3 was 54.1%. Multiple logistic regression analysis revealed that receiving RAI (OR: 4.922, 95% CI: 1.640 – 20.022, $p = 0.004$) was independently associated with a 5-fold higher risk for the presence of a presystolic wave.

Conclusions: RAI following total thyroidectomy in patients with DTC is associated with a five-fold increase in the presence of the presystolic wave, as a proxy marker for subclinical left ventricular dysfunction.

Keywords: Differentiated thyroid cancer, radioactive iodine, echocardiography, presystolic wave

Differentiated thyroid cancer (DTC), including papillary and follicular cancer, is one of the most common endocrine malignancies that accounts for about 90% of all types of thyroid cancer. Together with the advances in screening, early diagnosis, and treatment, the prognosis of DTC has considerably im-

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proved in the last few decades. The 10-year survival rate is 85% in DTC; however, 5% of the subjects develop metastatic disease despite aggressive treatment [1].

Treatment of DTC includes surgery, radioactive iodine (RAI), and TSH suppression in the majority of the cases [2]. Partial or total thyroidectomy followed by RAI to reduce residual disease and improve prognosis is the standard of care in most patients with DTC [3]. RAI is tolerated well in general; however, several complications may occur in the hematopoietic system, salivary glands, nasolacrimal apparatus, lungs, gonads, and gastrointestinal system [4]. Although these side effects are temporary and rarely life-threatening, they may still adversely influence health-related quality of life.

Despite the accumulation of data concerning the impact of RAI on the hematopoietic, genitourinary and gastrointestinal systems, its effects on the cardiovascular system have not yet been clearly identified. Ionizing radiation has been shown to cause arterial hypertension in addition to its inhibition of nitric oxide (NO)-mediated vasodilatation, which may further promote the development of vascular inflammation and atherosclerotic vascular disease [5]. However, evidence concerning the impact of RAI on LV function is lacking.

A presystolic wave, which is a late-diastolic event detected on Doppler examination of the left ventricular outflow tract (LVOT), has been shown to be associated with subclinical left ventricular dysfunction [6, 7]. We hypothesized that the presence of a presystolic wave could be predictive for the development or progression of cardiovascular dysfunction in subjects receiving RAI.

This study aimed to investigate the presence of the presystolic wave, which is predictive for subclinical left ventricular dysfunction, in patients with DTC who were treated with RAI after thyroidectomy.

METHODS

Study Design and Patient Selection

Patients aged 18-65 years who had undergone thyroidectomy or thyroidectomy and RAI treatment within 1-5 years were included in this cross-sectional study. Those with known atherosclerotic cardiovascular disorders, heart failure, resistant hypertension, diabetes mellitus, moderate-severe valvular dysfunction, non-sinus rhythm, 10-year cardiovascular risk of >1%, and chronic kidney or liver disease were excluded. Written informed consent was obtained from all subjects. The study was approved by the local ethics committee and was conducted in accordance with the Helsinki Declaration.

Subjects who had undergone thyroidectomy were categorized according to RAI administration. The first group included patients who received RAI following total thyroidectomy (Group 1, n = 33), the second group included patients who did not receive RAI following total thyroidectomy (Group 2, n = 34). Additionally, a group of age-matched subjects who underwent total thyroidectomy with suspicion of DTC and resulted in benign pathologies were selected as the third group (Group 3, n = 37). Cumulative dose of RAI was retrieved from patients' charts and was 100-150 mCi.

Data Collection

All subjects underwent transthoracic echocardiography in a standardized manner by the same cardiologist experienced in cardiovascular imaging in left lateral decubitis using Vivid 5 system (GE Vingmed Ultrasound AS, Horten, Norway) with a 2.5 MHz probe. 2-D measurements were carried out according to the American Society of Echocardiography guidelines [8]. Left ventricular dimensions were measured from the parasternal long-axis view: LV ejection fraction was measured by using the modified Simpson method. Doppler measurements, mitral E and A waves were determined by placing sample volume at mitral valve closure line. Pulse Doppler flow interrogation of LVOT flow was performed just proximal to aortic valve in the apical five-chamber view. The presence or absence of a presystolic wave preceding the LVOT flow was assessed in all patients.

Demographic characteristics, physical examination data and laboratory measurements were retrieved from patient charts. The primary outcome measure of this study was the difference in the presence of presystolic wave among subjects receiving and not receiving RAI. Identifying factors associated with the presence of the presystolic wave was the secondary outcome measure of the study.

Statistical Analysis

All analyses were performed according to a significance threshold of $p < 0.05$ and with use of the SPSS software, version 25 (IBM, Armonk, NY). Data are summarized as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution (assessed with histogram and Q-Q plots). Frequency and percentage (n, %) were used to summarize categorical variables. Continuous variables were analyzed with the Student's t-test or the Mann-Whitney U test depending on normality of distribution. Categorical variable distributions were compared with Pearson or Yate's chi-square tests or the Fisher's exact test. Multiple logistic regression analysis (forward conditional method) was utilized to identify factors independently associated with the presence of presystolic wave. All factors that demonstrated univariate significance were included in the logistic regression model.

RESULTS

A total of 67 subjects with DTC and 37 subjects with non-malingnant pathology were enrolled in the study. The mean time after surgery or radiotherapy was for group 1: 36.2 ± 1.8 months, for group 2: 31.9 ± 1.7 months and for group 3: 32.5 ± 1.7 months. The groups were similar with respect to age, sex, body mass index, systolic and diastolic blood pressure. Comparison of the group 1 vs group 2, and values of group 3 in terms of other characteristics is summarized

Table 1. Demographic features and laboratory measurements of the groups

Variables	Group 1 (n = 33)	Group 2 (n = 34)	Group 3 (n = 37)	*p* value
Age, years	44.82 ± 11.42	44.88 ± 10.46	45.38 ± 9.27	0.087
Female sex, n (%)	28 (84.8)	28 (82.4)	32 (86.5)	0.889
Smoking, n (%)	6 (18.2)	6 (17.6)	2 (5.4)	0.202
Body Mass Index, kg/m^2	29.27 ± 3.87	28.61 ± 3.91	30.13 ± 4.33	0.494
Waist circumference, cm	97.42 ± 10.71	95.21 ± 11.52	95.73 ± 9.72	0.418
Heart Rate, beats/min	82.64 ±12.49	74.97 ± 8.01	81.30 ± 13.35	**0.004**
Systolic Pressure, mmHg	132.15 ± 16.08	129.71 ± 21.89	128.97 ± 19.71	0.605
Diastolic Pressure, mmHg	79.55 ± 14.11	85.44 ± 13.95	81.92 ± 12.26	**0.032**
Fasting glucose, mg/dl	99 ± 7.62	98.32 ± 7.17	94.81 ± 8.09	**0.030**
Blood Urea Nitrogen	31.12 ± 8.99	27.58 ± 4.36	29.27 ± 6.58	0.084
Creatinine, mg /dl	0.75 (0.65 – 0.91)	0.73 (0.65 – 0.78)	0.80 (0.70 – 0.95)	0.087
Alanine transaminase	19.03 ± 5.70	19.52 ± 6.78	21.37 ± 8.23	0.746
Aspartate transaminase	19.96 ± 5.26	19.61 ± 5.30	20.40 ± 6.04	0.786
Total Cholesterol, mg/dl	192.54 ± 27.89	195.79 ± 39.30	186 ± 26.10	0.698
Leukocyte Count, ×10^3	7.17 ± 1.12	7.21 ± 1.16	6.94 ± 1.30	0.902
Hemoglobin,	13.09 ± 1.19	13.26 ± 2.01	13.43 ± 0.95	0.670
Hematocrit	39.20 ± 4.10	40.62 ± 5.08	39.27 ± 5.54	0.215
Platelet count, ×10^3	281.60 ± 68.03	273.41 ± 76.34	297.82 ± 51.25	0.645
TSH, mU/ml	1.03 (0.59 – 2.47)	0.86 (0.33 – 1.96)	1.40 (0.99 – 3.30)	0.061
fT4	1.27 ± 0.36	1.32 ± 0.24	1.54 ± 0.84	0.502

Data are given as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. *p* values in **bold** indicate statistical significance

Comparison was made between the group 1 and group 2.

in Table 1. Echocardiographic measurements of the study groups are presented in Table 2. E wave deceleration time (EDEC) of the subjects receiving RAI was significantly longer than that of those without RAI (189.2 ± 34.8 ms, vs 178.8 ± 33.2 ms, respectively, p = 0.013). Mitral E/A ratio was higher in subjects receiving RAI than that of those who had not received RAI (p = 0.009). Epicardial fat thickness was higher in subjects receiving RAI than that of those who had not received RAI (8.43 ± 1.75 mm vs. 7.87 ± 1.99, respectively, p = 0.039). Finally, presystolic wave was more common in subjects receiving RAI compared to those not receiving RAI (90.9% vs. 61.8%, respectively, p = 0.003) and its prevalence in Group 3 was 54.1%.

Variables with a p value < 0.05 were included in the multivariate analysis model. Multiple logistic regression analysis revealed that receiving RAI (OR: 4.922, 95% CI: 1.640 – 20.022, p = 0.004) was associated with a 5-fold higher likelihood of having a presystolic wave. Epicardial fat thickness (OR: 1.403, 95% CI: 1.110 – 2.265, p = 0.005), mitral E/A ratio (OR: 1.286, 95% CI: 1.102 – 1.719, p = 0.003), and heart rate (OR: 1.082, 95% CI: 1.006 – 1.128, p = 0.032) were also independently associated with the presence of presystolic wave (Table 3).

DISCUSSION

This study investigated whether RAI therapy in patients with DTC was associated with the presence of presystolic wave (as a proxy for cardiac function). Our findings indicate that subjects receiving RAI following total thyroidectomy more frequently exhibit presystolic wave compared to subjects with non-malignant pathology and to those who did not receive RAI following total thyroidectomy. Mitral E wave deceleration time is also prolonged in subjects who received RAI compared to those who did not receive RAI. Moreover, RAI treatment following total thyroidectomy was associated with a five-fold increase in the risk for the presence of presystolic wave.

Exposure to radiation has been established to increase reactive oxygen species, chemokines and cytokines in tissues, subsequently leading to the stimulation of inflammation [9]. Reactive oxygen species are associated with cardiac fibrosis, apoptosis and myocardial hypertrophy [10, 11]. Stimulated cytokines promote molecular and phenotypic changes in myocardial cells which may lead to contractile dysfunction [12]. However, although RAI may be expected to trigger such effects, it has been shown that there is a need for relatively high-dose radiation ex-

Table 2. Comparison of transthoracic echocardiography measurements among the groups

Variables	Group 1 (RAI (+) (n = 33)	Group 2 RAI (-) (n = 34)	Group 3 (n= 37)	*p* value
LVEF, %	60.2 ± 10.7	59.4 ± 9.9	59.6 ± 10.2	0.347
LVED diameter, mm	47.1 ± 8.6	47.7 ± 8.4	48.1 ± 9.2	0.402
LVES diameter, mm	33.6 ± 5.6	30.2 ± 5.1	29.5 ± 4.9	0.097
LA diameter, mm	42.9 ± 7.2	43.6 ± 7.6	44.2 ± 7.9	0.198
E peak rate, cm/sec	78.9 ± 16.8	77.1 ± 16.2	76.8 ± 16.4	0.326
A peak rate, cm/sec	60.7 ± 9.9	62.3 ± 10.8	62.4 ± 10.6	0.114
Mitral E deceleration time, msec	189.2 ± 34.8	178.8 ± 33.2	176.2 ± 31.8	**0.013**
Mitral E/A ratio	1.43 (1.02 – 2.09)	1.22 (0.72- 1.61)	1.24 (0.75 – 1.59)	**0.009**
Presystolic wave, n (%)	30 (90.9%)	21 (61.8%)	20 (54.1%)	**0.003**
Epicardial fat thickness, mm	8.43 ± 1.75	7.87 ± 1.99	7.82 ± 2.41	**0.039**
Aortic Velocity	103.09 ± 17.10	108.12 ± 17.84	120.68 ± 19.36	0.106

Data are given as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. p values in **bold** indicate statistical significance. Comparison was made between the group 1 and group 2.

Table 3. Univariate and multivariable analyses to detect parameters associated with the presence of presystolic wave

	Univariate		Multivariable	
	OR (%95 CI)	*p* value	OR (95 %CI)	*p* value
RAI treatment	6.190 (1.567 – 24.451)	**0.009**	4.922 (1.640 – 20.022)	**0.004**
Male sex	0.738 (0.243 – 2.236)	0.591		
Age	1.021 (0.982 – 1.061)	0.302		
LVEF	0.922 (0.827 – 1.046)	0.288		
LVED diameter	1.006 (0.989 – 1.012)	0.902		
LVES diameter	0.756 (0.402 – 1.308)	0.195		
LA diameter	1.011 (0.662 – 2.034)	0.624		
E peak rate	1.289 (1.085 – 2.106)	**0.021**	1.032 (0.992 – 1.907)	0.148
A peak rate	1.108 (0.902 – 2.644)	0.402		
Mitral E deceleration time	1.062 (1.008 – 1.640)	**0.003**	1.003 (0.982 – 1.548)	0.098
Mitral E/A ratio	1.361 (1.189 – 1.905)	**<0.001**	1.286 (1.102 – 1.719)	**0.003**
Epicardial fat thickness	1.398 (1.120 – 1.745)	**0.003**	1.403 (1.110 – 2.265)	**0.005**
Aortic Velocity	1.001 (0.979 – 1.022)	0.959		
Body mass index	1.013 (0.914 – 1.122)	0.804		
Fasting glucose	1.053 (0.994 – 1.115)	0.080		
Alanine transaminase	1.062 (0.991 – 1.137)	0.087		
Aspartate transaminase	1.100 (1.008 – 1.201)	**0.032**	0.982 (0.786 – 1.724)	0.663
LDL cholesterol	1.003 (0.994 – 1.013)	0.491		
HDL cholesterol	1.013 (0.975 – 1.053)	0.502		
Leukocyte count	1.128 (0.791 – 1.608)	0.507		
Hemoglobin	1.231 (0.915 – 1.656)	0.170		
Platelet count	0.999 (0.993 – 1.005)	0.711		
TSH	1.206 (0.935 – 1.555)	0.149		
fT4	0.536 (0.249 – 1.153)	0.110		
Heart Rate	1.050 (1.009 – 1.092)	**0.017**	1.082 (1.006 – 1.128)	**0.032**
Systolic pressure	1.004 (0.982 – 1.026)	0.731		
Diastolic pressure	0.998 (0.968 – 1.029)	0.881		

p values in **bold** indicate statistical significance. Variables with a *p* value <0.05 were included in the multivariate analysis model.

posure (30 Gy) in order to result in myocardial fibrosis. Nonetheless, radiation exposure may also complicate contractile function by accelerating the development of atherosclerosis through sustained inflammatory response, structural damage to the epicardial arteries or ultrastructural damage to capillary networks [13].

A presystolic wave, an easy-to-identify echocardiographic parameter, can be described as a late diastolic wave which can be observed through Doppler assessment of the LVOT. Kul *et al*. [7] have reported in their study enrolling 129 type 2 diabetic patients that presence of echocardiographic presystolic wave was related to subclinical LV dysfunction. Similarly, the study by Saylik *et al*. [14] revealed that echocardiographic presystolic waves were associated with sub-

clinical LV dysfunction, as measured by myocardial performance index, LV global longitudinal strain, and conventional Doppler. The role of the presence of presystolic wave was also assessed in hypertensive patients and it was determined that presystolic waves were an independent predictor associated with subclinical LV dysfunction in this patient subset [6]. Several studies have also reported an association between the presence of presystolic wave and arterial stiffness, systemic hypertension, and non-dipping [15-17]. There are also studies investigating potential relationships between coronary artery disease and the lack of presystolic waves. The absence of presystolic wave was found to be predictive for extensive coronary artery disease and higher Syntax score [18, 19]. Another study conducted by Saylik *et al*. [20] revealed that presystolic wave was a common occurrence among subjects with subclinical hypothyroidism and that presystolic waves were independently associated with subclinical LV dysfunction in subjects with subclinical hypothyroidism.

Current knowledge concerning the impact of radioiodine treatment on cardiovascular function is limited. A recent study conducted by Stanciu *et al*. [21] have investigated cardiovascular effects of radioiodine treatment in DTC in subjects with type 2 diabetes. The study revealed that cumulative 131I dosage was inversely correlated with systolic function in patients with type 2 diabetes but not in those without type 2 diabetes [21]. Although we did not assess diabetes in the present study, our results showed that fasting blood glucose levels were similar among DTC patients with and without RAI therapy. Considering that the RAI recipients were found to have a significantly higher likelihood of presystolic waves, it appears that the potential relationship between RAI and subclinical LV dysfunction (as determined by presystolic wave) was not associated with glucose levels. It should be also noted that the study conducted by Stanciu *et al*. [21] investigated the association between overt LV dysfunction as quantified by the measurement of the ejection fraction.

The present study is unique for addressing the impact of RAI on subclinical LV function through the utilization of a simple, feasible and readily available technique. Although there were no significant differences in LV ejection fraction among subjects with or without RAI in our groups, presystolic wave was more common among subjects who had received RAI, indicating the presence of subclinical systolic dysfunction in this patient subset.

Limitations

There are some limitations to be mentioned. The sample size is relatively small to reach a definitive conclusion regarding the role of RAI therapy on LV function. In addition, although the presystolic wave is largely accepted to be a proxy to detect subclinical LV dysfunction, the lack of longitudinal follow-up is another limitation.

CONCLUSION

In conclusion, RAI therapy following total thyroidectomy in patients with DTC was found to be associated with a five-fold higher likelihood for the presence of presystolic wave, which was utilized as a proxy for subclinical LV dysfunction. Subjects undergoing RAI subsequent to thyroid surgery may benefit from periodic transthoracic echocardiography for early detection of possible LV dysfunction.

Authors' Contribution

Study Conception: AV; OD; Study Design: AV, OD; Supervision: BS, OD; Funding: N/A; Materials: HB, SV; Data Collection and/or Processing: BS, SV; Statistical Analysis and/or Data Interpretation: OD, AV; Literature Review: OD, AV; Manuscript Preparation: AV, SV and Critical Review: AV, BS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Busaidy NL, Cabanillas ME. Differentiated thyroid cancer: management of patients with radioiodine nonresponsive disease. J Thyroid Res 2012;2012:618985.
2. Tuttle RM. Controversial issues in thyroid cancer management. J Nucl Med 2018;59:1187-94.

3. Pace-Asciak P, Russell JO, Tufano RP. The treatment of thyroid cancer with radiofrequency ablation. Tech Vasc Interv Radiol 2022;25:100825.
4. Fard-Esfahani A, Emami-Ardekani A, Fallahi B, Fard-Esfahani P, Beiki D, Hassanzadeh-Rad A, et al. Adverse effects of radioactive iodine-131 treatment for differentiated thyroid carcinoma. Nucl Med Commun 2014;35:808-17.
5. Soloviev AI, Kizub IV. Mechanisms of vascular dysfunction evoked by ionizing radiation and possible targets for its pharmacological correction. Biochem Pharmacol 2019;159:121-39.
6. Akyüz AR, Turan T, Gürbak İ, Korkmaz L, Ağaç MT, Çelik Ş. The relationship between presystolic wave and subclinical left ventricular dysfunction in asymptomatic hypertensive patients. Blood Press Monit 2016;21:277-81.
7. Kul S, Dursun İ, Ayhan S, Sayin MR, Üçüncü Ö, Bülbül NE, et al. Presystolic wave is associated with subclinical left ventricular dysfunction assessed by myocardial performance index in type 2 diabetes mellitus. Arq Bras Cardiol 2019;113:207-15.
8. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440-63.
9. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res 2004;94:1543-53.
10. Baselet B, Rombouts C, Benotmane AM, Baatout S, Aerts A. Cardiovascular diseases related to ionizing radiation: the risk of low-dose exposure (review). Int J Mol Med 2016;38:1623-41.
11. Puukila S, Lemon JA, Lees SJ, Tai TC, Boreham DR, Khaper N. Impact of ionizing radiation on the cardiovascular system: a review. Radiat Res 2017;188:539-46.
12. Schaue D, Micewicz ED, Ratikan JA, Xie MW, Cheng G, McBride WH. Radiation and inflammation. Semin Radiat Oncol 2015;25:4-10.
13. Darby SC, Cutter DJ, Boerma M, Constine LS, Fajardo LF, Kodama K, et al. Radiation-related heart disease: current knowledge and future prospects. Int J Radiat Oncol Biol Phys 2010;76:656-65.
14. Şaylık F, Akbulut T. A presystolic wave could easily detect subclinical left ventricular dysfunction in prediabetic patients with no history of hypertension. Echocardiography 2022;39:74-81.
15. Demiröz Ö, Öztürkeri B. The relationship of left ventricular outflow tract presystolic wave and 24-h ambulatory blood pressure measurements. Blood Press Monit 2022;27:378-83.
16. Kahyaoglu M, Bayam E, Gunay N, Gecmen C, Candan O, Yilmaz Y, et al. The relationship between presystolic wave and nondipper hypertension. Blood Press Monit 2020;25:142-6.
17. Korkmaz L, Akyüz AR, Gurbak I, Erkan H, Dursun I, Celik S. Presystolic A wave may predict increased arterial stiffness in asymptomatic individuals. Blood Press Monit 2016;21:144-8.
18. Alimi H, Hamidi F, Bigdelue L, Poorzand H, Ghaderi F, Fazlinejad A, et al. Prevalence of the presystolic wave in echocardiography and its association with the extent of coronary artery disease in patients with chronic stable angina. Echocardiography 2021;38:745-51.
19. Dursun I, Kul S, Sahin S, Kalaycioglu E, Akyuz AR, Korkmaz L. Relation of presystolic wave on doppler examination to syntax score in patients with acute myocardial infarction. Int J Cardiovasc Imaging 2018;34:569-76.
20. Saylik F, Akbulut T. The association of presystolic wave with subclinical left-ventricular dysfunction in patients with subclinical hypothyroidism. J Echocardiogr 2022;20:97-105.
21. Stanciu AE, Stanciu MM, Zamfirescu A, Gheorghe DC. Cardiovascular effects of cumulative doses of radioiodine in differentiated thyroid cancer patients with type 2 diabetes mellitus. Cancers (Basel) 2022;14:2359.

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Impact of COVID-19 disease on obstetric outcomes in the third trimester of pregnancy

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ABSTRACT

Objectives: The aim of this study is to evaluate the effect of coronavirus disease 2019 (COVID-19) diagnosed in the third trimester of pregnancy on maternal, fetal, and obstetric outcomes.

Methods: This retrospective study included 109 pregnant women hospitalized with a diagnosis of COVID-19 during the third trimester of pregnancy (28-40 weeks) in a tertiary center between March 1 and December 31, 2020. Demographic characteristics, clinical signs, and obstetric outcomes of the patients were searched for analysis. Laboratory and x-ray results were reported, and treatment methods were summarized. Finally, mother-newborn results were recorded.

Results: We included one hundred nine pregnant women in this study. We divided the patients into two groups as those with positive PCR test (n = 59) and negative PCR test and possible covid patients (n = 50) whose symptoms and histories meet the covid criteria. The mean age of the patients was 28.90 ± 6.21 years, and the mean week of gestation was 37.45 ± 2.29 weeks. Half of the patients were asymptomatic (n = 57, 47.7%), and 69% of all patients were delivered by cesarean section. The hospitalization time of antigen-positive cases was between 2-9 days. The mean lymphocyte count was 1.37 ± 0.45 ×103/mL in the PCR positive patient group, and this value was 1.67 ± 0.54 103/mL in the PCR negative patients ($p = 0.007$). While the mean neutrophil count was 8.13 ± 3.16 × 103/mL in the PCR positive patient group, this value was 10.99 ± 4.14 × 103/mL in the PCR negative patients ($p < 0.001$). Fifteen patients required intensive care unit follow-up, and 2 of them died while receiving mechanical ventilator support.

Conclusions: COVID-19 infection in the third trimester of pregnancy does not affect fetal and maternal outcomes if the disease is under control at an early stage. In hospitalized patients, symptoms are more precious than antigen testing.

Keywords: COVID-19, pregnancy and SARS CoV-2, prenatal diagnosis, third trimester

The new type of Coronavirus (COVID-19) has been defined as a deadly pandemic that spread to the world from Wuhan, China at the end of 2019, transmitted to a large number of people [1]. In January 2020, the World Health Organization (WHO) listed the new coronavirus pneumonia epidemic as a public health emergency of international concern. WHO defined the disease as "COVID-19" and the causative virus as "Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)" in March 2020 [2].

The incidence of adverse events caused by COVID-19 during pregnancy appears to be lower than

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previously reported rates for MERS and SARS infections, the same applies to maternal deaths: While the mortality rate in pregnant women affected by SARS and MERS varies between 25% and 30% [3, 4], the maternal mortality rate associated with COVID-19 is around 2% [5].

Due to physiological changes in the immune and cardiopulmonary systems, pregnant women are particularly susceptible to respiratory pathogens and severe pneumonia (for example, diaphragmatic elevation, increased oxygen consumption, and edema of the airway mucosa), which may render them intolerant to hypoxia [6]. Today, the clinical course of pregnant women diagnosed with COVID-19, delivery process, maternal and fetal outcomes are still important questions. Previous studies have reported high rates of adverse pregnancy outcomes such as preterm labor, premature rupture of membranes (PROM), and cesarean delivery (C/S) in pregnant women with COVID-19 infection [7, 8]. Therefore, pregnant women and their newborns should be considered as potential risk groups in the current COVID-19 pandemic.

The virus is mainly transmitted by respiratory droplets and/or contact [9]. The majority of patients (80%) present with a mild or asymptomatic clinical condition. Approximately 15% of them present with fever, cough, and shortness of breath, respiratory distress, and pneumonia. Approximately 5% of the patients require intensive care and respiratory support units due to 'Serious Acute Respiratory Syndrome' and multi-organ failure [10]. Laboratory findings usually include lymphopenia, thrombocytopenia, and abnormal liver enzymes [11]. Pneumonia is the most common serious symptom of infection and is usually seen with bilateral infiltration on thoracic imaging. Typical ground-glass opacities are seen in 56.4% of cases on computed tomography. Radiological findings are not seen in 17.9% of mild cases [12].

Diagnosis is based on the exposure history, clinical symptoms, laboratory test results, X-ray imaging findings, and a positive real-time reverse transcription-polymerase chain reaction (RT-PCR) result for COVID-19. RT-PCR test is the current gold standard for identifying SARS-CoV-2 from respiratory specimens with suspected COVID-19 patients [13].
The purpose of this article is to analyze the maternal, fetal, and obstetric outcomes of pregnancies diagnosed with COVID-19 in the third trimester [14, 15].

METHODS

This retrospective study was carried out on 109 pregnant women who were followed-up for COVID-19 infection in the third trimester (28-40 weeks) in a tertiary center and were treated in the hospital and then delivered in the same hospital. Fifty-nine of the patients were diagnosed as COVID-19 with positive PCR test and other fifty patients were defined as possible COVID-19. For the possible and confirmed Covid-19 diseases definition, the approaches of the World Health Organization and the Ministry of Health of the Republic of Turkey were accepted as a reference. The patients were diagnosed with COVID 19 infection based on real-time polymerase chain reaction (RT-PCR), pulmonary tomography results, and/or clinical status.

Bursa Yüksek İhtisas Training and Research Hospital ethics committee approved this study with numbered 2011-KAEK-25 2022/11-15, and informed consent was obtained for the study. The demographic, clinical characteristics, treatments, and obstetric outcomes of the patients were recorded by the researchers through patient files and hospital records. Eligibility criteria included laboratory-diagnosed COVID-19, third-trimester pregnancy at presentation. Pregnant women whose pregnancy follow-up or delivery data could not be obtained were excluded from the study.

Statistical Analysis

Statistical analyses were performed using SPSS version 23 (IBM Corp, Armonk, NY, USA). Mean or median values were used for descriptive variables according to data. Numbers and percentages were used for categorical data. The patients were grouped according to their PCR results. Shapiro Wilk test was used to determine the distribution and Mann Whitney U, or chi-square tests were applied to determine the differences between the groups. $P < 0.05$ was considered significant.

RESULTS

In Table-1, we presented the demographic and clinical

Table 1. Clinical characteristics and obstetric outcomes of groups

	COVID PCR positive n = 59	COVID PCR negative n = 50	*p value*
Age (years)	29.13 (6.49)	28.64 (5.93)	NS
BMI (kg/m^2)	27.98 (4.35)	27.85 (3.37)	NS
Gestational age (weeks)	37.50 (2.17)	37.40 (2.45)	NS
Route of delivery, n (%)			
Vaginal	17 (28.8)	16 (32.0)	NS**
C-section	42 (71.2)	34 (68.0)	
Pregnancy complications, n (%)			
Absent	43 (72.9)	37 (74.0)	NS**
Present	10 (16.9)	13 (26.0)	
Birth weight (g)	3039.79(585.66)	2942.40(654.48)	NS
Apgar score*			
1st min.	9 (0-9)	9 (6-9)	NS
5th min.	10 (0-10)	10 (8-10)	NS
Length of hospitalization (days), median (min-max)	5 (2-9)	5 (1-7)	**0.04**
Laboratory parameters			
Hemoglobine (g/dL)	10.99 (1.78)	10.68 (1.46)	NS
WBC (×10^9/L)	9.93 (3.59)	13.39 (4.19)	**< 0.001**
Lymphosyte (×10^3/mL)	1.37 (0.45)	1.67 (0.54	**0.007**
Neutrophil (×10^3/mL)	8.13 (3.16)	10.99 (4.14)	**< 0.001**
Platelets (×10^9/L)	220.59 (75.83)	248.22 (81.24)	NS
C-reactive protein (mg/L)	32.51 (35.19)	32.84 (37.07)	NS
D-dimer (µg/mL)	4.59 (10.37)	4.27 (7.54)	NS
Fibrinogen (mg/dL)	447.40 (109.18)	482.50 (115.17)	NS
Prothrombine Time (seconds)	11.44 (1.03)	11.27 (0.79)	NS
aPTT (seconds)	25.83 (4.49)	23.06 (3.22)	**0.001**
Troponin -T (ng/mL)	7.85 (21.92)	5.61 (6.30)	NS
Creatine kinase (ng/mL)	226.46 (281.67)	230.32 (365.65)	NS
FBG (mg/dL)	84.18 (25.08)	81.70 (13.41)	NS
AST (IU/L)	28.07 (19.69)	26.75 (17.23)	NS
ALT (IU/L)	17.77 (14.58)	14.55 (11.46)	NS
LDH (IU/L)	300.66 (257.52)	297.54 (98.43)	**0.03**
BUN (mg/dL)	19.49 (14.65)	16.09 (6.31)	NS
Creatinine (mg/dL)	0.56 (0.22)	0.50 (0.14)	NS

Data are given as mean (standard deviation) or median (min-max). WBC = white blood cell, aPTT = activated partial thromboplastin time, IU = international unit, FBG = fasting blood glucose, ALT = alanine aminotransferase. AST = aspartate aminotransferase, LDH = lactic dehydrogenase, BUN = blood urea nitrogen, NS = non-significant. *Mann Whitney U test, **Chi-square

characteristics of the patients. The mean age of all pregnant women was 28.9 ± 6.2 years. The mean week of delivery was 37.4 ± 2.3 weeks. Almost half of the patients were asymptomatic (n = 57, 47.7%). Computed tomography was performed on 41 patients, and 18 had severe involvement. Nineteen (17.5%) of the patients had severe disease, while 15 were followed in the intensive care unit, two patients died. Neonatal SARS CoV-2 was detected in only one baby. Preterm delivery and premature rupture of membranes were the most common pregnancy complications. Placental abruption developed in 5 patients. While the number of complications we have stated in the second table is 23, this number is seen as 40 in the first table. This difference is reflected in the fact that there is more than one complication in the same patient. All analysis is available in Table 1.

In Table 2, we analyzed the laboratory parameters and obstetric results of pregnant women with a diagnosis of COVID-19. In addition, we divided these patients into two groups with and without positive PCR tests and presented the analyzes.

The mean age of PCR-positive patients was 29.13 ± 6.49 years, while the mean age of PCR-negative patients was 28.64 ± 5.93 years. While 33 (30.2%) of 109 patients in the study delivered vaginally, the cesarean section rate of the other population was around 50% in the same period. The mode of delivery did not differ significantly between the two groups. While only 23 (21.1%) of all patients had pregnancy-related complications, this situation was not statistically different between the two groups with and without PCR positive (Table 2).

While the white blood cell count was 9.93 ± 3.59 ×109/L in the PCR positive patient group, it was 13.39 ± 4.19 ×109/L in the PCR negative patient group, and the situation differed between the two groups ($p < 0.001$). While the lymphocyte count was 1.37 ± 0.45 ×103/mL in the PCR positive patient group, this value was 1.67 ± 0.54 ×103/mL in the PCR negative patients, which was significant ($p = 0.007$). While the neutrophil count was 8.13 ± 3.16 ×103/mL in the PCR positive patient group, this value was 10.99 ± 4.14 ×103/mL in the PCR negative patients, which was significant ($p < 0.001$). Active partial thromboplastin time was significantly different in both groups ($p = 0.001$). While it was 25.83 ± 4.49 seconds in the PCR positive patient group, it was 23.06 ± 3.22 seconds in the neg-

Table 2. Demographic and clinical characteristics of patients (n = 109)

Variables	Values
Maternal age (years)	28.90 ± 6.21
Gestational age at delivery (weeks)	37.45 ± 2.29
Initial symptoms	
Asymptomatic	52 (47.7)
Symptomatic	57 (52.2)
Fever	24 (22.2)
Cough	23(21.1)
Headache	20 (18.3)
Sore throat	5 (4.5)
Dyspnea	18 (16.5)
Myalgia	6 (5.5)
Computed tomography (pulmonary)	
Normal	11 (10)
Pneumonia	12 (11)
Ground glass opacities	18 (16)
COVID-19 severity	
Mild	66 (60.6)
Moderate	24 (22.1)
Severe	19 (17.5)
Respiratory support	
Nasal oxygen therapy	10 (9.1)
High flow nasal cannula	8 (7.4)
Invasive mechanic ventilation	2 (1.9)
Route of delivery	
Vaginal	33 (30.3)
C-section	76(69.7)
Cesarean indications	
Previous cesarean delivery	33 (43.4)
Fetal distress	22 (28.9)
Cephalopelvic disproportion	6 (9.2)
Placenta previa	3(3.9)
Malpresenation	4(5.3)
Hypertension-Preeclampsia	6 (7.9)
Intrauterine exitus	2(1.9)
Pregnancy complications	
Gestational diabetes mellitus	2 (1.8)
Preeclampsia	6 (5.5)
Preterm delivery	17 (15.6)
Preterm rupture of membranes	10 (9.1)
Placental abruption	5 (4.6)
Neonatal Sars COv 2 positivity	1 (1)
Admission to NICU	15 (13.8)
Maternal mortality	2 (1.8)

Values are given as mean ± standard deviation or n (%).

ative patient group (Table 2). We present the complete analysis in Table 2.

DISCUSSION

This article represented the clinical findings, labor outcomes, obstetric outcomes, and pregnancy complications of hospitalized pregnant women due to SARS CoV-2 positivity in the third trimester of pregnancy. We also attempted to compare the PCR positive and negative of the disease with these outcomes.

In the present article, pregnant women who caught COVID-19 in the third trimester of pregnancy, in general, have a mild to moderate course of the disease. Whether the PCR result is positive or not, it is primarily uncomplicated. While approximately 70% of the patients delivered by cesarean section, most of these were due to COVID-19 unrelated reasons. Acute fetal distress developed in 20% of the pregnant women, but it was not unusual to determine their direct relationship with covid-19. Intrauterine fetal death occurred in 2 patients, but they were not neonatal SARS CoV-2. Although blood white blood cell, neutrophil, lymphocyte, aPTT, and LDH values were significantly different between PCR positive or negative groups, these values were not meaningful in pregnancy management.

In their study, Mahajan NN *et al*. reported the pregnancy complications due to first wave COVID-19 infection as 60.9%, while the complication rate due to the second wave was 72.2% [16]. Gajbhiye *et al*. [17] reviewed 441 cases and presented them as articles. Accordingly, preterm birth developed in 26% of the cases, fetal distress in 8% and premature rupture of membranes in 9% of cases. The most common comorbidities associated with pregnant women with COVID-19 were hypertensive disorders (10%), diabetes (9%), placental disorders (2%), coinfections (3%). Premature birth (25%), respiratory distress syndrome (8%), pneumonia (8%) were reported among newborns of COVID-19 mothers. In the article they published with a large patient group, Sun *et al*. [18] pointed out the complicated conditions of pregnancy that increased during the COVID-19 period. Accordingly, the most common adverse outcome in 152,903 deliveries examined during the COVID-19 pandemic period were premature rupture of membranes (10.3%), gestational diabetes (9.3%), and gestational hypertension (8.5%). Compared to the reference period, the pandemic period was characterized by statistically significantly higher rates of gestational diabetes, gestational hypertension, poor fetal growth, and preeclampsia.

Due to the vulnerable immune system of pregnant women, mothers and newborns are one of the weakest populations in pandemics [19]. There is insufficient literature on the approach and potential adverse effects of COVID-19 in different pregnancy trimesters [20-22]. Ideal pregnancy management in the COVID-19 pandemic is essential because pregnant women are at higher risk for a worsening clinical course, increased pregnancy complication rates, the efficacy of medications, the optimal delivery route, the safety of breastfeeding, and the risk of vertical transmission [23, 24]. The effectiveness of drugs, the optimal delivery route, the safety of breastfeeding, and the risk of vertical transmission are still controversial [25, 26].

According to our study, almost half of the pregnant women were asymptomatic. Those had generally mild symptoms. There were eighteen pregnant women describing dyspnea, and we had to start invasive mechanical ventilation for only two pregnant women. As mentioned above, the effects of COVID-19 infection in pregnant women are still controversial, and more extended studies are needed to understand the immune response. We provided mechanical ventilator support to 2 of the 15 patients we took to the intensive care unit, but both died. Considering this situation and the rate of asymptomatic patients, we assume that the progression of the disease is prevented at an early stage in terms of prognosis.

This study can claim that the mode of delivery is not affected by COVID-19 infection during the third trimester of pregnancy by looking at cesarean section indications. We also think that it is not related to neonatal SARS CoV-2. We also consider that it is not associated with neonatal SARS CoV-2.

According to the antigen test, there were no significantly different delivery modes or pregnancy complications in the definite and probable case groups. In this context, we should organize the follow-up and treatment of the pregnant according to the course of the disease, not the PCR antigen test. Keeping the disease under control at an early stage is the most critical factor, and it is still unclear at what stage and to what

extent the fetus will be affected.

Limitations

This study has some limitations. We could not report the final status of the infants and whether they had long-term diseases. Furthermore, it would be helpful to mention the mothers' long-term outcomes, the result of the disease, and its possible complications in more prolonged periods. On the other hand, there were also some limitations related to the data about the mother, like the starting and duration date of COVID-19 and vaccination status. However, this study supplied some critical information associated with the perinatal and neonatal results of COVID-19, and future well-designed meta-analyses can improve the awareness of this disease.

CONCLUSION

In conclusion, COVID-19 infection present in the third trimester of pregnancy may not affect pregnancy and delivery outcomes if the disease is under control at an early stage. Management may be more accurate in hospitalized patients based on patient symptoms than antigen testing.

Authors' Contribution

Study Conception: FB; Study Design: SSK; Supervision: SSK; Funding: FB; Materials: SSK; Data Collection and/or Processing: SSK; Statistical Analysis and/or Data Interpretation: FB; Literature Review: SSK; Manuscript Preparation: SSK, FB and Critical Review: SSK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Amankwah-Amoah J. COVID-19 pandemic and innovation activities in the global airline industry: a review. Environt Int 2021;156:106719.
2. Jogalekar MP, Veerabathini A, Gangadaran P. Novel 2019 coronavirus: genome structure, clinical trials, and outstanding questions. Exp Biol Med (Maywood) 2020;245:964-9.
3. Bhatti JS, Bhatti GK, Khullar N, Reddy AP, Reddy PH. Therapeutic strategies in the development of anti-viral drugs and vaccines against SARS-CoV-2 infection. Mol Neurobiol 2020;57:4856-77.
4. Allmer J. Special issue on COVID-19 data integration opportunities and vaccine development strategies. J Integr Bioinform 2021;18:1-2.
5. Di Mascio D, Khalil A, Saccone G, Rizzo G, Buca D, Liberati M, et al. Outcome of coronavirus spectrum infections (SARS, MERS, COVID-19) during pregnancy: a systematic review and meta-analysis. Am J Obstet Gynecol MFM 2020;2:100107.
6. Yan J, Guo J, Fan C, Juan J, Yu X, Li J, et al. Coronavirus disease 2019 in pregnant women: a report based on 116 cases. Am J Obstet Gynecol 2020;223:111.e1-111.e14.
7. Juan J, Gil MM, Rong Z, Zhang Y, Yang H, Poon LC. Effect of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcome: systematic review. Ultrasound Obstet Gynecol 2020;56:15-27.
8. Khalil A, Kalafat E, Benlioglu C, O'Brien P, Morris E, Draycott T, et al. SARS-CoV-2 infection in pregnancy: a systematic review and meta-analysis of clinical features and pregnancy outcomes. EClinicalMedicine. 2020;25:100446.
9. McIntosh K, Hirsch MS, Bloom A. COVID-19: Epidemiology, virology, and prevention. UpToDate. Dec 09, 2022. https://www.uptodate.com/contents/covid-19-epidemiology-virology-and-prevention
10. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
11. Cunningham FG. UpToDate. Aug 09, 2021. https://www.uptodate.com/contents/normal-reference-ranges-for-laboratory-values-in-pregnancy.
12. Moro F, Buonsenso D, Moruzzi MC, Inchingolo R, Smargiassi A, Demi L, . How to perform lung ultrasound in pregnant women with suspected COVID-19. Ultrasound Obstet Gynecol 2020;55:593-8.
13. Shimizu K, Kondo K, Osugi Y, Negita M, Mase H, Kondo T, et al. Early COVID-19 testing is critical to end the pandemic. J Gen Fam Med 2021;22:67.
14. Abaluck J, Kwong LH, Styczynski A, Haque A, Kabir MA, Bates-Jefferys E, et al. Impact of community masking on COVID-19: a cluster-randomized trial in Bangladesh. Science 2022;375:eabi9069.
15. Türk Toraks Derneği. COVID-19 Tanı ve Tedavisinde Kanıta Dayalı Öneriler ve Türk Toraks Derneği COVID-19 Görev Grubu Görüş Raporu. 2020;149-59.
16. Mahajan NN, Pophalkar M, Patil S, Yewale B, Chaaithanya IK, Mahale SD, et al. Pregnancy outcomes and maternal complications during the second wave of coronavirus disease 2019 (COVID-19) in India. Obstet Gynecol 2021;138:660-2.
17. Gajbhiye RK, Modi DN, Mahale SD. Pregnancy outcomes, newborn complications and maternal-fetal transmission of SARS-CoV-2 in women with COVID-19: a systematic review of

441 cases. MedRxiv 2020. doi: 10.1101/2020.04.11.20062356.
18. Sun S, Savitz DA, Wellenius GA. Changes in adverse pregnancy outcomes associated with the COVID-19 pandemic in the United States. JAMA Netw Open 2021;4:e2129560.
19. PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, et al. Immune mechanisms at the maternal-fetal interface: perspectives and challenges. Nat Immunol 2015;16:328-34.
20. la Cour Freiesleben N, Egerup P, Hviid KVR, Severinsen ER, Kolte AM, Westergaard D, et al. SARS-CoV-2 in first trimester pregnancy: a cohort study. Hum Reprod 2021;36:40-7.
21. Rizzo G, Mappa I, Maqina P, Bitsadze V, Khizroeva J, Makatsarya A, et al. Effect of SARS-CoV-2 infection during the second half of pregnancy on fetal growth and hemodynamics: a prospective study. Acta Obstet Gynecol Scand 2021;100:1034-9.
22. Fontanella F, Hannes S, Keating N, Martyn F, Browne I, Briet J, et al. COVID-19 infection during the third trimester of pregnancy: current clinical dilemmas. Eur J Obstet Gynecol Reprod Biol 2020;251:268-71.
23. Metz TD, Clifton RG, Hughes BL, Sandoval G, Saade GR, Grobman WA, et al. Disease Severity and Perinatal Outcomes of Pregnant Patients With Coronavirus Disease 2019 (COVID-19). Obstet Gynecol 2021;137:571-80.
24. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. BMJ 2020;370:m3320.
25. Huntley BJF, Mulder IA, Di Mascio D, Vintzileos WS, Vintzileos AM, Berghella V, et al. Adverse pregnancy outcomes among individuals with and without severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): a systematic review and meta-analysis. Obstet Gynecol 2021;137:585-96.
26. Woodworth KR, Olsen EO, Neelam V, Lewis EL, Galang RR, Oduyebo T, et al. Birth and infant outcomes following laboratory-confirmed SARS-CoV-2 infection in pregnancy - SET-NET, 16 Jurisdictions, March 29-October 14, 2020. MMWR Morb Mortal Wkly Rep 2020;69:1635-40.

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Investigating emotional regulation, aggression, and self-esteem in sexually abused adolescents

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ABSTRACT

Objectives: Child sexual abuse is a global problem with an increased risk of developing psychopathology. In recent studies, it was studied that children and adolescents who experienced sexual abuse have emotion regulation difficulties. We aimed to investigate some features of sexually abused adolescents by studying emotion regulation, aggression, and self-esteem.

Methods: One hundred four adolescents seeking treatment after sexual abuse to Bursa Yuksek Ihtisas Training and Research Hospital Child Monitoring Center and 91 control group were included. Participants were given to complete the personal information form, the adolescent emotion regulation scale, the reactive-proactive aggression scale, and the Rosenberg self-esteem scale

Results: It was determined that adolescents who were sexually abused used more internal dysfunctional and external dysfunctional emotion regulation while the control group used more external functional emotion regulation. While there was no difference between both groups regarding aggression, self-esteem was found to be lower in abused adolescents. Self-injurious behavior was higher in adolescent victims of sexual abuse. Aggression was found to increase as dysfunctional emotion regulation increased.

Conclusions: It was found that sexually abused adolescents had more difficulty in regulating their emotions and exhibited more self-injurious thoughts and behaviors but there was no difference between them and the control group in terms of aggression. We found that dysfunctional emotion regulation increased aggression in the study group.

Keywords: Sexual abuse, adolescent, emotion regulation, aggression

Child sexual abuse (CSA) is a common condition all over the World. Although the prevalence of CSA is estimated to be 11.8% worldwide, it is said that this rate is between 7.6% and 8.0% in men and 15.0% and 19.7% in women. The child's age, level of development, type of abuse, and social support can affect the level of psychological impact and the effects of abuse can last until adulthood [1].

Aggressive behavior occurs with different purposes and functions and is divided into reactive and proactive aggression. Aggression in the form of threat, provocation, and defensive response is defined as reactive aggression, while aggression aimed at achieving a goal or gain (for example, its material, physical, or

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social status) is defined as proactive aggression [2]. Anger in sexually abused children is seen even years after the abuse [3]. For this reason, aggressive behavior can be observed as a result of anger in abused children.

Emotions are affective elements that occur in people's lives as a result of complex interactions of biological, psychological, and environmental factors. It is also responsible for adapting to new situations. This also defines the ability to regulate emotion. This skill is influenced by temperament, neurological, physiological, and cognitive processes, and the family environment [4]. Emotion regulation is closely related to mental health in children and adults. Emotion regulation deficiencies or dysfunctions are theoretically and empirically closely related to psychopathology at all ages. For example, it is a risk factor for psychopathologies such as anxiety disorders, mood disorders, substance use, and post-traumatic stress disorder [5].

Sexual abuse in childhood and adolescence is associated with the severity of post-traumatic stress disorder, depressive symptoms, and emotional regulation difficulties [6]. Several studies investigating emotion regulation competencies in children who have been victims of interpersonal trauma have found that mistreatment is associated with a reduced ability to regulate one's emotions [7]. It has also been suggested that childhood sexual abuse can also inhibit a child's ability to identify and express emotions [8]. Sexual abuse has the potential to compromise socio-emotional development, which can lead to increased vulnerability to difficulties regulating emotions [9]. In addition, emotion regulation is thought to play a key role in several psychological disorders in victims of sexual abuse [10]. In this study, we aimed to contribute to existing knowledge by determining the self-esteem, emotion regulation skills, and aggression levels of sexually abused adolescents.

To our knowledge, there is no study investigating the relationship between emotional regulation and proactive and reactive aggression behaviors of sexual abuse experienced during adolescence. Also, different functional subtypes of aggressive behavior (eg, reactive or proactive) have not been systematically investigated. In this study, we aimed to examine the emotion regulation skills, self-esteem, and proactive and reactive aggression levels of sexually abused adolescents.

METHODS

Study Population

The sample group of the study consisted of 104 adolescents who were evaluated for sexual abuse at the Health Sciences University Bursa Yuksek Ihtisas Training and Research Hospital Child Monitoring Center (CİM) between February 2016 and July 2016. The control group of the study consisted of 91 adolescents from a high school in Bursa province, with similar characteristics to the patient group in terms of socioeconomic level, age, and gender. Verbal and written informed consent was obtained from the adolescents and their parents who agreed to participate in the study. Inclusion criteria in the study group were defined as being exposed to sexual abuse and accepting the study, and exclusion criteria were determined as having a psychotic and chronic physical illness. Inclusion criteria in the control group were defined as not having a known psychiatric illness or chronic physical illness and accepting the study.

Tools

Personal Information Form

It is a form prepared by the researchers for this study that includes sociodemographic characteristics of adolescents, such as age, gender, number of siblings, educational status of parents, occupation of parents, family type, income level, and occupation.

Emotion Regulation Scale for Adolescents

The original scale was developed by Phillips and Power (2007) to determine adolescents' emotion regulation skills. Consisting of four sub-dimensions, internal functional emotion regulation, external functional emotion regulation, internal dysfunctional emotion regulation, and external dysfunctional emotion regulation, the scale is a 5-point Likert type and consists of 18 items [11]. It is a scale suitable for use in adolescents. The Turkish validity and reliability study was conducted by Duy and Yildiz [12].

Reactive-Proactive Aggression Scale

The Reactive-Proactive Aggression Scale is a scale developed to measure reactive and proactive ag-

gression in children and adolescents. There are 23 items on the scale, 11 of them measure reactive aggression and 12 of them measure proactive aggression [2]. The Turkish validity and reliability study was conducted by Baş and Yurdabakan [13]. The Turkish adaptation of the scale was carried out with secondary and high school students.

Rosenberg Self-Esteem Scale

The scale, which was created by M. Rosenberg, and the Turkish validity and reliability study done by Çuhadaroğlu, assess self-esteem. It is a self-report scale consisting of 63 multiple-choice questions and has 12 subscales. In this study, only the first 10 items of the scale were used to assess self-esteem. If the total score obtained from the first 10 questions is 0-1, it indicates high self-esteem, 2-4 indicates medium and 5-6 indicates low self-esteem [14].

Ethics statement

The ethics committee approval of the research was given by the Clinical Research Evaluation Committee of SBU Bursa Yüksek İhtisas Training and Research Hospital. (Decision date / No: 27.01.2016/ 2011-KAEK-25 2016/02-09)

Statistical Analysis

The data obtained in this study were analyzed with the IBM SPSS Statistics version 22 package program. While investigating the normal distribution of the variables, Shapiro Wilks was used because of the number of units. While examining the differences between the groups, The Mann-Whitney U Test was used because the variables did not come from a normal distribution. Bonferroni test was used for pairwise comparison. Pearson correlation coefficient was calculated between variables. While interpreting the results, 0.05 was used as the significance level; It was stated that there was a significant difference in the case of $p < 0.05$ and there was no significant difference in the case of $p > 0.05$.

RESULTS

There was no statistical difference between the two groups in terms of age and gender among the adolescents included in the study. When the school attendance status of the adolescents was examined, it was seen that there was a significant difference between the two groups and that the whole healthy group attended school. Parents ' education levels were significantly lower in the study group than in the control group. Again, the number of divorced parents in the study group was significantly higher. In the study group, 23 (19.2%) adolescents had suicidal thoughts, 11 (9.2%) adolescents had suicide attempts, and 5 (4.2%) adolescents had self-destructive behaviors after the incident, while the control group had no suicidal thoughts, attempts, or self-destructive behaviors (Table 1).

Table 2 shows that there is a statistically significant difference between the groups in terms of "external dysfunctional emotion regulation" and "internal dysfunctional emotion regulation" scores ($p < 0.05$). The scores of the study group in both areas are significantly higher than that of the control group. In terms of "external functional emotion regulation" scores, the control group scored significantly higher than the research group ($p < 0.05$). It was found that the study group most often used “internal dysfunctional emotion regulation”, while the control group most often used “external functional emotion regulation” (Table 2).

There was a statistically significant difference between the groups in terms of self-esteem scores ($p < 0.05$). The "self-esteem" of the control group is significantly lower than that of the research group. There was no statistically significant difference between the groups in terms of aggression scale scores ($p > 0.05$) (Table 3).

When we look at the correlations of the study group between the scales; there was a moderate relationship between “external dysfunctional emotion regulation” and “proactive aggression”, and a strong and positive relationship between “reactive aggression” (($r = 0.502$; $p = 0.001$), ($r = 0.723$; $p = 0.001$) respectively). Finally, a moderately positive relationship was found between “internal dysfunctional emotion regulation” “reactive aggression” and “total aggression” ($r = 0.504$, $p = 0.001$ and $r = 0.531$, $p = 0.001$; respectively).

DISCUSSION

In our study, it was determined that adolescents who were victims of abuse used dysfunctional emotion reg-

Table 1. **Frequency distribution table of demographic information by groups**

	Study group (n = 120)	Control group (n = 113)	Total (n = 233)	*p* value
Age (years) (Mean ± SD)	14.75 ± 1.60	15.32 ± 3.99	15.03 ± 3.02	0.154
Gender, n (%)				0.627
Male	7 (5.8)	5 (4.4)	12 (5.2)	
Female	113 (94.2)	108 (95.6)	221 (94.8)	
School status, n (%)				**< 0.001**
Does not go to school	20 (16.7)	0 (0)	20 (8.6)	
Middle school	36 (30.0)	27 (23.9)	63 (27.0)	
High school	58 (48.3)	63 (55.8)	121 (51.9)	
Other	6 (5.0)	23 (20.4)	29 (12.4)	
Mother's educational status, n (%)				**< 0.001**
No education	27 (22.5)	7 (6.2)	34 (14.6)	
Primary school	73 (60.8)	85 (75.2)	158 (67.8)	
High school and above	20 (16.7)	21 (18.6)	41 (17.6)	
Father's educational status, n (%)				**< 0.001**
No education	17 (14.2)	2 (1.8)	19 (8.2)	
Primary school	76 (63.3)	64 (56.6)	140 (60.1)	
High school and above	27 (22.5)	47 (41.6)	74 (31.8)	
Family status, n (%)				**< 0.001**
Parents together	76 (63.3)	107 (94.7)	183 (78.5)	
Divorced	37 (30.8)	4 (3.5)	41 (71.6(	
Broken	7 (5.8)	2 (1.8)	9 (3.9)	
Suicidal ideation, n (%)	23 (19.2)	0 (0)	23 (9.9)	**< 0.001**
Suicide attempt, n (%)	11 (9.2)	0 (0)	11 (4.7)	**< 0.001**
Self-injurious behavior, n (%)	5 (4.2)	0 (0)	5 (2.1)	**0.060**

t test, Pearson Chi square, Fisher-exact test were used

Table 2. **The difference between groups in terms of the scale of emotion regulation**

	Study group (n = 120) Mean ± SD	Control group (n = 113) Mean ± SD	*p* value
Internal functional emotion regulation	12.83 ± 3.39	13.36 ± 1.56	0.124
External dysfunctional emotion regulation	9.19 ± 3.61	7.23 ± 1.51	**< 0.001**
Internal dysfunctional emotion regulation	14.01 ± 4.25	10.44 ± 2.38	**< 0.001**
External functional emotion regulation	9.33 ± 3.09	12.75 ± 1.98	**< 0.001**

The Mann-Whitney U test was used. SD = standard deviation

Table 3. The difference between groups in terms of aggression and the Rosenberg scale

	Study group (n = 120) Mean ± SD	Control Group (n = 113) Mean ± SD	*p* value
Self-esteem	1.69 ±0.94	1.18 ± 0.73	**< 0.001**
Proactive aggression	1.76 ± 2.80	1.88 ± 2.61	0.709
Reactive aggression	8.75 ± 4.80	8.14 ± 4.31	0.374
Total aggression	9.57 ± 6.26	9.96 ± 6.55	0.598

The Mann-Whitney U test was used. SD = standard deviation

ulation strategies more than the control group, their self-esteem was lower and there was no difference between them and the control group in terms of aggression levels. They also had a higher tendency to self-harm in the abuse group. We also found that dysfunctional emotion regulation strategies increase aggression levels.

When a child is exposed to maltreatment such as physical abuse, sexual abuse, and neglect, they may have difficulty regulating emotions. Difficulty regulating emotions is both a result of trauma and a predictor of psychopathology. The data of our study support the literature in this regard [15]. When the literature is examined, it has been shown in many studies that child, adolescent, and adult sexual abuse victims have difficulty regulating emotions [5, 16]. Langevin *et al*. [5] reported that preschool children who were sexually abused, especially boys, had lower emotional regulation competencies than children who were not abused. In another study, by Berzenski [17] it has been reported that children who have been sexually abused have poorer emotion regulation skills and exhibit more abstinence and social difficulties than children who have not been abused. It has been reported that emotion regulation competence is associated with social difficulty and withdrawal behavior [17].

Wolff *et al*. [18] emphasized that intense emotional dysregulation poses a high risk for non-suicidal self-harming behavior and therefore emotional dysregulation should be well understood in treatment. Therefore, therapeutic interventions that focus on emotional awareness, expression of emotions, anger management, and the ability to empathize with others will be more beneficial than treatments that focus solely on PTSD symptoms [19].

According to Paulus *et al*. [20], symptoms of child sexual abuse include irritability, temper tantrums, and aggressive behavior, especially offensive content in younger children's games. In another study, they reported that children who were sexually abused in preschool had deficiencies in emotional regulation. It has been found that this deficiency causes more internalized behavior problems, however, it partially mediates the relationship between externalized behavior problems [8]. This shows that no matter what developmental period children have been sexually abused, they develop emotion regulation skills and as a result related behavioral problems.

Abused children may be at risk for problems with aggression. It has been found that sexually abused adolescents are more aggressive in both their own and their parents' evaluations, the younger the child, the more negative life events and the higher the dysfunction in the family, the higher their aggression [21]. It has been suggested in studies that the aggressive tendencies of both men and women are linked to sexual abuse. While there are studies suggesting that women are more aggressive, there are also studies that find the opposite [22, 23]. In contrast to the findings in other studies, no difference was found between the groups in the current study. The fact that the victims experienced abuse in adolescence and that we did not question the control group's experiences of trauma other than sexual abuse may have revealed this finding.

Ford *et al*. [24] found results parallel to our study. In a sample group of 397 children with severe emotional disorders, it was determined that reactive aggression behaviors were associated with a history of physical abuse but not sexual abuse in children with reactive aggression behaviors. Girls are equally likely to be classified as reactively aggressive regardless of their history of physical abuse, but boys with a history of physical abuse are 50% more likely to be classified as reactively aggressive than boys without a history of

physical abuse. It was found that proactive aggression was not associated with a history of physical or sexual abuse [24]. The lack of association between sexual abuse and reactive or proactive aggression maybe because it is consistent with research suggesting that sexual abuse primarily involves internalization, resolution, and developmental problems, not aggression [25]. More research is needed to determine whether the risk or severity of reactive and proactive aggression changes if sexual abuse and physical abuse occur together. The findings of this study show that sexual abuse does not affect the relationship between reactive and proactive aggression. . There are studies in the literature showing that dysfunctional emotion regulation increases aggression [26-28]. It was also found that emotion regulation affected reducing aggression [29, 30], and those who could not functionally regulate their emotions resorted to violence more frequently [31]. The research findings are similar to the literature.

Many studies have shown that there is a relationship between sexual abuse and low self-esteem in children [32]. In our study, the self-esteem of the victims of sexual abuse was found lower than the control group. This finding is consistent with the results of many studies in the literature [23, 32].

Child sexual abuse poses a risk for emotional and behavioral disorders. It has been noted that this, in turn, occurs due to the stigma, shame, powerlessness, self-blame, and personal violation that accompany sexual victimization [22]. One of these problems is self-harming behavior. Exposure to sexual abuse in childhood, the number, and severity of abuse are associated with suicidal attempts at an advanced age. However, aggressive or hostile behavior can also be a result of the traumatic experience associated with high levels of emotional distress from the abuse. It has been found that almost all victims of sexual abuse have attempted suicide at least once in their lifetime and are 2.2 times more likely to attempt suicide in their lifetime than those who have not been sexually abused [33]. While suicidal ideation, suicide attempt, and self-harming behaviors were observed in abused adolescents in our study, such behaviors were not observed in the healthy group. It has been stated that young people exposed to sexual abuse may be at higher risk of post-traumatic stress symptoms, self-harming behaviors may be particularly striking and the role of emotional dysregulation in this relationship between sexual abuse and, self-harm has been emphasized [34].

Limitations

When the limitations of our study are examined; the use of self-report scales in the study may reduce the objectivity of the data. A scale questioning the traumatic experiences of the control group was not used and the intelligence levels of the adolescents participating in the study were evaluated by clinical observation. Another limitation of our study is that the characteristics of sexual abuse are not detailed. It is important that the emotional regulation difficulties of children who are victims of abuse are well evaluated and taken into account in treatment attempts. In this way, children's self-harming thoughts and behaviors can be reduced and their self-esteem can be increased.

CONCLUSION

It has been determined that adolescents with a history of sexual abuse have more difficulty in regulating their emotions and they have more self-harming thoughts and behaviors. However, it was determined that there was no difference between the group with a history of sexual abuse and the healthy group in terms of aggression. We found that dysfunctional emotion regulation increases adolescents' aggression levels.

Authors' Contribution

Study Conception: MEU, ZÇ; Study Design: ES, ZÇ; Supervision: N/A; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ES, ZÇ; Statistical Analysis and/or Data Interpretation: HŞ; Literature Review: MEU, HŞ; Manuscript Preparation: MEU, HŞ and Critical Review: ES.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Sanjeevi J, Houlihan D, Bergstrom KA, Langley MM, Judkins J. A review of child sexual abuse: impact, risk, and resilience in the context of culture. J Child Sex Abus 2018;27:622-41.
2. Kempes M, Matthys W, de Vries H, van Engeland H. Reactive and proactive aggression in children. A review of theory, findings and the relevance for child and adolescent psychiatry. Eur Child Adolesc Psychiatry 2005;14:11-9.
3. Estévez A, Ozerinjauregi N, Herrero-Fernández D. Maladaptive schemas as mediators in the relationship between child sexual abuse and displaced aggression. J Child Sex Abus 2016;25:449-65.
4. Thompson RA. Emotion dysregulation: a theme in search of definition. Dev Psychopathol. 2019;31:805-15.
5. Langevin R, Cossette L, Hébert M. Emotion regulation in sexually abused preschoolers. Child Psychiatry Hum Dev 2016;47:1-12.
6. Chang C, Kaczkurkin AN, McLean CP, Foa EB. Emotion regulation is associated with PTSD and depression among female adolescent survivors of childhood sexual abuse. Psychol Trauma 2018;10:319-26.
7. Ullman SE, Peter-Hagene LC, Relyea M. Coping, emotion regulation, and self-blame as mediators of sexual abuse and psychological symptoms in adult sexual assault. J Child Sex Abus 2014;23:74-93.
8. Langevin R, Hébert M, Cossette L. Emotion regulation as a mediator of the relation between sexual abuse and behavior problems in preschoolers. Child Abuse Negl 2015;46:16-26.
9. Mueller I, Tronick E. Early life exposure to violence: developmental consequences on brain and behavior. Front Behav Neurosci 2019;13:156.
10. Hébert M, Langevin R, Oussaïd E. Cumulative childhood trauma, emotion regulation, dissociation, and behavior problems in school-aged sexual abuse victims. J Affect Disord 2018;225:306-12.
11. Compas BE, Jaser SS, Bettis AH, Watson KH, Gruhnet MA, Dunbar JP, et al. Coping, emotion regulation, and psychopathology in childhood and adolescence: a meta-analysis and narrative review. Psychol Bull 2017;143:939-91.
12. Duy B, Yildiz A. [Adaptation of the regulation of emotions questionnaire (REQ) for Adolescents]. Türk Psikolojik Danışma ve Rehberlik Dergisi 2014;5:23-35. [Article in Turkish]
13. Baş AU, Yurdabakan I. Factor structure of the reactive-proactive aggression questionnaire in Turkish children and gender, grade-level, and socioeconomic status differences in reactive and proactive aggression. J Psychoeduc Assess 2012;30:284-97.
14. Kulu M, Özsoy F, Baykara S. [The effect of regular exercise on self-esteem in addiction]. Anadolu Psikiyatri Derg 2018;19:244-9. [Article in Turkish]
15. Thornback K, Muller RT. Relationships among emotion regulation and symptoms during trauma-focused CBT for school-aged children. Child Abuse Negl 2015;50:182-92.
16. Walsh K, DiLillo D, Scalora MJ. The cumulative impact of sexual revictimization on emotion regulation difficulties: an examination of female inmates. Violence Against Women. 2011;17:1103-18.
17. Berzenski SR. Distinct emotion regulation skills explain psychopathology and problems in social relationships following childhood emotional abuse and neglect. Dev Psychopathol 2019;31:483-96.
18. Wolff JC, Thompson E, Thomas SA, Nesi J, Bettis AH, Ransford B, et al. Emotion dysregulation and non-suicidal self-injury: a systematic review and meta-analysis. Eur Psychiatry 2019;59:25-36.
19. Choi JY, Oh KJ. Cumulative childhood trauma and psychological maladjustment of sexually abused children in Korea: Mediating effects of emotion regulation. Child Abuse Negl 2014;38:296-303.
20. Paulus FW, Ohmann S, Möhler E, Plener P, Popow C. Emotional dysregulation in children and adolescents with psychiatric disorders. A narrative review. Front Psychiatry 2021;12:628252.
21. Trask EV, Walsh K, Dilillo D. Treatment effects for common outcomes of child sexual abuse: a current meta-analysis. Aggress Violent Behav 2011;16):6-19.
22. Norton-Baker M, Wolff JM, Kolander TW, Evans M, King AR. Childhood sexual abuse and lifetime aggression. J Child Sex Abus 2019;28:690-707.
23. Gauthier-Duchesne A, Hébert M, Daspe MÈ. Gender as a predictor of posttraumatic stress symptoms and externalizing behavior problems in sexually abused children. Child Abuse Negl 2017;64:79-88.
24. Ford JD, Fraleigh LA, Connor DF. Child abuse and aggression among seriously emotionally disturbed children. J Clin Child Adolesc Psychol 2010;39:25-34.
25. Putnam FW. Ten-year research update review: child sexual abuse. J Am Acad Child Adolesc Psychiatry 2003;42:269-78.
26. Önder FC, Ayazseven ÖC. [Aggression in adolescents: the role of emotion regulation and emotional coping]. Kastamonu Educ J 2020;28:1198-208. [Article in Turkish]
27. Miles SR, Tharp AT, Stanford M, Sharp C, Menefee D, Kent TA. Emotion dysregulation mediates the relationship between traumatic exposure and aggression in healthy young women. Pers Individ Dif 2015;76:222-7.
28. Donahue JJ, Goranson AC, McClure KS, van Male LM. Emotion dysregulation, negative affect, and aggression: a moderated, multiple mediator analysis. Pers Individ Dif 2014;70:23-8.
29. Bowie BH. Understanding the gender differences in pathways to social deviancy: relational aggression and emotion regulation. Arch Psychiatr Nurs 2010;24:27-37.
30. Esmaeili L, Aghaei A, Abedi MR, Esmaeili M, Aghaei Ml. The efficacy of emotional regulation on the aggression of epileptic girls (14-18) in Isfahan. Procedia Soc Behav Sci 2012;46:2183-7.
31. Davidson RJ, Putnam KM, Larson CL. Dysfunction in the neural circuitry of emotion regulation--a possible prelude to violence. Science 2000;289:591-4.
32. Kim BN, Park S, Park MH. The relationship of sexual abuse with self-esteem, depression, and problematic internet use in Korean adolescents. Psychiatry Investig 2017;14:372-5.
33. Ferraz L, Portella MJ, Vállez M, Gutierrez F, Martin-Blanco

A, Martin-Santo R, et al. Hostility and childhood sexual abuse as predictors of suicidal behaviour in Borderline Personality Disorder. Psychiatry Res 2013;210:980-5.
34. Chaplo SD, Kerig PK, Bennett DC, Modrowski CA. The roles of emotion dysregulation and dissociation in the association between sexual abuse and self-injury among juvenile justice–involved youth. J Trauma Dissociation 2015;16:272-85.

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The effect of nasal steroids on retinal nerve fiber layer in patients with a family history of glaucoma

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ABSTRACT

Objectives: We aimed at analyzing the effect of nasal steroids on intraocular pressure (IOP) and retinal nerve fiber layer thickness (RNFL) in patients with a family history of glaucoma who also use fluticasone propionate (FP group) and mometasone furoate (MF group).

Methods: Patients with a family history of glaucoma and suitable for using nasal steroids were included in the study population. IOP, anterior chamber depth (ACD), axial length (AL) and central corneal thickness (CCT) and RNFL thickness measurements of the patients were carried out. Measurements were done on 3 levels, namely, one before starting the medication, the other 1 month after starting the medication and the last one 3 months after the medication. 3 groups were established in our study: patients who are using MF group and FP group and also C group (control group; healthy individuals who have a family history of glaucoma but not using any medication).

Results: The average age of patients in our study who were under medication was 33.2 ± 8.9 years. The study consisted of a total of 46 patients, 32 of whom were using nasal steroids and 14 belonging to the C group. It was found that global value in MF group decreased from 100.9 ± 7.7 to 99.6 ± 7.6 in the 3rd month and ACD in MF group decreased from 3.2 ± 0.4 mm to 2.9 ± 0.4 mm in the 1st month, both to be found statistically significant (p = 0.037 and p = 0.001 respectively). During the RNFL thickness measurements of patients, it was found that Temporal (T) segment in FP group decreased from 82.1 ± 13.8 to 81.7 ± 13.3 in the first month and T segment in MF group decreased from 72.8 ± 12.0 to 71.3 ± 10.2 in 3 months, both decreases to be found statistically significant (p = 0.047 and p = 0.003 respectively). It was found that IOP in FP group increased from 15.3 ± 3.6 mm Hg to 17.7 ± 4.1 mm Hg in the 3rd month hence found to be statistically significant (p = 0.006). CCT in FP patients was found to be significantly higher in the 3rd month (p = 0.025).

Conclusions: As a result of our study, it was found that nasal steroid usage in patients with a family history of glaucoma may cause an increase in IOP and thinning of the RNFL.

Keywords: Mometasone furoate, fluticasone propionate, intraocular pressure, retinal nerve fiber layer thicknes

Corticosteroids have been used in allergic rhinitis treatment first in 1950 in systemic and then in 1974 in intranasal topical form. Today, corticosteroids in the form of intranasal sprays are being used for various diseases such as allergic asthma, allergic rhinitis and nasal polyposis. The most commonly used second

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generation topical steroids are mometasone furoate (MF), budesonide, beclomethasone dipropionate (BP), fluticasone propionate (FP) and triamcinolone. Nasal congestion, nasal flow, sneezing and respiratory disorder complaints, which are typical symptoms of allergic rhinitis decline upon use of these molecules [1-4]. Among the nasal steroids, MF and FP are synthetic glucocorticosteroids [2-5].

In healthy people intraocular pressure (IOP) varies between 11-21 mm Hg based on the balance between production and drainage of aqueous humour. IOP has a diurnal rhythm, where values are higher in the mornings and relatively low in the afternoons and night time. In healthy individuals these fluctuations are lower than 5 mm Hg. Although the upper limit of normal IOP is 21 mm Hg, rim loss or visual field loss in the optic disc can be seen in cases of normotensive glaucoma even with values lower than 21 mm Hg. Steroids happen to be one of the external factors that might defect this balance. The changes in IOP depends on the way steroids are being used as well as their structure, duration of use and also the thickness of the cornea. Besides that, family history of glaucoma is the most important personal risk factor. It is suggested that even a low potent steroid may cause changes in the intraocular pressure within an average of 3-4 weeks of use [2, 5-8]. Generally accepted "cut-off" value in order to identify the IOP increase due to steroid use is defined as; an increase of 6 mm Hg in IOP since the beginning of steroid use or a IOP measurement higher than 21 mm Hg either before or after steroid and a subsequent decrease in IOP when the medication is discontinued [9]. Vision loss is important since it is related to IOP increase. Ischemic and mechanic theories are valid in the etiology of the retinal nerve fiber damage in cases of glaucoma. Ischemia of the optic nerve head and vascular pathologies dominate the ischemic theory whereas in mechanic theory, emphasis is on the direct damaging of retinal nerve fibers by the elevated IOP. This results with ganglion cell death in retina which in return causes optic nerve head cupping. All these eventually lead to vision loss or even blindness [10-13].

The most common side effect of steroids used as both systemic and topical eye drops is elevated IOP. It is known that similar side effect is seen during the use of steroids through intranasal or inhalation channels [10]. However, no previous study was done where the use of intranasal FP and MF medications on healthy individuals with no family history of glaucoma were compared. Therefore, in our study, we aimed to analyze the effect of nasal steroid treatment, both FP and MF, on IOP and retinal nerve fiber levels (RNFL) of patients who are diagnosed with nasal polyposis and allergic rhinitis and have a family history of glaucoma.

METHODS

In this prospective study, a total of 32 patients and 14 control patients using FP and MF nasal steroids were analyzed. Our study was conducted with the cooperation of clinic of ophthalmology and the clinic of otolaryngology, and all stages of the study were carried out in accordance with the Declaration of Helsinki. Düzce University, Medical Faculty Ethic Council approval was granted (Ethic Council number 20-15-37). Each patient was informed in detail before the study and related consent forms were approved. Patients who were found appropriate to use nasal steroids for at least 3 months due to their nasal polyposis or allergic rhinitis and had a family history of glaucoma were diverted to ophthalmology by the clinic of otolaryngology. Out of the total 49 patients, 18 patients were excluded from the study because 16 of them did not show up for the controls and 2 of them did not use the medication on a routine basis. All required measurements were completed before the medication.

Patients with a history of eye or nose surgery, retina or disc disease, smoking or drinking habit, systemic disease such as diabetes or hypertension, an intraocular pressure of 21 mm Hg or higher, amblyopia, diplopia and refractive defect of 1,5 D or higher as well as patients who are using systemic medication for the past 6 months due to allergies or using contact lenses were not included to the study. Right eye is used for all measurements. It is suggested that including both eyes of the patient into the study is not statistically appropriate considering the positive correlation of the results [14]. A single experienced ophthalmologist performed all the measurements in order to avoid any discrepancies. Each patient went through a detailed eye examination where corrected best visual acuity, dilate fundus examination with 90 (D) lens, IOP with Goldman applanation tonometry (GAT)

(mmHg), anterior chamber depth (ACD) (mm), axial length (AL) (mm), central cornea thickness (CCT) (μm) and RNFL(μm) assessment measurements were done on 3 levels, namely, one before starting the medication, the other 1 month after starting the medication and the last one 3 months after the medication. Patients were fully informed about the measurement method before the GAT measurement process where one drop of 0.5% proparacaine hydrochloride was applied to the eye and lower lid fornix was touched with the fluoresceine paper followed by asking the patient to look right across and while the cobalt blue light was in 60 degrees angle position, the knob on the device was turned by using the GAT mounted onto the biomicroscope until the inner sections of all circles were overlapping. IOP was then calculated by multiplying the result by 10 mmHg. This process was repeated for 3 times and the mean of all 3 was taken as the final result. (Considering that IOP might differ among the groups and individuals based on their CCT, "corrected IOP" values according to the CCTs were taken into account). ACD, AL and CCT measurements were done with Echoscan US 500 system (Nidek Co., Ltd., Aichi, Japan) device whereas retinal nerve fiber layer measurements were done with spectral domain optic coherence device (SD-OCT, Heildelberg Engineering, Heildelberg, Germany). All measurements were done between the hours of 9.00-11.00 in order to avoid any diurnal discrepancies. RNFL thickness was assessed through 7 points by manually drawing the optic disc outline (μm). RNFL thickness measurements were defined as global (G), temporal (T), superotemporal (ST), superonasal (SN), nasal (N), inferotemporal (IT) and inferonasal (IN). (Fig. 1). Reference points were adjusted during the first measurements where "register" specification of the device was used and recording was done after controlling that the reference line passes through the center of macula. Progression analysis was performed in the consecutive measurements by means of the device specifications hence preventing any inter measurement errors. All measurements were repeated for 3 times in order to avoid bias and reference points were taken into account in repeated measurements, entering the data based on the assumption that these reference points were correct. Segmentation of all shots were done by someone familiar with OCT reading. Ultimately, the mean of all 3 measurements were taken hence, securing the standardization in measurements.

3 groups were established in our study: (1) MF group: patients using MF; (2) FP group: patients using FP; and (3) C group: control group consisting of healthy individuals who are not on medication but have a family history of glaucoma.

Statistical Analysis

The results of the study were analyzed by SPSS 25.0 version software program. The distribution of data was displayed by descriptive analysis parameters (mean, standard deviation, minimum, maximum, frequency and percentage). Kolmogorov-Smirnov test was employed to analyze whether the data is consistent with the normal distribution. In comparison of the dependent groups based on time variable, ANOVA test was used for repeated measurements. In comparison of means between more than two independent groups, ANOVA test was used for data distributed evenly and Kruskal Wallis H Test was used for data not distributed evenly. In case any discrepancies were found in ANOVA and Kruskal Wallis H Test, Bonferroni Post Hoc test was employed in order to identify the group which was causing the discrepancy.

There were no previous studies on the effect of MF and FP medication on RNFL therefore it was not possible to calculate the sample size based on literature. However, an appropriate sample size was taken with respect to Cohen's description of standardized effect size as medium and large. When 0.3 was taken as the medium size standardized effect size and (α) 5% as risk of making a Type 1 error and (1-ß) %80 as power of the test, it was found out that 34 samples should be in the MF and FP medication groups. But when effect size was taken as 0.8 which is large standardized effect size, 16 samples were sufficient for the MF and FP medication groups [15].

There are three groups in the study as Fluticasone, Mometozone and Control groups. In the evaluation of the difference between the three groups as in the literature; In case the data are normally distributed, one-way analysis of variance (ANOVA), which is the parametric test, is used, and if the data is not normally distributed, the nonparametric Kruskal Wallis H test is used. The Mann Whitney U test requested by me is a nonparametric test used in comparisons between 2 groups and is not suitable for this study. If a significant difference is found as a result of one-way analysis of

variance and Kruskal Wallis H Test, the groups causing the difference are determined with the Posthoc test. In our study, the Bonferroni Posthoc test was used because the variances were homogeneous between the groups.

RESULTS

Table 1 shows the demographic data of the patients. The mean age of the patients in our study which are under medication is 33.2 ± 8.9 years. Our study consisted of 46 patients, 20 (43.5%) male and 26 (56.5%) female, which were distributed as 16 in MF group, 16 in FP group and 14 in C group.

Changes in FP, MF and C groups over time are shown in Table 2. It was found that global value in MF group decreased from 100.9 ± 7.7 to 99.6 ± 7.6 in the 3rd month and ACD in MF group decreased from 3.2 ± 0.4 mm to 2.9 ± 0.4mm in the 1st month, both to be found statistically significant (p = 0.037 and p = 0.001 respectively). According to the results, T segment of FP group during RNFL thickness measurements before the medication are higher than the MF group in a statistically significant level (before medication, p = 0.023). During the RNFL thickness measurements of patients, it was found that Temporal (T) segment in FP group decreased from 82.1 ± 13.8 to 81.7 ± 13.3 in the first month and T segment in MF group decreased from 72.8 ± 12.0 to 71.3 ± 10.2 in 3 months, both decreases to be found statistically significant (p = 0.047 and p = 0.003 respectively).

When Table 2 is reviewed, it was found that IOP in both MF and FP groups have increased in the 1st and 3rd months but this increase was not statistically significant. On the other hand, there was no increase in the control group. IOP in the FP group increased from 15.3 ± 3.6 mm Hg to 17.7 ± 4.1 mmHg in the 3rd month hence found to be statistically significant (p = 0.006; Table 3).

It was found that CCT of patients using FP was higher than the ones using MF in a statistically significant level (p = 0.06). CCT of patients using FP were found to be higher in the 3rd month compared to the ones using MF in a statistically significant level (p = 0.025). (IOP values adjusted to CCT was taken into consideration during IOP measurements of FP group since these patients start with an already high CCT which might affect IOP).

At the end of first month, it was found that AL of MF group were lower than the ones in C group in a statistically significant level (p = 0.033). At the 3rd of medication, AL parameter in FP and MF groups were found to be lower than C group in a statistically significant level (p = 0.013). As far as the other parameters are concerned, no further differences were found within the groups in a statistically significant level. Furthermore, no differences were found in AL parameter within the groups over time. In other words, when table was interpreted in terms of basal values of groups, it was found that AL value didn't show any statistically significant difference.

Table 1. Demographic specifications

	Data
Age (years) Mean ± SD (min-max)	**33.2 ± 8.9 (20-50)**
Gender, n (%)	
Male	20 (43.5)
Female	26 (56.5)
Group, n (%)	
Fluticasone	16 (34.8)
Mometasone	16 (34.8)
Control Group	14 (30.4)

DISCUSSION

Nasal steroids which are commonly used by people who have allergic asthma or allergic rhinitis reach eyes after being used, even before they are broken down in the kidneys or liver [16]. The effect of systemic use of steroids on the intraocular pressure is much less compared to the topical use of steroid eye drops. However, the rate of steroid use is quite high due to the high number of allergic asthma or allergic rhinitis patients, therefore, steroid-IOP relation is important [16]. Diverse theories are suggested for the mechanism of high IOP or glaucoma as a result of steroid use. Steroids cause electrolyte imbalance after binding to the steroid receptors in the cells of trabecular meshwork. Even-

Table 2. The alteration of parameters over time

	Group	Time			F	*p value*
		Premedication	1st month	3rd month		
		Mean ± SD	Mean ± SD	Mean ± SD		
T (µm)	**Fluticasone**	82.1 ± 13.8	81.7 ± 13.3	84.1 ± 13.9	0.238	0.790
	Mometasone	70.8 ± 9.5	72.8 ± 12.0	71.3 ± 10.2	1.033	0.349
	Control	71.6 ± 9.4	71.6 ± 9.4	69.2 ± 11.5	1.125	0.340
ST (µm)	**Fluticasone**	140.5 ± 29.0	150.5 ± 15.6	150.4 ± 16.8	1.736	0.205
	Mometasone	145.4 ± 15.9	145.1 ± 15.8	146.1 ± 16.2	0.423	0.659
	Control	140.1 ± 14.7	140.1 ± 14.7	139.4 ± 14.6	10.848	0.458
SN (µm)	**Fluticasone**	117.3 ± 17.5	116.4 ± 14.5	115.5 ± 20.1	11.267	0.146
	Mometasone	114.5 ± 19.1	115.2 ± 20.2	115.4 ± 19.0	3.396	0.271
	Control	113.5 ± 22.4	113.5 ± 22.4	113.6 ± 20.7	0.024	0.214
N (µm)	**Fluticasone**	77.8 ± 17.0	77.5 ± 15.3	74.7 ± 21.1	42.467	0.397
	Mometasone	74.9 ± 13.2	74.0 ± 14.7	75.2 ± 14.6	6.271	0.385
	Control	78.4 ± 15.4	78.4 ± 15.4	79.2 ± 15.2	3.090	0.063
IN (µm)	**Fluticasone**	104.4 ± 25.6	104.1 ± 20.6	107.2 ± 27.7	43.289	0.184
	Mometasone	112.6 ± 13.8	110.3 ± 16.4	113.2 ± 16.2	54.163	0.912
	Control	109.1 ± 20.8	109.1 ± 20.8	108.9 ± 20.3	0.214	0.218
IT (µm)	**Fluticasone**	142.3 ± 38.2	154.1 ± 23.4	150.9 ± 25.8	1.131	0.314
	Mometasone	142.4 ± 17.2	143.3 ± 17.7	142.5 ± 17.1	1.016	0.374
	Control	140.6 ± 11.8	140.6 ± 11.8	137.4 ± 14.0	0.830	0.447
G (µm)	**Fluticasone**	101.4 ± 14.9	105.3 ± 9.3	97.6 ± 25.5	0.750	0.442
	Mometasone	100.7 ± 7.3	100.9 ± 7.7	99.6 ± 7.6	4.531	**0.037**
	Control	99.1 ± 9.4	99.1 ± 9.4	92.1 ± 24.1	1.119	0.342
IOP (mmHg)	**Fluticasone**	15.3 ± 3.6	16.9 ± 3.7	17.7 ± 4.1	3.076	0.062
	Mometasone	14.8 ± 3.8	15.1 ± 3.9	15.5 ± 3.2	0.650	0.529
	Control	14.5 ± 1.0	14.0 ± 1.8	13.7 ± 1.9	1.049	0.365
CCT (µm)	**Fluticasone**	565.3 ± 48.2	562.4 ± 51.1	563.5 ± 52.2	0.092	0.779
	Mometasone	528.4 ± 40.6	518.1 ± 42.1	521.9 ± 43.3	1.710	0.198
	Control	546.3 ± 18.2	546.4 ± 18.1	546.3 ± 18.2	1.000	0.336
ACD (mm)	**Fluticasone**	3.2 ± 0.4	3.1 ± 0.4	3.0 ± 0.5	2.996	0.066
	Mometasone	3.2 ± 0.4	2.9 ± 0.4	2.9 ± 0.4	9.688	**0.001**
	Control	3.0 ± 0.5	3.0 ± 0.5	3.0 ± 0.5	0.204	0.817
AL (mm)	**Fluticasone**	22.4 ± 0.8	22.3 ± 0.8	22.3 ± 0.9	0.253	0.778
	Mometasone	22.3 ± 0.9	22.2 ± 0.8	22.1 ± 0.8	2.597	0.091
	Control	22.8 ± 0.7	22.8 ± 0.7	23.0 ± 0.7	2.125	0.140

IOP = Intraocular Pressure, ACD = Anterior Chamber Depth, AL = Axial Length, CCT = Central Cornea Thickness, RNFL = Retinal Nerve Fiber Level Thickness, G = Global, T = Temporal, ST = Superotemporal, SN = Superonasal, N = Nasal, IT = Inferotemporal, IN = Inferonasal, SD = standard deviation

Table 3. The assessment of the parameter differences within the 3 groups

	Time	Group			F/X2	*p value*	Difference
		Fluticasone Mean ± SD	Mometasone Mean ± SD	Control Mean ± SD			
T (µm)	**Premed.**	82.1 ± 13.8	70.8 ± 9.5	71.6 ± 9.4	7.564	**0.023**	FP-MF
	1st month	81.7 ± 13.3	72.8 ± 12.0	71.6 ± 9.4	3.280*	**0.047**	FP-MF FP- Control
	3rd month	84.1 ± 13.9	71.3 ± 10.2	69.2 ± 11.5	6.711*	**0.003**	FP-MF FP- Control
ST (µm)	**Premed.**	140.5 ± 29.0	145.4 ± 15.9	140.1 ± 14.7	0.843	0.656	-
	1st month	150.5 ± 15.6	145.1 ± 15.8	140.1 ± 14.7	4.045	0.132	-
	3rd month	150.4 ± 16.8	146.1 ± 16.2	139.4 ± 14.6	3.085	0.214	-
SN (µm)	**Premed.**	117.3 ± 17.5	114.5 ± 19.1	113.5 ± 22.4	0.806	0.958	-
	1st month	116.4 ± 14.5	115.2 ± 20.2	113.5 ± 22.4	0.149	0.928	-
	3rd month	115.5 ± 20.1	115.4 ± 19.0	113.6 ± 20.7	0.275	0.872	-
N (µm)	**Premed.**	77.8 ± 17.0	74.9 ± 13.2	78.4 ± 15.4	0.229*	0.796	-
	1st month	77.5 ± 15.3	74.0 ± 14.7	78.4 ± 15.4	0.361*	0.699	-
	3rd month	74.7 ± 21.1	75.2 ± 14.6	79.2 ± 15.2	0.298*	0.744	-rd
IN (µm)	**Pre-med**	104.4 ± 25.6	112.6 ± 13.8	109.1 ± 20.8	0.624*	0.540	-
	1st month	104.1 ± 20.6	110.3 ± 16.4	109.1 ± 20.8	0.441*	0.646	-
	3rd month	107.2 ± 27.7	113.2 ± 16.2	108.9 ± 20.3	0.310*	0.735	-
IT (µm)	**Pre-med**	142.3 ± 38.2	142.4 ± 17.2	140.6 ± 11.8	1.634	0.442	-
	1st month	154.1 ± 23.4	143.3 ± 17.7	140.6 ± 11.8	2.237*	0.119	-
	3rd month	150.9 ± 25.8	142.5 ± 17.1	137.4 ± 14.0	1.757*	0.185	-
G (µm)	**Pre-med**	101.4 ± 14.9	100.7 ± 7.3	99.1 ± 9.4	0.170*	0.844	-
	1st month	105.3 ± 9.3	100.9 ± 7.7	99.1 ± 9.4	1.916*	0.160	-
	3rd month	97.6 ± 25.5	99.6 ± 7.6	92.1 ± 24.1	1.621	0.445	-
IOP (mmHg)	**Premed.**	15.3 ± 3.6	14.8 ± 3.8	14.5 ± 1.0	0.466	0.792	-
	1st month	16.9 ± 3.7	15.1 ± 3.9	14.0 ± 1.8	2.760*	0.075	-
	3rd month	17.7 ± 4.1	15.5 ± 3.2	13.7 ± 1.9	5.705*	**0.006**	FP-Control
CCT (µm)	**Pre-med**	565.3 ± 48.2	528.4 ± 40.6	546.3 ± 18.2	3.610*	**0.036**	FP-MF
	1st month	562.4 ± 51.1	518.1 ± 42.1	546.4 ± 18.1	5.445	0.066	-
	3rd month	563.5 ± 52.2	521.9 ± 43.3	546.3 ± 18.2	4.051*	**0.025**	FP-MF
ACD (mm)	**Premed.**	3.2 ± 0.4	3.2 ± 0.4	3.0 ± 0.5	1.083*	0.348	-
	1st month	3.1 ± 0.4	2.9 ± 0.4	3.0 ± 0.5	0.562*	0.574	-
	3rd month	3.0 ± 0.5	2.9 ± 0.4	3.0 ± 0.5	0.355*	0.703	-
AL (mm)	**Premed.**	22.4 ± 0.8	22.3 ± 0.9	22.8 ± 0.7	4.644	0.098	-
	1st month	22.3 ± 0.8	22.2 ± 0.8	22.8 ± 0.7	6.796	**0.033**	MF- Control
	3rd month	22.3 ± 0.9	22.1 ± 0.8	23.0 ± 0.7	4.788*	**0.013**	FP- Control MF- Control

IOP = Intraocular Pressure, ACD = Anterior Chamber Depth, AL = Axial Length, CCT = Central Cornea Thickness, RNFL = Retinal Nerve Fiber Level Thickness, G = Global, T = Temporal, ST = Superotemporal, SN = Superonasal, N = Nasal, IT = Inferotemporal, IN = Inferonasal, Premed = premedication, SD = standard deviation

tually, mucopolysaccharide accumulates on trabecular meshwork and results in vasoconstriction of the episcleral veins. It is considered that this impact mechanism may lead to IOP increase [17].

There is no clear consensus in the literature related to the effect of nasal steroids on the IOP. Some studies show that the IOP increases whereas, some show that there was no alteration at all. Nasal steroids access the eye in 2 ways; namely direct access and indirect access. In direct access, metabolite absorbed from nasal mucosa reach and effect the final organ before it is broken down whereas in indirect access, steroids absorbed from the gastrointestinal system are broken down in liver hence a limited amount of active metabolite reaches the final organ. Various studies suggest that it reaches the eyes through both ways, causing an increase in IOP [17-19].

Some studies in literature suggest that BP increases IOP [12, 20, 21]. On the other hand, it was found that IOP did not change in the studies made by Öztürk *et al*. [18]with budesonide nasal spray and BP, Martino *et al*. [19] with dexamethasone and Yuen *et al*. [20] with beclomethasone. As a result of their 18-patient study with intranasal budesonide irrigation, Seiberling *et al*. [22] found that there was no increase in IOP with the exception of one patient.22 In their study with FP, MF and BP, Mohd Zain *et al*. [23] found that, similar to our study, IOP of the group under medication was higher in a statistically significant level when compared to the C group. Şimşek *et al*. [17], in their study with FP and MF users, found no statistically significant difference between two groups throughout the 24 week period. However, in our study, IOP of the patients under FP medication were found to be higher than the ones in C group in the 3rd month. Bross-Soriano *et al*. [24] who has used similar active agents as our study, found that there was no significant difference in IOP. However, the findings may naturally differ since not only the steroids being used but the reaction of the patients in each study also may vary hence effecting the results [25].

Only one single study related to the RNFL thickness measurement for the similar age group was found in the literature. In their study covering patients who are using nasal steroids, Marzouki *et al*. [26] found no statistically significant difference between the RNFL thickness and IOP values. The study generally based the comparisons on corticosteroids (patients under FP and MF medication have been analyzed in our study). Although the study was different from ours since it did not include the analysis of sub-segments of the RNFL, they both were still similar with respect to the final results. Furthermore, there was no difference in our studies in terms of C group with respect to the glaucoma optic disc values. The difference in our study was the variance in T segment of RNFL at the beginning of the study. We believe that this variance is the outcome of relatively small number of the study group as well as intragroup differences.

In our study, we found out significant differences in terms of CCT over time. Not only there were no studies in the literature on this subject covering the similar age group but the number of studies made on cornea was also very limited. In their studies conducted with children, Özkaya *et al*. [27] and Alsaadi *et al*. [7], similar to our study, suggested that there were no significant differences. We believe that the effect was not efficient since the steroid doses taken as nasal were insufficient to initiate any effect on the receptors of cornea.

In our study, we found that there was no statistical difference in terms of ACD parameter between 3 groups but there was a decrease in the MF group over time. We found that there was no significant difference in terms of AL in between 3 groups but over time, the AL parameter of MF group was lower than the C group in statistically significant level in 1st month and the AL parameters of the FP and MF groups were lower than the C group in statistically significant level in the 3rd month. Although no comparisons were done since there were no studies on ACD and AL in the literature, we assumed that the difference is the outcome of relatively small number of the study group as well as the variances among the groups in the beginning.

The strong aspect of our study is, being the first study in literature in terms of RNFL, ACD, AL and CCT with patients within the analyzed age group and who are using FP and MF and have a family history of glaucoma. On the other hand, the relatively limited number of patient group as well as relatively short monitoring period were the restricting aspects.

CONCLUSION

In our study, we analyzed adult individuals who do not

have systemic or ocular diseases but using FP and MP, in terms of IOP, RNFL thickness, ACD, CCT and AL. As a result of our study, we learned that FP and MF nasal steroids can be used safely for glaucoma in the short term, but the risk increases when the duration of use is prolonged. Therefore, if individuals with a family history of glaucoma are subject to long-term medication, we suggest that they continue their treatment under ophthalmologist control. Furthermore, an additional study with a high number of participants and a longer monitoring time would enable to reach a much more accurate, precise and reliable review.

Authors' Contribution

Study Conception: MTE; Study Design: MTE; Supervision: MTE, İÜ; Funding: İÜ; Materials: İÜ; Data Collection and/or Processing: İÜ, MTE; Statistical Analysis and/or Data Interpretation: MTE; Literature Review: MTE; Manuscript Preparation: MTE, İÜ and Critical Review: İÜ, MTE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Baroody FM. Allergic rhinitis: broader disease effects and implications for management. Otolaryngol Head Neck Surg 2003;128:616-31.
2. Naclerio RM, Baroody FM, Bidani N, De Tineo M, Penney BC. A comparison of nasal clearance after treatment of perennial allergic rhinitis with budesonide and mometasone. Otolaryngol Head Neck Surg 2003;128:220-7.
3. Salib RJ, Drake-Lee A, Howarth PH. Allergic rhinitis: past, present and the future. Clin Otolaryngol Allied Sci 2003;28:291-303.
4. Mygind N, Lund V. Intranasal corticosteroids for nasal polyposis : biological rationale, efficacy, and safety. Treat Respir Med 2006;5:93-102.
5. Man LX, Farhood Z, Luong A, Fakhri S, Feldman RM, Orlander PR, et al. The effect of intranasal fluticasone propionate irrigations on salivary cortisol, intraocular pressure, and posterior subcapsular cataracts in postsurgical chronic rhinosinusitis patients. Int Forum Allergy Rhinol 2013;3:953-7.
6. Bross Soriano D, Arrieta Gómez JR, Schimelmitz Idi J, Guzmán Urrutia R, Hanenberg Milver C. [Changes in intraocular pressure with the use of topical nasal mometasone furoate]. Rev Alerg Mex 2000;47:12-6. [Article in Spanish]
7. Alsaadi MM, Osuagwu UL, Almubrad TM. Effects of inhaled fluticasone on intraocular pressure and central corneal thickness in asthmatic children without a family history of glaucoma. Middle East Afr J Ophthalmol 2012;19:314-9.
8. Desnoeck M, Casteels I, Casteels K. Intraocular pressure elevation in a child due to the use of inhalation steroids--a case report. Bull Soc Belge Ophtalmol 2001;280:97-100.
9. Nuyen B, Weinreb RN, Robbins SL. Steroid-induced glaucoma in the pediatric population. J AAPOS 2017;21:1-6.
10. Kersey JP, Broadway DC. Corticosteroid-induced glaucoma: a review of the literature. Eye (Lond) 2006;20:407-416.
11. Halpern DL, Grosskreutz CL. Glaucomatous optic neuropathy: mechanisms of disease. Ophthalmol Clin North Am 2002;15:61-68.
12. Mitchell P, Cumming RG, Mackey DA. Inhaled corticosteroids, family history, and risk of glaucoma. Ophthalmology 1999;106:2301-6.
13. Rolle T, Dallorto L, Briamonte C, Penna RR. Retinal nerve fibre layer and macular thickness analysis with Fourier domain optical coherence tomography in subjects with a positive family history for primary open angle glaucoma. Br J Ophthalmol 2014;98:1240-4.
14. Zhang HG, Ying GS. Statistical approaches in published ophthalmic clinical science papers: a comparison to statistical practice two decades ago. Br J Ophthalmol 2018;102:1188-91.
15. Sullivan GM, Feinn R. Using effect size-or why the P value is not enough. J Grad Med Educ 2012;4:279-82.
16. Taner P, Bayar N, Akarsu C, Koç C. [Effect of topical nasal corticosteroids used for the treatment of allergic rhinitis to the intraocular pressure]. KBB ve BBC Dergisi 2001;9:21-4. [Article in Turkish]
17. Şimşek A, Bayraktar C, Doğan S, Karataş M, Sarıkaya Y. The effect of long-term use of intranasal steroids on intraocular pressure. Clin Ophthalmol 2016;10:1079-82.
18. Oztürk F, Yücetürk AV, Kurt E, Unlü HH, Ilker SS. Evaluation of intraocular pressure and cataract formation following the long-term use of nasal corticosteroids. Ear Nose Throat J 1998;77:846-848.
19. Martino BJ, Church CA, Seiberling KA. Effect of intranasal dexamethasone on endogenous cortisol level and intraocular pressure. Int Forum Allergy Rhinol 2015;5:605-9.
20. Yuen D, Buys YM, Jin YP, Alasbali T, Trope GE. Effect of beclomethasone nasal spray on intraocular pressure in ocular hypertension or controlled glaucoma. J Glaucoma 2013;22:84-7.
21. Opatowsky I, Feldman RM, Gross R, Feldman ST. Intraocu-

lar pressure elevation associated with inhalation and nasal corticosteroids. Ophthalmology 1995;102:177-9.
22. Seiberling KA, Chang DF, Nyirady J, Park F, Church CA. Effect of intranasal budesonide irrigations on intraocular pressure. Int Forum Allergy Rhinol 2013;3:704-7.
23. Mohd Zain A, Md Noh UK, Hussein S, Che Hamzah J, Mohd Khialdin S, Md Din N. The relationship between long-term use of intranasal corticosteroid and intraocular pressure. J Glaucoma 2019;28:321-4.
24. Bross-Soriano D, Hanenberg-Milver C, Schimelmitz-Idi J, Arrieta-Gomez JR, Astorga del Toro R, Bravo-Escobar G. Effects of three nasal topical steroids in the intraocular pressure compartment. Otolaryngol Head Neck Surg 2004;130:187-91.
25. Maeng MM, De Moraes CG, Winn BJ, Glass LRD. Effect of topical periocular steroid use on intraocular pressure: a retrospective analysis. Ophthalmic Plast Reconstr Surg 2019;35:465-468.
26. Marzouki HZ, AlThomali RK, Hefni L, Almarzouki N, Alhejailli F, Merdad M, et al. Effect of intranasal corticosteroids on intraocular pressure and nerve fiber layer thickness: a cross-sectional study at a tertiary care hospital in Western Saudi Arabia. Cureus 2021;13:e13146.
27. Ozkaya E, Mete F, Ozsutcü M, samanci N, Cekiç O. Evaluation impact of long-term usage of inhaled fluticasone propionate on ocular functions in children with asthma. Steroids 2011;76:548-52.

The European Research Journal 2023;9(2):231-236 Original Article

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Routine postoperative nasogastric or nasojejunal tube placement may be unnecessary after gastric resection

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ABSTRACT

Objectives: The purpose of this study was to investigate the effects of not using routine nasogastric (NG) or nasojejunal (NJ) tubes on postoperative complications in gastric cancer patients undergoing resection.

Methods: This study includes 250 patients who underwent gastric resection diagnosed with gastric adenocarcinoma between November 2011 and December 2021. The patients were divided into two groups: those who routinely use NG or NJ tube in the early postoperative period and those who do not. Postoperative complications and length of hospital stay were compared between the two groups.

Results: Demographic, surgical, and histopathological characteristics were similar between the two groups. Oral feeding was started earlier in the non- NG or NJ tube group. There was no difference between the two groups regarding the length of hospital stay ($p = 0.065$). Severe postoperative complications (Clavien Dindo ≥ 3) were significantly lower in patients who did not use a nasogastric or nasojejunal tube ($p = 0.001$). Two patients in the NG/NJ tube group and one in the non-NG/NJ tube group developed anastomotic leakage.

Conclusions: According to the results of our study, routine NG or NJ tube use does not reduce postoperative severe complications or length of hospital stay.

Keywords: Gastric adenocarcinoma, gastric resection, nasogastric or nasojejunal tube, postoperative complications

Gastric cancer is the fifth most commonly diagnosed malignancy and the third leading cancer-related death in the world among cancer patients [1]. Curative treatment is gastric resection; however, perioperative mortality and morbidity rates are high after gastric cancer surgery [2].

In patients with gastric cancer, a nasogastric (NG) tube after subtotal gastrectomy and a nasojejunal (NJ) tube after total gastrectomy are routinely used in the early postoperative period. The purpose of using an NG or NJ tube is to provide decompression, prevent distension, and reduce the risk of aspiration due to nausea and vomiting. Reducing postoperative distention due to paralytic ileus improves anastomotic safety. Most surgeons routinely use NG/NJ tubes in the early postoperative period.

However, the use of NG or NJ tubes has recently been questioned. Postoperative NG/NJ tube use does not help with recovery after gastric cancer surgery, or other abdominal surgery does not decrease paralytic ileus or the rate of postoperative complications and has an influence on patient comfort [3, 4].

The aim of this study is to investigate the effect of not using an NG/NJ tube in the postoperative period

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on early postoperative complications in gastric resection patients.

METHODS

This study is planned retrospective research. Between November 2011 and December 2021, Prof. Dr. Cemil Tascıoglu City Hospital reviewed 250 patients who underwent gastric cancer surgery and were diagnosed with gastric adenocarcinoma. The study includes patients over the age of 18 who had histological gastric adenocarcinoma and were undergoing gastric resection. Unresectable patients were excluded. D1 or D2 lymph node dissection was performed on all patients [5]. Continuity of the intestine Roux-en-Y esophagojejunostomy was performed in total gastrectomy patients, while Roux-en-Y gastrojejunostomy was performed in subtotal gastrectomy patients. Patients were given postoperative subcutaneous low molecular weight heparin for deep venous thrombosis prophylaxis and short-term perioperative antibiotic therapy.

Between November 2011 and December 2016, NG or NJ tubes were used as a routine treatment in patients undergoing gastric cancer resection. This group was defined as the NG/NJ tube group. The group that did not use routine NG/NJ tubes was determined to be between January 2017 and December 2021. The routine NG/NJ tube was not used in the postoperative period on this date. Depending on the surgeon's option, the NG/NJ tube used was 16 F or 14 F thick with a single lumen.

The tube was removed when the drainage was reduced to 150 ccs in patients who used NG/NJ tubes. Patients were given oral nutrition after the tube was removed. Parenteral or enteral nutrition (via jejunostomy) was started in patients who could not take oral food within seven days, depending on the type of complication. In patients who did not have NG/NJ tubes, the oral clear liquid diet was started on the first postoperative day.

In cases of severe vomiting and distension, NG/NJ tubes were prolonged or reinserted in clinically required patients. During the postoperative period, the time to begin oral nutrition and the length of hospital stay were recorded.

The hospital registration system was used to acquire patients' age, gender, comorbidities, body mass index (BMI), gastrectomy type, lymph node dissection (D1/D2), operation time, and tumor stages. The AJCC Cancer Staging Manual, 8th edition, was used to establish the stage [6].

The study's goal is to assess the impact of not using NG/NJ decompression on early postoperative complications. The first 30-days following surgery were considered early complications in the postoperative period. Intra-abdominal infections, wound infections, cardiac problems, pulmonary complications, anastomotic complications, pancreatic fistula, chylous leaks, intra-abdominal and intraluminal bleeding, and NG/NJ tube-related complications were described as postoperative complications. The Clavien-Dindo (CD) classification was used to grade complications [7]. When patients had more than one complication, the higher grade according to the CD classification was accepted. Severe complications were defined as CD grade 3 and above (CD $\geq$ 3). The data was collected from prospectively stored patient records.

The Ethics Committee of the University of Health Sciences, Prof. Dr. Cemil Tascioglu City Hospital (2022/18) approved this retrospective study.

Statistical Analysis

The patients were divided into two groups based on whether or not they used NG/NJ tubes. The Statistical Package for Social Sciences for Windows version 22.0 was used for statistical analysis (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corp. Armonk, NY, USA). For each variable, descriptive statistics were calculated. The Kolmogorov-Smirnov test was used to determine the normality of the data distribution. The median [minimum-maximum] was used to represent the results of continuous variables that did not have a normal distribution. The chi-square test for categorical data was used to assess statistically significant differences between groups. Nonparametric statistics (Mann-Whitney U) were used for continuous variables. *P* - values < 0.05 were considered statistically significant.

RESULTS

There were 165 males and 74 females among the 239 patients. The NG/NJ group had a median age of 61 (33-87) years, while the non-NG/NJ group had a me-

dian age of 63 (29-94). Subtotal gastrectomy was performed in 87 (36.4 %) patients. In both groups, there was no significant difference in tumor stage, type of lymph node dissection (D1/D2), or length of hospital stay. Table 1 summarizes the clinicopathological parameters of patients.

Table 2 evaluates the surgical duration of tube decompression, resuming oral feeding time after tube removal, patients reinserted after tube removal, and complications associated with tube usage in patients using NG/NJ tube. The average duration to remove the tube was three days, and the postoperative diet was allowed in four days. There were 18 patients who experienced complications as a result of using an NG/NJ tube (24.4 %). Three patients' NG tubes were removed and reinserted due to tube occlusion. In 3 patients, the tube dislocated spontaneously and had to be reinserted. There were 12 patients who developed tube intolerance. Due to tube irritation, these patients suffered severe sore throats, which improved when the tube was removed. There were no major complications, such as perforation, as a result of tube use. The number of patients in the NG/NJ tube group who had a reinserted tube after the tube was removed was 10. There was no patient in the non-NG/NJ tube group who needed tube insertion during the postoperative clinical course.

Table 3 shows a comparison of postoperative complications between the two groups. One patient in the NG/NJ tube group and 1 in the non-NG/NJ tube group developed anastomotic leakage. In either group, no patient suffered a mechanical intestinal obstruction. In patients who did not have the NG/NJ tube, an oral clear diet was started on the first postoperative day. 2 patients in the NG/NJ group developed an intra-abdominal abscess. The non-NG/NJ group had a greater rate of chylous leakage (8.6 % vs. 1.3 %). Severe postoperative complications were more common in the NG/NJ group ($p = 0.001$). There were 4 postoperative mortality in the NG/NJ tube group. There was

Table 1. Clinicopathological characteristics of patients

Patient characteristics	NG/NJ group (n = 77)	Non-NG/NJ group (n = 162)	*p* value
Age (years)	61 (33-87)	63 (29-94)	0.324**
Sex (Female/Male)	24/53	50/112	0.962*
BMI (kg/m^2)	26 (20-43)	26 (18-40)	0.576**
Type of Resection			**< 0.001***
Total gastrectomy	34 (44.2 %)	18 (72.8 %)	
Subtotal gastrectomy	43 (55.8%)	44 (27.2 %)	
Type of Reconstruction			**< 0.001***
Roux-en-y gastrojejunostomy	43 (55.8%)	44 (72.8 %)	
Roux-en-y esophagojejunostomy	34 (44.2%)	118 (27.2 %)	
Type of Lenfadenectomy			0.36*
D 1	9 (11.7 %)	13 (8 %)	
D 2	68 (88.3 %)	149 (92%)	
TNM Staging			0.159*
I	21 (27.3 %)	42 (25.9 %)	
II	20 (26 %)	50 (30.9 %)	
III	36 (46.8 %)	62 (38.3 %)	
IV	0 (0 %)	8 (4.9 %)	
Operation time (min)	210 (180-330)	240 (120-400)	**0.002****
LOS (day)	7 (5-50)	7 (6-152)	0.065**

Data are given as median (min-max) or n (%). LOS = Length of hospital stay, *Chi-Square Test, **Mann-Whitney U Test

Table 2. Postoperative follow-up of patients in the NG/NJ group according to gastrectomy types

	Total Gastrectomy (n = 34)	Subtotal Gastrectomy (n = 43)	*p* value
NG/NJ tube usage time (days)	3 (1-36)	3 (1-49)	0.263**
Reinserted tube	4 (11.8%)	6 (14%)	0.527*
Oral starting diet time (day)	4 (3-36)	4 (3-49)	0.83**
Tube occlusion	2 (5.9%)	1 (2.3%)	> 0.05*
Spontaneous tube disloged	1 (2.9 %)	2 (4.7%)	> 0.05*
Tube intolerance	6 (17.6%)	6 (14%)	> 0.05*

Data are given as median (min-max) or n (%). *Chi-Square Test, **Mann-Whitney U Test

no difference between the two groups in terms of postoperative hospital stay ($p = 0.065$).

DISCUSSION

Following gastric resection, an NG or NJ tube is commonly used for decompression. Tube usage still continues in the postoperative period because of the prolonged operation time and the possibility of paralytic ileus. Some studies have demonstrated that using NG/NJ tubes is not required in the postoperative period, but many surgeons have been discouraged from abandoning this practice [4, 8]. Patients who did not use tubes were given an oral diet earlier in the postoperative period, and no one required NG/NJ tube de-

Table 3. Postoperative complications

	NG/NJ group (n = 77)	Non-NG/NJ group (n = 162)	All (n = 239)	*p* value
All complication	13 (16.9%)	50 (30.9%)	63 (26.4%)	**0.022***
Intra- abdominal abscess	0	2	2	
Anastomotic leakage	2	1	3	
Pancreatic leakage	3	0	3	
Roux necrosis	1	0	1	
Wound infection	3	17	20	
Myocardial infarction	1	0	1	
Pneumonia	1	5	6	
COPD exacerbations	0	1	1	
Pulmonary embolism	1	0	1	
Intraluminal hemorrhage	0	4	4	
İntraabdominal hemorrhage	0	6	6	
Chylous leakage	1	14	15	
Severe complications	8 (10.4%)	5 (3.1 %)	13 (5.4 %)	**< 0.001***
Mortality	4	0	4 (1.7 %)	**0.01***
LOS (days)	7 (5-50)	7 (6-152)	7 5-152)	0.065**

Data are given as median (min-max). LOS = Length of hospital stay, *Chi-Square Test, **Mann-Whitney U Test

compression. There are various potential complications with NG/NJ tube placement. It can induce gastric, esophageal, and nasal trauma in patients, as well as nasal irritation and discomfort. The tube's inflammation can cause pharyngitis, sinusitis, or, in rare cases, nasal ala necrosis and otitis by blocking the eustachian canal [9]. It has the potential to cause aspiration and aspiration pneumonia by affecting the function of the epiglottis [10]. No serious complications associated with using an NG/NJ tube were detected in this study. NG/NJ group experiences delayed oral intake, and the patient suffers greatly from tube discomfort.

In this study, chylous leakage was higher (8.6% vs. 1.2%) in patients who did not use NG/NJ tubes. It has been reported that the rates of chylous leakage are higher in patients who start oral intake early [11-13]. Since oral intake was started in the early postoperative period in patients who did not use an NG/NJ tube, the rate of chylous leak is thought to be high. These patients were discharged without event, with oral fat-free diet recommendations.

During celiac dissection in radical gastric surgery, perigastric autonomic nerves are dissected. Postoperative distension owing to paralytic ileus is possible. By providing decompression, the placement of an NG/NJ tube is thought to minimize tension on the anastomosis and provides anastomotic safety. In recent randomized controlled studies, it was shown that postoperative complications and death rates were similar between NG/NJ tube-using and non-using patient groups [3, 14]. It has been demonstrated that there is no requirement to use NG/NJ tubes for decompression [3, 4, 10, 15]. Anastomotic leakage was found in 2 patients in NG/NJ group and one non-NG/NJ group, and severe postoperative complications (CD ≥ 3) were significantly lower in patients who did not use an NG/NJ tube in this study ($p = 0.001$).

Delaying oral intake in patients using NG/NJ tube may prolong the length of hospital stay [16]. In our study, there was no difference between the two groups.

According to research, all forms of gastric resection may be performed without the needing for an NG/NJ tube [3, 8, 17]. In our study, regardless of the extent of gastric resection and lymphadenectomy (D1/D2), the NG/NJ tube was not used in all patients in the non-NG/NJ tube group. Patients who do not use NG/NJ tubes may have better patient comfort [16]. In this trial, we observed patient mobilization and compliance were better which improved their quality of life in the non-NG/NJ group.

CONCLUSION

According to the findings of this investigation, the usage of NG/NJ showed no advantage in avoiding postoperative complications. After elective gastric cancer surgery, limiting the use of NG/NJ tubes, which have a detrimental impact on patient comfort, may be discussed. Gastric cancer surgery can be performed successfully without NG or NJ tube decompression.

Authors' Contribution

Study Conception: AA; Study Design: AA; Supervision: N/A; Funding: N/A; Materials: N/A; Data Collection and/or Processing: AA; Statistical Analysis and/or Data Interpretation: AA; Literature Review: AA; Manuscript Preparation: AA and Critical Review: MY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Kanda M. Preoperative predictors of postoperative complications after gastric cancer resection. Surg Today 2020;50:3-11. /
3. Pacelli F, Rosa F, Marrelli D, Morgagni P, Framarini M, Cristadoro L, et al. Naso-gastric or naso-jejunal decompression after partial distal gastrectomy for gastric cancer. Final results of a multicenter prospective randomized trial. Gastric Cancer 2014;17:725-32.
4. Chen CJ, Liu TP, Yu JC, Hsua S Der, Hsieh TY, Chu HC, et al. Roux-en-Y reconstruction does not require gastric decompression after radical distal gastrectomy. World J Gastroenterol 2012;18:251-6.
5. Japanese gastric cancer treatment guidelines 2018 (5th edition).

Gastric Cancer 20212021;24:1-21.
6. AJCC Cancer Staging Manual. Amin MB, Edge SB, Greene FL, et al. (eds), Springer Cham, 2017.
7. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004;240:205-13.
8. Wang Q, Wu Z, Shi J, Hou S, Shan F, Li S, et al. Omitting nasogastric tube placement after gastrectomy does not enhance postoperative recovery: a propensity score matched analysis. Langenbeck's Arch Surg 2022;407;113-22.
9. Sanaie S, Mahmoodpoor A, Najafi M. Nasogastric tube insertion in anaesthetized patients: a comprehensive review. Anaesthesiol Intensive Ther 2017;49:57-65.
10. Bergeat D, Merdrignac A, Robin F, Gaignard E, Rayar M, Meunier B, et al. Nasogastric decompression vs no decompression after pancreaticoduodenectomy: the Randomized Clinical IPOD Trial. JAMA Surg 2020;155:e202291.
11. Bhardwaj R, Vaziri H, Gautam A, Ballesteros E, Karimeddini D, Wu GY. Chylous ascites: a review of pathogenesis, diagnosis and treatment. J Clin Transl Hepatol 2018;6:105-13.
12. Lu J, Wei ZQ, Huang CM, Zheng CH, Li P, Xie JW, et al. Small-volume chylous ascites after laparoscopic radical gastrectomy for gastric cancer: results from a large population-based sample. World J Gastroenterol 2015;21:2425-32.
13. Kuboki S, Shimizu H, Yoshidome H, Ohtsuka M, Kato A, Yoshitomi H, et al. Chylous ascites after hepatopancreatobiliary surgery. Br J Surg 2013;100:522-7.
14. Doglietto GB, Papa V, Tortorelli AP, Bossola M, Covino M, Pacelli F. Nasojejunal tube placement after total gastrectomy: a multicenter prospective randomized trial. Arch Surg. 2004;139:1309-13.
15. Li C, Mei JW, Yan M, Chen MM, Yao XX, Yang QM, et al. Nasogastric decompression for radical gastrectomy for gastric cancer: a prospective randomized controlled study. Dig Surg 2011;28:167-72.
16. Hur H, Kim SG, Shim JH, Song KY, Kim W, Park CH, et al. Effect of early oral feeding after gastric cancer surgery: a result of randomized clinical trial. Surgery 2011;149:561-8.
17. Wang D, Li T, Yu J, Hu Y, Liu H, Li G. Is nasogastric or nasojejunal decompression necessary following gastrectomy for gastric cancer? A systematic review and meta-analysis of randomised controlled trials. J Gastrointest Surg 2015;19:195-204.

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Evaluation of patients with negative PCR tests after COVID-19 pneumonia in intermediate-level intensive care unit

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ABSTRACT

Objectives: The need for an intensive care unit has increased during the pandemic of coronavirus disease (COVID-19). For this reason, intermediate-level intensive care units (IICUs) were established in hospitals worldwide. This study aims to evaluate the data of patients that hospitalized in IICU.

Methods: Patients under treatment for COVID-19 were followed up in IICU after the negative polymerized chain reaction test. A total of 52 patients were evaluated retrospectively between August 24, 2020 and March 1, 2021. The patients were divided into two groups according to discharge status from IICU (Group 1: exitus, Group 2: transferred to clinic, or discharged home). Demographic data, comorbidities, Acute Physiology and Chronic Health Evaluation II (APACHE II), Glasgow Coma Scale (GCS), treatments and procedures, and complications were recorded.

Results: Seventeen (32.7%) of 52 patients who were followed up in IICU died. Thirty-five patients (67.3%) were transferred to the clinic or discharged home. The APACHE II scores at admission to IICU were higher in Group 1 (26.11 ± 5.86) than in Group 2 (23.43 ± 6.32) but not statistically significant. GCS was statistically significantly lower in Group 1 than in Group 2 (7.82 ± 2.42 and 10.25 ± 2.58, respectively, $p = 0.002$). Mechanical ventilation rate (82.3%) and the need for inotropic agents (76.5%) were higher in Group 1 ($p = 0,034$ and $p < 0.001$, respectively). Tracheostomy was applied to 5 of all patients, and percutaneous endoscopic gastrostomy was performed 4 of them.

Conclusions: We think that IICU created during the pandemic provides effective treatment for patients needing intensive care. We think IICU is beneficial in providing quick patient discharge in tertiary intensive care units.

Keywords: COVID-19, pneumonia, intensive care unit, intermediate, physiotherapy, mortality

In December 2019, acute respiratory distress syndrome (ARDS) caused by the SARS- CoV-2 virus in Wuhan, China, was defined as the new type of coronavirus disease, COVID-19 [1]. COVID-19 disease was reported as a pandemic by the World Health Organization (WHO) on March 2020, when the first case was seen in Turkey [2]. Due to the high virulence of COVID-19 disease, its rapid spread, and the lack of an effective treatment or vaccine, respiratory distress in patients has spread rapidly. These circumstances in-

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creased the need for ICU treatment. Hospitals all over the world changed their infrastructure and organization. Cooperation between different disciplines and new strategies were designed to manage the pandemic. New ICUs quickly entered service. IICUs reduced the overload of intensive care units during the current COVID-19 pandemic. Development, reorganization, and optimization of IICUs were achieved [3].

Patients with severe COVID-19 pneumonia receive ARDS treatment, viral infection treatment, and respiratory support treatment in the tertiary level ICU [4]. The duration of treatment is prolonged due to the presence of additional comorbidities and the development of complications. Intermediate intensive care units are needed for patients whose COVİD-19 PCR test is negative after treatment and who need intensive care follow-up. It is essential to determine the conditions encountered during the follow-up and treatment of these patients and the complications that develop after the disease.

This study aims to evaluate the clinical features and outcomes of patients who have negative PCR tests after treatment for COVID-19 pneumonia, continue to need ICU, and are hospitalized in the IICU.

METHODS

Ethics committee approval of the study was obtained from Bursa Yüksek İhtisas Training and Research Hospital with protocol number 2011-KAEK-25 2021/03-26. Between August 24, 2020 and March 1, 2021, 52 patients who were negative for the COVID-19 PCR test twice and followed up in the IICU were evaluated. Patients under 18 years old and who did not have COVID-19 pneumonia were excluded from the study. The patients were divided into two groups according to their exit from the IICU. Patients who died were classified as Group 1, and patients who were transferred to the clinic or discharged home were classified as Group 2. Demographic data, APACHE II scores and GCS, length of stay, type of respiratory support (mask/nasal oxygen, noninvasive ventilation, and high flow oxygen, mechanical ventilator), surgical procedures (tracheostomy and percutaneous endoscopic gastrostomy(PEG)), inotropic/vasoactive treatment, nutrition type, physical therapy, presence of pressure ulcers, and complications were recorded retrospectively from patient files and the hospital automation system.

Statistical Analysis

Data analysis was accomplished by IBM SPSS 23.0 statistical program. Descriptive statistics were specified as frequencies and percentages for qualitative data. Quantitative data were expressed as mean and standard deviation. The normal distribution was evaluated with the Kolmogorov-Smirnow test. In addition, Fisher's exact test was used for categorical variables, Mann Whitney U test was utilized for analysis of quantitative data. The statistical significance level was accepted as $p < 0.05$.

RESULTS

Seventeen (32.7%) of 52 patients who were followed

Table 1. Demographic data and comorbidities of patients

Parameters	All patients (n = 52)
Age (year)	70.55±11.40
Male, n (%)	22 (42.3)
Female, n (%)	30 (57.7)
BMI (kg/m^2)	28.55 ± 3.48
APACHE 2	24.32 ± 5.94
GCS	9.46 ± 2.76
Comorbidities, n (%)	
DM, COPD, CVD, HT	11 (21.2)
CAD, HT	10 (19.2)
CRF, ARF	5 (9.6)
DM	9 (17.3)
CVD	9 (17.3)
COPD	4 (7.7)
Malignancy	3 (5.8)
Substance Abuse	1 (1.9)

Data are shown as mean ± standard deviation or n (%). BMI = Body Mass Index, APACHE = Acute Physiology and Chronic Health Evaluation, GCS = Glasgow Coma Scale, DM = Diabetes mellitus, COPD = Chronic Obstructive Pulmonary Disease, CVD = Cerebrovascular Disease, CAD = Coronary Artery Disease, HT = Hypertension, CRF = Chronic Renal Failure, ARF = Acute Renal Failure

Table 2. Age, APACHE II and GCS scores of the groups, length of stay in Covid and intermediate ICUs

Parameters	Group 1 (n = 17)	Group 2 (n = 35)	*p* value
Age (year)	71.25 ± 9.71	69.11 ± 14.3	0.830
APACHE II	26.11 ± 5.86	23.43 ± 6.32	0.318
GCS	7.82 ± 2.42	10.25 ± 2.58	**0.002**
Length of stay in Covid ICU (day)	17.41 ± 25.27	15.60 ± 16.18	0.717
Length of stay in IICU (day)	14.64 ± 13.92	20.91 ± 17.33	0.133

Data are shown as mean±standard deviation, APACHE = Acute Physiology and Chronic Health Evaluation, GCS = Glasgow Coma Scale, ICU = Intensive Care Unit, IICU = Intermediate Intensive Care Unit

up and treated in IICU died. 35 (67.3%) patients were transferred to another clinic or discharged home. Demographic data and comorbidities are shown in Table 1. Eleven (21.2%) patients accepted to IICU had more than two comorbidities.

Age, APACHE II and GCS scores, tertiary ICU, and IICU length of stay were compared between groups (Table 2). The mean age in Group 1 was greater than in Group 2, but it was not statistically significant. The APACHE II score was higher but not statistically different in Group 1 patients. GCS was statistically lower in Group 1 than in Group 2 (p = 0.002). There was no statistically significance between the groups regarding the patient's length of stay in the tertiary level ICU and IICU.

Respiratory support applied to patients in IICU were mask/nasal oxygen, noninvasive ventilation (NIV) and, high flow oxygen (HFO), MV support (Table 3). MV support was higher in Group 1 than in Group 2 (82.3% vs. 48.6%, respectively, p = 0.034). Enteral nutritional support was administered to the patients via oral, nasogastric tube, and PEG. Parenteral nutrition support was applied to two (5.7%) patients in Group 2. The need for inotropic agents was 76.5% in Group 1 and 5.7% in Group 2 and it was statistically significant ($p < 0.001$) (Table 3). Physical therapy and

Table 3. Comparison of follow-up and treatments in IICU of the groups

Parameters	Group 1 (n = 17)	Group 2 (n = 35)
Airway		
Respiratory Support		
Mask-nasal O_2 (2-6 Lt/min) n (%)	1 (5.9)	15 (42.8)
HFO n(%)	1 (5.9)	2 (5.7)
NIV n(%)	1 (5.9)	1(2.9)
MV n(%)	14 (82.3)	17 (48.6)
Nutrition support		
Orally n(%)	6 (35.3)	22 (62.9)
Nasogastric tube-PEG n (%)	11 (64.7)	11 (31.4)
Parenteral n (%)	-	2 (5.7)
Inotropic agent requirement	13 (76.5)	2 (5.7)
Physical therapy and rehabilitation n(%)	9 (52.9)	28 (80.0)

HFO = High Flow Nasal Oxygen theraphy, NIV = Noninvaziv ventilation, MV = Mechanical ventilation, PEG = Percutaneous Endoscopic Gastrostomy

rehabilitation support was applied to 9 (52.9%) patients in Group 1 and 28 (80%) patients in Group 2 (Table 3). Among all patients in IICU, percutaneous tracheostomy and PEG were performed in 5 (9.6%) and 4 (7.7%) patients, respectively.

Complications that occurred in IICU were sepsis, multiple organ dysfunction syndromes (MODS), pressure ulcers, cardiac complications, peripheral neuropathy, cerebrovascular accident (CVO), and other infections (Table 4). Sepsis was observed in 35.3% in Group 1 and 22.9% of patients in Group 2. The incidence of MODS was 47.0% in Group 1 and 2.9% in Group 2. Pressure ulcers were present in 82.3% in Group 1 and 60% of patients in Group 2 (Table 4). Negative pressure wound treatment was applied to 5 patients (9.6%) due to pressure ulcers. In addition, 1 patient (1.9%) was treated surgically by debridement and tissue grafting.

DISCUSSION

In recent years, IICU units have been established for patients with respiratory problems in European countries. The primary use of intermediate care units before the pandemic is the treatment of hypercapnic or hypoxemic acute and chronic respiratory failure. In addition, the names of these units were reported as respiratory high dependency units [3]. The IICU is crucial because it is a transfer step between the ICU and the clinical service. In our study, 52 patients who were transferred from the 3rd Level ICU to the IICU after Covid-19 pneumonia were evaluated. 67.3% of the patients were discharged home or to the service. Over time, COVID-19 infection reveals persistent symptoms and a clinical condition such as MODS[5]. These prolong the ICU stay and increase the need for ICU. Therefore, IICUs were used for follow-up and treatment during the COVID-19 pandemic, and the studies conducted in these units contributed to the literature [6-8]. There was no IICU in our hospital before the pandemic. However, during the pandemic, a second-level IICU was created in our hospital for patients with comorbidities who continue to need intensive care. A team of intensive care specialists, nurses, dietitians, and physiotherapists served in this unit. All patients who were hospitalized and followed up in the IICU in our hospital were negative for PCR tests. In addition, our patients were admitted from the tertiary level ICU, whose infection continued and who needed supportive treatment due to additional comorbidities. Matute-Villacís *et al*. discussed the clinical features and outcomes of the patients during the pandemic in IICU [7]. They stated that 61% of the patients were accepted from the ICU, and the other patients were accepted from the ward or emergency room.

Carpagnano *et al*. [6] retrospectively evaluated 87 patients with a diagnosis of COVID-19 who were admitted to the IICU between March 11 and April 17, 2020. They administered NIV therapy to these patients. The patients were transferred to the tertiary level ICU in the necessary clinical conditions. They found that the mortality rate before March 29, 2020 (52.2%), was statistically higher than patients hospitalized after this date (17.1%). They emphasized the reasons for this as the advances in the treatments ap-

Table 4. Complications seen at the groups in IICU

Parameters	Group 1 (n = 17)	Group 2 (n = 35)
Sepsis, n (%)	6 (35.3)	8 (22.9)
MODS, n (%)	8 (47.1)	1 (2.9)
Pressure ulcers, n (%)	14 (82.3)	21(60)
Arrhythmia/heart failure, n (%)	1/1 (11.8)	1/3(11.4)
Peripheral neuropathy, n (%)	0 (0)	2 (5.7)
CVD, n (%)	1 (5.9)	0 (0)
Other Infections, n (%)	7 (41.2)	10 (28.6)

MODS = Multiple organ dysfunction syndrome, CVD = Cerebrovascular disease

plied, the location of IICUs closer to the tertiary level ICU, the creation of a multidisciplinary staff, the increase in the number of health professionals, and the advanced clinical experience. The mortality rate was 32.7% in 52 patients examined in our study. Our result has not been investigated periodically as in the study of Carpagnano *et al*. [6], and the mortality rate in our study was between 52.2% and 17.1%. At the same time period, the mortality rate of patients in the Covid-19 ICU in our hospital was approximately 61%.

Worse clinical outcomes and prolonged hospitalization are observed in patients with COVID-19 pneumonia and comorbidities [9]. Matute-Villacís *et al*. [7] established two new IICUs during the pandemic and discussed patients' clinical features and outcomes. They found that the mean age of the patients was 66 ± 12 years, and hypertension was the most common comorbidity [7]. The mean age of all patients in our study was 70.55 years. The most common comorbidity is multiple comorbidities. The rate of patients with more than two comorbidities (DM, COPD, CVD, and HT) is 21.2%. While Matute-Villacís *et al*. [7] reported the patients' hospital stay as 34 days and the duration of IICU hospitalization as 7 days [7], the IICU hospitalization period was 14.64 days in Group 1 and 20.91 days in Group 2 in our study. Therefore, we think that the length of IICU hospitalization in our study is due to multiple comorbidities in our patients and the long duration of clinical organization.

The mean APACHE 2 score and GCS score are important in determining the prognosis of intensive care unit patients. A high APACHE score and low GCS value are associated with poor prognosis [10]. In our patients, the mean APACHE 2 score was higher, and GCS was lower in the group, resulting in mortality (Group 1). Furthermore, the decrease in GCS value was found statistically significant.

Hernandez-Rubio *et al*. [8] evaluated the outcomes of COVID-19 patients admitted to IICU in their prospective cohort study. In their study, which included 70 patients, the respiratory supports applied to the patients were NIV, HFO, nasal oxygen therapy, and mechanical ventilation. They also reported that the rate of patients who underwent intubation was 37.1%. In addition, they found the mortality rate to be 53.8% in the intubated patient group and 6.8% in the non-intubated group. In our study, respiratory supports used in IICU were mask/nasal oxygen, NIV, HFO, and mechanical ventilation, in line with the literature. Mechanical ventilation support was higher in Group 1 in our study. In our IICU, respiratory support was performed on 4 patients with tracheostomy, and nutritional support was applied to 5 patients by PEG procedure.

There are recommendations regarding the nutrition of COVID-19 patients in the ICU in the literature. Enteral nutrition therapy is primarily recommended for patients requiring invasive mechanical ventilation in the ICU [11, 12]. In our study, enteral and oral nutrition was applied to the patients mostly, and parenteral nutrition was used to only two unsuitable patients.

The most common ICU complications related to COVID-19 are acute respiratory distress syndrome (ARDS) [13] and ICU-induced neuromuscular weakness [14]. Early onset physiotherapy in ICU is an important therapeutic tool to reduce the complications of immobilization. Rehabilitation has been widely recommended in people with COVID-19 pneumonia [15]. In a meta-analysis examining the effect of physical therapy on COVID-19-related disorders, it was reported that physical therapy had positive effects on lung function, physical function, and psychosocial function [16]. Another study concluded that respiratory physiotherapy and physical rehabilitation practices in COVID-19 patients in ICU reduced ICU-related complications and increased patients' quality of life [17]. In our study, physical therapy was applied to more patients in Group 2 than in Group 1 (52.9% vs. 80%, respectively). Because physical therapy was not performed on hemodynamically unstable patients in our hospital.

Pressure ulcers also affect the quality of life in the ICU. The hospital stay is prolonged due to wound care, debridement, and graft procedures [18]. In the literature, the most common areas of pressure ulcers due to supine positions are sacral (50%), heel (15%), and trochanteric (10%). The effect of tissue damage secondary to COVID-19 inflammation is also significant in the etiology of decubitus ulcers [19]. Recent epidemiological data from a primary health center in New York showed that the prevalence of pressure ulcers among COVID-19 patients requiring intensive care is three times higher than that of other non-COVID-19 patients requiring intensive care [20]. In a study, 445 COVID-19 patients were evaluated retro-

spectively for pressure ulcers, and the incidence of pressure ulcers was found to be 46.74%. They stated that age, Braden score, body mass index, comorbidity, DM, fecal incontinence, GCS, use of vasopressor agents, and length of hospital stay were significantly associated with pressure sores [21]. In a different study, including 111 patients hospitalized in the tertiary level ICU with the diagnosis of COVID-19, it was published that 56.8% of the patients had pressure injuries, and the average length of stay was 11.6±9.4 days. The same study stated that 77% of the patients had an additional disease other than COVID-19 [22]. In our study, the rate of pressure ulcers was 82.3% in Group 1 and 60% in Group 2. We attribute the reason why the rate of patients with pressure ulcers is slightly higher than in the literature to the fact that our patients were transferred from the tertiary level ICU and the length of their total ICU stay. In addition, physiotherapy could not be applied due to pandemic conditions in our tertiary level ICU. We think that a higher rate of patient's comorbidities is effective in the formation of pressure ulcers.

A study examining the results of COVID-19 pneumonia patients in the ICU treated with a standard protocol stated that the most common complication was neurological complications, with a rate of 37.66%. Other common complications in these patient groups were reported as infections (28.6% pneumonia, 16.9% sepsis, 14.3% urinary tract infection) and 11.7% as pneumothorax and pneumomediastinum [23]. Hernandez-Rubio *et al*. [8] stated that major medical complications (myocardial injury, hypertension, acute renal failure, bacteremia, septic shock, hospital pneumonia, bronchial obstruction, anemia, thrombocytopenia, and skin ulcers) occurred in 58.6% of patients diagnosed with COVID-19 and admitted to the IICU. In our study, sepsis, one of the most common complications in patients, was observed more frequently in Group 1 than in Group 2 (35.3% vs. 22.9%, respectively). MODS was more common in Group 1 (47.0%) than Group 2 (2.9%) (47.0% vs. 2.9%, respectively).

All patients in our study were admitted to the IICU from tertiary level Covid-19 ICU. Similarly, Matute-Villacís *et al*. [7] reported that most patients (61%) admitted to the IICU were transferred from the tertiary level ICU. They reported that the prominent role of an IICU during a pandemic is to provide rapid discharge and alleviate the tertiary level ICU burden. In addition, IICU is multidisciplinary care that shortens the length of stay in the intensive care unit and potentially shortens the hospital stay [7]. A multidisciplinary approach was applied for nutrition, physiotherapy and decubitus ulcer care in IICU.

Limitations

The limitations of our study are the number of patients and the retrospective nature. In addition, the long-term consequences of COVID-19 could not be evaluated.

CONCLUSION

Consequently, ensuring patient discharge from the tertiary level ICU for relieving high ICU load and allowing bed's turnover during the pandemic is essential. IICU provides effective treatment with multidisciplinary care and is needed to rehabilitate patients who continue to need intensive care.

Authors' Contribution

Study Conception: FA, CY; Study Design: FA, CY; Supervision: FA; Funding: ANB; Materials: TO, CSA; Data Collection and/or Processing: CSA, FA; Statistical Analysis and/or Data Interpretation: ÜK; Literature Review: BÖ, FA; Manuscript Preparation: FA, CY and Critical Review: CY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727−33.
2. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. Acta Biomed 2020;19;91:157-60.
3. Masa JF, Patout M, Scala R, Winck JC. Reorganizing the respiratory high dependency unit for pandemics. Expert Rev Respir Med 2021;15:1505-15.
4. T.C. Sağlık Bakanlığı Bilimsel Danışma Kurulu, COVID-19

(SARS-CoV-2 Enfeksiyonu) Ağır pnömoni, ARDS, Sepsis ve septik şok yönetimi. T.C.Sağlık Bakanlığı, 27 Mayıs 2021, Ankara. https://covid19.saglik.gov.tr/Eklenti/40781/0/covid19rehberiagirpnomoniardssepsisveseptiksokyontemipdf.pdf. Accessed May 30, 2021.
5. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A, et al. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Sci Rep 2021;11:16144.
6. Carpagnano GE, Migliore G, Grasso S, Procacci V, Resta E, Panza F, Resta O. More skilled clinical management of COVID-19 patients modified mortality in an intermediate respiratory intensive care unit in Italy. Respir Res 2021;22:16.
7. Matute-Villacís M, Moisés J, Embid C, Armas J, Fernández I, Medina M, et al. Role of respiratory intermediate care units during the SARS-CoV-2 pandemic. BMC Pulm Med 2021;21:228.
8. Carrillo Hernandez-Rubio J, Sanchez-Carpintero Abad M, Yordi Leon A, Doblare Higuera G, Garcia Rodriguez L, Garcia Torrejon C, et al. Outcomes of an intermediate respiratory care unit in the COVID-19 pandemic. PLoS One 2020;15:e0243968.
9. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al. China Medical Treatment Expert Group for COVID-19. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. Eur Respir J 2020;55:2000547.
10. Akelma FK, Altınsoy S, Çatalca S, Çaparlar CÖ, Sayın MM, Ergil J. Retrospective analysis of trauma patients in the intensive care unit. Gazi Med J 2021;32: 515-21.
11. Zhao X, Li Y, Ge Y, Shi Y, Lv P, Zhang J, et al. Evaluation of nutrition risk and its association with mortality risk in severely and critically ill COVID-19 patients. JPEN 2021;45:32-42.
12. Barazzoni R, Bischoff S C, Breda J, Wickramasinghe K, Krznaric Z, Nitzan D, et al. ESPEN expert statements and practical guidance for nutritional management of individuals with SARS-CoV-2 infection. Clin Nutr 2020;39:1631-8.
13. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
14. McWilliams D, Weblin J, Hodson J, Veenith T, Whitehouse T, Snelson C. Rehabilitation levels in patients with COVID-19 admitted to intensive care requiring invasive ventilation. An observational study. Ann Am Thorac Soc 2021;18:122-9.
15. Eggmann S, Kindler A, Perren A, Ott N, Johannes F, Vollenweider R, et al. Early physical therapist interventions for patients with COVID-19 in the acute care hospital: a case report series. Phys Ther 2021;101:pzaa194.
16. Debeuf R, Swinnen E, Plattiau T, De Smedt A, De Waele E, Roggeman S, et al. The Effect of physical therapy on impairments in COVID-19 patients from intensive care to home rehabilitation: a rapid review. J Rehabil Med 2022;54:jrm00242.
17. Rizvi MAR, Hanif H, Rasheed N, Simon NH. Effects of respiratory therapy and physical rehabilitation in a reduction of length of ICU stay among Covid-19 Patients – A narrative review; Int J Med Sci Health Res 2021;5:106-21.
18. Turgut N, Ak A, Ak E, Yakar N, Yakar N, Yılmaz B, et al. [Incidence, prevention and treatment of pressure ulcers in intensive care patients]. J Turk Soc Intens Care 2017;15:72-6. [Article in Turkish]
19. Gefen A, Ousey K. CoviD-19: pressure ulcers, pain and the cytokine storm. J Wound Care 2020;29:540-2.
20. Trevellini C. Challenges faced with morbidity obese patients during COVID-19. The 2020 Virtual Meeting of the European Pressure Ulcer Advisory Panel (EPUAP). https://www.epuap.org/wp-content/uploads/2020/09/epuap2020-virtual-abstract-book.qxp_sestava-1.pdf(accessed October 2020)
21. Amini M, Mansouri F, Vafaee K, Janbakhsh A, Mahdavikian S, Moradi Y, et al. Factors affecting the incidence and prevalence of pressure ulcers in COVID-19 patients admitted with a Braden scale below 14 in the intensive care unit: retrospective cohort study. Int Wound J. 2022;19:2039-54.
22. Kıraner E, Kaya H. [Pressure injuries in patients hospitalized in intensive care with the diagnosis of COVID-19 and retrospective analysis of risk factors]. Yoğun Bakım Hemşireliği Dergisi 2021;25:139-51. [Article in Turkish]
23. Demirel Kaya HS, Mammadov O, Dogan L, Sarıkaya ZT, Kısa Özdemir İ, Rasimoğlu S, et al. [Patients with COVID-19 pneumonia treated with the standard protocol: observational results of five intensive care units]. J Turk Soc Intens Care 2020;18:1-13. [Article in Turkish]

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Comparison of biomarkers of COVID-19 patients with the alpha variant (B.1.1.7), the delta variant (B.1.617), and no mutation detected

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ABSTRACT

Objectives: We aimed to compare biomarkers of COVID-19 patients with the Alpha variant (B.1.1.7), the Delta variant (B.1.617), and no mutation detected in our study.

Methods: A total of 600 patients with positive COVID PCR test and Alpha, Delta variant and no mutation detected with Covid PCR mutation test were included in the study. Troponin I, creatinine, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH), fibrinogen, D-dimer, ferritin, number of lymphocytes, lymphocytes (%), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), trombosite ratio in the blood (PCT), C-reactive protein (CRP) values were analyzed retrospectively. The age, gender, and hospitalization of the patients were evaluated concurrently.

Results: Age, troponin, creatinine, LDH, PLT, MPV, and D-dimer were laboratory parameters that vary significantly with COVID-19 virus mutation. Age, troponin, LDH, and MPV values were lower in patients with Delta variant according to patients with the Alpha variant. Lymphocytes (N) and lymphocytes (%) values were lower in hospitalized patients relative to outpatients while age, troponin, LDH, CRP, and D-dimer values were higher in hospitalized patients than outpatients irrespective of mutation. Creatinine values were higher only in hospitalized patients with no mutation detected while ferritin and fibrinogen values were higher in hospitalized patients with Delta variant and no mutation detected.

Conclusions: Age, troponin, creatinine, LDH, PLT, MPV, D-dimer, fibrinogen, ferritin, CRP, lymphocytes (N), and lymphocytes (%) values can guide to evaluate the diagnosis and hospitalization of patients with future different mutations.

Keywords: COVID-19, alpha, delta, mutation, biomarker

As SARS-COV-2 detection increases in the world and new mutant variants emerge, it is becoming necessary to monitor continuously and report quickly genetic changes to support public health control in the management of COVID-19 disease [1, 2]. Variant viruses mutated in the SARS-COV-2 infection can cause the infection to increase, increase in virulence, change in the clinical course of the disease, and decrease the protective effect of reinfections, infections, or antibodies after vaccination [2, 3]. The World

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Health Organization (WHO) classified variants as Variants of Concern (VOCs) which cause more severe disease show a widespread, have been proven to be more contagious, or have decreased neutralizing antibody levels resulting from post-vaccine and post-infectious infection [4].

Alpha variant (VOC202012/01) (UK variant) (B. 1.1.7) was first identified in Kent/England in September 2020. Mutations detected in the Alpha variant were found to be associated with cell attachment, cell entry, infection, and neutralization [5]. Spike protein gene mutation was reported to contain the target gene regions used in PCR tests, causing S-gene target failure andalso increasing the transmission by antagonizing natural immunity with nucleocapsid mutationsin the Alpha variant [6, 7]. By the second half of 2021, the Alpha variant was the variant responsible for the COVID infection in America and many European countries. The studies showed an evident increase in transmission, higher viral load, longer infection time, higher hospitalization rate, and distinct increase in mortality in the Alpha variant [5, 7].

The Delta variant (B. 1.617) was first seen in India in November 2020. Mutations detected in the Delta variant were shown to affect the affinity of connecting to the cell, infection, immunity evasion, vaccination, and neutralization resistance [5]. In June 2021, the WHO reported that the Delta variant spread in many countries, causing high infectious and high mortality [8]. Delta variant in 2021 showed that it was the most contagious variant and spread rapidly in unvaccinated individuals [9]. More infectivity, higher viral load, and higher pathogenicity were detected in the Delta variant than in other varials [10, 11].

Hematological, biochemical and inflammatory parameters that affect the course of the disease in COVID-19 have been investigated in several studies. High cardiac troponin level was an important biomarker in patients with or without underlying cardiovascular diseases [12, 13]. Increased concentrations of troponin levels in patients with COVID-19 disease were associated with disease severity and prognosis [14, 15]. Also, elevation in troponin levels in COVID-19 patients was associated with admission to the intensive care unit and a higher mortality rate [13]. Thrombocytopenia COVID-19 plays a role in disease development and severity [16-19]. MPV values show the size of the platelets and changes in MPV values can predict inflammation, sepsis, infective endocarditis, and pneumonia [20]. While some studies demonstrate that high MPV values were associated with mortality in intensive care unit patients, some publications indicate that the MPV values were lower [21, 22]. D-dimer and fibrinogen levels which indicate the activation of coagulation pathways and thrombosis were found to be higher in COVID-19 disease. Ferritin was demonstrated as the product of inflammation and defined as a pathogenic mediator in severe COVID-19 patients. D-dimer, fibrinogen, and ferritin values were determined higher in the critical severe patients, especially in the intensive care unit,and were admitted as a low prognostic factor [23-26]. Higher CRP values and lymphopenia were detected in COVID-19-positive patients and found to be associated with disease severity, clinical outcome, progression, and mortality [27-30]. Creatinine values were established higher in critically ill COVID-19 patients and associated with adverse outcomes [31-33]. High LDH values were determined for patients with severe COVID-19 disease [34-36].

In our study, we aimed to compare biomarkers of COVID-19 patients with the Alpha variant (B.1.1.7), the Delta variant (B.1.617), and no mutation detected.

METHODS

Our study was conducted retrospectively at Bursa Yuksek Ihtisas Training and Research Hospital, which serves the South Marmara region with a population of approximately 5 million. 200 patients with positive COVID-19 PCR test and detected Alpha variant (B. 1.1.7) (VOC202012/01) by COVID- 19 mutation test between February and April 2021were included in the study. 200 control patients with positive COVID PCR test with no mutations by COVID-19 mutation testbetween February and April 2021were included in the study.200 patients with positive COVID PCR test and detected Delta (B. 1,617) variant by COVID-19 mutation test were included in the study in August 2021 (600 patients in total). Troponin I, creatinine, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH), fibrinogen, D-dimer, ferritin, number of lymphocytes, lymphocytes (%), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), trombosite ratio in

the blood (PCT), C-reactive protein (CRP) were analyzed retrospectively. The age, gender, and hospitalization of the patients were evaluated concurrently.

COVID-19 manual RNA extraction was carried out from nasopharyngeal samples of suspected patients in the COVID-PCR laboratory of our hospital. The RNA amplification and COVID-19 PCR analysis were performed on the Qiagen Rotor gene device using the Real-time PCR method. The mutation tests for patients who tested positive for COVID-19 PCR were performed on the Qiagen Rotor equipment using the SARS-COV-2 Variant Plus kit and the SARS-COV-2 Emerging plus kit.

The CRP tests of the patients were analyzed in the immunonephelometry device (Siemens, Behring Nephelometer II, Dade Behring, Inc., Newark, DE, U.S.A.) in the Microbiology laboratory. The number of lymphocytes, lymphocytes (%), PLT, MPV, PDW, and PCT values were analyzed in the hematology analyzer device (Mindray, BC-6000, China)in the hematology laboratory. Troponin I, creatinine, AST, ALT, LDH, fibrinogen, D-dimer, and ferritin tests worked in COBAS 8000 (Roche, Germany) device between February and April 2021 and Architect Plus (Abbott Diagnostics, U.S.A) device in August 2021 in Biochemistry laboratory.

Statistical Analysis

Data were expressed by frequency or related percent values. Normality analyzes were done for data (N > 50) with the Kolmogorov-Smirnov test. Comparison of the two groups was done with the independent sample T-test for normally distributed parameters and Mann Whitney U- test for non-normally distributed parameters. Comparison of more than two groups was done with One way ANOVA and Welch tests for normally distributed parameters. Bonferroni and Dunnet'c tests were used for the analysis of the difference. Comparison of more than two groups was done with Kruskal-Wallis tests for non-normally distributed pa-

Table 1. Comparison of biomarkers of patients with the Delta variant, the Alpha variant, and no mutation detected

	Delta variant	Alpha variant	Control (No mutation)	*p value*
Age (years)	41 ± 17	50 ± 17	46 ± 18	**< 0.001[a]**
Troponin (ng/L)	8.4 ± 55.7	19.9 ± 117.8	24.8 ± 169.8	**< 0.001[a]**
Creatinine (mg/dL)	1.01 ± 1.00	0.98 ± 0.74	0.95 ± 0.70	**0.014[a]**
ALT (U/L)	25 ± 22	29 ± 38	34 ± 115	0.909[a]
AST (U/L)	26 ± 18	34 ± 57	39 ± 181	0.313[a]
LDH (U/L)	231 ± 99	277 ± 176	275 ± 418	**0.009[a]**
Fibrinogen (mg/dL)	147.67 ± 234.60	184.54 ± 268.19	214.96 ± 471.85	0.341[a]
Lymphocyte (N)	1.59 ± 0.77	1.50 ± 0.66	2.82 ± 14.95	0.063[a]
Lymphocyte (%)	26.56 ± 11.16	25.03 ± 11.26	25.76 ± 12.59	0.399[a]
PLT (mcl)	212 ± 59	225 ± 149	229 ± 68	**0.019[a]**
MPV (fL)	10.5 ± 1.1	10.7 ± 1.2	10.3 ± 1.3	**0.002[a]**
PDW (%)	16.2 ± 0.4	16.2 ± 0.4	16.2 ± 0.4	0.204[a]
PCT (%)	0.22 ± 0.06	0.24 ± 0.16	0.23 ± 0.07	0.063[a]
CRP (mg/L)	17.45 ± 28.50	26.97 ± 51.50	19.31 ± 33.49	0.163[a]
D-Dimer (µg/mL)	0.52 ± 0.44	0.98 ± 3.34	1.00 ± 5.72	**0.011[a]**
Ferritin (ng/mL)	147.67 ± 234.60	184.54 ± 268.19	214.96 ± 471.85	0.341[a]

Data are shown as mean±standard deviation. ALT = Alanine Aminotransferase, AST = Aspartate Aminotransferase, LDH = Lactate Dehydrogenase, PLT = platelet, MPV = mean platelet volume, PDW = platelet distribution width, PCT = trombosite ratio in the blood, CRP = C-reactive protein

[a]Kruskal-Wallis Test

rameters. Analysis was done in the SPSS program. $P < 0.05$ and $p < 0.01$ were accepted as statistically significant.

RESULTS

Biomarkers of patients with the Delta variant, the Alpha variant,and no-mutation detected (control group) were compared in Table 1. There was a significant difference in age, troponin, creatinine, LDH, PLT, MPV, and D-dimer values of the patients ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.01$, $p < 0.05$, $p < 0.01$, and $p < 0.05$; respectively). The age, troponin, LDH, and MPV values were detected significantly lower in patients with Delta variant according to patients with the Alpha variant ($p < 0.01$, $p < 0.01$, $p < 0.01$, and $p < 0.05$; respectively). Age, troponin, and PLT values were determined significantly lower in patients with Delta variant according to the control patient group ($p < 0.05$, $p < 0.01$, and $p < 0.05$; respectively). Creatinine values were found to be significantly higher in patients with Delta variant according to the control patient group ($p < 0.01$). The age and MPV values were found to be significantly higher in patients with the Alpha variant than in the control patients ($p < 0.01$ and $p < 0.01$; respectively). PLT and D-dimer values were detected significantly lower in patients with the Alpha variant relative to control patients ($p < 0.05$ and $p < 0.01$; respectively).

Biomarkers of patients with the Delta variant, the Alpha variant, and no mutation detected were compared according to gender in Table 2. Troponin, creatinine, ALT, AST, fibrinogen, lymphocyte (N), PDW and ferritin values were found to be significantly higher in males than females in patients with the Delta variant ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p <0.05$, $p <0.01$, $p < 0.05$, $p < 0.05$, and $p < 0.01$; respectively). PCT and D-dimer values were higher in females than malesfor patients with the Delta variant ($p = 0.01$ and $p < 0.01$; respectively). Troponin, creatinine, ALT, AST, fibrinogen, and CRP values were determined higher in males than females for patients with the Alpha variant ($p < 0.01$, $p < 0.01$, $p < 0.01$, $p = 0.01$, $p < 0.01$, and $p < 0.01$; respectively). PLT and PCT values were higher in females than malesfor patients with the Alpha variant ($p < 0.01$, and $p < 0.01$; respectively). Creatinine, fibrinogen, PDW, CRP, and ferritin values were found higher in males than females for non-mutation detected control group patients ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.05$, and $p < 0.01$; respectively). Troponin, ALT, AST, PLT, PCT, and D-dimer values were found to be higher in females than males for non-mutation detected control group patients ($p < 0.05$, $p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.01$, and $p < 0.01$; respectively).

Biomarkers of patients with the Delta variant, the Alpha variant,and no mutation detected were compared according to the hospitalization in Table 3. Age, troponin, LDH, fibrinogen, CRP, D-dimer, and ferritin values were found to be higher in hospitalized patients with the Delta variant than in outpatients with the Delta variant ($p < 0.05$, $p < 0.05$, $p < 0.01$, $p < 0.05$, $p < 0.01$, $p < 0.01$, and $p < 0.05$; respectively). Lymphocytes (N) and lymphocytes % values were significantly lower in hospitalized patients with the Delta variant according to outpatients with Delta variant ($p < 0.01$, and $p < 0.05$; respectively). Age, troponin, LDH, CRP, and D-dimer values were found to be significantly higher in hospitalized patients with the Alpha variant according to outpatients with the Alpha variant ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$, and $p < 0.01$; respectively). Lymphocytes (N) and lymphocytes % values were determined significantly lower in hospitalized patients with the Alpha variant according to outpatients with the Alpha variant ($p < 0.01$, and $p < 0.01$; respectively). Age, troponin, creatinine, LDH, fibrinogen, CRP, D-dimer, and ferritin values were found to be significantly higher in hospitalized patients according to outpatients for non-mutation detected control group patients ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.01$, and $p < 0.01$; respectively). Lymphocyte (N) and lymphocyte % values were detected significantly lower in hospitalized patients according to outpatients for non-mutation detected control group patients ($p < 0.01$ and $p < 0.01$; respectively).

DISCUSSION

Our study shows that the Delta variant spread more in the younger age groups than the Alpha variant similar to other studies [1, 37, 38]. According to the determination of the Delta variant which was mostly detected in younger patients in our study was the dominant mu-

Table 2. **Comparison of biomarkers of patients with the delta variant, the alpha variant, and no mutation detected compared to gender**

	Delta variant		*p value*	Alpha variant		*p value*	Control (No mutation)		*p value*
	Female	**Male**		**Female**	**Male**		**Female**	**Male**	
Age (years)	42 ± 17	41 ± 17	0.758[b]	52 ± 17	49 ± 17	0.346[b]	45 ± 19	47 ± 18	0.399[b]
Troponin (ng/L)	2.0 ± 4.1	14.1 ± 76.3	**0.044[b]**	6.4 ± 8.9	33.6 ± 166.1	**0.003[b]**	30.4 ± 225.1	18.3 ± 59.6	**0.022[b]**
Creatinine (mg/dL)	0.91 ± 1.19	1.10 ± 0.78	**< 0.001[b]**	0.83 ± 0.80	1.12 ± 0.65	**< 0.001[b]**	0.84 ± 0.62	1.07 ± 0.76	**< 0.001[b]**
ALT (U/L)	23 ± 22	27 ± 22	**0.002[b]**	22 ± 20	36 ± 49	**0.004[b]**	35 ± 155	32 ± 29	**< 0.001[b]**
AST (U/L)	25 ± 20	27 ± 15	**0.013[b]**	27 ± 22	41 ± 77	**0.010[b]**	48 ± 246	28 ± 15	**0.030[b]**
LDH (U/L)	223 ± 88	237 ± 107	0.429[b]	262 ± 161	291 ± 189	0.073[b]	289 ± 561	259 ± 130	0.360[b]
Fibrinogen (mg/dL)	81.17 ± 122.05	207.20 ± 289.63	**< 0.001[b]**	95.95 ± 153.59	274.05 ± 324.70	**< 0.001[b]**	148.29 ± 448.48	292.61 ± 488.76	**< 0.001[b]**
Lymphocyte (N)	1.49 ± 0.80	1.69 ± 0.74	**0.015[b]**	1.48 ± 0.60	1.52 ± 0.71	0.827[b]	3.82 ± 20.37	1.65 ± 0.76	0.504[b]
Lymphocyte (%)	27.22 ± 11.14	25.97 ± 11.19	0.432[a]	25.84 ± 10.60	24.23 ± 11.87	0.315[a]	26.76 ± 13.07	24.60 ± 11.97	0.229[a]
PLT (mcl)	217 ± 62	207 ± 56	0.208[b]	249 ± 192	202 ± 83	**0.001[b]**	238 ± 69	217 ± 66	**0.013[b]**
MPV (fL)	10.7 ± 1.1	10.3 ± 1.2	0.072[b]	10.7 ± 1.1	10.7 ± 1.3	0.792[a]	10.4 ± 1.2	10.2 ± 1.3	0.249[b]
PDW (%)	16.2 ± 0.3	16.3 ± 0.4	**0.013[b]**	16.2 ± 0.4	16.3 ± 0.4	0.065[a]	16.1 ± 0.3	16.3 ± 0.4	**0.036[b]**
PCT (%)	0.23 ± 0.06	0.21 ± 0.05	**0.010[b]**	0.26 ± 0.20	0.21 ± 0.07	**0.001[b]**	0.25 ± 0.07	0.22 ± 0.07	**0.006[b]**
CRP (mg/L)	14.96 ± 18.21	19.68 ± 35.20	0.586[b]	16.14 ± 40.70	37.59 ± 58.53	**< 0.001[b]**	16.27 ± 29.04	22.81 ± 37.85	**0.044[b]**
D-Dimer (µg/mL)	0.57 ± 0.41	0.47 ± 0.46	**0.002[b]**	0.75 ± 1.22	1.21 ± 4.56	0.330[b]	1.36 ± 7.77	0.58 ± 0.90	**0.007[b]**
Ferritin (ng/mL)	81.17 ± 122.05	207.20 ± 289.63	**< 0.001[b]**	95.95 ± 153.59	274.05 ± 324.70	**< 0.001[b]**	148.29 ± 448.48	292.61 ± 488.76	**< 0.001[b]**

Data are shown as mean ± standard deviation. ALT = Alanine Aminotransferase, AST = Aspartate Aminotransferase, LDH = Lactate Dehydrogenase, PLT = platelet, MPV = mean platelet volume, PDW = platelet distribution width, PCT = trombosite ratio in the blood, CRP = C-reactive protein

[a]Independent T test, [b]Mann-Whitney U test

Table 3. Comparison of the biomarkers of patients with the delta variant, the alpha variant, and no mutation detected according to hospitalization

	Delta variant		*p value*	Alpha variant		*p value*	Control (No mutation)		*p value*
	Outpatient	Hospitalized Patients		Outpatient	Hospitalized Patients		Outpatient	Hospitalized Patients	
Age (years)	41 ± 17	61 ± 26	**0.049**[b]	49 ± 17	59 ± 13	**0.032**[b]	44 ± 17	65 ± 15	**< 0.001**[b]
Troponin (ng/L)	3.4 ± 11.5	203.2 ± 314.1	**0.013**[b]	8.9 ± 26.1	141.2 ± 390.5	**0.007**[b]	9.5 ± 34.1	168.2 ± 527.7	**< 0.01**[b]
Creatinine (mg/dL)	1.01 ± 1.01	0.94 ± 0.47	0.431[b]	0.91 ± 0.44	1.59 ± 1.80	0.092[b]	0.87 ± 0.48	1.58 ± 1.52	**0.028**[b]
ALT (U/L)	24 ± 19	61 ± 67	0.082[b]	27 ± 24	53 ± 97	0.386[b]	25 ± 22	105 ± 347	0.696[b]
AST (U/L)	25 ± 13	73 ± 67	0.052[b]	30 ± 23	73 ± 168	0.223[b]	26 ± 14	150 ± 554	0.493[b]
LDH (U/L)	225 ± 90	450 ± 177	**0.001**[b]	254 ± 129	486 ± 343	**< 0.001**[b]	236 ± 110	632 ± 1,273	**0.001**[b]
Fibrinogen (mg/dL)	139.15 ± 225.21	478.30 ± 369.98	**0.046**[b]	158.44 ± 202.80	451.60 ± 571.15	0.091[b]	127.91 ± 186.55	1,030.43 ± 1,142.49	**< 0.001**[b]
Lymphocyte (N)	1.62 ± 0.77	0.62 ± 0.31	**0.001**[b]	1.54 ± 0.65	1.11 ± 0.55	**0.004**[b]	3.01 ± 15.80	1.19 ± 1.28	**< 0.001**[b]
Lymphocyte (%)	26.84 ± 10.93	15.68 ± 15.71	**0.027**[a]	26.01 ± 10.88	16.21 ± 10.95	**< 0.001**[a]	27.28 ± 12.10	12.85 ± 8.74	**< 0.001**[a]
PLT (mcl)	212 ± 56	203 ± 127	0.537[b]	213 ± 70	330 ± 414	0.264[b]	225 ± 62	260 ± 102	0.137[b]
MPV (fL)	10.5 ± 1.1	11.1 ± 1.4	0.365[b]	10.7 ± 1.2	10.5 ± 1.3	0.539[a]	10.3 ± 1.3	10.6 ± 1.5	0.238[b]
PDW (%)	16.2 ± 0.4	16.3 ± 0.4	0.349[b]	16.2 ± 0.4	16.1 ± 0.5	0.169[a]	16.2 ± 0.3	16.3 ± 0.6	0.752[b]
PCT (%)	0.22 ± 0.05	0.21 ± 0.12	0.581[b]	0.23 ± 0.07	0.34 ± 0.44	0.287[b]	0.23 ± 0.07	0.27 ± 0.09	0.073[b]
CRP (mg/L)	14.91 ± 20.72	115.96 ± 83.95	**< 0.001**[b]	17.65 ± 25.90	109.01 ± 114.70	**< 0.001**[b]	15.70 ± 30.03	52.90 ± 44.92	**< 0.001**[b]
D-Dimer (µg/mL)	0.49 ± 0.37	1.50 ± 1.31	**0.006**[b]	0.82 ± 3.33	2.49 ± 3.17	**0.007**[b]	0.51 ± 0.55	5.88 ± 18.56	**< 0.001**[b]
Ferritin (ng/mL)	139.15 ± 225.21	478.30 ± 369.98	**0.046**[b]	158.44 ± 202.80	451.60 ± 571.15	0.091[b]	127.91 ± 186.55	1,030.43 ± 1,142.49	**< 0.001**[b]

Data are shown as mean ± standard deviation. ALT = Alanine Aminotransferase, AST = Aspartate Aminotransferase, LDH = Lactate Dehydrogenase, PLT = platelet, MPV = mean platelet volume, PDW = platelet distribution width, PCT = trombosite ratio in the blood, CRP = C-reactive protein

[a]Independent T test, [b]Mann-Whitney U test

tant type in the period (in the second half of 2021) after the Alpha variant (in the first half of 2021) in our country, our study emphasizes the importance of vaccination in the young population [39-41]. According to our study, the result of hospitalized patients with the Delta variant, the Alpha variant, and no mutation was detected were older ages than outpatients indicating that the older age was an important criteria at hospitalization regardless of mutation. In similar studies, hospitalization was increasing in older patients with Delta mutant, Alpha mutant and no mutation was detected [42-45].

Troponin values were determined lower in patients with the Delta variant than in patients with the Alpha variant and no mutation was detected in our study. Troponin values were established higher in male patients with Delta and Alpha variants than in females while found to be higher in female patients with non-mutation COVID-19 virus detected than in males. The fact that troponin values were found to be higher in hospitalized patients compared to outpatients with Delta variant, Alpha variant, and no mutation, according to our study, highlights that high troponin level is a parameter that indicates the risk of hospitalization independently of the mutation.

Lower PLT values in patients with the Delta variant and Alpha variant indicate that the risk of serious infections of patients with mutant variants is higher than those of non-mutation detected control group patients in our study.

MPV values were determined higher in patients with the Alpha variant, compared to patients with the Delta variant and control group patients in our study. In contrast to some studies, no difference was found between hospitalized patients and outpatients in platelet count and platelet parameters in COVID patients in our study [46].

D-dimer values in patients with the Alpha variant were determined lower than the control group in our study. D-dimer levels were determined higher in hospitalized patients than in outpatients regardless of mutation in our study. Fibrinogen and ferritin values were established higher in hospitalized patients with the Delta variant and no mutation than in outpatients with the Delta variant and no mutation. But fibrinogen and ferritin values were not differentiated between hospitalized and outpatients with the Alpha variant detected. In conclusion, according to our study, D-dimer, ferritin, and fibrinogen values in COVID-19 patients can be used as a guide to determine the severity of the patient's condition and can be used as hospitalization criteria especially independently of mutation.

In our study, similar to other studies, CRP values were determined higher and lymphocyte (N, %) values were established lower in hospitalized patients than in outpatients independent of mutation.

In our study, while creatinine values were lower in control group patients with no mutation detected according to patients with Delta variant, creatinine values were higher in hospitalized control group patientsthan control group outpatients. According to our study, the detection of higher creatinine values in patients with Delta and Alpha variants was not considered a significant parameter during hospitalization. LDH values were found lower in patients with the Delta variant compared to patients with the Alpha variant in our study,. In addition, in our study, similar to other studies, LDH values were higher in hospitalized patients than in outpatients independent of mutation.

The lack of our study was that the analysis can be determined not only by the laboratory data but with the clinical data of the patients. It was another lack of ability to consult the ICU mortality data to determine the prognosis of patients.

Limitations

The limitations of our study were the inability to evaluate the clinical data of the patients, since the study was retrospective, and the inability to evaluate the development of mortality due to COVID-19 infection, especially in hospitalized patients.

In conclusion, age, troponin, creatinine, LDH, PLT, MPV, D-dimer, fibrinogen, ferritin, CRP, lymphocytes (N), and lymphocytes (%) values can guide to evaluate the diagnosis and hospitalization of patients with future different mutations.

CONCLUSION

As a result; lymphocytes (N) and lymphocytes %, values were determined lower in hospitalized patients relative to outpatients while age, troponin, LDH, CRP, and D-dimer values were established higher in hospitalized patients than outpatients irrespective of mutation. In addition, creatinine values were found higher

only in hospitalized patients with no mutation detected while ferritin and fibrinogen values were determined higher inhospitalized patients with Delta variant and no mutation detected.

Authors' Contribution

Study Conception: SKG; Study Design: SKG; Supervision: SKG; Funding: SKG; Materials: SKG; Data Collection and/or Processing: SKG; Statistical Analysis and/or Data Interpretation: SKG; Literature Review: SKG; Manuscript Preparation: SKG and Critical Review: SKG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Twohig KA, Nyberg T, Zaidi A, Thelwall S, Sinnathamby MA, Aliabadi S, et al. Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B.1.617.2) compared with alpha (B.1.1.7) variants of concern: a cohort study. Lancet Infect Dis 2022;22:35-42.
2. Gravagnuolo AM, Faqih L, Cronshaw C, Wynn J, Klapper P, Wigglesworth M. High throughput diagnostics and dynamic risk assessment of SARS-CoV-2 variants of concern. EBioMedicine 2021;70:103540.
3. Hemmer CJ, Löbermann M, Reisinger EC. COVID-19: epidemiology and mutations: an update. Radiologe 2021;61:880-7.
4. WHO. SARS- CoV-2 variants of concern and variants of interest. World Health Organization https://www.who. int/en/activities/tracking- SARS-CoV-2-variants/ 2021.
5. Thye AYK, Law JWF, Pusparajah P, Letchumanan V, Chan KG, Lee LH. Emerging SARS-CoV-2 variants of concern (VOCs): an impending global crisis. Biomedicines 2021;9:1303.
6. Volz, E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, et al. Assessing transmissibility of SARS- CoV-2 lineage B.1.1.7 in England. Nature 2021;593:266-9.
7. Tao K, Tzou PL, Nouhin J, Gupta RK, Oliveira T, Kosakovsky Pond SL, et al. The biological and clinical significance of emerging SARS-CoV-2 variants. Nat Rev Genet 2021;22:757-73.
8. Essa RA, Ahmed SK, Bapir DH, Rasul SA, Khdir AA, Abubakr CP. Clinical features and laboratory findings first case of B. 1.617.2 (delta) variant concern (VOC) in Iraq. Ann Med Surg (Lond) 2021;69:102814.
9. Bernal JL, Andrews N, Gower C, Public Health England Delta Variant Vaccine Effectiveness Study Group. Effectiveness of Covid-19 vaccines against the B.1.617. 2 (Delta) variant. N Engl J Med 2021;385:585-94.
10. Butt AA, Dargham SR, Chemaitelly H, Khal AA, Tang P, Hasan MR, et al. Severity of illness in persons infected with the SARS-CoV-2 delta variant vs beta variant in Qatar. JAMA Intern Med 2021;22;e217949.
11. Xia Y, Qi W, Li X, Yang Y, Cao J. Clinical efficacy and safety of QingshuYiqi decoction as a complementary and alternative therapy for COVID-19 with Delta variant: a protocol for systematic review and meta-analysis. Medicine (Baltimore) 2021;100:e28184.
12. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62.
13. Varmani SG, Chowhan RK, Sharma I, Narang R. COVID-19 and cardiovascular disease: Clinical implications of biochemical pathways. J Pract Cardiovasc Sci 2021;7:97-107.
14. Zaninotto M, Mion MM, Padoan A, Babuin L, Plebani M. Cardiac troponin I in SARS-CoV-2-patients: the additional prognostic value of serial monitoring. Clinica Chimica Acta 2022; 511:75-80.
15. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Napoli RD. Features, Evaluation, and Treatment of Coronavirus (COVID-19). Treasure Island (FL): StatPearls Publishing; 2022.
16. Henry BM, Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med 2020;58:1021-8.
17. Violetis OA, Chasouraki AM, Giannou AM, Baraboutis IG. COVID-19 infection and haematological involvement: a review of epidemiology, pathophysiology and prognosis of full blood count findings. SN Compr Clin Med 2020;2:1089-93.
18. Wool GD, Miller JL. The impact of COVID-19 disease on platelets and coagulation. Pathobiology 2021; 88:15-27.
19. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. Clin Chim Acta 2020;506:145-8.
20. Yıldırım Ö, Bayram M, Özmen RS, Soylu B, Dündar AS, Köksal AR. Evaluation of hematological indices in terms of COVID-19 related mortality and ICU admission. J Health Sci Med 2021;4:666-70.
21. Ozcelik N, Ozyurt S, Yilmaz Kara B, Gumus A, Sahin U. The value of the platelet count and platelet indices in differentiation of COVID-19 and influenza pneumonia. J Med Virol 2021;93:2221-6.
22. Ceyhan MA, Tümer M, Gürü S, Kadı S, Demir GG. Mean platelet volume and red cell distribution width values in patients with COVID-19 admitted to intensive care units or wards from emergency department. Arch Curr Med Res 2021;2:88-92.
23. Hassan S, Sheikh F N, Jamal S, Ezeh JK, Akhtar A. Coronavirus (COVID-19): a review of clinical features, diagnosis, and treatment. Cureus 2020;12:e7355.

24. Rostami M, Mansouritorghabeh H. D-dimer level in COVID-19 infection: a systematic review. Expert Rev Hematol 2020;13:1265-75.
25. Di Micco P, Russo V, Carannante N, Imparato M, Cardillo G, Lodigiani C. Prognostic value of fibrinogen among COVID-19 patients admitted to an emergency department: an Italian cohort study. J Clin Med 2020;9:4134.
26. Cheng L, Li H, Li L, Liu C, Yan S, Chen H, et al. Ferritin in the coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. J Clin Lab Anal 2020;34:e23618.
27. Kunal S, Sharma SM, Sharma SK, Gautam D, Bhatia H, Mahla H, et al. Cardiovascular complications and its impact on outcomes in COVID-19. Indian Heart J 2020;72:593-8.
28. Birben B, Birben OD, Akın T, Akkurt G, Surel AA, Yakısık E, et al. Efficacy of the delta neutrophil index in predicting 30-day mortality in COVID-19 patients requiring intensive care. Int J Clin Pract 2021;75:e13970.
29. Frater JL, Zini G, d'Onofrio G, Rogers HJ. COVID-19 and the clinical hematology laboratory. Int J Lab Hematol 2020;42:11-8.
30. Mao J, Dai R, Du RC, Zhu Y, Shui LP, Luo XH. Hematologic changes predict clinical outcome in recovered patients with COVID-19. Ann Hematol 2021;100: 675-89.
31. Wu J, Shi L, Zhang P, Wang Y, Yang H. Is creatinine an independent risk factor for predicting adverse outcomes in COVID-19 patients? Transpl Infect Dis 2021;23:e13539.
32. Alfano G, Ferrari A, Fontana F, Mori G, Ligabue G, Giovanella S, et.al. Twenty-four-hour serum creatinine variation is associated with poor outcome in the novel coronavirus disease 2019 (COVID-19) patients. Kidney Res Clin Pract 2021;40:231-40.
33. Malik P, Patel U, Mehta D, Patel N, Kelkar R, Akrmah M, et al. Biomarkers and outcomes of COVID-19 hospitalizations: systematic review and meta-analysis. BMJ Evid Based Med 2021;26:107-8.
34. Zhang Y, Zheng L, Liu L, Zhao M, Xiao J. Liver impairment in COVID-19 patients: a retrospective analysis of 115 cases from a single center in Wuhan city, China. Liver Int 2020;40:2095-103.
35. Li C, Ye J, Chen Q, Hu W, Wang L, Fan Y, et al. Elevated lactate dehydrogenase (LDH) level as an independent risk factor for the severity and mortality of COVID-19. Aging (Albany NY) 2020;12:15670-81.
36. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: a pooled analysis. Am J Emerg Med 2020;38:1722-6.
37. Acer Ö, Bahce YG, Özüdoğru O. Association of viral load with age, gender, disease severity, and death in severe acute respiratory syndrome coronavirus 2 variants. J Med Virol 2022;94:3063-9.
38. Chun JY, Jeong H, Kim Y. Age-varying susceptibility to the delta variant (B.1.617.2) of SARS-CoV-2. JAMA Network Open 2022;5:e223064.
39. Cetin M, Balcı PO, Sivgin H, Cetin S, Ulgen A, Demir HD, et al. Alpha variant (B.1.1.7) of SARS-CoV-2 increases fatality-rate for patients under age of 70 years and hospitalization risk overall. Acta Microbiol Immunol Hung 2021;3:153-61.
40. Özüdoğru O, Acer O, Bahçe YG. Risks of catching COVID-19 according to vaccination status of healthcare workers during the SARS-CoV-2 Delta variant dominant period and their clinical characteristics. J Med Virol 2022;94:3706-13.
41. Anadolu agency.'Turkiye, Health', 20.08.2021. https://www.aa.com.tr/en/health/over-90-of-covid-cases-in-turkey-are-delta-variant-health-minister/2341624.
42. Stokes EK, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, et al. Coronavirus disease 2019 case surveillance - United States, January 22-May 30, 2020. MMWR Morb Mortal Wkly Rep 2020;69:759-65.
43. Veneti L, Salamanca BV, Seppälä E, Starrfelt J, Storm ML, Bragstad K, et al. No difference in risk of hospitalization between reported cases of the SARS-CoV-2 delta variant and alpha variant in Norway. Int J Infect Dis 2022;115:178-84.
44. Ryu BH, Hong SI, Lim SJ, Cho Y, Hwang C, Kang H, et al. Clinical features of adult COVID-19 patients without risk factors before and after the nationwide SARS-CoV-2 B.1.617.2 (Delta)-variant outbreak in Korea: experience from Gyeongsangnam-do. J Korean Med Sci 2021;36:e341.
45. Odabasi MS, Ozkaya G, Serin E, Akkus A, Yilmaz P, Sayan I. Laboratory findings in predicting intensive care need and death of COVID-19 patients. Int J Med Biochem 2021;4:77-84.
46. Barrett TJ, Bilaloglu S, Cornwell M, Burgess HM, Virginio VW, Drenkova K, et al. Platelets contribute to disease severity in COVID-19. J Thromb Haemost 2021;19:3139-53.

The European Research Journal 2023;9(2):253-263 Original Article

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The effect of CT-based cardiothoracic ratio on survival of COVID-19 patients

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ABSTRACT

Objectives: We aimed to investigate the relationship between computed tomography (CT)- based cardiothoracic ratio (CTR) with mortality rates of COVID-19 patients.

Methods: Our study was a single-center retrospective analysis of 484 patients (aged ≥ 18) who were admitted to our hospital's emergency department. We included only laboratory-confirmed COVID-19 patients who underwent chest CT. Data of demographic information, laboratory findings, survivals, and chest CT imaging findings were recorded. The radiologist calculated CTR by dividing the greatest transverse cardiac diameter by the greatest transverse thoracic diameter on the initial chest CT. Cardiomegaly was defined if "CTR > 0.5".

Results: Thirty (6.2%) patients were treated as outpatients, and 135/484 (%27.9) patients were treated in the intensive care unit (ICU). A total of 147 /484 (30.4%) patients died. We found a statistical association between cardiomegaly with mortality rates ($p < 0.001$) and ICU admission ($p = 0.008$). In multivariate analysis, older age was 1.07-fold ($p < 0.001$), cardiomegaly 1.75-fold ($p = 0.015$), history of cerebrovascular diseases 2.929-fold ($p = 0.018$), and elevated serum LDH level 1.003-fold ($p = 0.011$) associated with higher risks of mortality.

Conclusions: Since the presence of cardiomegaly on chest CT is associated with a worse prognosis for COVID-19 patients, more caution should be exercised in the evaluation, follow-up, and treatment of COVID-19 patients with cardiomegaly.

Keywords: COVID-19, computed tomography, chest, cardiothoracic ratio

The Coronavirus disease 2019 (COVID-19) pandemic has become a serious public health threat to millions of people worldwide [1]. In COVID-19 patients, the reverse transcriptase-polymerase chain reaction (RT-PCR) test is the standard for diagnosis. But in some cases, it may give a false-negative result, especially in the early stages of the disease, possibly due to insufficient viral samples in the sample or technical problems during nucleic acid extraction [2, 3]. As a result, chest computed tomography (CT) has become one of the main methods for diagnosing COVID-19 and assessing disease severity [4]. Typical chest CT imaging findings of COVID-19 are multifocal ground-glass opacities (GGOs) with or without consolidations that predominate in the posterior areas and reticulations in the lung region near the visceral pleural surface [5].

Emergency department healthcare professionals had a great role in the assessment and treatment of COVID-19 patients during the pandemic, as they are

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at the forefront of the management of patients with acute illness [6]. COVID-19 patients should be evaluated for risk factors at their first admission to the emergency department for appropriate treatment planning. Respiratory involvement in patients with COVID-19 patients' may be a mild flu-like illness or progress to potentially fatal acute respiratory distress syndrome (ARDS) or fulminant pneumonia. Patients with pre-existing cardiovascular disease (CVD) are more vulnerable to developing COVID-19 and have more severe diseases with worse prognoses [7-10]. Also, COVID-19 may worsen the underlying CVD and can precipitate new cardiac complications. In patients with comorbidities such as diabetes, cardio-cerebrovascular disease, and hypertension, the risk of developing serious disease and the need for intensive care unit (ICU) admission increases [8].

The CTR is noted as a reliable marker for cardiomegaly on computed tomography (CT). For many years, first chest radiography and then CT has been used to evaluate CTR in clinical practice. The CTR is obtained by dividing the maximal diameter of the chest by the cardiac maximal diameter and CTR > 0.5 is defined as cardiomegaly [11]. The CTR measurement is a non-invasive and easily available method used to evaluate cardiomegaly and cardiac volume [12].

In the literature, it has been shown that imaging-based cardiac indices predict an increased risk of mortality and morbidity in various non-COVID-19 acute and chronic diseases. They reported that an increase in the cardiothoracic ratio (CTR) is associated with severity in patients with respiratory diseases [13, 14]. Also, Eslami *et al*. [15] investigated the relationship between CTR values with CT severity scores and mortality rates in hospitalized COVID-19 patients with a small sample size.

In the current study, we aimed to investigate the association of the CT-based CTR with the ICU admission and survival of COVID-19 patients with large sample size.

METHODS

This study was approved by the Ethical Committee of Amasya University, Faculty of Medicine, and was conducted according to the Declaration of Helsinki and Good Clinical Practice (8 July 2021, number:126), and the requirement for informed consent was waived.

Study Population and Data Collection

Our study was a single-center retrospective analysis conducted on an original cohort of 484 patients (≥ 18 years) who applied to our hospital's emergency department between September and December 2020. We included only laboratory-confirmed patients which were determined by positive RT-PCR tests. All patients underwent at least one chest CT scan in our hospital's Radiology Department. Patients with negative RT-PCR tests and pre-existing chest wall abnormalities were also excluded from the study.

We collected the data from our hospital's electronic records including demographic characteristics, laboratory findings, lengths of hospitalization or ICU stays, and clinical outcomes of the patients.

RT-PCR tests were performed on all patients' oropharyngeal and nasopharyngeal swab specimens according to WHO interim guidelines. Laboratory tests include complete blood count (CBC), serum biochemistry parameters, inflammatory markers such as C-reactive protein (CRP), lactate dehydrogenase level (LDH), erythrocyte sedimentation rate (ESR), ferritin, coagulation markers such as D-Dimer, International Normalized Ratio (INR), fibrinogen were recorded at admission.

CT Protocol

The non-contrast chest CT examinations were performed in a supine position with the multidetector CT scanner 128- slice GE Healthcare Revolution EVO CT (GE Medical Systems; Milwaukee, WI). The acquisition and reconstruction parameters were as follows: tube potential, 120kV; tube current, 100–450 mA; beam collimation, 64 mm × 0.625 mm; beam pitch, 1.375; gantry rotation, 0.4 seconds; acquisition direction, caudocranial; reconstruction kernel, standard; slice thickness, 0.625 mm; and section overlap, 0.625 mm. All chest CT scans were assessed at a lung window of 1500 WW and −450 WL and a mediastinal window of 400 WW and 40 WL.

Image Analysis

A radiologist with more than 15 years of experience in chest CT imaging, performed the CT image analysis in a standard clinical picture archiving and di-

agnostic system (PACS) workstation, blinded to the clinical data and laboratory indicators.

The parenchymal and mediastinal imaging findings such as GGOs, consolidation, crazy paving pattern, pleural, pericardial effusion were reported by the radiologist. The severity of COVID-19 pulmonary involvement was evaluated by the semi-quantitative CT severity scoring system visually that was previously reported by Pan *et al*. [16]. Depending on the extent of lobe involvement in COVID-19 pneumonia, each lobe is scored from 0 to 5: score 0, 0% involvement; score 1, less than 5% involvement; score 2, 5% to less than 25 % involvement; score 3, 25 % to less than 50 % involvement; score 4, 50 % to less than 75 % involvement; and score 5, 75 % or greater involvement. The total score is obtained by summing the lober scores (range 0-25).

Also, the maximum transverse cardiac diameter (from outer to outer myocardium) and the maximum transverse thoracic diameter were measured manually on axial CT images. CTR was calculated as the greatest transverse cardiac diameter divided by the greatest transverse thoracic diameter (Fig. 1) [15]. CTR > 0.50 was defined as an indicator of cardiomegaly [11].

Statistical Analysis

Statistical analysis was applied using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Released 2017. Armonk, NY). The conformity of the variables to the normal distribution was examined using Kolmogorov-Smirnov. In descriptive analyses, normally distributed variables were represented as mean and standard deviation (SD), and non-normally distributed variables were represented as median [interquartile range (IQR)]. Pearson's chi-squared or Fisher tests were used to compare categorical variables according to cardiomegaly groups (in cases where the values displayed in the cells did not meet the assumptions of the chi-squared test). In the comparison of continuous variables according to cardiomegaly, the Student's t-test was used for those with normal distribution and the Mann-Whitney U test for those who were not normally distributed. The main factors related to mortality were evaluated by univariate binary logistic regression analysis. Explanatory variables with a $p < 0.25$ in the univariate logistic regression analysis were included in the multivariate logistic regression analysis [17]. The Hosmer-Lemeshow test was used for model fit. $p < 0.05$ was considered statistically significant.

RESULTS

Demographic features of the study population

The study population included 484 patients with

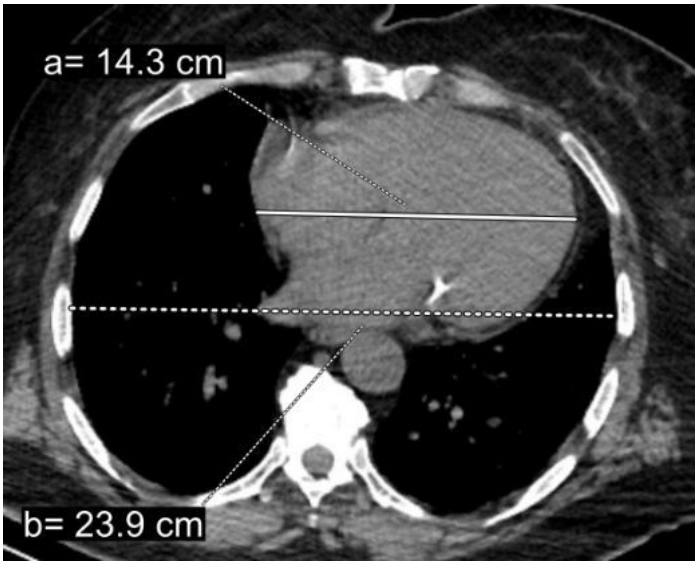

Fig. 1. Axial image of chest CT shows the measurement of maximum transverse thoracic diameter (dashed line) and maximum transverse cardiac diameter (straight line) for calculating the cardiothoracic ratio. The cardiothoracic ratio was calculated as "0.60" by dividing the maximal diameter of the chest by the cardiac maximal diameter and there was cardiomegaly.

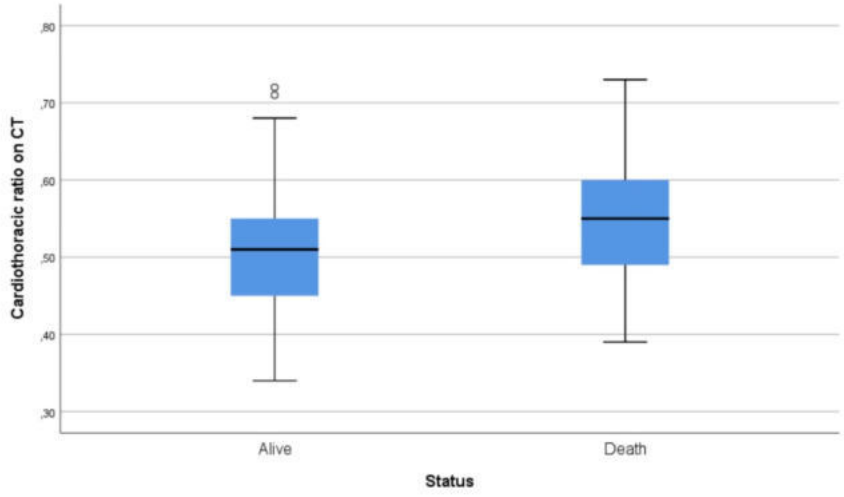

Fig. 2. Statistical graphic shows the cardiothoracic ratio values of alive and deceased patients.

a median value of age was 66 (IQR 55-75), 284 (58.7%) were male. 287/484 (59.2%) patients had cardiomegaly in their CT imaging. The median age of patients with cardiomegaly was 70 (IQR 62-78) and the median age of patients without cardiomegaly was 57 (IQR 47-68.5). Cardiomegaly was statistically associated with older age ($p < 0.001$). 148 (59.1%) patients were male in patients with cardiomegaly ($p < 0.001$). There was a statistical relationship between cardiomegaly and gender ($p < 0.001$). Of the patients 30/484 (6.2%) were treated as outpatients, 135/484 (%27.9) were treated in ICU. 147 /484 (30.4%) patients died in the total study population. 95/135 (70.4%) patients who were treated in ICU had cardiomegaly. 107/147 (72.8%) deceased patients had cardiomegaly. So, we found a statistical association between cardiomegaly with mortality rates ($p < 0.001$) and ICU admission ($p = 0.008$) (Fig 2, Fig 3). According to the chest CT imagings, cardiomegaly was statistically associated with GGOs ($p = 0.014$), consolidation, pleural, pericardial effusion, crazy paving pattern, and mediastinal lymph node enlargement (all $p < 0.001$) in the CT imaging. Most common comorbidities of the study population were hypertension (HT) 239/484 (49.4%), diabetes mellitus (DM) 162/484 (33.5%), hyperlipidemia 130/484 (26.9%) and coronary artery disease (CAD) 88/484 (18.2%). There was a significant statistical association between

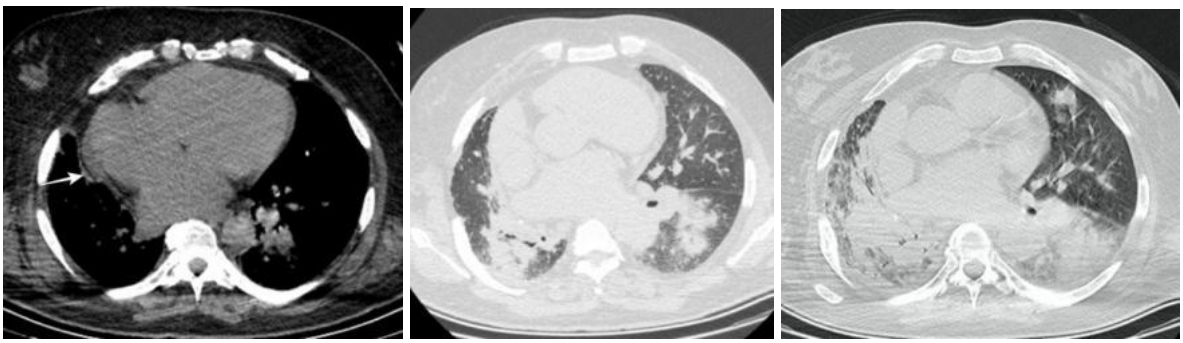

Fig. 3. A 52-year-old man with a history of chronic heart disease was admitted to our hospital with complaints of fever, cough and sore throat. His RT-PCR test was positive. He was hospitalized and treated in the service for 14 days and in the intensive care unit for 6 days. But he died in the intensive care unit. (a) Axial mediastinal window of non-contrast chest CT shows an excessive enlargement of cardiothoracic ratio with minimal pericardial effusion (white arrow), (b) Axial lung window of initial non-contrast chest CT shows bilateral consolidations and ground-glass opacities in the lower lobes. CT-SS = 9 and (c) Axial lung window of second non-contrast chest CT shows bilateral extensive consolidations and ground-glass opacities. Consolidations and CT-SS increased compared to previous CT. CT-SS = 21.

Table 1. Comparison of demographic and chest CT findings according to the presence of cardiomegaly

		Cardiomegaly			
		Absent n (%)	Present n (%)	Total	*p* value
Gender	Male	136 (40.9)	148 (59.1)	284	**< 0.001**
	Female	61 (58.6)	139 (41.4)	200	
Mortality or surviving	Alive	157 (48.2)	180 (51.8)	337	**< 0.001**
	Death	40 (48.5)	107 (51.5)	147	
Intensive care unit	ICU	42 (31.1)	93 (68.9)	135	**0.008**
	Non-ICU	155 (44.4)	194 (55.6)	349	
Ground-glass opacities	Absent	42 (53.2)	37 (46.8)	79	**0.014**
	Present	155 (38.3)	250 (61.7)	405	
Consolidation	Absent	157 (46.9)	178 (53.1)	335	**< 0.001**
	Present	40 (26.8)	109 (73.2)	149	
Crazy paving pattern	Absent	131 (48.5)	139 (51.5)	270	**< 0.001**
	Present	66 (30.8)	148 (69.2)	214	
Pericardial effusion	Absent	184 (44.3)	231 (55.7)	415	**< 0.001**
	Present	13 (18.8)	56 (81.2)	69	
Pleural effusion	Absent	189 (44.4)	237 (55.6)	426	**< 0.001**
	Present	8 (13.8)	50 (86.2)	58	
Diabetes mellitus	Absent	139 (43.2)	183 (56.8)	322	0.120
	Present	58 (35.8)	104 (64.2)	162	
Chronic heart diseases	Absent	190 (43.5)	247 (56.5)	437	**< 0.001**
	Present	7 (14.9)	40 (85.1)	47	
Hypertension	Absent	123 (50.2)	122 (49.8)	245	**< 0.001**
	Present	74 (31)	165 (69)	239	
Hyperlipidemia	Absent	157 (44.4)	197 (55.6)	354	**0.007**
	Present	40 (30.8)	90 (69.2)	130	
Cardiovascular diseases	Absent	165 (44.1)	209 (55.9)	374	**0.005**
	Present	32 (29.1)	78 (70.9)	110	
Chronic pulmonary disease	Absent	169 (41.5)	238 (58.5)	407	0.398
	Present	28 (36.4)	49 (63.6)	77	
Neurological diseases	Absent	189 (41.3)	269 (58.7)	458	0.289
	Present	8 (30.8)	18 (69.2)	26	
Kidney diseases*	Absent	191 (40.3)	283 (59.7)	474	0.329*
	Present	6 (60)	4 (40)	10	

Pearson's chi-squared or Fisher tests (*) were used to compare categorical variables according to cardiomegaly groups. Fisher's test was used in the Chi-square analysis of categorical variables with less than 5 data in cells.

Table 2. Comparison of age, CT-SS and laboratory findings according to the presence of cardiomegaly

			Mean	Std. Deviation	Minimum	Maximum	*p* value
Age	Absent	197	57		47	68.50	**< 0.001***
	Present	287	70		62	78	
	Total	484	66		55	75	
Cardiothoracic ratio	Absent	197	0.45	0.03	0.34	0.50	**< 0.001**
	Present	287	0.57	0.05	0.51	0.73	
	Total	484	0.52	0.07	0.34	0.73	
CT severity score	Absent	197	10		3	16	0.473*
	Present	287	10		4	17	
	Total	484	10		4	17	
Length of stay in hospital	Absent	177	11		8	17.50	0.042*
	Present	276	13		8	20	
	Total	453	12		8	19	
Length of stay in ICU	Absent	42	10.50		6	20.25	0.803*
	Present	94	9.50		7	19	
	Total	136	10		6.25	19	
WBC ($\times 10^9$/L)	Absent	197	7.39	6.58	1.42	66.65	0.482
	Present	287	7.74	4.30	2.25	30.47	
	Total	484	7.60	5.34	1.42	66.65	
Neutrophil count ($\times 10^9$/L)	Absent	197	4.98	5.30	0.34	69	**0.025**
	Present	287	5.97	4.34	1.44	33.32	
	Total	484	5.57	4.77	0.34	69	
Lymphocyte count ($\times 10^9$/L)	Absent	197	1.45	0.70	0.29	4.52	**0.012**
	Present	287	1.28	0.77	0.14	5.60	
	Total	484	1.35	0.75	0.14	5.60	
Triglycerides (mg/dL)	Absent	188	151.95	99.74	42.00	881	0.111
	Present	276	138.09	85.77	3	822	
	Total	464	143.70	91.84	3	881	
Total cholesterol (mg/dL)	Absent	174	153.84	45.09	68	262	**0.004**
	Present	259	142.42	37.00	57	297	
	Total	433	147.01	40.78	57	297	
LDH (U/L)	Absent	195	290.46	135.81	140	1422	**< 0.001**
	Present	285	346.18	148.28	121	1167	
	Total	480	323.54	145.80	121	1422	
ALP (U/L	Absent	175	74.50	35.01	14	30	0.307

Table 2 contunied. Comparison of age, CT-SS and laboratory findings according to the presence of cardiomegaly

			Mean	Std. Deviation	Minimum	Maximum	*p* value
	Present	259	77.91	32.46	20	245	
	Total	434	76.53	33.51	14	309	
CRP (mg/L)	Absent	197	41.90	45.80	0.06	273.10	**< 0.001**
	Present	287	64.34	60.53	0.29	304.19	
	Total	484	55.20	56.05	0.06	304.19	
	Present	284	60.49	61.46	6.00	979.00	
	Total	479	53.46	50.97	6.00	979.00	
Fibrinogen (mg/dL)	Absent	194	498.76	137.58	30.70	987.00	**< 0.001**
	Present	282	551.28	155.97	216	985	
	Total	476	529.88	150.83	30.70	987	
INR	Absent	185	1.04	0.10	0.87	1.63	**0.028**
	Present	281	1.09	0.34	0.09	4.73	
	Total	466	1.07	0.27	0.09	4.73	
D-dimer (µg/mL)	Absent	194	0.88	1.67	0.02	16.79	**0.034**
	Present	283	1.50	3.85	0.05	46.60	
	Total	477	1.25	3.16	0.02	46.60	

WBC = White blood cell, LDH = lactate dehydrogenase, ALP = Alkaline Phosphatase, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, INR = International Normalized Ratio. In the comparison of continuous variables according to cardiomegaly, the Student's t-test was used for those with normal distribution and the Mann-Whitney U test for those who were not normally distributed (*).

cardiomegaly with comorbidities such as hypertension ($p < 0.001$), hyperlipidemia ($p = 0.007$), cardiovascular diseases ($p = 0.005$), and chronic heart disease ($p < 0.001$) (Table 1).

The mean value of CTR was 0.57±0.05 in patients with cardiomegaly, there was a statistical difference between the groups with and without cardiomegaly ($p < 0.001$). The median value of CT- SS of the group with cardiomegaly was 10 (IQR 4-17), and without cardiomegaly was 10 (IQR 3-16). No statistical difference was found between the presence of cardiomegaly with the initial CT-SS ($p = 0.473$). In the total study population, the median value of the length of stay in service of the hospital was 12 (IQR 8-19), and the median value of the length of stay in ICU was 10 (IQR 6.25-19). No statistical difference was found between the presence of cardiomegaly with the length of stay in the hospital or ICU. Statistical associations were found between cardiomegaly with laboratory findings such as elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) ($p < 0.001$), high coagulation factors such as fibrinogen ($p < 0.001$), D-Dimer ($p = 0.034$) and the other serum parameters that are shown in Table 2.

Multivariate analysis of risk factors for mortality

In multivariate analysis, older age 1.07-fold (95% CI: 1.043-1.097, $p < 0.001$), cardiomegaly 1.752-fold (95% CI: 1.114-2.756, $p = 0.015$), history of cerebrovascular diseases 2.929-fold (95% CI: 1.203-7.135, $p = 0.018$) and elevated serum LDH levels 1.003-fold (95% CI: 1.001-1.005, $p = 0.011$) associated with higher risks of mortality (Table 3).

DISCUSSION

In this retrospective analysis, we investigated the impact of the presence of cardiomegaly on ICU admission, and mortality rates of COVID-19 patients. Our

Table 3. Univariate- multivariate logistic regression analysis of predicting the risk of mortality in COVID-19 patients

Mortality	Univariate				Multivariate			
	p-value	OR	95% CI for OR		*p*-value	OR	95% CI for OR	
			Lower	Upper			Lower	Upper
Age	**< 0.001**	1.08	1.06	1.10	**< 0.001**	1.07	1.043	1.097
Gender	0.959	1.010	0.682	1.497				
CT Severity Score	0.466	0.991	0.967	1.016				
WBC	0.02	1.05	1.01	1.09	0.996	1.000	0.937	1.067
Hemoglobin	0.01	0.87	0.79	0.97	0.806	1.018	0.882	1.176
Neutrophil count	**< 0.001**	1.07	1.02	1.13	0.472	1.029	0.951	1.114
Lymphocyte count	**0.04**	0.74	0.56	0.99	0.287	1.423	0.743	2.727
GGT	0.90	1.00	1.00	1.00				
ALP	**0.01**	1.01	1.00	1.01	0.089	1.007	0.999	1.015
LDH	**< 0.001**	1.00	1.00	1.01	**0.011**	1.003	1.001	1.005
CRP	**< 0.001**	1.01	1.00	1.01	0.903	1.000	0.995	1.006
Ferritin	0.14	1.00	1.00	1.00	0.14	1.00	1.00	1.00
ESR	0.56	1.00	1.00	1.01	0.56	1.00	1.00	1.01
Diabetes mellitus	0.56	1.13	0.75	1.70	0.56	1.13	0.75	1.70
Hypertension	**< 0.001**	2.45	1.64	3.66	0.049	1.584	1.002	2.503
Asthma	0.12	1.64	0.88	3.04	0.12	1.64	0.88	3.04
Cardiomegaly	**< 0.001**	2.33	1.53	3.55	**0.015**	1.752	1.114	2.756
Hyperlipidemia	**< 0.001**	1.99	1.31	3.04	0.235	1.361	0.819	2.260
Chronic heart disease	**0.012**	2.462	1.22	4.97	0.110	1.843	0.871	3.900
Chronic obstructive pulmonary disease	0.611	1.217	0.571	2.594	0.611	1.217	0.571	2.594
Cerebrovascular diseases	**0.002**	3.84	1.62	9.08	**0.018**	2.929	1.203	7.135
Chronic kidney disease	0.063	2.525	0.951	6.703				

WBC = White Blood Cell, AST: aspartate aminotransferase, ALT = alanine transaminase, GGT = gamma-glutamyl transferase, LDH = lactate dehydrogenase, ALP = Alkaline Phosphatase, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, INR = International Normalized Ratio. Hosmer and Lemeshow Test= 0.301 Accuracy= 73.8%

study found that the presence of cardiomegaly was associated with a higher risk of the requirement of an ICU treatment or mortality in patients with COVID-19. In multivariate analysis, cardiomegaly 1.752-fold had higher risks of mortality. Our results showed no association between the presence of cardiomegaly with CT-SS, since we only calculated CT-SS in CTs at the time of admission, and lung involvement increased mostly in follow-up CTs of the patients.

COVID-19 symptoms are more severe in patients with CVD. The probable reason for this is the increase in ACE-2 secretion in COVID-19 patients [18]. While pre-existing CVD appears to be associated with increased mortality and worse outcomes in COVID-19 patients [9, 10, 19–21]. COVID-19 itself can cause cardiovascular disorders, such as arrhythmia, acute coronary syndrome, venous thromboembolism, and myocardial injury [22-24]. Also, morbidity and mor-

tality are higher in patients with COVID-19 and CV risk factors such as HT and DM [19, 22]. According to the literature, older patients with comorbidities including HT, CVD, and DM may have a severe prognosis in COVID-19 [25]. In our study, mortality rates of the study population statistically increased with older age, cardiomegaly, history of cerebrovascular disease, and elevated serum LDH levels.

Although there is insufficient evidence to establish a direct relationship between cardiac injury and underlying CVD in COVID-19 patients, it may be thought that patients with CAD or heart failure are more susceptible to heart damage. If severe pneumonia occurs in these patients, they are more prone to myocardial ischemia or heart failure. In addition, acute inflammatory responses may lead to ischemia in patients with pre-existing CVD. Also, inflammation may cause endothelial dysfunction, thereby increasing the procoagulant activity of the blood [26]. Based on these reasons, Shie *et al* speculated that an intensive inflammatory response superimposed on CVD may precipitate the observed heart damage in patients with COVID-19 [22]. Similar to the literature we found increased mortality risk for patients with cardiomegaly.

Previous studies have shown that imaging-based cardiac indices predict an increased risk of mortality and morbidity in various acute and chronic diseases. The increase in the CTR value in patients with non-COVID-19 respiratory diseases is associated with poor clinical outcomes and the severity of the disease [11, 12]. Cardiomegaly is related to an increased risk of CVD. Eslami V. *et al*. reported that a high CTR was associated with increased mortality in hospitalized COVID-19 patients with a small sample size [15]. In the study of Mehrabi Nejad *et al*. [27], cardiomegaly was significantly more common in the deceased group ($p = 0.005$). Our study is the most comprehensive study in the literature, with the largest number of patients, comparing CTR values measured on thorax CT with ICU admission and mortality rates in patients with COVID-19. In accordance with the literature, we also found that patients with cardiomegaly have a higher risk of mortality than patients with COVID-19. Unlike previous studies, we also investigated the effects of cardiomegaly on the need for ICU admission. More COVID-19 patients with cardiomegaly were admitted to ICU than those without cardiomegaly.

It is very important to evaluate COVID-19 patients for cardiomegaly as a risk factor at the time of the first admission to the emergency department. Since they are the first to encounter COVID-19 patients, emergency department health professionals have important contributions to the management of the diagnosis and treatment of their patients. It should be known that COVID-19 patients with cardiomegaly detected on CT or radiography may have a high risk of needing ICU treatment or mortality and more care should be taken in the follow-up, consultation, follow-up, and treatment of these patients.

Limitations

There were some limitations in our study. First, our study was a single-center, retrospective study. So, a multicenter study with large sample size is needed for more validation. Second, CTR was measured on chest CTs of patients in our study, and the measurements may be affected by changes in cardiac motion and breathing. Third, we evaluated the initial chest CT images of the patients. We did not use follow-up CTs, so we cannot review late period CT finding changes.

Key points

-Our study is the most comprehensive study in the literature, with the largest number of patients, comparing CTR values measured on chest thorax CT with ICU admission and mortality rates of COVID-19 patients.

-Presence of cardiomegaly was associated with a high risk of ICU admission and mortality.

-Evaluation of the presence of cardiomegaly on initial chest CT can provide useful prognostic information and assist physicians and nurses in the triage and follow-up of patients with COVID-19

CONCLUSION

We found that the presence of cardiomegaly is associated with the high risk of ICU admission and mortality and may predict survival in patients with COVID-19. Therefore, evaluation of the presence of cardiomegaly on thoracic CT can provide useful prognostic information and assist physicians in the triage and follow-up of patients with COVID-19.

Authors' Contribution

Study Conception: BA, ATK; Study Design: BA, ATK; Supervision: BA, ATK; Funding: N/A; Materials: BA, ATK; Data Collection and/or Processing: BA, ATK; Statistical Analysis and/or Data Interpretation: BA, ATK, ŞÇ; Literature Review: BA; Manuscript Preparation: BA and Critical Review: BA, ATK, ŞÇ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

REFERENCES

1. Majumder J, Minko T. Recent developments on therapeutic and diagnostic approaches for COVID-19. AAPS J 2021;23:14.
2. Kanji JN, Zelyas N, MacDonald C, Pabbaraju K, Khan MN, Prasad A, et al. False negative rate of COVID-19 PCR testing: a discordant testing analysis. Virol J 2021;18:13.
3. Ding X, Xu J, Zhou J, Long Q. Chest CT findings of COVID-19 pneumonia by duration of symptoms. Eur J Radiol 2020;127:109009.
4. Wang Y, Dong C, Hu Y, Li C, Ren Q, Zhang X, et al. Temporal changes of CT findings in 90 patients with COVID-19 pneumonia: a longitudinal study. Radiology 2020;296:E55-64.
5. Nair AV, Kumar D, Yadav SK, Nepal P, Jacob B, Al-Heidous M. Utility of visual coronary artery calcification on non-cardiac gated thoracic CT in predicting clinical severity and outcome in COVID-19. Clin Imaging 2021;74:123-30.
6. Spoorthy MS, Pratapa SK, Mahant S. Mental health problems faced by healthcare workers due to the COVID-19 pandemic - A review. Asian J Psychiatr 2020;51:102119.
7. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020;323:1239-42.
8. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clin Res Cardiol 2020;109:531-8.
9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
10. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
11. Jotterand M, Faouzi M, Dédouit F, Michaud K. New formula for cardiothoracic ratio for the diagnosis of cardiomegaly on post-mortem CT. Int J Legal Med 2020;134:663-7.
12. Ogata H, Kumasawa J, Fukuma S, Mizobuchi M, Kinugasa E, Fukagawa M, et al. The cardiothoracic ratio and all-cause and cardiovascular disease mortality in patients undergoing maintenance hemodialysis: results of the MBD-5D study. Clin Exp Nephrol 2017;21:797-806.
13. Zouk AN, Gulati S, Xing D, Wille KM, Rowe SM, Wells JM. Pulmonary artery enlargement is associated with pulmonary hypertension and decreased survival in severe cystic fibrosis: a cohort study. PLoS One 2020;15:e0229173.
14. Wells JM, Morrison JB, Bhatt SP, Nath H, Dransfield MT. Pulmonary artery enlargement is associated with cardiac injury during severe exacerbations of COPD. Chest 2016;149:1197-204.
15. Eslami V, Abrishami A, Zarei E, Khalili N, Baharvand Z, Sanei-Taheri M. The association of CT-measured cardiac indices with lung involvement and clinical outcome in patients with COVID-19. Acad Radiol 2021;28:8-17.
16. Pan F, Ye T, Sun P, Gui S, Liang B, Li L. Time course of lung changes at chest CT during recovery. Radiology 2020;295:715-21.
17. Menard S. Applied logistic regression analysis. Quantitative Applications in Social Sciences, Vol. 106, 2nd ed., Sage Publications, Inc., 2002.
18. Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol 2020;17:259-60.
19. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.
20. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62.
21. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 2020;46:846-8.
22. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020;5:802-10.
23. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol 2020;5:811-8.
24. Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. Eur Heart J 2020;41:2070-9.
25. Wei PF. Diagnosis and treatment of pneumonia caused by novel coronavirus (trial version 7). General office of the Chinese health commission 2020. Chin Med J 2020;133:1087-95.
26. Corrales-Medina VF, Musher DM, Shachkina S, Chirinos JA.

Acute pneumonia and the cardiovascular system. Lancet 2013;381:496-505.
27. Mehrabi Nejad M-M, Abkhoo A, Salahshour F, Salehi M, Gity M, Komaki H, et al. Chest CT scan features to predict COVID-19 patients' outcome and survival. Radiol Res Pract 2022;2022:4732988.

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Mothers' perspective on human papillomavirus vaccine in Eastern Turkey

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ABSTRACT

Objectives: The rates of vaccination with human papillomavirus (HPV) vaccine, which can prevent many cancers, rank the last place when compared to other childhood vaccination rates. The purpose of the present study was to evaluate the knowledge levels of mothers living in our region about HPV vaccine and their perspectives regarding having the vaccine.

Methods: The study was conducted between 15 July and 15 August 2022 in the Department of Obstetrics and Gynecology, Faculty of Medicine, Kafkas University with the mothers of 377 girls. The SPSS 21 program was used and $p < 0.05$ was accepted as significant.

Results: No statistically significant differences were detected between the desire of the mother to have her child vaccinated against HPV and the age of her child ($p = 0.740$), income level of the household ($p = 0.590$) and the working status of the mother ($p = 0.419$). There were statistically significant differences between the desire of the mother to have her daughter vaccinated with HPV, the place where the mother lived ($p = 0.001$), age of the mother ($p = 0.037$), family type ($p = 0.001$), number of people living at home ($p = 0.027$), education year of the mother ($p = 0.004$), father's approval for vaccination ($p = 0.001$), and mother's hearing about the Smear Test ($p = 0.001$).

Conclusions: Even if the HPV vaccine is given free of charge, there is resistance in mothers to the vaccine. It is necessary to inform mothers about HPV vaccination for their daughters and to make the vaccination more widespread.

Keywords: Human papillomavirus (HPV) vaccine, vaccine hesitancy, cervical cancer, genital wart

Although the Human Papillomavirus (HPV) is a common infection worldwide, it is the most common infection among sexually transmitted infections. It is also the most common known cause of cervical, vulva, anus and penile cancers. It was announced by the Center for Disease Control that more than 75% of the society will face HPV at least once in their sexual lives [1-3]. The incidence of HPV-related infection was found to be more than 14 million per year. Cervical cancer is the second most common cause of cancer-related mortality in women with more than 528.000 new cases and more than 300.000 deaths on an annual scale [4, 5].

In Turkey, the national HPV screening program was initiated on August 1, 2008. When the results of this program on 10 January 2019 were examined, the

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HPV positivity rate was determined as 187312 (4.3%) in our country [6].

There are multiple HPV types. The most common oncogenic types known are HPV 16, 18, playing active roles in approximately 94% of cervical cancer [7]. HPV vaccines were developed aside from early diagnosis treatment strategies to prevent the extremely common HPV infection and its effects on society [8, 9]. Vaccination provides 75% protection against cervical cancer, but for greater protection, vaccination is recommended by the World Health Organization to apply before the first sexual contact [10, 11]. The most effective factor is parents' attitude towards vaccination in adolescent vaccination [12].

The prevalence of the infections that are preventable with vaccination is decreasing, and many diseases are no longer seen with the vaccination of the entire population. When the literature data were reviewed, the adolescent vaccination rate in the United States was found to be 14% in girls [13, 14]. The rate of parents vaccinating their children is very low despite the very high efficacy of the vaccine.

The rates of vaccination with HPV vaccine, which can prevent many cancers, are in the last place when compared to other childhood vaccination rates. For this reason, the purpose of the present study was to examine the knowledge levels of mothers living in our region about HPV vaccine and their perspectives against having the vaccine.

METHODS

In Turkey, childhood vaccination rates vary between 64% and 82% according to TDHS 2018 data. In a study that was conducted in the city of Kars, which is located in the Northeast of Turkey, where the study was conducted, in 2020, the knowledge level of mothers about childhood vaccines was found to be 94.9%, and the rate of mothers who knew all vaccines was 27.7%. However, the study only included the vaccines included in the national immunization schedule [15].

The Type of Study

The study had a hospital-focused cross-sectional design.

The Population of the Study

The number of women who applied to the Obstetrics and Gynecology Department of the Kafkas University, Faculty of Medicine, in 2021 was used to determine the study population. In this respect, the total number of applicants was 9658 in 2021. Considering that the same number of people would apply in 2021, the study population was determined as 9658.

The Sample of the Study

The number of mothers of children to be sampled was calculated with the formula "n = Nt2 p q/d2 (N-1) + t2 p q" because the universe of the study was known. Here, N refers to the number of individuals in the universe, **n** is the number of individuals to be sampled; **p** is the incidence (probability) of the event under investigation; **q** is the frequency (probability) of the event under investigation; t is the theoretical value in the table t at a given degree of freedom and detected error level; d is the desired ± deviation according to the prevalence of the event. In this respect, when *p* = ; q = 0.80; t = 1.96; d = 0.05, the sample size was determined as 183 mothers [16]. However, the study was continued with 377 mothers to increase its quality.

Ethics Committee Approval

The study was reviewed by the Ethics Committee of University of Kafkas (approval data and number:28.06.2022/06) and was conducted in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. Also, written consent was obtained from the patients who would participate in the study.

Data Collection

The data of the study were collected by the researcher between 15 July and 15 August 2022 in the Obstetrics and Gynecology Clinic by using face-to-face interview technique. Dependent Variable of the Study: The status of mothers of girls who had HPV vaccination to their children. Independent Variables of the Study: The sociodemographic, biodemographic, and socioeconomic characteristics.

Preliminary Implementation of the Study: It was conducted with the mothers of 11 children who applied to the clinic. Necessary adjustments were made in the data collection form.

Statistical Analysis

The Chi-Square Analyzes were used for the comparisons of the data that were determined by counting. The variables that were significant in the Chi-Square Analyzes were included in the Logistic Regression Analysis (Backward:LR) and $p < 0.05$ was considered significant.

RESULTS

A total of 377 mothers who had adolescent girls participated in the study. The question "*Would you have the HPV vaccine if it were free of charge?*" was asked to these women.

The level of mothers who did not want to have their children vaccinated was found to be 65.0% in the study (Table 1).

Table 1 provides the results of the binary analyzes and logistic regression analysis. When the table is followed, it is seen that no statistically significant differences were found between the mother's desire to have her child vaccinated against HPV and the age of the child ($p = 0.740$), income level of the household being adequate for living ($p = 0.590$), and working status of

Table 1. Distribution and logistic regression analysis results of socio demographic characteristics on the mother's desire to have her child vaccinated against HPV

Independent variables		Request to vaccinate your child		Total	X^2	*p value*			
		Yes	No				OR	CI %95	*p value*
		n (%)*	n (%)*	n (%)**					
Where the woman lives	Town	13 (13.7)	82 (86.3)	95 (25.2)	25.392	**0.001**	4.057	1.710-9.623	**0.001**
	City	119 (42.2)	163 (57.8)	282 (74.8)			1 (reference)		
Woman's age (years)	40 ≥	52 (29.5)	124 (70.5)	176 (46.7)	4.337	**0.037**	3.390	1.733-6.633	**0.001**
	39 ≤	80 (39.8)	121 (60.2)	201 (53.3)			1 (reference)		
Child's age (months)	11.50 ≤	72 (34.3)	138 (65.7)	210 (55.7)	0.110	0.740			
	11.51 ≥	60 (35.9)	107 (64.1)	167 (44.3)					
Family type	Extended	9 (9.3)	88 (90.7)	97 (25.7)	38.014	**0.001**	13.566	5.507-33.422	**0.001**
	Nuclear	123 (43.9)	157 (56.1)	280 (74.3)			1 (reference)		
Number of people living in the house	5 ≥	56 (42.4)	76 (57.6)	132 (35.0)	4.903	**0.027**	5.749	2.801-11.802	**0.00**1
	4 ≤	76 (31.0)	169 (69.0)	245 (65.0)			1 (reference)		
Mother's education year	8 ≤	81 (30.5)	185 (69.5)	266 (70.6)	8.264	**0.004**	0.497	0.210-1.180	0,113
	9 ≥	51 (45.9)	60 (54.1)	111 (29.4)			1 (reference)		
Mother's working status	Working	29 (31.5)	63 (68.5)	92 (24.4)	0.652	0.419			
	Not working	103 (36.1)	182 (74.3)	285 (75.6)					
Father's consent to have his child vaccinated against HPV	Confirms	9 (11.5)	69 (88.5)	78 (20.7)	23.818	**0.001**	4.615	1.848-11.524	**0.001**
	Disapproving	123 (41.1)	176 (71.8)	299 (79.3)			1 (reference)		
Total income entering the house	It is enough	39 (37.1)	66 (62.9)	105 (27.9)	0.290	0.590			
	It is not enough	93 (34.2)	179 (65.8)	272 (72.1)					
Mother's job	Working	29 (31.5)	63 (68.5)	92 (24.4)	0.652	0.419			
	Not working	103 (36.1)	182 (63.9)	285 (75.6)					
Has the mother heard of the smear test?	No	99 (54.1)	84 (45.9)	183 (48.5)	56.928	**0.001**	3.898	2.012-7.551	**0.001**
	Yes	33 (17.0)	161 (83.0)	194 (51.5)			1 (reference)		
Total		132 (35.0)	245 (65.0)	377 (100.0)					

*row percentage, **column percentage, ***OR = odds ratio, CI = confidence interval

the mother (p = 0.419). Also, there was a statistically significant difference between the mother's desire to have her child vaccinated with HPV, place where the mother lived (p = 0.001), age of the mother (p = 0.037), family type (p = 0.001), number of people living at home (p = 0.027), education year of the mother (p = 0.004), father's approval for vaccination (p = 0.001), and mother's hearing about the Smear Test (p = 0.001).

On the other hand, when the age group of 39 years and younger was taken as a reference, the status of the mother not being vaccinated was 3.390-foldmore (CI: 1.733-6.633) in the group aged 40 and over;when those living in the city were taken as reference, it was 4.057-fold more (CI: 1.710-9.623) in the group living in rural areas; when elementary families were taken as reference, it was 13.566-fold more (CI: 5.507-33.422) in those living in extended families; when the number of people living at home was taken as 4 and below was taken as reference, it was 5.749-fold more (CI: 2.801-11.802) in families with 5 people and above;when those who did not have the father's consent to vaccinate children were taken as reference, it was 4.615-fold more (CI: 1.848-11.524) in those who had consent; and when mothers who had heard of the Smear Test were taken as references, it was 3.898-fold more (CI: 2.012-7.551) in those who had not heard of the Smear Test (Table 1).

DISCUSSION

It is known in our present day that some infectious diseases can be prevented or even eliminated through vaccination. However, vaccination programs are delayed due to vaccine hesitation and people continue to become sick. Factors that may affect the spread of vaccination must be examined in detail and measures should be taken against these [17].

A total of 65.0% of the mothers participating in the study did not want their children to be vaccinated. In previous studies, the rates of requesting HPV vaccination to their children in Turkey were found to be 51.7%, 62%, 49% and 24.1%, respectively. The fact that there was such a difference between studies was because of the questions asked to the participants of the study. These questions were in the form of "Would you have the vaccine if it were covered by the state?", or "Would you have it if you paid a fee for the vaccine?" [18]. When a worldwide literature review was conducted, it was found that the willingness to vaccinate was higher if the vaccine was administered free of charge [19, 20].

In the present study, when the age group 39 and below was taken as reference, the rate of mothers not having their children vaccinated was found to be 3.390-fold more (CI: 1.733-6.633) in the group aged 40 and above. When the literature studies are examined, we think that the reason for this is the increased health literacy rates as the mothers come into contact with more sexually transmitted diseases as their age increases, they go to gynecological examinations more, and therefore, they are more informed by the physicians [8].

In the present study, in accordance with the literature data, when those living in the city were taken as a reference, there was a desire to have the vaccine for children by 4.057-fold more (CI: 1.710-9.623) with HPV vaccine in those living in rural areas. In some studies in the literature, it was reported that this is the opposite. In these studies, it was explained that women living in urban areas might want to be vaccinated more because they had easier access to healthcare centers, and therefore, are more informed by physicians [21]. In rural areas, where the present study was conducted, especially the family structure is extended family and the region has a patriarchal structure. For this reason, mothers both receive information about HPV, genital warts, and cervical cancer from their elder relatives at home, and see the problems they experience when they have one of these, and their desire to vaccinate their children increases in this way [22, 23].

When the nuclear family is taken as reference, the desire of mothers to have HPV vaccine increased 13.566-fold (CI: 5.507-33.422) among those living in extended families. Elderly individuals who faced diseases such as HPV-based cancer or pre-cancerous lesions in families knew how dangerous it was and did not want their children to experience the same pain [24-26]. When the number of people living at home is 4 or less, the desire to have the vaccinate increased 5.749-fold more (CI: 2.801-11.802) in families with 5 people or more. The reason for this is thatmothers want to have their daughters vaccinated for fear of more cervical cancer in line with the literature data [27]. In fact, Texas Children's Hospital prepared a

book containing the stories of those who had cervical cancer in their families, trying to increase the rate of vaccination against HPV through exposure [28].

There is a reverse relationship between the increased education levels of mothers and the desire to have their children vaccinated against HPV, in line with the literature data. The reason for this is that women who have higher education levels want to do more research themselves, instead of receiving information from healthcare professionals. These mothers read wrong information especially on social media and accept this as correct, and therefore, their desire to have their children vaccinated decreases [29].

In the present study, when the father's consent to vaccinate children was taken as reference, it was shown that the desire of the mother to be vaccinated increased 4.615-fold (CI: 1.848-11.524) in those who did, in line with the literature data. This is because of the fact that the structure of the region is more patriarchal [30].

In line with the literature data, when mothers who have heard about the Smear Test are taken as reference, there is a desire to have HPV vaccination 3.898-fold more (CI: 2.012-7.551) in those who have not heard of the Smear Test. The reason for this is that patients are informed about why the test is done and the diseases that may occur as a result before the Smear Test, as well as the methods of protection from these diseases. In this regard, women who have Smear Tests become more knowledgeable about HPV, their risk perception increases, and they tend to have the vaccinate to protect their children [27].

Limitations

The single-center nature of the study was the limitation of the study. The strength of the study was that it contained the first data of the region.

CONCLUSION

Inconclusion, health policies should be developed to provide detailed information on the diseases that HPV might cause in the region where the study was conducted.

Authors' Contribution

Study Conception: ÜATA, ÖYS; Study Design: ÜATA, ÖYS, AEY; Supervision: ÜATA, SK, ÖYS, AEY; Funding: ÜATA, SK; Materials: ÜATA, SK, ÖYS; Data Collection and/or Processing: ÜATA, SK; Statistical Analysis and/or Data Interpretation: ÜATA, SK, ÖYS, AEY; Literature Review ÜATA, SK, ÖYS, AEY; Manuscript Preparation: ÜATA, SK, AEY and Critical Review: ÜATA, SK, ÖYS, AEY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. STD Facts - HPV and Men. Avaible at: http://www.cdc.gov/std/hpv/std-fact-hpv-and-men.htm. Accessed date: September 24, 2022.
2. Ho GY, Studentsov Y, Hall CB, Bierman R, Beardsley L, Lempa M, et al. Risk factors for subsequent cervico vaginal human papillomavirus (HPV) infection and the protective role of antibodies to HPV-16 virus-like particles. J Infect Dis 2002;186:737-42.
3. Koutsky L. Epidemiology of genital human papilloma virus infection. Am J Med 1997;102(5A):3-8.
4. Mabeya H, Odunga J, Broeck DV. Mothers of adolescent girls and human papilloma virus (HPV) vaccination in Western Kenya. Pan Afr Med J 2021;38:126.
5. Caballero-Pérez P, Tuells J, Rementería J, Nolasco A, Navarro-López V, Arístegui J. [Acceptability of the HPV vaccine among Spanish university students in the pre-vaccine era: a cross-sectional study]. Rev Esp Quimioter 2015;28:21-8. [Article in Spanish]
6. Turkey cancer statistics. Avaible at: https://hsgm.saglik.gov.tr/depo/birimler/kanser- Accessed date: September 24, 2022.
7. Seoud M, Tjalma WA, Ronsse V. Cervical adenocarcinoma: moving towards better prevention. Vaccine 2011; 29 (49):9148–5.
8. Patel PR, Berenson AB. Sources of HPV vaccine hesitancy in parents. Hum Vaccin Immunother. 2013;9(12):2649-53.
9. Bosch FX, Lorincz A, Muñoz N, Meijer CJ, Shah KV. The causal relation between human papilloma virus and cervical cancer. J Clin Pathol 2002;55:244-65.
10. HPV Vaccına- WHO. . Avaible at: http://www.who.int/immunization/hpv/learn/comprehensive_cervical_cancer_who_2013.pdf. Accessed date: September 24, 2022.
11. Low EL, Simon AE, Lyons J, Romney-Alexander D, Waller J. What do British women know about cervical cancer symptoms and risk factors? Eur J Cancer 2012;48):3001-8.
12. Wamai RG, Ayissi CA, Oduwo GO, Perlman S, Welty E, Manga S, et al. Assessing the effectiveness of a community-based

sensitization strategy in creating awareness about HPV, cervical cancer and HPV vaccine among parents in North West Cameroon. J Community Health 2012;37:917-26.
13. Laz TH, Rahman M, Berenson AB. An update on humanpapillomavirus vaccine uptake among 11-17 year old girls in the United States: National Health Interview Survey, 2010. Vaccine 2012;30:3534-40.
14. Laz TH, Rahman M, Berenson AB. Human papillomavirus vaccine uptake among 9-17 year old males in the United States: the National Health Interview Survey, 2010. Hum Vaccin Immunother 2013;9:874-8.
15. Evran M, Bozkurt HB. [Knowledge status and affecting factors about the current childhood vaccines of mothers of children applying to state and university hospital pediatric clinics in Kars]. Kafkas J Med Sci 2020;10:173-9. [Article in Turkish]
16. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JH. Basic and Clinical Biostatistics (Lange Medical Books) 2nd edition by Dawson-Saunders, Beth, Trapp, Robert G. 1994.
17. Adverse Effects of Vaccines: Evidence and Causality. Committee to Review Adverse Effects of Vaccines; Institute of Medicine; Stratton K, Ford A, Rusch E, et al., eds. Washington (DC):National Academies Press (US); 2011 Aug 25.
18. Monsonego J, Bosch FX, Coursaget P, Cox JT, Franco E, Frazer I. Cervical cancer control, priorities and new directions. Int J Cancer 2004;108:329-33.
19. Walhart T. Parents, adolescents, children and the human papilloma virus vaccine: a review. Int Nurs Rev 2012;59:305-11.
20. Özdemir S, Akkaya R, Karaşahin KE. Analysis of community-based studies related with knowledge, awareness, attitude, and behaviors towards HPV and HPV vaccine published in Turkey: a systematic review. J Turk Ger Gynecol Assoc 2020;21:111-23.
21. Rosenthal SL, Rupp R, Zimet GD, Meza HM, Loza ML, Short MB, et al. Uptake of HPV vaccine: demographics, sexual history and values, parenting style, and vaccine attitudes. J Adolesc Health 2008;43:239-45.
22. Dahlström LA, Tran TN, Lundholm C, Young C, Sundström K, Sparén P. Attitudes to HPV vaccination among parents of children aged 12-15 years-a population-based survey in Sweden. Int J Cancer 2010;126:500-7.
23. Ferris DG, Waller JL, Miller J, Patel P, Jackson L, Price GA, et al. Men's attitudes toward receiving the humanpapillomavirus vaccine. J Low Genit Tract Dis 2008;12:276-81.
24. Omer SB, Salmon DA, Orenstein WA, deHart MP, Halsey N. Vaccine refusal, mandatory immunization, and the risks of vaccine-preventable diseases. N Engl J Med 2009;360:1981-8.
25. Zimet GD, Mays RM, Sturm LA, Ravert AA, Perkins SM, Juliar BE. Parental attitudes about sexually transmitted infection vaccination for their adolescent children. Arch Pediatr Adolesc Med 2005;159:132-7.
26. Ritvo P, Wilson K, Willms D, Upshur R, Goldman A, Kelvin D, et al. CANVAC Socio behavioural Study Group. Vaccines in the public eye. Nat Med 2005;11(4 Suppl):S20-4.
27. Olshen E, Woods ER, Austin SB, Luskin M, Bauchner H. Parental acceptance of the human papilloma virus vaccine. J Adolesc Health 2005;37:248-51.
28. Patel PR, Berenson AB. Sources of HPV vaccine hesitancy in parents. Hum Vaccin Immunother 2013;9:2649-53.
29. Burton-Jeangros C, Golay M, Sudre P. [Compliance and resistance to child vaccination: a study among Swiss mothers]. Rev Epidemiol Sante Publique 2005;53:341-50. [Article in French]
30. Cassell JA, Leach M, Poltorak MS, Mercer CH, Iversen A, Fairhead JR. Is the cultural context of MMR rejection a key to an effective public health discourse? Public Health 2006;120:783-94.

The European Research Journal 2023;9(2):270-276 Original Article

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The systemic immune-inflammation index (SII) in predicting postoperative systemic inflammatory response syndrome (SIRS) after ureteroscopy

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ABSTRACT

Objectives: The predictive value of some preoperative inflammatory markers for postoperative infective complications in urinary system stone disease has been demonstrated. We investigated how valuable the systemic immune-inflammation index (SII) is as a marker for the development of postoperative systemic inflammatory response syndrome (SIRS) in patients undergoing ureteroscopy.

Methods: The data of 495 patients who met the study criteria were evaluated retrospectively. The patients were divided into groups according to whether they developed SIRS postoperatively or not. The clinical and demographic data of the patient groups as well as blood parameters were evaluated.

Results: Postoperative systemic inflammatory response syndrome was detected in 105 (21.2%) of the patients. Age, diabetes, cardiovascular disease, preoperative urinary culture positivity, SII, neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR) and platelet-lymphocyte ratio (PLR) were found to be significant for the development of SIRS. When the areas under the curve were measured in the ROC analysis, it was seen that the SII, NLR, LMR and PLR had high predictive values for the development of SIRS (area under the curve values of 0.73, 0.76, 0.72, 0.67, respectively). In the multivariate logistic regression analysis, among the risk factors, age, cardiovascular disease, preoperative urinary culture positivity and NLR were determined as independent risk factors.

Conclusions: Study revealed that only NLR among inflammatory markers is an independent risk factor for the development of SIRS. Although there is a relationship between the preoperative SII and the development of SIRS, it is not an independent risk factor. Along with many other variables, it may contribute to the development of SIRS.

Keywords: Biomarkers, systemic inflammatory response syndrome, ureterolithiasis, ureteroscopy

The incidence of urinary tract stones depends on geographical, ethnic, dietary and genetic factors. The risk of recurrence in stone disease is associated with the underlying disorder. The prevalence of urinary system stone disease is up to 20% with regional differences. The prevalence is higher in North American and European countries with high living standards. Management of ureteral stones is determined by the location and size of the stone and the clinical characteristics of the patient. In the current urology guideline, treatment options for ureteral stones are medical expulsive therapy, shock wave lithotripsy

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(SWL), and ureteroscopy (URS) [1]. Infectious complications are observed at a rate of 9-25% after URS. These infections can progress to serious conditions such as urosepsis and septic shock [2]. Therefore, it is of great importance to be able to predict infectious complications before surgery. Systemic inflammatory response syndrome (SIRS) can occur for many reasons such as infection, trauma and surgery. It refers to the clinical condition, which can lead to organ damage, caused by an excessive inflammatory response. In the literature, the relationship between biomarkers such as neutrophil- lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR) and platelet-lymphocyte ratio (PLR) and inflammatory response has been reported [4]. In some studies, these markers have been shown to have predictive value for postoperative SIRS after urinary stone surgery [4,5]. The systemic immune-inflammation index (SII) (platelet x neutrophil/lymphocyte) is another marker showing high inflammatory processes. The role of SII in many malignant diseases has been evaluated [6-8]. In this study, the predictive value of preoperative SII in the development of SIRS after URS was investigated.

METHODS

Patients who underwent URS in our clinic between January 2021 and September 2022 were evaluated retrospectively (Ethics committee approval number: E2-22-2677). Oncology patients, immunosuppressive patients, patients with hematological disease, patients with previous ureteral stent, patients with active infection and patients with preoperative SIRS criteria were not eligible for the study. Of 655 patients who underwent URS in our clinic, 495 were counted in the study. Detailed medical histories of all patients were obtained. The comorbidities of the patients were questioned. Those with diabetes mellitus, hypertension, and cardiovascular disease were noted. Patients with atherosclerotic heart disease such as previous myocardial infarction or coronary bypass surgery were included in the cardiovascular disease group. The size and localization of the stones were evaluated with direct urinary system radiography (DUSG) and non-contrast computed tomography (CT). Patients were given 1 g of cefazolin as routine perioperative antibiotic prophylaxis.

Patients who met two or more of the following criteria were diagnosed with SIRS: body temperature < 36 °C or > 38 °C, heart rate > 90 bpm, respiratory rate > 20 breaths/min or PaCO2 < 32 mmHg and white blood cell count > 12×109 cells/ L or < 4×109 cells/L.

The patients were divided into groups according to whether they developed SIRS postoperatively or not. The groups were statistically compared in terms of demographic data and preoperative inflammatory markers, and it was evaluated whether there was a significant relationship.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS 25.0 software package. Normality of distribution for continuous variables was evaluated with the One-Sample Kolmogorov-Smirnov Test. Mann-Whitney U test was used to analyze the quantitative variables of the groups that did not show normal distribution, and the Chi-Square test was used to analyze the categorical variables. $P < 0.05$ was considered to be significant. The optimal threshold value was calculated by performing ROC analysis for the variables that were found to be significant. The diagnostic values of the biomarkers were determined by calculating the areas under the curve. Variables found to be significant were evaluated using a multivariate logistic regression model to identify independent risk factors for the development of SIRS after URS.

RESULTS

All clinical characteristics and laboratory values are shown in Table 1. One hundred and sixty-eight (33.9%) of the patients were female and 327 (66.1%) were male. SIRS were detected in 105 (21.2%) of the patients. The incidence of SIRS was found to be 19% (32/168) in women and 22% (73/327) in men. There was no difference between the groups with and without SIRS in terms of gender, stone size, stone localization, hydronephrosis grade, and serum platelet count. The age of the group with SIRS was significantly higher ($p < 0.001$). Further, SIRS was found to be significantly higher in those treated for urinary tract infection in the preoperative period ($p < 0.001$). In patients who were evaluated in the emergency outpatient clinic, SIRS was significantly higher than those oper-

Table 1. Comparison of clinical characteristics and laboratory values between SIRS group and non-SIRS group

	No SIRS n = 390 (78.8%)	SIRS, n = 105 (21.2%)	*p* value
Age (years), median (min-max)	50 (20 - 88)	56 (20 - 88)	**< 0.001****
Gender, n (%)			0.419*
Male	254 (65.1)	73 (69.5)	
Female	136 (34.9)	32 (30.5)	
Hypertension, n (%)	60 (15.4)	24 (22.9)	0.07*
Diabetes mellitus, n (%)	67 (17.2)	32 (30.5)	**0.002***
Cardiovascular disease, n (%)	31 (7.9)	25 (23.8)	**< 0.001***
Outpatient, n (%)			**0.001***
Elective	364 (93.3)	87 (82.9)	
Emergency	26 (6.7)	18 (17.1)	
Stone size (mm), median (min-max)	8 (5-20)	8 (5-20)	0.34**
Stone Localization, n (%)			0.41*
Proximal ureter	129 (33.1)	28 (26.7)	
Mid ureter	106 (27.2)	29 (27.6)	
Distal ureter	155 (39.7)	48 (45.7)	
Hydronephrosis grade, n (%)			0.78*
1	159 (40.8)	41 (39)	
2	155 (39.7)	40 (38.1)	
3	76 (19.5)	24 (22.9)	
Preoperative urinary culture positivity, n (%)	46 (11.8)	29 (27.6)	**< 0.001***
Preoperative laboratories, median (min-max)			
Leukocyte (10^9/L)	7.94 (4.24-20.06)	8,65 (4.17-20.96)	**< 0.001****
Neutrophil (10^9/L)	4.84 (1.8-15.84)	6,06 (1.89-16.94)	**< 0.001****
Lymphocyte (10^9/L)	2.08 (0.53-4.86)	1.68 (0.56-3.8)	**< 0.001****
Monocyte (10^9/L)	0.4 (0.12-2.03)	0.45 (0.15-2.04)	**< 0.001****
Platelet (10^9/L)	285.5 (116-632)	290 (108-599)	0.57**
Preoperative inflammatory markers, median (min-max)			
NLR	2.26 (0.44-18.55)	3.81 (0.73-15.91)	**< 0.001****
LMR	5.25 (0.95-16.06)	3.78 (0.78-12.72)	**< 0.001****
PLR	134.56 (52.12-575.47)	171.59 (67.47-688.51)	**< 0.001****
SII	658.53 (121.45-5656.89)	1038.71 (182.85-7353.24)	**< 0.001****

SIRS = systemic inflammatory response syndrome, NLR = neutrophil-lymphocyte ratio, LMR = lymphocyte-monocyte ratio, PLR = platelet-lymphocyte ratio, SII = systemic immune-inflammation index

* Chi-Square Test, **Mann-Whitney U Test

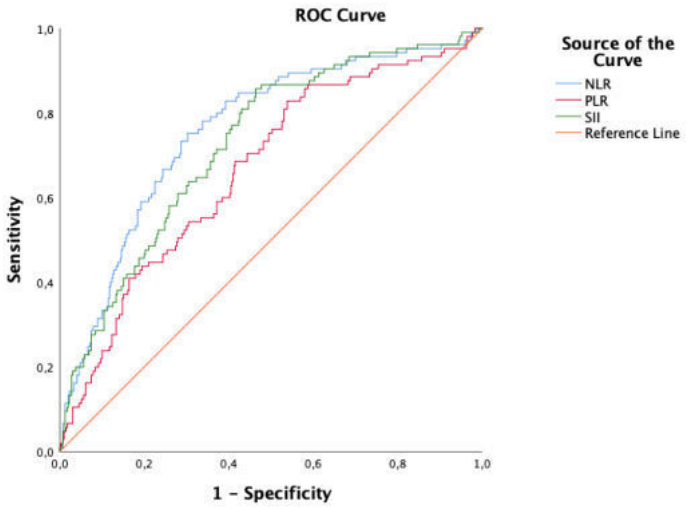

Fig. 1. ROC curve analysis of NLR, PLR and SII according to SIRS.

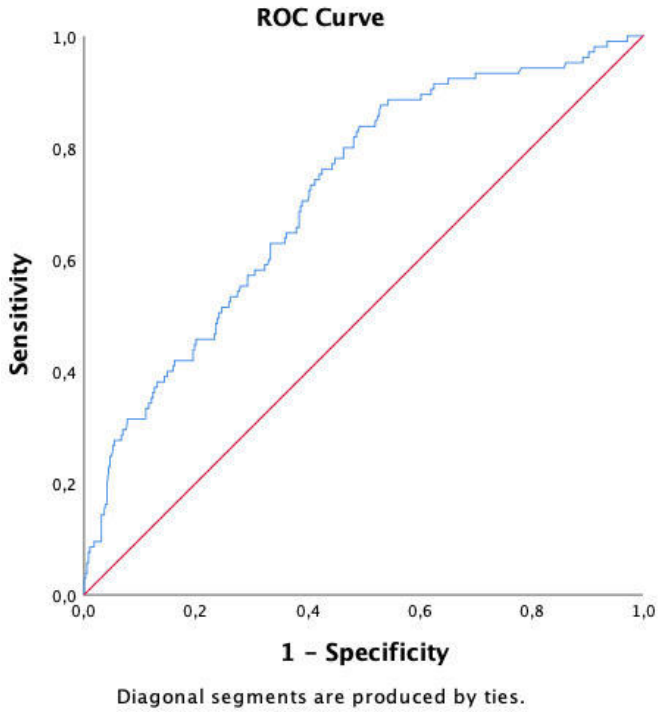

Fig. 2. ROC curve analysis of LMR according to SIRS.

ated under elective conditions ($p = 0.001$). There were significant differences in serum leukocyte, neutrophil, lymphocyte and monocyte counts and NLR, LMR, PLR and SII values between the two groups ($p < 0.001$ in all) (Table 1). In the ROC curve analysis (Figs. 1 and 2), the optimal cut-off (AUC, sensitivity, specificity) values for SII, NLR, LMR and PLR were found to be 816.16 (0.73, 66.7%, 65.1%), 2.98 (0.76, 73.3%, 71.3%), 4.5 (0.72, 64.8%, 63.8%), and 152.02 (0.67, 60%, 61.5%), respectively (Table 2). Besides, multivariate logistic regression analyses showed that age (OR 1.022, 95% CI 1.003-1.04; $p = 0.02$), cardiovascular disease (OR 2.684, 95% CI 1.335-5.397; $p = 0.006$), preoperative urinary culture positivity (OR 2.919, 95% CI 1.588-5.366; $p < 0.001$), and NLR (OR 6.347, 95% CI 2.976-13.537; $p < 0.001$) were found to be independent risk factors for the development of postoperative SIRS (Table 3).

DISCUSSION

Today, the most commonly used procedure in the treatment of ureteral stones is URS. Although URS is among the minimally invasive procedures, it is associated with many complications. Infective complications may present with clinical presentations ranging from bacteremia to sepsis. Although sepsis and septic shock are very rare, they are closely associated with perioperative mortality [9]. Therefore, there is a need for biomarkers to predict postoperative infective complications. Until now, many predictive factors have been studied in the definition of infective complications after URS. Studies with large series have shown

Table 2. ROC curve analysis for NLR, PLR, SII and LMR

	AUC	Sensitivity	Specificity	Cut-off	*p* value
NLR	0.76	73.3	71.3	>2.98	**< 0.001**
PLR	0.67	60	61.5	>152.02	**< 0.001**
SII	0.73	66.7	65.1	>815.16	**< 0.001**
LMR	0.72	64.8	63.8	<4.5	**< 0.001**

AUC = area under curve, NLR = neutrophil-lymphocyte ratio, LMR = lymphocyte-monocyte ratio, PLR = platelet-lymphocyte ratio, SII = systemic immune-inflammation index

Table 3. Multivariate analysis of factors that predict SIRS

Multivariate logistic regression analyses		
	OR (CI 95%)	***p* value**
Age	1.022 (1.003-1.04)	**0.02**
Diabetes mellitus	1.243 (0.682-2.263)	0.38
Cardiovascular disease	2.684 (1.335-5.397)	**0.006**
Outpatient type	1.415 (0.692-2.895)	0.37
Preoperative urinary culture positivity	2.919 (1.588-5.366)	**< 0.001**
NLR	6.347 (2.976-13.537)	**< 0.001**
PLR	1.076 (0.586-1.975)	0.81
SII	0.842 (0.389-1.823)	0.66
LMR	1.264 (0.702-2.274)	0.44

NLR = neutrophil-lymphocyte ratio, LMR = lymphocyte-monocyte ratio, PLR = platelet-lymphocyte ratio, SII = systemic immune-inflammation index

that comorbidities such as preoperative pyuria, preoperative urine culture positivity, prolonged operation time, high Charlson Comorbidity index, and diabetes mellitus are associated with postoperative fever and SIRS [10-12]. In our study, a relationship was found between age, diabetes mellitus, cardiovascular disease, preoperative urine culture positivity and the development of SIRS. Among these, age, cardiovascular disease, and preoperative urine culture positivity were found to be independent predictors for the development of postoperative SIRS in multivariate analysis.

Atherosclerosis, the main underlying pathology for ischemic heart diseases, is a chronic inflammatory process initiated and maintained by leukocytes infiltrating the subendothelial space. The accumulation of pro-inflammatory leukocytes drives progression of atherosclerosis. Many studies with animal experiments support this theory [13]. The fact that cardiovascular disease was an independent risk factor for the development of SIRS in our study may be related to this inflammatory background.

Although it could not be shown in the multivariate analysis, the risk of developing SIRS was higher in patients who were evaluated and operated in the emergency outpatient clinic compared to elective patients. This association may possibly be related to higher preoperative urine culture positivity in emergency patients compared to elective patients (16.8% vs. 29.4%).

URS is not applied to any patient whose urine culture is not sterile in our clinic. We found a significantly higher rate of postoperative SIRS in patients who received treatment for urinary tract infection in the preoperative period. Although preoperative urine culture positivity was found to be higher in women compared to men in previous studies, no such difference was found in our study. The propensity for infection in female gender in the population may be related to the higher incidence of phosphate stones in women, which are directly related to postoperative infection [14, 15]. Parallel to this, while female gender has been reported as a risk for the development of SIRS in the literature, such a relationship could not be shown in our study. This difference may be related to many factors such as ethnicity, geography and genetics.

In fact, the development of SIRS in patients with sterile preoperative urine cultures may seem confusing, but several mechanisms may be explanatory. In the presence of a ureteral stone that does not allow urinary passage, the infection may not be detected in a urethral sample and may cause infective complications after URS. In a study by Singh *et al*. [16], the fact that intraoperative renal pelvic urine culture taken during the percutaneous nephrolithotomy (PCNL) operation was found to be associated with postoperative SIRS also supports this view. In the aforementioned study, the correlation of preoperative urine culture with intraoperative pelvic urine culture and stone culture was found in only 18% of cases [16]. Most stones are known to carry bacteria under their layers. These bacteria that emerge with the lithotripsy procedure can be a source of infection. In a study evaluating patients who underwent PCNL, it was reported that infective complications that occur when urine cultures are sterile are due to bacteria released during lithotripsy [17]. In our study, no relationship was found between stone size and postoperative SIRS. This finding may be associated with less stone burden in those who underwent URS compared to those who underwent PCNL. In the literature, long operation time has also been found to be associated with infective complications. These complications are usually attributed to a longer duration of irrigation fluid reflux [18]. However, long operation time may be associated with excessive stone

burden. Increased stone burden may also cause more bacteria to emerge and infective complications.

It is known that high neutrophil and low lymphocyte count in the circulation prepare a suitable environment for kidney stone formation via inflammatory mediators [4]. On the other hand, vascular permeability factors arising from activated platelets also contribute to the inflammatory response by causing leukocyte chemotaxis [5]. The relationship between this inflammatory response, which predisposes to stone formation, and postoperative infective complications is not clear. So far, NLR, LMR and PLR have been studied to evaluate the development of SIRS after stone surgery. The predictive value of these markers in the development of SIRS after both URS and PCNL surgery has been demonstrated [4, 5, 19]. Hu *et al*. [20] first described a new inflammatory index, the SII, defined as platelet, which includes absolute blood counts. In the same study, it was found to be a prognostic indicator for poor outcome in patients with hepatocellular carcinoma [20]. It has also been shown to have prognostic significance for other gastrointestinal cancers and prostate cancer in subsequent studies [6, 7]. SII as an inflammatory marker have not been used so far to assess the risk of developing SIRS after URS. Our study is the first research in the literature in this respect. Preoperative SII, NLR, and PLR value were found to be significantly higher and LMR value significantly lower in the group of patients who developed SIRS. In the ROC analysis, we revealed that SII, NLR, LMR and PLR have predictive value for the development of postoperative SIRS. When these inflammatory markers were categorically grouped according to their cut-off values and evaluated by multivariance analysis, only NLR was detected as an independent risk factor. If the cut-off value for NLR is taken as > 2.98, we can predict the development of postoperative SIRS with 73.3% sensitivity and 71.3% specificity. With this result, it can be thought that preoperative NLR may help the clinician to predict high-risk patients. Therefore, patients with a high preoperative NLR value should be closely monitored for postoperative infective complications. Although previous studies have reported that SII and LMR are independent risk factors for the development of postoperative SIRS, our study reveals that they cannot be used alone as independent predictors. It can be thought that these, along with other clinical and laboratory variables, contribute to the development of postoperative SIRS.

Limitations

Unfortunately, our study has some limitations. First of all, our regional patient population may not reflect the whole society due to its single-center nature. In addition, due to its retrospective nature, we could not obtain data that could contribute to our study such as operation time, perioperative urine culture and stone culture.

CONCLUSION

Our study revealed that only NLR among inflammatory markers is an independent risk factor for the development of postoperative SIRS. Although SII has been reported to be an independent risk factor in some other diseases, we found no such relationship in terms of postoperative SIRS development in patients who underwent ureteroscopy. Elderly patients, patients with cardiovascular disease, patients receiving urinary infection treatment in the preoperative period, and patients with high preoperative NLR values should be closely monitored for postoperative infective complications. Especially, patients receiving preoperative infection treatment have a high risk of encountering infective complications even if the urine culture is sterile. It should be ensured that these patients receive adequate antibiotic therapy. Larger-scale, prospective, multicenter studies may provide more precise information to support the predictive value of inflammatory markers in the future.

Authors' Contribution

Study Conception: SY, KC; Study Design: SY, KC, AD; Supervision: SY; Funding: N/A; Materials: SY, KC; Data Collection and/or Processing: KC; Statistical Analysis and/or Data Interpretation: SY; Literature Review: SY, KC; Manuscript Preparation: SY and Critical Review: SY, KC.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Skolarikos A, Neisius A, Petřík A, Somani B, Thomas K, Gambaro G, et al. EAU Guidelines on Urolithiasis. European Association of Urology, 2022. Available at https://uroweb.org/guidelines/urolithiasis. Accessed September 10, 2022.
2. Fan S, Gong B, Hao Z, Zhang L, Zhou J, Zhang Y, et al. Risk factors of infectious complications following flexible ureteroscope with a holmium laser: a retrospective study. Int J Clin Exp Med 2015;8:11252-9.
3. Lucca I, de Martino M, Hofbauer SL, Zamani N, Shariat SF, Klatte T. Comparison of the prognostic value of pretreatment measure ments of systemic inflammatory response in patients undergoing curative resection of clear cell renal cell carcinoma. World J Urol 2015; 33:2045-52.
4. Tang K, Liu H, Jiang K, Ye T, Yan L, Liu P, et al. Predictive value of preoperative inflammatory response biomarkers for metabolic syndrome and post-PCNL systemic inflammatory response syndrome in patients with nephrolithiasis. Oncotarget 2017;8:85612-27.
5. Peng C, Li J, Xu G, Jin J, Chen J, Pan S. Significance of preoperative systemic immune-inflammation (SII) in predicting postoperative systemic inflammatory response syndrome after percutaneous nephrolithotomy. Urolithiasis 2021;49:513-9.
6. Wang K, Diao F, Ye Z, Zhang X, Zhai E, Ren H, et al. Prognostic value of systemic immuneinfammation index in patients with gastric cancer. Chin J Cancer 2017;36:75.
7. Lolli C, Caffo O, Scarpi E, Aieta M, Conteduca V, Maines F, et al. Systemic immuneinfammation index predicts the clinical outcome in patients with mCRPC treated with abiraterone. Front Pharmacol 2016;7:376.
8. Man YN, Chen YF. Systemic immune-inflammation index, serum albumin, and fibrinogen impact prognosis in castration resistant prostate cancer patients treated with first-line docetaxel. Int Urol Nephrol 2019;51:2189-99.
9. Somani BK, Giusti G, Sun Y, Osther PJ, Frank M, De Sio M, et al. Complications associated with ureterorenoscopy (URS) related to treatment of urolithiasis: the Clinical Research Office of Endourological Society URS Global study. World J Urol 2017;35:675-81.
10. Southern JB, Higgins AM, Young AJ, Kost KA, Schreiter BR, Clifton M, et al. Risk factors for postoperative fever and systemic inflammatory response syndrome after ureteroscopy for stone disease. J Endourol 2019;33:516-22.
11. Mitsuzuka K, Nakano O, Takahashi N, Satoh M. Identification of factors associated with postoperative febrile urinary tract infection after ureteroscopy for urinary stones. Urolithiasis 2016;44:257-62.
12. Kazan HO, Cakici MC, Efiloglu O, Cicek M, Yildirim A, Atis RG. Clinical characteristics of postoperative febrile urinary tract infections after ureteroscopic lithotripsy in diabetics: impact of glycemic control. Arch Esp Urol 2020;73:634-42.
13. Marchini T, Mitre LS, Wolf D. Inflammatory cell recruitment in cardiovascular disease. Front Cell Dev Biol 2021;9:635527.
14. King BJ, Seufert CJ, Okhunov Z, Khater N, Baldwin DD, Callas PW, et al. Profile of metabolic and infectious stone-formers in a contemporary PCNL cohort. Can J Urol 2017;24:8641-5.
15. Daudon M, Donsimoni R, Hennequin C, Fellahi S, Le Moel G, Paris M, et al. Sex- and age-related composition of 10 617 calculi analyzed by infrared spectroscopy. Urol Res 1995;23:319-26.
16. Singh P, Yadav S, Singh A, Saini AK, Kumar R, Seth A, et al. Systemic inflammatory response syndrome following percutaneous nephrolithotomy: assessment of risk factors and their impact on patient outcomes. Urol Int 2016;96:207-11.
17. Mariappan P, Smith G, Bariol SV, Moussa SA, Tolley DA. Stone and pelvic urine culture and sensitivity are better than bladder urine as predictors of urosepsis following percutaneous nephrolithotomy: a prospective clinical study. J Urol 2005;173:1610-4.
18. Wang Y, Jiang F, Wang Y, Hou Y, Zhang H, Chen Q, et al. Post-percutaneous nephrolithotomy septic shock and severe hemorrhage: a study of risk factors. Urol Int 2012;88:307-10.
19. Kutluhan MA, Unal S, Ozayar A, Okulu E, Kayigil O. Predictive value of preoperative high-sensitive C-reactive protein (hs-CRP)/albumin ratio in systemic inflammatory response syndrome (SIRS) after semi-rigid ureteroscopy. Cureus 2022;14:e23117.
20. Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, et al. Systemic immuneinflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin Cancer Res 2014;20:6212-22.

The European Research Journal 2023;9(2):277-284 Original Article

DOI: 10.18621/eurj.1173859 *Medical Services and Techniques*

Investigation of nasal *Staphylococcus aureus* carriage by real-time PCR in patients receiving hemodialysis treatment

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ABSTRACT

Objectives: *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), and methicillin-resistant *S. aureus* (MRSA), which are significant nosocomial pathogens, have become a growing global problem because their carriage and diseases have become resistant to many antibiotics. This study aimed to investigate and determine the rate of MRSA carriage among patients receiving hemodialysis treatment using molecular methods.

Methods: In the 254 hemodialysis patients, the nasal carriage rates, susceptibility and resistance to *S. aureus*, CoNS and MRSA were examined using culture and real-time PCR methods. Nasal samples from hemodialysis patients were examined using real-time PCR. Microscopic examination was performed using the Gram staining method, and *S. aureus* was identified using catalase and coagulase. The strains were then tested for antibiotic susceptibility. Staphylococci was isolated from 231 of the 254 patients.

Results: *S. aureus* carriage was detected in 50 patients, MRSA in 16, methicillin-susceptible *S. aureus* (MSSA) in 33, CoNS in 66, methicillin-resistant CoNS (MR-CoNS) in 38, and methicillin-susceptible CoNS (MS-CoNS) in 28. *S. aureus* and MRSA strains exhibited 100% susceptibility to nitrofurantoin, and vancomycin. MSSA strains showed the highest susceptibility to chloramphenicol, clindamycin (84.8%), and co-trimoxazole (36.4%). CoNS showed 100% susceptibility to vancomycin, and 16.7% susceptibility to ampicillin. Vancomycin was found to be the most effective antibiotic against *S. aureus*, CoNS, and MRSA pathogens identified in patients undergoing hemodialysis, whereas penicillin resistance was found.

Conclusions: It can be concluded that one of the most effective ways to prevent the formation of antibiotic resistant strains is the hygiene of the hospital and hospital staff. Real-time PCR is very important for analyzing with high sensitivity.

Keywords: *Staphylococcus aureus*, nasal carriage, antibiotic resistant, infection, real-time PCR

Staphylococci have had a significant majority among pathogens due to the diseases they have caused in humans and animals since 1881, when they were defined as infectious agents [1]. *Staphylococcus aureus*, which is resistant to methicillin-resistant *S. aureus* (MRSA), and coagulase-negative staphylococci (CoNS), is among the most common nosocomial infectious agents. It is critical that multiple drug resist-

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ance, particularly in MRSA infections and strains, reduces treatment options [2, 3]. Depending on the origin of the disease, MRSA can be nosocomial or community acquired. Community-acquired MRSA strains cause invasive infections, as well as skin and soft tissue infections, at an accelerating rate. Nosocomial MRSA infections can lead to severe disease and death in humans [4]. CoNS is a clinical concern because it is commonly isolated from foreign body infections, such as invasive catheters, prosthetic heart valves, and joint prostheses. Staphylococci (*S. aureus*, CoNS) inhabit the nasal mucosa and are responsible for approximately 1/3 of bloodstream infections. Approximately 80% of *S. aureus* isolated from the bloodstream were identical to those isolated from nose [5]. MRSA can spread through contaminated hands and inorganic materials after active nasal colonization, putting the carriers and others around them at risk [6]. Medical personnel and students colonized with *S. aureus* and MRSA pose risks in terms of the development and transmission of nosocomial infections [7]. Hospitalization, surgery, dialysis, indwelling catheters or percutaneous medical devices (tracheostomy tubes, gastrostomy tubes, or Foley catheters), long-term care facility residency, and positive culture for MRSA are risk factors for patients in terms of MRSA infection [8].

One of the greatest challenges in the treatment of nosocomial infections is the resistance of MRSA isolates to multiple antibiotics in hospitals [9, 10]. MRSA strains are more easily transmitted than others in hospitals, and they are often difficult to eliminate once they have established themselves [11]. Because MRSA is resistant to all types of penicillin (methicillin, oxacillin, nafcillin, cloxacillin, and dicloxacillin), cephalosporins, and a wide range of antibiotics, including clindamycin, erythromycin, tetracycline, and aminoglycosides, treatment options for infections are limited and can be fatal [12]. According to previous studies, approximately 30% of the general population is colonized by SA, and approximately 3% of these strains are methicillin-resistant [13, 14]. In another study, methicillin resistance was observed in 10.9% of SA strains, and 38.8% of CoNS [15]. Colonization was found in 45% of patients admitted to intensive care units in European hospitals, with nosocomial MRSA accounting for 21% of them [16].

Although methicillin-resistant staphylococci infections are difficult to treat, they are also a leading cause of morbidity and mortality. *S. aureus*, CoNS, and MRSA, which are predominantly nosocomial, have become major global health problems around the world.

METHODS

Study Design

Using nasal swab samples taken from 254 hemodialysis patients who visited the Mardin Training and Research Hospital on a regular basis between March 2019, and May 2021, the present study aimed to assess the methicillin carriage and multiple antibiotic resistances of nasal *S. aureus*, and CoNS strains. 254 hemodialysis patients, the nasal carriage rates, susceptibility and resistance to *S. aureus*, CoNS and, MRSA were examined using culture and real-time PCR methods.

Isolation of Staphylococci and Culture-Identification

Microscopic examination was performed on one of the two samples taken from each patient's nasal cavity using the Gram staining method and *S. aureus* identification was performed using catalase and coagulase operations in order to be employed in culture processes.

The collected nasal swab samples were followed by phenotypic identification procedures. It was inoculated on commercially obtained (Oxoid) 5% sheep blood agar and incubated at 37°C for 24 hours. Growing bacteria were evaluated in terms of colony morphology and the identification of *S. aureus* suspicious colonies was made by catalase test, gram stain, coagulase test and mannitol fermentation test. The presence of coagulase was demonstrated by a commercial latex agglutination test, the Staphylase test kit (Oxoid Limited, England). Catalase and coagulase tests are positive and staphylococci that grow on Mannitol Salt agar by forming a yellow zone are interpreted as *S. aureus*.

Antibiotic Susceptibility

The antibiotic susceptibility of the *S. aureus* and CoNS strains was determined using oxacillin, penicillin, rifampicin, tetracycline, and vancomycin discs.

After 18 h of incubation at 37 °C, the inhibition zone diameters of the strains were measured. Results were interpreted using the criteria specified by the Clinical and Laboratory Standards Institute (CLSI) [4]. The control strains used in this study were ATCC 25923 (MSSA), ATCC 43300 (MRSA) and *S. aureus* ATCC 25923 (*S. aureus*). Suspensions of *S. aureus* and MRSA strains diluted in PBS were transferred to 100 µl sterile microcentrifuge tubes and heated at 95 °C, 7 min to generate DNA [17]. In line with this protocol, DNA quantity was measured using spectrophotometry at 260-280 nm.

Determination of the presence of bacteria strains using real-time PCR

To identify *S. aureus* and CoNS using real-time PCR, the femB gene-specific SA detection kit was used according to the manufacturer's instructions. The final volume of the real-time PCR procedure was 20µL with 5µL of DNA. A LightCycler 480-II instrument was used for amplification. The protocol was as follows: initial denaturation at 95°C for 2min, 50cycles of 10 s at 95°C, 1min at 60°C, with a single reading.

MecA1(5′-GCA ATC GCT AAA GAA CTA AG-3′) and MecA2(5′-GGG ACC AAC ATA ACC TAA TA-3′), which are mecA gene-specific primers, were utilized to identify MRSA using real-time PCR [18]. The final volume for real-time PCR was set to 20µL, which contained 2µL of DNA. Real-time PCR was performed on a LightCycler 480-II using the LightCycler FastStart DNA Master SYBR Green-I kit according to the manufacturer's instructions. With a single readout, real-time PCR reaction was set to 35cycles of 10min at 95°C for initial denaturation, 0s at 95°C, 5s at 55°C, and 8 s at 72°C. Following this, The single-cycle melting curve program consisted of heating at 95°C with a hold time of 0s, heating at 58°C, 60s, and heating at 95°C, 0s.

Statistical Analysis

Statistical analyzes were performed using the SPSS 24.00 program. Categorical data are presented as numbers and percentages. Pearson Chi-Square test was used in categorical data analysis. Statistical significance was accepted as $p < 0.05$.

RESULTS

The study included 254 patients of whom 145 (57%) were male and 109 (43%) were female. In terms of age, 72 (28%) participants were 50 years or older, 124 (49%) were 30-49 years old, and 58 (23%) were under the age of 29. Of the patients, 159 (63%) were admitted for less than a year, whereas 95 (37%) were admitted for more than a year (Table 1).

The presence of staphylococci in nasal samples from 231 of 254 participants was evaluated. Nasal *S. aureus* carriage was discovered in 50 (22%) of the pa-

Table 1. Demographic features of 254 hemodialysis patients

Variables	n	%
Gender		
Male	145	57
Female	109	43
Age		
29 and under	58	23
30-49	124	49
50 and over	72	28
Duration of hemodialysis admittance		
Less than 1 year	159	63
More than 1 year	95	37

Table 2. Distribution of the bacteria strains isolated from cultures

Variables	n	%
Gender		
Male	145	5
Female	109	4
Age		
29 and under	58	2
30-49	124	4
50 and over	72	2
Duration of hemodialysis admittance		
Less than 1 year	159	6
More than 1 year	95	3

tients, MRSA 16(7%), MSSA 33 (14%), CoNS 66 (29%), MR-CoNS 38 (16%), MS-CoNS 28 (12%) (Table 2).

Antibiotic susceptibilities of the *S. aureus* strains shown in Table 3. The highest susceptibility antibiotics was nitrofurantoin, and vancomycin at a rate of 50 (100%) followed by 47 (94%) to gentamicin, 45(90%) to rifampicin, and 16 (32%) to co-trimoxazole, and erythromycin (indicating that they had developed strong resistance to these antibiotics). The lowest susceptibility 4(8%) and so highest resistance were to penicillin (Table 3).

Antibiotic susceptibilities of the CoNS strains shown in Table 4. The strains demonstrated the highest resistance to vancomycin at a rate of 100% and did not gain further resistance. CoNS strains exhibited high susceptibility and low resistance to rifampicin 54(81.8%), nitrofurantoin 51 (77.3%), ciprofloxacin and fusidic acid 48 (72.7%). The lowest susceptibility was observed for ampicillin with 11 (16.7%). The strains showed low susceptibility/high resistance 14 (21.2%) to penicillin (Table 4).

In Table 5, it was observed that MSSA (n = 33) strains showed the highest susceptibility and the least resistance. The highest susceptibility antibiotics was 28 (84.8%) to chloramphenicol and clindamycin followed by 25 (75.8%) to cefotaxime and ciprofloxacin, 18 (54.5%) toerythromycin and 12 (36.4%) to co-trimoxazole. The MSSA strains showed the lowest susceptibility and the highest resistance to ampicillin with five strains (15.2%) (Table 5).

When the distribution of the antibiotic susceptibilities of MRSA strains in Table 6 was examined, it was observed that the highest susceptibility antibiotics was 16 (100%) to nitrofurantoin and vancomycin (they did not exhibit resistance) followed by 11 (68.8%) to rifampicin, and 9 (56.3%) to clindamycin. The MRSA strains showed the lowest susceptibility 1 (6.3%) to ampicillin, and cefotaxime, indicating that they gained strong resistance to these antibiotics. Additionally, they demonstrated low susceptibility and so high resistance to erythromycin, and fusidic acid in 2 strains (12.5%) (Table 6).

Table 3. Distribution of the antibiotic susceptibilities of *Staphylococcus aureus* strains (n = 50)

Staphylococcus aureus	**n (%)**	
	S	I
Ampicillin	7 (14)	-
Cefotaxime	26 (52)	5 (10)
Chloramphenicol	36 (72)	5 (10)
Ciprofloxacin	39 (78)	1 (2)
Clindamycin	39 (78)	1 (2)
Co-trimoxazole	16 (32)	4 (8)
Erythromycin	16 (32)	10 (20)
Fusidic acid	26 (52)	-
Gentamicin	47 (94)	-
Nitrofurantoin	50 (100)	-
Oxacillin	33 (66)	-
Penicillin	4 (8)	-
Rifampicin	45 (90)	1 (2%)
Tetracycline	12 (24)	-
Vancomycin	50 (100)	-

S = Susceptible, I = Intermediate

Table 4. Distribution of the antibiotic susceptibilities of CoNS strains

CoNS (n = 66)	**n (%)**	
	S	**I**
Ampicillin	11 (16.7)	-
Cefotaxime	46 (69.7)	-
Chloramphenicol	30 (45.5)	2 (3)
Ciprofloxacin	48 (72.7)	-
Clindamycin	42 (63.6)	2 (3)
Co-trimoxazole	52 (78.8)	1 (1.5)
Erythromycin	36 (54.5)	-
Fusidic acid	48 (72.7)	-
Gentamicin	38 (57.6)	-
Nitrofurantoin	51 (77.3)	-
Oxacillin	26 (39.4)	-
Penicillin	14 (21.2)	-
Rifampicin	54 (81.8)	-
Tetracycline	39 (59.1)	-
Vancomycin	66 (100)	-

S = Susceptible, I = Intermediate

DISCUSSION

Real-time PCR, which is a quick and reliable procedure, was used. The real-time PCR method has been favored in previous studies because it detects MRSA, an important pathogen, faster and more precisely than the traditional culture and latex agglutination methods [19, 20].

Males comprised 57% of the 254 hemodialysis patients in the present study, while females accounted for 43%. 28% of the patients were over the age of 50, 49% were between 30-49 years, and 23% were under the age of 29. 63% of patients were admitted for one year or less, and 37% of the patients were admitted for more than one year. There was no correlation between MRSA nasal carriage and age or sex in this study (Table 1). In a similar study, nasal carriage of MRSA was not significantly related to gender or age group [21].

The determination and prevention of nasal carriage in the development of nosocomial *Staphylococcus* are of great importance in terms of infection control. The nose is the primary site of *S. aureus* colonization, but it can also be isolated from other regions of the body. The present study investigated the presence of *S. aureus*, MRSA, MSSA, CoNS, MR-CoNS, and MS-CoNS in nasal swab samples from 231 of 254 individuals. *S. aureus* carriage was found in 50 (22%) patients, MRSA in 16 (7%), MSSA in 33 (14%), CoNS in 66 (29%), MR-CoNS in 38 (16%), and MS-CoNS carriage in 28 (12%) patients, according to the data. This led to the conclusion that dialysis patients are exposed to these strains throughout the dialysis process, which increases the frequency of resistant strains (Table 2). In a previous study of catheter-related bloodstream infections [22] discovered that MR-CoNS developed in 23 (21.1%) of 109 patients, MS-CoNS in 1(0.9%), MRSA in 15 (13.8%), and MSSA in 23 (21.1%). *S. aureus* was found in 39.6% (345/871) of the samples taken from clinical samples in a study conducted by [23]. MRSA was detected in 18.1%, and MSSA in 21.5%. In another study [24], 42 of 100 staphylococcal strains isolated from diverse clinical specimens were *S. aureus* strains, and 58 were CoNS strains. In another study, 54 of 139 strains isolated from diverse clinical specimens were *S. aureus* strains, while 94 were CoNS strains [25]. In certain studies, nasal *S. aureus* carriage and MRSA rates were reported as 19.3% and 2.4% in Jordan [26], 24.7% and 0.3% in China [27], 30.8% and 6.6% in Ireland [28], respectively. According to another study, over 30% of the US population carries *S. aureus* in their anterior nostrils and 1-2% carries MRSA in their nostrils [8]. When the findings of previous studies conducted on a

Table 5. Distribution of the antibiotic susceptibilities of MSSA strains (n = 33)

MSSA	n (%)	
	S	I
Ampicillin	5 (15.2)	-
Cefotaxime	25 (75.8)	5 (15.2)
Chloramphenicol	28 (84.8)	4 (12.1)
Ciprofloxacin	25 (75.8)	1 (3.0)
Clindamycin	28 (84.8)	-
Co-trimoxazole	12 (36.4)	3 (9.1)
Erythromycin	18 (54.5)	10 (30.3)

S = Susceptible, I = Intermediate

Table 6. Distribution of the antibiotic susceptibilities of MRSA strains

MRSA (n = 16)	n (%)	
	S	I
Ampicillin	1 (6.3)	-
Cefotaxime	1 (6.3)	-
Chloramphenicol	6 (37.5)	1 (6.3)
Ciprofloxacin	2 (12.5)	-
Clindamycin	9 (56.3)	1 (6.3)
Co-trimoxazole	4 (25)	1 (6.3)
Erythromycin	2 (12.5)	-
Fusidic acid	2 (12.5)	-
Gentamicin	14 (87.5)	-
Nitrofurantoin	16 (100)	-
Oxacillin	-	-
Penicillin	-	-
Rifampicin	11 (68.8)	-
Tetracycline	5 (31.3)	-
Vancomycin	16 (100)	-

S = Susceptible, I = Intermediate

global scale were compared, it was clear that the transmission rates of *S. aureus*, MRSA, and CoNS varied by region, population, and hospital. The carriage that occurs as a result of colonization of healthcare workers' skin and nasal mucosa is significant because it is a possible source of infection and plays a vital role in nosocomial epidemics.

S. aureus strains were found to have the highest susceptibility to nitrofurantoin and vancomycin, with a 100% rate of susceptibility, indicating that they did not develop any resistance to these two antibiotics. They showed a high susceptibility to gentamicin, and rifampicin (94% and 90%, respectively). They were also susceptible to clindamycin and ciprofloxacin in 78% of the cases, and chloramphenicol in 72%. They demonstrated 16% susceptibility to co-trimoxazole and erythromycin, indicating that they developed significant resistance to these medicines, according to the findings. Although the strains were 14% susceptible to ampicillin, they were only 8% susceptible to penicillin, indicating that they had the highest antibiotic resistance to penicillin. (Table 3) Penicillin resistance was found to be most common (85%) in a similar study, as well [29].

CoNS is one of the leading foreign body infections and nosocomial bacteremia [30]. CoNS strains were found to have the highest susceptibility to vancomycin at a rate of 100% and did not acquire any resistance. CoNS strains showed high susceptibility and low resistance to rifampicin (81.8%), nitrofurantoin (77.3%), ciprofloxacin, fusidic acid (72.7%). The lowest susceptibility was observed for ampicillin (16.7%. The strains exhibited low susceptibility and high resistance to penicillin, at a rate of 21.2% (Table 4). CoNS strains were found to be more antibiotic-resistant than *S. aureus* strains [15]. In another study, MR-CoNS strains were more resistant to antibiotics than MS-CoNS, while all of them were susceptible to vancomycin and teicoplanin [24]. In a previous study, 84.2% of 172 CoNS strains were found to be resistant to penicillin, 38.8% to methicillin, 54.8% to erythromycin, 44.4% to clindamycin, 42.2% to co-trimoxazole, 25% to ciprofloxacin, 28.1% to fusidic acid; however, no vancomycin resistance was reported [15].

Table 5 showed that MSSA strains had the highest susceptibility to chloramphenicol and clindamycin (84.8%). The strains exhibited susceptibility at a rate of 75.8% to cefotaxime, 54.5% to erythromycin, and 36.4% to co-trimoxazole. The MSSA strains showed the lowest susceptibility and highest resistance to ampicillin at a rate of 15.2% (Table 5). When the distribution of antibiotic susceptibility of MRSA strains was examined, it was determined that they were the most susceptible to nitrofurantoin and vancomycin at a rate of 100%, indicating that they did not demonstrate any resistance. Susceptibilities to gentamicin, rifampicin, and clindamycin were 87.5%, 68.8%, and 56.3%, respectively. MRSA strains showed low susceptibility and high resistance to erythromycin and fusidic acid, at a rate of 12.5%. MRSAs showed the lowest sensitivity to ampicillin and cefotaxime antibiotics at a rate of 6.3%, indicating that they gained high resistance to these antibiotics (Table 6). In a previous study, the MRSA resistance rates were reported to be 29.9% for trimethoprim-sulfamethoxazole, 60.8% for clindamycin, 71.8% for erythromycin, 7.7% for teicoplanin, 90.1% for gentamicin, 88.8% for ofloxacin, 88.1% for norfloxacin and 100% for penicillin. Additionally, all the isolates were reported to be susceptible to vancomycin [23].

Consistent with the findings, the most efficient antibiotic against *S. aureus,* CoNS, MRSA was vancomycin, which had a 100% susceptibility rate. Vancomycin and nitrofurantoin susceptibilities were 100% for the SA and MRSA strains. The MRSA strains were resistant to penicillin and showed no signs of susceptibility. With the exception of vancomycin, CoNS showed no susceptibility to other antibiotics and was confirmed to be resistant.

CONCLUSION

In conclusion, multidrug resistance in *S. aureus*, CoNS, and MRSA, all of which are highly significant nosocomial infections, has become a major global health issue. Furthermore, this condition results in significant issues such as non-responsiveness and an increase in treatment costs. As treating MRSA is difficult and proper safeguards are not taken today, these bacteria may create major health concerns in the future. As a result of their close contact with healthcare professionals and members of the general public, sick, and resistant bacteria can spread quickly to community members and hospitalized hemodialysis patients. Therefore, it is suggested that patient circulation in

hospitals should be limited, to ensure hygiene and carrier rates should be identified and monitored.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki, and the ethics committee approval for the study was obtained from the Ethics Committee of Gazi Yasargil Training and Research Hospital on 2022/59. In addition, in order to carry out the study, Permission was obtained from the TR Ministry of Health / Mardin Provincial Health Directorate.

Authors' Contribution

Study Conception: SNK; Study Design: SNK, SU, AD; Supervision: SNK, SU; Funding: N/A; Materials: SU; Data Collection and/or Processing: AD, MB, SU; Statistical Analysis and/or Data Interpretation: MB, AD; Literature Review: SNK, MB, AD; Manuscript Preparation: SNK, AD and Critical Review: SNK, MB, SU.

Conflict of interest

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REFERENCES

1. Abroo S, Jazani NH, Sharifi Y. Methicillin-resistant Staphylococcus aureus nasal carriage between healthy students of medical and nonmedical universities. Am J Infect Control 2017;45:709-12.
2. Ansari S, Jha RK, Mishra, SK, Tiwari BR, Asaad AM. Recent advances in Staphylococcus aureus infection: focus on vaccine development. Infect Drug Resist 2019;12:1243.
3. Siddiqui AH, Koirala J. Methicillin resistant Staphylococcus aureus. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2020.
4. Kollef MH, Micek ST. Methicillin-resistant Staphylococcus aureus: a new community-acquired pathogen. Curr Opin Infect Dis 2006;19:161-8.
5. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteremia. N Engl J Med 2001;344:11-6.
6. Cimolai N. MRSA and the environment: implications for comprehensive control measures. Eur J Clin Microbiol Infect Dis 2008;27:481-93.
7. Atalik K, Selale DS, Dinc HO, Aydinli A, Babaoglu UT, Kaya AD, et al. [Investigation of Staphylococcus aureus nasal carriage in nursing students]. Value Health Sci 2022;12:104-7. [Article in Turkish]
8. Bagheri A, Kumar S. Methicillin-resistant Staphylococcus aureus (MRSA) carriage certainly poses a risk in pregnant women. Cukurova Med J 2015;40:28-35.
9. Aribas E, Ozcan M, Altindis M. [The antibiotics resistance rates of Staphylococci isolated from various clinical specimens]. Turkish J Infect 2001;15:73-7. [Article in Turkish]
10. Leclercq R. [Staphylococci resistant to antibiotic therapy]. Ann Fr Anesth Reanim 2002;21:375-83. [Article in French]
11. Ayliffe G. The progressive intercontinental spread of methicillin-resistant Staphylococcus aureus. Clin Infect Dis 1997;24:74-9.
12. Altındiş M. Hemşireler için mikrobiyoloji. Nobel Tıp Kitabevi, 2010.
13. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al. Staphylococci and related Gram-positive cocci. Color Atlas and Textbook of Diagnostic Microbiology. Philadelphia, Lippincott Williams and Wilkins, 2006.
14. Kirecci E, Ozer A, Aral M, Miraloglu M. A research of nasal methicillin resistant/sensitive Staphylococcus aureus and pharyngeal beta-haemolytic Streptococcus carriage in midwifery students in Kahramanmaras, Eastern Mediterranean Region Turkey. Ethiop J Health Dev 2010;24:57-60.
15. Aydin N, Gultekin B, Eyigor M, Gurel M. [The antibiotic resıstance of Staphylococci isolated of clinical specimens]. ADÜ Týp Fakültesi Dergisi 2001;2:21-6. [Article in Turkish]
16. Oguzkaya-Artan M, Baykan Z, Artan C. Nasal carriage of Staphylococcus aureus in healthy preschool children. Jpn J Infect Dis 2008;61:70-2.
17. Kilic A, Li H, Stratton CW, Tang YW. Antimicrobial susceptibility patterns and staphylococcal cassette chromosome mec types of, as well as Panton-valentine leukocidin occurrence among, methicillin-resistant Staphylococcus aureus isolates from children and adults in middle Tennessee. J Clin Microbiol 2006;44:4436-40.
18. Fang H, Hedin G. Rapid screening and identification of methicillin-resistant Staphylococcus aureus from clinical samples by selective-broth and real-time PCR assay. J Clin Microbiol 2003;41:2894-9.
19. Grisold AJ, Kessler HH. Use of hybridization probes in a real-time PCR assay on the LightCycler® for the detection of methicillin-resistant Staphylococcus aureus. Methods Mol Biol 2006;345:79-89.
20. Dulger D, Ekici S, Albuz O, Pakdemirli A. Investigation of nasal Staphylococcus aureus carriage in hospital employees and rapid detection of PVL and mecA genes by RT-PCR. Etlik Vet Microbiol Derg 2020;31:47-51.
21. Kirecci E, Ali O, Mustafa G, Tanis H, Sucakli MH. [Nasal MRSA carriages in nursing home residents]. Kocatepe Med J 2013;14:77-82. [Article in Turkish]
22. Yuksel E, Safak K, Gunay E. [Evaluation of catheter-related bloodstream infections in hemodialysis patients]. Dicle Med J 2020;47:665-70. [Article in Turkish]
23. Savas L, Duran N, Onlen Y, Savas N, Erayman M. Prospec-

tive analysis of antibiotic susceptibility patterns of MRSA in a Turkish University Hospital. Turk J Med Sci 2005;35:323-7.
24. Tetik T, Eryilmaz M, Ahmet A. [Investigation of the relation between slime production and antibiotic resistance of Staphylococci isolated from various clinical specimens]. J Fac Pharm Ankara 2010;39:187-94. [Article in Turkish]
25. Yasar KK, Bilir YA, Pehlivanoglu F, Sengoz G. [The relations between slime factor positivity, methicillin and antibiotic resistance in Staphylococci]. Ankem Derg 2011;25:89-93. [Article in Turkish]
26. Al-Tamimi M, Himsawi N, Abu-Raideh J, Al-jawaldeh H, Mahmoud SAH, Hijjawi N, et al. Nasal colonization by methicillin-sensitive and methicillin-resistant Staphylococcus aureus among medical students. J Infect Dev Ctries 2018;12:326-35.
27. Chen BJ, Xie XY, Ni LJ, Dai XL, Lu Y, Wu XQ, et al. Factors associated with Staphylococcus aureus nasal carriage and molecular characteristics among the general population at a Medical College Campus in Guangzhou, South China. Ann Clin Microbiol Antimicrob 2017;16:28.
28. Budri PE, Shore AC, Coleman DC, Kinnevey PM, Humpreys H, Fitzgerald-Hughes D. Observational cross-sectional study of nasal staphylococcal species of medical students of diverse geographical origin, prior to healthcare exposure: prevalence of SCCmec, fusC, fusB and the arginine catabolite mobile element (ACME) in the absence of selective antibiotic pressure. BMJ Open 2018;8:e020391.
29. Sezgin FM, Sarihan S, Turkoglu HN, Yagmur M, Bucak G, Buyuktatar NS, et al. Nasal carriage of Staphylococcus aureus by medical students: assessment of antibiotic susceptibility and risk factors. Cumhur Med J 2020;42:259-70.
30. Domenico P, Baldassarri L, Schoch PE, Kaehler K, Sasatsu M, Cunha BA. Activities of bismuth thiols against staphylococci and staphylococcal biofilms. Antimicrobl Agents Chemother 2001;45:1417-21.

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Evaluation of optic nerve by histogram analysis on MRI in patients with isolated optic neuritis

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ABSTRACT

Objectives: We aimed to evaluate the Magnetic Resonance Imaging (MRI) histogram texture analyzis of the optic nerve by comparing patients of isolated optic neuritis with a healthy control group and to provide objective information without using contrast in the diagnosis of the disease.

Methods: A total of 40 patients, including 20 patients with isolated optic neuritis (13 females, 7 males) and 20 healthy controls (11 females, 9 males), were included in the study. Non-contrast brain MR images of the patient and control groups were analyzed retrospectively. In the coronal T2-weighted MRI sequence of both groups, the Region of Interest (ROI) was placed in the extraocular anterior 1/3 of the optic nerve of both eyes. Numerical data were obtained using histogram analysis and the data were evaluated in the MATLAB program. The data were compared statistically, in addition, sensitivity and specificity were determined by Receiver Operating Characteristic (ROC) curve analysis.

Results: As a result of histogram analysis, a significant difference was found between the mean values in the healthy and affected eye of the patients with isolated optic neuritis and the mean values of the control group ($p < 0.05$). A significant difference was found in standard deviation, minimum, maximum, median, variance values between both groups. ROC analysis was performed for mean value, AUC = 0.943 and when threshold value was selected as 354.258 Haunsfield Unit, two groups could be differentiated with 84.2% of sensitivity and 92.1% of specificity. We can say that patients with isolated optic neuritis also have histological effects on the clinically asymptomatic eye.

Conclusions: Histogram analysis can be used in the diagnosis of the patients with isolated optic neuritis without the need to use contrast in their MRI. In addition, histological effect can be detected in the eye that does not show clinical symptoms with histogram analysis.

Keywords: Optic neuritis, histogram analysis, texture analysis

Optic neuritis (ON) is characterized by inflammation of the optic nerve which is mainly idiopathic demyelination. Also, many causes including ischemic, metabolic, nutritional, hereditary, toxic, traumatic, paraneoplastic and radiation induced events [1]. Clinical presentation of the optic neuritis is mostly visual loss, periocular pain and dyschromatopsia of triple semptoms, and some patients may suffer from visual

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field defects and swelling or atrophy of the optic nerve [2, 3].

Treatment planning and prognosis will depend on the etiology in patients with optic neuritis. In order to identify differential diagnosis, clinical history, visual field perimetry, optical coherence tomography (OCT), Magnetic Resonance Imaging (MRI) and serologic testing are used [4].

MRI is an important test that can be used in the diagnosis of the disease, and it excludes other diagnoses. MRI findings are shown enlarged of the affected segment of the optic nerve, high-signal-intensity lesions in the optic nerve on T2-weighted MRI and typically as contrast uptake in a contrast-enhanced T1 sequence with fat suppression [5].

The texture analysis technique is a mathematical calculation of the the signal properties, the position and density of pixels in an digital images and how many pixels in the image have a certain gray level value. This provides objective information about the basic structure and pathological process of tissue [6]. Histogram analysis is part of the tissue analysis method that is increasingly used in recent years. This has been successfully applied in the differentiation of pathological tissues from many intact tissues such as brain, liver, breasts, thyroid and lungs [7-11]. In addition, histogram texture feature analyzis was used to evaluate the optic nerve in patients with Multiple Skleroz (MS) [12].

In this study, we aimed to evaluate the MRI histogram texture analysis of the optic nerve in patients with isolated optic neuritis without MS, having an objective knowledge in the diagnosis of the disease without using contrast.

METHODS

This study was conducted retrospectively, and informed consent from the patients wasn't used because MRI is used in the diagnosis of the disease in clinical practice at our hospital. An ethical approval (approval number-date 13/18.03.2020) was obtained from our hospital ethical committee.

Our study was designed as a case control, and the patient group will consist of patients who applied to our hospital's ophthalmology clinic and diagnosed with isolated optic neuritis.

The control group consists of patients with brain MR images who have applied to our hospital for any reason. A total of 40 patients including 20 patients with isolated optic neuritis diagnosis and 20 healthy control group were included in the study. The diagnosis of isolated optic neuritis was made in the ophthalmology clinic according to the history and examination findings, considering the age of the patient. The exclusion criteria of patients, Multiple sclerosis, behçet, inflammatory syndromes, glaucoma, ophthalmic surgery history, and orbital mass.

Study Design and Image Processing

Optic nerves were evaluated from coronal T2-weighted MRI sequence in brain MRI images in patients and control groups.

For standardization, the extraocular anterior 1/3 part of the optic nerve was measured. A round-shaped Region of Interest (ROI) was inserted by a single radiologist, by manual drawing, surrounding the optic nerve (Workstation: 27 inch iMac computer, Apple Inc. Cupertino, 88 California, using ROI from). ROI was placed in the optic nerve of the other intact eye, as well as the clinically affected eye of the patients (Fig. 1).

Similarly in the control group, an ROI was placed

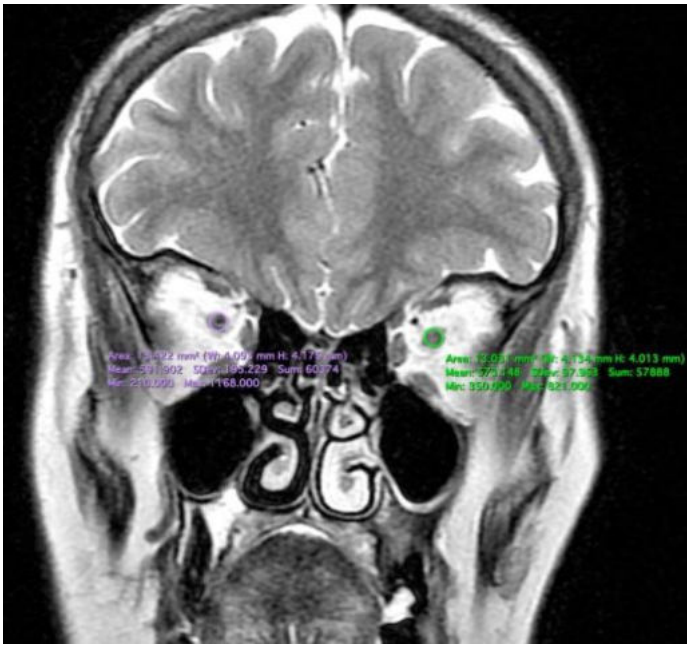

Fig. 1. A round-shaped Region of Interest (ROI) was inserted by a single radiologist, by manual drawing, surrounding the optic nerve.

in the extraocular anterior 1/3 of the optic nerve in two eyes.

The area of all ROI's ranged from 10 mm^2 to 20 mm^2 on the optic nerve. The ROI calculation was exported to an XML (Extensible Markum Language) file.

MRI Examination

Cranial MRI examinations were performed using 1.5T MRI with a cranial coil (Philips Ingenia 1.5T, Eindhoven 2015, the Netherlands); The following parameters were used in coronal T2-weighted: time of repetition (TR) 4827 ms, time of echo (TE) 100 ms, slice thickness 5 mm, interslice gap 1 mm, field of view 23×23 cm, matrix 356×221, and number of excitations (NEX) 2.00.

Statistical Analysis

Histogram analysis from ROI's were performed using a (Matrix laboratory, MathworksInc, Natick, ABD) computer program. As a result of the analysis, mean, standard deviation (SD), minimum, maximum, median, variance, entropy (disorder), uniformity (homogeneity), skewness and kurtosis parameters were calculated from these values. ROI values were transferred numerical data and Mann Whitney u test was used to compare between the patient and control group. The specificity and sensitivity were determined by performing Receiver Operating Characteristic (ROC) curve analysis. Chi-square test was used for gender comparison between the two groups, and the mann withney u test was used for age comparison. All statistical analyses were made with SPSS, version 25.0 (IBM Statistics for Windows, version 25, IBM Corporation, New York, U.S.A.). Statistically, $p < 0.05$ was considered significant.

RESULTS

In the patient group, 18 patients had clinical symptoms in one eye, while 2 patients had symptoms in both eyes.

The mean age of the study population (40 patients) was 40.5 years (min: 14, max: 57). There was no statistically significant difference between the ages of the patient and control groups ($p > 0.05$).

The patient group (13 females, 7 males) and the control group (11 females, 9 males), no significant difference was found between the two groups in terms of gender ($p > 0.05$).

In the histogram analysis performed on the optic nerve in both eyes in the patient group, the mean was 603.81 + -177.62 Haunsfield Unit (HU), in the healthy control group, the mean was 255.38 + 54.71 HU, and mean values in optic neuritis patients were found to be significantly higher compared to the control group ($p < 0.05$). As shown in Table 1, a significant differ-

Table 1. The analysis of histogram parameters of the normal and optic neuritis

	Optic neuritis (n = 38) mean ± SD	Control (n = 38) mean ± SD	*p value*
Mean *	603.81 ± 177.62	255.38 ± 54.71	**< 0.001**
Standart Deviation *	164.83 ± 51.54	61.36 ± 20.88	**< 0.001**
Minimum*	305.45 ± 143.36	142.61 ± 64.160	**< 0.001**
Maximum*	1069.29 ± 312.01	427.18 ± 100.43	**< 0.001**
Median*	578.07 ± 172.71	249.69 ± 53.17	**< 0.001**
Variance*	29758.87±15984.47	4190.02 ± 2700.07	**< 0.001**
Entropy	6.08 ± 0.24	6.06 ± 0.14	0.736
Size%L	14.14 ± 3.77	15.32 ± 3.41	0.182
Size%U	16.16 ± 3.08	15.26 ± 2.57	0.238
Size%M	69.68 ± 5.56	69.40 ± 5.04	0.823
Kurtosis	3.32 ± 1.38	3.31 ± 0.97	0.506
Skewness	0.59 ± 0.47	0.44 ± 0.50	0.245
Uniformity	0.21 ± 0.05	0.21 ± 0.06	0.625

SD = Standard Deviation

*Statistically significant difference, $p < 0.005$

ence was found in mean, standard deviation, minimum, maximum, median, variance values between both groups ($p < 0.05$).

No significant difference was found between the optic nerve histogram analysis values in the right and left eyes in both groups. In addition, ROC curve analysis was performed for mean value, AUC = 0.943, and when the threshold value was selected as 354.258 HU, 84.2% of sensitivity and 92.1% of specificity could be differentiated from patient-normal (Fig. 2).

DISCUSSION

Texture analysis allows the pixels in the image to be evaluated with mathematical expressions, independent of the experience of the radiologist [13, 14]. Reaching the diagnosis with objective information increases the accuracy of the diagnosis. The use of histogram analysis, which is a new method in patients with isolated optic neuritis, will contribute to the early diagnosis and the course of the prognosis. In our study, we can distinguish the patients from the healthy patient group with high sensitivity and specificity with mean histogram values.

However, in studies in the literature, similar parameters such as entropy, skewness, kurtosis were examined using histogram analysis in patients with isolated optic neuritis but mean values were not mentioned. Mean values measure the overall grayscale intensity and increase with higher signal intensity [15].

In histogram analysis, radiological modalities such as ultrasonography, computed tomography and magnetic resonance imaging can be used. MRI, one of the radiological modalities, is frequently used in histogram analysis due to its high tissue resolution [6].

In the literature, MRI was used in histogram analysis studies in neuroradiologically brain tumors, migraine, acute stroke, focal cortical dysplasia, Alzheimer's, epilepsy and multiple sclerosis patients [16, 17]. We used MRI images in our study in accordance with the literature.

Entropy shows the irregularity in pixel distribution. Liu *et al*. [18] it is observed that patients with optic neuritis can differentiate with high sensitivity and specificity in entropy and energy parameters by performing histogram analysis of the optic nerve in contrast-enhanced and non-contrast MR images [18]. In our study, we kept the study design different by not including contrast images, so we think that our entropy values did not show difference.

There is a close relationship between optic neuritis and MS. Some of the patients diagnosed with isolated optic neuritis develop MS in the following years [19,

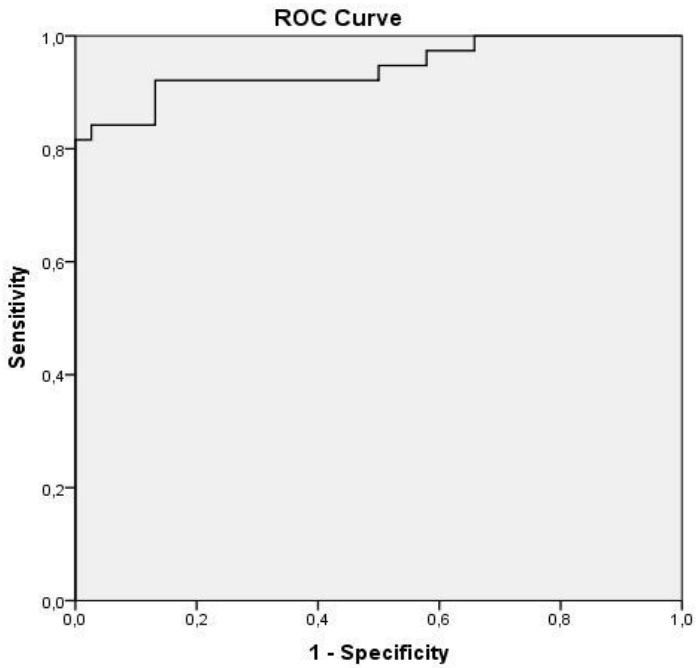

Fig. 2. ROC analysis of mean values.

20]. Even in 70% of the patients diagnosed with MS, acute optic neuritis is seen as the first symptom [21]. Therefore, early diagnosis of optic neuritis is very important in MS diagnosis, treatment planning and prognosis.

Contrast-enhanced MR is used in routine practice in the diagnosis of patients with optic neuritis, but the use of contrast is an invasive method. It is difficult to get the correct diagnosis in optic neuritis without using contrast. Tissue analysis, which is a non-invasive method, can be helpful in diagnosis by showing early histological changes. Histogram analysis was very successful in distinguishing the affected eye from the healthy eye. Hence the use histogram analysis is an important advantage in the patients whose use of contrast is contraindicated.

In addition, with histogram analysis, we can observe histological changes that are invisible to the naked eye. In patients with isolated optic neuritis, it can often cause symptoms in one eye clinically. When the opposite eye is viewed with the naked eye on the MRI images of these patients at the time of diagnosis, it is usually seen as normal. In the literature, there is a study done by adding diffusion tensor imaging to MRI to show the effect of the opposite eye [22]. However, taking additional sequences to routine imaging increases the duration and cost of the exposure. In our study, we can say that although there was no symptom in the contralateral eye at the time of diagnosis, the optic nerve in the contralateral eye was subclinically affected when histogram analysis was performed. This shows that although there is no clinical symptom, early initiation of treatment is important for the prognosis of the disease.

Limitations

The study has some limitations. Since the study is retrospective, the number of patients was limited.

CONCLUSION

Histogram analysis can be used in the diagnosis of patients with isolated optic neuritis without the use of contrast.

Authors' Contribution

Study Conception: SNK; Study Design: SNK, SU, AD; Supervision: SNK, SU; Funding: N/A; Materials: SU; Data Collection and/or Processing: AD, MB, SU; Statistical Analysis and/or Data Interpretation: MB, AD; Literature Review: SNK, MB, AD; Manuscript Preparation: SNK, AD and Critical Review: SNK, MB, SU.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Kanda T, Miyazaki A, Zeng F, Ueno Y, Sofue K, Maeda T, et al. Magnetic resonance imaging of intraocular optic nerve disorders: review article. Pol J Radiol 2020;85:e67-81.
2. Dooley MC, Foroozan R. Optic neuritis. J Ophthalmic Vis Res 2010;5:182-7.
3. Hoorbakht H, Bagherkashi F. Optic neuritis, its differential diagnosis and management. Open Ophthalmol J 2012;6:65-72.
4. Bennett JL. Optic Neuritis. Continuum (Minneap Minn) 2019;25:1236-64.
5. Petzold A, Wattjes MP, Costello F, Flores-Rivera J, Fraser CL, Fujihara K, et al. The investigation of acute optic neuritis: a review and proposed protocol. Nat Rev Neurol 2014;10:447-58.
6. Castellano G, Bonilha L, Li LM, Cendes F. Texture analysis of medical images. Clin Radiol 2004;59:1061-9.
7. Jian ZC, Long JF, Liu YJ, Hu XD, Liu JB, Shi XQ, et al. Diagnostic value of two dimensional shear wave elastography combined with texture analysis in early liver fibrosis. World J Clin Cases 2019;7:1122-32.
8. Kwon MR, Shin JH, Hahn SY, Oh YL, Kwak JY, Lee E, et al. Histogram analysis of grey scale sonograms to differentiate between the subtypes of follicular variant of papillary thyroid cancer. Clin Radiol 2018;73:591.e1-591.e7.
9. Chitalia RD, Kontos D. Role of texture analysis in breast MRI as a cancer biomarker: a review. J Magn Reson Imaging 2019;49:927-38.
10. Cauley KA, Fielden SW. A Radiodensity histogram study of the brain in multiple sclerosis. Tomography 2018;4:194-203.
11. Chabat F, Yang GZ, Hansell DM. Obstructive lung diseases: texture classification for differentiation at CT. Radiology 2003;228:871-7.
12. Dogan A, Baykara M. The evaluation of the optic nerve in multiple sclerosis using MRI histogram analysis. Ann Med Res 2020;27:780-3.
13. Lubner MG, Smith AD, Sandrasegaran K, Sahani DV, Pickhardt PJ. CT texture analysis: definitions, applications, biologic correlates, and challenges. Radiographics 2017;37:1483-503.

14. Zhang S, Chiang GC, Magge RS, Fine HA, Ramakrishna R, Chang EW, et al. Texture analysis on conventional MRI images accurately predicts early malignant transformation of low-grade gliomas. Eur Radiol 2019;29:2751-9.
15. Razik A, Goyal A, Sharma R, Kandasamy D, Seth A, Das P, et al. MR texture analysis in differentiating renal cell carcinoma from lipid-poor angiomyolipoma and oncocytoma. Br J Radiol. 2020;93:20200569.
16. Shin HJ, Kwak JY, Lee E, Lee MJ, Yoon H, Han K, et al. Texture analysis to differentiate malignant renal tumors in children using gray-scale ultrasonography images. Ultrasound Med Biol 2019;45:2205-12.
17. Kassner A, Thornhill RE. Texture analysis: a review of neurologic MR imaging applications. Am J Neuroradio 2010;31:809-16.
18. Liu HJ, Zhou HF, Zong LX, Liu MQ, Wei SH, Chen ZY. MRI histogram texture feature analysis of the optic nerve in the patients with optic neuritis. Chin Med Sci J 2019;34:18-23.
19. Rizzo JF, III, Lessell S. Risk of developing multiple sclerosis after uncomplicated optic neuritis: a long-term prospective study. Neurology 1988;38:185-90.
20. Francis DA, Compston DA, Batchelor JR, McDonald WI. A reassessment of the risk of multiple sclerosis developing in patients with optic neuritis after extended follow-up. J Neurol Neurosurg Psychiatry 1987;50:758-65.
21. Leocani L, Guerrieri S, Comi G. Visual evoked potentials as a biomarker in multiple sclerosis and associated optic neuritis. J Neuroophthalmol 2018;38:350-7.
22. Chen Z, Lou X, Liu M, Huang D, Wei S, Yu S, et al. Assessment of optic nerve impirment in patients with neuromyelitis optica by MR diffusion tensor imaging. PLoS One 2015;10:e0126574.

The European Research Journal 2023;9(2):291-300 Original Article

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Pulmonary and cardiac involvement in patients with rheumatoid arthritis and ankylosing spondylitis

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ABSTRACT

Objectives: Our aim is to study the disease activity status, laboratory data and to determine the frequency of cardiac and pulmonary findings in patients with Rheumatoid arthritis (RA) and ankylosing spondylitis (AS).

Methods: Forty-five AS patients and 78 RA patients took part in the study. The C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, creatinine, ALT, leukocyte, neutrophil ratio, hemoglobin, platelet, rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) levels were measured. In addition, the patients' thoracic computed tomography, high-resolution computed tomography (HRCT), echocardiography (ECHO), and pulmonary function test (PFT) results were evaluated. Disease activity was assessed with the Disease Activity Score-28 (DAS-28) in RA patients, whereas the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was used in patients with AS.

Results: The relationship between the anti-CCP value and gender was significant in RA patients. Significant differences were present between gender and hemoglobin value in RA and AS patients, whereas no significant differences were determined between CRP, ESR, platelet, and DAS-28 values. There was a significant difference between RF and ESR values in RA patients, whereas no significant difference was present between CRP, leukocyte, DAS-28, hemoglobin, and platelet values. Pulmonary involvement was determined in 35.7% of RA patients and 4.2% of AS patients. The difference between these was statistically significant.

Conclusions: The most common pulmonary involvement is millimetric nonspecific nodule and the most common lesion among ECHO findings was left ventricular diastolic dysfunction in RA and AS patients. In RA, no significant difference was determined between PFT and DAS-28 results, as a result, the disease activity will not directly indicate pulmonary involvement.

Keywords: Rheumatoid arthritis, ankylosing spondylitis, high-resolution computed tomography

Rheumatoid arthritis (RA) is a chronic, autoimmune, multisystemic, inflammatory disease with unknown etiology and characterized by joint destruction due to synovial cellular proliferation and inflammation [1-3]. Despite treatment, it usually has a clinical course with exacerbations, resulting in progressive joint destruction, deformity, disability, and sometimes early mortality. In addition, the disease's extra-articular manifestations are also common, and sometimes with a severe clinical course [4, 5]. Females are affected 2-3 times more commonly than males. On the other hand, RA may manifest at any age and is most common between 40-50 years of age.

Even though RA is a disease primarily affecting

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the joints, it is a systemic disorder that might involve every organ system [6]. RA's extra-articular manifestations are more common in patients with active severe articular manifestations and in Rheumatoid factor (RF) (+) cases [7]. The serum concentration of CRP increases rapidly and dramatically following the inflammatory stimulus, in line with the amount of tissue damage [8].

Pulmonary involvement in RA is more common in males than females. The primary predisposing factors for pulmonary involvement in RA are middle age, male gender, severe destructive arthritis, excessively elevated RF titers, subcutaneous nodules, and extra-articular involvements [9]. The most classical type of parenchymal involvement in RA is diffuse interstitial fibrosis, and its most frequent finding is bilateral basilar interstitial involvement on posteroanterior chest X-rays, often presenting asymmetrically. Involvement starts with a patchy alveolar infiltration and then proceeds to a reticulonodular pattern. The pulmonary disorder may also occur because of direct pulmonary toxicity due to drugs or other agents used in RA treatment or infection secondary to immunosuppressive use (e.g., glucocorticoids, antimetabolites, anti-cytokine treatment).

Clinically overt pericarditis or myocarditis is not commonly observed in RA's cardiac involvement. Instead, coronary artery disease, heart failure, and atrial fibrillation are more common in RA patients [9, 10]. The clinical features of pericarditis are present in less than 10% of patients. Pericardial effusion is detected in echocardiographic examination with no clinical signs in approximately 30% of patients [11]. Myocarditis may be granulomatous or interstitial-type, rare in RA, and associated with an active articular disease or other non-articular involvements [6]. Valvular failures and heart blocks may occur because of rheumatoid nodules, which can develop in pericardium, myocardium, and valvular structures [7].

Ankylosing spondylitis (AS) is a chronic, progressive, systemic, inflammatory disease with unknown etiology, primarily involving the sacroiliac joints and axial skeleton, which may also manifest peripheral articular involvement. It is the prototype of spondyloarthritis (SpA)-group disorders. [12]. Even though AS manifests more commonly in the second and third decades of life, it is distributed in a wide age range, including the pediatric and geriatric age groups [13]. Males are more commonly affected than females, with a ratio of approximately 2-3/1. Erythrocyte sedimentation rate (ESR) and C-Reactive Protein (CRP) levels are not well-correlated with the axial disease activity, but they may better correlate with peripheral arthritis. Normochromic normocytic anemia is common and is typical in the very active disease form [14, 15].

The risks for aortic insufficiency and cardiovascular involvement are increased in AS. The incidence of aortic insufficiency is 6-10%, and conduction-type problems are met in 3-33% of patients [16-19]. Moreover, the risks for other cardiovascular diseases are also increased in AS.

Pulmonary problems occur due to musculoskeletal disease-related restrictive changes, pulmonary interstitial nodules, and parenchymal changes. Restrictive pulmonary disease is usually associated with reduced thoracic wall and spinal mobility. As a result, the vital capacity is reduced, whereas the functional residual capacity is increased. In a small number of patients, approximately 1.3-1.5%, signs such as apical pulmonary fibrosis are observed in plain chest x-ray [20-23]. Apical fibrosis is usually asymptomatic and associated with prolonged disease duration. The mosaic pattern, subpleural nodules, and parenchymal bands are high-resolution computed tomography (HRCT) findings that can also be observed in the disease's early period [22, 23].

METHODS

Study Design and Patients

The study was conducted on a total of 123 patients [RA 63.4% (n = 78), AS 36.6% (n = 45)] who were admitted to the Outpatient Immunology / Rheumatology Clinic of Karadeniz Technical University Medical Faculty. Forty-five AS patients, consisting of 26 (57.8%) females, and 19 (42.2%) males, aged between 18-82 years, diagnosed and followed up according to the modified New York 1984 criteria, and 78 RA patients, consisting of 57 (73.1%%) females, and 21 (26.9%) males, aged between 26-86 years, diagnosed and followed up according to the 2010 ACR/EULAR criteria were included in the study.

The patients' medical histories and physical exam-

ination results were reviewed. In addition, the computed chest tomography, HRCT, echocardiography (ECHO), and pulmonary function test (PFT) results were evaluated and recorded. FEV1 (1-second forced expiratory volüme) (%, L), FVC (forced vital capacity (%, L), and FEV1/FVC ratio were recorded. In addition, CRP, ESR, albümin, creatinine, ALT, leukocyte, neutrophil %, hemoglobin, platelet count, RF, and anti-CCP were recorded. In RA patients' disease activity assessment with DAS-28, for joint examination, two shoulders, two elbows, two wrists, ten metacarpophalangeal (MCP) joints, ten proximal interphalangeal (PIP) joints, and two knee joints were evaluated regarding tenderness and swelling. The tender joint count (TJC) and swollen joint count (SJC) were calculated. For DAS-28 scoring, the erythrocyte sedimentation rate (ESR) and 43-general health status (GHS) values were required, and the following formula was used; all these parameters and calculators, prepared specifically for DAS-28, were used with a constant formula- DAS-28 = (0.56×TJC) + (0.28×√SJC) + (0,70 log ESR) + (0.014×GHS). In AS patients, BASDAI was used for disease activity assessment in clinical evaluation.

Inclusion and Exclusion Criteria and Definitions

Among RA patients, those who were seropositive, and among AS patients, those who were HLA-B27 positive were included in the study. The study's exclusion criteria were determined as age under 18 years, not being seropositive for RA patients, and lack of HLA-B27 positivity for AS patients.

Statistical Analysis

Descriptive statistics of evaluation results were presented as the number and percentage for categorical variables and the mean, standard deviation, minimum and maximum for quantitative variables. The conformity of quantitative variables with a normal distribution was evaluated with the Kolmogorov-Smirnov test. Regarding comparisons of quantitative variables between two independent groups, the Student T-test was used when prerequisites for normal distribution were met, and the Mann-Whitney U test was used when they were not. Differences between the ratios of categorical variables in independent groups were tested with Chi-Square Analysis. Spearman's test calculated correlation coefficients and statistical significance for the relationships between quantitative variables. Data were analyzed with SPSS 13.0 statistical package software. $P < 0.05$ was considered the level of statistical significance.

RESULTS

Forty-five AS patients, consisting of 26 (57.8%) females, and 19 (42.2%) males, aged between 18-82 years, and 78 RA patients, consisting of 57 (73.1%%) females, and 21 (26.9%) males, aged between 26-86 years, were included in the study. The mean age was 57.60 ± 13.124 years in RA patients and 45 ± 14.618 years in AS patients. The gender distributions of AS patients and RA patients were presented in Table 1.

RA- Signs of PulmonaryIinvolvement

In the patients with RA, the HRCT findings were as follows: millimetric nonspecific nodule - 15 (34.1%) cases; the atelectatic band – 8 (18.2%) cases; fibrosis – 7 (15.9%) cases; atelectasis – 6 (14.3%) cases; ground glass appearance – 5 (11.4%) cases; tubular bronchiectasis – 3 (7.1%) cases; emphysematous changes – 3 (6.8%) cases; tractional bronchiectasis – 2 (4.5%) cases; honeycomb – 2 (4.5%) cases; subpleural cyst – 1 (2.4%) case; chronic interstitial pulmonary disease – 1 (2.4%) case; septal thickening – 1 (2.4%) case.

The chest CT findings of the RA patients were as follows: multiple lymph nodes sized < 10 mm – 27 (50.9%) cases; a nodule sized < 5 mm – 17 (32.1%) cases; the atelectatic band – 17 (32.1%) cases; millimetric nonspecific nodule – 11 (20.8%) cases; fibrotic

Table 1. Participant characteristics

Parameter	Rheumatoid arthritis	Ankylosing spondylitis
Gender, n (%)		
Female	57 (73.1)	26 (57.8)
Erkek	21 (26.9)	19 (42.2)
Age (years)		
Minimum	26	18
Maximum	86	82
Mean ± SD	57.60 ± 13.124	45 ± 14.618

SD = standard deviation

Table 2. **The pulmonary function test results in the patients with RA and AS**

	RA/AS	Min-Max (RA/AS)	Mean ± SD (RA/AS)
FEV1/FVC	37/28	48/71-100/98	77.89 ± 11.983/ 86.68 ± 8.010
FEV1 (L)	37/28	0.78/1.45-4.21/4.48	2.277 ± 0.838/ 2.962 ± 0.814
FVC (L)	37/28	1.25/1.81-5.10/5.67	2.960 ± 1.014/ 3.462 ± 1.041
FEV1 (%)	37/28	40/62-144/153	98.46 ± 23.246/ 106.43 ± 21.488
FVC (%)	37/28	53/53-135/159	96.89 ± 20.795/ 97.54 ± 24.406

AS = ankylosing spondylitis, RA = rheumatoid arthritis, FEV1 = 1-second forced expiratory volüme, FVC = forced vital capacity, SD = standard deviation, Min =minimum, Max = maximum

Table 3. **The PFT results and HRCT findings in RA patients**

	n	Mean ± SD	*p* value
FEV1/FVC			0.78
Pulmonary Finding (+)	18	74.8± 12.25	
Pulmonary Finding (-)	10	76.22± 12.698	
FEV1 (L)			0.87
Pulmonary Finding (+)	18	2.32± 0.811	
Pulmonary Finding (-)	10	2.26± 0.841	
FVC (L)			0.71
Pulmonary Finding (+)	17	3.16± 1.14	
Pulmonary Finding (-)	10	3.01± 0.931	
FEV1 (%)			0.9
Pulmonary Finding (+)	18	96.7± 20.49	
Pulmonary Finding (-)	10	97.89± 25.254	
FVC (%)			0.91
Pulmonary Finding (+)	18	98.1± 25.062	
Pulmonary Finding (-)	10	97.17± 18.03	
DAS-28			0.5
Pulmonary Finding (+)	15	3.238± 1.3791	
Pulmonary Finding (-)	27	2.975± 0.6801	

RA = rheumatoid arthritis, PFT = pulmonary function test, HRCT = high-resolution computed tomography, DAS-28 = disease activity score-28, FEV1 = 1-second forced expiratory volüme, FVC = forced vital capacity, SD = standard deviation

retraction/change – 8 (15.1%) cases; lymphadenopathies sized > 10 mm – 7 (13%) cases; emphysematous changes – 4 (7.5%) cases; cardiomegaly – 2 (3.8%) cases.

AS- Signs of Pulmonary Involvement

In the patients with AS, the HRCT findings were identified as follows: the atelectatic band – 7 (28%) cases; the millimetric nonspecific nodule – 7 (28%) cases; atelectasis – 1 (4.2%) case; tractional bronchiectasis – 1 (4.2%) case.

The distribution of chest CT findings in AS patients was as follows: the millimetric nonspecific nodule – 8 (24.2%) cases; a nodule sized < 5 mm – 8 (24.2%); the atelectatic band – 7 (21.2%) cases; fibrotic retraction/change – 7 (21.2%) cases; lymphadenopathies sized > 10 mm – 4 (12.1%) cases; multiple lymph nodes sized <10 mm – 3 (9.1%) cases; emphysematous changes – 2 (6.1%) cases; cardiomegaly – 2 (6.1%) cases.

PFT Results

PFT results, consisting of values for FEV1/FVC, FEV1 L, FVC L, FEV1 %, FVC % minimum, maximum, mean, and standard deviation, in the patients with RA and AS were presented in Table 2.

The PFT Results and HRCT Findings in RA Patients

The pulmonary function test results were compared to HRCT findings in RA patients. No significant differences regarding the FEV1/FVC, FEV1 L, FVC L, FEV1 %, FVC % values, and the DAS-28 score were determined between the groups with and without pulmonary involvement (p = 0.776, p = 0.868, p = 0.709, p = 0.900, p = 0.910, and p = 0.497, respectively). The PFT results and HRCT findings in RA patients were presented in Table 3.

The HRCT findings were compared according to gender in the patients with RA. No pulmonary finding was present in 37 (84.1%) female patients, whereas seven (15.9%) patients presented with pulmonary findings. On the other hand, while no pulmonary finding was present in 13 (59.1%) male patients, nine (40.9%) patients presented with pulmonary findings. However, the difference between male and female groups was not statistically significant (p = 0.054).

The patients with RA were compared to those with AS regarding their HRCT findings. Pulmonary involvement was not identified in 64.3%, whereas it was present in 35.7% of RA patients. In AS patients, no pulmonary involvement was identified in 95.8% of them, whereas only 4.2% of AS patients manifested pulmonary involvement. The difference between the RA and AS groups was statistically significant (p = 0.01). The HRCT findings in RA and AS patients were presented in Table 4.

Table 4. The HRCT findings in RA and AS patients

	Pulmonary Finding (-)	Pulmonary Finding (+)	Total
RA			
n	27	15	42
%	64.3	35.7	100
AS			
n	23	1	24
%	95.8	4.2	100

AS = ankylosing spondylitis, RA = rheumatoid arthritis, HRCT = high-resolution computed tomography, p = 0.01

Table 5. The ECHO results of the patients with RA and AS

Finding	RA n (%)	AS n (%)
Left ventricular hypertrophy	22 (39.3)	8 (25.8)
Left atrial dilatation	10 (18.9)	3 (9.7)
Valvular disease	12 (22.2)	8 (45.2)
Left ventricular systolic dysfunction	5 (9.3)	0 (0)
Left ventricular diastolic dysfunction	42 (75)	14 (45.2)
Dilatation of pulmonary artery	2 (3.8)	3 (9.7)
Dilatation of ascending aorta	15 (26.8)	4 (12.9)
Right ventricular systolic dysfunction	3 (5.4)	0 (0)
Pericardial effusion	0 (0)	0 (0)
IVS hypertrophy	1 (1.8)	1 (3.2)
Sinus of valsalva aneurysm	1 (1.8)	1 (3.2)

AS = ankylosing spondylitis, RA = rheumatoid arthritis, ECHO = echocardiography

RA/AS Findings of Cardiac Involvement

The most common lesion among ECHO findings was left ventricular diastolic dysfunction in RA and AS patients, with ratios of 75% and 45.2, respectively. The distributions of ECHO findings in RA and AS patients were presented in Table 5.

Laboratory Findings

RA patients were grouped according to their RF values being low (< 10.2) and high (> 10.2) and compared regarding gender. While seven female patients had a low RF value, in 49 females, the RF level was high. Only one had a low RF value in male patients, whereas 20 males had high RF values. The difference was not statistically significant ($p = 0.435$).

The patients diagnosed with RA were grouped according to their anti-CCP values being low (< 0.5) and high (> 0.5), and then, the two groups were compared regarding gender.

While 14 of the female patients were determined to have a low anti-CCP value, in 43 females, the level of anti-CCP was high. None of the males had a low anti-CCP value, while it was high in 20 males. The difference between the two genders was statistically significant ($p = 0.016$).

The RA patients, divided into two groups according to their anti-CCP values as low and high, were compared regarding the CRP value. Sixty-two patients were in the anti-CCP-high group and had a mean CRP value of 1.48, whereas the anti-CCP-low group had fourteen patients with a mean CRP level of 2.05. However, the difference between the two groups was not statistically significant ($p = 0.288$).

In RA patients, the CRP, ESR, hemoglobin, platelet, leukocyte, and DAS-28 values were compared according to gender. While there was a significant difference between the hemoglobin value and gender, there were no significant differences in other parameters ($p = 0.550$, $p = 0.850$, $p = 0.011$, $p = 0.360$, $p = 0.084$, and $p = 0.729$, respectively).

In patients with AS, the CRP, ESR, hemoglobin, platelet, leukocyte, and BASDAI values were compared according to gender. While there was a significant difference between the hemoglobin value and gender, there were no significant differences in other parameters ($p = 0.174$, $p = 0.301$, $p < 0.001$, $p = 0.519$, $p = 0.689$, and $p = 0.089$, respectively).

RF values in RA patients were grouped as < 10.2 low, 10.2-720 high, > 720 very high. Anti-CCP values were grouped as < 0.5 low, 0.5-200 high, > 200 very high. The RF and Anti-CCP values in RA patients were presented in Table 6.

Table 6. RF and Anti-CCP values distribution in RA patients

Parameter	RF n (%)	Anti-CCP n (%)
Low	8 (10.4)	14 (18.2)
High	63 (81.8)	41 (53.2)
Very high	6 (7.8)	22 (28.6)
Total	77 (100)	77 (100)

RF, Rheumatoid factor, anti-CCP = anti-cyclic citrullinated peptide

DISCUSSION

The most common pulmonary involvement is millimetric nonspecific nodule and the most common lesion among ECHO findings was left ventricular diastolic dysfunction in RA and AS patients. No significant differences regarding the PFT values, and the DAS-28 score were determined between the groups with and without pulmonary involvement in RA patients. The HRCT findings were compared according to gender in the patients with RA. However, the difference between male and female groups was not statistically significant. Pulmonary involvement was not identified in 64.3%, whereas it was present in 35.7% of RA patients. In AS patients, no pulmonary involvement was identified in 95.8% of them, whereas only 4.2% of AS patients manifested pulmonary involvement.

There is no specific indicator of the disease activity in ankylosing spondylitis. The investigators' views on this subject are controversial. Numerous methods have been developed to evaluate the disease activity-related symptoms and signs of AS. For this reason, the AS patients are assessed together with their clinical features, laboratory results [erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)], and radiological findings. On the other hand, ESR and CRP are weakly correlated with BASDAI, and their predictive

values are low [24]. Bostan *et al*. [25], in their study assessing the disease activity with various clinical scales, stated that it was better correlated with CRP than other laboratory parameters.

The literature review revealed that high ESR and CRP levels were not typical in AS. Besides, some publications have stated that they were not well-correlated with clinical activity and radiological progression [24, 26].

In the study published by Pinheiro *et al*. [27] in 2003, an anti-CCP positivity was present in 120 of 150 RA patients with a mean disease duration of 12 years, and anti-CCP was negative in 30 patients. In addition, they determined a strong correlation between anti-CCP positivity and the DAS-28 score (r = 0.82). They showed that the presence of anti-CCP was associated with high disease activity [27]. Anti-CCP positive RA patients were reported to have higher disease activity and DAS-28 scores than anti-CCP negative RA patients. However, the same investigators could not determine an association between anti-CCP positivity and clinical activity in another study. On the other hand, they found that the IgM RF-positive patients had higher clinical activity [28, 29]. Our study did not determine a significant difference between the DAS-28 score and HRCT findings of RA patients.

In a review article on the pleuro-pulmonary involvement in AS, written by El Maghrauoi and Dehhaoui [30] in 2012, it was emphasized that abnormalities identified on thoracic HRCT images might have been commonly detected without signs of respiratory system disease as well as in early periods of the disease. The common result of all studies conducted to evaluate potential changes in respiratory functions due to reduced thoracic cage mobility was that a restrictive-type respiratory dysfunction was present in AS patients [31-33]. Another significant finding was the FEV1/FVC ratio being normal despite reductions occurring in FVC and FEV1 values [33, 34]. In addition, the mosaic pattern, subpleural nodules, and parenchymal bands are findings that might be detected in early periods of the disease [22, 23]. Even though the incidence of pleuro-pulmonary involvement has been reported within a wide range of 1.3-30%, the most frequently defined and well-known pathology is apical fibrosis [35]. Until recently, plain radiographs and PFT have been used in studies performed to determine pulmonary parenchymal changes. However, using HRCT to evaluate pleuropulmonary involvement has recently become more common in AS patients [36, 37]. It has been suggested that the etiology of the interstitial lung disease identified with HRCT in AS patients was a consequence of the disease's inflammatory process rather than the mechanical effects [38]. In addition to thoracic involvement, the pleuropulmonary involvement, presenting as an extra-articular manifestation, might also be a significant factor for respiratory dysfunctions [6]. In the studies conducted to test this hypothesis, PFT has been performed together with chest x-ray and HRCT for pleuropulmonary involvement in AS patients. It was determined that no association was present between pulmonary functions and pulmonary radiological findings, and PFT was determined to be insensitive in identifying pulmonary interstitial changes. However, HRCT is still recommended in patients with restrictive-type disorders that mechanical factors cannot explain alone [36, 37].

Even though the actual prevalence of pulmonary involvement in RA has not been identified, it has been reported in 1-40% of cases [39]. In RA, pulmonary involvement (9.9%) is the third most common cause of mortality, following infections (23.5%) and cardiovascular disorders (17.3%) [17]. The clinical course is adversely affected mainly by pulmonary fibrosis development. Even though RA is more common in females, pılmonary involvement is more common in males [39]. In numerous studies investigating pulmonary pathologies with HRCT in RA patients, different results have been reported regarding the pathologies' types and prevalences. It has been reported that such differences might have been due to various disease-unrelated factors like smoking, air pollution, and exposure to adverse environmental factors, or disease-related factors like seropositivity, disease duration, drug type, and the duration of its use. Pleural involvement has been determined as the most common type of pulmonary involvement in RA. McDonagh *et al*. reported the interstitial lung disease as the most frequent finding, whereas Cortet *et al*. reported that they most frequently determined bronchiectasis [40, 41]. Regarding the distribution of our RA patients' HRCT findings, the most common lesions were the atelectatic band (18.2%) and fibrosis (15.9%). Regarding the dis-

tribution of chest CT findings, the most common lesions were multiple lymph nodes smaller than 10 mm in size (50.9%), a nodule less than 5 mm in size (32.1%, and the atelectatic band (32.1%).

Even though the pulmonary function test is a valuable method frequently used in clinical practice, it is less sensitive than bronchoscopic and radiographic methods for diagnosing interstitial pulmonary disease in patients with RA. Nevertheless, PFT is an important test for diagnosing pulmonary hypertension and diffuse interstitial lung disease and the response to treatment in rheumatologic disorders, particularly scleroderma [40, 42]. No significant difference was determined between the pulmonary function tests and HRCT findings of RA patients in our study.

Limitations

Our study has various limitations, such as the relatively small patient number, lack of a control group, and being retrospective in nature. These limitations might have led our results to be consistent with or different from the studies published in the literature. Besides, ESR and CRP, which are among nonspecific laboratory parameters affected by many diseases, might be considered factors that could affect the study results. Moreover, clinical, laboratory and radiological features, age, gender, environmental factors, the age of disease onset, harmful habits like smoking, psychological and mental status, socioeconomic level, and drug use being effective on patient assessment might have led to different results compared to other studies. Therefore, we suggest that our study has provided additional information to the literature with these differences.

CONCLUSION

RA, which is one of the most common autoimmune disorders, leads to joint deformities and systemic involvement when diagnosis and treatment are delayed. Therefore, early diagnosis of RA is essential regarding damage prevention. For this reason, specific and sensitive serologic tests are required for early diagnosis. In addition, because pulmonary involvement significantly affects morbidity and mortality in RA, it is necessary to determine pulmonary involvement and arrange the treatment modality in the early period. The pulmonary functions of AS patients are affected due to chest wall restriction and/or primary lung tissue involvement. A restrictive-type pulmonary disorder causing decreased exercise tolerance develops due to reduced chest wall mobility and/or primary lung tissue involvement. Pulmonary involvement in HRCT was more common in RA than AS in our study.

In conclusion, it can be suggested that BASDAI can be used in AS patients in addition to ESR and CRP, which are the most significant indicators of disease activity in clinical practice, whereas ESR, CRP, anti-CCP, and DAS-28 can be used in RA patients. Furthermore, the idea that respiratory dysfunction develops because of thoracic involvement-related mechanical problems rather than pulmonary parenchymal invoılvement occurring during the disease process was supported.

Authors' Contribution

Study Conception: AHT; Study Design: AHT; Supervision: AHT; Funding: AHT; Materials: AHT; Data Collection and/or Processing: AHT; Statistical Analysis and/or Data Interpretation: AHT; Literature Review: AHT; Manuscript Preparation: AHT and Critical Review: AHT.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Çalgüneri M. Romatoid Artrit. In: Yasavul Ü, Ed., Hacettepe İç Hastalıkları Kitabı Ankara: Prestij Basımevi; 2003: pp. 1477-95.
2. Lipsky PE. Rheumatoid Arthritis. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL editors. Harrison's Principles of Internal Medicine. New York: McGraw-Hill; 2005. pp. 1968-77.
3. Hellmann DB, Stone JH. Rheumatoid Arthritis. In: Tierney LM, McPhee SJ, Papadakis MA editors. Current Medical Diagnosis & Treatment. New York: McGraw-Hill; 2005; pp. 801-7.
4. Fuchs HA, Sergent SJ. Rheumatoid arthritis the clinical picture. In: Koopman WJ (ed). Arthritis and Allied conditions, 13th ed. Pennsylvania: Williams and Wilkins, 1997: pp. 1041-70.

5. Firestein GS. Etiology and pathogenesis of rheumatoid arthritis. In: Ruddy S, Haris ED, Sledge CB (eds). Kelley's Textbook of Rheumatology, sixth ed. Philadelphia: WB Saunders, 2001: pp. 921-1000.
6. Harris ED. Clinical features of rheumatoid arhritis. In: Ruddy S, Harris ED, Sledge CB (eds). Texbook of Rheumatology, 6th ed. Philadelphia: WB Saunders, 2001: pp. 967-1000.
7. Halla JT, Schrohenloher RE, Koopman WJ. Local Ġmmüno responses in certain extraarticular manifestations of rheumatoid arthritis. Ann Rheum Dis 1992;51:698-701.
8. Gray S. Romatoid Artrit. In Edward D. Harris JR, Ralph C, Clement B (ed)s. Kelley's Textbook of Rheumatology. çeviri: Arasıl T. Kelley Romatoloji 7. baskı. Ankara: Güneş Kitabevi, 2006:996-1078.
9. Hamuryudan V. Romatoid artrit. Sempozyum 2007;55:69-86.
10. Tanoue LT. Pulmonary manifestations of rheumatoid arthritis. Clinic Chest Med 1998;19:667-85.
11. Holmqvist ME, Neovius M, Eriksson J, Mantel A, Wallberg-Jonsson S, Jacobsson LTH, et al. Risk of venous thromboembolism in patients with rheumatoid arthritis and association with disease duration and hospitalization. JAMA 2012; 308:1350-6.
12. Özgöçmen S. Tanı ve Sınıflama Kriterleri. Ankilozan spondilit ve spondiloartropatiler (çeviri), çeviri ed: Özgöçmen S. Birinci baskı. Veri medikal yayıncılık, İstanbul, 2008: pp. 24-6.
13. Onel D. Romatizmal Hastalıklar. Tayf Ofset, İstanbul, 1994: pp. 326-7.
14. Sieper J, Braun J, Rudwaleit M, Boonen A, Zink A. Ankylosing spondylitis: an overview. Ann Rheum Dis 2002; 61 Suppl 3:iii8-18.
15. Niccoli L, Nannini C, Cassarà E, Kaloudi O, Cantini F. Frequency of anemia of inflammation in patients with ankylosing spondylitis requiring anti-TNFα drugs and therapy-induced changes. Int J Rheum Dis 2012;15:56-61.
16. Slobodin G, Naschitz JE, Zuckerman E, Zisman D, Rozenbaum M, Boulman N, et al. Aortic involvement in rheumatic diseases. Clin Exp Rheumatol 2006;24:41-7.
17. Eder L, Sadek M, McDonald-Blumer H, Gladman DD. Aortitis and spondyloarthritis-- an unusual presentation: case report and review of the literature. Semin Arthritis Rheum 2010;39:510-4.
18. Vinsonneau U, Brondex A, Mansourati J, Saraux A, Cornily JC, Arles F, et al. Cardiovascular disease in patients with spondyloarthropathies. Joint Bone Spine 2008;75:18-21.
19. Brunner F, Kunz A, Weber U, Kissling R. Ankylosing spondylitis and heart abnormalities: do cardiac conduction disorders, valve regurgitation and diastolic dysfunction occur more often in male patients with diagnosed ankylosing spondylitis for over 15 years than in the normal population? Clin Rheumatol 2006;25:24-9.
20. Boushea DK, Sundstrom WR. The pleuropulmonary manifestations of ankylosing spondylitis. Semin Arthritis Rheum 1989;18:277-81.
21. Kchir MM, Mtimet S, Kochbati S, Zouari R, Ayed M, Gharbi T, et al. Bronchoalveolar lavage and transbronchial biopsy in spondyloarthropathies. J Rheumatol 1992;19:913-6.
22. Quismorio FP Jr. Pulmonary involvement in ankylosing spondylitis. Curr Opin Pulm Med 2006;12:342-5.
23. Kiris A, Ozgocmen S, Kocakoc E, Ardicoglu A, Ogur E. Lung findings on high resolution CT in early ankylosing spondylitis. Eur J Radiol 2003;47:71-6.
24. Spoorenberg A, van der Heijde D, de Klerk E, Dougados M, de Vlam K, Mielants H, et al. Relative value of erythrocyte sedimentation rate and C-reactive protein in assessment of disease activity in ankylosing spondylitis. J Rheumatol 1999;26:980-4.
25. Bostan EE, Borman P, Bodur H, Barça N. Functional disability and quality of life in patients with ankylosing spondylitis. Rheumatol Int 2003;23:121-6.
26. Ruof J, Stucki G. Validity aspects of erythrocyte sedimentationrate and Creactive protein in ankylosing spondylitis: a literatüre review. J Rheumatol 1999;26:966-70.
27. Pinheiro GC. Anti-cyclic citrullinated peptide antibodies in advanced rheumatoid arthritis. Ann Intern Med 2003;139:234-5.
28. Bas S, Genevay S, Meyer O and Gabay. Anti-cyclic citrullinated peptide antibodies IgM and IgA rheumatoid factors in the diagnosis and prognosis ofrheumatoid arthritis. Rheumatology (Oxford) 2003;42:677-80.
29. Bas S, Perneger TV, Seitz M, et al. Diagnostic tests for rheumatoid arthritis:comparision of anti-cyclic citrulinated peptide antibodies, antikeratinantibodies and IgM rheumatoid factors. Rheumatology (Oxford) 2002;41:809-14.
30. El Maghrauoi A, Dehhaoui M. Prevalence and characteristics of lung involvement on high resolution computed tomography in patients with ankylosing spondylitis: a systematic review. Pulm Med 2012;2012:965956.
31. Miller JM, Sproule BJ. Pulmonary function in ankylosing spondylitis. Am Rev Respir Dis 1964;90:376-82.
32. Feltius N, Hedenström H, Hillerdal G, Hallgren R. Pulmonary involvement inankylosing spondylitis. Ann Rheum Dis 1986;45:736-40.
33. Seçkin Ü, Bölükbası N, Gürsel G, Eröz S, Sepici V, Ekim N. Relationship between pulmonary function and exercise tolerance in patients with ankylosing spondylitis. Clin Exp Rheumatol 2000;18:503-6.
34. Sahin G, Çalıkoglu M, Özge C, İncel N, Biçer A, Ulsubas B, et al. Respiratory muscle strength but not BASFI score relates to diminished chest expansion in ankylosing spondylitis. Clin Rheumatol 2004;23:199-202.
35. Rosenow EC, Strimlan CV, Muhm JR, Ferguson RH. Pleuropulmonary manifestations of ankylosing spondylitis. Mayo Clin Proc 1977;52:641-9.
36. Casserly LP, Fenlon HM, Breatnach E, Sant SM. Lung findings on high resolution computed tomography in idiopathic ankylosing spondylitis-correlation with clinical findings, pulmonary function testing and plain radiography. Br JRheumatol 1997;36:677-82.
37. Ayhan-Ardıç FF, Oken Ö, Yorgancıoglu ZR, Üstün N, Gökharman FD. Pulmonary involvement in life long non-smoking patients with rheumatoid arthritis and ankylosing spondylitis without respiratory symptoms. Clin Rheumatol 2006;25:213-8.
38. Walker WC, Wright V. Pulmonary lesions and rheumatoid arthritis. Medicine 1968;47:501-20.
39. Horton MR. Rheumatoid arthritis associated interstitiallung

disease. Crit Rev Comput Tomogr 2004;45:429-40.
40. Cortet B, FLipo RM, Remy-Jardin M, Coqueralla P, Duquesnoy B, Remy J, et al. Use of high resolution computed tomography of the lungs in patients with rheumatoid arthritis. Ann Rheum Dis 1995;54: 815-9.
41. Mc Donagh J, Greaves M, Wright AR, Heycock C, Owen JP, Kelly CA. High resolution computed tomography of the lungs in patients with rheumatoid arthritis and interstitial lung disease. Br J Rheumatol 1994;33:118-22.

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Bone turnover markers and bone mineral density in patients with type 2 diabetes

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ABSTRACT

Objectives: This study was designed in order to evaluate bone and mineral metabolism in type 2 diabetic patients and its relationship with bone mineral density and diabetic microvascular complications.

Methods: Forty-two type 2 diabetic patients and 23 healthy cases were included in the study. Serum osteocalcin, procollagen type I C-peptide (PICP), total and bone-specific alkaline phosphatase (bone ALP), urinary deoxypyridinoline (free DPD), parathormone (PTH), serum and urinary calcium and phosphorus levels were measured. Bone mineral densities of all subjects were studied in lumbar vertebra and femur region using dual X-ray absorptiometry (DXA).

Results: Serum osteocalcin and bone ALP levels of the diabetics were found to be significantly lower and total alkaline phosphatase and calcium levels were higher in diabetic patients compared to healthy controls, but PICP and free DPD levels were not different between these two groups. There was a positive correlation between PTH levels and urinary DPD excretion. Among diabetics, serum osteocalcin levels increased with the impairment of renal functions. Bone mineral densities were lower in diabetics with worse renal functions.

Conclusions: Bone turnover is slow in type 2 diabetes and there is no prominent bone loss related to this condition. PTH is an important factor determining the rate of bone resorption in diabetics. Renal functional impairment is the most important factor affecting bone mass in type 2 diabetic patients.

Keywords: Diabetes, bone mineral density, bone turnover, diabetic complications

Bone mineral density is an important marker of the pathophysiological condition of bone and is accepted to be a reliable index for the risk of future fractures. Alterations in the rate of both bone formation and resorption can lead to changes in bone mass. Biochemical markers of bone turnover enable to determine the alterations in the metabolic activity of bone. There are many reports suggesting the existence of altered bone and mineral metabolism in diabetes mellitus. Low bone mineral content has been described for type 1 diabetes, but the relationship between type 2 diabetes and bone mass is less clear. The bone mass has been reported to be normal, increased, or decreased in type 2 diabetes [1-6]. Also bone formation and turnover seem to be decreased in both types of diabetes [7-10]. But the chronic complications of diabetes and even the type of therapy may also affect bone turnover and bone mineral density [10-13]. There are

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also publications regarding the increased risk of fractures despite normal or high bone mineral density [14, 15].

In this study, we aimed to evaluate bone and mineral metabolism in type 2 diabetic patients with biochemical markers of bone turnover and its relationship with bone mineral density and diabetic microvascular complications.

METHODS

Twenty-seven females and 15 males total of 42 type 2 diabetic patients (mean age: 56.2 ± 1.65 years) were studied. Thirteen females and 10 males, total 23 healthy subjects (mean age: 53.9 ± 1.81 years) comprised the control group. The study was conducted in the Endocrinology outpatient Department of Eskisehir Osmangazi University Faculty of Medicine Hospital. None of the subjects in both groups had a condition that could affect bone and mineral metabolism and none of the subjects were taking medications known to interfere with bone metabolism. All of the women were in the postmenopausal stage. The mean duration of diabetes was 9.46 ± 1 years. Twenty-six of the diabetic patients were receiving oral hypoglycemic medications, and 16 were receiving insulin. None of the patients were on thiazolidinediones.

Blood was drawn after 12 hours of fasting from all subjects and serum calcium (Ca), albumin, phosphorus (P), creatinine (Cr), and total alkaline phosphatase (ALP) levels were studied. Parathormone (PTH) was studied with Immulite (DPC, Los Angeles USA) solid phase two-site chemiluminescence immunometric assay in fasting blood samples.
In all subjects serum osteocalcin (OC), procollagen type I C-terminal propeptide (PICP) and bone-specific alkaline phosphatase (Bone ALP) levels were measured in fasting blood samples by ELISA (Metra Biosystems, Mountain View, Ca). Osteocalcin and PICP levels were expressed as ng/ml and bone-specific alkaline phosphatase levels as U/L.

Creatinine and calcium levels were studied in 24-hour urine collections. Based on these results creatinine clearance (Ccr) and 24-hour calcium excretion (calciuria) were determined. Urinary free deoxypyridinoline crosslink excretion (free DPD) was measured by ELISA (Pyrilinks-D; Metra Biosystems) in morning urine specimens. Results were expressed as nanomol deoxypyridinium / milimol creatinine (nM DPD /mM cr). Also, urinary calcium and creatinine levels were studied in second void morning urine specimens and urinary calcium/creatinine ratios were calculated. HbA1c levels of the diabetics were measured by BM-Hitachi 911 autoanalyzer using Boehringer Mannheim kits and the results were expressed as %. Body mass indices (BMI) of all subjects were calculated.

Bone mineral densities of all subjects were measured with Hologic- Q DR 4500 W Fan beam X-ray densitometer using the Dual photon X-ray absorptiometry (DXA) method. Values regarding lumbar and femoral regions were determined as g/cm^2.

Diabetic patients were evaluated for the chronic complications of diabetes. Retinal examinations were performed using direct and indirect ophthalmoscopy and fluorescein angiography were performed if necessary. Patients were classified according to these findings as patients with normal retinal examinations and patients with retinopathy. In order to determine diabetic neuropathy, patients were questioned for the symptoms regarding peripheral diabetic neuropathy. The superficial and deep sensation was examined and electroneuromyography (ENMG) were performed to all diabetics. According to the findings patients with and without peripheral neuropathy were determined. Twenty-four hour proteinuria levels were determined with BM-Hitachi 911 biochemical autoanalyzer using Boehringer Mannheim kits. Patients were divided into 2 groups according to their creatinine clearance as Ccr < 70 mL/min and Ccr ≥ 70 mL/min.

Informed written consent was obtained from all of the subjects recruited to the study. The study was approved by the local ethical committee of Kutahya Dumlupinar University (Reference number: 2017-6/2). The work was carried out in accordance with Declaration of Helsinki.

Statistical Analysis

Statistical significance was checked by Student's "t" test for paired and when appropriate, Pearson correlation analysis and variance analysis were used. Data were presented as mean ± SEM. $P < 0.05$ were considered to be statistically significant.

RESULTS

Characteristics of the study population are given in Table 1. Serum levels of OC and bone ALP were lower and total alkaline phosphatase levels were higher in the diabetics than in the healthy controls. Serum PICP and urinary-freeDPD levels did not differ significantly between these two groups. Serum mean Ca levels were higher in diabetics compared to healthy controls, but were in the normal range. All the patients and the controls had albumin levels in the normal range. Mean BMI of the patients with diabetes was higher than the control group. Mean calculated creatinin clearence values of the diabetics were lower than the healthy subjects. There was no significant difference in the other parameters between these groups.

Bone mineral densities of the diabetics were found to be slightly lower than of the healthy controls at the intertrochanteric and total hip regions (Fig. 1). Among diabetics, there was a positive correlation between serum osteocalcin levels and proteinuria levels (r: 0.331, $p < 0.05$) and a negative correlation between serum osteocalcin levels and Ccr values (r: -0.382, $p < 0.05$). There was a strong positive correlation between urinary DPD levels and serum PTH levels in diabetic subjects (r: 0.625, $p < 0.001$). Further, a slight negative correlation was observed between DPD and calciuria levels (r: -0.408, $p < 0.05$). There was no significant correlation between turnover markers and other parameters.

Correlations found to be significant between bone turnover markers and bone mineral densities are shown in Table 2. Serum OC correlated with L1, L2, L3, and L1-4 vertebrae bone mineral densities negatively in diabetic subjects. In contrast, a negative correlation was found between urinary-free DPD levels and bone mineral densities of the femoral site.

Diabetics treated with oral hypoglycemic drugs were compared with diabetics treated with insulin. Serum OC of the patients treated with insulin were

Table 1. Characteristics of the study population

	Diabetics (n = 42)	Controls (n = 23)	*p* value
Gender (Female)	64.2% (27/42)	%56.5 (13/23)	> 0.05
Age (years)	56.2 ± 1.65	53.9 ± 1.81	> 0.05
Duration of diabetes (years)	9.46 ± 1 (n = 41)		
HbA1c (%)	8.84 ± 0.5 (n = 41)		
Serum Ca (mg/dL)	9.72 ± 0.08	9.25 ± 0.19	**< 0.01**
Serum P (mg/dL)	3.75 ± 0.09	3.66 ± 0.15	>0.05
ALP (U/L)	208.21 ± 17.1	140.73 ± 10.2	**< 0.01**
Bone ALP (U/L)	12.73 ± 1.25	22.52 ± 2.52	**< 0.001**
Osteocalcin (ng/mL)	7.407 ± 0.42	14.336 ± 1.13	**< 0.001**
PICP (ng/mL)	81.732 ± 4.11	94.550±7.9	> 0.05
DPD (nM/mMcr)	11.60 ± 1.27	10.79 ± 1.18	> 0.05
Proteinuria (mg/d)	384.7 ± 151.4 (n = 39)	134.34 ± 20.9	> 0.05
Ccr (ml/min)	85.8 ± 5.5 (n = 39)	106 ± 5.4	**< 0.05**
BMI (kg/m^2)	30.24 ± 0.91(n = 28)	28.44 ± 0.90	**< 0.05**
PTH (pg/ml)	60.2 ± 10.3 (n = 28)	37.89 ± 4.88	> 0.05
Calciuria (mg/d)	180.1 ± 24.6(n = 33)	212.26 ± 33	> 0.05

Data are given as mean ± standard deviation or n (%). HbA1c = glycated hemoglobin, Ca =calcium, ALP = alkaline phosphatase, PICP = procollagen type I C-peptide, DPD = deoxypyridinoline, CCr = creatinine clearance, BMI = body mass index, PTH = parathormone

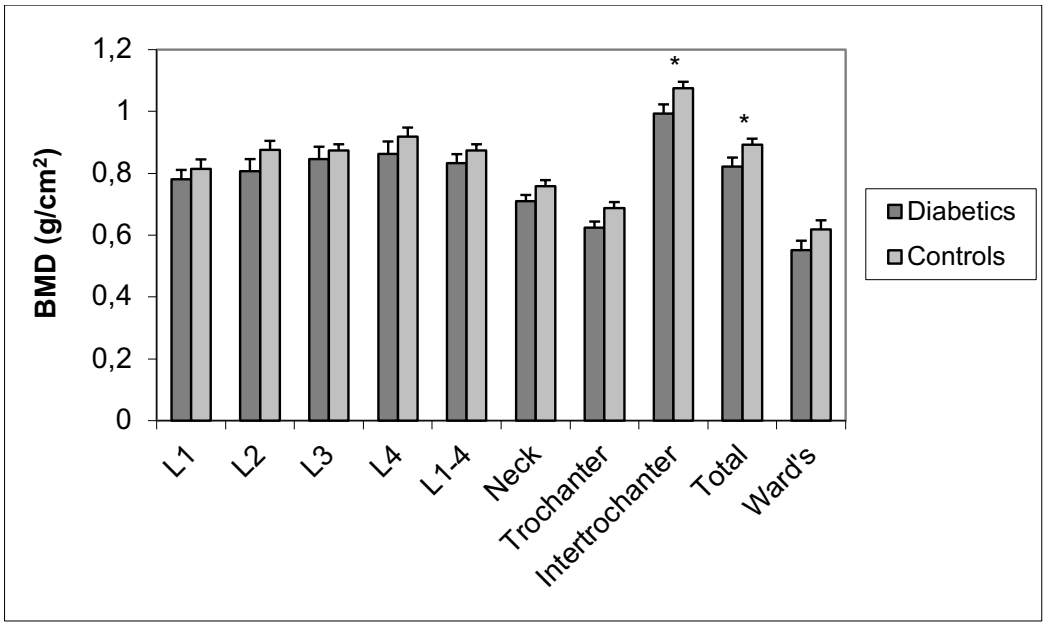

Fig. 1. Bone mineral densities (BMDs) of the diabetics and of the control group. * $p < 0.05$.

found to be higher than those treated with oral hypoglycemic drugs (9.10 ± 0.80 vs. 6.31 ± 0.36, $p < 0.01$). Mean BMI of the group treated with insulin was significantly higher than the group treated with oral drugs (34.21 ± 1.51 vs. 27.98 ± 0.85, $p < 0.001$). There was no other significant difference between patients treated with insulin and oral hypoglycemic drugs. Bone mineral densities did not differ between these groups.

Serum OC levels of the diabetics with retinopathy were higher than those without retinopathy (8.36 ± 0.69 vs. 6.58 ± 0.5, $p < 0.05$). But, the mean Ccr of the diabetics with retinopathy was also significantly lower than the diabetics with normal retinal examinations (66.83 ± 5.72 vs. 102.14 ± 7.41, $p < 0.01$). There was no other significant difference between patients with and without retinopathy. Likewise, bone mineral densities of these two groups, although slightly lower in patients with retinopathy, did not show any significant difference.

Biochemical markers of bone turnover and bone mineral densities did not differ between patients with and without peripheral neuropathy.

Bone mineral densities of the diabetics that have a Ccr < 70 mL/min were significantly lower than those that have a Ccr ≥ 70 mL/min (Table 3).

Table 2. Correlations found to be significant between bone mineral densities and biochemical markers of bone turnover in diabetic patients

	L_{1-4} (g/cm²) (n = 29)	L_1 (g/cm²) (n = 29)	L_2 (g/cm²) (n = 29)	L_3 (g/cm²) (n = 29)	L_4 (g/cm²)	Neck (g/cm²) (n = 29)	Trochanter (g/cm²) (n = 29)	Inter-trochanter (g/cm²) (n = 29)	Total hip (g/cm²) (n = 29)
OC (ng/ml)	r:-0.44	r:-0.37	r:-0.42	r:-0.40					
p-value	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**					
Free DPD (nM/mMcr)						r:-0.49	r:-0.44	r:-0.42	r:-0.42
p-value						**< 0.01**	**< 0.05**	**< 0.05**	**< 0.05**

L = Lumbar spine, OC = Osteocalcin, DPD = deoxypyridinoline

Table 3. Bone mineral densities of the diabetic patients classified according to Ccr levels

	Ccr < 70 (n = 8)	Ccr ≥ 70 (n = 21)	*p* value
L_{1-4} (g/cm^2)	0.699 ± 0.07	0.883 ± 0.02	**< 0.01**
L_1 (g/cm^2)	0.697 ± 0.06	0.809 ± 0.03	> 0.05
L_2 (g/cm^2)	0.644 ± 0.10	0.867 ± 0.03	**< 0.01**
L_3 (g/cm^2)	0.705 ± 0.08	0.900 ± 0.03	**< 0.05**
L_4 (g/cm^2)	0.781 ± 0.04	0.894 ± 0.05	>0.05
Neck (g/cm^2)	0.642 ± 0.04	0.735 ± 0.0	**< 0.05**
Trochanter (g/cm^2)	0.536 ± 0.06	0.657 ± 0.01	**< 0.05**
Intertrochanter (g/cm^2)	0.887 ± 0.06	1.033±0.02	**< 0.05**
Total (g/cm^2)	0.719 ± 0.05	0.860±0.02	**< 0.05**

Data are given as mean ± standard deviation. L = Lumbar spine, CCr = creatinine clearance

DISCUSSION

In this study, bone formation markers serum osteocalcin and bone specific alkaline phosphatase levels were found to be lower in type 2 diabetic patients than the healthy controls. Serum PICP levels, another bone formation marker, were not significantly different in diabetic patients compared to nondiabetic subjects. PICP is an early osteoblastic marker and the finding of normal PICP levels while the other formation markers are depressed may suggest that bone formation rate is slow in type 2 diabetes and the disorder of osteoblastic functions is primarily a maturation defect [16].

In contrast, ALP levels of the diabetics were higher than of the healthy controls. As the levels of ALP are highly affected by the extraosseous contribution, higher than normal levels found in type 2 diabetics is probably not an indicator of bone formation rate. Total alkaline phosphatase levels were reported to be elevated in diabetics. The evaluation of plasma ALP isoenzymes revealed that liver isoenzyme was elevated profoundly in diabetics [15]. Furthermore, body mass indices of the diabetics evaluated in our study were higher than nondiabetic controls which suggests that obesity and liver steatosis might help total alkaline phosphatase levels to rise.

There was no significant difference between urinary-free DPD levels of the diabetics and of the healthy controls which might indicate that bone resorption was not markedly increased in type 2 diabetes [16, 17].

According to the study data, serum Ca levels tend to be slightly higher in type 2 diabetics compared to healthy controls. There are various reports showing disturbances in Ca homeostasis in diabetics [18, 19]. In long-term animal models of diabetes, Ca hyperabsorption and low bone turnover was demonstrated [20]. Pedrazzoni *et al*. [10] found increased levels of serum Ca levels in diabetics and concluded subsequent PTH suppression to be responsible from the reduction of bone turnover.

Bone mineral densities of the diabetic patients were slightly lower than the healthy controls only on intertrochanteric and total lumbar regions. However, if it is taken into account that the diabetics were more obese and the controls had better renal functions, lower bone mineral densities seen in diabetics can not be attributed solely to diabetes itself.

Serum osteocalcin levels increased with declining renal functions in our study. It seems likely that the increase in osteocalcin levels accompanying the glomerular and tubular damage does not show the increase in bone formation rate but its retention due to decrease in its clearence. Increased osteocalcin levels were reported in children with renal failure [20].

Serum osteocalcin levels of type 2 diabetics treated with insulin were higher than those treated with oral hypoglycemic drugs. This finding may suggest

the higher insulin levels to slow bone turnover rate. But at the same time, insulin treated diabetic patients were more obese than the patients receiving oral hypoglycemic drugs. As this condition can reflect the effect of insulin on bone, it can also be related with the contribution of obesity. No significant difference was observed regarding bone mineral densities between these two treatment groups. In accordance with our study, Gregorio *et al*. [5] found osteocalcin levels to be higher in insulin treated than oral hypoglycemic treated type 2 diabetics, but could not show any significant effect of the type of treatment on bone mineral density.

Serum osteocalcin levels of the diabetics with retinopathy were found to be higher than those without retinopathy. But, also renal functions of the diabetics with retinopathy were significantly lower than the patients without retinopathy. Kidneys are the main route of the degradation of osteocalcin and significant retention in serum can be observed in renal failure [21, 22]. The increase observed in osteocalcin levels of the patients with retinopathy may indicate the decline in its clearence due to accompanying nephropathy. Bone mineral densities were not affected by the presence of retinopathy.

No correlation could be shown between the presence or the degree of diabetic peripheral neuropathy with either bone mineral density or biochemical markers of bone turnover.

A significant correlation was found between urinary deoxypyridinium levels and serum PTH levels among diabetics. This finding may show PTH levels to be the primary factor determining the rate of bone resorption in type 2 diabetics. It can be concluded that there is an increase in bone resorption only in diabetics with elevated PTH levels [23].

There was a negative correlation between femoral region bone mineral densities and DPD excretion rate. It seems likely that the increase in bone resorption rate effects mainly femoral region bones that consist primarily cortical bone. Also, a negative correlation was found between serum osteocalcin levels and lumbar spine bone mineral density.

Bone mineral densities were lower in diabetics with impaired glomerular filtration rates. This condition was evident on both lumbar spine and hip bones. Although, mean Ccr levels of the diabetics with retinopathy were also lower than the patients without retinopathy, the difference in their bone densities was not significant. This may be because only some of the patients with retinopathy have also nephropathy. Among the diabetic microvascular complications, the one that effects the bone mass most negatively seems to be the diabetic nephropathy. It appears that there is no osteopenia attributable to type 2 diabetes per se. But the conditions accompanying diabetes such as nephropathy may lead to bone loss. In another study no association was found between parathormone levels and glucose homeostasis in type 2 diabetes [24]. In our study we observed that the increase in osteocalcin levels with the deterioration of renal functions were associated with bone mineral loss in lumbar spine consisting mostly trabecular bone. But, in diabetics with renal function impairment bone loss was detected on both lumbar vertebrae and on hip bones. It was determined that DPD levels increased with increasing PTH and bone loss reflected by DPD excretion rate affected the hip site bones negatively. This finding may suggest that, renal functional impairment seen in diabetics may be operative in bone loss in two ways; by secondary hyperparathyroidism that influences mostly the cortical bone mass and by another way, might be uremia, that is effective mostly on the trabecular bone mass. Renal function impairment is the most important factor affecting the bone mass negatively in type 2 diabetics.

Limitations

However, there are some limitations of our study. First of all, our study population is small and including larger number of patients would have increased the power of our results. Although we tried to compose an age and gender matched control group, BMI of the diabetics were higher than the control group. Since obesity can influence bone metabolism and bone mineral density it would be better to form a BMI matched control group. Although a small study our study shows the importance of renal impairment on bone loss among the other diabetic complications and gives a clue about the types of bone loss in diabetics.

CONCLUSION

Our findings suggest that bone turnover is suppressed especially on the side of formation in diabetic patients

and bone loss seen in diabetics seems to be related mostly with the renal functional impairment. Besides our results suggests that the formation defect seen in diabetics preferentially shows itself on trabecular compartment of the bone and the increased resorption probably mediated by the excess parathormon affects the femoral site which is composed largely of cortical bone.

Authors' Contribution

Study Conception: AA, FBE, İÖA; Study Design: AA, KO, GY; Supervision: GY; Funding: N/A; Materials: AA, FBE; Data Collection and/or Processing: GY, KO; Statistical Analysis and/or Data Interpretation: AA, İÖA; Literature Review: AA, KO; Manuscript Preparation: AA, GY and Critical Review: AA, FBE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Hu Y, Li X, Yan X, Huang G, Dai R, Zhou Z. Bone mineral density spectrum in individuals with type 1 diabetes, latent autoimmune diabetes in adults, and type 2 diabetes. Diabetes Metab Res Rev 2021;37:e3390.
2. Li J, Li C. Relationship between abnormal glucose metabolism and osteoporosis in Han Chinese men over the age of 50 years. Clin Interv Aging 2019;14:445-51.
3. Zhao C, Liu G, Zhang Y, Xu G, Yi X, Liang J, et al. Association between serum levels of bone turnover markers and bone mineral density in men and women with type 2 diabetes mellitus. J Clin Lab Anal 2020;34:e23112.
4. Auwerx J, Dequeker J, Bouillon R, Geusens P, Nijs J. Mineral metabolism and bone mass at peripheral and axial skeleton in diabetes mellitus. Diabetes 1988;37:8-12.
5. Gregorio F, Cristallini S, Santeosanio F, Filipponi P, Fumelli P. Osteopenia associated with non-insulin-dependent diabetes mellitus: what are the causes? Diabetes Res Clin Pract 1994;40:75-9.
6. Zhang YX, Min SX. [Analysis of clinical factors correlating with osteoporosis in patients with type 2 diabetes mellitus]. Nan Fang Yi Ke Da Xue Xue Bao 2016;36:1546-9. [Article in Chinese]
7. Yao X, Xu X, Jin F, Zhu Z. The correlation of type 2 diabetes status with bone mineral density in middle-aged adults. Diabetes Metab Syndr Obes 2020;13:3269-76.
8. Miyake H, Kanazawa I, Sugimoto T. Association of Bone Mineral Density, Bone Turnover Markers, and Vertebral Fractures with All-Cause Mortality in Type 2 Diabetes Mellitus. Calcif Tissue Int 2018;102:1-13.
9. Yamamoto M, Yamaguchi T, Nawata K, Yamauchi M, Sugimoto T. Decreased PTH levels accompanied by low bone formation are associated with vertebral fractures in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab 2012;97:1277-84.
10. Pedrazzoni M, Ciotti G, Pioli G, Girasole G, Davoli L, Palummeri E, Passeri M. Osteocalcin levels in diabetic subjects. Calcif Tissue Int 1989;45:331-6.
11. Paschou SA, Dede AD, Anagnostis PG, Vryonidou A, Morganstein D, Goulis DG. Type 2 diabetes and osteoporosis: a guide to optimal management. J Clin Endocrinol Metab 2017;102:3621-34.
12. Dede AD, Tournis S, Dontas I, Trovas G. Type 2 diabetes mellitus and fracture risk. Metabolism 2014;63:1480-90.
13. Xu H, Wang Z, Li X, Fan M, Bao C, Yang R, et al. Osteoporosis and osteopenia among patients with type 2 diabetes aged ≥50: role of sex and clinical characteristics. J Clin Densitom 2020;23:29-36.
14. Compston J. Type 2 diabetes mellitus and bone. J Intern Med 2018;283:140-53.
15. Tibi L, Collier A, Patrick AW, Clarke BF, Smith AF. Plasma alkaline phosphatase isoenzymes in diabetes mellitus. Clinica Chimica Acta 1988;177:147-56.
16. Cloos C, Wahl P, Hasslacher C, Traber L, Kistner M, Jurkuhn K, et al. Urinary glycosylated, free and total pridinoline and free and total deoxypyridinoline in diabetes mellitus. Clin Endocrinol (Oxf) 1998;48:317-23.
17. Suh S, Bae JC, Jin SM, Jee JH, Park MK, Kim DK, et al. Serum calcium changes and risk of type 2 diabetes mellitus in Asian population. Diabetes Res Clin Pract 2017;133:109-14.
18. Shahwan MJ, Khattab MH, Jairoun AA. Association of serum calcium level with waist circumference and other biochemical health-care predictors among patients with type 2 diabetes. J Pharm Bioallied Sci 2019;11:292-8.
19. Hough S, Russel JE, Teitelbaum SL, Avioli LV. Calcium homeostasis in chronic streptozotosin-induced diabetes mellitus in the rat. Am J Physiol 1983;242:E451-6.
20. Cole DEC, Carpenter TO, Gundberg CM. Serum osteocalcin concentrations in children with metabolic bone disease. J Pediatr 1985;106:770-6.
21. Hauscka P, Lian JB, Cole DEC, Gundberg CM. Osteocalcin and matrix Gla protein: Vitamin K dependent proteins in bone. Physiol Rev 1989;69:990-1046.
22. Li Y, Chen JP, Duan L, Li S. Effect of vitamin K2 on type 2 diabetes mellitus: a review. Diabetes Res Clin Pract 2018;136:39-51.
23. Bora K, Ruram AA. No association of 25-hydroxyvitamin D

and parathormone levels with glucose homeostasis in type 2 diabetes - a study from Shillong, Meghalaya. Int J Vitam Nutr Res 2019;89:285-92.

24. Dhaliwal R, Cibula D, Ghosh C, Weinstock RS, Moses AM. Bone quality assessment in type 2 diabetes mellitus. Osteoporos Int 2014;25:1969-73.

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Here is the metastasis - find the primary carcinoma for it: a large biopsy study

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ABSTRACT

Objectives: According to estimates, cancer of unknown primary origin (CUP) accounts for approximately 3 to 5% of new cancer cases annually in the United States. With early diagnosis, many CUP patients can achieve long-term remission. The aim of this study was to document the organ tropism for metastasis for tumors with a definite primary cancer. In addition, we sought to contribute to understanding organ tropism in tumors of unknown origin.

Methods: Retrospectively, biopsies of 492 metastatic cancers were evaluated. The required immunohistochemical panel was applied to determine the tumors' origins.

Results: A total of 492 patients with metastases were included in the study. The most common origins of metastasizing tumors were lung (25.4%), colon (18.9%), breast (15.4%) and stomach cancers (10.6%). The least metastatic tumors were the salivary gland, neuroendocrine, adrenal, tubal, nasopharynx, rectum and esophagus cancers (0.22% for all). The most common cancers that metastasized to the liver were colon cancer (29.0%), stomach cancer (18.6%) and pancreatic cancer (18.6%). The most common cancers that metastasized to the bone were lung cancer (58.0%), breast cancer (29.0%), and prostate cancer (13.9%). The most common types of tumors that metastasized to the lungs, the kidney (35.1%), colon (29.7%), and breast cancers (16.2%), were the most common. The most common tumors that metastasized to the brain were the lung (61.1%), the breast (16.7%), and kidney cancers (8.7%). The most common tumors metastasizing to the skin were lung (31.0%), breast (27.6%), and colon (13.8%) cancers.

Conclusions: Primary cancers have different metastatic patterns, which may help determine their causes. By involving distant anatomical structures and disrupting their function, tumor metastases increase the morbidity and mortality associated with the disease; thus, they affect staging, prognosis, and treatment. Metastases (or cancers from unknown primary sites) usually require extensive investigation to determine their primary source. Whether the cancer is tracked prospectively or retrospectively, a detailed observational template highlighting expected metastatic patterns would greatly assist in diagnosis and treatment.

Keywords: Cancer of unknown primary origin; organ tropism; metastasis

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Cancer of unknown primary origin (CUP) is cancer that cannot be traced back to its point of origin. It is one of the most common cancers, accounting for 3-5% of all cancer diagnoses [1, 2]. It is estimated that carcinomas with unknown primary origin (CUP) account for 10% of all cancers. Most CUP cases are diagnosed in patients over 50, with the median age at diagnosis being 65 years [3]. The most common sites of metastatic carcinoma are the lungs, liver, and brain. The lungs are the most common site of metastatic disease, accounting for approximately 50% of all cases. The liver is the second most common site of metastasis, accounting for about 30% of all cases. The brain is the third most common site of metastasis, accounting for approximately 20% of all cases [3, 4]. While the cause of CUP is unknown, it is believed to be the result of a mutation in a cell's DNA [5].

Several factors determine the distribution of metastatic carcinoma. The most crucial factor is the type of primary cancer. For example, breast cancer typically metastasizes to the bones, while lung cancer often spreads to the brain or liver [5]. Other factors include the stage of the tumor (early-stage cancers are more likely to spread), the tumor's size, and the primary tumor's location. Metastatic carcinoma can also influence the patient's age, gender, and overall health [3].

The prognosis for CUP patients varies depending on the cancer diagnosis stage. The outcome of patients with CUP relies on diagnosing the primary cause. A patient with CUP who can attribute the cause to a specific site has a better prognosis than a patient whose origin cannot be determined. Metastatic carcinoma is difficult to treat, and the chances of survival are often low. The best hope for a cure is early detection and treatment of the primary tumor. However, even with early treatment, the chances of survival are only about 50% [5]. A five-year survival rate of 25% is achieved by CUP patients who can identify the primary tumor site [6]. In contrast, only 5% is completed by patients unable to determine the primary tumor site. The use of immunohistochemistry is one of the most reliable, cost-effective, and widely available approaches to evaluating these lesions. An epidemiology and morphology-driven approach to tumor diagnosis and site of origin assignment cannot be completed without immunohistochemistry [5]. With early diagnosis and treatment, many patients with CUP can achieve long-term remission [6]. It is crucial to know where metastases occur in unknown primary tumors so that additional clinical and pathological studies can be guided.

Our study aimed to describe the occurrence and location of metastases in many carcinomas with known primary carcinomas, seeking to drive cases with only metastases detected at the time of diagnosis and cases with unknown primary carcinomas for possible primary cancers.

METHODS

In this study, biopsies of 492 metastatic cancer patients between 2015 and 2022 were retrospectively analyzed. Samples of the cases included in the survey include biopsies taken from metastases that developed during the follow-up of the patients, metastasectomy materials, and metastasectomy materials performed in addition to the operation during the primary operation. The age, gender, and clinical information of the patients were obtained from the hospital information system. Routine follow-up was applied to all biopsies, embedded in paraffin, and stained with hematoxylin-eosin (HE). When cases are deemed necessary for each patient to determine tumors origins (pan-cytokeratin, vimentin, cytokeratin 7, cytokeratin 20, CDX2, CD45, TTF-1, estrogen receptor, progesterone receptor, chromogranin, synaptophysin, CD56, MELAN-A, S-100, HMB-45, thyroglobulin, HEPPAR, p40) were selected and an immunohistochemical panel was applied.

The method used in the immunohistochemical studies: Four micron-thick sections from tissues in appropriate paraffin blocks for each antibody were taken on poly-L-lysine coated slides. The antigen retrieval technique was used in IHC studies; the avidin-biotin-peroxidase complex method was applied. Antibodies were stained on a Leica band max automated immunohistochemical staining device. A Bond Polymer Refine Detection kit (Leica, DS9800) was used for each antibody. The necessary staining procedure was performed according to the datasheet of each antibody, and appropriate positive and negative controls were used for each antibody. After covering them with a coverslip ultra-mount, the prepared samples were examined under an Olympus BX51 model microscope. The origin of metastatic carcinoma, which was determined by evaluating histomorphological, immunohis-

Table 1. Metastasizing organs for primary cancers

Metastasis location	n	%
Liver	210	42.7
Bone	116	23.6
Lung	37	7.5
Brain	36	7.3
Skin	29	5.9
Bone marrow	14	2.8
Ovary	13	2.6
Surrenal	9	1.8
Colon	6	1.2
Pleura	5	1.0
Other	17	3.5
Total	**492**	100.0

tochemical, and clinical findings in pathology reports, was recorded.

The Clinical Research Ethics Committee approved the study (number: 2022/369. dated 01.07.2022).

Statistical Analysis

The PASW Statistics 18.0 package program was used for statistical analysis. In descriptive statistics, categorical data are shown as percentage frequency, and continuous variables as mean, standard deviation, minimum, and maximum.

RESULTS

A total of 492 patients with metastases were included in the study. The mean age of the patients was 59.97 ± 11.73 years of age (min: 27-max: 97). In total, 57.1% (n = 282) of the patients were males. Tumors were detected by excisional methods in 52.3% (n = 272) and incisional methods in 44.7% (n = 220) of the patients. Metastases were detected primarily in the liver (47.2%), bone (23.6%), and lung (7.5%). The amounts detected in other organs (kidneys (0.4%), endometrium (0.4%), and stomach (0.4%) were minimal. Among the bone metastases, 56.9% (n = 66) were in the vertebrae, 22.4% (n = 26) in the femur, 6.9% (n = 8) in the humerus, and 3.4% (n = 4) in the ribs. The organs of the patients with metastases are listed in frequency in Table 1.

The most common origins of metastasizing tumors were lung (25.4%), colon (18.9%), breast (15.4%), and stomach cancers (10.6%). Among other tumors that metastasized the least was the salivary gland (0.22%), neuroendocrine (0.22%), adrenal (0.22%), tuba (0.22%), nasopharynx (0.22%), rectum (0.22%) and esophagus cancers (0.22%). Men's most common metastasizing tumors were lung, colon, stomach, and pancreas, while in women, breast, colon, stomach, and lung were observed. Table 2 shows the frequency distribution of primary tumors that metastasize by gender.

Table 3 summarizes the origins of tumors that metastasize to the liver and bone based on frequency.

Table 2. Origin of primary metastasizing tumors and the distribution by gender

Origin	Total		Male		Female	
	n	%	n	%	n	%
Lung	125	25.4	107	85.6	18	14.4
Colon	93	18.9	54	58.1	39	41.9
Breast	76	15.4	3	3.9	73	96.1
Stomach	52	10.6	32	61.5	20	38.5
Pancreas	43	8.7	27	62.8	16	37.2
Kidney	32	6.5	19	59.4	13	40.6
Prostate	22	4.5	21	95.5	1	4.5
Gall bladder	11	2.2	2	18.2	9	81.8
Bladder	10	2.0	6	60.0	4	40.0
Ovary	7	1.4	0	0.0	7	100.0
Liver	5	1.0	4	80.0	1	20.0
Thyroid	4	0.8	1	25.0	3	75.0
Endometrium	3	0.6	0	0.0	3	100.0
Other	9	1.8	5	55.6	4	44.4

Table 3. Cancers most commonly metastasizing to the liver and the bone

Metastasis to the liver			Metastasis to the bone		
Metastasis origin	**n**	**%**	**Metastasis origin**	**n**	**%**
Colon	61	29.5	Lung	50	58.0
Stomach	39	18.6	Breast	25	29.0
Pancreas	39	18.6	Prostate	12	13.9
Lung	31	14.8	Kidney	9	10.4
Breast	17	8.1	Stomach	4	4.6
Gall bladder	7	3.3	Colon	3	3,5
Bladder	4	1.9	Pancreas	3	3,5
Kidney	3	1.4	Thyroid	3	3,5
Ovary	3	1.4	Ovary	2	2.3
Prostate	2	1.0	Gall bladder	2	2.3
Neuroendocrine	1	0.5	Endometrium	1	1,2
Surrenal	1	0.5	Bladder	1	1,2
Tubal	1	0.5	Nasopharynx	1	1,2
Total	**210**	**100.0**	**Total**	**116**	**100.0**

The most common cancers that metastasized to the liver were colon cancer (29.0%), stomach cancer (18.6%), and pancreatic cancer (18.6%). The most common cancers that metastasized to the bone were lung cancer (58.0%), breast cancer (29.0%), and prostate cancer (13.9%).

A list of the most common tumor origins that metastasize to the lung and the brain is presented in Table 4. Among the most common types of tumors that metastasized to the lungs, the kidney (35.1%), colon (29.7%), and breast cancers (16.2%) were the most common. The most common tumors that metastasized to the brain were the lung (61.1%), the breast (16.7%), and kidney cancers (8.7%).

The most common tumor origins that metastasize to the skin and bone marrow are given in Table 5. The most common tumors metastasizing to the skin were lung (31.0%), breast (27.6%), and colon (13.8%) cancers. The tumors that most frequently metastasized to the bone marrow were breast (28.6%), lung (21.4%), and stomach cancers (21.4%), respectively.

DISCUSSION

Several questions arise when discussing metastasis, many of which remain unanswered. What causes metastasis? Does the primary tumor need a certain size

Table 4. Cancers most commonly metastasizing to the lung and the brain

Metastasis to the lung			Metastasis to the brain		
Metastasis origin	**n**	**%**	**Metastasis origin**	**n**	**%**
Kidney	13	35.1	Lung	22	61.1
Colon	11	29.7	Breast	6	16.7
Breast	6	16.2	Kidney	3	8.3
Bladder	2	5.4	Liver	2	5.6
Endometrıum	1	2.7	Bladder	2	5.6
Liver	1	2.7	Prostate	1	2.8
Stomach	1	2.7			
Prostate	1	2.7			
Testis	1	2.7			
Total	**37**	**100**	**Total**	**36**	**100**

Table 5. Cancers most commonly metastasizing to the skin and the bone marrow

Metastasis to the skin			Metastasis to the bone marrow		
Metastasis origin	**n**	**%**	**Metastasis origin**	**n**	**%**
Lung	9	31.0	Breast	5	35.8
Breast	8	27.6	Stomach	3	21.4
Colon	4	13.8	Lung	3	21.4
Kidney	3	10.3	Prostate	2	14.3
Stomach	2	6.9	Pancreas	1	7.1
Endometrıum	1	3,4			
Bladder	1	3.4			
Salivary gland	1	3.4			
Total	**29**	**100.0**	**Total**	**14**	**100.0**

to be able to leave and migrate? Do tumor cells move randomly from the tumor or are these selective clones genetically different from the dominant clone? Do carcinoma cells move individually or collectively? These issues have been extensively discussed in the literature. There have been many studies that have examined the metastatic patterns of different types of cancers. No recent studies, however, provide quantitative analyses of such patterns arising from a broad range of cancers based primarily on primary surgical and biopsy-diagnostical tissue analyses. To guide further clinical and pathological studies, it is essential to know where metastatic sites occur in unknown primary tumors. To guide patients with only metastases found at the time of diagnosis and those with unknown primary carcinomas on possible primary cancers, our study described the occurrence and locations of metastases in many carcinomas with known primary carcinomas. In this study, we attempted to contribute to understanding organ tropism in tumors of unknown origin.

Our review of data from biopsies performed between 2015 and 2022 on 492 metastatic cancer patients, comprising 20 different primary cancers and 13 different metastatic sites, demonstrated that: Based on the data collected during the 29 years during which a postmortem study conducted by Disibio and French [5], findings provided a comprehensive analysis of metastatic behaviors in both common and uncommon cancers. In addition to providing a sensitive, quantitative baseline of metastatic patterns among the analyzed malignancies, these findings were derived from actual postmortem tissue analyses from the largest cohort of autopsies reported to date in the medical literature [5].

In our study, the most common primary cancers were, in descending order, lung (25.4%), colon (18.9%), breast (15.4%), stomach (10.6%), and pancreas (8.7%). Several large clinical and autopsy studies have reported a different ranking. Presumably, essential characteristics of primary cancers and population-specific differences will need to be studied. According to a large autopsy study conducted in the USA on metastatic patterns of primary cancers, the most common primary cancers were reported as rectum (11.4%), breast (11.3%), cervix (10.9%), stomach (9.1%), and prostate (5%) [5]. The timeline as well as the population is a determining factor for the distribution of primary cancers. According to the results of the first large series autopsy study of 1000 cases presented in 1950 from New York Montefiore Hospital, the most common primary cancers were reported as breast (16.7%), lung (16.0), stomach (11.9%), colon (11.6%) and rectum (8.9%).

As a primary neoplasm, lung cancer was the primary lead cancer, accounting for 25.4% of all the cases and 85.65% for males. Cancers of the lung are another common metastatic site for breast, melanoma, and thyroid cancers. The rate of lung tropism in our study was 7.5%. The physiology of the lung makes it ideal for colonization and metastasis. The broad surface area and numerous capillaries allow cancer cells to adhere, extravasate and colonize.

Our study found colon cancer to be the second

most frequently metastasizing cancer type, with a rate of 18.9%. In cancers with known primary sites, the rate of metastasis to the colon was 1.2%. In their large autopsy study, Disibio and French [6] reported colon metastasis rates of approximately 4% and metastasis to the large intestine as < 1%. The difference may be because that study separated rectal cancer: the rate for rectal cancers was 11%.

As a primary neoplasm, breast cancer was the third common type and accounted for 15.4% of our study, and as a metastatic target, it accounted for only 0.7% of all metastases. According to the literature, the ratio differs from the second to third most common malignancy [5, 6]. Worldwide, breast cancer is the fifth most common cause of death due to cancer (502 000 deaths per year), followed by lung (1.3 million deaths per year), stomach (1 million deaths per year), liver (662 000 deaths per year), and colon (655 000 deaths per year) [7, 8]. In this study, breast cancer metastasized robustly: Both accounted for a high proportion (15.4%) of all primary malignancies and generated many metastases. As a result, breast cancer contributed 15.4% to all metastases, the highest number among all analyzed malignancies. Consistent with the difficulty of treating primary breast cancer and identifying breast primaries among cancers that initially manifest as metastases from unknown primary sites, this tendency toward widespread metastasis occurs.

The breast was a rare metastatic target in this data set, receiving only 0.7% of all metastases. Yet 87.1% of all metastases to the breast were classified as having originated from the breast itself in the archival data set. Because it is difficult at present to determine whether breast lesions are metastases from contralateral primary malignancies or secondary (ipsilateral) primary malignancies, this finding is of interest. Retrospectively, it appears likely that the former assumption was made when the data were collected, and caution should be exercised in evaluating them.

The liver is a favorite metastatic site for solid tumors such as breast, lung, and gastrointestinal cancers [9, 10]. Similarly, in our study, 42.7% of all metastases were to the liver.

Different types of cancer disseminate to bone at different rates. Cancers of the breast and prostate are the most common cancers that metastasize to the bones. The other rates of primary metastasis of cancers are as follows prostate 90.1%, breast 71%, melanoma 48.6%, renal 44.5%, lung 34%, pancreas 25%, thyroid 13 %, colon 8%, liver 8% and ovarian 3% [10-12]. The bone ranked second among metastatic organotropism, with a rate of 23.6% of all metastasis targeting the bone. Like other studies, our study determined that the cancers that metastasized to the liver most frequently were cancers of colorectal origin. However, in our study, this rate was found to be 29% and relatively lower; in other studies, it is 46.1% and 48%, respectively [5, 13]. Our research suggests that ethnic, geographical, and genetic factors strongly influence the rate of metastasis. Cancer research should consider the specific characteristics of each race and region based on this experiment. Our study's second most common cancers metastasizing to the liver were the stomach and pancreas, while the pancreas and breast metastasized in another study [5].

There has been much interest in bone cancer in terms of organ tropism and metastatic niche. After the liver and lung, bone is the most common metastatic site [14], but our study found it to be the second most common site after the liver. The first three cancers metastasizing to the bone most frequently were lung, breast, and prostate [15]. Similarly, the first three cancers that metastasized to the bone most frequently in our study were lung, breast, and prostate. Nevertheless, our study's percentage of patients with metastatic lung cancer was significantly higher: 58% developed bone metastases. According to the previous study [15], bladder-derived metastases ranked fourth after kidney-derived metastases. Our study, however, found gastrointestinal metastases more frequent than bladder metastases. Endometrium, bladder, and nasopharynx metastasized to bone the least in our study.

Most cancers metastasize to the lungs as the third organ in the chain. Colorectal tumors commonly metastasise to the parenchyma and the endobronchial nich [15, 16]. Breast cancer usually metastasizes to the bone, liver, and lung, and the rate of metastasis to these organs is around 25% [16]. Like nowhere else in the body, the brain consists of two major microenvironments, a variety of parenchymal cells and leptomeninges filled with cerebrospinal fluid. The two microenvironments differed both cellular and metabolically. A cancer cell must cross and adapt to both environments to metastasize to the brain [16]. Adaptation may even lead to the acquisition of brain-like

characteristics [17]. As tumor cells cross the blood-brain barrier, they also carry inflammatory cells. There is also the possibility that metastatic tumor cells can adapt to the brain as an immune microenvironment [18]. Lung, breast, malignant melanoma, and kidney tumors are the most common cancers that metastasize to the brain [19, 20]. According to our study, lung, and breast cancers were the first to metastasize to the brain. A small percentage of hepatocellular carcinomas metastasize to the brain; two of our cases were hepatocellular carcinomas (5.6%). Detection of prostate cancer metastatic to the brain from autopsies and case series in the literature is extremely rare [21]; its incidence ranges from 0.4 to 2% [22]. In our study, one out of 36 cases (2.8%) of prostate adenocarcinoma metastasized to the brain. Metastasis of solid organ cancers to the skin is rare. Skin metastasis may be the first sign of advanced cancer or an indicator of cancer recurrence. Metastasis to the skin is rare; its rate varies between 0.2-9% among all metastases [17, 18]. In our study, the rate of skin metastasis was 5.9%, which was consistent with the literature. Among the organs that metastasize to the skin, the breast was the most common primary origin in some studies, and the lung in some studies [22]. In our study, breast carcinoma was the two most common organs that metastasized to the skin after lung carcinoma. In our study, metastases originating from the colon and kidney, breast, and lung were at a later frequency. In the later order of frequency and among cancers that metastasize to the skin, the cancers with the least origin are quite different [18]. Endometrial cancer and salivary gland-derived cancers are present in some studies but not at all in some studies [19]. Our study detected salivary gland metastasis, which is a rare report.

One of the organs in which the concepts of the metastatic niche are best defined is the bone marrow [20]. Breast carcinomas are the most common tumors metastasizing to the bone marrow [20]. Sometimes, surprisingly, even sarcomas can metastasize to the skin: in some studies, gastric carcinomas are the most frequently metastasized cancers [21, 22]. In our study, the breast was found to be the most common among primary cancers that metastasized to the bone marrow. According to the previous study of our team on bone marrow metastases, their chances of receiving effective chemotherapy decrease when a patient is diagnosed with bone marrow cancer [16]. Cancer patients do not show direct evidence of bone marrow involvement. Despite the common involvement of bone marrow in solid tumors bone marrow tumors are uncommonly diagnosed. Solid malignancy staging does not routinely use bone marrow biopsy. A clinical warning sign is unexplained hematological findings and thrombocytopenia. In the diagnosis of solid tumor-related bone marrow involvement, as well as in the detection of hematological malignancies, bone marrow biopsy is an effective, inexpensive, and applicable method.

Despite the uniqueness of each distant organ, certain general principles underlying organotropism emerge. The prerequisite for understanding the molecular level of these mechanisms knows the most common metastasis tropisms of primary tumours.

Our study is definitive proof of which organ is the primary focus in metastatic cancers. Because the cases in our study were those whose primary cancer was known, followed up and biopsied from the metastasis. In addition, the primary organ was supported with an immunohistochemical panel. Cases with diffuse metastases who did not undergo biopsy were not included in our study.

CONCLUSION

As expected, different primary cancers tended to metastasize to different sites with a different frequency. By looking at these varying metastatic patterns, it may be possible to deduce the origins of cancers whose primary sites are unclear at the presentation. Furthermore, this baseline provides a foundation for further studies that will clarify the clinical and molecular characteristics of specific malignancies cited in this study, especially those that occur less frequently.

Authors' Contribution

Study Conception: ÇÖ, MB, HD; Study Design: ÇÖ, MB; Supervision: YS, MB; Funding: N/A; Materials: MOA, ÇÖ; Data Collection and/or Processing: MOA, ÇÖ; Statistical Analysis and/or Data Interpretation: YŞ; Literature Review: ÇÖ; Manuscript Preparation: ÇÖ, MB and Critical Review: HD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Seyfried TN, Huysentruyt LC. On the origin of cancer metastasis. Crit Rev Oncog 2013;18:43-73.
2. Pavlidis N, Pentheroudakis G. Cancer of unknown primary site. Lancet. 2012;379:1428-35.
3. Gupta GP, Massagué J. Cancer metastasis: building a framework. Cell 2006;127:679-95.
4. Fidler IJ. Critical determinants of metastasis. Semin Cancer Biol 2002;12:89-96.
5. Disibio G, French SW. Metastatic patterns of cancers: results from a large autopsy study. Arch Pathol Lab Med 2008;132:931-9.
6. Riihimäki M, Thomsen H, Sundquist K, Sundquist J, Hemminki K. Clinical landscape of cancer metastases. Cancer Med 2018;7:5534-42.
7. Koşaloğlu-Yalçın Z, Blazeska N, Vita R, Carter H, Nielsen M, Schoenberger S, et al. The Cancer Epitope Database and Analysis Resource (CEDAR). Nucleic Acids Res 2023;51(D1):D845-D52.
8. Sokolova A, Johnstone KJ, McCart Reed AE, Simpson PT, Lakhani SR. Hereditary breast cancer: syndromes, tumour pathology and molecular testing. Histopathology 2023;82:70-82.
9. Holmes FF, Fouts TL. Metastatic cancer of unknown primary site. Cancer 1970;26:816-20.
10. Gao Y, Bado I, Wang H, Zhang W, Rosen JM, Zhang XH-F. Metastasis organotropism: redefining the congenial soil. Dev Cell 2019;49:375-91.
11. Mortezaee K. Organ tropism in solid tumor metastasis: an updated review. Future Oncol 2021;17:1943-61.
12. Obenauf AC, Massagué J. Surviving at a distance: organ-specific metastasis. Trends Cancer 2015;1:76-91.
13. de Ridder J, de Wilt JH, Simmer F, Overbeek L, Lemmens V, Nagtegaal I. Incidence and origin of histologically confirmed liver metastases: an explorative case-study of 23,154 patients. Oncotarget 2016;7:55368.
14. Coleman R. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat Rev 2001;27:165-76.
15. Ulucaköy C, Yapar A, Şeyhoğlu MC, Tokgöz MA, Atalay İB, Güngör BŞ. [Demographic Features of Metastatic Bone Tumors]. Acta Oncol Tur 2021;54:98-102. [Article in Turkish]
16. Yavaşoğlu F, Özdemir Ç. [Patients with carcinoma metastasis diagnosed by bone marrow biopsy]. Osmangazi Tıp Dergisi 2022;44:211-6. [Article in Turkish]
17. Teyateeti P, Ungtrakul T. Retrospective review of cutaneous metastasis among 11,418 patients with solid malignancy: a tertiary cancer center experience. Medicine 2021;100:e26737.
18. Wong CY, Helm MA, Helm TN, Zeitouni N. Patterns of skin metastases: a review of 25 years' experience at a single cancer center. Int J Dermatol 2014;53:56-60.
19. Dumlao JKG, Cubillan ELA, Villena JPDS. Clinical and histopathologic profile of patients with cutaneous metastasis in a tertiary hospital in the Philippines. Dermatopathology (Basel) 2022;9:392-407.
20. Sanmartin MC, Borzone FR, Giorello MB, Pacienza N, Yannarelli G, Chasseing NA. Bone marrow/bone pre-metastatic niche for breast cancer cells colonization: the role of mesenchymal stromal cells. Crit Rev Oncol Hematol 2021;164:103416.
21. Germans SK, Weinberg OK. Metastatic angiosarcoma in a bone marrow biopsy. Blood2022;140:2001.
22. Ozkalemkas F, Ali R, Ozkocaman V, Ozcelik T, Ozan U, Ozturk H, et al. The bone marrow aspirate and biopsy in the diagnosis of unsuspected nonhematologic malignancy: a clinical study of 19 cases. BMC Cancer 2005;5:144.

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Comparing artificial intelligence based diagnosis with expert results in SARS-COV-2 RT-qPCR

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ABSTRACT

Objectives: Reverse transcription and real-time polymerase chain reaction (RT-qPCR) based on the SARS-CoV-2 viral RNA demonstration is the gold standard in diagnosis. Data files obtained from PCR devices should be analysed by a specialist physician and results should be transferred to Laboratory Information Management System (LIMS). CAtenA Smart PCR (Ventura, Ankara, Türkiye) program is a local bioinformatics software that assess PCR data files with artificial intelligence, submits to expert approval and transfers the approved results to LIMS. The aim of this study is to investigate its accuracy and matching success rate with expert analysis.

Methods: A total of 9400 RT-qPCR test results studied in Ankara Provincial Health Directorate Public Health Molecular Diagnosis Laboratory were compared with respect to expert evaluation and CAtenA results.

Results: It was determined that the preliminary evaluation results of the CAtenA matched 86% of the negative and 90% of the positive results provided by expert analysis. 987 tests which CAtenA determined as inconclusive and suggested repeating PCR were found either negative or positive by expert analysis. A significant difference between positive and negative matching success rates and artificial intelligence (AI) based software overall accuracy was found and associated with the missed tests of the AI.

Conclusions: As a result, it was suggested there is a low risk of confirming false positive results without expert analysis and test repetitions would cause losing time along with extra test costs. It was agreed that the PCR analysis used in CAtenA should be improved particularly in terms of test repetitions.

Keywords: SARS-CoV-2, RT-qPCR, expert analysis, CAtenA, artificial intelligence

Coronavirus disease-2019 (COVID-19), emerged in Wuhan, China in late 2019 and became a pandemic, is an infectious disease caused by SARS-CoV2 (Severe Acute Respiratory Syndrome Coronavirus-2) that affects the respiratory tract [1]. World Health Organization (WHO) declared COVID-19 pandemic on March 11, 2020 [2]. The effectiveness of variant viruses is considered as one of the reasons for increasing SARS-CoV-2 infection which is spreading steadily as is the case worldwide. Five variants in the SARS-CoV-2 have been identified as Variant of Concern (VOC) category so far including the WHO's last weekly status report published on 06 April 2022 [3]. These variants are termed as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) [3]. WHO reported that the standard diagnosis of SARS-Cov-2 will be made by nucleic acid amplification method [4]. The most widely used

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among these methods is the SARS-CoV-2 real time polymerase chain reaction test [5]. In our country, SARSars-CoV-2 VOCs are studied by microbiology laboratories determined by the TR Ministry of Health [6] with one-step reverse transcription and real-time polymerase chain reaction (RT-qPCR) tests based on the detection of mutated regions in viral RNA vNAT (viral nucleic acid extraction buffer) transfer tubes, also provided by the Turkish Ministry of Health, are used for the transport and storage of nasopharyngeal swab samples for this purpose [7]. PCR data files should be analysed by the expert in the laboratory and the results should be transferred to the web-based Laboratory Information Management System after SARS-CoV-2 RT-qPCR test. Testing services are carried out 24/7 and an intense workflow continues in the COVID-19 PCR diagnostic laboratories which are opened with the pandemic. The rapid completion of these tests and their error-free transfer to the system are very important steps for the diagnosis, treatment, and follow-up of COVID-19 [8]. Programs with artificial intelligence such as machine learning and deep learning contribute to the development of the health system by enabling the analysis of complex and large data sets processed in clinical laboratories [9, 10]. Artificial intelligence (AI) has started to take its place in image analysis and various data sources and applications in Microbiology laboratories recently [11, 12]. Matrix-assisted laser desorption-ionization/time of flight mass spectrometry and processing of all gene analysis data with artificial intelligence has opened a new era in microbiology field [13].

CAtenA Smart PCR (Ventura, Ankara, Turkey) is a web-based bioinformatics software that evaluates PCR data files with artificial intelligence, submits them to expert approval and can transfer the approved results to the Laboratory Information Management System [14]. CatenA aims to assist the specialist physician in the rapid conclusion of the tests. The aim of this study is to investigate compatibility of the preliminary evaluation results carried out by the CatenA Smart PCR program with specialist physician analysis result.

METHODS

A hundred PCR studies, each containing 94 patient samples and two internal quality control samples, were performed at Ankara Provincial Health Directorate Public Health Molecular Diagnosis Laboratory between April 15, 2021, and November 15, 2021 and uploaded to the online CatenA (Ventura, Ankara, Turkey) artificial intelligence system. A medical virologist analyzed PCR data through the program. The preliminary evaluation results of the program and the expert results were recorded in electronic environment. Negative and positive controls which are internal quality control were excluded from the assessment. Thus, 9400 PCR test results were included in the study.

Ethical approval was obtained from Yıldırım Beyazit University Yenimahalle Training and Research Hospital Scientific Research Ethics Committee (Decision No: E-2022-22) to conduct this study. Samples were obtained after written informed consent had been obtained, and all procedures were performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical Analysis

Analysis of PCR Tests

The statistical evaluation and accuracy calculation were performed by utilizing classification matrix also known as confusion matrix. The assessments and their justification are detailed in the following section under results.

RESULTS

It was determined that the preliminary evaluation results of the CatenA Smart PCR program matched 94% of the negative and 92.5% of the positive results provided by expert analysis. 91.2% of the 987 test results for which CatenA suggested repeating PCR were found to be negative by expert analysis while 8.8% of them were positive. It has been determined that the samples for which CatenA recommends retesting have 0% match with the expert analysis.

The matching rates of CAtenA with expert analysis results with respect to negative and positive classification were almost 86% and 90% respectively (Table 1). Precise false positives (73 patients for whom unnecessary quarantine and treatment will be applied) and false negatives (166 patients who requires quar-

Table 1. Comparison of PCR test results with CAtenA smart PCR program and expert analysis.

Method	Negative	Positive	Retest	Total
Expert	6907	2493	0	9400
CAtenA	5934	2240	987 *	
Coherence	0.859	0.898	0	

*Number of patients which could not classified as either positive or negative by CAtenA AI is 987. This yields a %10 fail rate. Expert classified all these patients. AI requires more re-sampling and re-testing. Therefore, it is suggested that software needs calibration and further adaptation to reduce and eliminate inconclusive status if possible.

antine and treatment are to be discharged and cause further spreading the infection), obtained by direct comparison with expert analysis result lists, were considered to achieve a more refined analysis. These statistics do not contribute to the total number of patients in classification matrix (Table 2) which is provided as n=8413 since CatenA missed 987 patients and declared inconclusive which in return to be interpreted as a retest. They were inherently included in the calculation. The overall accuracy of CatenA is almost 97 % based on the classification matrix.

There is a substantial difference between positive and negative matching success rates and overall accuracy. Test repetition, also termed as fail rate in this study, is believed to be the main reason for this difference. AI based software seems to have a high accuracy when fail rate after initial run have been omitted but retesting requires re-sampling of patients in doubt and re-testing by AI and/or expert which will cause further use of PCR test kit, lab material and lab expert workhours. The outcome of the retest will affect the accuracy of as there is a possibility of more false positives and negatives. Even though accuracy of Catena AI based software have been declared as 97.96% according to the results of the preliminary study carried out by the manufacturer [14] and similarly calculated in this study, this accuracy rate is believed to be synthetic and do not reflect real life usage without considering repeated test results.

Table 2. Expert analysis and CAtenA smart PCR results classification summary matrix

Number of Patients (n = 8413)		Expert Results	
		Positive	Negative
CAtenA Results	Positive	2313	73
	Negative	166	6100

DISCUSSION

When the test results were grouped as negative, positive and retest, 86% matching was observed between the negative results, 90% between the positive results, and 0% matching was found between the analyszes since all the PCR tests that the artificial intelligence recommended to repeat were concluded as negative or positive by the expert. CAtenA Smart PCR software has been put into use by evaluating more than 4 million PCR test results studied in various COVID-19 diagnostic laboratories located in Turkey, Italy and Uzbekistan for trial purposes [14]. It was reported that the results of the preliminary evaluation of CAtenA were matching expert analysis at a rate of 97.96% according to the results of the preliminary study carried out by the manufacturer [14].

Even though the overall accuracy (compliance rate) we obtained was found to be similar the compliance rate reported by the company based on the comparison results in this study, it is required to inspect the fail rate and include the results of test repetitions. Overall accuracy is believed to decrease after an additional run for missed patients since the expert classified them as positive and negative. A proper comparison of AI based software and expert results stipulates covering the full set of patients.

The increased workload due to the pandemic may cause disruptions in the rapid evaluation and transfer of test results to the system in COVID-19 PCR laboratories. CAtenA Smart PCR software which is developed for this reason can help the user with fast and accurate results. It is a great advantage that the tests

performed by a specialist physician can be analysed with artificial intelligence which can offer recommendations according to the preferences of the specialist physician. On the other hand, it seems that CAtenA needs improvement on the retest recommendation considering the findings of comparison we made approximately one year after the launch of the program. In addition, the software may suggest false positive results according to our findings, albeit at a low rate. False positive results cause negative effects such as unnecessary treatment, quarantine and contact tracing, thus unnecessary economic costs and loss of workforce. Therefore, it was emphasized that artificial intelligence should also be enhanced in terms of positive result suggestions. The worst-case scenario when classifying PCR tests is false negative results. False-negative results can cause important problems such as the patient receiving inappropriate treatment and the continuation of the contagiousness of asymptomatic positive individuals in the community.

The absence of false negative results among the test recommendations provided by CAtenA Smart PCR program in our study increases the reliability of the software. It has been shown that the COVID-19 PCR test results must be checked by a specialist physician before they can be approved by utilizing the bioinformatics program when all of our findings are evaluated. Another advantage of the CAtenA Smart PCR software is the ability of transferring test results LIMS without error and therefore post-analytical errors in this regard can be avoided. Manually uploading test results to LIMS can be performed with patient-based or batch approval options. Health centers with a high-test load prefer to use the collective approval option. It is necessary to apply extreme caution and to control the accuracy of the data at every stage to avoid any mistakes in manual data entry. CAtenA Smart PCR program allows patient results to be transferred directly to LIMS without the need for any manual entry with the barcoding method included in the worklists.

Although PCR data analysis is performed with certain rules, the results are closely related to the test kit, consumables, device experience and working quality of the microbiologist [15]. Many experts using the CAtenA smart PCR program may evaluate the same study differently for this reason. For example, a sample thought to be low positive may have been run again. Comparing more analysis of PCR data by more experts will yield more precise results.

CONCLUSION

It was determined that CAtenA smart PCR, an artificial intelligence-based bioinformatics data analysis program software, was 86% successful in predicting negative results. All the samples for which CAtenA recommends a PCR retest were evaluated as negative or positive by the expert. It is thought that there is a risk of confirming false results in test approvals without expert analysis, and test repetitions will cause additional costs and loss of time considering the findings we have obtained. It has been suggested that the PCR analysis used in CAtenA, particularly in terms of false positive results and test repetitions, should be improved. In lights of these observations, it is suggested that an AI based software can be considered an asset and facilitation tool in a pandemic environment which requires rapid evaluation and firm decision making to guide quarantine processes, treatment protocols and patient follow-up. Human expert supervision would not be optional and highly recommend if/when AI based software is deployed.

Authors' Contribution

Study Conception: BGG, GGA; Study Design: BGG, GGA; Supervision: BGG, GGA; Funding: N/A; Materials: BGG; Data Collection and/or Processing: GGA; Statistical Analysis and/or Data Interpretation: BGG; Literature Review: GGA; Manuscript Preparation: BGG and Critical Review: BGG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Eng J Med 2020;382:727-33.
2. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19, 11 March 2020. (online). Available from: https://www.who.int/ director-general/speeches/detail/who-directorgeneral-s-opening-remarks-at-the-media-briefing-oncovid-19---11-march-2020) (cited 14 April 2022).
3. World Health Organization, Tracking SARS-CoV-2 variants (online). Available from: https://www.who.int/en/activities/tracking-sars-cov-2-variants/ (cited 14 April 2022).
4. Worl Health Organization. Laboratory testing of 2019 novel coronavirus (2019-nCoV) in suspected human cases: interim guidance, 17 January 2020. (online). Available from: https://www.who.int/publications/i/item/laboratory-testingof-2019-novel-coronavirus-(-2019-ncov)-in-suspectedhuman-cases-interim-guidance-17-january-2020). (cited 14 April 2022).
5. Sule, WF, Oluwayelu, DO. Real-time RT-PCR for COVID-19 diagnosis: challenges and prospects. Pan Afr Med J 2020;35(Suppl 2):121.
6. The Turkish Ministry of Health, COVID-19 information page (online). Available from: https://covid19.saglik.gov.tr/TR 68720/covid-19-yetkilendirilmis-tani-laboratuvarlari-listesi.html. (cited 14 April 2022).
7. Petruzzi G, De Virgilio A, Pichi B, Mazzola F, Zocchi J, Mercante G, et al. COVID-19: nasal and oropharyngeal swab. Head Neck 2020;42:1303-4.
8. Sreepadmanabh M, Sahu AK, Chande A. COVID-19: advances in diagnostic tools, treatment strategies, and vaccine development. J Biosci 2020;45:148.
9. Tasdelen A, Sen B. A hybrid CNN-LSTM model for pre-miRNA classification. Sci Rep 2021;11:14125.
10. Peiffer-Smadja, N, Dellière S, Rodriguez C, Birgand G, Lescure F. X, Fourati S, et al. Machine learning in the clinical microbiology laboratory: has the time come for routine practice? Clin Microbiol Infect 2020;26:1300-9.
11. Smith KP, Wang H, Durant TJS, Mathison BA, Sharpeh SE, Kirby JE, et al. Application of artificial intelligence in clinical microbiology diagnostic testing. Clin Microbiol Newsletter 2020;42:61-70.
12. Asada K, Kaneko S, Takasawa K, Machino H, Takahashi S, Shinkai N, et al. Integrated analysis of whole genome and epigenome data using machine learning technology: toward the establishment of precision oncology. Front Oncol 2021;11:666937.
13. van Oosten LN, Klein CD. Machine learning in mass spectrometry: a MALDI-TOF MS approach to phenotypic antibacterial screening. J Med Chem 2020;63:8849-56.
14. Ventura. CAtenA Smart PCR. (online). Available from: https://ventura.com.tr/catena (cited 14 April 2022).
15. Kuang J, Yan X, Genders AJ, Granata C, Bishop DJ. An overview of technical considerations when using quantitative real-time PCR analysis of gene expression in human exercise research. PloS One 2018;13:e0196438.

The European Research Journal 2023;9(2):322-327 Original Article

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Pre-conception folic acid intake and attention deficit and hyperactivity disorder in children

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ABSTRACT

Objectives: Attention Deficit Hyperactivity Disorder (ADHD) is a common disorder in children. It is thought to occur due to the interaction of many genetic and environmental factors during early development. Many studies have been conducted to investigate the etiology of ADHD. Some have investigated neurobiology, and others have investigated malnutrition and trace element deficiency. To investigate the effect of folic acid intake during the pre-conception period in terms of ADHD development.

Methods: Five hundred and ninety-eight participants, 246 children with ADHD, and 352 healthy controls were enrolled. A questionnaire was completed, including socio-demographic information and the use of folic acid and other supplements such as a multivitamin, iron, and omega 3 during pregnancy. Data were examined to determine associations with ADHD. Mann-Whitney U tests for continuous variables, Pearson's chi-square, and Fisher's exact tests for categorical variables were used to compare groups.

Results: The use of folic acid during pre-pregnancy among mothers who had a child with ADHD was 13% and this was 31% in the control group ($p < 0.001$). There was no statistically significant difference between the groups' use of folic acid in pregnancy ($p = 0.617$). Other situations related to ADHD were advanced maternal age ($p < 0.001$ for both groups), abnormal double screening test results in pregnancy (27% vs. 5%, $p < 0.001$) and omega 3 use in pregnancy ($p < 0.001$).

Conclusions: The use of folic acid before and during pregnancy is important for neurological development. However, there is little data on use before pregnancy in the literature. This study shows that folic acid taken before pregnancy may prevent ADHD in childhood. The present study recommends folic acid usage in planned pregnancy to prevent ADHD in the child.

Keywords: Pre-pregnancy, folic acid, attention deficit hyperactivity disorder, children

The use of folic acid before and during pregnancy is known to be important for neurological development. Folic acid supplementation during the pre-conception period and first trimester of pregnancy reduces the incidence of neural tube defects (NTDs). Studies show that the supplementation of 0.4 mg folic acid during the periconceptional period prevents 50-70% of NTDs [1].

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Just as it prevents the development of NTDs, lack of folate has been found to be associated with the development of many neurodevelopmental disorders [2]. For example, aggression [3], impaired cognitive functions [4] and reduced memory function [5] have found to be associated with folate/homocysteine levels.

Attention deficit hyperactivity disorder (ADHD) is a common neurodevelopmental disorder in children, with a prevalence estimated from 5% to 7% across cultures. There may be accompanying comorbid diseases and problems affecting life. Individuals with this disorder have lower self-esteem, social functioning, academic and economic achievement [6]. Medical problems include smoking, obesity, nonadherence, and comorbid medical illnesses [7]. Though the etiology is not fully identified, it is thought to be multifactorial [6]. The majority view is that environmental and genetic factors like toxins, pregnancy/birth problems, brain damage, and immune system problems, play a role together. Exposure of the baby to trauma during pregnancy and birth (e.g., asphyxia, infection, toxic-metabolic-mechanic trauma) may cause minimal brain damage or functional disorders in the baby's brain [8]. This functional disorder might cause ADHD accompanied by neurological soft signs [9]. Lower folate status in early pregnancy was associated with impaired fetal brain development and affects hyperactivity/inattention and peer problems in childhood [10]. To our knowledge, there is no study or contribution relating to the association of prepregnancy folate usage and ADHD development in the literature. The present study aimed to investigate the effect of folic acid intake during the pre-conception period in terms of possible relation with ADHD development.

METHODS

A case-control study was carried out. The study included 251 children with ADHD and their parents, and 450 healthy children and their parents. Exclusion criteria were as follows: (1) Premature babies; (2) Those with known neurological disease like mental motor retardation and epilepsy in mother and child and known accompanying chronic disease (cardiovascular, endocrinological, psychiatric); (3) Those who used substances such as alcohol; (4) Having a child with any neurologic disease such as neural tube defect; and (5) Mother and children who did not wish to be included in the study.

As a result, the sample included 246 children who were diagnosed as ADHD by the Child and Adolescent Psychiatry out-patient clinic and 352 healthy children who were chosen from the pediatric policlinic as control group. In AD/HD group, diagnosis was based on criteria from the Diagnostic and Statistical Manual of Mental Disorders (Fourth edition, Washington, DC: American Psychiatric Association, 1994) by Child and Adolescent Psychiatry doctors.

Parents and children provided written informed consent for participation and the necessary legal approvals and University Ethical Committee approval were obtained. Demographic characteristics (age, educational level, socioeconomic situation), obstetric history (preeclampsia, vaginal bleeding during pregnancy, gestational diabetes mellitus), and the use of folic acid during the pre and periconceptional period were questioned. Folic acid supplement use was categorized in three groups: (i) starting preconception, (ii) starting within the first 10 weeks of pregnancy or (iii) no use. Other supplemental treatments such as multivitamin and omega 3 intake during pregnancy, were also questioned. Socioeconomic status was self-reported and defined into three terms as good, moderate, or poor.

Ethical Approval

Ethical approval for the study was obtained from the ethics committee of Şevket Yılmaz Training and Research Hospital on 04.03.2015 with the decision number 2015/05-03.

Statistical Analysis

The data was examined by the Shapiro Wilk test whether or not it presents normal distribution. The results were presented as median (minimum-maximum) or frequency and percentage. Mann Whitney U tests were used for nonnormally distributed data. Categorical variables were compared using Pearson's chi-square test and Fisher's exact test between groups. $p < 0.05$ was considered as significance levels. Statistical analyses were performed with IBM SPSS ver.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

RESULTS

Five hundred and ninety-eight participants, 246 children with ADHD and 352 healthy controls, were enrolled in the study. Characteristics of the groups are given in Table 1.

The use of preconception folic acid appeared to be statistically significantly low in the ADHD group compared to the control group. This situation is shown in Fig 1. Folic acid use during pregnancy was not related to ADHD (Fig 2). Similarly, using multivitamins and iron during pregnancy was not related to ADHD (p = 0.223 and p = 0.228; respectively). In pregnancy, the omega 3 use was 11% in the ADHD group, while this rate was 31% in the control group ($p < 0.001$).

T he groups were statistically similar in terms of socioeconomic status ($p = 0.036$). In both groups, though the number of mothers smoking preconception and during pregnancy was low, there was no difference between the groups ($p = 0.806$). There was a statistically significant difference between the groups in terms of maternal age. In the ADHD group the mean maternal age was 35, while in the control group the mean maternal age was 32 ($p < 0.001$). Similarly, in the ADHD group the age of fathers appeared to be older (mean age of 38 vs. 36 years, $p < 0.001$).

There was no difference between the groups in terms of situations like gestational diabetes mellitus experienced during pregnancy by the mother (n=15 vs. n = 49), preeclampsia (n = 13 vs. n = 32), and progesterone requirements due to vaginal bleeding (n = 44 vs. n = 49). The number of abnormal double-screening tests in the first trimester was higher in the ADHD group, and this was significant statistically (27% vs. 5%, $p < 0.001$). This was linked to advanced maternal age.

DISCUSSION

This study showed that the use of folic acid before pregnancy might be effective at preventing the development of ADHD. Maternal nutrition affects fetal brain development [11]. Some micronutrients have been shown to be linked with the maladjusted behavior of the offspring later in life [12]. Folic acid affects neural stem cell proliferation and differentiation, decreases apoptosis, and alters DNA biosynthesis [13].

Table 1. Characteristics of the groups

		ADHD group (n = 246)	Non-ADHD group (n = 352)	*p* value
Age (year)		10 (6.25-15.75)	9,13 (6-15)	**< 0.001***
Gender	Female	60 (24.3%)	121 (34.3%)	0.09**
	Male	186 (75.7%)	231 (65.7%)	
Delivery Type	NVD	118 (47.9%)	179 (50.8%)	0.518**
	C/S	127 (52.1%)	173 (49.2%)	
Birth weight (gram)		3290 (2550-4300)	3250 (2500-4500)	0.635*
Birth order		2 (1-5)	2 (1-7)	0.958*
Maternal age (year)		35 (20-52)	32 (20-49)	< 0.001*
Paternal age (year)		38 (23-60)	36 (22-56)	**< 0.001***
Maternal education	< 8years	109 (44.3%)	159 (45.1%)	0.835**
	> 8years	137 (45.7%)	193 (54.9%)	
Paternal education	< 8years	186 (75.6%)	124 (35.2%)	0.946**
	> 8years	160 (24.4%)	228 (64.8%)	

Data are given median (minimum-maximum) or frequency (n) with percentage (%). NVD = Normal Vaginal Delivery, C/S = Cesarian section

*Mann-Whitney U test, **Pearson chi-Square test

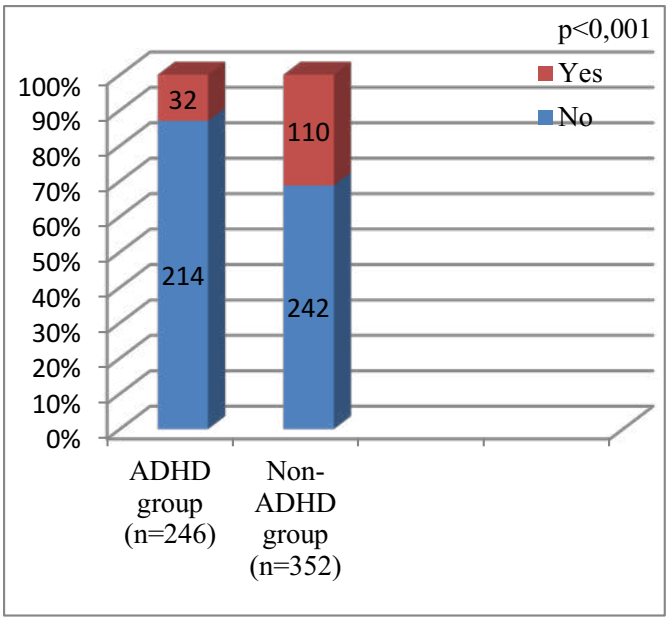

Fig. 1. Cumulative bar graph of folic acid intake during pre-pregnancy period (ADHD = Attention Deficit Hyperactivity Disorder) (Statistically significant difference was found between ADHD and Non-ADHD groups using Pearson chi-square test).

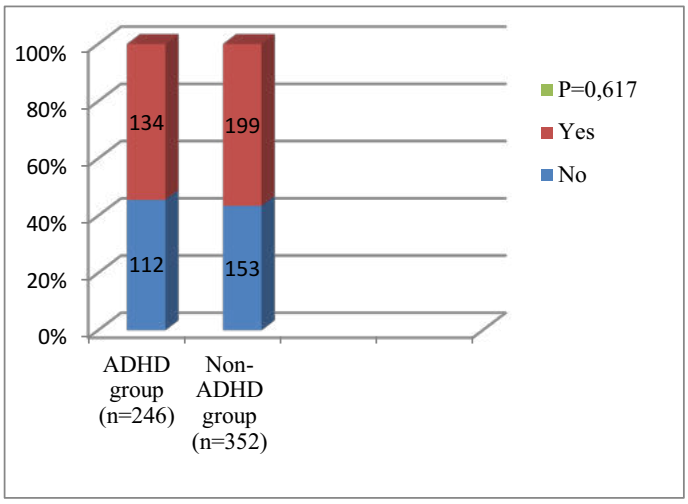

Fig. 2. Cumulative bar graph of folic acid intake during pregnancy (ADHD = Attention Deficit Hyperactivity Disorder) (Statistically significant difference was not found between ADHD and Non-ADHD groups using Pearson chi-square test).

Folate deficiency during gestation can impair cellular growth and replication in the fetus [14] and has been demonstrated to cause a net reduction of cells in the fetal brain [15], a loss of progenitor cells in the brain, as well as reduced brain weight [16] in mice. Such brain structure alterations may be long-lasting and contribute to behavioral difficulties later in life [17]. For example, abnormal folate transport into the fetal central nervous system has been found to be related to cerebral folate deficiency associated developmental delays, cognitive impairment and reduced memory function [4], while higher plasma folate showed a correlation with better cognitive performance [18]. A strong association was reported between neural tube defect (NTD) and folate-related gene polymorphisms [19]. The risk of NTD recurrence in subsequent pregnancy is 2-3% [20]. In these patients, though of multifactorial origin, in light of our study, we believe the use of folate before pregnancy should be recommended before planned pregnancy and for women with risks of having an ADHD child. A study questioned maternal use of folate before pregnancy and assessed the correlation with autism in women with blood folate levels examined in the first trimester. In conclusion, similar to our study, they identified a correlation not with pregnancy folate levels but with pre-conception folate use. However, it should be taken into account that the patient and control groups were not the same age in our study [21]. That may indicate that maternal folate's potential effect on offspring's brain development might occur earlier.

Fatty acids, in particular omega-3 fatty acids, have a substantial impact on human brain development and function and have been found to affect behavior and cognition [22]. It is unclear whether a deficit in intake or metabolism of long chain polyunsaturated fatty acids (LC-PUFA) may play a major role in the pathogenesis of ADHD. Increasing evidence indicates that LC-PUFA imbalance or deficiencies may be associated with ADHD through involvement in the dopaminergic corico-striatal metabolism. In studies, the omega 3 levels in children with ADHD were shown to be lower [23]. Moreover dietary supplementation with ω-3 PUFAs was found to improve ADHD symptoms [24]. In our study, we questioned the omega 3 situation and use it in children with ADHD; however we found the omega 3 use during pregnancy by mothers with ADHD children was clearly low. On the other hand, the mothers' fish consumption was not questioned, which may be considered one of the limitations of the present study. Due to the antioxidant properties of omega 3 affects the neuroinflammatory process, and as it is thought to play a role in ADHD development, dietary supplementation is recommended during pregnancy [25].

Both young and old mothers are considered to belong to an obstetric risk group, according to clinical experience and research [26]. Lower maternal age has been addressed as a risk factor for ADHD in children [27]. Contrary to this, in our study we identified a correlation between advanced maternal age and ADHD. Additionally, the age of fathers in the ADHD group was older. Studies have reported associations between paternal age and ADHD, with conflicting results [28].

Limitations

There are some limitations of our study. The study represents one region in Turkey. Another limitation is that the study is not prospective. If the study was prospective and folic acid levels in the blood of both mother and child were examined, and a correlation was found, this would be a more valuable study. Other limitations are the lack of comparison in terms of gender and ADHD subtypes and the age difference between the patient and control groups. However, this study notes the preconception use of folic acid and is useful in attracting attention to this topic for families and health care professionals.

CONCLUSION

Folic acid is important for the growing fetus and deficiency may lead to irreversible or treatment-requiring situations. This study showed that folic acid use before pregnancy might prevent childhood ADHD. In conclusion, according to our study, using preconceptional folic acid supplement seems to reduce the risk of ADHD and neural tube NTDs.

Authors' Contribution

Study Conception: MEU, NK; Study Design: NK, HA, MEU; Supervision: N/A; Funding: N/A; Materials: N/A; Data Collection and/or Processing: MEU, ESG; Statistical Analysis and/or Data Interpretation: HA, FB, HŞ; Literature Review: HŞ, FB; Manuscript Preparation: MEU, NK, HŞ and Critical Review: HA, FB, ESG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Friel JK, Frecker M, Fraser FC. Nutritional patterns of mothers of children with neural tube defects in Newfoundland. Am J Med Genet 1995;55:195-9.
2. Lakhan SE, Vieira KF. Nutritional therapies for mental disorders. Nutr J 2008;7:2.
3. Chan A, Tchantchou F, Graves V, Rozen R, Shea TB. Dietary and genetic compromise in folate availability reduces acetylcholine, cognitive performance and increases aggression: Critical role of S-adenosyl methionine. J Nutr Health Aging 2008;12:252-61.
4. Moretti P, Peters SU, del Gaudio D, Sahoo T, Hyland K, Bottiglieri T, et al. Brief report: Autistic symptoms, developmental regression, mental retardation, epilepsy, and dyskinesias in CNS folate deficiency. J Autism Dev Disord 2008;38:1170-77.
5. Antai-Otong, D, Zimmerman ML. Treatment approaches to attention deficit hyperactivity disorder. Nurs Clin North Am 2016;51:199-211.
6. Nigg JT. Attention-deficit/hyperactivity disorder and adverse health outcomes. Clin Psychol Rev 2013;33:215-28.
7. Biederman J. Attention-deficit/hyperactivity disorder: a selective overview. Biol Psychiatry 2005;57:1215-20.
8. Biederman J, Faraone SV. Attention-deficit hyperactivity disorder. Lancet. 2005;366:237-48.
9. Schlotz W, Jones A, Phillips DIW, Gale CR, Robinson SM, Godfrey KM. Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. J Child Psychol Psychiatry 2010;51:594-602.
10. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr 2007;85:614S-20S.
11. Gale CR, Robinson SM, Godfrey KM, Law CM, Schlotz W, O'Callaghan FJ. Oily fish intake during pregnancy–association with lower hyperactivity but not with higher full−scale IQ in offspring. J Child Psychol Psychiatry 2008;49:1061-8.
12. Zeisel SH. Importance of methyl donors during reproduction. Am J Clin Nutr 2009;89:673S-77S.
13. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr 2000;71:1295S-303S.
14. Xiao S, Hansen DK, Horsley ET, Tang YS, Khan RA, Stabler SP, et al. Maternal folate deficiency results in selective upregulation of folate receptors and heterogeneous nuclear ribonucleoprotein−E1 associated with multiple subtle aberrations in fetal tissues. Birth Defects Res A Clin Mol Teratol 2005;73:6-28.

15. Middaugh LD, Grover TA, Blackwell LA, Zemp JW. Neurochemical and behavioral effects of diet related perinatal folic acid restriction. Pharmacol Biochem Behav 1976;5:129-34.
16. Castellanos FX, Lee PP, Sharp W, et al. Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. JAMA 2002;288:1740-48.
17. De Lau LM, Refsum H, Smith AD, Johnston C, Breteler MM. Plasma folate concentration and cognitive performance: Rotterdam Scan Study. Am J Clin Nutr 2007;86:728-34.
18. Pangilinan, F, Molloy, AM, Mills JL, Troendle JF, Parle-McDermott A, Signore C, et al. Evaluation of common genetic variants in 82 candidate genes as risk factors for neural tube defects. BMC Med Genet 2012;13:62.
19. Hall JG, Solehdin F. Genetics of neural tube defects. Mental Retard Dev Disabil Res Rev 1998;4:269-81.
20. Steenweg-de Graaff J, Ghassabian A, Jaddoe VW, Tiemeier H, Roza SJ. Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study. Eur J Publ Health 2015;25:431-33.
21. Bos DJ, van Montfort SJ, Oranje B, Durston S, Smeets PA. Effects of omega-3 polyunsaturated fatty acids on human brain morphology and function: what is the evidence? Eur Neuropsychopharmacol 2016;26:546-61.
22. Crippa A, Agostoni C, Mauri M, Molteni M, Nobile M. Polyunsaturated fatty acids are associated with behavior but not with cognition in children with and without ADHD: an Italian study. J Atten Disord 2018;22:971-83.
23. Bloch MH, Qawasmi A. Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 2011;50:991-00.
24. Strickland AD. Prevention of cerebral palsy, autism spectrum disorder, and attention deficit-hyperactivity disorder. Med Hypotheses 2014;82:522-8.
25. Reichman NE, Pagnini DL. Maternal age and birth outcomes: data from New Jersey. Fam Plann Perspect 1997;29:268-95.
26. Gustafsson P, Källén K. Perinatal, maternal, and fetal characteristics of children diagnosed with attention−deficit-hyperactivity disorder: results from a population−based study utilizing the Swedish Medical Birth Register. Dev Med Child Neurol 2011;53:263-68.
27. Chudal R, Joelsson P, Gyllenberg D, Lehti V, Leivonen S, Hinkka-Yli-Salomäki S, et al. Parental age and the risk of attention-deficit/hyperactivity disorder: a nationwide, population-based cohort study. J Am Acad Child Adolescent Psychiatry 2015;54:487-94.e1.

The European Research Journal 2023;9(2):328-337 Original Article

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The role of diffusion-weighted imaging in the diagnosis of early kidney damage

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ABSTRACT

Objectives: This study aimed to determine a threshold value for distinguishing early-stage chronic kidney disease (CKD) from moderate and advanced stages as well as patients with early-stage CKD from those with normal renal function using apparent diffusion coefficient (ADC) and normalized ADC values.

Methods: This retrospective study enrolled 257 patients. Diffusion-weighted images were obtained with a set of b = 50,400,800 values. In each patient, six ADC values were measured from upper, middle, and lower areas of both kidneys, and three ADC values were measured from the spleen. Patients with CKD were classified into five subgroups and healthy patients were classified into two subgroups according to their glomerular filtration rate (GFR).

Results: The renal ADC values were found to be positively correlated with GFR (r = 0.790, $p < 0.001$) and negatively correlated with creatinine levels (r = − 0.709, $p < 0.001$). The mean ADC values of the stage 1 and 2 CKD groups were found to be significantly higher than those of advanced-stage CKD groups ($p < 0.001$), and these values were significantly lower in the stage 1 and 2 CKD groups than in the healthy group ($p < 0.001$). With a cut-off value of ≥ 1.791 for ADC, the sensitivity was 76.5% and the specificity was 85% while distinguishing between patients with early- and advanced-stage CKD.

Conclusion: Renal and normalized ADC values are strongly correlated with CKD stages, and with the use of appropriate threshold values, the difference between early and advanced stages of CKD can be predicted.

Keywords: Diffusion-weighted imaging, apparent diffusion coefficient, chronic kidney disease

Chronic kidney disease (CKD) is a progressive illness that causes decrease in renal function as well as subsequent kidney tissue damage and uremia [1]. The main goal in managing CKD is to slow down the progression of CKD through early diagnosis and appropriate treatment selection based on the underlying pathology [2]. Another parameter that affects the treatment approach is the stage of CKD. Serum creatinine and blood urea nitrogen (BUN) levels as well as estimated glomerular filtration rate (eGFR) are the most commonly used markers during the follow-up of patient with CKD and evaluation of renal function [3]. As serum creatinine level begins to rise when the loss of renal function exceeds 50%, it is not a reliable marker for the early diagnosis of CKD [4]. GFR, which provides information about renal function, is calculated by measuring 24-hour urinary and serum creatinine clearance. In general, serum creatinine clearance-based eGFR levels are calculated according to the Cockcroft-Gault formula and are used for CKD

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staging. However, in cases where eGFR is > 60 mL/min/1.73 m^2, the dietary measures to be adopted during the disease is relatively uncertain. Although serum markers help assess overall renal function, they do not provide information about the morphology of the kidney and whether there is a difference between the two kidneys. Ultrasonography (US) examination is insufficient to detect early changes in renal function and is generally used in diagnosis when morphological and anatomical changes are evident. With the use of imaging techniques, such as intravenous pyelography (IVP), computed tomography (CT), or magnetic resonance imaging (MRI), it is possible to obtain information about functions of the kidneys as well as their morphological structure using contrast material. However, especially in patients with reduced renal functions, there is a risk of nephrotoxicity with IVP and CT urography and systemic nephrogenic fibrosis with MR urography. Therefore, the use of these imaging techniques is limited in such patients [5]. Scintigraphy is widely accepted and provides a quantitative result in the evaluation of kidney functions; however, the magnitude of the radiation exposure and low spatial resolution are its important disadvantages. Therefore, there is a need for a noninvasive and quantitative radiological method to detect kidney damage at an early stage. Currently, the diffusion-weighted imaging (DWI) technique, which basically reveals the Brownian motion of water molecules in biological tissues, as well as microcirculation and diffusion are used appropriately without contrast material. In this technique, diffusion in biological tissues can be expressed quantitatively by measuring the apparent diffusion coefficient (ADC). Promising results have been obtained in some studies on this subject [6-8].

In this study, we aimed to investigate the relationship between conventional ADC values obtained using DAG/DWI MR and normalized ADC values (nADC) obtained by using the spleen as a reference organ, with CKD stage, eGFR and serum creatinine level, and to compare them with previous studies.

METHODS

Patient Selection

Overall, 243 patients with CKD who underwent MRI of the upper abdomen for any reason at our hospital between 2018 and 2022 were included in this study; in addition, 100 patients without a history of CKD were included in the control group. Of 243 patients with CKD, 86 were excluded because of the following reasons: apparent atrophic renal parenchyma or absence of organs to be examined (n = 34); absence of serum biomarkers concomitant with MRI scan (n = 24); dense artifacts that may interfere with MRI measurements or inadequate image quality (n = 22); and presence of a mass lesion or multiple cysts of immeasurable extent in the renal parenchyma (n = 6). Accordingly, 157 patients with CKD and 100 patients without a history of CKD (control group) were included in this study. The study protocol was approved by the research ethics committee of our hospital, and the requirement of informed consent from the patients was waived. Patients in the CKD group were classified into five subgroups based on the severity of the disease according to the Kidney Disease Outcomes Quality Initiative (K/DOQI CKD) guidelines [9]: Stage 1: eGFR ≥ 90 mL/min/1.73 m^2 (kidney damage with normal or elevated eGFR), Stage 2: eGFR = 60-89 mL/min/1.73 m^2 (kidney damage with mild decrease in eGFR), Stage 3: eGFR = 30-59 mL/min/1.73 m^2 (moderate decrease in eGFR), Stage 4: eGFR = 15-29 mL/min/1.73 m^2 (severe decrease in eGFR), and Stage 5: eGFR < 15 mL/min/1.73 m^2 (renal failure). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula as follows: eGFR cr = 142 × min (S cr /κ, 1) α × max (S cr /κ, 1) − 1,200 × 0.9938 Age × 1.012 [if female] [10]. Patients in the control group were classified into two subgroups: those with eGFR ≥ 90 mL/min/1.73 m^2 (n = 59) and those with eGFR = 60-89 mL/min/1.73 m^2 (n = 41).

MRI Technique and Analysis

MRI and DWI examinations of the patients were performed using a sixteen-channel body coil and a 1.5 T MR device (Optima MR360 Advance, GE Medical Systems, Milwaukee, Wisconsin, USA).

First, coronal T2-weighted single-shot fast spin-echo (SSFSE) (TR = 1800 ms, TE = 70 ms, flip angle = 90°, field of view = 40 × 40 cm, matrix = 200 × 192, breath holding), axial T2-weighted single-shot fast spin-echo (SSFSE) (TR = 1700 ms, TE = 110 ms, flip angle = 90°, field of view = 40 × 40 cm, matrix = 320 × 224, breath holding), axial fs-FSE T2-weighted (TR = 2200 ms, TE = 85 ms, thickness = 5.5 mm, pitch = 1 mm,

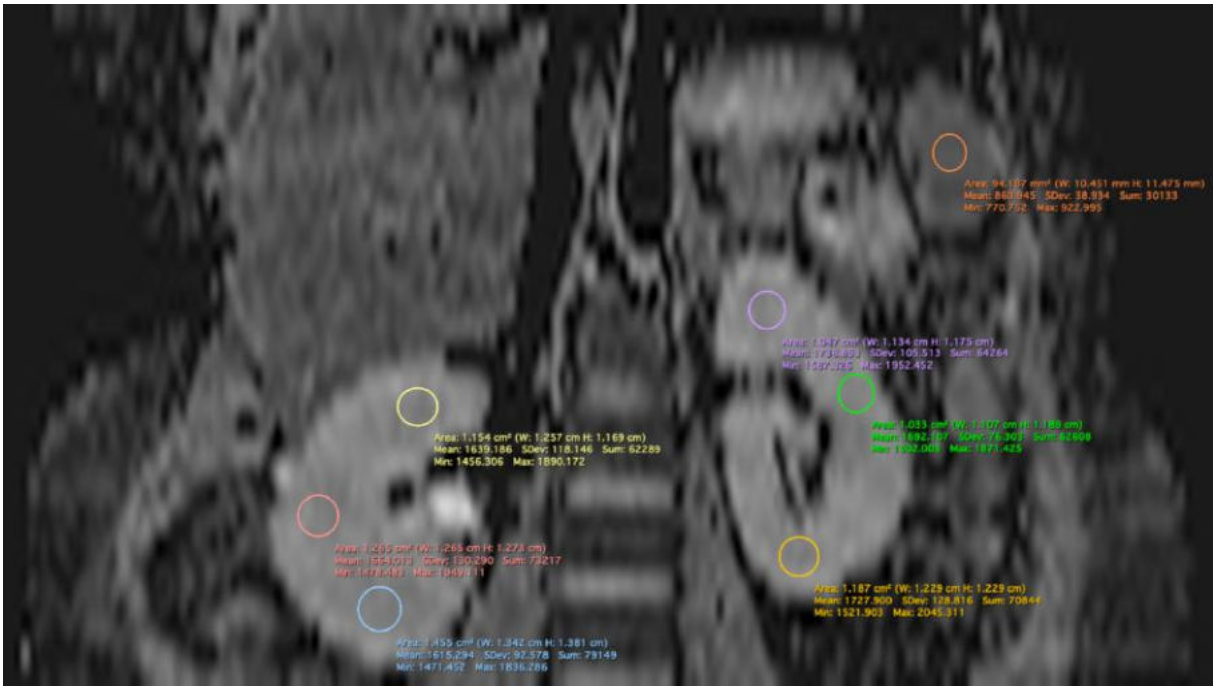

Fig. 1. ADC measurement sites in both kidney parenchyma are shown.

rotation angle = 90°, matrix = 320 × 224, mean number = 1), axial fat-suppressed FIESTA (TR = 4.2 ms, TE = 2.1 ms, thickness = 5.5 mm, pitch = 1 mm, flip angle = 75°, matrix = 192 × 288, mean number = 1), and axial 3D DualEcho (TR = 6.4 ms, TE = 2.1 and 4.3 ms, thickness = 5.5 mm, spacing = 1 mm, flip angle = 12°, matrix = 320 × 224) sequences were obtained. Subsequently, axial DWI was analyzed using a single-shot echo-planar imaging array (TR = 5,000 ms, TE = 75 ms, field of view = 41 × 41 cm; matrix = 160 × 160, NEX = 2, slice thickness = 5.0 mm, slice space = 1.0 mm, b-values 50, 400 and 800 s/mm2, acquisition time = 100 sec). The DWI sequence was triggered by breathing using the navigator-trigger prospective acquisition correction technique (PACE), and the position of the diaphragm was periodically evaluated using navigator echoes. ADC maps were created on a different workstation (Advantage workstation 4.4-GE Medical Systems) using a software (FuncTool). The images of patients were obtained using picture archiving and communication system, and evaluation was made at the workstation after the images were transferred. In the axial ADC map, a region of interest (ROI) ranging from 60 to 100 mm^2 was placed in the renal parenchyma of both kidneys for the measurement of ADC values without any preference for cortex or medulla. Overall, six ROIs were placed in the renal parenchyma of each patient, including one each in the upper pole, interpolar space, and lower pole of each kidney (Fig. 1). The mean values and standard deviations for each kidney and each patient were analyzed separately. In addition, three ROIs were placed in the parenchymal region in the upper and lower poles of the spleen and at the hilum level on the ADC map in each patient, and the mean values were calculated. In each patient, nADC was calculated by dividing the mean ADC values obtained from the renal parenchyma by the mean ADC value obtained from the spleen parenchyma.

Statistical Analysis

SPSS version 23.0 was used for the analysis of the collected data. After analyzing the normal distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests, descriptive statistical values, such as frequency and percentage, were used for categorical variables, and mean and standard deviation (SD), were used for the continuous variables. The Pearson's chi-square or Fisher's exact test was used for comparison between the categorical groups. For nonparametrically distributed data, the Kruskal-Wallis test was used to compare more than two groups; subsequently, the Mann-Whit-

ney U test was used to identify differences between the groups. Correlations between continuous variables were analyzed using the Pearson's correlation analyses. Receiver operating characteristic (ROC) analysis was performed to calculate the area under the curve for determining the cut-off value to be used to distinguish between Stage 1-2 and Stage 3-5 CKD groups. In addition, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were obtained according to the cut-off value calculated in both groups. The variability in ADC values measured from three different areas in both kidneys was evaluated using the intraclass correlation coefficient (ICC). The interpretation of the degree of fit for different ICC values is as follows: ICC ≤ 0.20, poor; 0.2 < ICC ≤ 0.4, poor-to-moderate; 0.4 < ICC ≤ 0.6, moderate; 0.6 < ICC ≤ 0.8, substantial; and ICC ≥ 0.8, almost perfect [11]. Multiple linear regression analysis was performed to examine the effect of serum creatinine level, GFR, BUN, and patient age on ADC value. During comparison between more than two groups, $p < 0.005$ was considered significant after Bonferroni correction, and $p < 0.05$ was considered significant in all other statistical tools.

RESULTS

This study included 157 patients with CKD of different stages and 100 patients without CKD or any other known chronic disease affecting the renal parenchyma. Of these, there were 84 (53.5%) females and 73 (46.5%) males in the CKD group, whereas there were 69 (69%) females and 31 (31%) males in the healthy group. The mean age of the participants was 53.6 ± 9.0 (range, 18-79) years in the CKD group, whereas it was 48.4 ± 11.0 (range, 18-78) years in the healthy group. The mean creatinine value was 1.78 ± 1.6 mg/dL (range, 0.5-9.4) in the CKD group and 0.79 ± 0.4 mg/dL (range 0.5–1.0) in the healthy group. The mean ADC value for the right and left kidney in patients with impaired renal function was found to be 1.831 ± 0.133 and 1.835 ± 0.127 (×10−3mm^2/s), respectively, and it was 1.833 ± 0.129 (×10−3mm^2/s) when both kidneys were considered. The mean ADC value was 2.115 ± 0.115 (× 10 − 3mm^2/s) in the healthy control group, and it was significantly higher than that in the Stage 1 and 2 CKD groups ($p < 0.001$). Patients with renal dysfunction were classified according to the K/DOQI CKD classification, and demo-

Table 1. Demographic, biochemical and imaging findings of groups formed according to eGFR levels.

	Group 1 (n = 38)	Group 2 (n = 45)	Group 3 (n = 37)	Group 4 (n = 20)	Group 5 (n = 17)
Age (years), mean (min, max)	38.5 (18.0-69.0)	59.7 (19.0-76.0)	57.9 (18.0-79.0)	55.4 (35.0-68.0)	60.1 (46.0-74.0)
Gender (female), n (%)	23 (60.5)	23 (51.1)	20 (54.1)	9 (45.0)	9 (52.9)
Mean ADC values					
Right Kidney (×10^{-3} mm^2/s)	1.957 ± 0.114	1.868 ± 0.101	1.799 ± 0.092	1.706 ± 0.049	1.669 ± 0.054
Left Kidney (×10^{-3}mm^2/s)	1.955 ± 0.110	1.871 ± 0.102	1.810 ± 0.065	1.713 ± 0.044	1.672 ± 0.058
Mean (×10^{-3} mm^2/s)	1.956 ± 0.110	1.869 ± 0.100	1.804 ± 0.075	1.709 ± 0.046	1.670 ± 0.056
Spleen (×10^{-3} mm^2/s)	0.836 ± 0.059	0.863 ± 0.077	0.858 ± 0.069	0.865 ± 0.032	0.856 ± 0.049
Normalised ADC	2.348 ± 0.194	2.179 ± 0.188	2.115 ± 0.190	1.978 ± 0.088	1.956 ± 0.105
BUN (mg/dL)	22.3 ± 7.541	34.9 ± 10.159	45.9 ± 12.964	85.3 ± 28.882	138.3 ± 52.107
Creatinin (mg/dL)	0.83 ± 0.146	1.01 ± 0.163	1.43 ± 0.323	2.69 ± 0.718	5.60 ± 1.705
eGFR (mL/min/1.73 m^2)	102.9± 9.446	75.0 ± 8.079	50.2 ± 7.158	25.550 ± 4.322	10.41 ± 3.202

eGFR = Estimated glomerular filtration rate, ADC = Apparent diffusion coefficient, BUN = Blood urea nitrogen, Group 1 = eGFR; ≥ 90 mL/min/1.73m^2, Group 2 = eGFR; 60-89 mL/min/1.73 m^2, Group 3 = eGFR; 30-59 mL/min/1.73 m^2, Group 4 = eGFR;15-29 mL/min/1.73 m^2, Group 5 = eGFR; < 15 mL/min/1.73 m^2.

Table 2. Agreement o ADC values measured from three separate areas in the right and left kidney.

Variable	ADC value ICC (95% CI)		ICC	*p value*
	Lower limit	Upper limit		
Right Kidney	0.973	0.982	0.978	**< 0.001**
Left Kidney	0.980	0.987	0.984	**< 0.001**

ADC = Apparent diffusion coefficient, ICC = intraclass correlation coefficient, CI= confidence interval

graphic, laboratory, and ADC measurement data for each group were evaluated in terms of mean value and SD (Table 1). In these patients, the compatibility of three ADC measurements from each kidney was evaluated using ICC, and the result was found to be almost perfect ($p < 0.001$) (Table 2). Serum creatinine level, GFR, BUN, and age were identified as independent determinants of ADC values measured in patients with renal dysfunction, and these were analyzed using stepwise multiple linear regression method. The established regression model revealed statistical significance ($F = 66.219$, $p < 0.001$). Of the independent determinants, a significant linear regression relationship was observed only between the eGFR level and ADC value ($p < 0.001$) (Table 3). In the same group, the correlation between the ADC values and eGFR, creatinine, and BUN levels was evaluated. ADC was found to be positively correlated with eGFR ($r = 0.790$, $p < 0.001$) and negatively correlated with creatinine and BUN levels ($r = -0.709$, $p < 0.001$; $r = -0.704$, $p < 0.001$, respectively). Moreover, a statistically significant difference was found in comparison using Kruskal–Wallis test in terms of mean ADC values and nADC coefficient of groups representing different stages of CKD (x2: 88.963, $p < 0.001$; x2: −65.085, $p < 0.001$, respectively) (Table 4). In the pairwise comparison of five different groups of patients with CKD using the Mann–Whitney U test, there was no significant difference between the groups representing Stage 4 and 5 kidney diseases ($p = 0.067$); however, the difference between the other groups was statistically significant ($p < 0.001$) (Table 5). A statistically significant difference was found in pairwise comparisons between the Stage 1-2 CKD and healthy groups, which were divided into two groups in terms of eGFR levels ($p < 0.001$) (Table 5). Regarding the ROC analysis performed to determine a cut-off value to distinguish moderate and severe stages from early stages and the control group from the CKD group, while detecting Stage 1 or 2 CKD in patients with an ADC value of ≥ 1791, sensitivity, specificity, PPV, and NPV were found to be 76.3%, 85%, 89.2%, and

Table 3. Multiple linear regression analysis of ADC values with , eGFR, serum creatinine levels, BUN and age.

Variable	Unstandardized		Bootstrapping BCa 95% CI		Standardized		R^2	$F_{(4-152)}$	*p value*
	B	SE_B	Lower limit	Upper limit	*β*	*t*			
(Constant)	1.672	0.049	1.574	1.770		33.856			**< 0.001**
Age	-0.001	0.000	-0.002	0.001	-0.069	-1.255			0.211
eGFR	0.003	0.000	0.002	0.004	0.759	8.414	0.582	66.219	**< 0.001**
BUN	0.001	0.000	-0.001	0.001	0.062	0.727			0.468
Creatinin	-0.006	0.007	-0.020	0.008	-0.072	-0.811			0.418

ADC = Apparent diffusion coefficient, eGFR = Estimated glomerular filtration rate, BUN = Blood urea nitrogen.

Table 4. Comparison of the mean ADC and nADC values of groups representing the five stages of CKD established according to the K/DOQI CKD classification

Variable	ADC ($\times 10^{-3}$ mm^2/s) mean ± SD	Mean rank	X^2 *p value*	nADC mean ± SD	Mean rank	X^2 *p value*
Group 1 (n = 38)	1.956 ± 0.110	120.87		2.348 ± 0.194	118.74	
Group 2 (n = 45)	1.869 ± 0.100	94.40		2.179 ± 0.188	86.14	
Group 3 (n = 37)	1.804 ± 0.075	69.92	88.963 **< 0.001**	2.115 ± 0.190	73.28	65.085 **< 0.001**
Group 4 (n = 20)	1.709 ± 0.046	31.15		1.978 ± 0.088	36.75	
Group 5 (n = 17)	1.670 ± 0.056	20.71		1.956 ± 0.105	33.41	

ADC = Apparent diffusion coefficient, nADC = Normalised apparent diffusion coefficient, CKD = Chronic kidney disease, K/DOQI = kidney disease outcomes quality initiative, SD = standard deviation, Group 1 = eGFR; ≥ 90 mL/min/1.73 m^2, Group 2 = eGFR; 60-89 mL/min/1,73 m^2, Group 3 = eGFR; 30-59 mL/min/1.73 m^2, Group 4 = eGFR; 15-29 mL/min/1.73 m^2, Group 5 = eGFR;< 15 mL/min/1.73 m^2

*Kruskal Wallis H

Table 5. ADC values of CKD patients at different stages and healthy group

Groups	n	Mean ADC	Mean Rank	Sum of Ranks	*U*	*r*	*p value*
Group1 vs 2	38	1.956	52.76	2005.00	446.000	-0.42	**< 0.001**
	45	1.869	32.91	1481.00			
Group 2 vs 3	45	1.869	49.02	2206.00	494.000	-0.35	**0.002**
	37	1.804	32.35	1197.00			
Group 2 vs 4	45	1.869	42.00	1890.00	45.000	-0.71	**< 0.001**
	20	1.709	12.75	255.00			
Group 3 vs 4	37	1.804	36.05	1334.00	109.000	-0.58	**< 0.001**
	20	1.709	15.95	319.00			
Group 4 vs 5	20	1.709	22.00	440.00	110.000	-0.30	0.067
	17	1.670	15.47	263.00			
Group 1 vs N1	38	1.956	25.14	956.00	215.000	-0.68	**< 0.001**
	59	2.144	64.36	3797.00			
Group 2 vs N2	45	1.869	26.67	1200.00	165.000	-0.71	**< 0.001**
	41	2.073	61.98	2541.00			

ADC = Apparent diffusion coefficient, CKD = Chronic kidney disease, Group 1= eGFR; ≥ 90 mL/min/1.73 m^2, Group 2 = eGFR; 60-89 mL/min/1,73 m^2, Group 3 = eGFR; 30-59 mL/min/1.73 m^2, Group 4 = eGFR; 15-29 mL/min/1.73 m^2, Group 5 = eGFR; < 15 mL/min/1.73 m^2, N1 = Control group (eGFR > 90 ml/min/1.73m^2), N2 = Control group (90 < eGFR < 60 mL/min/1.73m^2).

It was considered statistically significant for $p < 0.005$ with Bonferroni correction.

Table 6. Comparison of area under curve (AUC) to predict the CKD stage and patients with normal renal function

	AUC (95%CI)	Cut-off levels	Sensitivity	Specificity	PPV	NPV	*p* value
CKD Stage 1-2							
ADC ($\times 10^{-3} mm^2/s$)	0.872 (0.817-0.927)	1.791	0.763	0.850	0.892	0.689	**< 0.001**
nADC	0.798 (0.728-0.869)	2.155	0.803	0.728	0.735	0.797	**< 0.001**
Normal renal function							
ADC ($\times 0^{-3} mm^2/s$)	0.940 (0.913-0.968)	1.944	0.740	0.954	0.940	0.790	**< 0.001**
nADC	0.883 (0.840-0.926)	2.296	0.697	0.877	0.830	0.771	**< 0.001**

CKD = Chronic kidney disease, PPV = Positive predictive value, NPV= Negative predictive value, ADC =Apparent diffusion coefficient, nADC= Normalised apparent diffusion coefficient.

68.9%, respectively. Moreover, the area under the ROC curve was found to be 87.2% (Table 6).

DISCUSSION

The results of the present study indicated that the ADC values measured from the renal parenchyma and the normalized ADC coefficient obtained using the spleen as a reference organ can effectively represent the stages of CKD. DWI is based on the principle of detecting the random motion (Brownian motion) occurring as a result of the induction of basic water molecules, and the degree of diffusion in an organ is quantitatively expressed using ADC [12]. There are many factors that affect the diffusion-weighted images of the kidneys. These include the water content of the kidneys, renal perfusion, blood flow and blood flow volume of the kidney, the amount of intrarenal tubular flow, and the water content of the tubules [12-14]. Therefore, each factor that causes a change in DWI affects the quantitative indicator ADC. In the early stages of CKD, especially in Stage 1, minor changes are commonly seen in terms of the structure and function of the kidney. At this stage, detection of the disease is crucial because early treatment can prevent or decrease the rate of possible functional and structural loss [2, 15, 16]. In our study, we found that the mean renal ADC and nADC values of patients with Stage 1 CKD differed significantly from those with Stage 2 CKD and healthy controls with an eGFR of ≥ 90. Similarly, there was a significant difference between Stages 2-3 and 3-4 in terms of mean ADC and nADC values. However, there was no significant difference between Stages 4 and 5 in terms of mean ADC and nADC values. This may be due to the following reaons: small number of patients with CKD Stages 4 and 5, functional and structural changes in the renal parenchyma, and case-by-case perfusion differences. Emre *et al*. compared creatinine clearance and renal ADC values in a retrospective study that involved 62 patients with CKD, and they found a significant difference between ADC values of Stage 1 and 2 CKD groups; notably, this finding was similar to that of our study [17]. Moreover, Emre *et al*. revealed significant differences between all stage groups in terms of mean ADC values. In a recent study by Arora *et al*. [18] that compared 60 patients with CKD to 60 healthy individuals without a history of CKD and high creatinine levels, the mean ADC values significantly differed in the CKD group at different stages. In addition, they found that the mean ADC values in the CKD group were significantly lower than those in the healthy control group. Şafak *et al*. [19] examined 110 patients, including 95 patients with CKD and 15 healthy volunteers, in terms of ADC values and serum creatinine and eGFR levels. They found that the mean ADC values of Stage 1-2 groups were significantly higher than

those of Stage 3-5 groups, and these values were significantly lower in the CKD group than in the healthy group. Carbone *et al*. [20] examined the relationship between renal ADC values and CKD stages in 14 patients (including 9 with CKD and 5 healthy controls). Although no significant difference was found between the Stage 1-2 and 2-3 groups in terms of mean ADC values, they found that mean ADC values of Stage 3-4 groups were significantly different from healthy and Stage 1-2 groups. This result that indicates significant difference between the early-stage and healthy groups but no difference within the early-stage groups in terms of mean ADC values could be attributed to the relatively small sample size and the relative uncertainty of Cockcroft–Gault formula at GFR > 60/mL/min/1.73 m^2. Decreased water diffusion and possibly reduced perfusion owing to renal function loss and structural deterioration in early-stage renal parenchymal disease may explain the lower ADC values in these patients. In addition, in cases of glomerulosclerosis and tubular atrophy, wherein the movement of water molecules in the intracellular and extracellular components is restricted, a decrease in diffusion and ADC values can be expected.

Serum creatinine level and the degree of correlation between creatinine clearance and renal ADC values are among the most frequently investigated topics in the relevant literature. In our study, we found a significantly strong correlation between eGFR, serum creatinine level, and BUN and ADC levels, as indicators of creatinine clearance ($p < 0.001$). In a study by Namimoto *et al*. that examined 34 patients, a significant correlation was found between serum creatinine levels and ADC values measured in the renal cortex [21]. Similarly, in a study by Xu *et al*. [22], the kidneys of patients with mild, moderate, and severe renal impairment as well as those of healthy volunteers were evaluated. Based on the b-values ranging from 0 to 500 s/mm^2 in these four groups, they found a positive correlation between measured ADC values and GFR and a negative correlation between ADC values and serum creatinine level. They also found a positive correlation between CKD stages and renal ADC. Goyal *et al*. [23] examined 22 patients with renal dysfunction and 66 patients with preserved kidney function, and they reported that the mean ADC values obtained using b = − 0 – 500 s/mm^2 values differed significantly in different stages of CKD, and the ADC levels tended to decrease with increasing stage of CKD. In the same study, they found a negative correlation between the ADC value and serum creatinine level and a positive correlation between the ADC value and GFR level. In a meta-analysis published by Haitian *et al*. [24] in 2018, DWI was reported as a useful method for demonstrating renal function; moreover, the meta-analysis reported that there was a significant correlation between GFR level and ADC and that DWI can distinguish patients with early-stage CKD from those with normal renal function. The main reason for the decrease in ADC levels in renal parenchymal damage can be explained by decreased diffusion of water and decreased perfusion. This could explain why the measured mean ADC values were significantly lower in patients with impaired renal function than in those with normal renal function.

Accurate prediction of renal damage and thereby CKD stages is critical for early prediction of outcome and provision of individualized treatments [2]. Studies have particularly focused on the distinction of early stages from moderate and severe stages of CKD. In our study, we performed ROC analysis to differentiate between early-stage CKD and moderate-to-severe-stage CKD. We found that CKD stages 3 and higher could be predicted with 76.3% sensitivity and 85% specificity for measurements below the cut-off value of 1.791 (× 10 − 3mm^2/s) for ADC. While predicting CKD stages 3 and higher, the sensitivity was 80.3% and the specificity was 72.8% with the measurements below the cut-off value of 2.155 (× 10 − 3mm^2/s) for nADC. We found that individuals with normal renal function can be differentiated from patients with dysfunction with 69.7% sensitivity and 87.7% specificity for measurements above the cut-off value of 1.944 (× 10 − 3mm^2/s). Notably, Arora *et al*. [18] found the cut-off value of 2.000 (× 10 − 3mm^2/s) as an indicator of renal dysfunction. They reported that with the currently used cut-off value, the sensitivity is 56% and the specificity is 91%. However, in their study, the cut-off value was not calculated to distinguish the early stages from the moderate and advanced stages. Some studies have reported similar threshold ADC values for patients with normal renal function [25-27]. In studies evaluating normal and impaired renal functions, the mean ADC values measured for both normal kidneys and different stages of CKD differed [6, 8, 19, 20, 23, 28]. This difference may be due to different pa-

rameters in the MR devices used for imaging, the device being of 1.5 or 3 Tesla, the selection of different b-values, and the heterogeneity of the patient population included in the study. In particular, the selected b-value has a direct effect on the used measurements. ADC values measured at low b-values are prone to perfusion, and this leads to an increase in ADC values. With higher b-values, the effect of perfusion is strongly suppressed, and the measured ADC values reflect tissue diffusion more accurately [29]. However, as b-value increases, image quality may decrease due to TE elongation, T2-weight dominance, and weaker signal strength. In a study involving 100 patients, Kim *et al*. [30] calculated the mean ADC values from the liver, spleen, pancreas, and kidney parenchyma using six different combinations of b = 0.50,400,800 s/mm^2 and nADC values using the spleen as a reference organ. In their study, they reported higher ADC values at lower b-values (b2 = 0.800 and b4 = 0,50,800 s/mm^2) and significantly lower ADC values at higher b-values (b5 = 50,800 and b6 = 50,400,800 s/mm^2). They calculated the mean nADC value of the kidney parenchyma as 2.310 when a set of b = 50,400,800 s/mm^2 was used. In our study, we found the nADC value to be 2.155 in CKD group and 2.512 in the control group, with a set having the same b-values (b = 50,400,800 s/mm^2).

Limitations

Our study had some limitations. First, ADC measurements from the kidney and spleen parenchyma were performed by manual ROI insertion, and this method can lead to some inaccurate measurements. Second, the study did not consider some parameters, such as edema or dehydration, water restriction, or diuretic use, before imaging. Hence, the ADC values measured in patients with water restriction and diuretic use may differ. Finally, the sample size of the study is relatively small; therefore, the results may contain a certain margin of error.

CONCLUSION

In conclusion, DWI is a useful method to evaluate renal function, differentiate patients with early-stage CKD from those with normal renal function, and perform staging, treatment, and follow-up of patients with CKD. The use of the ADC and nADC cut-off values may be beneficial in distinguishing healthy individuals from patients with CKD and early-stage CKD from moderate-to-advanced CKD.

Authors' Contribution

Study Conception: ŞK; Study Design: ŞK; Supervision: ŞK; Funding: ŞK; Materials: ŞK; Data Collection and/or Processing: ŞK; Statistical Analysis and/or Data Interpretation: ŞK; Literature Review: ŞK; Manuscript Preparation: ŞK and Critical Review: ŞK.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Iino Y. Nihon rinsho. Jpn J Clin Med 2008;66:1645-9.
2. Kiberd BA, Clase CM. Cumulative risk for developing end-stage renal disease in the US population. J Am Soc Nephrol 2002;13:1635-44.
3. Prigent A. Monitoring renal function and limitations of renal function tests. Semin Nucl Med 2008;38:32-46.
4. Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney Int 1985;28:830-8.
5. Deo A, Fogel M, Cowper SE. Nephrogenic systemic fibrosis: a population study examining the relationship of disease development to gadolinium exposure. Clin J Am Soc Nephrol 2007;2:264-7.
6. Wang WJ, Pui MH, Guo Y, Wang LQ, Wang HJ, Liu M. 3T magnetic resonance diffusion tensor imaging in chronic kidney disease. Abdom Imaging 2014;39:770-5.
7. Zhao J, Wang ZJ, Liu M, Zhu J, Zhang X, Zhang T, et al. Assessment of renal fibrosis in chronic kidney disease using diffusion-weighted MRI. Clin Radiol 2014;69:1117-22.
8. Feng Q, Ma Z, Wu J, Fang W. DTI for the assessment of disease stage in patients with glomerulonephritis--correlation with renal histology. Eur Radiol 2015;25:92-8.
9. National Kidney Foundation K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and

stratification. Am J Kidney Dis 2002;39:S1-266.
10. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al; Chronic Kidney Disease Epidemiology Collaboration. New creatinine- and cystatin C-based equations to estimate GFR without race. N Engl J Med 2021;385:1737-49.
11. Donner A. Eliasziw M. Sample size requirements or reliability studies. Stat Med 1987;6:441-8.
12. Prasad PV, Priatna A. Functional imaging of the kidneys with fast MRI techniques. Eur J Radiol 1999;29:133-48.
13. Muller MF, Prasad PV, Bimmler D, Kaiser A, Edelman RR. Functional imaging of the kidney by means of measurements of the apparent diffusion coefficient. Radiology 1994;193:711-5.
14. Prasad PV, Thacker J, Li LP, Haque M, Li W, Koenigs H, et al. Multi-parametric evaluation of chronic kidney disease by MRI: a preliminary cross-sectional study. PLoS One 2015;10:e0139661.
15. Nangaku M. Hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. Nephron Exp Nephrol 2004;98:e8-e12.
16. Palm F, Nordquist L. Renal tubulointerstitial hypoxia: cause and consequence of kidney dysfunction. Clin Exp Pharmacol Physiol 2011;38:474-80.
17. Emre T, Kiliçkesmez Ö, Büker A, İnal BB, Doğan H, Ecder T. Renal function and diffusion-weighted imaging: a new method to diagnose kidney failure before losing half function. Radiol Med 2016;121:163-72.
18. Arora V, Khatana J, Singh K. Does diffusion-weighted magnetic resonance imaging help in the detection of renal parenchymal disease and staging/prognostication in chronic kidney disease? Pol J Radiol 2021;86:e614-9.
19. Yalçin-Safak K, Ayyildiz M, Ünel SY, Umarusman-Tanju N, Akça A, Baysal T. The relationship of ADC values of renal parenchyma with CKD stage and serum creatinine levels. Eur J Radiol Open 2015:9;3:8-11.
20. Carbone SF, Gaggioli E, Ricci V, Mazzei F, Mazzei MA, Volterrani L. Diffusion-weighted magnetic resonance imaging in the evaluation of renal function: a preliminary study. Radiol Med 2007;112:1201-10.
21. Namimoto T, Yamashita Y, Mitsuzaki K, Nakayama Y, Tang Y, Takahashi M. Measurement of the apparent diffusion coefficient in diffuse renal disease by diffusion-weighted echo-planar MR imaging. J Magn Reson Imaging 1999;9:832-7.
22. Xu Y, Wang X, Jiang X. Relationship between the renal apparent diffusion coefficient and glomerular filtration rate: preliminary experience. J Magn Reson Imaging 2007;26:678-81.
23. Goyal A, Sharma R, Bhalla AS, Gamanagatti S, Seth A. Diffusion-weighted MRI in assessment of renal dysfunction. Indian J Radiol Imaging 2012;22:155-9.
24. Liu H, Zhou Z, Li X, Li C, Wang R, Zhang Y, et al. Diffusion-weighted imaging for staging chronic kidney disease: a meta-analysis. Br J Radiol 2018;91:20170952.
25. Lavdas I, Rockall A, Castelli F, Sandhu RS, papadaki A, Honeyfield L, et al. Apparent diffusion coefficient of normal abdominal organs and bone marrow from whole-body DWI at 1.5 T: the effect of sex and age. AJR Am J Roentgenol 2015;205:242-50.
26. Ries M, Jones RA, Bassesu F, Moonen CT, Grenier N. Diffusion tensor MRI of the human kidney. J Magn Reson Imaging 2001;14:42-9.
27. Thoeny HC, Zumstein D, Simon-Zoula S, Eisenberger U, De Keyser F, Hofmann L, et al. Functional evaluation of transplanted kidneys with diffusion-weighted and BOLD MR imaging: initial experience. Radiology 2006; 241:812-21.
28. Toya R, Naganawa S, Kawai H, Ikeda M. Correlation between estimated glomerular filtration rate (eGFR) and apparent diffusion coefficient (ADC) values of the kidneys. Magn Reson Med Sci 2010;9:59-64.
29. Thoeny HC, De Keyzer F, Oyen RH, Peeters RR. Diffusion-weighted MR imaging of kidneys in healthy volunteers and patients with parenchymal diseases: initial experience. Radiology 2005;235:911-7.
30. Kim BR, Song JS, Choi EJ, Hwang SB, Hwang HP. Diffusion-weighted imaging of upper abdominal organs acquired with multiple B-value combinations: value of normalization using spleen as the reference organ. Korean J Radiol 2018;19:389-96.

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Immature granulocyte in prediction of the short-term and long-term mortality of patients with acute myocardial infarction

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ABSTRACT

Objectives: Despite great advances in the treatment of acute myocardial infarction (AMI), it is still the most common cause of death in the world. Therefore, predicting mortality in advance is clinically very important. In this study, we aimed to investigate the role of immature granulocyte (IG) and other hematological markers in predicting short- and long-term mortality in patients with AMI.

Methods: Laboratory information system (LIS) data of a tertiary hospital were used in this study. Of the 298 patients who were admitted to the coronary intensive care unit with the diagnosis of myocardial infarction, 258 recovered after treatment and were discharged. 40 of them died. It was determined that 36 of these 258 patients, who were followed up retrospectively, died within 15 months after discharge.

Results: The mean age of 298 people who participated in this retrospective study was 73.26 ± 8.6 years, and 53.3% were male. Moderate and high predictive property in receiver operating characteristic (ROC) analysis for short-term mortality, white blood cell (WBC) area under curve (AUC) = 0.802), neutrophil count (AUC = 0.817), IG count (AUC = 0.841), neutrophil/lymphocyte ratio (NLR) (AUC = 0.701), and C-reactive protein (CRP) (AUC = 0.758) tests detected. For long-term mortality, a moderate predictive feature was observed in the age (AUC = 0.712) parameter.

Conclusions: IG is a marker that does not require extra cost, provides rapid results and has high predictive value in predicting death in the short term in patients with acute myocardial infarction. It is ineffective in predicting long-term mortality.

Keywords: Immature granulocyte count, acute myocardial infarction, mortality, inflammation, hematological markers

Acute myocardial infarction (AMI) is still the most common cause of death in the world, despite all the advances over the past three decades in medical treatments [1]. AMI is triggered by the rupture of atherosclerotic plaques in the coronary arteries. As a result, a thrombotic process begins. Unstable angina or non-ST-segment elevation myocardial infarction (NSTEMI) occurs if the coronary artery lumen is partially occluded, and ST-elevation myocardial infarction (STEMI) occurs if the arterial lumen is

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completely occluded [2-5].

Many pathophysiological factors, including inflammation, trigger this process. Inflammation is effective in initiating atherosclerosis and facilitating its progression [6]. Inflammatory markers such as white blood cell (WBC), C-reactive protein (CRP), neutrophil/lymphocyte ratio (NLR), and WBC to mean platelet volume (MPV) ratio (WMR) have been investigated in many studies as potential prognostic markers in the prediction of short-term and long-term mortality [7-9].

Immature granulocyte (IG), a parameter that is poorly recognized by most clinicians, indicates the immature granulocyte fraction in peripheral blood. The IG count, a indicator of local and systemic inflammation, can be measured using the complete blood count (CBC). In recent years, it has been shown to be useful in predicting the severity and mortality of many disease such as acute pancreatitis, sepsis and cancer [10-12]. It is seen that there are only a few studies in the literature investigating the relationship between the number of IGs and AMI mortality. Therefore, our study aimed to evaluate the importance of IG number in predicting short- (hospital mortality) and long-term (> 12 mounth) [13] mortality in AMI.

METHODS

This retrospective study was conducted in a tertiary education and research hospital. Hospital information processing management system and laboratory information system (LIS) were used to obtain the data, and they were scanned between January 2020 and August 2021. A total of 298 patients who were admitted to the hospital and admitted to the coronary intensive care unit with the diagnosis of AMI (both NSTMI and STMI) were included in the study. Of these, 258 constituted the group that recovered after treatment, while 40 of them formed the group that died despite treatment. It was determined that 36 of these 258 patients, who were followed up retrospectively, died within 15 months after discharge. Pregnant women, patients under the age of 18, trauma patients, those who continued their treatment in another hospital, and patients without hemogram data were excluded from the study. Hemogram tests of both deceased and surviving groups were measured in XN 1000 (Sysmex, Kobe, Japan) device, CRP test was measured in DxC 700AU (Beckman Coulter, Brea, CA, USA) device and hs troponin I test was measured in Unicel Dxi 600 (Beckman Coulter, Brea, CA, USA) devices. Thus, it was tried to estimate the short and long term mortality of the patients from the first blood results obtained when they applied to the hospital.

In order to carry out this retrospective study, necessary permissions were obtained from the Kastamonu University Clinical Research Ethics Committee with the decision dated 20.04.2002 and numbered 2022-KAEK-41.

Statistical Analysis

"Statistical Package for Social Sciences 18.0 for Windows" (SPSS Inc., Chicago, USA) program was used for statistical analysis of the data. Descriptive statistics of the obtained data were given as number and % for categorical variables, and median (25 Percentiles, 75 Percentiles) for numerical variables. The Mann Whitney U test was used to compare the data between the surviving and deceased groups, since the results did not fit the normal distribution. Chi-square test was performed to find out whether there was a significant difference between the two groups in terms of nominally distributed conditions such as gender, hypertension (HT), diabetes mellitus (DM), chronic kidney disease (CKD), smoking, chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and coronary artery disease (CAD). Receiver Operating Characteristic (ROC) analysis was performed and Youden's index was used to determine area under the ROC curve (AUC), cut-off, sensitivity and specificity values. A p value of < 0.05 was considered statistically significant. Univariate and multivariate cox regression analysis were performed for short-term survival analysis. Univariate and multivariate logistic regression analysis were performed for long-term survival analysis.

RESULTS

Data of 298 AMI patients, 142 (47.7%) females and 156 (53.3%) males, were used in the study. The mean age of the patients was 73.26 ± 8.6 years. As additional diseases, 92 (30.8%) patients had HT, 54 (18.1%) had DM, 15 (5%) had CKD, 9 (3.5%) had COPD, 14

Table 1. Comparison of demographic, clinical and hemogram parameters of patients with AMI who died and survived in short-term mortality

Parameters	Deceased (n = 40)	Survival (n = 258)	*p value*
Age (years)	75 (66-80)	67 (63-75)	**< 0.001**
Male gender, n (%)	19 (47.5)	168 (52.7)	0.461
HT, n (%)	9 (22.5)	83 (32.1)	0.192
DM, n (%)	6 (15)	48 (18.6)	0.547
CKD, n (%)	6 (15)	9 (3.4)	**0.002**
Smoker, n (%)	8 (20)	48 (17.4)	0.735
COPD, n (%)	2 (5)	7 (2.7)	0.478
CVD, n (%)	8 (20)	6 (2.3)	**< 0.001**
CAD, n (%)	12 (30)	52 (20.1)	0.179
WBC ($\times10^3$/µL)	15.2 (11.7-18.3)	9.8 (7.9-11.8)	**< 0.001**
RBC (10^6/µL)	4.59 (3.69-4.82)	4.81 (4.42-5.23)	**< 0.001**
Hb (g/dL)	12.2 (10.7-13.7)	13.5 (12.5-15.0)	**< 0.001**
Hct (%)	39.4 (32.2-43.3)	41.0 (37.7-44.9)	**0.003**
MCHC (g/dL)	32.2 (31.4-33.1)	33.1 (32.2-33.9)	**0.001**
RDW-SD (fL)	43.3 (41.2-47.5)	41.2 (39.0-44.1)	**< 0.001**
RDW-CV (%)	13.8 (12.8-15.0)	13.1 (12.5-13.8)	**< 0.001**
PLT ($\times10^3$/µL)	269 (235-339)	245 (201-280)	**0.011**
MPV (fL)	10.4 (10.0-11.1)	10.0 (9.5-10.6)	**< 0.001**
P-LCR (%)	28.3 (25.0-33.7)	24.8 (20.4-29.9)	**< 0.001**
PCT (%)	0.30 (0.24-0.37)	0.22 (0.19-0.27)	**< 0.001**
Neutrophil count ($\times10^3$/µL)	11.6 (8.9-15.5)	6.9 (5.3-9.1)	**< 0.001**
Lymphocyte count ($\times10^3$/µL)	1.61 (1.14-3.31)	1.49 (0.93-2.12)	**0.048**
IG count ($\times10^3$/µL)	0.09 (0.06-0.21)	0.04 (0.02-0.06)	**< 0.001**
IG (%)	0.65 (0.50-1.27)	0.40 (0.30-0.50)	**< 0.001**
NLR	7.8 (4.0-3.9)	3.8 (2.4-7.1)	**0.001**
WMR	1.3 (1.0-1.7)	0.9 (0.8-1.2)	**< 0.001**
Length of stay (day)	2 (1-3.7)	4 (3-4)	**< 0.001**
Hs troponin I (ng/L)	4317 (319-14265)	504 (89-2352.5)	**< 0.001**
CRP (mg/L)	18.9 (7.1-78.5)	5.5 (2.6-11.4)	**< 0.001**

Data are shown as medians (25^{th}-75^{th} percentile) or n (%). AMI = acute myocardial infarctus, HT = hypertension, DM = diabetes mellitus, CKD = chronic kidney disease, COPD = chronic obstructive disease, CVD = cardiovascular disease, CAD = coronary artery disease, WBC = White blood cell, RBC = red blood cell, Hb = hemoglobin, Hct =hematocrit, MCHC = mean corpuscular hemoglobin concentration, RDW-SD = red blood cell distribution width-standard deviation, RDW-CV = red blood cell distribution width- coefficient of variation, P-LCR = platelet large cell ratio, PCT = procalcitonin, IG = immature granulocyte, NLR= neutrophil/lymphocyte ratio, WMR = WBC to mean platelet volume (MPV) ratio, Hs = high-sensitive, CRP = C-reactive protein

(4.6%) had CVD and 64 (21.4%) CAD, and 56 (18.7%) were smokers. When we evaluate the medians (25th-75th percentile) of hematological parameters and then it is recommended WBC (×103/µL) 8.4 (6.6-10.2), red blood cell distribution width-standard deviation RDW-SD (fL) 43.4 (40.4-46.6), RDW-coefficient of variation (RDW-CV) (%) 13.5 (13.0-14.6), neutrophil count (×103/µL) 5.45 (4.19-7.81), IG count (×103/µL) 0.03 (0.02-0.05), IG (%) 0.4 (0.3-0.5) and NLR were found 3.23 (2.07-5.58).

When the demographic, clinical and hemogram data of the patients who died and survived in the short term (Table 1), it will be seen that 40 people constitute the deceased group and 258 the survivor group. When the demographic, clinical and hemogram data of the patients who died and survived in the long term (Table

Table 2. Comparison of demographic, clinical and hemogram parameters of patients with AMI who died and survived in long-term mortality

Parameters	Deceased (n = 36)	Survival (n = 222)	*p value*
Age (years)	73.5 (66.5-78)	66 (61-72)	**< 0.001**
Male gender, n (%)	21 (58.3)	147 (66.2)	0.347
HT, n (%)	12 (46.1)	69 (31)	0.970
DM, n (%)	7 (19.4)	40 (18)	0.946
CKD, n (%)	2 (0.5)	7 (3.1)	0.652
Smoker, n (%)	3 (8.3)	45 (20.2)	**0.048**
COPD, n (%)	2 (0.5)	5 (2.2)	0.340
CVD, n (%)	4 (11.1)	2 (1.3)	**0.001**
CAD, n (%)	14 (38)	69 (31)	0.970
WBC ($\times10^3$/µL)	9.9 (8.02-12.0)	10.2 (8.2-12.7)	0.735
RBC (10^6/µL)	4.52 (4.12-5.00)	4.87 (4.47-5.26)	0.008
Hb (g/dL)	12.7 (11.2-14.0)	13.8 (12.6-15.0)	**0.005**
Hct (%)	38.5 (34.3-42.7)	41.7 (38.2-45.0)	0.007
MCV (fL)	87.3 (83.5-90.9)	85.3 (82.1-88.8)	**0.029**
MCHC (g/dL)	32.4 (31.8-33.0)	33.3 (32.2-34.0)	**0.003**
RDW-SD (fL)	43.6 (40.2-47.7)	41.0 (38.9-43.3)	**0.002**
RDW-CV (%)	13.6 (12.8-15.0)	13.0 (12.4-13.7)	**0.001**
IG count ($\times10^3$/µL)	0.04 (0.02-0.06)	0.04 (0.02-0.06)	0.547
IG (%)	0.40 (0.30-0.575)	0.40 (0.20-0.60)	0.145
NLR	3.76 (3.14-7.04)	3.92 (2.24-7.11)	0.348
WMR	1.00 (0.77-1.21)	0.98 (0.82-1.29)	0.642
Length of stay (day)	4 (3-5.75)	4 (3-4)	0.173
Hs troponin I (ng/L)	1381 (174-4565)	469 (76-2188)	0.052
CRP (mg/L)	8.4 (2.8-20.3)	4.6 (2.3-10.5)	**0.005**

Data are shown as medians (25th-75th percentile) or n (%). AMI = acute myocardial infarctus, HT = hypertension, DM = diabetes mellitus, CKD = chronic kidney disease, COPD = chronic obstructive disease, CVD = cardiovascular disease, CAD = coronary artery disease, WBC = White blood cell, RBC = red blood cell, Hb = hemoglobin, Hct =hematocrit, MCV = mean corpuscular valume, MCHC = mean corpuscular hemoglobin concentration, RDW-SD = red blood cell distribution width-standard deviation, RDW-CV = red blood cell distribution width- coefficient of variation, IG = immature granulocyte, NLR= neutrophil/lymphocyte ratio, WMR = WBC to mean platelet volume (MPV) ratio, Hs = high-sensitive, CRP = C-reactive protein

Table 3. ROC analysis values of some hematological parameters in AMI patients in short-term mortality

Parameters	Cut-off	AUC	95% CI	*p value*	Sensitivity (%)	Specifity (%)
WBC (×10^3/µL)	11.38	.802	0.73-0.88	**< 0.001**	85	66
Neutrophil count (×10^3/µL)	8.68	.817	0.75-0.89	**< 0.001**	85	67
IG count (×10^3/µL)	0.045	.841	0.77-0.91	**< 0.001**	92	62
IG (%)	0.45	.806	0.74-0.88	**< 0.001**	82	70
NLR	6.75	.701	0.61-0.807	**< 0.001**	64	74
WMR	1.01	.754	0.68-0.83	**< 0.001**	90	51
Hs troponin I (ng/L)	2381	.688	0.595-0.79	**< 0.001**	64	75
CRP (mg/L)	11.4	.758	0.68-0.84	**< 0.001**	64	75

AMI = acute myocardial infarctus, ROC = receiver operating characteristic, AUC = area under curve, IG = immature granulocyte, NLR= neutrophil/lymphocyte ratio, WMR = WBC to mean platelet volume (MPV) ratio, Hs = high-sensitive, CRP = C-reactive protein

2), it will be seen that 36 people constitute the deceased group and 222 the survivor group.

In the ROC analysis, it was determined that they showed moderate-high predictive properties with IG count (cut off: 0.045, AUC = 0.841) and IG (%) (cut off: 0.45, AUC = 0.806) (Table 3, Fig. 1). Age was found to be the most predictive marker for long-term mortality (Table 4, Fig. 2). In univariate and multivariate cox regression analyzes RDW-CV, platelet large cell ratio (P-LCR), WBC, hemoglobin concentration (Hb) and IG were found to be independent markers for short-term mortality (Table 5). In univariate and mul-

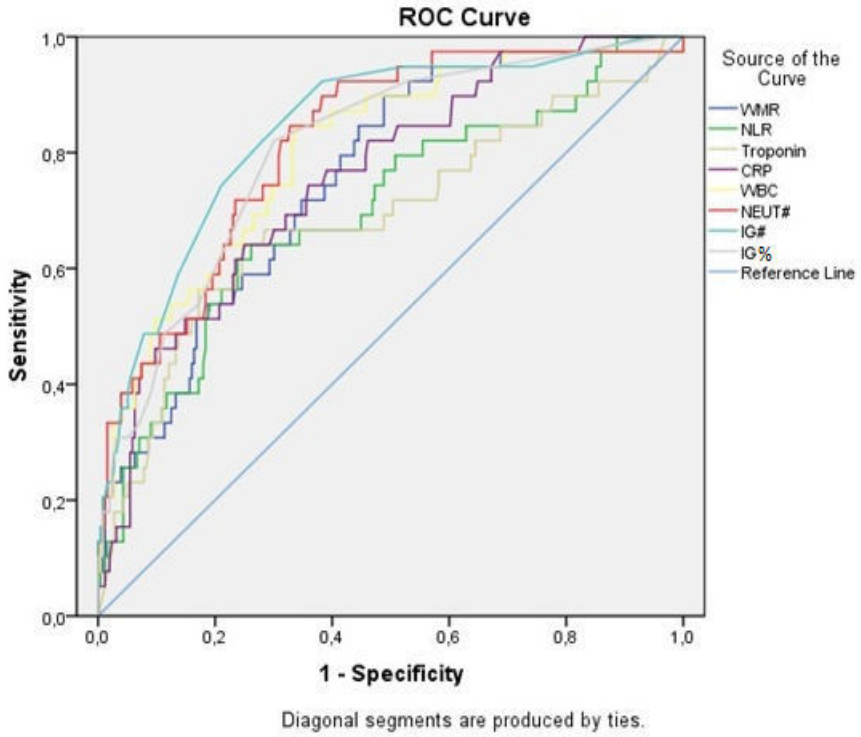

Fig. 1. ROC curve analysis of some hematological data in AMI patients in short-term mortality. AMI = acute myocardial infarctus, ROC = receiver operating characteristic.

Table 4. ROC analysis values of age parameter in AMI Patients in long-term mortality

Parameters	Cut-off	AUC	95% CI	*p value*	Sensitivity (%)	Specifity (%)
Age (years)	68.5	.712	0.63-0.80	**< 0.001**	72	64

AMI = acute myocardial infarctus, ROC = receiver operating characteristic, AUC = area under curve

tivariate logistic regression analyzes, age was found to be independent predictors of long-term mortality (Table 6).

DISCUSSION

The most striking result in our study is the demonstration that IG is a fast, non-expensive and predictive marker for predicting short-term mortality in patients with AMI. In addition, it showed a higher predictive feature than many other parameters that predict mortality in the literature. IG, which has been shown to be an important biomarker in predicting mortality and the severity of the disease in many studies in other diseases, is also promising in patients with AMI. However, it is ineffective in predicting long-term mortality in AMI patients.

he effect of inflammation in the initiation and even progression of coronary atherosclerosis is significant. [14]. In this relationship, which has been known for many years, [2] intensely inflamed atherosclerotic plaques increase the susceptibility to acute coronary syndrome [14]. Inflammation plays an active role in many stages of atherosclerosis, including endothelial dysfunction, leukocyte recruitment and even platelet activation. Neutrophils produce many proteolytic enzymes that cause tissue destruction, such as acid phosphatase, myeloperoxidase, and elastase [16-18]. Moreover, inflammation is highly associated with possible complications and prognosis of AMI [19]. Increasing the intensity of inflammation increases the likelihood of atherosclerotic plaque, which in turn causes MI [20].

Studies have shown that haematological parameters (e.g. WBC, NLR, neuthrophil count, etc.) in AMI have been of great interest as they can provide independent information on prognosis and risk stratifica-

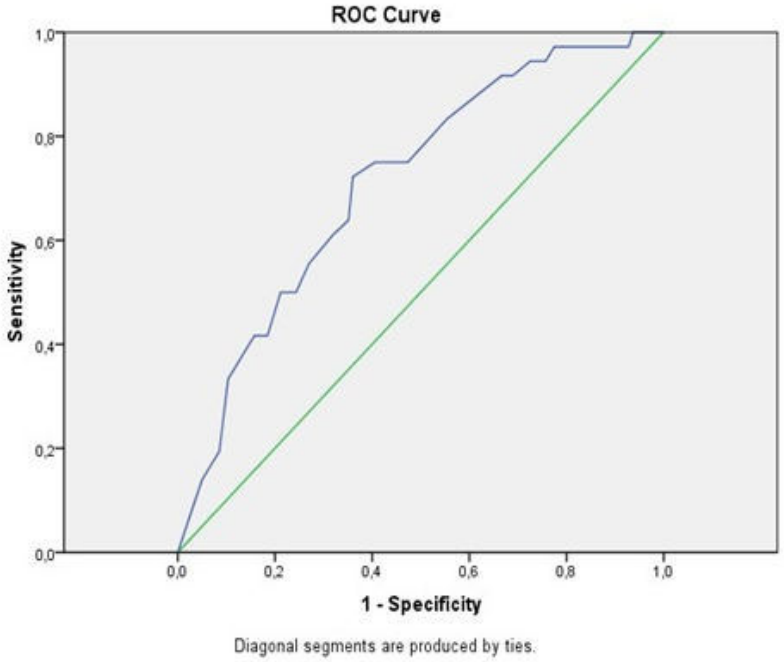

Fig. 2. ROC curve analysis of age parameter in AMI patients in long-term mortality. AMI = acute myocardial infarctus, ROC = receiver operating characteristic.

Table 5. Cox regression in short-term mortality in AMI patients

	Univariate HR (95% CI)	*p value*	Multivariate HR (95% CI)	*p value*
Sex	0.502 (0.266-0.947)	**0.033**	1.038 (0.498-2.166)	0.920
Age	1.059 (1.019-1.101)	**0.004**	1.010 (0.970-1.053)	0.625
RDW-CV (%)	1.270 (1.108-1.457)	**0.001**	1.297 (1.075-1.566)	0.007
P-LCR (%)	1.072 (1.029-1.117)	**0.001**	1.083 (1.032-1.136)	**0.001**
WBC (×10^3/µL)	1.139 (1.083-1.197)	**< 0.001**	1.111 (1.052-1.173)	**< 0.001**
Hb (g/dL)	0.748(0.645-0.868)	**< 0.001**	0.783 (0.658-0.931)	0.006
IG count (×10^3/µL)	1.643(1.385-1.949)	**< 0.001**	1.319 (1.036-1.679)	**0.025**

AMI = acute myocardial infarctus, RDW-SD = red blood cell distribution width-standard deviation, RDW-CV = red blood cell distribution width- coefficient of variation, WBC = White blood cell, Hb = hemoglobin, IG = immature granulocyte

tion [21]. In addition, troponin, CRP, and NLR were used both in clinical scoring and as prognostic indicators [22].

NLR is one of the parameters that have been published in many articles in recent years to predict short- and long-term mortality in patients with STEMI and NSTEMI [23-25]. There are clinical and meta-analysis studies showing that NLR can be independent factor in determining AMI [9, 26, 27]. In a study by Basem Azab *et al*. [23], in which they looked at the long-term mortality prediction rate in NSTEMI patients, it was shown that NLR showed a better predictive value of 4.7 than all other hemogram parameters. In our study, NLR showed short-term mortality, the cut-off value was 6.75, AUC value was 0.701, sensitivity was 64% and specificity was 74%. This value was found to be the cut-off point for the estimation of mortality in patients who had a heart attack. In addition, the neuthrophil count cut-off value was 8.68, the AUC value was 0.817, the sensitivity was 85%, and the specificity was 67%. With these values, neuthrophil count shows a high predictive feature in mortality in AMI patients. In our study, NLR was found to be ineffective in demonstrating long-term mortality in contrast to some studies in the literature [23, 28]. This may be because they are examining a much longer period of time than 15 months, or the NLR they calculated is not the NLR at the time of first admission to the hospital (Tables 3 and 4).

WBC has been an important prognostic marker in demonstrating short-term and mid-term mortality in patients with AMI [29]. Clinical evidence that WBC can be independent factor in determining mortality and meta-analysis studies are accessible [9, 26, 27]. Çiçek *et al*. [9] showed that WBC is an important marker in demonstrating mortality and found a cut-off value of

Table 6. Logistic regression analysis in long-term mortality in AMI patients

	Univariate HR (95% CI)	*p value*	Multivariate HR (95% CI)	*p value*
Sex	0.714 (0.348-1.465)	0.359	1.662 (0.686-4.027)	0.261
Age	1.095 (1.043-1.149)	**< 0.001**	1.074 (1.022-1.129)	**0.005**
RDW-CV (%)	1.378 (1.122-1.692)	**0.002**	1.336 (1.027-1.738)	**0.031**
P-LCR (%)	1.021 (0.969-1.075)	0.437	1.017 (0.960-1.078)	0.565
WBC (×10^3/µL)	0.993(0.893-1.103)	0.894	1.061 (0.941-1.196)	0.332
Hb (g/dL)	0.744 (0.611-0.905)	**0.003**	0.859 (0.660-1.117)	0.257
IG count (×10^3/µL)	1.316 (0.631-2.744)	0.463	0.956 (0.414-4.027)	0.917

AMI = acute myocardial infarctus, RDW-SD = red blood cell distribution width-standard deviation, RDW-CV = red blood cell distribution width- coefficient of variation, WBC = White blood cell, Hb = hemoglobin, IG = immature granulocyte

14.4 (57.8% sensitivity; 90.3% specificity). In their study, Avcı *et al*. [30] showed that WBC values above 10,8 were a strong predictor of mortality with 68.4% sensitivity and 62.5% specificity. In our study, with a cut-off value of 11.4, AUC of 0.827, sensitivity of 85% and specificity of 73%, WBC is an important biomarker of short-time mortality in patients with AMI. It was found to be ineffective in demonstrating 15-month mortality (Tables 3 and 4).

Recently, Dehghani *et al*. [31] investigated a new parameter called WMR as a predictor of long-term outcomes in patients with NSTEMI. They suggested that WMR is a better predictor of worse outcomes than WBC and mean platelet volume (MPV) in patients with NSTEMI. Çiçek *et al*. [9] showed that WMR is a good predictor of long-term mortality in a large number of STEMI patients undergoing primary percutaneous coronary intervention (PPCI) [9]. In our study WMR 1.01 cut-off value, 0.754 AUC value, was shown to be a moderate independent predictor of short-term mortality in patients with heart attack, but it was found to be ineffective in demonstrating 15-month mortality (Tables 3 and 4).

Age was quite impressive in short- and long-term mortality rates after AMI, as demonstrated in the study by Goldberg *et al*. [32]. In a study by Haller *et al*. [33], age was an independent predictor of both short-term and long-term mortality in STEMI patients. In our study, age had a predictive value in both short-term and long-term mortality (Table 3 and 4). In fact, age was the most predictive predictor of long-term mortality in our study.

In a study by Vrsalovic *et al*. [34], low hemoglobin and high CRP values were shown to be predictive markers for short-term mortality in STEMI patients. In another study by Padayachee *et al*. [35], patients with CRP > 3 mg/L were found to be effective in predicting both long-term and short-term mortality. It has been suggested that it increases the risk of death due to coexistence [36]. In our study, although the hemoglobin values of the deceased group in short-term mortality were lower, the predictive feature of hemoglobin was not high enough. CRP, on the other hand, was more successful in predicting short-term mortality than long-term (Tables 3 and 4).

The left shift of the granulocyte shift, which means by the presence of IG, indicates that the bone marrow is active. Based on the cell morphology in IG peripheral smear, it is classified as promyelocytes, myelocytes, metamyelocytes and band forms. However, IG counting is difficult with this way of spreading. However, almost all of today's hemogram analysers measure IG accurately [37].

IG, on the other hand, is a parameter that is little known by physicians and is already measured in many hemogram devices together with other parameters. In other words, it does not require an extra effort, cost and time. Several recent studies have shown that IG can be used to predict short-term mortality [38, 39]. In a recent study, it was shown that IG number predicts mortality in upper gastrointestinal system diseases with a cut-off value of 0.95 with a sensitivity of 66.7% and a specificity of 75.7% [11]. In another recent study, IG predicted mortality in patients with peritonitis with a cut-off value of 1.05 [39]. Similarly, in a study by Korkut *et al*. [21], it was shown that there is a significant relationship between high IG values and mortality in STEMI patients. In this study, IG was shown to be a moderately predictive marker in predicting hospital mortality [21]. In our study, the cut-off point of the IG number was 0.045 and the AUC value of 0.841, and the sensitivity was 92% and the specificity was 62%. The percentage of IG was AUC = 0.806, the sensitivity was 82% with a cut-off point of 0.45 and the specificity was 70%. Both parameters were highly effective in predicting short-term mortality. However, they were ineffective in demonstrating long-term mortality (Tables 3 and 4).

Limitations

Nevertheless, the study presented here has some limitations. It was originally designed as a single-center, retrospective study. In addition, delta IG measurements were not made. These measurements can be much more useful for mortality estimation. Prospective multicenter studies are needed to demonstrate the relationship between AMI and IG.

CONCLUSION

We think that estimating the patient group whose condition is serious from the hemogram blood at the first admission to the hospital will provide clinicians with a serious advantage in terms of managing the treatment of the patients. With this patient group, we be-

lieve that clinicians can reduce mortality rates, perhaps by increasing the number of visits, or by starting effective treatment early. In this context, IG will be very useful for clinicians in the short-term process management of AMI patients, as it does not require extra costs, provides fast results, can be studied even in the smallest laboratories, and has a high predictiveness in predicting mortality after AMI. However, it is ineffective in demonstrating long-term mortality.

Authors' Contribution

Study Conception: SG, NCÇ; Study Design: SG, NCÇ, FMKG; Supervision: SG, NCÇ, FMKG; Funding: N/A; Materials: SG, SA; Data Collection and/or Processing: SG, SA; Statistical Analysis and/or Data Interpretation: SG, NCÇ, FMKG; Literature Review: SG, SA; Manuscript Preparation: SG, SA and Critical Review: FMKG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Omer MA, Tyler JM, Henry TD, Garberich R, Sharkey SW, Schmidt CW, et al. Clinical characteristics and outcomes of STEMI patients with cardiogenic shock and cardiac arrest. JACC Cardiovasc Interv 2020;13:1211-9.
2. White PD, Mallory GK, Salcedo-Salgar J. The speed of healing of myocardial infarcts. Trans Am Clin Climatol Assoc 1936;52:97-104.1.
3. Bursi F, Weston SA, Killian JM, Gabriel SE, Jacobsen SJ, Roger VL. C-reactive protein and heart failure after myocardial infarction in the community. Am J Med 2007;120:616-22.
4. Fuster V. Lewis A. Conner Memorial Lecture. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. Circulation 1994;90:2126-46.
5. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340:115-26.
6. Moriya J. Critical roles of inflammation in atherosclerosis. J Cardiol 2019;73:22-7.
7. Sari I, Sunbul M, Mammadov C, Durmus E, Bozbay M, Kivrak T, et al. Relation of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio with coronary artery disease severity in patients undergoing coronary angiography. Kardiol Pol 2015;73:1310-6.
8. Silvestre-Roig C, Braster Q, Ortega-Gomez A, Soehnlein O. Neutrophils as regulators of cardiovascular inflammation. Nat Rev Cardiol 2020;17:327-40.
9. Çiçek G, Açıkgöz SK, Yayla Ç, Kundi H, İleri M. White blood cell count to mean platelet volume ratio: a novel and promising prognostic marker for ST-segment elevation myocardial infarction. Cardiol J 2016;23:225-35.
10. Bedel C, Korkut M, Aksoy F, Kuş G. Usefulness of immature granulocytes to predict high coronary SYNTAX score in acute coronary syndrome: a cross-sectional study. Arch Acad Emerg Med 2020;8:e73.
11. Bedel C, Korkut M, Avcı A, Uzun A. Immature granulocyte count and percentage as new predictors of mortality in patients with upper gastrointestinal bleeding. Indian J Crit Care Med 2020;24:794-8.
12. Korkut M, Bedel C, Selvi F. Are immature granulocytes and derivatives early predictors of acute appendicitis and acute complicated appendicitis in adults? Formosan J Surg 2020;53:123-7.
13. Lee W, Lee J, Woo SI, Choi SH, Bae JW, Jung S, et al. Machine learning enhances the performance of short and long-term mortality prediction model in non-ST-segment elevation myocardial infarction. Sci Rep 2021;11:1-14.
14. Altun B, Turkon H, Tasolar H, Beggi H, Altun M, Temiz A, et al. The relationship between high-sensitive troponin T, neutrophil lymphocyte ratio and SYNTAX Score. Scand J Clin Lab Invest 2014;74:108-15.
15. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685-95.
16. Reichlin T, Socrates T, Egli P, Potocki M, Breidthardt T, Arenja N, et al. Use of myeloperoxidase for risk stratification in acute heart failure. Clin Chem 2010;56:944-51.
17. Tousoulis D, Antoniades C, Koumallos N, Stefanadis C. Pro-inflammatory cytokines in acute coronary syndromes: from bench to bedside. Cytokine Growth Factor Rev 2006;17:225-33.
18. Mehta J, Dinerman J, Mehta P, Saldeen TG, Lawson D, Donnelly WH, et al. Neutrophil function in ischemic heart disease. Circulation 1989;79:549-56.
19. Gul I, Cerit L, Senturk B, Alkan MB, Kemal H, Cerit Z, et al. The importance of intra-aortic pulse pressure after anterior ST-segment elevation myocardial infarction. Braz J Cardiovasc Surg 2018;33:579-87.
20. Mozos I, Malainer C, Horbańczuk J, Gug C, Stoian D, Luca CT, et al. Inflammatory markers for arterial stiffness in cardiovascular diseases. Front Immunol 2017;8:1058.
21. Monteiro Júnior JGDM, Torres DDOC, da Silva MCFC, Martins CMDH, da Silva IK, do Nascimento, et al. Prognostic value of hematological parameters in patients with acute myocardial infarction: intrahospital outcomes. PloS One 2018;13:e0194897.
22. Korkut M, Bedel C, Sivil R, Arslan MA, Selvi F, Kuş G, et al. Usefulness of immature granulocytes as a prognostic factor in ST-elevation myocardial infarction. Braz J Cardiovasc Surg 2022;37:893-9.
23. Azab B, Zaher M, Weiserbs KF, Torbey E, Lacossiere K, Gaddam S, et al. Usefulness of neutrophil to lymphocyte ratio in predicting short- and long-term mortality after non-ST-elevation myocardial infarction. Am J Cardiol 2010;106:470-6.

24. Kurtul S, Sarli B, Baktir AO, Demirbas M, Saglam H, Doğan Y, et al. Neutrophil to lymphocyte ratio predicts SYNTAX score in patients with non-ST segment elevation myocardial infarction. Int Heart J 2015;56:18-21.
25. Gazi E, Bayram B, Gazi S, Temiz A, Kirilmaz B, Altun B, et al. Prognostic value of the neutrophil-lymphocyte ratio in patients with ST-elevated acute myocardial infarction. Clin Appl Thromb Hemost 2015;21:155-9.
26. Bajari R, Tak S. Predictive prognostic value of neutrophil-lymphocytes ratio in acute coronary syndrome. Indian Heart J 2017;69 Suppl 1(Suppl 1):S46-S50.
27. Bhat T, Teli S, Rijal J, Bhat H, Raza M, Khoueiry G, et al. Neutrophil to lymphocyte ratio and cardiovascular diseases: a review. Expert Rev Cardiovasc Ther 2013;11:55-9.
28. Sawant AC, Adhikari P, Narra SR, Srivatsa SS, Mills PK, Srivatsa SS. Neutrophil to lymphocyte ratio predicts short- and long-term mortality following revascularization therapy for ST elevation myocardial infarction. Cardiol J 2014;21:500-8.
29. Libby P, Simon DI. Inflammation and thrombosis: the clot thickens. Circulation 2001;103:1718-20.
30. Avci BŞ, Avci A, Dönmez Y, Kaya A, Gülen M, Özer Aİ, et al. The effectiveness of neutrophil-lymphocyte artio in predicting in-hospital mortality in non-ST-elevation myocardial infarction. Emerg Med Int 2020;2020:8718304.
31. Dehghani MR, Rezaei Y, Taghipour-Sani L. White blood cell count to mean platelet volume ratio as a novel non-invasive marker predicting long-term outcomes in patients with non-ST elevation acute coronary syndrome. Cardiol J 2015;22:437-45.
32. Goldberg RJ, McCormick D, Gurwitz JH, Yarzebski J, Lessard D, Gore JM. Age-related trends in short- and long-term survival after acute myocardial infarction: a 20-year population-based perspective (1975-1995). Am J Cardiol 1998;82:1311-7.
33. Haller PM, Jäger B, Farhan S, Christ G, Schreiber W, Weidinger F, et al. Impact of age on short- and long-term mortality of patients with ST-elevation myocardial infarction in the VIENNA STEMI network. Wien Klin Wochenschr 2018;130:172-81.
34. Vrsalovic M, Pintaric H, Babic Z, Pavlov M, Presecki AV, Getaldic B, et al. Impact of admission anemia, C-reactive protein and mean platelet volume on short term mortality in patients with acute ST-elevation myocardial infarction treated with primary angioplasty. Clin Biochem 2012;45:1506-19.
35. Padayachee L, Rodseth RN, Biccard BM. A meta-analysis of the utility of C-reactive protein in predicting early, intermediate-term and long term mortality and major adverse cardiac events in vascular surgical patients. Anaesthesia 2009;64:416-24.
36. Al Falluji N, Lawrence-Nelson J, Kostis JB, Lacy CR, Ranjan R, Wilson AC. Effect of anemia on 1-year mortality in patients with acute myocardial infarction. Am Heart J 2002;144:636-41.
37. Bhansaly P, Mehta S, Sharma N, Gupta E, Mehta S, Gupta S. Evaluation of immature granulocyte count as the earliest biomarker for sepsis. Indian J Crit Care Med 2022;26:216-23.
38. Bedel C, Korkut M, Selvi F, Zortuk Ö. The immature granulocyte count is a new predictor of the 30-day mortality in intracerebral haemorrhage patients: preliminary study. Indian J Neurosurg 2021;10:114-20.
39. Sinaga RH, Utariani A, Wardhani P. Immature granulocyte and mean platelet volume as a predictor of 30-day postoperative mortality in patients with sepsis caused by peritonitis. Bali J Anesth 2020;4:166-71.

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Effect of caffeine supplementation during treadmill exercise on hippocampal genes expression levels in adolescent rats

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ABSTRACT

Objectives: The usage of caffeine, a psychostimulant that is included in many foods and drinks, is rising, especially among young people. Also, caffeine works as an ergogenic substance during exercise to improve performance, and strength. In this study, our aim was to investigate the effect of caffeine consumption and exercise on hippocampal learning and memory functions in early life.

Methods: Postnatal 28 days old Wistar albino male rats (n = 28) were randomly divided into 4 groups; control group (C), caffeine group (Cf), exercise group (E), caffeine+exercise group (CfE). Caffeine was dissolved in drinking water (0.3 g/L) and the treadmill exercise was applied 3 days a week. Following, the rats were applied to Morris Water Maze Test (MWMT) and open field test. N-methyl-D-aspartate (NMDA) receptors NR2A, NR2B, and brain-derived neurotrophic factor (BDNF) gene expression levels were investigated in hippocampus tissue by RT-PCR.

Results: In MWMT, there was no significant difference in terms of learning and memory functions and hippocampal gene expression levels of the groups ($p > 0.05$). In the open field test, the time spent in the center was decreased in the CfE group, and the number of entries to the center was decreased in the E and CfE groups compared to the control group ($p < 0.05$).

Conclusions: We assumed that caffeine given with exercise application caused anxiety behavior but did not affect learning and memory. There is a need for new studies investigating the effect of caffeine on exercise with different doses and durations depending on age.

Keywords: Caffeine, exercise, hippocampus, learning, memory

Caffeine is a psychostimulant substance that is frequently consumed all over the world and increases alertness by showing a stimulant effect on the peripheral and central nervous systems [1]. Caffeine affects central nervous system stimulation, increases metabolism and respiratory rate, induces diuresis, and increases blood pressure by antagonizing adenosine receptors. Thus, it prevents drowsiness and increases alertness [2]. Caffeine performs its psychostimulant effect through the adenosine A1 receptor. Adenosine A1 receptors are abundant in some brain regions, such as the hippocampus, cortex, cerebellum, and hypothalamus [3]. It is known that caffeine has a neuroprotective effect in various neurodegenerative diseases and

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is protective against neurotoxicity in Alzheimer's. Moreover, adenosine and N-methyl-D-aspartic acid (NMDA) receptors may be responsible for this neuroprotective mechanism [4]. For instance, Oliveriora and colleagues showed that subchronic caffeine treatment can reduce the hyperlocomotion and cognitive deficits caused by NMDA receptor antagonists in mice [5]. Together with caffeine, physical activity has positive effects on cognition and brain function at various levels.

Exercise can improve cognitive functioning (such as executive function in old age or mental ability in children with educational disabilities), reduce stress levels, and ward off feelings of anxiety and depression [6]. Exercise is individually planned and known as a structured physical activity that is done voluntarily to be fit and healthy [7]. Exercise protects neurons from various brain injuries, activates neuronal cells, promotes neurogenesis, increases brain plasticity, and improves cognitive function [8]. Exercise increases NMDA receptor expression in the hippocampus [9, 10]. In a study with Alzheimer's transgenic mice, they found that the NMDA receptor subunit NR2B levels were decreased. They also reported that NR2B levels increased significantly after exercise administration, thereby attenuating NMDA receptor damage and improving cognitive memory [11]. Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophic factor and stimulates the brain with exercise [12]. It has been reported that BDNF prolongs the lifespan of neurons, preserves their integrity, stimulates neurogenesis, strengthens learning, and protects cognitive functions during aging [13]. Exercise affects BDNF expression through both direct and indirect mechanisms [14].

Caffeine is an ergogenic supplement that increases cognitive function and ameliorates the performance of exercise by increasing endurance and strength [15, 16]. It has been reported that caffeine consumption and physical exercise that is applied during the adolescent development period have the potential for improving behavioral disorders and stimulating neuroplasticity in attention deficit hyperactivity disorder (ADHD) [17]. Human studies investigate the behavioral effects of caffeine in adults in particular [18]. However, there are fewer studies in the literature evaluating the safety and effects of caffeine in children and adolescents compared to adults. Based on this information, the purpose of this study was to look into the effects of caffeine and/or physical exercise on learning and memory in adolescent rats using behavioral tests and hippocampal NR2A, NR2B, and BDNF gene expression levels.

METHODS

Experimental Protocol

All experimental procedures were carried out according to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. The ethical approval of the study was taken from the Experimental Animal Ethics Committee of Mersin University (Approval No: 2021/21). Wistar albino male rats (n = 28, 4 weeks old postnatal) were randomly assigned into four groups: control group (C), caffeine group (Cf), exercise group (E), caffeine + exercise group (CfE). The rats were fed with tap water and Purina rodent chow ad-lib. All applications and tests were carried out in the physiology laboratory of the Mersin University Medicine Faculty. During the experiment, the temperature of the room where the animals were kept was adjusted to 23 ± 2ºC. The rats' consumed the amount of water measured daily, and their weight and the amount of feed were followed up weekly.

Caffeine Treatment

Caffeine is an anhydrous form (Sigma Aldrich, Saint Louis, MO, USA, lot: r068K8730V) was administered via drinking water (0.3 g⁄L) chronically for 4 weeks and prepared daily [19, 20]. The daily consumption of caffeine by each rat was ensured to be nearly 16-20 mg. The amount of water consumed by rats in the caffeinated groups for 4 weeks was found similar to the control group.

Treadmill Exercise Application

Exercise application was started from the 28th day of postnatal period (PND). The exercise protocol was performed on a treadmill apparatus with 2 sections, 3 times a week for 4 weeks. Exercise intensity is ~ 0.5 km/h for 4 weeks; slope increase was not applied [21]. Before starting the exercise application, the rats were kept on the treadmill for 10-15 minutes to familiarize themselves with the environment, and then the exer-

cise application was started. Exercise application was performed as 20 min/day in the first week, 50 min/day in the second week, 60 min/day in the 3rd week, and 60 min/day in the 4th week. The learning memory performances of the rats whose exercise application was completed were measured with the Morris Water Maze Test (MWMT), and their anxiety levels were measured with the open field test.

Testing Spatial Learning and Memory Using the Morris Water Maze Test (MWMT)

A circular stainless-steel tank that has a diameter of 1.5 m and 0.6 m in depth was used in the MWMT. The tank was filled with water to a depth of 0.5 m. The water and room temperature were adjusted to the temperature of 22 ± 1°C. A curtain ornamented with several marked visual cues surrounded the tank. Inside the tank, there was a circular platform that has a diameter of 15 cm. To record the swimming track of the test animals, a camera was used placed above the tank. The behavioral data were recorded and analyzed by a visual analysis system (EthoVision, Noldus Information Technology, Wageningen, NL). The parameters recorded during the experiments included latency, the path length of rats to reach the platform in meters (m), swimming velocity in cm/s, and time spent in the targeted quadrant. All experiments were conducted between 9:00 a.m. to 13:00 for 6 days [20].

MWMT was performed with 4 days of learning, with the first day of habituation, and a memory test on the last day. The visible platform (1.5 cm above the water surface) on the first day of the experiment, the hidden platform at 2-5 days (1.5 cm below the water surface), and the platform was not used on the 6th day. On the habituation day, the rats were released to the tank, facing the wall. The rat was expected to find the platform within 60 seconds. The rats that could not find the platform were guided by the researcher with the help of a plastic stick, and they were allowed to find the platform and stay on the platform for 5 seconds. In the learning days (2-5). The platform was fixed to the southeast quadrant. The rats were released into the water 4 times a day from 5 different locations and were expected to find the platform in 60 seconds. During the learning days, the time to find the platform (latency, sec), total distance traveled (cm) and swimming speed (cm/sec) were recorded and analyzed. On the 6th day, the last day of the experiment, the platform was removed from the water and the rats were released into the water with a single shot in the north direction, and recording was made for 60 s. At the end of this period, the ratio of the average time spent by the rats in the southeast quadrant, where the platform was previously located, to the total time was calculated.

Testing Anxiety Level Using the Open-Field Test

The open field test is used to measure locomotor activity and anxiety behavior of experimental animals entering a new environment [22]. The open-field apparatus (100 × 100 × 40 cm) was a black opaque plexiglass open area that was divided into the central zone and the peripheral zone using the software. On the test day, rats were placed in the center to explore the environment for 10 min. At the end of the test, the animal was returned to its home cage and the maze was thoroughly cleaned with 20 % ethanol. The total distance moved and time spent in the center was measured with the Noldus Ethovision tracking system. The room temperature was maintained at 21 ± 2 ◦C, and behavioral tests were performed at this temperature [23].

Determination of Hippocampal NR2A, NR2B, and BDNF Gene Expression Level

The rats in all groups were decapitated under ketamine xylazine anesthesia and their brain tissues were removed. The brain was placed in PBS (Phosphate Buffered Saline) solution and hippocampus tissue was isolated. Isolated right and left hippocampus tissues were stored at -80°C to determine gene expression levels by RT-PCR. Expression levels of NR2A, NR2B, and BDNF genes in the hippocampus were investigated.

Total RNA was isolated from the hippocampus tissues with RNeasy Lipid Tissue Kit (Qiagen Inc., Valencia, CA) / TRIZOL (Invitrogen). Isolated RNA was dissolved in an RNase-free solution for cDNA synthesis. 4 µl total RNA extraction was used as a template for the synthesis of cDNA. cDNA was obtained by using a High-Capacity cDNA Reverse Transcription Kit (LifeTech Cat. no. 4368814) Individual reactions were carried out using thermal conditions (Bioer, Gene Pro Thermal Cycler): 25 ◦C for 10 min, 37 ◦C for 120 min, 85 ◦C for 5 s, and 4 ◦C for 1 min. RT- PCR was run on an Applied Biosystems Viia 7 using Taqman GE Master Mix. β-actin was used as a housekeeping gene that is generally preferred in neurological re-

search. The reaction was carried out using 40 amplification cycles of 50 ◦C for 2 min, 95 ◦C for 10 min, 95 ◦C for 15 s, 60 ◦C, for 1 min, and 40 ◦C for 30 s. The relative expression of genes was calculated by the comparative 2–ΔΔCt method using peptidylprolyl isomerase A (PPIA) RNA levels as an internal control.

Statistical Analysis

Shapiro Wilk ($p > 0.05$) test examined conformity for normal distribution in each group. The data are expressed as mean values ± SD. For normally distributed data, a one-way analysis of variance (ANOVA) was conducted for the probe trial of the MWMT and gene expression followed by Tukey post hoc comparisons (significance determined as $p < 0.05$). Within-subject measurements, such as escape latencies, distance move, and velocity across trials (1., 2., 3., 4., days) in the MWMT, were analyzed using repeated-measures ANOVA. For each day in water maze testing, to test the difference between groups for variables (distance move, latency, and velocity), one-way analysis of variance (ANOVA) was conducted followed by Tukey post hoc comparisons.

RESULTS

The weights of the rats were measured weekly throughout the experimental procedure. There is no significant difference in weight between the groups ($F_{3.28} = 0.924$, $p = 0.444$). The amount of water consumed by the rats daily and the amount of feed consumed weekly were monitored. There was no significant difference between the groups in terms of the amount of water and feed consumed ($F_{3.28} = 2.829$, $p = 0.060$ and $F_{3.28} = 0.712$, $p = 0.554$; respectively).

Morris Water Maze Test

In the MWMT, there was a significant difference in the total distance move between the groups in the 4-day learning phase ($F_{1.28} = 26.958$, $p = 0.000$). There was a significant difference between days 1st compared to the 2nd and 3rd ($p < 0.001$, $p = 0.009$ and $p < 0.001$; respectively), and between days 4th compared to the 2nd and 3rd ($p = 0.001$ and $p < 0.001$, respectively). In the learning phase, the total distance traveled was decreased from the 1st to the 4th day.

There was a significant difference in the time spent

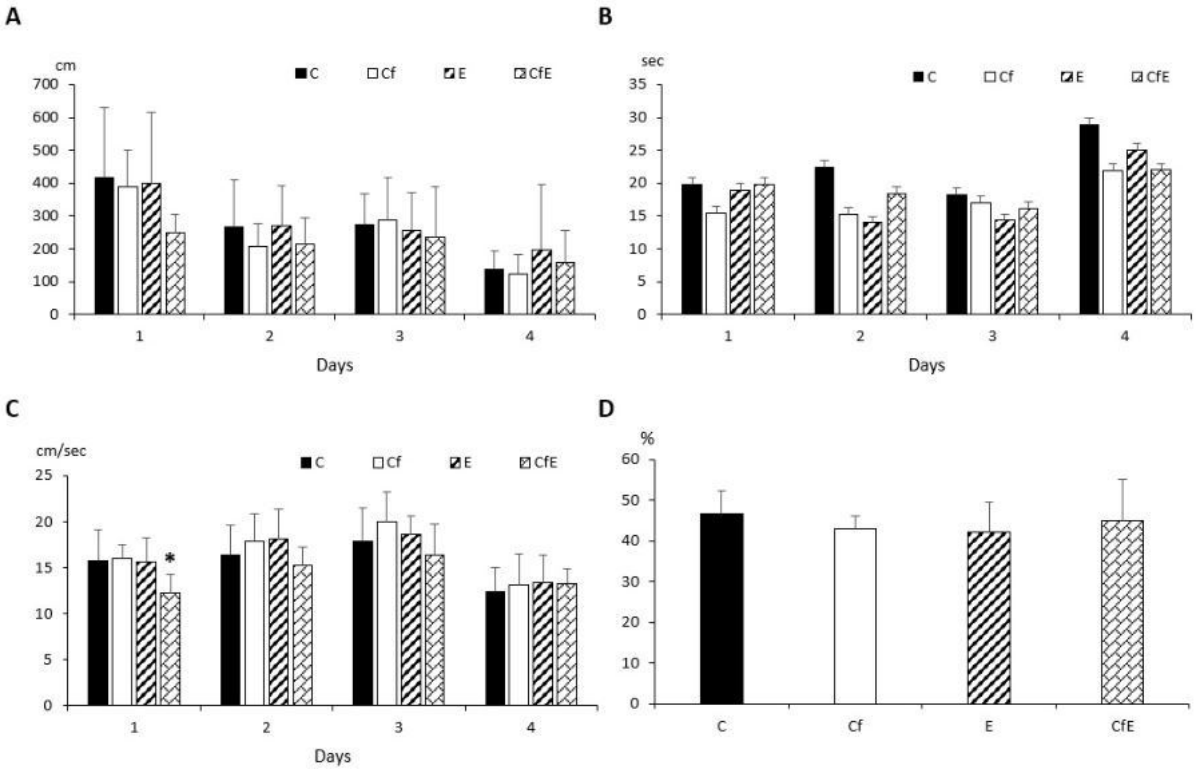

Fig. 1. Mean distance move (cm) (A), escape latency (sec) (B), swimming velocity (cm/sec) (C), and time spent in targeted quadrant (%) (D) in Morris Water Maze Test for C, Cf, E and CfE groups. All values represent the mean ± standard derivation from 7 male rats in each group. * $p < 0.05$, compared with CfE and C, Cf (C).

finding the platform between days. ($F_{1.28} = 5.768$, $p = 0.023$). The time required to find the platform was different from day 1st, 2nd and 3rd day compared to 4th ($p = 0.014$, $p = 0.002$ and $p < 0.001$; respectively). The time required to find the platform was increased on day 4 compared to other days.

There was a significant difference in the swimming speeds between days ($F_{1.28} = 4.360$, $p = 0.046$). Swimming speed has differed significantly between days 1st compared to the 2nd, 3rd and 4th ($p = 0.003$, $p < 0.001$ and $p = 0.009$; respectively), and between days 2nd compared to the 3rd and 4th ($p = 0.005$ and $p < 0.001$, respectively) and between 3rd and 4th days ($p < 0.001$). Swimming speed was decreased on the 4th day.

According to the between-group analysis, there was no significant difference in the total distance move and time to find the platform in the learning phase between groups ($p > 0.05$) (Figs. 1A and 1B). When the swimming speeds of the groups were compared in the learning phase, there was a significant difference only on the 1st day ($F_{3.28} = 3.779$, $p = 0.024$). According to the post-doc analysis, there was a significant difference between the CfE compared to the C and Cf ($p = 0.05$ and $p = 0.038$, respectively). On the 1st day of the learning phase, the swimming speed of the CfE was decreased (Fig. 1C).

When the percentage of time spent in the quadrant with the platform in the test phase was compared, there was no significant difference between the groups ($F_{3.28}= 0.524$, $p = 0.670$) (Fig. 1D).

Open Field Test

In the open field test, there was no significant difference in the total distance move between groups ($F_{3.28} = 2.482$, $p = 0.085$). There was a significant difference in the time spent in the center between groups ($F_{3.28} = 4.402$, $p = 0.014$). CfE spent less time in the center compared to the control group ($p = 0.009$) (Fig. 2A). There was a significant difference in the number of entries to the center between groups ($F_{3,28} = 6.122$, $p = 0.003$). The number of entries to the center was significantly different between the C compared to the E and the CfE. The number of entries was decreased in the E ($p = 0.023$) and CfE ($p = 0.003$) compared to the control group (Fig. 2B).

NR2A, NR2B, and BDNF Gene Expression Levels

NR2A, NR2B, and BDNF gene expression levels in rat hippocampus tissue were determined by the RT-PCR method. In the comparison between groups, there was no significant difference in NR2A ($F_{3.28} = 0.410$, $p = 0.748$), NR2B ($F_{3.28} = 0.017$, $p = 0.997$), and BDNF gene expression levels between the groups ($F_{3.28} = 0.723$, $p = 0.553$) (Figs. 3A, 3B and 3C).

DISCUSSION

Caffeine is an ergogenic supplement that is often preferred because of its stimulant feature in exercise applications, increasing the use of fatty acids and the thought that it will increase performance. In this investigation, the effects of caffeine supplementation on anxiety behavior, hippocampus learning memory function, and NR2A, NR2B, and BDNF levels in rats were examined in a manner that is comparable to the level of moderately taken caffeine in humans. Our results showed that caffeine administered during exer-

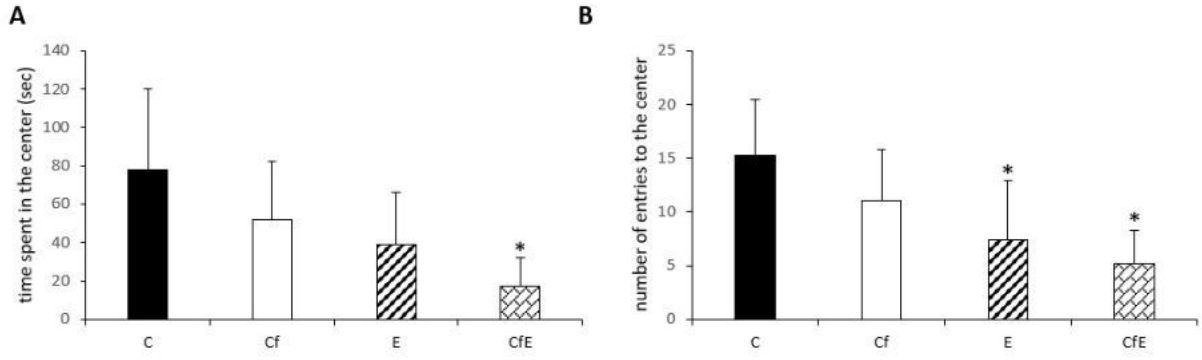

Fig. 2. Comparison of the time spent in the center between groups in the open field test (A). Comparison of the number of entries to the center between groups in the open field test (B). * ($p < 0.05$) compared to the control group. Values were given as mean ± standard deviation.

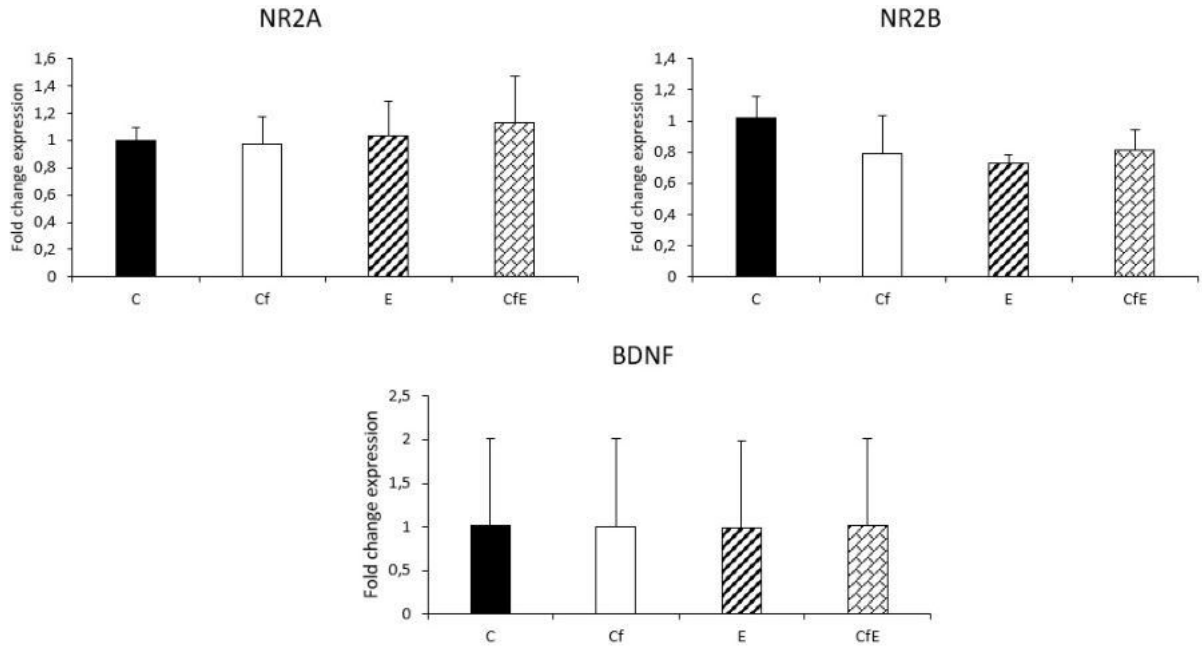

Fig. 3. Graphs showing the levels of fold change gene expression NR2A, NR2B and BDNF. Values were expressed as a percentage of the cage control value (100%) from five male rats in each group. Values were given as mean ± standard deviation; significance level was accepted as $p < 0.05$.

cise caused anxiety behavior but had no significant impact on learning, memory function, or hippocampus gene expressions.

Caffeine can regulate body weight since it is thought to increase energy consumption and affect thermogenesis, fat oxidation, and energy balance [24]. In the caffeine-treated group of obese rats fed a high-carbohydrate, high-fat diet, there was a reduction in body fat (0.5 g/kg caffeine in the final 8 weeks of the 16-week procedure) [25]. In another study, it was concluded that the body weights of rats fed both a high-fat diet and caffeine were not different from the group fed with standard feed and not taken caffeine [26]. In this study, it was determined that given caffeine and applied exercise did not affect the body weight of adolescent male rats. According to another study that supported the findings of the present investigation, caffeine did not affect body weight increase [27]. The effect of caffeine on body weight may be due to differences in administered caffeine dose, nutritional content, gender, body weight, or body fat composition. The response to caffeine may create gender-specific differences depending on circulating steroid hormones [28]. To better understand our findings, more specific studies using gender-specific and individual caffeine dosages are required.

The effects of caffeine on appetite and energy balance are conflicting. Some studies indicate that caffeine has a mild anorectic effect [29]. On the contrary, others demonstrate that caffeine increases appetite in a dose-dependent manner. Low-dose caffeine has been found to significantly increase appetite compared to high-dose caffeine [30]. Furthermore, it has also been demonstrated that caffeine does not affect appetite or food intake [31]. The results of our investigation showed that caffeine did not affect the feed and water intake of the rats.

Differences in caffeine dosage, dietary factors, gender, body weight, or body fat composition may all have an impact on how much caffeine affects body weight. Caffeine, which is widely consumed by humans, affects behavior [32]. Caffeine consumption, especially when excessive, might be problematic for sensitive people. In human tests, caffeine in high (400 mg/day) and moderate (200 mg/day) levels helped people remember more words than caffeine in low doses [33]; beneficial effects were also seen as a caffeine supplement sped up writing [34]. However, a study with university students revealed that caffeine harmed memory since the group that received caffeine remembered fewer words when they took the auditory-verbal learning test [35]. Caffeine administration

to postnatal rats has been proven in studies to have positive benefits on spatial learning [36]. In addition, it was noted that the offspring of rats exposed to caffeine (20 mg/kg twice a day) during pregnancy may suffer from cognitive damage [37]. This study revealed that caffeine did not affect learning and memory performance in MWMT. Similarly, it has been shown that caffeine does not affect learning performance in the new object recognition test [38]. In MWMT, it was found that caffeine treatment (0.3-10 mg/kg) after training increased memory retention while caffeine administration (0.3-10 mg/kg) before training did not affect the performance of the animals [39]. The study by Angelucci *et al*. [39] offers a different perspective on discrepancies in the literature by demonstrating that caffeine improves memory retention but not memory acquisition.

It is well established that physical activity has beneficial benefits on both learning and healthy aging processes. Exercise has been demonstrated to improve the hippocampus and learning and memory processes [40-43]. On the other hand, it has been noted that there is an inverse correlation between memory performance and the intensity of treadmill activity of different intensities [44]. In our study, although it was not statistically significant in MWMT, the time spent in the water to find the platform was the least and the swimming speed was the highest in the exercise group. Our results show that neither caffeine nor exercise had any discernible impact on cognitive function. Similar to our findings, research has demonstrated that caffeine (6 mg/day or 9 mg/day) and exercise have no impact on cognitive function [45], and exercise has no direct impact on cognition [46]. The fact that exercise must be done three days a week and is required may have prevented the potential benefits of exercise from being noticed. Additionally, it's possible that the rats' tolerance was brought on by the administration of caffeine in moderate dosages throughout the experiment.

The open field test is a procedure that assesses an animal's locomotor activity and anxiety by measuring the time they spend in the center and at the margins [47]. The results of a study examining the behavioral effects of regular caffeine consumption in adolescent male rats showed that low (0.1 mg/mL), medium (0.3 mg/mL), and high (1.0 mg/mL) dosages of caffeine had no influence on activity but did have anxiogenic effects, similar to those in our study [48]. In studies examining the effect of caffeine on behaviors like anxiety, the situations such as the dose of caffeine consumed and the sensitivity of the person to caffeine should also be taken into account. In a study, it was demonstrated that caffeine had a dose-dependent effect on anxiety; a high caffeine dose induced anxiety, whereas a low caffeine dose had no such effect [49]. Similarly, other studies have shown that high doses of caffeine cause anxiety symptoms such as inducing panic attacks in Parkinson's patients [50, 51]. Treadmill exercise reduces oxidative stress and anxiety-like behaviors in brain tissue in rats [52, 53]. On the other hand, voluntary wheel exercise was found to be ineffective in reducing anxiety-like behaviors in rats in the study by Jones *et al*. [54]. According to the findings of our study, the time spent in the center of the CfE in the open field test was found to be significantly decreased than the control. In addition, the number of entrances and exits to the center was found to be decreased in the E and CfE compared to the control. This result shows that caffeine alone does not cause anxiety, but there are signs of anxiety in exercise groups. The treadmill used for exercise and the compulsory of exercise may be the cause of anxiety in exercise groups.

NMDA receptors are known to play a role in cognitive functions [55]. It has been determined that chronic use of NR2A antagonists or caffeine supplementation starting at puberty prevents delayed memory deficit and related synaptotoxicity [56]. NMDA receptors have a curative effect on behavioral disorders brought on by caffeine withdrawal [57]. In our study, withdrawal did not occur in rats due to caffeine administration until the experiment was over and there were no alterations in the NMDA receptors. Additionally, there are also conclusions that NMDA-type glutamate receptors do not play a significant role in mediating the locomotor stimulating effects of caffeine or its tolerance to these effects [58].

Several studies are showing that exercise can activate NMDA receptors in the hippocampus [59-61]. The negative effects of maternal stress on depressive-like behaviors in adult rats are mitigated by voluntary wheel exercise during adolescence; stressed rats exhibit an increase in the expression of the NR2A subunit of NMDA receptors in the hippocampus, and its antidepressant-like effects can reduce NR2A expression [62]. In our study, neither caffeine nor exercise changed hippocampal NR2A and NR2B gene expres-

sion levels. These results suggest that wheel exercise may activate NMDA receptors in the hippocampus, which in turn may increase BDNF production and neurogenesis [63].

According to certain research, caffeine improves memory functions by increasing BDNF levels in the hippocampus [64, 65]. On the other hand, exposure to caffeine (20 mg/day twice daily) during pregnancy has been observed to result in decreased BDNF levels in the offspring [37]. Although voluntary chronic exercise is known to increase BDNF levels in rats, the effects of compulsive wheel exercise are not clear. The intensity of the run is another component that contributes to this. In a study where acute wheel exercise was used at different running intensities, it was found that low-intensity (15 m/min) wheel exercise which generates minimum stress, can increase BDNF and hippocampus functioning in comparison to more severe versions [66]. It has been observed that compulsory exercise (starting with a 3-minute warm-up at 8 m/min and gradually increasing to 12 m/min for 30 minutes on training days after 10 m/min in 10 minutes in the first sessions) which were subjected to pregnant rats increased hippocampal BDNF levels [67]. Additionally, it was found that moderate-intensity treadmill activity (2 weeks, 20 minutes per day) had no effect on the BDNF levels in the regions of the brain under investigation [68]. Our findings on hippocampus NR2A, NR2B, and BDNF appear to be in line with the conclusion that MWMT does not affect memory and learning. A moderate dose of caffeine and compulsory exercise does not affect learning and memory performance and related gene expression level either alone or together.

CONCLUSION

According to our findings, chronic exposure to caffeine and compulsory exercise in the early life do not have a significant effect on learning and memory functions. Caffeine and compulsive exercise appear to have a major effect on anxiety behavior. In our study result of, caffeine supplementation and exercise have no effect on learning and memory behavior in MWMT and no effect on learning-related genes in the hippocampus are consistent findings with each other. The chronic administration of moderate doses of caffeine and the obligatory 3 days per week of exercise are thought to be the main factors affecting the results of the study. As a result, it is noteworthy that the interactions between caffeine and exercise are unpredictable and the complexity of their effects on memory functions. In order to eliminate the acute effect of exercise after chronic exercise, tests should be performed at least 24 hours after the last application. In conclusion, to better understand how caffeine and exercise affect memory and learning, more studies are required that simultaneously address a variety of variables, including caffeine dose, chosen age group, gender, exercise to be performed (compulsory/voluntary), exercise duration, tests to be subjected, and other parameters.

Authors' Contribution

Study Conception: SMK, FB; Study Design: SMK, FB, LS; Supervision: SMK; Funding: SMK; Materials: SMK, FB, OSC, LS; Data Collection and/or Processing: SMK, FB, LS, OSC; Statistical Analysis and/or Data Interpretation: SMK, FB, LS; Literature Review: SMK, FB; Manuscript Preparation: SMK, FB, OSC, LS and Critical Review: SMK, LS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Barry RJ, Rushby JA, Wallace M, Clarke AR, Johnstone SJ, Zlojutro I. Caffeine effects on resting-state arousal. Clin Neurophysiol 2005;116:2693-700.

2. Bonita JS, Mandarano M, Shuta D, Vinson J. Coffee and cardiovascular disease: invitro, cellular, animal, and human studies. Pharmacol Res 2007;55:187-98.
3. Mitchell DC, Knight CA, Hockenberry J, Teplansky R, Hartman TJ. Beverage caffeine intakes in the U.S. Food Chem Toxicol 2013;63:136-42.
4. Alhaider IA, Alkadhi KA. Caffeine treatment prevents rapid eye movement sleep deprivation-induced impairment of late-phase long-term potentiation in the dentate gyrus. Eur J Neurosci 2015;42:2843-50.
5. Oliveira RV, Dall'Igna OP, Tort AB, Schuh JF, Neto PF, Santos Gomes MW, et al. Effect of subchronic caffeine treatment on MK-801-induced changes in locomotion, cognition and ataxia in mice. Behav Pharmacol 2005;16:79-84.
6. Sun YL, Wang J, Yao JX, Ji CS, Dai Q, Jin YH. Physical exercise and mental health: cognition, anxiety, depression and self-concept. Sheng Li Ke Xue Jin Zhan 2014;45:337-42.
7. Türkiye Endokrinoloji ve Metabolizma Derneği (TEMD)-2020. Tıbbi beslenme ve egzersiz metabolizması kılavuzu. 2021. Available at: https://file.temd.org.tr/Uploads/publications/guides/documents/20210104143105-2021tbl_kilavuzada2d60e7b.pdf. Accessed January 23, 2023.
8. Collins A, Hill LE, Chandramohan Y, Whitcomb D, Droste SK, Reul JM. Exercise improves cognitive responses to psychological stress through enhancement of epigenetic mechanisms and gene expression in the dentate gyrus. PLoS One 2009;4:e4330.
9. Park JK, Lee SJ, Kim TW. Treadmill exercise enhances NMDA receptor expression in schizophrenia mice. J Exerc Rehabil 2014;10:15-21.
10. Dietrich MO, Mantese CE, Porciuncula LO, Ghisleni G, Vinade L, Souza DO, et al. Exercise affects glutamate receptors in postsynaptic densities from cortical mice brain. Brain Res 2005;1065:20-5.
11. Zhang L, Qin Z, Sharmin F, Lin W, Ricke KM, Zasloff MA, et al. Tyrosine phosphatase PTP1B impairs presynaptic NMDA receptor-mediated plasticity in a mouse model of Alzheimer's disease. Neurobiol Dis 2021;156:105402.
12. Leal G, Afonso PM, Salazar IL, Duarte CB. Regulation of hippocampal synaptic plasticity by BDNF. Brain Res 2015;1621:82-101.
13. Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 2002;25:295-301.
14. Loprinzi PD, Frith EA. Brief primer on the mediational role of BDNF in the exercise-memory link. Clin Physiol Func Imaging 2019;39:9-14.
15. Ganio MS, Klau JF, Casa DJ, Armstrong LE, Maresh CM. Effect of caffeine on sport-specific endurance performance: a systematic review. J Strength Cond Res 2009;23:315-24.
16. Tunnicliffe JM, Erdman KA, Reimer RA, Lun V, Shearer J. Consumption of dietary caffeine and coffee in physically active populations: physiological interactions. Appl Physiol Nutr Metab 2008;33:1301-10.
17. França AP, Schamne MG, Souza BS, Luz Scheffer D, Bernardelli AK, Corrêa T, et al. Caffeine consumption plus physical exercise improves behavioral impairments and stimulates neuroplasticity in spontaneously hypertensive rats (SHR): an animal model of attention deficit hyperactivity disorder. Mol Neuro 2020;57:3902-19.
18. Metro D, Cernaro V, Santoro D, Papa M, Buemi M, Benvenga S, et al. Beneficial effects of oral pure caffeine on oxidative stress. J Clin Trans Endocrinol 2017;10:22-7.
19. Keloglan SM, Sahin L, Cevik OS. Chronic caffeine consumption improves the acute sleep deprivation-induced spatial memory impairment while altering N-methyl-D-aspartate receptor subunit expression in male rats. Int J Dev Neurosci 2022;82:596-605.
20. Han ME, Park KH, Baek SY, Kim BS, Kim JB, Kim HJ, et al. Inhibitory effects of caffeine on hippocampal neurogenesis and function. Biochem Biophys Res Commun 2007;356:976-80.
21. Batista DF, Gonçalves AF, Rafacho BP, Okoshi K, Paiva SAR, Zornoff LAM. Delayed rather than early exercise training attenuates ventricular remodeling after myocardial infarction. Int J Cardiol 2013;170:3-4.
22. Küçük A, Gölgeli A. [Anxiety models in experimental animals and evaluation of anxiety]. Sağlık Bilim Derg 2005;14:209-17. [Article in Turkish]
23. Li C, Liu Y, Yin S, Lu C, Liu D, Jiang H, et al. Long-term effects of early adolescent stress: dysregulation of hypothalamic-pituitary-adrenal axis and central corticotropin releasing factor receptor 1 expression in adult male rats. Behav Brain Res 2015;288:39-49.
24. Harpaz E, Tamir S, Weinstein A, Weinstein Y. The effect of caffeine on energy balance. J Basic Clin Physiol Pharmacol 2017;28:1-10.
25. Panchal SK, Wong WY, Kauter K, Ward LC, Brown L. Caffeine attenuates metabolic syndrome in diet-induced obese rats. Basic Nutr Invest 2012;28:1055-62.
26. Moy GA, McNay EC. Caffeine prevents weight gain and cognitive impairment caused by a high-fat diet while elevating hippocampal BDNF. Physiol Behav 2013;109:69-74.
27. Pettenuzzo LF, Noschang C, Toigo EP, Fachin A, Vendite D, Dalmaz C. Effects of chronic administration of caffeine and stress on feeding behavior of rats. Physiol Behav 2008;95:295-301.
28. Temple JL, Ziegler AM. Gender differences in subjective and physiological responses to caffeine and the role of steroid hormones. J Caffeine Res 2011;1:41-48.
29. Moore RHS, Franko DL, Thompson D, Barton B, Schreiber GB, Daniels SR. Caffeine intake in eating disorders. J Eat Disord 2006;39:162-5.
30. Sweney P, Levack R, Watters J, Xu Z, Yang Y. Caffeine increases food intake while reducing anxiety-related behaviors. Appetite 2016;101:171-7.
31. Correa M, Miguel NS, Lopez-Cruz L, Carratala-Ros C, Olivaras-Garcia R, Salamone JD. Caffeine modulates food ıntake depending on the context that gives access to food: comparison with dopamine depletion. Front Psychiatry 2018;9:411.
32. Smith A. Effects of caffeine on human behavior. Food Chem Toxicol 2002;40:1243-55.
33. Loke WH. Effects of caffeine on mood and memory. Physiol Behav 1988;44:367-72.
34. Traxler PA, Saho RJ, Wistner MB. The acute effects of caffeine on reaction time, memory performance, and reaction time. Onu Student Res Colloquium 2022;43.

35. Terry WS, Phifer B. Caffeine and memory performance on the AVLT. Psychodynamics and Psychopathology. J Clin Phychol 1986;42:863-3.
36. Singh AB, Xu Y, Wang H, Kumar VHS. The beneficial effects of postnatal caffeine on spatial learning in adult mice J Caffeine Adenosine Res 2009;9:64-8.
37. Li Y, Zhang W, Shi R, Sun M, Zhang L, Li Na, et al. Prenatal caffeine damaged learning and memory in rat offspring mediated by ARs/PKA/CREB/BDNF pathway. Physiol Res 2018;67:975-83.
38. Turgeon SM, Townsend SE, Dixon RS, Hickman ET, Lee SM. Chronic caffeine produces sexually dimorphic effects on amphetamine-induced behavior, anxiety and depressive-like behavior in adolescent rats. Pharmacol Biochem Behav 2016;143:26-33.
39. Angelucci MEM, Cesário C, Hiroi RH, Rosalen PL, Cunha CD. Effects of caffeine on learning and memory in rats tested in the Morris water maze. Braz J Med Biol Res 2002;35:1201-8.
40. Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 2005;25:8680-5.
41. Grace L, Hescham S, Kellaway LA, Bugarith K, Russsell VA. Effect of exercise on learning and memory in a rat model of developmental stress. Metab Brain Dis 2009;24:643-57.
42. Alaei HA, Moloudi R, Sarkaki AR. Effects of treadmill running on mid-term memory and swim speed in the rat with Morris water maze test. J Bodyw Mov Ther 2008;12:72-5.
43. Albeck DS, Sano K, Prewit GE, Dalton L. Mild forced treadmill exercise enhances spatial learning in the aged rat. Behav Brain Res 2006;168:345-8.
44. Wang XQ, Wang WG. Effects of treadmill exercise intensity on spatial working memory and long-term memory in rats. Life Sci 2016;149:96-103.
45. Hogervorst E, Bandelow S, Schmitt J, Jentjens R, Oliveira M, Allgrove J, et al. Caffeine improves physical and cognitive performance during exhaustive exercise. Med Sci Sports Exerc 2008;40:1841-51.
46. Brown BA, Frost N, Rainey-Smith SR, Doecke J, Markovic S, Gordon N, et al. High-intensity exercise and cognitive function in cognitively normal older adults: a pilot randomised clinical trial. Alzheimers Res Ther 2021;13:33.
47. Prut I, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463:3-33.
48. Ardais AP, Borges MF, Rocha AS, Sallaberry C, Cunha RA, Porciuncula LO. Caffeine triggers behavioral and neurochemical alterations in adolescent rats. Neurosci 2014;270:27-39.
49. Childs E, Hohoff , Deckert J, Xu K, Badner J, Wit H. Association between ADORA2A and DRD2 polymorphisms and caffeine-induced anxiety. Neuropsychopharmacol 2008;33:2791-800.
50. Klevebrant L, Frick A. Effects of caffeine on anxiety and panic attacks in patients with panic disorder: a systematic review and meta-analysis. Gen Hosp Psychiatry 2022;74:22-31.
51. Anderson NL, Hughes N. Increased emotional reactivity in rats following exposure to caffeine during adolescence. Neurotoxicol Teratol 2008;30:195-201.
52. Seo JH. Treadmill exercise alleviates stress-induced anxiety-like behaviors in rats. J Exerc Rehabil 2018;14:724-30.
53. Patki G, Li L, Allam F, Solanki N, Dao AT, Alkadhi K, et al. Moderate treadmill exercise rescues anxiety and depression-like behavior as well as memory impairment in a rat model of post-traumatic stress disorder. Physiol Behav 2014;130:47-53.
54. Jones AB, Gupton R, Curtis KS. Estrogen and voluntary exercise interact to attenuate stress-induced corticosterone release but not anxiety-like behaviors in female rats. Behav Brain Res 2016;311:279-86.
55. Hansen KB, Yi F, Perszyk RE, Furukawa H, Wollmuth LP, Gibb AJ, et al. Structure, function, and allosteric modulation of NMDA receptors. J Gen Physiol 2018;150:1081-105.
56. Cognato GP, Agostinho PM, Hockemeyer J, Müller CE, Souza DO, Cunha RA. Caffeine and an adenosine A(2A) receptor antagonist prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life. J Neurochem 2010;112:453-62.
57. Sukhotina IA, Zvartau EE, Danysz W, Bespalov AY. Caffeine withdrawal syndrome in social interaction test in mice: effects of the NMDA receptor channel blockers, memantine and neramexane. Behav Pharmacol 2004;15:207-14.
58. Powel KR, Holtzman SG. Lack of NMDA receptor involvement in caffeine-induced locomotor stimulation and tolerance in rats. Pharmacol Biochem Behav 1998;59:433-8.
59. Yu Q, Li X, Wang J, Li Y. Effect of exercise training on long term potentiation and NMDA receptor channels in rats with cerebral infarction. Exp Ther Med 2013;6:1431-6.
60. Ke Z, Hu S, Cui W, Sun J, Zhang S, Mak S, et al. Bis(propyl)-cognitin potentiates rehabilitation of treadmill exercise after a transient focal cerebral ischemia, possibly via inhibiting NMDA receptor and regulating VEGF expression. Neurochem Int 2019;128:143-53.
61. Şahin L, Çevik SÖ, Koyuncu DD, Kocahan S. Caffeine as a potential arousal enhancer: altered NMDA subunit gene expression without improving cognitive performance in REM sleep deprived rats. Cell Mol Biol 2019;65:63-8.
62. Masrour FF, Peeri M, Azarbayjani M, Hosseini MJ. Voluntary exercise during adolescence mitigated negative the effects of maternal separation stress on the depressive-like behaviors of adult male rats: role of NMDA receptors. Neurochem Res 2018;43:1067-1074.
63. Kitamura T, Mishina M, Sugiyama H. Enhancement of neurogenesis by running wheel exercises is suppressed in mice lacking NMDA receptor epsilon 1 subunit. J Neurosci Res 2003;47:55-63.
64. Costa MS, Botton PH, Mioranzza S, Ardais AP, Moreira JD, Souza DO, et al. Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immunocontent in the hippocampus. Neurochem Int 2008;53:89-94.
65. Sallaberry C, Nunes F, Costa MS, Fioreze GT, Ardais AP, Botton PHS, et al. Chronic caffeine prevents changes in inhibitory avoidance memory and hippocampal BDNF immunocontent in middle-aged rats. Neuropharmacol 2012;64:153-9.
66. Soya H, Nakamura T, Deocaris CC, Kimpara A, Iimura M, Fujikawa T, et al. BDNF induction with mild exercise in the rat

hippocampus. Biochem Biophys Res Commun 2007;358:961-7.
67. Akhavan MM, Gorji HM, Abarghoie ME, Safari M, Moghaddam BS, Vafaei AA, et al. Maternal voluntary exercise during pregnancy enhances the spatial learning acquisition but not the retention of memory in rat pups via a TrkB-mediated mechanism: the role of hippocampal BDNF expression. Iran J Basic Med Sci 2013;16:955-61.
68. Cechetti F, Fochesatto C, Scopel D, Nardin P, Gonçalves CA, Netto CA, et al. Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. Brain Res 2008;1188:182-8.

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Postoperative changes in nonspecific low back pain after atlantoaxial stabilization surgery

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ABSTRACT

Objectives: Posterior atlantoaxial stabilization can be technically challenging. Postoperative C2 nerve dysfunction occurs as a complication of this procedure or when a lateral mass is exposed and sacrificed for screw placement. Patients with cervical pathology sometimes complain of low back pain simultaneously. In this study, we aimed to investigate the results of C1-C2 posterior stabilization and C2 nerve root sacrifice on postoperative lumbar complaints.

Methods: Twenty-six patients who underwent multicentric posterior C1-C2 stabilization due to atlantoaxial dislocation were retrospectively included in the study. Those that were not sacrificed the C2 nerve root during stabilization were considered group 1 (n = 12), and those that were sacrificed the C2 nerve root were considered group 2 (n = 14). A visual Analogue Scale (VAS) was used for the neck, arm, low back, and leg pain. The EQ-5D Index was used as the overall quality of life scale.

Results: Six months postoperatively, both groups showed significant ($p < 0.01$) improvement in VAS neck, arm, and low back scores between preoperative and postoperative. In addition, there was no significant improvement in the VAS leg and EQ-5D score in either of the groups. While no significant decrease was found in occipital neuralgia in group 1, it showed significant ($p < 0.01$) improvement in occipital neuralgia in group 2.

Conclusions: Although the exact pathophysiology is unknown, cervical stabilization surgery and sacrification of the C2 nerve root in this surgery can, directly and indirectly, improve low back pain.

Keywords: Cervical vertebra, cervical instability, C2 nerve root, low back pain

C1 and C2 are unique in their anatomical and functional differences that do not conform to the typical features of vertebrae (atypical vertebrae). In addition, the C1 and C2 vertebrae cover the entire brain stem. Therefore, it is an essential bone for the survival and functionality of the human system. Although trauma is the most common cause of atlantoaxial instability, rheumatological diseases, inflammatory diseases, malignancy, and congenital malformations cause instability [1]. In atlantoaxial joint instability, most cases require C1-C2 fixation to preserve vertebral column alignment and prevent neuronal compression. Atlantoaxial stabilization is technically challenging due to the increased range of motion and anatomical relationships. Harms developed Goel's work on atlantoaxial screw fixation in 2001 and de-

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scribed the posterior C1-C2 stabilization technique using polyaxial screws. This technique was named the "Goel-Harms method" [2]. Posterior C1-C2 is an important structure in stabilization because there are vascular plexuses around the C2 nerve root and they pass under the posterior arch of C1. Preservation of the C2 nerve root in stabilization complicates the operation and impairs the quality of life by causing entrapment neuropathy. With C2 neurectomy, C1 screws are placed much more easily and perioperative blood loss is reduced. However, sensory deficits and occipital ulcers can be seen due to C2 neurectomy. Therefore, the clinical benefits of sacrificing the C2 nerve are still controversial [3, 4].

Animal studies have shown connections between neurons of the C2 spinal cord and the thalamus, hypothalamus, anterior cingulate cortex, and amygdala. Thus, the C2 spinal cord segment shows that it is directly connected to most areas of the pain matrix [5, 6].

Studies have shown that decompression of the cervical canal can improve patients' symptoms of myelopathy or radiculopathy. Studies are showing that only cervical surgery can lead to improvement in both cervical and lumbar symptoms as well as the overall quality of life [7-9].

However, there is no study between the C2 nerve root and lumbar symptoms. Accordingly, in this study, we investigated the effect of sacrificing the C2 nerve root in posterior C1-C2 stabilization on both cervical and lumbar symptoms as well as the overall quality of life.

METHODS

Patient Population

This study was approved by the İstinye University ethics committee (3/2022.G-180). 26 patients (between 18-65 years old) who underwent posterior C1-C2 stabilization due to atlantoaxial dislocation, and who were treated at our hospital between January 2015 and January 2022, were included in the retrospective study. Patients with terminal cancer, multiple traumas, chronic organ failure, patients with a history of spinal surgery, and patients with chronic rheumatic disease were excluded from the study.

Hospital electronic medical records, patients with posterior C1-C2 stabilization, and lumbar complaints were retrospectively reviewed. After identification, demographic, clinical, and radiological information about each patient was collected. Cervical and lumbar pathologies were evaluated with magnetic resonance imaging (MRI) preoperatively, both clinically and radiographically.

Study Design

The patients were divided into two groups: those who did not sacrifice the C2 nerve root in C1-C2 stabilization surgery were named group 1; those who sacrificed the C2 nerve root in C1-C2 stabilization surgery were named group 2.

The Visual Analogue Scale (VAS) was used for the neck, arm, low back, and leg pain, and the EQ-5D Index was used as the overall quality of life scale. VAS pain score was evaluated as "painless" (score = 0) and

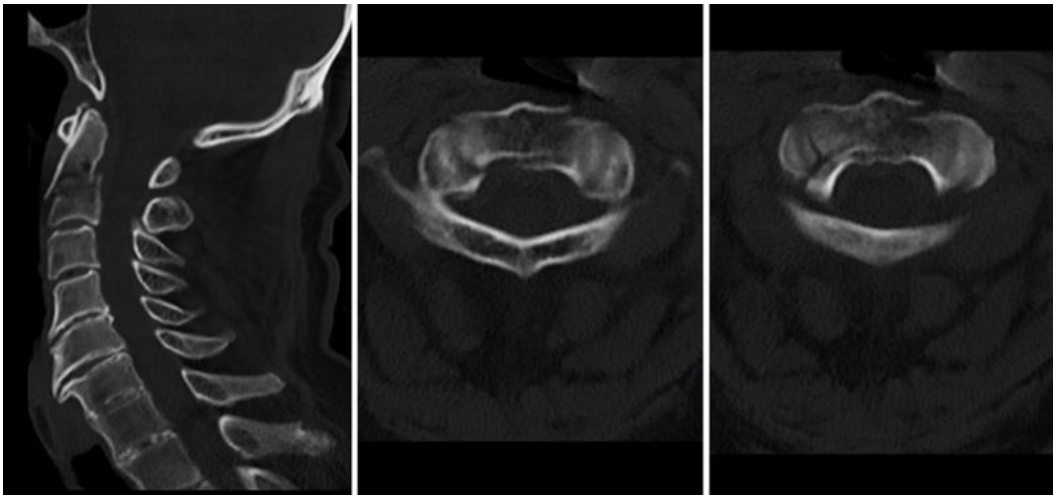

Fig. 1. Preoperative cervical CT images of a patient who has a C2 vertebral fracture.

"worst pain" (score = 10).

EQ-5D Index calculates an index score ranging from -0.59 to 1 from 5 dimensions of the scale. A value of 1 indicates perfect health, while negative values indicate conditions such as unconsciousness, and confinement to bed.

All patients were operated on in the prone position by experienced spinal surgeons using the Mayfield® nail head restraint. Posterior C1-C2 fusion was performed using the standard posterior midline approach and the Goel-Harms technique. Confirmed using fluoroscopy (Figs. 1 and 2). Operational techniques such as mobilization and preservation of the C2 nerve root by retraction (group 1) or sacrificing the C2 nerve root (Fig. 3) by deliberate cauterization with bipolar (group 2) were applied.

Both groups were mobilized with the postoperative cervical orthosis and discharged. Patients received drug therapy (eg, nonsteroidal anti-inflammatory drugs, weak opioids, muscle relaxants) and physiotherapy during follow-up. The patients were evaluated in terms of VAS, EQ-5D Index values, and occipital neuralgia at preoperative, postoperative 1st, and 6th months.

Statistical Analysis

Demographic variables among cohorts were disaggregated using Fisher's test. Continuous data between cohorts were compared with Student's t-test and post hoc paired t-tests using one-way ANOVA analyses and Tukey's test. All p values < 0.01 were considered statistically significant to adjust with multiple comparisons.

RESULTS

Study Population

Analysis was performed with 26 patients with at least 6 months of follow-up. Twelve patients in group 1 and 14 patients in group 2 were included in the study. The mean age of the patients was 49.7 (age range: 18-65

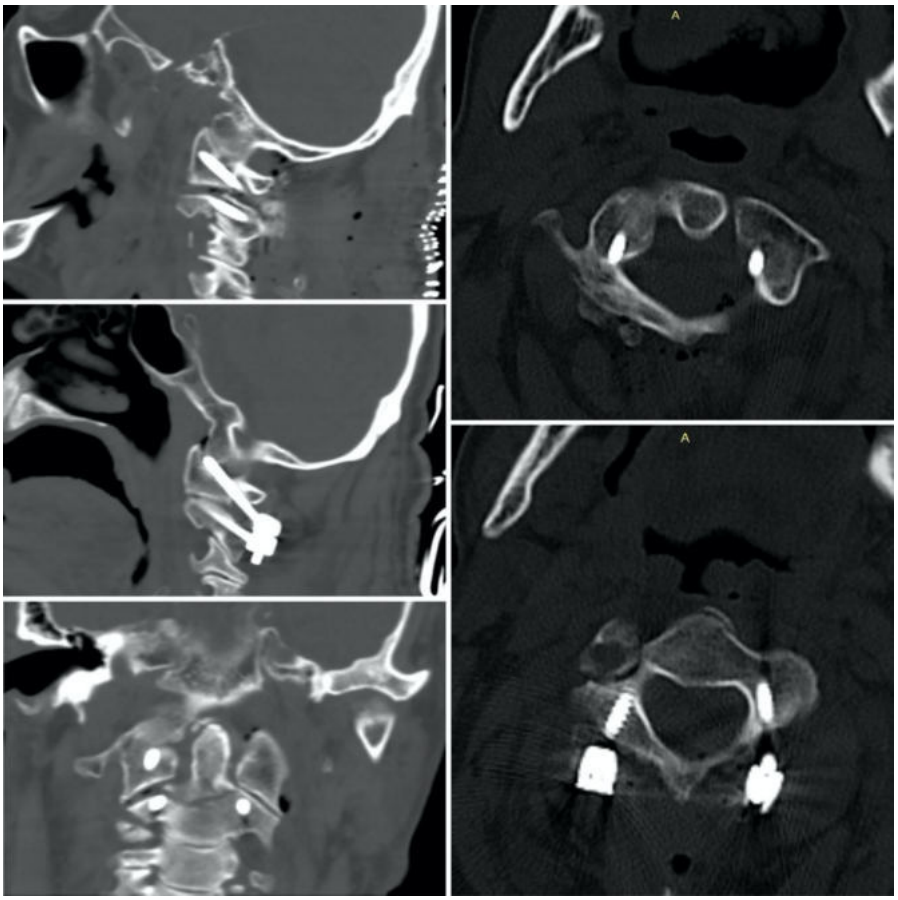

Fig. 2. Postoperative cervical CT images of a patient who has a C2 vertebral fracture.

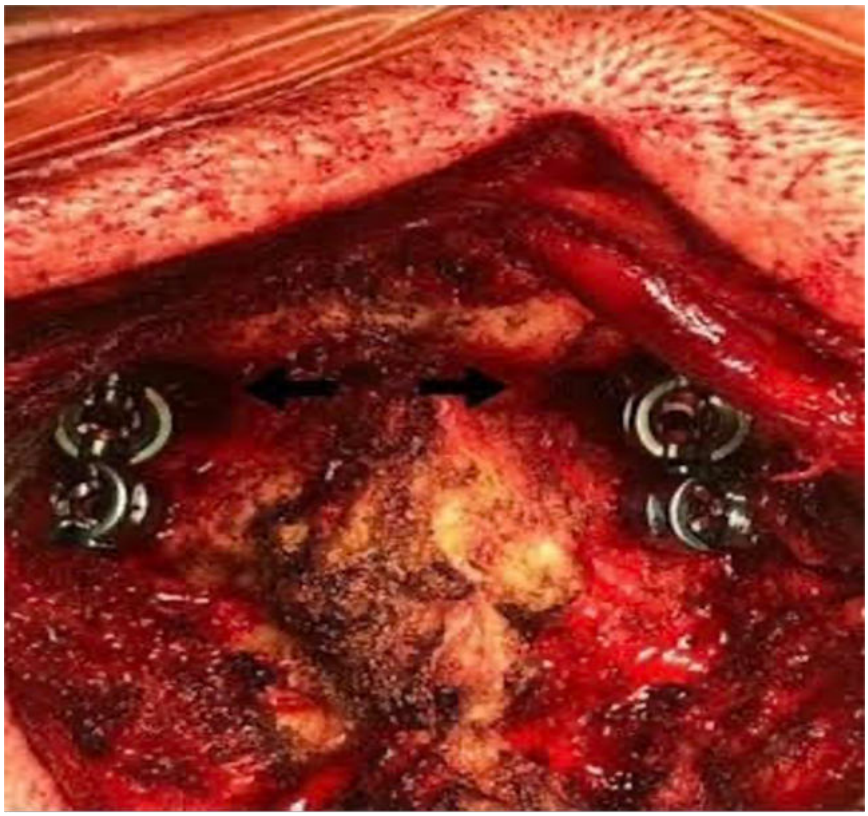

Fig. 3. Sacrified C2 nerve root is indicated by arrows.

years). Nine (34.6%) of the patients were female and 17 (65.4%) were male. The demographic and clinical data of both groups are summarized in Table 1. While there was no statistically significant difference in age ($p = 0.17$), the difference was statistically significant in terms of gender ($p < 0.01$). The mean hospital stay was 6.2 days (4-12) in group 1 and 5.9 days (3-18) in group 2. The difference was not statistically significant ($p = 0.34$).

The most common etiology for the operation of C1-C2 fusion was trauma (84.6%), the most common indication for instrumentation was type 2 dens fracture (68%), and the most common symptom was axial (mechanical) neck pain (87.3%). The most common pathology in the lumbar was degenerative disc disease (69.2%). The most common symptom was low back pain (94.2%).

Posterior C1-C2 fusion was applied to all patients using the Goel-Harms technique. Screws were placed properly in instrumentation in all patients on postoperative imaging. In three patients, the C2 pars screw breached the vertebral artery foramen without complications. No patient had evidence of instrumentation failure or nonunion based on postoperative routine standard standing radiograms and computed tomography scans. No special treatment (medication or physiotherapy) was applied to any patient due to lumbar pathology.

Patient Characteristics

There was a significant decrease in VAS Arm (3.6, 3.3), VAS Neck (3.7, 3.1), and VAS Low Back (3.1, 2.6) in the early postoperative period. Despite this, there was no significant decrease in VAS Leg (3.1, 2.5), even a slight increase in group 1 (Table 2). However, these decreases in VAS scores for both groups were statistically significant ($p < 0.01$). The changes in the preoperative and postoperative 6th-month scores of the patients were as follows. VAS Arm (-2.4, -3.1), VAS Neck (-3.7, -4.3), VAS Low Back (-0.3, -0.9), VAS Leg (0.1, -0.2) and EQ-5D (0, 0.01). At the end of 6 months, VAS was found to be statistically significant in both surgical cohorts ($p < 0.01$). However, there was no statistically or clinically significant im-

Table 1. Patient Characteristics

	Group 1 (n = 12)	Group 2 (n =14)	*p* value*
Age (years)	47.3 ± 10.6	51.7 ± 6.7	0.17
Female sex, n (%)	4 (33.3)	5 (35.7)	0.01
Smoker, n (%)	5 (41.6)	6 (42.8)	0.73
Diabetic, n (%)	4 (33.3)	5 (35.7)	0.64
BMI	25.7 ± 6.3	26.1 ± 1.4	0.68
Cervical disease type, n (%)			
Trauma	10 (83.3)	12 (85.7)	–
Degenerative	2 (16.7)	2 (14.3%)	
Lumbar disease type, n (%)			
DDD	8 (66.7)	10 (71.4)	–
LSS	4 (33.3)	4 (28.6)	

Data are shown as mean ± standard deviation or n (%).
BMI = Body Mass Index, LSS = lumbar spinal stenosis, DDD = degenerative disc disease

provement in EQ-5D ($p = 0.42$).

While 3 patients (25%) had occipital neuralgia preoperatively in group 1, occipital neuralgia was seen in 3 patients (21.4%) in group 2. In the office visits made 1 month and 6 months after the operation, 4 (33.3%) of 12 patients in group 1 complained of occipital neuralgia, while 1 (7.1%) of 14 patients in group 2 complained of occipital neuralgia.

The difference between the two groups in terms of occipital neuralgia was statistically significant ($p < 0.01$). While only 2 (12.5%) of 12 patients in postoperative group 1 had occipital numbness, 4 (33.3%) of 14 patients in group 2 had occipital numbness. While 1 (8.3%) of these patients in group 1 had C2 paresthesia, 3 (21.4%) in group 2 had C2 paresthesia. During the follow-up, none of the patients had earache, speech, or swallowing difficulties. After 6 months, 1 patient (8.3%) in group 1 and 4 patients (28.6%) in group 2 complained of symptomatic drowsiness.

No patients in either group had postoperative neurological deterioration, CSF leakage, or vascular injury. A deep wound infection developed in one patient, intravenous antibiotic therapy was given, and the instrumentation was removed. Another patient developed a superficial subcutaneous infection that resolved with intravenous antibiotics. No patient developed neuropathic ulcers in the occipital region.

DISCUSSION

Sacrificing the C2 nerve root in C1-C2 stabilization has advantages such as increasing the adequate visualization of the C1 facets [10], reducing blood loss, and reducing the operation time secondary to avoiding C2 dissection [11, 12]; It also has disadvantages such as developing dysesthesia in the C2 dermatome area or causing postoperative numbness in the occiput and retro auricular areas. Few researchers have examined the effects of the C2 nerve in C1-C2 instrumentation, and although some studies have been reported on this topic, it is still unclear whether it affects patients' quality of life [11-16].

The primary aim of this study was to demonstrate the effect of the sacrification of the C2 nerve root in atlantoaxial instrumentation on quality of life and postoperative changes in low back pain. Although VAS Low Back decreased in both groups, it decreased significantly in group 2.

Studies suggest that the prevalence of both cervical and lumbar lesions (Degenerative disc disease, spinal stenosis) is high. Its prevalence was found to be 1-5.5 percent in studies with cadavers [17]. Its prevalence was found to be 11% in a study with random MRI in the patient population [18]. In some studies, the prevalence of its association was found to be 9-

Table 2. Quality of Life Outcomes at 6 months follow-up

	Group 1	Group 2	*p* value
VAS Arm			
Pre-op	5.7 ± 1.5	5.9 ± 2.2	
Post-op/1m	3.6 ± 2.9	3.3 ± 2.6	< 0.01
Post-op/6m	3.3 ± 3.0	2.8 ± 2.5	
VAS Neck			
Pre-op	6.8 ± 2.7	6.7 ± 2.2	
Post-op/1m	3.7 ± 1.9	3.1 ± 2.5	< 0.01
Post-op/6m	3.1 ± 3.1	2.4 ± 1.8	
VAS Low Back			
Pre-op	3.2 ± 3.1	3.4 ± 2.6	
Post-op/1m	3.1 ± 2.5	2.6 ± 2.2	< 0.01
Post-op/6m	2.9 ± 3.1	2.5 ± 2.8	
VAS Leg			
Pre-op	2.9 ± 2.5	2.7 ± 2.5	
Post-op/1m	2.8 ± 2.9	2.5 ± 2.3	< 0.01
Post-op/6m	3.0 ± 3.0	2.5 ± 2.1	
EQ-5D Index			
Pre-op	0.52 ± 0.21	0.54 ± 0.2	
Post-op/1m	0.53 ± 0.18	0.55 ± 0.17	0.42
Post-op/6m	0.52 ± 0.19	0.55 ± 1.8	
Occ Neur, n (%)			
Pre-op	3 (25)	3 (21.4)	
Post-op/1m	4 (33.3)	1 (7.1)	< 0.01
Post-op/6m	4 (33.3)	1 (7.1)	

Data are shown as mean ± standard deviation or n (%). VAS = visual analogue scale, EQ-5D = Euro QOL-5 Dimensions, Occ Neur = occipital neuralgia

60% [19, 20]. Therefore, the coexistence of cervical and lumbar lesions should be considered.

Although the cause of non-specific low back pain in patients with cervical spinal cord compression is not known, several hypotheses have been proposed regarding its pathophysiology. First, cervical cord compression may affect the spinothalamic tract, limiting activity by causing central sensitization, and indirectly causing low back pain [21-23]. Second, due to cervical cord compression, inhibitory systems and central nociceptive sensation may alter the integrity of endogenous descending pain, causing central tenderness in the lumbar region, resulting in a low back pain response [22, 23]. Third, cervical cord compression may cause low back pain by disrupting the spinal sagittal axis [21, 22]. Fourth, findings such as pain, sensory impairment, and spasticity caused by cervical pathology may cause decreased physical activity and thus indirectly cause low back pain [24]. For these reasons, it is thought that it may affect low back pain directly or indirectly.

Kawakita *et al*. [25] showed that 43% of patients' low back pain complaints improved after cervical surgery in 28 patients with cervical cord compression.

Our study included patients mostly with lumbar degenerative changes, similar to this study. However, in our study, we found that the complaints of VAS-Leg improved slightly in the postoperative period in both groups, and even worsened after improvement in group 2, ie vice versa. This may be due to the small number of patients included in both studies.

Studies by Kim *et al*. [24] and Epstein *et al*. [26] thought that physical activity might help improve low back pain by causing patients with cervical decompression surgery to be more active. In our study, we found that although the VAS Low Back was statistically significantly decreased in both groups (VAS Low Back (-0.3, -0.9)), it was statistically significantly decreased in group 2 ($p < 0.01$).

Alvin *et al*. [9] showed significant improvement in EQ-5D in their 1-year study on 84 patients and Dagi *et al*. [27] in a 22-month retrospective study with 19 patients. In our study, no significant difference was found in VAS Leg and EQ-5D. It may be related to the severity of cervical pathology or lumbar pathology. It may also be a coincidental finding due to the small number of patients in the study.

In C1-C2 instrumentation, visualizing the C1 lateral mass requires mobilization of the C2 nerve root. In the preservation of the C2 nerve root, the pressure of the screw on the C2 nerve root is a possible cause of this neuralgia. Dewan *et al*. [13] showed that it may be associated with occipital neuralgia in approximately 35% of patients in instrumentation with the preserved C2 nerve root. This may explain the increase in postoperative occipital neuralgia in group 1 and the decrease in neuralgia in Group 2 in our study. A larger and longer follow-up study is required to clarify the pathophysiology of low back pain after cervical instrumentation surgery.

Limitations

Due to the small number of patients included in this study, it did not have a sufficiently large comparison group in which the C2 nerve root was preserved, and it has several limitations, including being retrospective. Because its pathophysiology is unclear, this study may identify an incidental finding. We speculate that the reduction of low back pain symptoms is due to increased mobilization. However, the activity was not implemented and evaluated systematically.

CONCLUSION

Improvement of lumbar symptoms following atlantoaxial instrumentation surgery was determined by clinical findings rather than imaging findings. Although the exact pathophysiology cannot be demonstrated, it can, directly and indirectly, ameliorate low back pain. There was no improvement in the quality of life of the patients in our study. Future prospective studies are needed to confirm these findings and examine the effect of sacrificing the C2 nerve root on patients with low back pain.

Authors' Contribution

Study Conception: CS, RA; Study Design: CS, RA; Supervision: CS, RA; Funding: CS, RA; Materials: CS, RA; Data Collection and/or Processing: CS, RA; Statistical Analysis and/or Data Interpretation: CS, RA; Literature Review: CS, RA; Manuscript Preparation: CS, RA and Critical Review: CS, RA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Rezvani M, Sourani A, Nikzad H. Postoperative complications of Goel-Harms C1-C2 screw-rod fixation technique with for C1-C2 instability after C2 nerve sacrifice, a prospective study over two years follows up. J Clin Neurosci 2021;88:52-6.
2. Bourdillon P, Perrin G, Lucas F, Debarge R, Barrey C. C1-C2 stabilization by harms arthrodesis: Indications, technique, complications and outcomes in a prospective 26-case series. Orthop Traumatol Surg Res 2014;100:221-7.
3. Florman JE, Cushing DA, England EC, White E. How to transect the C2 root for C1 lateral mass screw placement: case series and review of an underappreciated variable in outcome. World Neurosurg 2019;127:e1210-4.
4. Salunke P, Karthigeyan M, Futane S. Pros and cons of C2 nerve sectioning/preservation in posterior fusion for congenital atlantoaxial dislocation. World Neurosurg 2018;118:e925-32.
5. Edvinsson L. Tracing neural connections to pain pathways with relevance to primary headaches. Cephalalgia 2011;31:737-47.
6. De Ridder D, Plazier M, Menovsky T, Kamerling N, Vanneste

S. C2 subcutaneous stimulation for failed back surgery syndrome: a case report. Neuromodulation 2013;16:610-3.
7. Eskander MS, Aubin ME, Drew JM, Eskander JP, Balsis SM, Eck J, et al. Is there a difference between simultaneous or staged decompressions for combined cervical and lumbar stenosis? Clin Spine Surg 2011;24:409-13.
8. Kikuike K, Miyamoto K, Hosoe H, Shimizu K. One-staged combined cervical and lumbar decompression for patients with tandem spinal stenosis on cervical and lumbar spine: Analyses of clinical outcomes with minimum 3 years follow-up. J Spinal Disord Tech 2009;22:593-601.
9. Alvin MD, Alentado VJ, Lubelski D, Benzel EC, Mroz TE. Cervical spine surgery for tandem spinal stenosis: The impact on low back pain. Clin Neurol Neurosurg 2018;166:50-3.
10. Goel A, Kaswa A, Shah A, Rai S, Gore S, Dharurkar P. Extraspinal-interdural surgical approach for C2 neurinomas - Report of an experience with 50 cases. World Neurosurg 2018;110:575-82.
11. Kang MM, Anderer EG, Elliott RE, Kalhorn SP, Frempong-Boadu A. C2 nerve root sectioning in posterior C1-2 instrumented fusions. World Neurosurg 2012;78:170-7.
12. Elliott RE, Kang MM, Smith ML, Frempong-Boadu A. C2 nerve root sectioning in posterior atlantoaxial instrumented fusions: a structured review of literature. World Neurosurg 2012;78:697-708.
13. Dewan MC, Godil SS, Devin CJ, McGirt MJ. C2 nerve root transection during C1 lateral mass screw fixation: does it affect functionality and quality of life? Spine J 2013;13:3-4.
14. Hamilton DK, Smith JS, Sansur CA, Dumont AS, Shaffrey CI. C-2 neurectomy during atlantoaxial instrumented fusion in the elderly: patient satisfaction and surgical outcome. J Neurosurg Spine 2011;15:3-8.
15. Squires J, Molinari RW. C1 lateral mass screw placement with intentional sacrifice of the C2 ganglion: Functional outcomes and morbidity in elderly patients. Eur Spine J. 2010;19:1318-24.
16. Sezer C, Gokten M, Sezer A, Gezgin İ, Binboga AB, Onay M. Role of craniectomy versus Craniotomy via the retrosigmoid approach in decreasing the incidence of postoperative headache. Int Surg 2022;106:32-8.
17. Bajwa NS, Toy JO, Ahn NU. Is congenital bony stenosis of the cervical spine associated with congenital bony stenosis of the thoracic spine? An anatomic study of 1072 human cadaveric specimens J Spinal Disord Tech 2013;26:24-9.
18. Nagata K, Yoshimura N, Hashizume H, Ishimoto Y, Muraki S, Yamada H, et al. The prevalence of tandem spinal stenosis and its characteristics in a population-based MRI study: the Wakayama Spine Study. Eur Spine J 2017;26:2529-35.
19. Lee SH, Kim KT, Suk KS, Lee JH, Shin JH, So DH, et al. Asymptomatic cervical cord compression in lumbar spinal stenosis patients: a whole spine magnetic resonance imaging study. Spine (Phila Pa 1976) 2010;35:2057-63.
20. Kawaguchi Y, Oya T, Abe Y, Kanamori M, Ishihara H, Yasuda T, et al. Spinal stenosis due to ossified lumbar lesions. J Neurosurg Spine 2009;3:262-70.
21. Ochiai H, Yamakawa Y, Minato S, Nakahara K, Nakano S, Wakisaka S. Clinical features of the localized girdle sensation of mid-trunk (false localizing sign) appeared in cervical compressive myelopathy patients. J Neurol 2002;249:549-53.
22. Roussel NA, Nijs J, Meeus M, Mylius V, Fayt C, Oostendorp R. Central sensitization and altered central pain processing in chronic low back pain: Fact or myth? Clin J Pain 2013;29:625-38.
23. Correa JB, Costa LO, de Oliveira NT, Sluka KA, Liebano RE. Central sensitization and changes in conditioned pain modulation in people with chronic nonspecific low back pain: a case-control study Exp Brain Res 2015;233:2391-9.
24. Kim CH, Chung CK, Lee U, Choi Y, Park SB, Jung JM, et al. Postoperative changes in moderate to severe nonspecific low back pain after cervical myelopathy surgery. World Neurosurg 2018;116:e429-35.
25. Kawakita E, Kasai Y, Uchida A. Low back pain and cervical spondylotic myelopathy. J Orthop Surg (Hong Kong) 2009;17:187-9.
26. Epstein N, Epstein J, Carras R, Murthy V, Hyman R. Coexisting cervical and lumbar spinal stenosis: diagnosis and management. Neurosurgery 1984;15:489-96.
27. Dagi TF, Tarkington MA, Leech JJ. Tandem lumbar and cervical spinal stenosis. J Neurosurg 2009;66:842-9.

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The effects of low-dose sorafenib on epithelial-mesenchymal transition and multidrug resistance markers in HepG2 cell line

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ABSTRACT

Objectives: Sorafenib is an orally administered tyrosine kinase inhibitor in hepatocellular cancer. Low sorafenib concentrations are attained during pharmacotherapy due to pharmacokinetic profile and patient inadherence. Resistance to treatment is a limitation to improving survival. Underlying mechanisms include epithelial-mesenchymal transition. The aim of the study was to evaluate epithelial-mesenchymal transition and multidrug resistance-related parameters in HepG2 cells following low-dose and short-term sorafenib treatment.

Methods: Epithelial-mesenchymal transition and multidrug resistance-related markers were examined by quantitative PCR, flow cytometry, and confocal laser scanning microscopy.

Results: An increase in epithelial marker E-cadherin and downregulation of mesenchymal markers Vimentin and Snail1 were detected by gene expression analysis. While P-glycoprotein expression increased, multidrug resistance protein 1, and breast cancer resistance protein mRNA levels did not alter after sorafenib treatment. The accumulation of the ABC transporter substrate rhodamine 123 in the cells increased following the treatment, corresponding to a less efficient efflux of rhodamine 123 and a possible effect on other transporters and mechanisms.

Conclusions: The results indicate a protective effect of sorafenib against epithelial-mesenchymal transition and upregulation in P-glycoprotein expression, which is, however, not sufficient to cause less intracellular rhodamine 123 accumulation. The effects of low-dose and short-term sorafenib on epithelial-mesenchymal transition and multidrug resistance-related markers might contribute to enlightening new treatment strategies in hepatocellular cancer

Keywords: Sorafenib, hepatocellular cancer, HepG2, multidrug resistance, epithelial-mesenchymal transition

Sorafenib is an orally administered tyrosine kinase inhibitor that increased overall survival in hepatocellular cancer (HCC). After oral intake, peak plasma concentrations are achieved within 3 hours, half-life varies between 25-48 hours [1]. Steady-state plasma concentrations are achieved in seven days. The drug

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concentration is reduced by 29% when it is taken with high-fat meals. Sorafenib is highly protein-bound (99.5%) [2], which leads to drug-drug interaction potential with lower sorafenib concentrations.

Sorafenib therapy might exert adverse effects. Gastrointestinal, dermatological, and cardiovascular adverse effects are frequently reported [3]. Therefore, dose reduction is considered an applicable strategy in clinical practice [4]. As therapeutic drug monitoring is not established for sorafenib, dose adjustment due to toxicity is the standard approach [5]. Moreover, though it is the best pharmacological approach, most HCC cases develop resistance to sorafenib therapy. Possible underlying mechanisms include epithelial-mesenchymal transition (EMT) [1].

EMT is defined as the transformation of the epithelial cells in cells with a mesenchymal phenotype. E-cadherin, Vimentin, N-cadherin, Twist, and Snail1 are prominent markers [6]. The loss of E-cadherin, acquisition of Vimentin, and upregulation of Snail-1 are reported in the progression of various cancers [7]. A worse prognosis is correlated with Twist and Snail expression in about 40%-70% of HCC cases with adherens junction disruption [8].

Multidrug resistance (MDR) is a multifactorial process, where cancer cells are unresponsive to the treatment. Increased drug efflux, decreased drug uptake, sequestration of drugs, changes in drugs metabolism, altered expression of non-coding RNAs, inhibition of apoptosis, alterations in the tumor environment, and expression of cancer stem cell (CSC) markers are considered among the mechanisms of MDR [9]. The ATP-binding cassette (ABC) transporters are membrane transport proteins, responsible to remove the anti-neoplastic drugs from the cells to achieve insufficient intracellular concentration and bioavailability. Twelve members of this large family are highly expressed in normal and neoplastic hepatocytes [10]. P-glycoprotein (ABCB1, MDR1, P-gp), multidrug resistance protein 1 (ABCC1, MRP1), and breast cancer resistance protein (BCRP) are the prominent efflux transporters associated with chemotherapy resistance and have been reported to impact the clinical outcomes in HCC treatment with sorafenib [11]. Inhibiting efflux transporters is a major strategy to resensitize the HCC cells and increase the response to sorafenib [12].

The presence of EMT within tumors is linked to CSCs that are also implicated in drug resistance [13]. Previously, our group observed that low-dose sorafenib (4 µM) treatment results in low cytotoxicity in HepG2 cells, yet also induces CSC-related changes [14]. This study is based on the known link between the CSCs and the occurrence of EMT and MDR within tumors [13, 15]. The purpose of the study is to investigate the effect of low-dose and short-term sorafenib (4 µM) treatment on EMT and/or MDR-related markers by gene expression analysis and functional tests in HepG2 cells.

METHODS

Cell Culture and Sorafenib Treatment

HepG2 cells (passage #15; American Type Culture Collection, USA) were maintained in Dulbecco's modified Eagle medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, and % 1 (v/v) penicillin (10000 U/mL) and streptomycin (10 mg/ml) at 37 0C in a humidified incubator with 5% CO_2.
Sorafenib (LC Laboratories, USA) was dissolved in dimethyl sulfoxide to prepare a 10 mM stock. The cells were treated with 4 µM sorafenib for 72 hours, which was shown to be associated with approximately 75% cell proliferation with the cell counting kit-8 (CCK-8) assay previously by our group [14].

Total RNA Isolation, Reverse Transcription and Quantitative PCR (RT- qPCR)

Total RNA was isolated following sorafenib treatment for 72 hours by using GeneJET RNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions. After confirming the RNA integrity, cDNA was synthesized from 1 µg total RNA with oligo dT primers and RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). QPCR was carried out with RealQ Plus Master Mix Green Without ROX (Ampliqon, Denmark) and the gene-specific primers for detection of E-cadherin, Vimentin, Snail1, P-glycoprotein (ABCB1, MDR1, P-gp), multidrug resistance protein 1 (ABCC1, MRP1), breast cancer resistance protein (ABCG2, BCRP; aforementioned primers from BM Lab, Turkey) mRNA levels and GAPDH as an internal control (Thermo Scientific) in a LightCycler® 96 instrument (Roche Diagnostics International, Switzerland). Delta

delta Ct ($2^{-\Delta\Delta Ct}$) relative quantitation method was employed for quantification of gene expression [16].

Gene-specific primers are as following: 5'-CACTATGCCGCGCTCTTTC-3' and 5'-GGTCGTAGGGCTGCTGGAA-3' for Snail [17]; 5'-AGGCAAAGCAGGAGTCCACTGA-3' and 5'-ATCTGGCGTTCCAGGGACTCAT-3' for Vimentin (#HP206907, OriGene Technologies, USA); 5'-GCCTCCTGAAAAGAGAGTGGAAG-3' and 5'-TGGCAGTGTCTCTCCAAATCCG-3' for E-Cadherin (#HP207683, OriGene Technologies, USA); 5'-GGGAGCTTAACACCCGACTTA-3' and 5'-GCCAAAATCACAAGGGTTAGCTT3' for P-gp; 5'-TGTGGGAAAACACATCTTTGA-3' and 5'-CTGTGCGTGACCAAGATCC3' for MRP1; 5'-AGATGGGTTTCCAAGCGTTCAT-3' and 5'-CCAGTCCCAGTACGACTGTGACA-3' for BCRP. GAPDH primers were included in the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific).

Determination of Rhodamine 123 (Rh123) Accumulation by Flow Cytometry

The cells were treated with sorafenib for 72 hours. Next, they were trypsinized and centrifuged at 500 g for 5 minutes. After washing with phosphate-buffered saline (PBS), 10^6 cells per data point were resuspended in DMEM at pH 7.8 and 37 °C, containing Rh123 (Sigma-Aldrich). The cells were loaded with Rh123 at a final concentration of 0.2 µg/mL (0.53 µM) at 37 °C for 30 minutes in a water bath and taken into ice at the end of the incubation to terminate the loading. After chilling in ice, the cells were washed twice with ice-cold DMEM, pH 7.4. Finally, they were resuspended in ice-cold DMEM, pH 7.4 for measuring the Rh123 fluorescence in FL-1 channel in an Accuri C6+ flow cytometry (BD Biosciences, USA). The tubes were kept in ice during the measurements. A minimum of 20,000 events were recorded and Rh123 median fluorescence values were used for generation of the bar graphs.

Determination of Rhodamine 123 (Rh123) Accumulation by Confocal Laser Scanning Microscopy

The cells were harvested with trypsinization, counted and seeded at a concentration of 1×105 cells to 24-well plates containing autoclaved coverslips. After an overnight incubation, sorafenib was added to the cells and the cells were further incubated for 72 hours. Next, they were washed with PBS and incubated with Rh123 (final concentration of 1 µM). After washing with PBS, they were counterstained with 1 mg/mL of Hoechst 33258 (Thermo Scientific) and the images were obtained with a Zeiss LSM 700 confocal scanning microscope (Germany).

Statistical Analysis

All data are expressed as the mean ± standard deviation. Student's t-test was used the compare the groups. GraphPad Prism V.8.01 (GraphPad Software, USA) was employed for generating the bar graphs and performing the statistical analyses. An α of 0.05 was used as the cut off for significance.

RESULTS

Effects of Low-Dose Sorafenib Treatment on EMT-Related Gene Expression

To investigate whether low-dose sorafenib treatment alters the expression EMT-related genes and the cell migration, we analysed the mRNA levels of Vimentin, Snail1 and E-cadherin. In response to 4 µM sorafenib

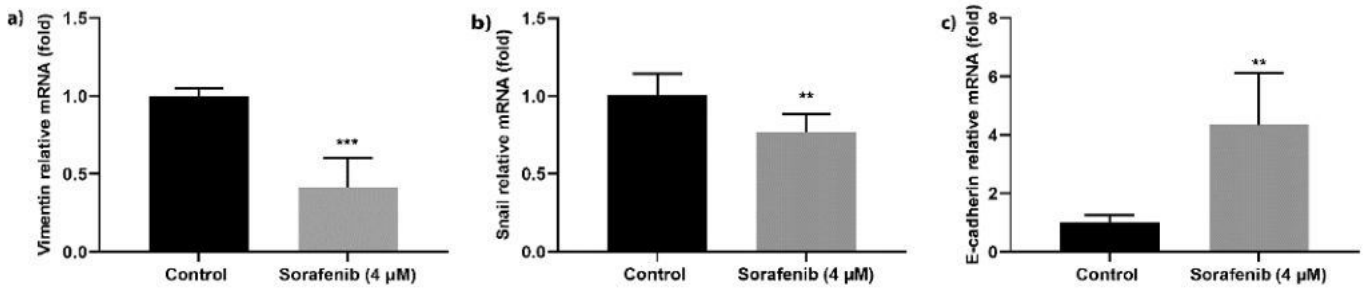

Fig. 1. Gene expression levels of (a) Vimentin, (b) Snail1 and (c) E-cadherin in response to 4 µM sorafenib treatment for 72 hours. Student's t-test was used to find significance. **$p < 0.01$, ***$p < 0.001$ vs control group.

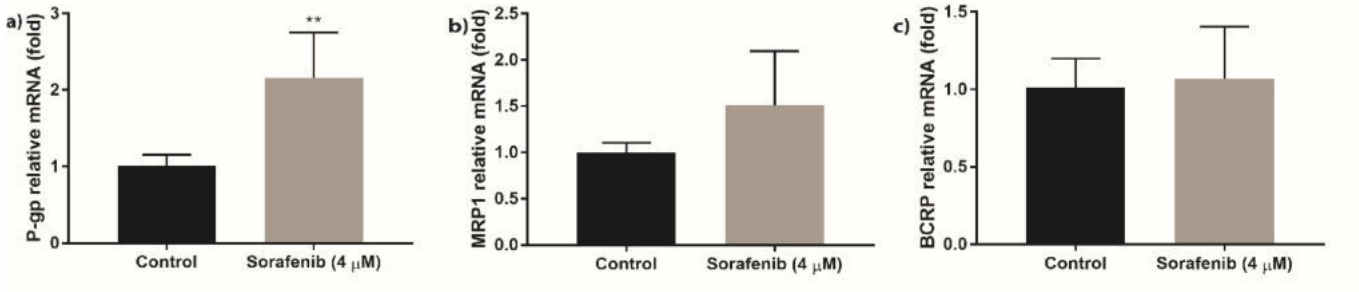

Fig. 2. Gene expression levels of (a) P-gp, (b) MRP1 and (c) BCRP in response to 4 μM sorafenib treatment for 72 hours. Student's t-test was used to find significance. **$p < 0.01$ vs control group.

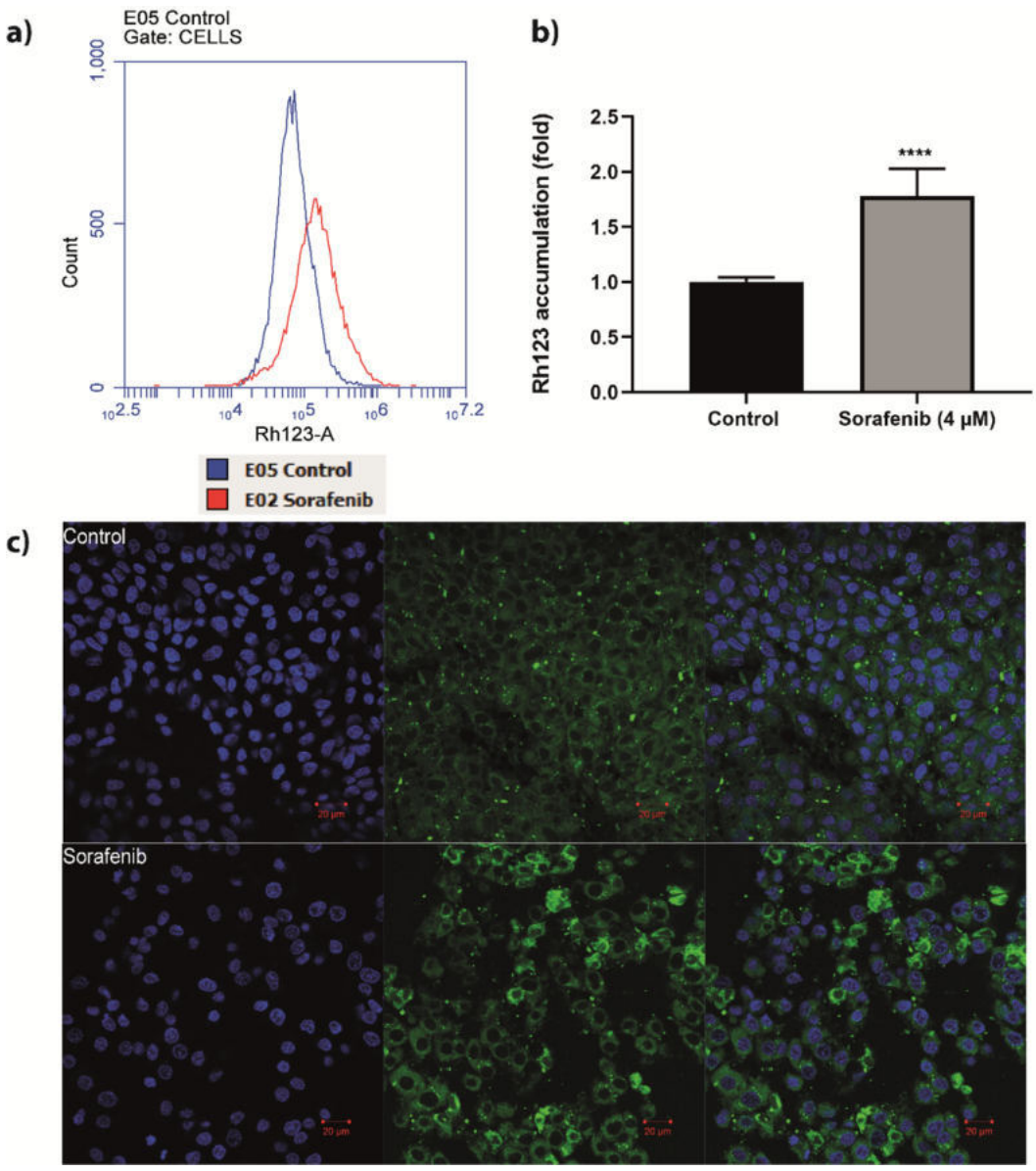

Fig. 3. Rh123 accumulation assays. (a) Histogram demonstrating the Rh123 fluorescence of control and sorafenib treated groups measured in FL-1 channel. (b) Bar graph showing fold change in Rh123 accumulation. Student's t-test was used to find significance. ****$p < 0.0001$ vs control group. (c) Confocal laser scanning microscopy of the control and sorafenib treated groups showing Rh123 accumulation (middle panel). Nuclei were counterstained with Hoechst 33258 (left panel). Merged images (right panel).

treatment for 72 hours, Vimentin and Snail1 expressions decreased significantly (0.42 fold, $p = 0.0009$ and 0.77 fold, $p = 0.0087$), while E-cadherin expression increased dramatically (4.36 fold, $p = 0.0010$) (Fig. 1).

Effects of Low-Dose Sorafenib Treatment on MDR-Related Gene Expression and Rh123 Accumulation

The association of sorafenib with multidrug resistance is a major obstacle for the treatment of HCC [18]. We explored whether low-dose sorafenib induces any changes in the mRNA levels of MDR-related genes and the accumulation of Rh123, which is effluxed by mainly P-gp and also to a lesser extent by MRP1. As shown in Fig. 2, while P-gp gene expression increased significantly (2.16 fold, $p = 0.0012$), no significant change was detected in the mRNA levels of MRP1 and BCRP (1.51 fold, $p = 0.0634$; 1.07 fold, $p = 0.7106$, respectively).

To examine if increased P-gp mRNA levels correspond to a change in cellular Rh123 accumulation, we measured and visualized Rh123 accumulation by flow cytometry (Figs. 3a and b) and confocal laser scanning microscopy (Fig. 3c), respectively. We demonstrated a 1.78 fold increase in Rh123 accumulation following sorafenib treatment for 72 hours ($p < 0.0001$). Similarly, sorafenib treated cells accumulated more Rh123 as evident by confocal laser scanning microscopy images.

DISCUSSION

Sorafenib is an approved agent in the treatment of advanced unresectable HCC. Sorafenib appears beneficial only approximately in 30% of patients. Unfortunately, this population develops drug resistance within 6 months [18]. Moreover, clinically significant toxicities develop in about 50% of the patients [3]. Adverse events such as diarrhea, weight loss, hypertension and hand and foot skin reactions generally occur within 2-6 weeks of the treatment [19]. Due to the high frequency and severity of the adverse effects, the patients generally undergo a dose reduction or drug discontinuation [3]. A recent study performed by Tak *et al*. [4] focused on progression-free survival, overall survival, duration of the treatment, cumulative dose, adverse effects, and drug discontinuation in HCC patients treated with sorafenib. The researchers recommended dose reduction to prolong survival and to improve the treatment efficiency with a higher cumulative dose and longer duration by increasing patient tolerance and adherence.

The aim of this study was to evaluate whether low-dose sorafenib treatment results in any EMT or MDR-related changes in HepG2 cells. Previously, our group evaluated the cytotoxicity of sorafenib in HepG2 cells and demonstrated approximately 75% cell proliferation following the treatment with 4 μM sorafenib [14]. In the same study, we also reported altered expression of the cancer stem cell (CSC) markers CD44, CD90, and CD133. The known link between the CSCs and the occurrence of EMT and MDR within tumors prompted us to examine the effects of low-dose and short-term sorafenib (4 μM) treatment on EMT and MDR by gene expression analysis and functional tests [13, 15].

EMT is a developmental process characterized by the loss of epithelial cell polarity, weakening of E-cadherin-related cell-cell adhesion and, the acquisition of mesenchymal markers such as Vimentin and N-cadherin through EMT-inducing transcription factors including Snail1 [6]. The clinical significance of EMT has been demonstrated in HCC. Reduced E-cadherin and overexpression of Snail1 were identified in 60.2% and 56.9% of primary HCC samples, respectively and the alterations of the two markers were shown to be associated with worse prognosis [20]. We found significantly lower mRNA levels of the mesenchymal markers Vimentin and Snail in response to low-dose sorafenib. On the other hand, the expression of the epithelial marker E-cadherin increased significantly, indicating a protective effect of low-dose sorafenib against EMT in gene expression level. Similarly, several studies reported that sorafenib inhibits EMT in a variety of experimental settings [21-23]. Moreover, sorafenib resistance is often associated with changes in the tumor microenvironment, and sorafenib-resistant cells were shown to exhibit EMT [24-26].

EMT and drug resistance with the overexpression of ABC transporters appear to correlate strongly [27-29]. A recent study reported that sorafenib-resistant HCC cells with the overexpression of Snail also exhibit increased levels of P-gp expression. Moreover,

the same study demonstrated the association of the reversal of resistance with the up-regulation of E-cadherin and simultaneous down-regulation of Snail, Vimentin and P-gp [24]. To investigate whether low-dose sorafenib alters MDR-related gene expression, we analyzed the mRNA levels of P-gp, MRP1 and BCRP. We obtained an about 2-fold increase in P-gp expression, while no change was detected in the expression levels of MRP-1 and BCRP. We also performed Rh123 accumulation analysis to measure the functionality of the efflux pumps. Rh123, a fluorescent substrate, is effluxed by mainly P-gp and also to a lesser extent by MRP1 [30]. Despite the increased P-gp expression, we showed an increase in Rh123 accumulation, corresponding to a lower efflux rate and a possible involvement of other molecular mechanisms. Rh123 was identified as a high-affinity substrate also for other transporters including for organic cation transporters 1 and 2 [31], and the increased P-gp expression might not be sufficient alone for an increased Rh123 efflux. Moreover, the interaction of sorafenib with ABC transporters is inconclusive. While sorafenib was suggested to have a moderate affinity for P-gp and negligible for BCRP [32], others propose that BCRP is rather important for sorafenib sensitivity [33, 34]. The measurement of Rh123 to determine the activity of ABC transporters is a limitation of this study, as the fluorescent molecule is also a high-affinity substrate for organic cation transporters 1 and 2. Another limitation is the lack of protein expression assays.

Sorafenib dose reductions or discontinuations because of the intolerable adverse effects or acquired or primary drug resistance are common during HCC treatment. Currently, combination therapies involving sorafenib at lower doses are hotspots in research and are expected to provide beneficial clinical outcomes [35]. Several studies were performed to examine if the dose reductions compromise the treatment outcomes, and the researchers suggest dose modifications for maximizing patient adherence and outcomes [4, 36-38].

CONCLUSION

In the current study, we examined the effects of low-dose sorafenib, yielding 75% cell proliferation, on EMT and MDR in HepG2 cells. We obtained an increase in epithelial marker E-Cadherin and downregulation of mesenchymal markers Vimentin and Snail1, indicating a protective effect of sorafenib against EMT. Moreover, we demonstrated upregulated P-gp expression, yet this change in mRNA levels did not result in a reduction in Rh123 accumulation in the cells. Other mechanisms are possibly involved and require further investigation. Understanding the EMT and MDR-related changes might contribute to lower sorafenib dose and enlighten new treatment strategies to overcome drug resistance.

Authors' Contribution

Study Conception: YDÇ, ZGÖ; Study Design: YDÇ, ZGÖ; Supervision: YDÇ, ZGÖ; Funding: YDÇ; Materials: YDÇ; Data Collection and/or Processing: YDÇ, ZA, GD; Statistical Analysis and/or Data Interpretation: YDÇ, RGA, ZGÖ; Literature Review: YDÇ, ZA, GD, ZGÖ; Manuscript Preparation: YDÇ, ZGÖ and Critical Review: YDÇ, ZA, GD, RGA, ZGÖ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Keating GM. Sorafenib: a review in hepatocellular carcinoma. Target Oncol 2017;12:243-53.
2. Karbownik A, Szkutnik-Fiedler D, Czyrski A, Kostewicz N, Kaczmarska P, Bekier M, et al. Pharmacokinetic interaction between sorafenib and atorvastatin, and sorafenib and metformin in rats. Pharmaceutics 2020;12:600.
3. Pang Y, Eresen A, Zhang Z, Hou Q, Wang Y, Yaghmai V, et al. Adverse events of sorafenib in hepatocellular carcinoma treatment. Am J Cancer Res 2022;12:2770-82.
4. Tak KY, Nam HC, Choi JY, Yoon SK, Kim CW, Kim HY, et al. Effectiveness of sorafenib dose modifications on treatment outcome of hepatocellular carcinoma: analysis in real-life settings. Int J Cancer 2020;147:1970-8.
5. Labeur TA, Hofsink Q, Takkenberg RB, van Delden OM, Mathôt RAA, Schinner R, et al. The value of sorafenib trough levels in patients with advanced hepatocellular carcinoma - a substudy of the SORAMIC trial. Acta Oncol 2020;59:1028-35.
6. Gurzu S, Kobori L, Fodor D, Jung I. Epithelial mesenchymal

and endothelial mesenchymal transitions in hepatocellular carcinoma: a review. BioMed Res Int 2019;2019:2962580.
7. Myong NH. Loss of E-cadherin and acquisition of Vimentin in epithelial-mesenchymal transition are noble indicators of uterine cervix cancer progression. Korean J Pathol 2012;46:341-8.
8. Jou J, Diehl AM. Epithelial-mesenchymal transitions and hepatocarcinogenesis. J Clin Invest 2010;120:1031-4.
9. Duan B, Huang C, Bai J, Zhang YL, Wang X, Yang J, et al. Multidrug resistance in hepatocellular carcinoma. In: JEE T-P, ed. Hepatocellular carcinoma [Internet]. Chapter 8, Brisbane (AU); 2019.
10. Fornari F, Giovannini C, Piscaglia F, Gramantieri L. Elucidating the molecular basis of sorafenib resistance in hcc: current findings and future directions. J Hepatocell Carcinoma 2021;8:741-57.
11. Cabral LKD, Tiribelli C, Sukowati CHC. Sorafenib resistance in hepatocellular carcinoma: the relevance of genetic heterogeneity. Cancers (Basel) 2020;12:1576.
12. Marin JJG, Monte MJ, Macias RIR, Romero MR, Herraez E, Asensio M, et al. Expression of chemoresistance-associated abc proteins in hepatobiliary, pancreatic and gastrointestinal cancers. Cancers (Basel) 2022;14:3524.
13. Chen YA, Ho CL, Ku MT, Hwu L, Lu CH, Chiu SJ, et al. Detection of cancer stem cells by EMT-specific biomarker-based peptide ligands. Sci Rep 2021;11:22430.
14. Dönmez Çakıl Y, Sitar ME, Özünal ZG, Kayalı D, Gülhan Aktas R. Flow cytometric evaluation of cancer stem cell markers in HepG2 cells following sorafenib treatment. Int J Med Biochem 2021;4:200-4.
15. Cho Y, Kim YK. Cancer stem cells as a potential target to overcome multidrug resistance. Front Oncol 2020;10:764.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402-8.
17. Wang H, Chirshev E, Hojo N, Suzuki T, Bertucci A, Pierce M, et al. The epithelial-mesenchymal transcription factor SNAI1 represses transcription of the tumor suppressor miRNA let-7 in cancer. Cancers (Basel) 2021;13:1469.
18. Tang W, Chen Z, Zhang W, Cheng Y, Zhang B, Wu F, et al. The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutic aspects. Signal Transduct Target Ther 2020;5:87.
19. Zhou J, Sun H, Wang Z, Cong W, Wang J, Zeng M, et al. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (2019 Edition). Liver Cancer 2020;9:682-720.
20. Yang MH, Chen CL, Chau GY, Chiou SH, Su CW, Chou TY, et al. Comprehensive analysis of the independent effect of twist and snail in promoting metastasis of hepatocellular carcinoma. Hepatology 2009;50:1464-74.
21. Chen YL, Lv J, Ye XL, Sun MY, Xu Q, Liu CH, et al. Sorafenib inhibits transforming growth factor β1-mediated epithelial-mesenchymal transition and apoptosis in mouse hepatocytes. Hepatology 2011;53:1708-18.
22. Ha TY, Hwang S, Moon KM, Won YJ, Song GW, Kim N, et al. Sorafenib inhibits migration and invasion of hepatocellular carcinoma cells through suppression of matrix metalloproteinase expression. Anticancer Res 2015;35:1967-76.
23. Dong S, Kong J, Kong F, Kong J, Gao J, Ji L, et al. Sorafenib suppresses the epithelial-mesenchymal transition of hepatocellular carcinoma cells after insufficient radiofrequency ablation. BMC Cancer 2015;15:939.
24. Dong J, Zhai B, Sun W, Hu F, Cheng H, Xu J. Activation of phosphatidylinositol 3-kinase/AKT/snail signaling pathway contributes to epithelial-mesenchymal transition-induced multi-drug resistance to sorafenib in hepatocellular carcinoma cells. PloS One 2017;12:e0185088.
25. Tian X, Yan T, Liu F, Liu Q, Zhao J, Xiong H, et al. Link of sorafenib resistance with the tumor microenvironment in hepatocellular carcinoma: Mechanistic insights. Front Pharmacol 2022;13:991052.
26. van Malenstein H, Dekervel J, Verslype C, Van Cutsem E, Windmolders P, Nevens F, et al. Long-term exposure to sorafenib of liver cancer cells induces resistance with epithelial-to-mesenchymal transition, increased invasion and risk of rebound growth. Cancer Lett 2013;329:74-83.
27. Jiang ZS, Sun YZ, Wang SM, Ruan JS. Epithelial-mesenchymal transition: potential regulator of ABC transporters in tumor progression. J Cancer 2017;8:2319-27.
28. Saxena M, Stephens MA, Pathak H, Rangarajan A. Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. Cell Death Dis 2011;2:e179.
29. Choi HS, Kim YK, Yun PY. Upregulation of MDR- and EMT-related molecules in cisplatin-resistant human oral squamous cell carcinoma cell lines. Int J Mol Sci 2019;20:3034.
30. Saengkhae C, Loetchutinat C, Garnier-Suillerot A. Kinetic analysis of rhodamines efflux mediated by the multidrug resistance protein (MRP1). Biophys J 2003;85:2006-14.
31. Jouan E, Le Vee M, Denizot C, Da Violante G, Fardel O. The mitochondrial fluorescent dye rhodamine 123 is a high-affinity substrate for organic cation transporters (OCTs) 1 and 2. Fundam Clin Pharmacol 2014;28:65-77.
32. Hu S, Chen Z, Franke R, Orwick S, Zhao M, Rudek MA, et al. Interaction of the multikinase inhibitors sorafenib and sunitinib with solute carriers and ATP-binding cassette transporters. Clin Cancer Res 2009;15:6062-9.
33. Lagas JS, van Waterschoot RA, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Breast cancer resistance protein and P-glycoprotein limit sorafenib brain accumulation. Mol Cancer Ther 2010;9:319-26.
34. Huang WC, Hsieh YL, Hung CM, Chien PH, Chien YF, Chen LC, et al. BCRP/ABCG2 inhibition sensitizes hepatocellular carcinoma cells to sorafenib. PloS One 2013;8:e83627.
35. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7:6.
36. Bolondi L, Craxi A, Trevisani F, Daniele B, Di Costanzo GG, Fagiuoli S, et al. Refining sorafenib therapy: lessons from clinical practice. Future Oncol 2015;11:449-65.
37. Nishikawa H, Osaki Y, Endo M, Takeda H, Tsuchiya K, Joko K, et al. Comparison of standard-dose and half dose sorafenib therapy on clinical outcome in patients with unresectable hepa-

tocellular carcinoma in field practice: a propensity score matching analysis. Int J Oncol 2014;45:2295-302.
38. Reiss KA, Yu S, Mamtani R, Mehta R, D'Addeo K, Wileyto EP, et al. Starting dose of sorafenib for the treatment of hepatocellular carcinoma: a retrospective, multi-institutional study. J Clin Oncol 2017;35:3575-81.

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The relationship of bone marrow fibrosis at diagnosis with prognosis and survival in childhood acute lymphoblastic leukemia

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ABSTRACT

Objectives: Our aim in this study is to investigate the effect of fibrosis at diagnosis on treatment and survival in childhood acute lymphoblastic leukemia (ALL).

Methods: This study is retrospective. We evaluated the relationship between patients' age, white blood cell count at diagnosis, morphological blast percentage and flow cytometric blast percentage at diagnosis, day 15th and day 33th, absolute blast count in peripheral smear on day 8th, and the degree of fibrosis in bone marrow biopsy at diagnosis in 36 pediatric patients. The fibrosis degree in biopsy on the thirty-third day after induction therapy was measured.

Results: Twenty-eight (77.8%) cases were diagnosed B-ALL and 8 T-ALL (22.2%). There was no statistically significant difference between the groups with and without fibrosis in terms of any parameter measured at the time of diagnosis, 8th day, 15th day, and 33th day. No significant difference was found between the groups according to overall survival (OS): the mean OS was 50.22 ± 5.44 months in the fibrosis group and 49.70 ± 3.96 months in the non-fibrosis group (p = 0.557).

Conclusions: There is a high detection rate of bone marrow fibrosis in ALL pediatric cases at the time of diagnosis. Nevertheless, fibrosis does not affect survival.

Keywords: Leukemia, fibrosis, bone marrow, childhood, lymphoblastic, microenvironment

Acute lymphoblastic leukemia (ALL) is childhood cancer's most common type. Although cure rates for ALL are 80-90% with advanced chemotherapy protocols and supportive treatment, 10-20% of patients will die due to disease recurrence or treatment-related complications [1]. Patients at high risk of developing resistance to chemotherapy and relapse; are defined as patients under one-year-old, patients with BCR/ABL translocation, those with KMT2A/AFF re-arrangement, those with hypodiploidy, those with t (17;19) (q23; p13) (TCF3/HLF), IKZF1 plus mutations, those with high minimal residual disease (MRD) during induction and re-induction, and those with T immunophenotype [2].

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The cancer microenvironment is now recognized as a significant factor in cancer progression and response to treatment as it is in solid organ cancers. Consequently, the cancer microenvironment should be considered among cancer treatment targets [3-5]. Similarly, leukemia cells and the microenvironment (or niche) play a substantial role in resistance to chemotherapy and treatment failure [6]. Bone marrow microenvironment (BMM) consists of a highly complex cell population, such as mesenchymal stem cells [7], endothelial cells [8], osteoblasts [9], osteoclasts [10], adipocytes [11] and stromal cells [7]. It is important to note that the vascular and endosteal niches formed by cells in the BMM are anatomically and functionally distinct. The endosteal niche makes leukemic stem cells quiescent and chemo-resistant; in contrast, the vascular niche makes them more active and mature [6]. There is no clear understanding of the pathogenesis of bone marrow fibrosis; however, pathway-activating mutations in the JAK-STAT pathway affect the expression of cytokines produced by leukemia cells and those created by cells in the microenvironment, which leads to the development of fibrosis [12]. There is limited evidence that bone marrow fibrosis in childhood ALL is high at the time of diagnosis [13]. It is believed that fibrosis disappears during treatment in ALL pediatric cases with marrow fibrosis at diagnosis [14]. However, there still needs to be more clarity regarding the relationship between bone marrow fibrosis at the diagnosis and prognosis and treatment response in pediatric acute leukemia.

This study was aimed to make a comparison between patients treated according to the ALLIC BFM 2009 Chemotherapy protocol, which had bone marrow fibrosis detected or not detected at the time of diagnosis based on ALL risk groups and the effect of ALL treatment on bone marrow responses on days 15 and 33 and its impact on mortality.

METHODS

Thirty-six patients diagnosed with ALL were included in this study. Flow cytometric (FC) analysis, immunohistochemical studies on bios, and peripheral smears were used to diagnose ALL. All cases were divided into two groups, B-ALL and T-ALL.

The bone marrow biopsies were processed after 10% formaldehyde fixation, followed by routine tissue examination after decalcification, and embedded in paraffin. Bone marrow biopsy fibrosis was evaluated using reticulin and Masson's trichrome histochemical staining. A 4-micron thick section of a polylyzed slide was taken and stained with the ready kit by the instructions provided in the paint's instruction manual. Reticular fiber grading in the bone marrow was based on the European Consensus Report of Bone Marrow Fibrosis Grading. Accordingly, the degree of bone marrow fibrosis was evaluated between 0 and 3 (Table 1). [15]: 0 and 1 were considered as having no significant fibrosis, and 2 and 3 were supposed to have significant fibrosis. ALL-Inter Continental Berlin-Frankfurt-Münster (ALL-IC BFM) 2009 protocol was followed in all cases (Table 2) [16].

The study investigated the age of the patients, the number of white blood cells at diagnosis, morphological blast percentage at diagnosis, flow cytometric blast percentage at diagnosis, absolute blast number in peripheral blood smear on the eighth day, bone marrow morphological blast percentage on day 15 (M1; < 5%, M2; ≥ 5 – < 25, M3 ≥ 25) [16], flow cytometric

Table 1. European Consensus Report of Bone Marrow Fibrosis grading [15]

Grade	Description
Grade 0	Scattered linear reticulin with no intersections (cross-overs) corresponding to normal bone marrow
Grade 1	Loose network of reticulin with many intersections, especially in perivascular areas
Grade 2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis
Grade 3	Diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis

Table 2. Parameters used to determine the risk group of patients [16]

Standard Risk Group (SR)*	Intermediate Risk Group (IR)	High Risk Group (HR)**
PB day 8: < 1,000 blasts/µL	All patients who are not stratified to SR or HR are intermediate risk patients.	IR and, if available FC MRD >10% or M3 marrow on day 15
Age ≥ 1 yr – < 6 yr		SR if available FC MRD >10%
Initial WBC < 20,000/µL		Periferik Blood on day 8: ≥ 1,000 blasts/µL
FC MRD < 0,1% or M1/ M2 marrow on day 15		M2 or M3 marrow on day 33
M 1 marrow on day 33		Translocation t(9;22) [BCR/ABL] or t(4;11) [MLL/AF4]
*All criteria must be fulfilled		Hipodiploidy ≤ 44
		**At least one criterion must be fulfilled.

PB = Peripheral Blast, SR = Standard Risk Group, IR = Intermediate Risk Group, HR = High Risk Group, FC = Flow Cytometri, MRD = Minimal Residual Disease

blast percentage on day 15, bone marrow morphological blast percentage on day 33, flow cytometric blast percentage and evaluated their relation with the degree of fibrosis in the bone marrow at diagnosis. Additionally, the extent of bone marrow fibrosis on day 33 and the effect of fibrosis on OS were evaluated.

Statistical Analysis

As descriptive statistics, numbers and percentages were used for qualitative data, and arithmetic means and standard deviation were used for quantitative data. The Mann-Whitney U test was used to compare the two groups since the Shapiro-Wilk test indicated that the continuous data were not normally distributed. The Spearman correlation coefficient was used to evaluate the correlation of quantitative data. Overall survival (OS) was estimated by the Kaplan-Meier method. Observational survival was calculated from the date of the first diagnosis to the date of any death from any cause and censored at the final follow-up date for event-free and viable patients. The log-rank test was used to compare different groups. The statistical significance level was taken as $p < 0.05$, SPSS 18.0 package program was used to evaluate the data.

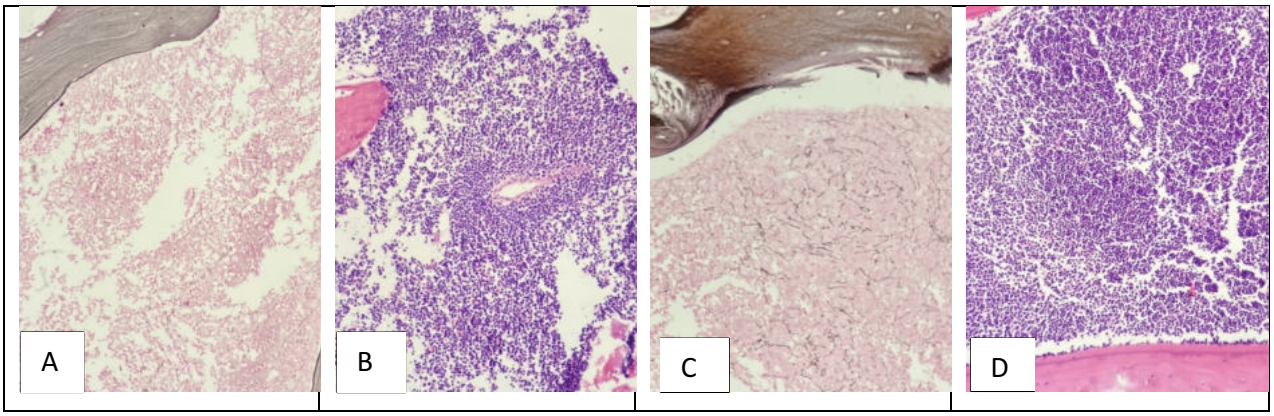

Fig. 1. Very thin short reticun fiber in the intertrabecular area, more prominent around the vessel, Grade 0 (A, ×200), infiltration of leukemia cells in the same biopsy (B, ×200 HE). Loose usually unconnected thin reticulin fiber in the intertrabecular space, Grade 1 (C, ×200), infiltration of leukemia cells in the same biopsy (D, ×200 HE).

RESULTS

Thirty-six pediatric ALL patients were included in the study. The median age at diagnosis was 4.5 years (2-18 years). Sixteen were girls (44.4%), and 20 were boys (55.6%). Twenty-eight patients were B-ALL (77.8%), and 8 were T-ALL (22.2%). According to the bone marrow biopsy examination fibrosis evaluation in diagnosis, 4 of the patients were grade 0 (11.1%), 13 patients were grade 1 (36.1%), 14 patients were grade 2 (38.9%), and five patients were grade 3 (13.9%) fibrosis. Totally 55.9% (n = 19) of the patients had fibrosis: 57.1% (n = 16) of the B-ALL patient group, and 37.5% (n = 3) of the T-ALL patient group had fibrosis (Figs. 1 and 2).

Fourteen of the patients were in the high-risk group (HR) (38.9%), 20 of them in the intermediate risk group (IR) (56.5%), and 2 of them in the standard risk (SR) (5.6%) group. Five of the patients in the HRG group were classified as T ALL (three of them were steroid unresponsive on the eighth day, and the bone marrow morphology was M3 on the 15th day, one was steroid unresponsive on the eighth day, one was steroid unresponsive on the eighth day, and M2 bone marrow was detected on the 33rd day.), nine of them were B ALL (three were evaluated as HR due to positive detection of t (9:22), M3 bone marrow detection on day 15 in four, and detection of M2 bone marrow on day 33 in two of them). According to the morphological classification made from the bone marrow aspiration smear on the 15th day, 69.4% (n = 25) were M1, 11.1% (n = 4) M2, and 19.5 (n = 7) M3. According to the morphological classification performed on the 33rd day, 94.3% (n = 33) were M1, and 5.7% (n = 2) were M2. Since a patient with reticulin fibrosis three at the diagnosis died on the 25th day, the bone marrow could not be evaluated on the 33th day. Diagnostic white blood cell count of the patients, morphological blast percentage in the bone marrow aspiration smear at diagnosis, and flow cytometric at diagnosis. Blast percentage, absolute blast number on day eight, morphological blast percentage on day 15, flow cytometric on day 15 blast percentage, morphological blast percentage on day 33, and flow cytometric on day 33 blast percentage averages are given in Table 3.

Blasts at the diagnosis (p = 0.961), the diagnosis flow cytometric between the group with and without fibrosis in the bone marrow biopsy examination at the time of diagnosis. Blast percentage (p = 0.501), absolute blast number on the eighth day (p = 0.086), morphological blast percentage on the 15th day (p = 0.856), flow cytometric on the 15th day blast percentage (p = 0.296), morphological blast percentage on day 33 (p = 0.754), flow cytometric on day 33 No statistically significant difference was found between the rate of blasts (p = 0.192) (Table 4). There was no statistically significant correlation between these values and the reticulin value (p > 0.05).

According to the leukemia type of the patients (with B-ALL fibrosis (n = 16, 57.1%), (n = 3, 37.5%) with T-ALL fibrosis (p = 0.326), according to the risk group of the patient (SR and There was no statistically

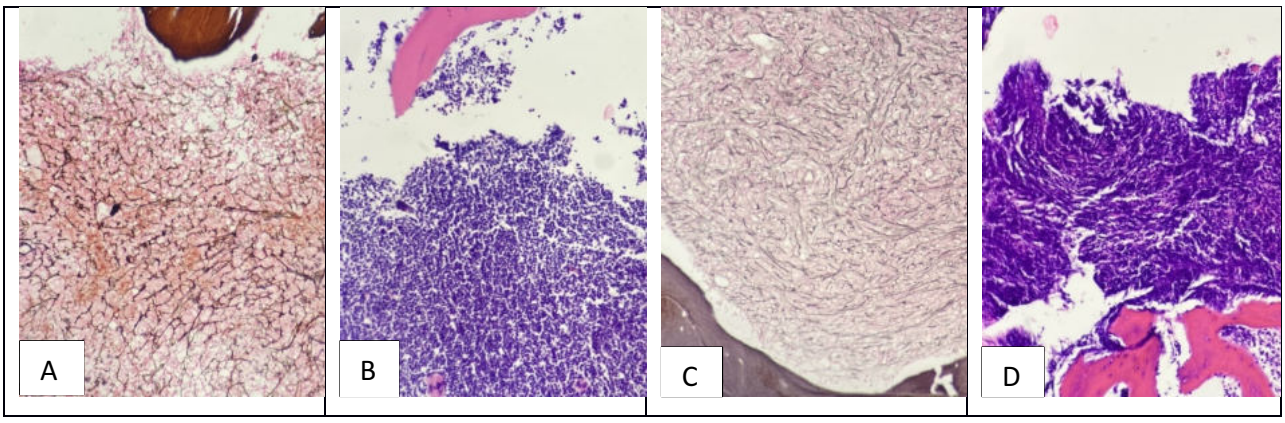

Fig. 2. Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen, Grade 2 (A, ×200), crush artifact is observed in leukemia cells compared to biopsy with lower grade reticulin (B, ×200 HE). Diffuse and very dense, thick collagen fibers, Grade 3 (C, ×200), significant crush artifact is observed in leukemia cells (D, ×200 HE).

Table 3. Demographic and laboratory characteristics of the patients

Patient data	Mean ± SD	Median	Min-Max
Diagnosis age (years)	6.78 ± 5.13	4.50	2.00-18.00
Number of white blood cell at diagnosis ($\times 10^3$)	45.7 ± 98.00	15.96	1.00-548.00
Blast rate in diagnosis (Flow cytometry) (%)	79.19 ± 19.18	85.00	29.00-99.00
Blast rate in diagnosis (Bone marrow dissemination) (%)	92.89 ± 6.15	95.00	70.00-99.00
Absolute blasts on day 8 (Peripheral spread)	23.82 ± 20.67	20.00	0.00-72.00
15. day blast rate (Bone marrow spread) (%)	11.92 ± 19.35	2.00	0.00-70.00
15. day blast rate (Flow cytometry) (%)	10.63 ± 18.11	2.54	0.00-68.00
33. day blast rate (Bone marrow spread) (%)	1.74 ± 1.62	1.00	0.00-8.00
Day 33 blast rate (Flow cytometry) (%)	4.54 ± 9.21	0.89	0.00-33.30

SD = Standard deviation, Min = Minimum, Max = Maximum

significant difference in terms of the degree of fibrosis among patients with IR fibrosis (n = 11, 50.0%), HR fibrosis (n = 8, 57.1%)) ($p = 0.676$). According to bone marrow examination, 70.6% (n = 12) of those without fibrosis were M1, 5.9 (n = 1) were M2, 2, 3.9% (n = 4) were M3, and 68.4% of those with fibrosis. It was observed that morphological blasts of the patients on the 33rd day were M1, 15.8% (n = 3) M2, and 15.8% M3, and there was no statistically significant difference between their distributions ($p = 0.764$). According to the percentages of bone marrow thinning, 100% (n = 17) of those without fibrosis were M1, 88.9% (n = 16) of those with fibrosis were M1, and 11.1% (n = 2) were M2. No significant difference was found ($p = 0.486$).

The median follow-up period was 20.15 months (min: 1.5, max: 60.67). The 18-month OS was 84.1% ± 6.7% (Fig. 3, on the left). In comparing OS between the groups with and without fibrosis, the mean OS was 50.22 ± 5.44 months in the fibrosis group and 49.70 ± 3.96 months in the non-fibrosis group. There was no statistically significant difference between them ($p = 0.557$) (Fig. 3, on the right).

While 31 patients were alive and in complete remission, five died. Two patients who died during treatment were T-ALL, and three were B-ALL. It was observed that one of the T-ALL patients was in the HR risk group and did not have fibrosis (grade 1), and the second in the IR risk group had fibrosis (grade 3). It was observed that one of the three patients with B-ALL had IR and fibrosis (grade 3), the other had IR and fibrosis (grade 1), and another had HR and fibrosis (grade 0). One of the two patients with fibrosis died due to intracranial hemorrhage after falling during induction therapy (during the first 33 days of treatment), and one died due to sepsis during the re-induction period.

In the evaluation of bone marrow fibrosis in the bone marrow biopsy on the 33rd day, one patient could not be evaluated because he died before the 33rd day. Therefore, fibrosis could be assessed on the 33rd day of 18 patients with fibrosis at the time of diagnosis, and fibrosis did not improve in only one of these patients. This patient had grade 3 fibrosis at the time of diagnosis and grade 2 fibrosis on the 33rd day of treatment.

DISCUSSION

The bone marrow is filled with leukemia cells, so much in children with acute lymphoblastic leukemia that they weaken the bone speculum [17]. As cellularity increases, reticulin fibrosis increases and serves as a support and connection between leukemia cells and bone marrow [18]. A greater degree of fibrosis prevents chemotherapy from reaching the blasts and reduces the effectiveness of chemotherapy [19]. Moreover, reticulin fibrosis has decreased adult survival [20].

Studies have reported the rate of fibrosis in ALL in childhood between 38.1% and 57% [21, 22]. Fibrosis was found in 52.8% of our patients at diagnosis, which is consistent with the literature. The incidence of fi-

Table 4. Relationship between fibrosis and other parameters

	Mean ± SD	Median	Min-Max	*p value*
Age				0.749
Fibrosis	6.8 ± 4.9	5.5	2.0-18.0	
No fibrosis	6.8 ± 5.5	4.0	2.0-17.0	
Number of white globes at diagnosis ($\times 10^3$)				0.296
Fibrosis	56.4 ± 122.3	21.7	3.0-548.0	
No fibrosis	33.8 ± 122.3	12.0	1.0-260.0	
Blast rate in diagnosis (Flow cytometry) (%)				0.501
Fibrosis	76.8 ± 20.8	83.5	29.0-95.0	
No fibrosis	81.7 ± 17.7	90.0	32.0-99.0	
Blast rate at diagnosis (Bone marrow smear) %				0.961
Fibrosis	93.3 ± 4.9	95.0	80.0-99.0	
No fibrosis	92.4 ± 7.4	95.0	70.0-98.0	
8. day white globe number ($\times 10^3$)				0.398
There is fibrosis	3.8 ± 6.8	1.6	0.5-27.5	
No fibrosis	3.9 ± 5.2	1.9	1.0-20.0	
Absolute blasts on day 8 (PS)				0.086
Fibrosis	321.8 ± 278.6	300.0	1.0-1120.0	
No fibrosis	609.2 ± 22.1	600.0	35.0-1400.0	
15. day blast (Bone marrow smear) (%)				0.856
Fibrosis	10.0 ± 16.2	2.0	0.0-60.0	
No fibrosis	14.1 ± 22.7	1.0	0.0-70.0	
15. day blast (Flow cytometry (%)				0.296
Fibrosis	7.3 ± 11.0	1.4	0.0-38.0	
No fibrosis	14.3 ± 23.5	5.0	0.0-68.0	
33. day blast (Bone marrow smear) (%)				0.754
Fibrosis	1.8 ± 2.0	1.0	0.0-8.0	
No fibrosis	1.6 ± 1.1	1.0	1.0-4.0	
33. day blast (Flow cytometry) (%)				0.192
Fibrosis	0.027 ± 0.043	0.5	0.0-12.3	
No fibrosis	0.061 ± 0.119	1.0	0.0-33.3	

SD = Standard deviation, Min = Minimum, Max = Maximum, PS= Peripheral Smear

brosis is higher in B-ALL than in T-ALL [23, 24], and B cell markers have been suggested as being the cause of this phenomenon [22]. It has also been reported that cytokines released by megakaryocytes and platelets increase fibrosis in acute megakaryoblastic leukemia. According to the same study, reticulin synthesis is thought to be stimulated by cytokines secreted by CD34 and HLA-DR expressing leukemic cells in diseases characterized by abnormal megakaryocytes [25]. According to our study, 57.1% of B-ALL patients and 37.5% of T-ALL patients had fibrosis; our findings are like those of previous studies.

There were 38.9% of HR cases in our study, similar to 33.3% of 81 cases in a previous study [24]. Fi-

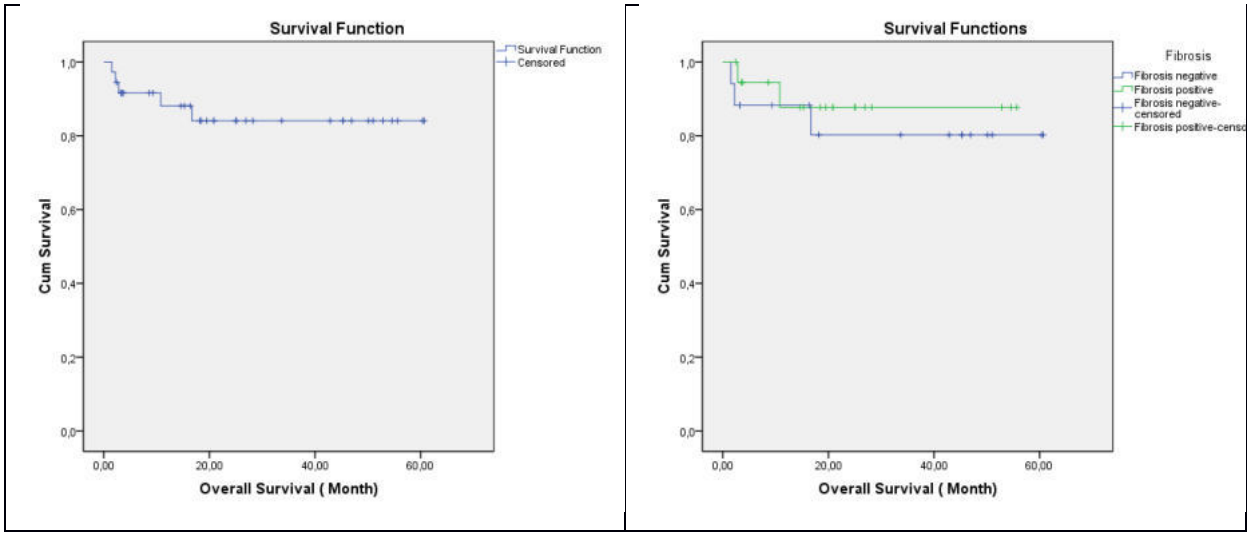

Fig. 3. Overall survival of the study population (on the left) and Overall survival of the study population according to the fibrosis (on the right).

brosis was detected in 57.1% of HR patients; in 50.0% of SR and IR patients; these findings are consistent with those from prior studies [23, 24]. We found no statistically significant difference between HR and other risk groups in bone marrow fibrosis ($p = 0.676$); however, some reports state a significantly higher level of fibrosis in the HR group [13]. This is because leukemia patients have easier access to haemato-oncologists, and treatment is initiated early in the disease process before fibrosis develops.

In our study, the median white blood cell count at diagnosis was 21.7 (×103) in the group with fibrosis, whereas it was 12.0 (×103) in the group without fibrosis. There was no significant difference ($p = 0.296$) or correlation ($p > 0.05$) between these two groups. In a previous study by Hann *et al*. [23], a negative correlation was found between the number of white blood cells at the time of diagnosis and high reticulin fiber density. Similarly, a negative correlation was found between the number of white blood cells at diagnosis and reticulin fiber density in 146 precursor B-ALL patients (r= -0.22, $p = 0.008$) [13]. We believe that our study's results depend on early access to haemato-oncologists by patients with leukemia.

An earlier study found that bone marrow fibrosis and blasts in the peripheral blood were negatively correlated (Spearman's correlation r= -0.278, $p = 0.018$) [23]. In a second study, a negative correlation was found between the number of blasts that filled the blood vessels in the bone marrow and the density of bone marrow fibrosis [18]. Nevertheless, we found that the median value of blasts in flow cytometry at diagnosis was lower in the group with fibrosis than in the group without fibrosis, 83.5% and 90.0%, respectively; neither the difference nor the correlation was significant between the two groups ($p = 0.501$, $p > 0.05$). There may be a connection between the low number of blasts detected in flow cytometry and the fact that fibrosis traps leukemia cells.

The literature reports a correlation between marrow fibrosis rate and the number of blasts, suggesting that fibrosis traps leukemia cells leading to the increase in blasts [13]. According to our study, the median blast value was 95.0% in the presence or absence of fibrosis, which was neither statistically significant ($p = 0.961$) nor correlated ($p > 0.05$).

The literature reports that high diagnostic reticulin fiber density and minimal residual disease (MRD) level on the 29th day after induction therapy are correlated and prognostic for BCP-ALL patients. Accordingly, patients with MRD values greater than 10-4 were found to have a higher degree of reticulin fibrosis (RFD) at diagnosis [13]. We did not assess MRD in our study; however, in the bone marrow examination, 100% (n = 17) of patients without fibrosis were M1, 88.9% (n = 16) of patients with fibrosis were M1, and 11.1% (n = 2) were M2. They did not differ statistically significantly ($p = 0.486$).

A total of 31 patients in our study were in remission and healthy, while five died. Fibrosis was present in only two of the Ex-patients. Among the patients with and without fibrosis, the mean OS of the patients with fibrosis was 50.22 ± 5.44 months, and the mean OS of the patients without fibrosis was 49.70 ± 3.96 months; the difference was insignificant ($p = 0.557$). The survival analysis of our study was similar to that of the previous study of 84 pediatric ALL patients: no difference was found between the two groups ($p = 0.108$) [13].

In opposite to our study, in a study of 44 adults, ALL patients found that patients with profound reductions of bone marrow fibrosis after induction therapy had longer survivals (39 months vs. 12 months) compared to patients without fibrosis or those without profound reduction at diagnosis. Reduction of fibrosis appears to be associated with better relapse-free survival [20].

The bone marrow fibrosis almost entirely returned to normal after induction treatment in other studies reported in the literature; in one study, bone marrow fibrosis was significantly reduced on the 15th day compared to when it was diagnosed. A study found that only 2.6% of patients had bone marrow fibrosis after induction therapy. Similarly, the bone marrow fibrosis of the patients in our study returned to normal except for one patient (5.5%) [14, 26].

Limitations and Strengths

Biopsy is generally not performed in the world in the diagnosis and follow-up of childhood leukemia. However, in leukemia, the bone marrow microenvironment is constantly questioned. Our study contains rare data in the world that clarifies this issue. Minimal residual disease was not detected when evaluating the response to treatment, which is the limitation of our study.

CONCLUSION

Bone marrow fibrosis can be detected during diagnosis in ALL pediatric cases. It is unclear whether fibrosis interferes with treatment or is prognostic since very few studies have clarified these issues, and their results contradict each other. Few studies have reported that bone marrow fibrosis is resolved in almost all pediatric ALL patients after induction therapy. As far as we know, this is our country's first study of its kind. In our research, the fibrosis in almost all patients returned to normal after initiating induction therapy, which suggests that the presence of fibrosis at the time of diagnosis does not contribute to predicting prognosis or survival. Based on the almost complete normalization of bone marrow fibrosis with treatment and the lack of association with prognosis, it has been hypothesized that childhood acute lymphoblastic leukemias have a different pathophysiology than childhood myeloid leukemias. The disappearance of leukemia cells with treatment and the almost normalization of fibrosis in pediatric ALL can be attributed to some cytokines expressed from leukemia cells as the cause of fibrosis. Conducting studies on a larger patient population would be necessary to uncover these factors.

Ethics Committee Approval

The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (July 2, 2021/429).

Authors' Contribution

Study Conception: ÇÖ; Study Design: ÇÖ; Supervision: YDK, İE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: NE, HSŞ; Statistical Analysis and/or Data Interpretation: YŞ; Literature Review: NE, HSŞ; Manuscript Preparation: ÇÖ, İE and Critical Review: YDK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Kato M, Manabe A. Treatment and biology of pediatric acute lymphoblastic leukemia. Pediatr Int 2018;60:4-12.
2. Brown P, Inaba H, Annesley C, Beck J, Colace S, Dallas M, et

al. Pediatric acute lymphoblastic leukemia, version 2.2020, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2020;18:81-112.
3. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 2012;21:309-22.
4. Bissell MJ, Radisky D. Putting tumours in context. Nat Rev Cancer 2001;1:46-54.
5. Bader JE, Voss K, Rathmell JC. Targeting metabolism to improve the tumor microenvironment for cancer immunotherapy. Mol Cell 2020;78:1019-33.
6. Wu Z, Chen R, Wu L, Zou L, Ding F, Wang M, et al. Bone marrow fibrosis at diagnosis predicts survival for primary acute myeloid leukemia. Clin Transl Oncol 2017;19:1462-8.
7. Hughes AM, Kuek V, Kotecha RS, Cheung LC. The bone marrow microenvironment in B-cell development and malignancy. Cancers (Basel) 2022;14:2049.
8. Chen J, Hendriks M, Chatzis A, Ramasamy SK, Kusumbe AP. Bone vasculature and bone marrow vascular niches in health and disease. J Bone Miner Res 2020;35:2103-20.
9. Visnjic D, Kalajzic Z, Rowe DW, Katavic V, Lorenzo J, Aguila HL. Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. Blood 2004;103:3258-64.
10. Mansour A, Anginot A, Mancini SJ, Schiff C, Carle GF, Wakkach A, et al. Osteoclast activity modulates B-cell development in the bone marrow. Cell Res 2011;21:1102-15.
11. Żelechowska P, Brzezińska-Błaszczyk E, Kusowska A, Kozłowska E. The role of adipokines in the modulation of lymphoid lineage cell development and activity: an overview. Obes Rev 2020;21:e13055.
12. Hasselbalch HC. The role of cytokines in the initiation and progression of myelofibrosis. Cytokine Growth Factor Rev 2013;24:133-45.
13. Norén-Nyström U, Roos G, Bergh A, Botling J, Lönnerholm G, Porwit A, et al. Bone marrow fibrosis in childhood acute lymphoblastic leukemia correlates to biological factors, treatment response and outcome. Leukemia 2008;22:504-10.
14. Nguyen T-V, Melville A, Nath S, Story C, Howell S, Sutton R, et al. Bone marrow recovery by morphometry during induction chemotherapy for acute lymphoblastic leukemia in children. PloS One 2015;10:e0126233.
15. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 2005;90:1128-32.
16. Campbell M, Castillo L, Riccheri C, Janic D, Jazbec J, Kaiserova E, et al. A Randomized Trial of the I-BFM-SG for the Management of Childhood non-B Acute Lymphoblastic Leukemia. ALL IC-BFM, August 2009.
17. Leeuw JA, Koudstaal J, Wiersema-Buist J, Kamps WA, Timens W. Bone histomorphometry in children with newly diagnosed acute lymphoblastic leukemia. Pediatr Res 2003;54:814-8.
18. Nath SV, Nicholson I, Tapp H, Zola H, Zannettino AC, Revesz T. Reticulin fibres anchor leukaemic blasts in the marrow of patients with acute lymphoblastic leukaemia. Med Hypotheses 2011;77:333-5.
19. Døsen-Dahl G, Munthe E, Nygren MK, Stubberud H, Hystad ME, Rian E. Bone marrow stroma cells regulate TIEG1 expression in acute lymphoblastic leukemia cells: role of TGFbeta/BMP-6 and TIEG1 in chemotherapy escape. Int J Cancer 2008;123:2759-66.
20. Cooke A, Montante-Montes D, Zúñiga-Tamayo D, Rivera M, Bourlon C, Aguayo Á, et al. Bone marrow fibrosis as prognostic marker in adult patients with acute lymphoblastic leukemia. J Hematopathol 2019;12:75-84.
21. Wallis JP, Reid MM. Bone marrow fibrosis in childhood acute lymphoblastic leukaemia. J Clin Pathol 1989;42:1253-4.
22. Hann IM, Evans DI, Marsden HB, Jones PM, Palmer MK. Bone marrow fibrosis in acute lymphoblastic leukaemia of childhood. J Clin Pathol 1978;31:313-5.
23. Bharos, A, AJ de Jong, Nicholas Manton, N Venn, Colin Story, G Hodge, et al. Bone marrow fibrosis and vascular density lack prognostic significance in childhood acute lymphoblastic leukaemia. Leukemia 2010;24:1537-8.
24. Norén-Nyström U, Roos G, Bergh A, Forestier E. Prognostic impact of vascular density and fibrosis in the bone marrow of children with high-risk acute lymphoblastic leukemia. Leukemia 2005;19:1998-2001.
25. Abou Dalle I, Nassif S, Bazarbachi A. Acute promyelocytic leukemia with increased bone marrow reticulin fibrosis: description of three cases and review of the literature. Hematol Oncol Stem Cell Ther 2018;11:99-104.
26. Ayyanar P, Kar R, Dubashi B, Basu D. Post-chemotherapy changes in bone marrow in acute leukemia with emphasis on detection of residual disease by immunohistochemistry. Cureus 2021;13:e20175.

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Complicated and uncomplicated acute appendicitis in pregnancy: a single center experience

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ABSTRACT

Objectives: Acute appendicitis is the most common non-obstetric pathology requiring emergency surgery on pregnant women. The aim of the study is to show the effect of uncomplicated and complicated acute appendicitis on pregnancy.

Methods: This study was conducted retrospectively at the Department of General Surgery of Konya City Hospital. Pregnant patients who underwent surgery for acute appendicitis from January 1, 2020, through December 31, 2021, were included in the study. The cases were divided into two groups as complicated and uncomplicated appendicitis and compared. The demographic, clinical, and laboratory findings of the patients were obtained by screening the patient files. The cases were divided into two groups as complicated and uncomplicated appendicitis and compared.

Results: The study included 46 pregnant patients with a mean age of 25.9 years. The mean gestational age of the patients at the time of surgery was 16.5 weeks. Of the patients, 47.8% were in the first trimester. Open appendectomy was performed in 35 (76.1%) patients, and laparoscopic appendectomy in eight (17.4%). Seven (15.2%) appendicitis cases were complicated. A negative appendectomy was performed in two (4.3%) patients. Preterm labor was seen at a rate of 28.6% in the complicated appendicitis group and 5.1% in the uncomplicated appendicitis group ($p = 0.04$), and the mean operative times were 65.8 and 46.1 minutes, respectively ($p < 0.001$).

Conclusions: Both postoperative complications due to surgery and obstetric complications increase in patients with complicated appendicitis; therefore, it is particularly important to closely follow up these cases.

Keywords: Appendicitis, complication, laparoscopy, pregnancy, preterm labor

Acute appendicitis (AA) is the most common non-obstetric pathology requiring emergency surgery in pregnant women (PW). It has been reported to affect 0.1-0.3% of PW every year [1]. It is especially common in the second trimester [2]. AA is seen less frequently in PW compared to non-pregnant women. Therefore, it is considered that pregnancy may have a protective effect against AA through hormonal changes [3].

In patients who have undergone surgery for non-obstetric reasons, AA is the cause with the highest rate of premature birth and fetal loss. In AA, the rate of

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fetal loss has been reported to be 2.6%, while this rate increases up to 10.9% in the cases of complicated AA with peritonitis [4]. Fetal loss usually occurs within the first postoperative week [5]. In PW, negative appendectomy rates have been reported to be in the range of 23-33% [6, 7]. Obstetric complications are more common in those who have undergone a negative appendectomy; therefore, utmost care should be taken when diagnosing AA in this patient population.

The first and most important step in the diagnosis of AA in the general population is a physical examination (PE). Although PE findings are also important in PW, they may be altered, especially in the later stages of pregnancy, with the enlargement of the uterus and the displacement of the intra-abdominal organs. In these patients, ultrasonography (USG) is usually the first-line imaging method used. However, magnetic resonance imaging (MRI) remains the most important imaging modality to exclude appendicitis [6, 8].

Laparoscopy appendectomy (LA) and open appendectomy (OA) are performed in the surgical treatment of AA. Some authors have indicated 26-28 weeks of gestation as the upper limit for laparoscopic interventions in PW [1]. However, recent studies have reported that there is no such upper limit, and that laparoscopy can be used in any suitable patient [9]. Although research suggests that OA is safer, many studies have reported that LA is a safe procedure that does not increase the risk of maternal complications with open surgery [10-17]. However, laparoscopy also has certain disadvantages, such as the possibility of uterine injury, technical difficulties due to uterine enlargement, and the risk of carbon dioxide absorption due to impaired uterine blood flow [18].

AA can be classified into two groups as complicated and uncomplicated. Perforated, gangrenous and plastron appendicitis are classified in the complicated group. Studies have shown that surgical and obstetric complications are higher in complicated pregnant appendicitis cases compared to uncomplicated appendicitis [19].

This study aimed to examine the clinical data of AA in a PW, the surgical methods used in treatment, the problems that occurred in the postoperative period, and the effects of AA on pregnancy. In addition, the effect of complicated appendicitis on pregnancy was also investigated.

METHODS

Trial Design

This study was conducted retrospectively at the Department of General Surgery of Health Sciences University Konya City Hospital. Approval was obtained from the Ethics Committee of KTO Karatay University Faculty of Medicine (decision number: 50517, date: December 30, 2022), and written informed consent from all participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Participants and Eligibility Criteria

PW who underwent surgery for AA between January 1, 2020, and December 31, 2021, were included in the study. Inclusion criteria: Being aged 18 and over, being pregnant, and undergoing surgery with a pre-diagnosis of AA. Exclusion criteria: Age under 18 years, undergoing surgery for reasons other than AA, patients whose information cannot be reached from hospital records or by phone. According to the surgical findings and final pathology results, the cases were divided into two groups as complicated and uncomplicated appendicitis. Perforated, gangrenous and plastron appendicitis were included in the complicated appendicitis group.

Outcomes

The files of the patients included in the study were examined, and demographic characteristics and clinical data, such as preoperative imaging methods, surgical methods applied, operative time, postoperative complications, length of hospital stay, and follow-up information about pregnancy and pregnancy outcomes were recorded.

Pregnancy outcomes were obtained from the hospital records. For those who gave birth in another healthcare center, the related data were obtained by calling the patients. Data on patients who terminated their pregnancy voluntarily for reasons other than medical necessity were also recorded.

The primary outcome of the study was determined as preterm labor or the premature rupture of mem-

branes, defined as deliveries within one month after surgery. The limit for preterm labor was accepted as 37 weeks. The secondary outcome was clinical data, including leukocyte count at admission, C-reactive protein (CRP) value, time interval of onset of symptoms to admission to hospital, time interval of admission to hospital to surgery time, surgical procedures performed, length of hospital stay, operative time, postoperative complication rates, complicated appendicitis rates, and pathology results. The recorded data were compared between the complicated and uncomplicated appendicitis groups.

Statistical Analysis

Prior to the statistical analysis, the Kolmogorov-Smirnov and Shapiro-Wilk normality tests were performed. If normality could not be achieved in even one of the groups, non-parametric test methods were preferred. Then, the Mann-Whitney U test was conducted to compare the variables obtained by measurement between the groups. The chi-square and Fisher's exact tests were used to analyze the relationship or differences between the groups in terms of categorical variables. Comparative results between the groups and other demographic characteristics were presented using the rates of qualitative variables. Quantitative variables were shown with mean and median (minimum-maximum) values. The Statistical Package for the Social Sciences (SPSS), version 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, and a p value of < 0.05 was accepted as the statistical significance limit.

Table 1. Demographic and clinical data

	Value
Maternal age (years), mean ± SD (range)	25.9 ± 4.9 (18-37)
Gestational week at surgery (week), mean ± SD (range)	16.5 ± 7.6 (3-30)
Trimester, n (%)	
First	22 (47.8)
Second	16 (34.8)
Third	8 (17.4)
Nulliparity, n (%)	26 (56.5)
Multiparity, n (%)	20 (43.5)
History of miscarriage, n (%)	5 (10.8)
Comorbidity (HT, DM, or CHD), n (%)	3 (6.5)
WBC ($\times 10^3$/uL), mean ± SD (range)	14.3 ± 4.1 (7-25)
CRP (mg/dL), mean ± SD (range)	23.9 ± 31.1 (3-157)
Diagnosis method, n (%)	
Ultrasonography	35 (76.1)
Magnetic resonance imaging	11 (23.9)
Length of hospital stay (day), mean ± SD (range)	2.4 ± 1.5 (1-7)
Readmission, n (%)	5 (10.8)
Complications, n (%)	5 (10.8)
Superficial SSI	1 (2.2)
Deep SSI	2 (4.3)
Spinal syndrome	1 (2.2)
Non-specific abdominal pain	1 (2.2)

Data are presented as mean ± standard deviation, range (minimum-maximum), and frequency. SD = standard deviation, WBC = white blood cell, CRP = C-reactive protein, HT = hypertension, DM = diabetes mellitus, CHD = coronary heart disease, SSI = surgical site infection

RESULTS

Between 1 January 2020 and 31 December 2021, there were 53 PW who were operated on with the diagnosis of AA. Seven patients whose hospital records or data could not be reached by telephone were excluded from the study. Thus, 46 patients were included in the study. The mean age of these patients was 25.9 years. It was the first pregnancy of 26 (56.5%) of the patients. There were five (10.8%) patients with a previous history of miscarriage, and three (6.5%) patients with the comorbidities of hypertension, diabetes mellitus, and coronary heart disease (Table 1).

The mean gestational age of the patients at the time of surgery was 16.5 weeks. Twenty-two (47.8%) patients were in the first trimester, 16 (34.8%) were in the second trimester, and eight (17.4%) were in the third trimester.

The mean time interval of onset of symptoms to admission to hospital was 7.4 hours in all patients. The mean time interval of admission to hospital to surgery time was 3.1 hours. (Table 2).

According to the blood tests performed before surgery, the mean white blood cell (WBC) count was 14.3 ×103/uL, and the mean CRP value was 23.9 mg/dL. The AA diagnosis was made using USG in 35 (76.1%) of the patients and MRI in 11 (23.9%). OA was performed in 35 (76.1%) patients, and LA in eight (17.4%). Conversion from laparoscopy to open appendectomy was required in three (6.5%) cases. Seven (15.2%) AA cases were complicated. A negative appendectomy was performed in two (4.3%) patients.

Table 2. Surgical and clinical data

	Value
Time interval of admission to hospital to surgery time (hour), mean ± SD (range)	3.1 ± 1.1 (1-5)
Time interval of onset of symptoms to admission to hospital (hour), mean ± SD (range)	7.4 ± 1.8 (5-12)
Preterm labor, n (%)	4 (8.7)
Term delivery, n (%)	39 (84.8)
Cesarian section	12 (26.1)
Normal vaginal delivery	27 (58.7)
Spontaneous abortion, n (%)	3 (6.5)
Fetal loss, n (%)	3 (6.5)
Surgical procedure, n (%)	
Open appendectomy	35 (76.1)
Laparoscopic appendectomy	8 (17.4)
Conversion to open surgery	3 (6.5)
Operative time (minute), mean ± SD (range)	49.4 ± 13.1 (28-76)
Complicated appendicitis, n (%)	7 (15.2)
Negative appendectomy, n (%)	2 (4.3)
Pathology result, n (%)	
Appendicitis	38 (82.6)
Normal appendix	2 (4.3)
Neuroendocrine tumor	3 (6.5)
Mucinous cystadenoma	1 (2.2)
Benign lymphoid hyperplasia	2 (4.3)

Data are presented as mean ± standard deviation, range (minimum-maximum), and frequency.

The mean operative time was 49.4 minutes, and the mean length of hospital stay was 2.4 days (Tables 1 and 2).

In the postoperative period, five (10.8%) patients were readmitted to the hospital due to the development of complications. One of these patients had a superficial surgical site infection, two had deep surgical site infections, one had spinal syndrome, and the other had non-specific abdominal pain. All patients were treated medically. These complications were only seen in the patients who underwent OA, with no postoperative complication occurring in the cases in which a LA was performed. There was no maternal mortality in our study. Thirty-nine (84.8%) patients had term deliveries, while preterm labor was seen in four (8.7%) patients and spontaneous abortion occurred in three (6.5%). Of the term deliveries, 58.7% were delivered through the vaginal route and 26.1% were delivered through a cesarean section. The pathology results were AA in 38 (82.6%) patients, normal appendix tissue in two (4.3%), neuroendocrine tumors in three (6.5%), benign lymphoid hyperplasia in two (4.3%), and mucinous cystadenoma in one (2.2%) (Table 2).

When the complicated and uncomplicated appendicitis groups were compared, there was no significant difference in terms of WBC count, CRP, postoperative complications, the rate of spontaneous abortion, length of hospital stay, readmission rate, and gestational week at surgery. There was no significant difference between the two groups on the time interval of onset of symptoms to admission to hospital and the time interval of admission to hospital to surgery time. Preterm labor was observed in 28.6% of the patients in the complicated appendicitis group and 5.1% of those in the uncomplicated appendicitis group ($p = 0.04$). The mean operative time was 65.8 minutes in the complicated appendicitis group and 46.1 minutes in the uncomplicated appendicitis group ($p < 0.001$) (Table 3).

DISCUSSION

AA is one of the most common causes of emergency surgery in PW [1]. The rates of premature birth and fetal loss increase significantly in patients that have undergone surgery with this diagnosis. In the literature, it has been reported that AA is particularly more frequent in the second trimester [2]. However, in the

Table 3. Comparison of the complicated and uncomplicated appendicitis groups

	Complicated appendicitis (n = 7)	Uncomplicated appendicitis (n = 39)	*p value*
Gestational week at surgery, mean ± SD (range)	19.8 ± 8.4 (5-30)	15.5 ± 7.1 (3-30)	0.15
Time interval of onset of symptoms to admission to hospital (hour), mean ± SD (range)	8.3 ± 2.2 (6-12)	7.3 ± 1.8 (5-10)	0.29
Time interval of admission to hospital to surgery time (hour), mean ± SD (range)	3.6 ± 0.9 (2-5)	3.1 ± 1.1 (1-5)	0.24
WBC (10^3/mm^3), mean ± SD (range)	16.3 ± 5.1 (8-23)	13.8 ± 3.8 (7-25)	0.16
CRP (mg/dL), mean ± SD (range)	36.1 ± 54.3 (4-157)	19.7 ± 22.3 (3-75)	0.24
Postoperative complication, n (%)	2 (28.6)	3 (7.7)	0.10
Preterm labor, n (%)	2 (28.6)	2 (5.1)	**0.04**
Spontaneous abortion, n (%)	0	3 (7.7)	0.5
Fetal loss, n (%)	0	3 (7.7)	0.5
Length of hospital stay (day), mean ± SD (range)	3 ± 1.6 (1-6)	2.1 ± 1.3 (1-6)	0.14
Readmission, n (%)	2 (28.6)	3 (7.7)	0.10
Operative time (minute), mean ± SD (range)	65.8 ± 10.6 (47-76)	46.1 ± 10.7 (28-75)	**< 0.001**

Data are presented as mean ± standard deviation, range (minimum-maximum), and frequency. SD = standard deviation, WBC = white blood cell, CRP = C-reactive protein

current study, it was more common among the patients in the first trimester (47.8%).

The imaging method used as the first choice in diagnosis is usually USG. However, USG can be non-diagnostic many times. For this reason, MRI is the safest method to exclude AA [6, 8]. In our study, USG was used most frequently (76.1%), but in cases where USG could not make a diagnosis, MRI was used in 23.9% of the cases. Despite the PE and imaging methods, negative appendectomy can be performed frequently in PW. This rate has been reported in the range of 23-33% [6, 7]. In our study, this rate was lower than in the literature and was found to be 4.3%.

The negative effect of AA on fetal loss and preterm labor is known. In the literature, the fetal loss rate was reported as 2.6% in uncomplicated cases and 10.9% in complicated cases [4]. Fetal loss rate was determined as 6.5% in all patients in our study. In a systematic review, the rate of preterm labor in AA cases was reported as 8.1% [20]. All patients in our study, this rate was found to be 8.7%, which is consistent with the literature. In addition, this rate was found to be significantly higher in complicated appendicitis cases (28.6%-5.1%) ($p = 0.04$). This supports the knowledge that obstetric problems are higher in complicated cases, as stated in the literature. In the literature, the rate of postoperative complications varies between 5% and 20%, depending on the complexity of the cases [19]. In our study, the postoperative complication rate was found to be 10.8%, which is consistent with the literature. These complications were surgical site infections and spinal syndrome, which were treated medically. While no postoperative complications were found in patients who underwent LA, all complications were found in patients who underwent OA. This suggests that laparoscopy is superior both in terms of wound infection and spinal syndrome due to spinal anesthesia.

Surgery for AA can be performed with the laparoscopic or open method [1, 10, 12-15]. In our study, open surgery was performed in 76.1% of the patients, and laparoscopy in 17.4%. In three (6.5%) cases, conversion to open surgery was required due to the complicated nature of appendicitis. As stated in the literature, both laparoscopy and open surgery can be applied to PW [10].

In our study, the mean operative time was 49.4 minutes, which is consistent with the literature [14]. As expected, the operative time was significantly longer in the complicated appendicitis group ($p < 0.001$). The length of hospital stay was 2.4 days, which is similar to the value reported by a systemic review (3 days) [11].

The final pathology results were consistent with AA in 38 (82.6%) patients, a normal appendix in two (4.3%), and other conditions, such as neuroendocrine tumors, mucinous cystadenomas, and benign lymphoid hyperplasia in six (13%). In a previous study conducted in Turkey, the incidence of non-appendicitis pathology results was reported to be 8.3% , indicating a higher rate in our study [20].

Limitations

The most important limitation of this study concerns its retrospective design and small number of cases.

CONCLUSION

AA is one of the most common causes of emergency surgery in PW. In this patient population, the risk of preterm delivery and fetal loss is high, especially in complicated appendicitis cases. Therefore, when AA is suspected in PW, it is important to make a rapid diagnosis using USG and MRI methods, and to perform surgical treatment with a laparoscopic or open method without delay.

Authors' Contribution

Study Conception: İH; Study Design: MEU; Supervision: İH; Funding: MEU; Materials: İH; Data Collection and/or Processing: MEU; Statistical Analysis and/or Data Interpretation: İH; Literature Review: MEU; Manuscript Preparation: İH and Critical Review: MEU.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Barnes SL, Shane MD, Schoemann MB, Bernard AC, Boulanger BR. Laparoscopic appendectomy after 30 weeks pregnancy: report of two cases and description of technique. Am Surg 2004;70:733-6.
2. Hee PP, Viktrup L. The diagnosis of appendicitis during pregnancy and maternal and fetal outcome after appendectomy. Int J Gynecol Obstet 1999;65:129-35.
3. Andersson REB, Roland EB, Lambe M. Incidence of appendicitis during pregnancy. Int J Epidemiol 2001;30:1281-5.
4. Cohen-Kerem R, Railton C, Oren D, Lishner M, Koren G. Pregnancy outcome following non-obstetric surgical intervention. Am J Surg 2005;190:467-73.
5. Corneille MG, Gallup TM, Bening T, Wolf SE, Brougher C, Myers JG, et al. The use of laparoscopic surgery in pregnancy: evaluation of safety and efficacy. Am J Surg 2010;200: 363-7.
6. Lemieux P, Rheaume P, Levesque I, Bujold E, Brochu G. Lemieux, Patrice, et al. Laparoscopic appendectomy in pregnant patients: a review of 45 cases. Surg Endosc 2009;23:1701-5.
7. McGory ML, Zingmond DS, Tillou A, Hiatt JR, Ko CY, Cryer HM. Negative appendectomy in pregnant women is associated with a substantial risk of fetal loss. J Am Coll Surg 2007;205:534-40.
8. Pedrosa I, Levine D, Eyvazzadeh AD, Siewert B, Ngo L, Rofsky NM. MR imaging evaluation of acute appendicitis in pregnancy. Radiology 2006;238:891-9.
9. Jackson H, Granger S, Price R, Rollins M, Earle D, Richardson W, et al. Diagnosis and laparoscopic treatment of surgical diseases during pregnancy: an evidence-based review. Surg Endosc 2008;22:1917-27.
10. Cheng HT, Wang YC, Lo HC, Su LT, Soh KS, Tzeng CW, et al. Laparoscopic appendectomy versus open appendectomy in pregnancy: a population-based analysis of maternal outcome. Surg Endosc 2015;29:1394-9.
11. Walsh CA, Tang T, Walsh SR. Laparoscopic versus open appendicectomy in pregnancy: a systematic review. Int J Surg 2008;6:339-44.
12. Palanivelu C, Rangarajan M, Parthasarathi R. Laparoscopic appendectomy in pregnancy: a case series of seven patients. JSLS 2006;10:321.
13. Rizzo AG. Laparoscopic surgery in pregnancy: long-term follow-up. J Laparoendosc Adv Surg Tech A 2003;13:11-5.
14. Lyass S, Pikarsky A, Eisenberg VH, Elchalal U, Schenker JG, Reissman P. Is laparoscopic appendectomy safe in pregnant women? Surg Endosc 2001;15:377-9.
15. Wu JM, Chen KH, Lin HF, Tseng LM, Tseng SH, Huang SH. Laparoscopic appendectomy in pregnancy. J Laparoendosc Adv Surg Tech A 2005;15: 447-50.
16. Kirshtein B, Perry ZH, Avinoach E, Mizrahi S, Lantsberg L. Safety of laparoscopic appendectomy during pregnancy. World J Surg 2009;33:475-80.
17. Sadot E, Telem DA, Arora M, Butala P, Nguyen SQ, Divino CM. Laparoscopy: a safe approach to appendicitis during pregnancy. Surg Endosc 2010;24:383-9.
18. Fatum M, Rojansky N. Laparoscopic surgery during pregnancy. Obstet Gynecol Survey 2001;56:50-9.
19. Humes DJ, Simpson J. Acute appendicitis. BMJ 2006;333:530-4.
20. Yilmaz M, Akbulut S, Kutluturk K, Sahin N, Arabaci E, Ara C, et al. Unusual histopathological findings in appendectomy specimens from patients with suspected acute appendicitis. World J Gastroenterol 2013;19:4015-22.

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Analgesic efficacy and safety of epidural and paravertebral blocks in thoracotomy surgery

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ABSTRACT

Objectives: Epidural anesthesia, once considered a gold standard, has significant complications: hypotension, urinary retention, and catastrophic neurological damage in rare cases. While paravertebral analgesia can provide similar analgesic effectiveness to epidural analgesia, it is associated with fewer side effects. To compare the perioperative and postoperative effects of epidural (ED) and ultrasound-guided paravertebral block (PVB) applications in thoracotomy surgeries.

Methods: Fifty-two patients underwent elective thoracotomy; Group 1 (ED, n = 23) and Group 2 (PVB, n = 29) were evaluated. A comparison of hemodynamic parameters and complications preoperatively and postoperatively, postoperative analgesia requirements, visual analog scale (VAS) pain scores within 24 hours at specified times, the amount of analgesic used, the time to mobilize, the time to discharge, and the VAS values measured at three months was made.

Results: There was no difference between the study groups regarding demographic and ASA values, most comorbid chronic diseases, obesity, and smoking. The number of attempts, the postoperative 2nd hour VAS score, and the doses of paracetamol and tramadol used was significantly higher in Group 1 than in Group 2 ($p = 0.002$, $p = 0.002$, $p = 0.012$, and $p = 0.022$, respectively). There was no statistical difference between the groups in terms of postoperative 6, 12, 24 hours, and 3 months VAS scores, first mobilization and discharge, time to remove the thorax tube, intensive care (PACU) need, and perioperative and postoperative complications ($p > 0.05$).

Conclusions: Ultrasound-guided PVB can be considered a safe and effective alternative to ED in thoracotomy surgery.

Keywords: Epidural block, paravertebral block, postoperative pain, chronic pain

Thoracotomy pain is one of the most severe complications associated with surgery. The pain is caused by damage to the pleura, muscles, intercostal nerves, and costovertebral joints. It can also result in adverse outcomes such as respiratory complications, prolonged hospital stays, poor quality of life, and chronic pain syndrome [1]. Pain after thoracotomy may vary between 25% and 91%; it shows heterogene-

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ity depending on the underlying pathophysiology, type of injury, pain classification methods, and clinical evaluation. Acute pain intensity is also associated with chronic pain [2].

A goal to minimize the complications that may accompany anesthesia and to prevent acute and chronic pain has led to the search for an ideal anesthetic method. For patients with postoperative pain, a multimodal approach combining systemic and regional anesthesia is most effective in enhancing analgesia [3]. Generally, thoracic epidural blocks (ED) are preferred as analgesia methods in thoracic surgery: it is usually considered the most appropriate pain management technique following cardiothoracic surgery. There is, however, a contraindication to its use in patients with coagulopathy. Additionally, it may cause some adverse effects, such as hypotension and nerve damage. The PVB, on the other hand, can prevent respiratory side effects (atelectasis, infection, pulmonary failure) and hypotension caused by respiratory muscle weakness [4].

With this study, we aimed to evaluate and compare the perioperative and postoperative effects of multimodal ED and ultrasound-guided PVB techniques in thoracotomy surgeries in our clinic.

METHODS

A total of 52 thoracotomy surgery patients who underwent USG-guided PVB and EP were included. Permission of local ethics committee date and numbered 2011- KAEK-25 2022/10-06, and the written consent was obtained.

Inclusion criteria were unilateral elective pulmonary surgery, age ≥ 16, and agreeing to cooperate with physicians for a postoperative analgesic regimen. Patients who did not agree to participate in the study, who were allergic to anesthetic drugs in the past, who currently have psychological problems, or who have a local infection at the surgery site were excluded. Patients were excluded from the analysis if they were on mechanical ventilator support 4 hours after the operation, need for reoperation and if pain scores could not be reached by phone after 3 months postoperatively (Fig. 1). The demographic characteristics (age, gender, body mass index [BMI], ASA values), comorbidities (Diabetes Mellitus [DM], Hypertension [HT], Chronic obstructive lung disease [COPD], coronary artery disease [CAD], Obesity [BMI > 30 kg/m^2]), smoking were recorded. Patients were divided into two groups: those who underwent thoracic epidural catheterization before anesthesia (Group 1) and who underwent ultrasound-guided thoracic PVB in the surgically appropriate lateral position after induction of general anesthesia (Group 2).

The number of attempts, hemodynamic data at preoperatively determined times, minor intraoperative complications (desaturation [SpO_2 < 90%], hypotension [20% decrease in MAP compared to baseline], hypertension, arrhythmia), and major complications (atelectasis, respiratory distress, bleeding) and postoperative complications, pain scores (between 0 and 10 points) recorded with Visual Analogue Scale (VAS) at postoperative 2, 6, 12, 24 hours, need for postoperative intensive care unit (PICU), first mobilization time and discharge times, used analgesic amounts were observed retrospectively from patient files. Three months after the operation, patients were contacted by phone to determine their VAS scores. Blind anesthesiologists made the blocks prospectively. Tramadol 1 mg/kg iv was given to patients with VAS > 4 as routine rescue analgesia in the surgical service, and paracetamol 1 gr iv was given to patients with VAS > 2.

Thoracic Epidural Block

Following routine monitoring, 2 mg midazolam was administered intravenously to patients, and 1 mL of 2% prilocaine was injected with a needle at the T5-6 vertebra level subcutaneously and in a sitting position. A Tuohy needle was used to enter the epidural space using either the hanging drop technique or the loss of resistance technique. The catheter was fixed at an appropriate level after aspiration. 8 mL of 0.5% bupivacaine was injected with 100 µg of fentanyl and 10 cc of SF through a 15 mL catheter.

Paravertebral Block

A high-frequency linear USG probe (5-13 MHz; GE Healthcare Logiq P5, USA) was placed longitudinally in the lateral decubitus position. At the level of T5-6 vertebrae, a transverse process (TP) and hyperechoic pleura were found 2-2.5 cm lateral to the spinous processes, and 1 mL of 2% prilocaine was injected into the subcutaneous tissue. We used a 22-gauge, 50 mm scale peripheral block needle (Stimuplex A®; B Braun,

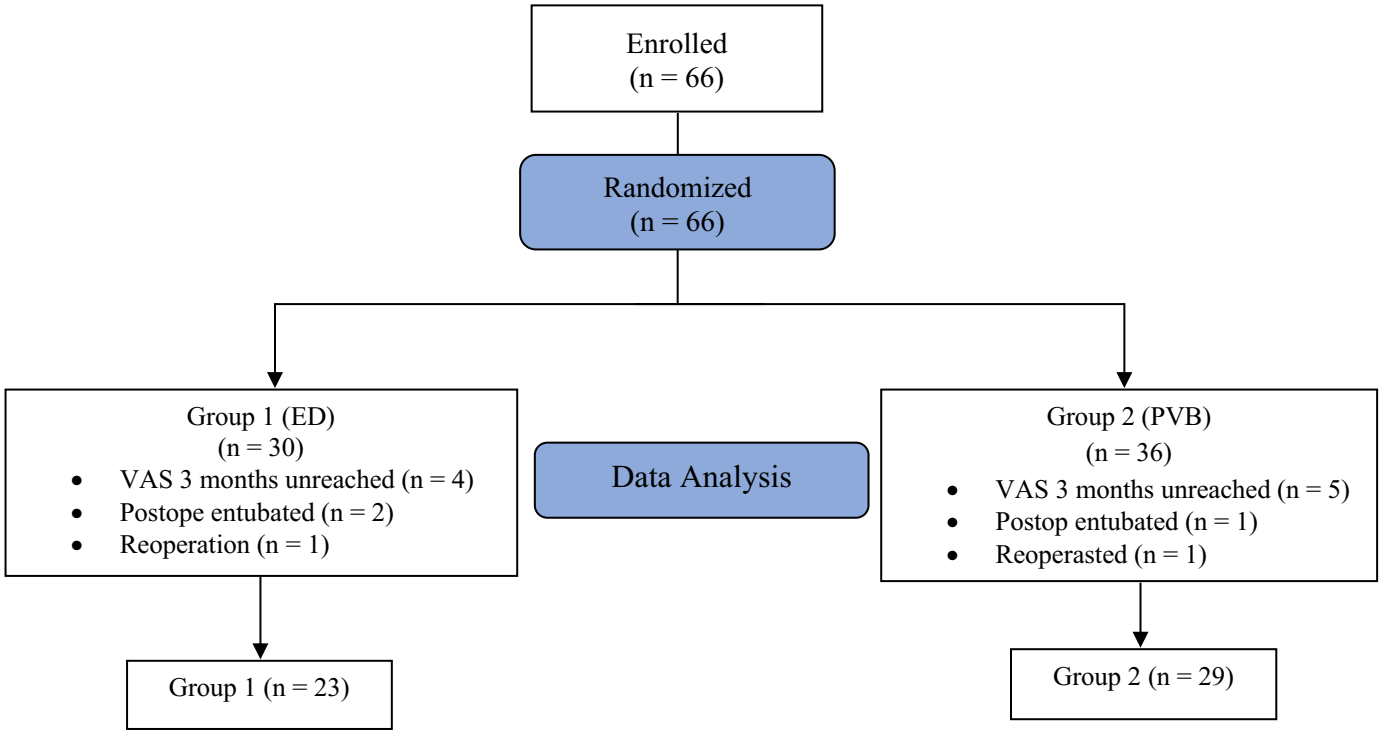

Fig. 1. Flow Chart of Procedure. ED = epidural, PVB = paravertebral block, VAS = visual analog scale

Melsungen, Germany) to make all the blocks. The needle's plane technique advanced the selected paravertebral area by passing through the trapezius, rhomboids, erector spina muscles, and superior costotransverse ligament. A total of 15 mL of 0.5% bupivacaine, 5 mL of 2% lidocaine and 10 cc SF solution was injected bilaterally after negative aspiration. The parietal pleura shifted downward in each procedure.

Primary and Secondary Outcomes

Analgesic doses in 24 hours and VAS values at postoperative 24 hours and three months were the primary outcomes. Secondary outcomes included perioperative hemodynamics, side effects, complications, mobilization, thoracic tube removal, and intensive care requirement.

Statistical Analysis

Descriptive data are presented as numbers and percentages, and measurement data are presented as mean + standard deviation and median (minimum-maximum). The assumption of normal distribution was tested using the Shapiro-Wilk test and histograms. The Chi-square test was used to compare categorical data. The Mann-Whitney U test was used to compare normally distributed measurements, and the Spearman Correlation test was used to correlate two variables. $P < 0.05$ was accepted as statistically significant. SPSS 20 was used for all analyses.

RESULTS

A comparison of 52 patients showed no statistically significant differences in age, ASA, DM, HT, COPD, obesity, or smoking. Group 2 had a statistically significant higher CAD rate than Group 1 ($p = 0.048$) (Table 1).

There were no statistically significant differences between the groups regarding MAP, HR, and SpO_2 at the measurement times determined during surgery (Table 2). Three patients in both groups experienced desaturation, responsive to increasing oxygen flow rate.

The number of interventions was higher in Group 1 ($p = 0.002$). Group 1 had significantly higher postoperative 2 hour VAS scores ($p = 0.002$) (Table 3). There was no statistical difference between the groups

Table 1. The demographic data of the patients

	Group 1 (n = 23)	Group 2 (n = 29)	*p value*
Gender (Female/male)	11/12	14/15	0.974
Age (years) (mean ± SD)	57.35 ± 7.36	60.29 ± 7.91	0.178
ASA II/III	18/5	18/11	0.209
HT, n (%)	12 (52.1)	12 (41.3)	0.438
DM, n (%)	9 (39.1)	12 (41.3)	0.870
CAD, n (%)	5 (21.7)	14 (48.2)	**0.048***
Obesity, n (%)	2 (8.6)	6 (20.6)	0.234
Smoking, n (%)	12 (52.1)	12 (41.3)	0.438
COPD, n (%)	5 (21.7)	9 (31.03)	0.453

ASA = American Society of Anesthesiologists, HT = Hypertension, DM = Diabetes Mellitus, CAD = Coronary Artery Disease, COPD = Chronic Obstructive Pulmonary Disease, SD = standard deviation

$p < 0.05$, Mann-Whitney U testi

regarding VAS 6, 12, 24 hours, and 3-month VAS scores.

There was no statistical difference between the groups regarding the first mobilization and discharge time, thorax tube removal time, and need in PICU ($p > 0.05$) (Table 3).

Postoperative paracetamol and tramadol doses were significantly higher in Group 1 ($p = 0.012$ and $p = 0.022$, respectively) (Table 3).

Correlation between VAS 24th hour and thoracic tube removal and discharge time showed no significant difference between the groups (r: 0.102, $p = 0.473$ and r: 0.097, p = 0.494, respectively).

A patient in Group 1 developed hypotension, and three patients in Group 2 suffered arrhythmia and hypertension as postoperative complications.

DISCUSSION

In our study comparing ED with PVB for multimodal analgesia in thoracotomy surgeries, the postoperative second-hour VAS, the number of attempts, and paracetamol and tramadol doses were higher in the ED group. All other hemodynamic parameters, postoperative VAS values at 6, 12, 24 hours, and 3 months, initial mobilization, time to discharge, time to remove thoracic tubes, PICU need, and complications were similar between the groups.

As part of the multimodal approach to analgesia for thoracic surgery, regional techniques may reduce the likelihood of developing postoperative complications and chronic pain. The use of both ED and PVB in the management of thoracic surgery has been deemed appropriate in systematic reviews and meta-analyses over the past 20 years [5-7]. Evidence suggests that the effects of ED versus thoracic PVB for postoperative analgesia are comparable. PVB is associated with fewer side effects when used for unilateral or bilateral thoracic surgery and video-assisted thoracoscopy. Furthermore, there is evidence that thoracic PVB is an appropriate and safer alternative to thoracic epidural placement when anticoagulation is a contraindication [5]. Meta-analysis of 10 randomized studies comparing epidural and paravertebral block analgesia after thoracotomy demonstrated adequate and similar analgesia and pain scores [8]. Our study observed a similar relationship between analgesic effects and VAS scores, except for the postoperative 2nd hour VAS score. There is a possibility that this may be explained by the fact that the local anesthetic absorption from the epidural veins is higher in ED patients due to the central block, as well as the higher dose of paracetamol and tramadol used since the analgesic efficacy passes more quickly in these patients.

Both interventions are believed to affect hemodynamics during and after surgery based on their mechanisms of action. In meta-analyses comparing thoracic

Table 2. Measurements of perioperative hemodynamics and saturation

	Group 1	Group 2	*p* value
MAP1	88.13 ± 11.25	87.38 ± 11.91	0.818
MAP2	78.48 ± 7.74	76.48 ± 9.93	0.433
MAP3	70.65 ± 6.30	70.86 ± 7.48	0.915
MAP4	69 ± 5.02	68.72 ± 6.75	0.871
MAP5	67.09 ± 5.11	67.07 ± 5.15	0.990
MAP6	65.74 ± 4.63	66.66 ± 4.93	0.498
HR1	82.26 ± 10.45	81.24 ± 10.54	0.730
HR2	76.21 ± 7.82	75.68 ± 9.38	0.830
HR3	75.48 ± 6.59	74.83 ± 7.71	0.749
HR4	74.87 ± 7.40	74.03 ± 7.18	0.683
HR5	74.83 ± 6.90	73.86 ± 6.46	0.607
HR6	74 ± 6.57	73.55 ± 6.22	0.802
SPO2-1	95.17 ± 0.93	95.31 ± 0.92	0.603
SPO2-2	93.78 ± 1.75	92.82 ± 2.53	0.131
SPO2-3	88.21 ± 1.78	88.27 ± 2.71	0.929
SPO2-4	92.47 ± 2.06	92.37 ± 2.51	0.880
SPO2-5	94.52 ± 1.59	94.48 ± 2.27	0.945
SPO2-6	95.21 ± 0.95	95.06 ± 1.33	0.655

Data are shown as mean ± standard deviation. MAP = Mean Arterial Pressure, HR = Heart Rate, SpO_2 = Oxygen Saturation

Table 3. Results of postoperative VAS scoring, number of interventions, hospital stay time, PICU requirements, and analgesia dosage

	Group 1	Group 2	*p value*
VAS1 median (min-max)	2.82 (2-5)	2.13 (1-4)	**0.002***
VAS2 median (min-max)	3.08 (1-6)	2.58 (1-4)	0.123
VAS3 median (min-max)	3.82 (2-6)	3.27 (2-6)	0.064
VAS4 median (min-max)	3.82 (3-6)	3.48 (2-5)	0.160
VAS5 median (min-max)	2.69 (1-5)	2.62 (1-4)	0.782
Attempt number (min-max)	1.73(1-3)	1.20(1-2)	**0.002***
Mobilization time (h)	17.65 ± 3.60	16.79 ± 4.13	0.435
Discharge time (h)	97.73 ± 13.31	96.72 ± 16.53	0.812
TT withdrawal (h)	24.95 ± 7.74	24.72 ± 7.46	0.913
PACU, n (%))	9 (39.1)	11 (37.9)	0.930
Paracetamol dose (mg)	230.43 ± 76.48	186.20 ± 44.11	**0.012***
Tramadol dose (mg)	134.78 ± 126.52	68.96 ± 71.23	**0.022***

Data are shown as mean ± standard deviation or median (minimum-maximum) or n (%). VAS = Visual Analog Scale, VAS1 = Postoperative 2nd hour VAS, VAS2: Postoperative 6th hour VAS, VAS3: Postoperative 12th hour VAS, VAS4: Postoperative 24th hour VAS, VAS5: Postoperative 3rd month VAS, TT = Thorax tube, PACU = Postoperative anesthetic care unit

*$p < 0.05$ Mann-Whitney U test

PVB and ED analgesia for pain relief after thoracotomy, short-term and transient hypotension was defined in the ED group [9, 10]. Since local anesthetics were administered unilaterally close to the somatic roots, the paravertebral block was found to have a lower incidence of hypotension. Paravertebral anesthesia has a minor effect on blood pressure and heart rate, making it safe for patients with cardiovascular comorbidities. Furthermore, the paravertebral block provides greater perioperative hemodynamic stability, requiring a smaller amount of intravenous colloid and less vasopressor than the ED [11, 12]. A similar hemodynamic profile was observed between the groups during the surgery in our study. However, PVB was preferred, especially in patients with coronary artery disease with an expected risk of hemodynamic instability and hypotension, resulting in heterogeneity in the comparison of groups.

In recent years, enhanced recovery after surgery (ERAS) has also gained popularity in thoracic surgery. The ERAS protocol is designed to provide an immediate termination. It aims to reduce complications by using less invasive surgical techniques and providing effective analgesia [13, 14]. Likewise, the chest tube facilitates rapid recovery following thoracic surgery. Many studies have focused on the postoperative chest tube approach in thoracic surgery [15]. According to these studies, long-term chest tube use can prolong hospital stays and increase complications. The timing of chest tube removal is under debate, but commonly it is decided based on volume plus exit thresholds. According to some surgeons, higher daily volume outputs (450-500 cc) are acceptable thresholds for removing chest tubes [16]. In most cases, patients are discharged the same day after chest tube removal. However, some may need to remain in the hospital for longer.

Furthermore, chronic co-morbidities, obesity, and smoking history contribute to chest tube lengthening. Shortening the discharge time by early removing the chest tube; it may be an appropriate approach to a center-based situation [15]. Operation time, pain scores, and postoperative respiratory movements were evaluated as factors affecting chest tube withdrawal time and discharge time. Our study found no significant difference in groups between 24th hour VAS scores and the time of discharge or removal of thoracic tubes. Low VAS scores in the postoperative period indicate that effective analgesia was provided. The complications caused by pain were reduced since almost all patients received effective analgesia. In our study length of hospital stay after thoracic tube removal exceeded 24 hours, which we attribute to clinic procedures rather than complications.

In a study comparing PVB and ED in thoracotomy cases, including upper gastrointestinal surgery, PVB showed a lower risk of minor complications. Perioperative complications were similar between the two groups in our study. Three patients in both groups experienced desaturation during one-lung ventilation responsive to an increase in oxygen flow rate. PVB was as effective as thoracic ED for the control of acute pain. There was no difference in 30-day mortality, major complications, or length of hospital stay [1, 17]. Similarly, in our study, there were no differences in postoperative complications or length of hospital stay between the groups.

ICD-11 defines post-surgical chronic pain as pain that persists beyond the healing process, at least 3 months after surgery or a tissue injury [18]. Chronic pain can develop following any surgery but is more likely to occur following an amputation, thoracotomy, or mastectomy. A low-quality evidence study suggests that preventive epidural analgesia before thoracotomy incision reduces acute pain intensity and chronic pain incidence after thoracotomy [7]. In the present study, postoperative VAS values in the 3rd month postoperatively were similar between the two groups and compatible with acute pain scores.

CONCLUSION

Our study demonstrated that both block applications were sufficient and adequate for postoperative analgesia in thoracotomy patients, did not impair perioperative hemodynamics, and had low complications. We consider ultrasound-guided PVB a safe, effective alternative to ED for managing acute and chronic pain in thoracotomy procedures.

Authors' Contribution

Study Conception: TO, AO; Study Design: TO; Supervision: ŞEÖ; Funding: ŞEÖ; Materials: AD, ŞEÖ; Data Collection and/or Processing: AO, TO; Sta-

tistical Analysis and/or Data Interpretation: AO, ME; Literature Review: AD, ÜK; Manuscript Preparation: TO, ÜK and Critical Review: ÜK, ME.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Yeung JH, Gates S, Naidu BV, Wilson MJ, Smith FG. Paravertebral block versus thoracic epidural for patients undergoing thoracotomy. Cochrane Database Syst Rev 2016;2:CD009121.
2. Arends S, Böhmer AB, Poels M, Schieren M, Koryllos A, Wappler F, et al. Post-thoracotomy pain syndrome: seldom severe, often neuropathic, treated unspecific, and insufficient. Pain Rep 2020;5:e810.
3. Marshall K, McLaughlin K. Pain management in thoracic surgery. Thorac Surg Clin 2020; 30:339-46.
4. Scarci M, Joshi A, Attia R. In patients undergoing thoracic surgery is paravertebral block as effective as epidural analgesia for pain management? Interact Cardiovasc Thorac Surg 2010; 10:92-6.
5. D'Ercole F, Arora H, Kumar PA. Paravertebral block for thoracic surgery. J Cardiothorac Vasc Anesth 2018;32:915-27.
6. Feray S, Lubach J, Joshi GP, Bonnet F, Van de Velde M;PROSPECT Working Group *of the European Society of Regional Anaesthesia and Pain Therapy. PROSPECT guidelines for video-assisted thoracoscopic surgery: a systematic review and procedure-specific postoperative pain management recommendations. Anaesthesia 2022;77:311-25.
7. Park SK, Yoon S, Kim BR, Choe SH, Bahk JH, Seo JH. Preemptive epidural analgesia for acute and chronic post-thoracotomy pain in adults: a systematic review and meta-analysis. Reg Anesth Pain Med 2020;45:1006-16.
8. Davies RG, PS Myles, Graham J. A comparison of the analgesic efficacy and side-effects of paravertebral vs epidural blockade for thoracotomy -- a systematic review and meta-analysis of randomized trials. BJA: Br J Anaesth 2006;96:418-26.
9. Mohta M, Ophrii EL, Sethi AK, Agarwal D, Jain BK. Continuous paravertebral infusion of ropivacaine with or without fentanyl for pain relief in unilateral multiple fractured ribs. Indian J Anaesth 2013;57:555-61.
10. Grider JS, Mullet TW, Saha SP, Harned ME, Sloan PA. A randomized, double-blind trial comparing continuous thoracic epidural bupivacaine with and without opioid in contrast to a continuous paravertebral infusion of bupivacaine for post-thoracotomy pain. J Cardiothorac Vasc Anesth 2012;26:83-9.
11. Baidya DK, Khanna P, Maitra S. Analgesic efficacy and safety of thoracic paravertebral and epidural analgesia for thoracic surgery: a systematic review and meta-analysis. Interact Cardiovasc Thorac Surg 2014;18:626-35.
12. Ng A Swanevelder J. Pain relief after thoracotomy: is epidural analgesia the optimal technique? Br J Anaesth 2007;98:159-62.
13. Markham T, Wegner R, Hernandez N, Lee JW, Choi W, Eltzschig HK, et al. Assessment of a multimodal analgesia protocol to allow the implementation of enhanced recovery after cardiac surgery: retrospective analysis of patient outcomes. J Clin Anesth 2019;54:76-80.
14. Batchelor TJ, Rasburn NJ, Abdelnour-Berchtold E, Brunelli A, Cerfolio RJ, Gonzalez M, et al. Guidelines for enhanced recovery after lung surgery: recommendations of the Enhanced Recovery After Surgery (ERAS®) Society and the European Society of Thoracic Surgeons (ESTS). Eur J Cardiothorac Surg 2019;55:91-115.
15. Asban A, Xie R, Abraham P, Kirklin JK, Donahue J, Wei B. Reasons for extended length of stay following chest tube removal in general thoracic surgical patients. J Thorac Dis 2020; 12:5700-8.
16. Cerfolio RJ, Bryant AS, Skylizard L and Minnich DJ. Optimal technique for the removal of chest tubes after pulmonary resection. J Thorac Cardiovasc Surg 2013;145:1535-9.
17. Terkawi AS, Tsang S, Sessler DI, Terkawi RS, Nunemaker MS, Durieux ME, et al. Improving analgesic efficacy and safety of thoracic paravertebral block for breast surgery: a mixed-effects meta-analysis. Pain Physician 2015;18:E757-80.
18. Scholz J, Finnerup NB, Attal N, Aziz Q, Baron R, Bennett MI, et al. The IASP classification of chronic pain for ICD-11: chronic neuropathic pain. Pain 2019;160:53-9.

The European Research Journal 2023;9(2):398-406 Original Article

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The relationship of pain severity with anxiety, depression, somatic symptoms, personality, and alexithymia in the early period after total knee arthroplasty

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ABSTRACT

Objectives: This study aims to investigate the relationship between pain severity in the early postoperative period and preoperative psychometric factors in individuals undergoing total knee arthroplasty (TKA).

Methods: This research was designed as a cross-sectional and descriptive study. Fifty participants undergoing TKA were inpatients at a private hospital's Orthopedics and Traumatology Clinic were included in the study. The same anesthesia protocol (spinal anesthesia) and the same surgical technique were performed on all patients. In data collection, Visual Analogue Scale (VAS), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Somatic Amplification Scale (SAS), Toronto Alexithymia Scale (TAS), Eysenck Personality Questionnaire Revised-Abbreviated (EPQR-A), and Sociodemographic Data Form were used.

Results: The mean age of the participants in the study was 64.80 ± 8.70 years, and 45 of the cases (90.0%) were women. Considering the Single Factor ANOVA Analysis, only psychoticism subscale scores were observed to be statistically significant in the change in VAS-Static scores. When all psychometric properties were controlled, the significant change in VAS-Dynamic scores was shown to continue at low effect.

Conclusions: According to these findings, the psychoticism dimension was concluded to be more associated with an antisocial personality disorder or aggressive patients, and the high level of pain in these patients may be related to low levels of serotonin and cortisol from a neurobiological point of view.

Keywords: Knee arthroplasty, pain, depression, anxiety, alexithymia, personality, somatic symptoms

Total knee arthroplasty is one of the most commonly performed orthopedic surgical procedures today; however, postoperative pain control is still one of the most significant issues [1]. Although postoperative pain occurs in all operated patients, it cannot be of the same severity in all. Whether there are individual psychological factors underlying different postoperative pain levels in patients undergoing the same surgical procedure by the same surgeon seems to be an issue that should be investigated.

According to the International Association for the Study of Pain (IASP), acute pain is a sensory and emotional experience that occurs with tissue damage [2]. Chronic pain is defined as pain that lasts longer than

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3-6 months. Pain seen in psychiatric disorders is chronic pain. Autonomic physiological arousal, activation of proinflammatory processes, and the stress response of the hypothalamic-pituitary-adrenal system play a crucial role in pain occurring in somatoform disorders and depression [3]. This leads to increased sensation of existing pain or increased sensitivity to somatic sensation [4].

The relationship between postoperative pain severity and preoperative psychological factors has been studied in the literature, and conflicting results have been obtained. Some studies indicate that approximately 25% of patients undergoing total joint arthroplasty experience psychological symptoms in the pre-operative period [5].

Although the relationship between preoperative anxiety and depression symptoms and the pain was insufficient in one study, other studies found a significant relationship between anxiety, depression symptoms, and postoperative pain, which has been proven in different surgical disciplines [6, 7].

Alexithymia is defined as the inability to identify one's own and other people's emotions and has been reported to increase pain sensitivity [8]. It has also been associated with somatization, depression, and anxiety [9]. Recent studies have revealed new data that patients' personality traits may be associated with postoperative pain severity [10]. In the current study, we have tried to find an answer to the question of why the severity of pain in the early postoperative period is different, although the same surgical technique and the same anesthesia method were performed by the same physician. In this study, the hypothesis that preoperative psychological factors play an significant role in the prediction of early postoperative severe pain in patients undergoing total knee arthroplasty was tested. We think that severe post-operative pain can be predicted by determining the psychometric risk factors before the operation and can be improved with an individual-specific treatment approach.

METHODS

This study is a prospective clinical study. Fifty participants fulfilling the study criteria and undergoing total knee arthroplasty as inpatients at the Orthopedics and Traumatology Clinic of a private hospital were included in the study. As a result of orthopedic examination and knee radiographic imaging of the participants, patients diagnosed with primary osteoarthritis and advanced stage gonarthrosis, having surgical intervention indication, being over 18 years old, being literate, signing the consent form to participate in the study, and undergoing TKA under spinal anesthesia were included. Patients, having mental retardation, neurological disease history, severe mental disorder, acute or chronic pain situations, more than 20 degrees flexion contractures before surgery, less than 90 degrees knee flexion and more than 20 degrees varus alignments, secondary osteoarthritis, inflammatory arthritis, and patients in whom spinal anesthesia is not performed were excluded from the study. Patients with epidural catheters or general anesthesia, a history of uncontrolled diabetes, a history of deep vein thrombosis or severe venous insufficiency, chronic polyneuropathies in the lower extremities or neurologic deficits, undergoing peripheral nerve block for pain control, and receiving intravenous or epidural patient-controlled analgesia were also in the exclusion group. The same anesthesia protocol (spinal anesthesia) was managed, and a standard medial parapatellar arthrotomy was performed followed by arthroplasty using the same prosthesis with a fixed insert cutting the posterior cruciate ligament for all patients. The patella was not changed in any patient. The periarticular modified Ranawat cocktail was given to all patients during surgery. After coming patients out of anesthesia, all were mobilized and early rehabilitation was started. IV Paracetamol 1gr (three times per day) was administered in routine analgesia. At the 2nd, 4th, 8th, 12th, 24th, and 48th hours after the operation, the pain intensity of patients was evaluated by the service nurse using the Visual Pain Scale (VAS), during rest (static) and while exercising (dynamic) managed by the healthcare personnel. 1mg/kg pethidine was added to routine treatment in patients with VAS > 5. The narcotic analgesic needs of the them were also recorded.

Our study was approved by the local ethics committee on July 7th, 2021 with the number 2011-KAEK-25 2021/07-17.

Assessment Tools

Psychometric questionnaires were conducted on the participants in the preoperative period. The Visual Analogue Scale (VAS) was used to measure the pain

intensity of the participants. The psychometric characteristics of the participants were evaluated with the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), The Somatosensory Amplification Scale (SAS), Toronto Alexithymia Scale (TAS), and Eysenck Personality Questionnaire Revised-Abbreviated (EPQR-A). The sociodemographic characteristics of the participants were determined using the Sociodemographic Data Form prepared by the authors.

Beck Depression Inventory (BDI)

It was developed by Beck *et al*. [11]. in 1988 to measure the risk and severity of depression in individuals. BDI is a self-reporting scale consisting of twenty-one Likert-type questions, in which each question is scored between 0-3. The total score of BDI is between 0-63. As the scores increase, the severity of depression increases. The Cronbach alpha value of the scale is 0.86. The validity and reliability study in our country was conducted by Hisli in 1988 [12].

Beck Anxiety Inventory (BAI)

BAI was developed to measure the presence and severity of anxiety in individuals. BAI is a self-reporting scale consisting of twenty-one Likert-type questions, and each question is scored between 0-3. The total score of BAI is between 0-63. The Cronbach's alpha value for BAI was specified as 0.93. As the scores increase, the severity of anxiety also increases. The validity and reliability study in our country was conducted by Ulusoy *et al*. [13].

The Somatosensory Amplification Scale (SAS)

To measure an individual's sensitivity to somatic sensation, it was developed by Barsky *et al*. [14]. This scale aims to measure the tendency of the individual to amplify the physical symptoms noticed in his/her body. The scale, a five-point Likert-type scale with 10 items, questioned somatic sensations, not indicating a particular disease. The amplification score is obtained by summing the score for each item and there is no cutoff score. The Turkish reliability study was performed by Güleç *et al*. [15].

Toronto Alexithymia Scale (TAS-20)

It was developed by Bagby *et al*. [16,17]. in 1994 to evaluate alexithymia characteristics in individuals. The questions of the 20-item Likert-type self-report questionnaire, are scored between 1-5. An increase in the scores obtained from the scale means that the level of alexithymia also increases. The validity and reliability study of the scale in Turkey was carried out by Güleç *et al*. [18]. The total scale Cronbach's alpha value was found to be 0.78, and the subscales were between 0.57 and 0.80.

Visual Analogue Scale (VAS)

To determine the severity of pain in patients, it was developed by Price *et al*. [19]. The test is easy to perform. The patient is asked to mark the point corresponding to the pain on a 10 cm long line. With this scale, the numerical equivalent of pain is determined. It is a reliable test performed all over the world.

Eysenck Personality Questionnaire Revised-Abbreviated (EPQR-A)

It is one of the scales developed to measure personality traits and was developed by Eysenck [20]. Following the personality theory created by Eysenck [20], the scale evaluating the characteristics within the scope of the theory was revised by Francis *et al*. [21], reduced to 24 items, and named Eysenck Personality Inventory-Revised Abbreviated Form. The Turkish validity and reliability study was performed by Karancı *et al*. [22]. The scale consists of 4 sub-dimensions: extraversion, psychoticism, neuroticism, and lie. Each sub-dimension consists of 6 items and a maximum of 6 points can be obtained from each sub-dimension.

Sociodemographic Data Form

The form was created by the researchers to evaluate sociodemographic characteristics such as age, gender, education level, marital status, employment and social status, and clinical characteristics such as pain regions and duration of pain.

Statistical Analysis

Demographic and clinical characteristics of the cases participating in the study were evaluated with descriptive statistical methods such as number, percentage, mean, and standard deviation. The change in VAS scores was analyzed by Single Factor ANOVA Analysis for Repeated Measures. Besides, the results of the Greenhouse Geisser Analysis were used in the

study by the reason of the unequal change between VAS score measurements (Static and Dynamic). The effect of EPQR-A, TAS, BDI, and BAI scores on the change in VAS scores was analyzed using ANOVA Analysis. The significance level for all analyzes was determined as $p < 0.05$. The hypothesis for normal distribution was confirmed in the Single Factor ANOVA Analysis for Repeated Measures. The conformity of the data to the normal distribution was evaluated considering the kurtosis and skewness coefficients (± 1.5). IBM SPSS 22.0 program was used for the analysis.

RESULTS

Sociodemographic Characteristics

The mean age of the cases evaluated in the study was 64.80±8.70 years, and 45 cases (90.0%) were women. Forty-four (88.0%) of the cases were primary school graduates, 4 (8.0%) secondary school, and 2 (4.0%) high school graduates. Of the cases, 2 (4.0%) were single, 39 (78.0%) were married, and 9 (18.0%) were divorced. Of the cases, 47 (94.0%) were not working, 11 (22.0%) had a low monthly income, 33 (66.0%)

Table 1. Sociodemographic characteristics of the participants

Gender, n (%)	**Female**	**45 (90.0)**
	Male	**5 (10.0)**
Education Level, n (%)	**Primary school graduate**	**44 (88.0)**
	Secondary school graduate	**4 (8.0)**
	High-school graduate	**2 (4.0)**
	University graduate	**0 (0.0)**
Marital status, n (%)	**Single**	**2 (4.0)**
	Married	**39 (78.0)**
	Divorced	**9 (18.0)**
Employment status, n (%)	**Employee**	**3 (6.0)**
	Nonemployee/student	**47 (94.0)**
Monthly Income, n (%)	**Low**	**11 (11.0)**
	Medium	**33 (66.0)**
	Good	**6 (12.0)**
	High	**0 (0.0)**
Place of residence, n (%)	**City Center**	**24 (48.0)**
	District Center	**22 (44.0)**
	Village Center	**4 (8.0)**
Habits, n (%)	**No**	**47 (94.0)**
	Smoking	**1 (2.0)**
	Alcohol consumption	**0 (0.0)**
	Substance-use	**0 (0.0)**
	Narcotic analgesic	**1 (2.0)**
	Smoking +alcohol consumption	**1 (2.0)**
Analgesic consumption, n (%)	**No**	**50 (100.0)**
	Yes	**0 (0.0)**
BMI (kg/m^2) (mean ± SD)		**33.97±6.00**
Duration of the joint disorder (days) (mean ± SD)		**8.56 ± 5.12**

BMI = Body mass index.

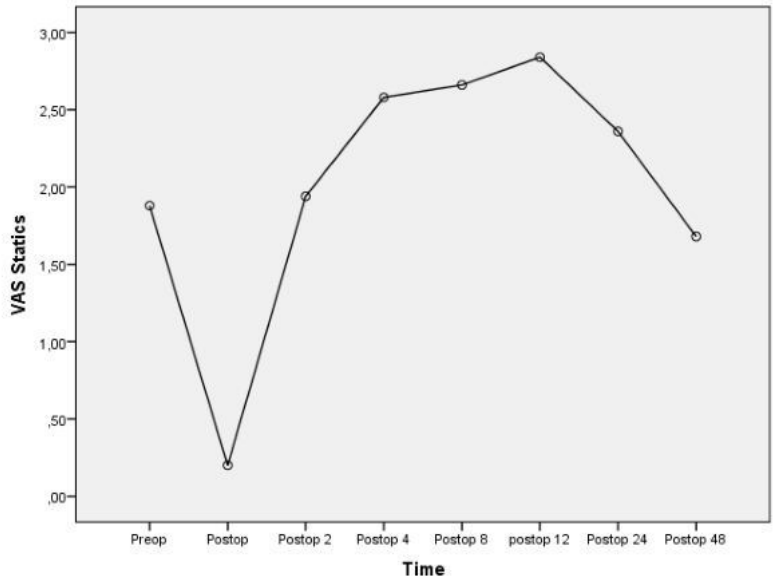

Fig. 1. The change in VAS-Static scores. (VAS = Visual Analogue Scale, Preop = Preoperative, Postop = Postoperative, F = 40.40, p < 0.001, η2 = 0.45).

had a medium income, 6 (12.0%) had a good income. Twenty-four (48.0%) of them were living in the city center, 22 (44.0%) in the district center, and 4 (8.0%) in the village center. Besides, 1 (2.0%) was a smoker, and 1 (2.0%) stated to use cigarettes and alcohol. Twenty-seven (54.0%) of the participants evaluated in the study were determined to have comorbidities, the mean BMI for all was 33.97 ± 6.00, and the mean duration of joint disease was 8.56 ± 5.12 years. The sociodemographic characteristics of the patients were summarized in Table 1.

Clinical Characteristics

Results Related to VAS Scores: Considering the Single-Factor ANOVA Analysis for Repeated Measures, the VAS-Static score means were found to show a statistically significant change (F = 40.40, $p < 0.001$) (Fig. 1) (Table 2).

According to the Single-Factor ANOVA Analysis for Repeated Measures, the scores of Extraversion (F = 0.28, $p = 0.962$), Lie (F = 0.41, $p = 0.896$), Neuroticism (F = 0.76, $p = 0.622$), Psychoticism (F = 1.32, $p = 0.242$), TAS (F = 1.45, $p = 0.182$), BDI (F = 1.15, p

Table 2. The efficiency of psychometric properties in the change in VAS-Static scores

	F	*p value*	*η2*
Extraversion	0.28	0.962	0.007
Lie	0.41	0.896	0.010
Neuroticism	0.76	0.622	0.018
Psychoticism	1.32	0.242	0.031
TAS	1.45	0.182	0.034
BDI	1.15	0.332	0.027
BAI	0.72	0.658	0.017
SAS	0.79	0.599	0.019
Duration of the joint disorder	1.66	0.119	0.039

VAS = Visual Analogue Scale, TAS = Toronto Alexithymia Scale, BDI = Beck Depression Inventory, BAI =Beck Anxiety Inventory, SAS = Somatosensory Amplification Scale

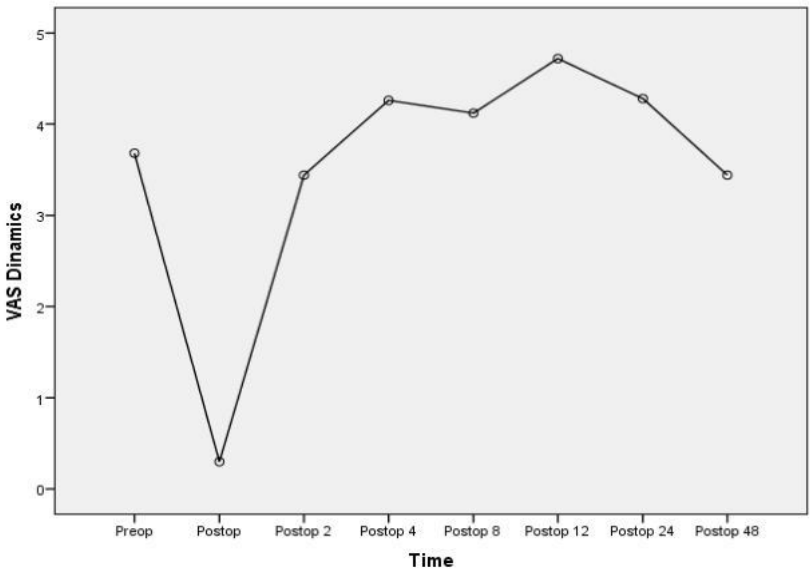

Fig. 2. The change in VAS-Dynamic scores. (VAS = Visual Analogue Scale, Preop = Preoperative, Postop= Postoperative, F = 79.19, p < 0.001, η2 = 0.618).

= 0.332), BAI (F = 0.72, p = 0.658), and SAS (F = 0.79, p = 0.599) were found not to be statistically significant due to the non-homogeneous distribution of the data for the change in VAS-Static scores. Besides, when the effects of these psychometric properties were controlled, the change in VAS-Static scores was found not to be statistically significant (F = 1.66, p = 0.119).

According to the Single Factor ANOVA Analysis for Repeated Measurements, the VAS-Dynamic mean score was showed a statistically significant change (F = 79.19, p < 0.001) (Fig. 2) (Table 3).

According to the Single-Factor ANOVA Analysis for Repeated Measures, only psychoticism subscale scores were found to have a statistically significant effect on the change in VAS-Static scores (F = 2.39, p = 0.040), but the level of this effect was around 5.5%. Moreover, considering the controlled psychometric properties evaluated in the study, the significant change in VAS-Dynamic scores was detected to continue (F = 2.95, p = 0.014), however, the effect size of the variation decreased from 61.8% to 6.7%.

DISCUSSION

In this study, there was found not to be a significant relationship between the psychometric characteristics of the patients before the surgical intervention and the VAS levels in the early postoperative period.

The relationship between preoperative psychometric factors and postoperative pain level has been analyzed in the literature, but conflicting results have been reported. A total of 37 articles of medium quality were examined in the systematic review study of Hernández *et al*. [23]. A strong correlation was found between postoperative pain and female gender, lower socioeconomic status, higher preoperative pain, comorbidities, low back pain, poor preoperative functional status, and psychological factors (depression, anxiety).

In a prospective study with 124 participants undergoing knee and hip arthroplasty, Pinto *et al*. [24] analyzed the demographic characteristics, clinical status, and psychological factors of the patients. They assessed pain levels, anxiety, and analgesic consumption 24 hours before and 48 hours after surgery. As a result, using a multivariate model, psychological factors were stated to be determinative in the prediction of pain at the 48th hour after surgery.

In the systematic review by Vissers *et al*. [25], 35 studies were examined. High preoperative pain scores have been reported to affect postoperative pain in patients undergoing total knee arthroplasty, on the other hand, the effect of psychological factors has been reported to be limited, has given contradictory results, or there is no evidence of its effectiveness.

In the current study, preoperative anxiety, depres-

Table 3. The efficiency of psychometric properties in the change in VAS-Dynamic scores

	F	*p value*	*η2*
Extraversion	0.39	0.854	0.09
Lie	0.73	0.602	0.017
Neuroticism	0.58	0.712	0.014
Psychoticism	2.39	**0.040**	0.055
TAS	1.97	0.086	0.046
BDI	1.62	0.157	0.038
BAI	0.63	0.673	0.015
SAS	1.43	0.184	0.036
Duration of the joint disorder	2.95	**0.014**	0.067

VAS = Visual Analogue Scale, TAS = Toronto Alexithymia Scale, BDI = Beck Depression Inventory, BAI =Beck Anxiety Inventory, SAS = Somatosensory Amplification Scale

sion, somatosensory amplification, alexithymia, and personality traits were shown not to affect the pain in the first 48 hours postoperatively. Only psychoticism subscale scores were, however, detected to be statistically significant in the change in VAS-Static scores according to the Single-Factor ANOVA Analysis for Repeated Measures, and the level of this effect was around 5.5%. Besides, when the psychometric properties evaluated in the study were controlled, the significant change in VAS-Dynamic scores was found to continue, but the effect size of the change decreased from 61.8% to 6.7%.

In the current study, although psychoticism was an effective factor in the change in postoperative VAS-Dynamic scores, this effect size was small. People with high psychoticism scores were thought to might interpret somatosensorial stimuli differently. The psychoticism dimension has been shown to have a pain-increasing effect on dynamic pain scores. Individuals with psychoticism might be, therefore, inferred to could perceive pain more after the intervention for the patient.

There is not much information about the biological basis of the psychoticism dimension. Higher psychoticism scores were obtained among psychopaths and criminals in the literature. There has been reported to be a strong relationship between the psychoticism dimension and antisocial behaviors [26, 27].

Basal cortisol levels were found to be low in individuals with antisocial personality disorders. Cortisol has a pain-reducing effect due to its anti-inflammatory effect. In the literature, there has been reported to be a negative correlation between psychopathy and this level [28]. Individuals displaying antisocial and aggressive behaviors were observed to have high testosterone levels [29]. Testosterone and cortisol have a reciprocal antagonistic effect. These people may have low cortisol when testosterone levels rise.

Surgery is one of the strongest stimuli of adrenocorticotropic hormone (ACTH) and cortisol. Cortisol and ACTH release after surgical trauma are sensitive indicators of the stress response. Basal cortisol and ACTH levels increase in the acute period [30]. Arthroplasty operations have been reported to lead to significant surgical stress response [31]. The cortisol secretion from the beginning of these operations may contribute to the reduction of acute pain. In our study, the basal cortisol levels of patients with psychoticism after surgical trauma were thought to may be lower than those without psychoticism.

Abnormalities in the serotonergic system have been detected in individuals with antisocial personality behavior (ASPD) [32]. The serum-free tryptophan level was determined to be higher in individuals with antisocial behaviors, impulsivity, and violent behaviors compared to healthy controls [33]. As a result of the stimulation of serotonergic neurons in the central nervous system, serotonin exerts an analgesic effect in the posterior horn of the medulla spinalis [34].

In a study conducted by Davis *et al*. [35] on uni-

versity students, psychological pain was stated to be significantly associated with self-harm and psychoticism. However, the relationship between physical pain and psychoticism has not been reported in the literature.

In many studies, the pain has been associated with the dimension of neuroticism [36]. However, these studies have been evaluated in relation to chronic pain or in the late postoperative period. In our study, acute pain severity in the early postoperative period was evaluated and the neuroticism dimension was not associated with pain in this period.

Neuroticism is a personality trait with a reported effect on somatization and can be defined as the tendency to react negatively to social and psychological stressors [37]. Somatization, considered a subset of neuroticism, has been reported to be associated with the number of somatic symptoms and coping with these symptoms [38, 39]. In a study by Atay *et al*. [40], a positive correlation was found between high pain scores in the late-postoperative period and somatosensory amplification in patients undergoing total knee arthroplasty and those were consistent with the results of previous studies. There is a significant relationship between psychometric symptoms and pain in patients with chronic pain. However, early-postoperative period was evaluated in our study and this relationship was not detected.

CONCLUSION

No significant relationship was determined between preoperative psychometric factors and postoperative acute pain scores in our study. Considering the Single Factor ANOVA Analysis, only psychoticism subscale scores were, however, deduced to have a statistically significant effect on the change in VAS-Static scores. The significant change in VAS-Dynamic scores was shown to continue at a low effect with the control of all psychometric features. The psychoticism dimension was concluded to be more related to ASPD or aggressive patients, and the high level of pain might be related to the neurobiologically low serotonin and cortisol levels. Thus, we think that conducting new studies investigating the effects of psychoticism on ongoing post-surgical pain symptoms may contribute to the literature.

Authors' Contribution

Study Conception: ÇT, AK; Study Design: ÇT, AK; Supervision: ÇT, AK; Funding: ÇT, AK; Materials: ÇT, AK; Data Collection and/or Processing: ÇT, AK; Statistical Analysis and/or Data Interpretation: ÇT; Literature Review: ÇT, AK; Manuscript Preparation: ÇT, AK and Critical Review: ÇT, AK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Sommer M, de Rijke JM, van Kleef M, Kessels AG, Peters ML, Geurts JW, et al. The prevalence of postoperative pain in a sample of 1490 surgical inpatients. Eur J Anaesthesiol 2008;25:267-4.
2. Merskey HE, Bogduk N. Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. Pain Suppl 1986;3:S1-226.
3. Rief W, Barsky AJ. Psychobiological perspectives on somatoform disorders. Psychoneuroendocrinology 2005;30:996-2.
4. Rief W, Hennings A, Riemer S, Euteneuer F. Psychobiological differences between depression and somatization. J Psychosom Res 2010;68:495-2.
5. Lingard EA, Riddle DL. Impact of psychological distress on pain and function following knee arthroplasty. J Bone Joint Surg Am 2007;89:1161-9.
6. Utrillas-Compaired A, De la Torre-Escuredo BJ, Tebar-Martínez AJ, Asúnsolo-Del Barco Á. Does preoperative psychologic distress influence pain, function, and quality of life after TKA? Clin Orthop Relat Res 2014;472:2457-5.
7. Granot M, Ferber SG. The roles of pain catastrophizing and anxiety in the prediction of postoperative pain intensity: a prospective study. Clin J Pain 2005;21:439-5.
8. Tesio V, Di Tella M, Ghiggia A, Romeo A, Colonna F, Fusaro E, et al. Alexithymia and depression affect quality of life in patients with chronic pain: a study on 205 Patients with fibromyalgia. Front Psychol 20184;9:442.
9. Hosoi M, Molton IR, Jensen MP, Ehde DM, Amtmann S, O'Brien S, et al. Relationships among alexithymia and pain intensity, pain interference, and vitality in persons with neuromuscular disease: considering the effect of negative affectivity. Pain 2010;149:273-7.
10. Zheng H, Schnabel A, Yahiaoui-Doktor M, Meissner W, Van Aken H, Zahn P, et al. Age and preoperative pain are major confounders for sex differences in postoperative pain outcome: a

prospective database analysis. PLoS One 2017;12:e0178659.
11. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561-71.
12. Hisli N. Reability and validity of Beck Depression Inventory among university students. J Turk Psychol 1989;7:3-13.
13. Ulusoy M, Sahin NH, Erkmen H. Turkish version of the Beck Anxiety Inventory: psychometric properties. J Cogn Psychother 1998;12:163.
14. Barsky AJ, Wyshak G, Klerman GL. The somatosensory amplification scale and its relationship to hypochondriasis. J Psychiatr Res 1990;24:323-34.
15. Güleç H, Sayar K. Reliability and validity of the Turkish form of the Somatosensory Amplification Scale. Psychiatry Clin Neurosci 2007;61:25-30.
16. Bagby RM, Parker JD, Taylor GJ. The twenty-item Toronto Alexithymia Scale-I. Item selection and cross-validation of the factor structure. J Psychosom Res 1994;38:23-2.
17. Bagby RM, Taylor GJ, Parker JD. The Twenty-item Toronto Alexithymia Scale-II. Convergent, discriminant, and concurrent validity. J Psychosom Res 1994;38:33-40.
18. Güleç H, Köse S, Güleç MY, Çitak S, Evren C, Borckardt J, at al. Reliability and factorial validity of the Turkish version of the 20-item Toronto alexithymia scale (TAS-20). Bull Clin Psychopharmacol 2009;19:214-20.
19. Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 1983;17:45-56.
20. Eysenck HJ, Eysenck SB. Manual of the Eysenck Personality Questionnaire (Junior and Adult). London: Hodder & Stoughton. 1975.
21. Leslie Francis J, Brown LB, Philipchalk R. The development of an abbreviated form of 61 the revised Eysenck personality questionnaire (EPQR-A): its use among students in England, Canada, the U.S.A. and Australia. Pers Individ Dif 1992;13:443-49.
22. Karancı AN, Dirik G, Yorulmaz O. Turkish Adaptation of the Eysenck Personality Questionnaire-Revised Shortened Form (EKA-GGK). Turk J Psychiatr 2007;18:1-8.
23. Hernández C, Díaz-Heredia J, Berraquero ML, Crespo P, Loza E, Ruiz Ibán MÁ. Pre-operative predictive factors of postoperative pain in patients with hip or knee arthroplasty: a systematic review. Reumatol Clin 2015;11:361-80.
24. Pinto PR, McIntyre T, Ferrero R, Almeida A, Araújo-Soares V. Predictors of acute postsurgical pain and anxiety following primary total hip and knee arthroplasty. J Pain 2013;14:502-15.
25. Vissers MM, Bussmann JB, Verhaar JA, Busschbach JJ, Bierma-Zeinstra SM, Reijman M. Psychological factors affecting the outcome of total hip and knee arthroplasty: a systematic review. Semin Arthritis Rheum 2012;41:576-88.
26. Docherty M, Boxer P, Huesmann LR, O'Brien M, Bushman BJ. Exploring primary and secondary variants of psychopathy in adolescents in detention and in the community. J Clin Child Adolesc Psychol 2016;45:564-78.
27. Romero E, Luengo MA, Sobral J. Personality and antisocial behaviour: Study of temperamental dimensions. Pers Individ Dif 2001:31:329-48.
28. van Honk J, Schutter DJ. Unmasking feigned sanity: a neurobiological model of emotion processing in primary psychopathy. Cogn Neuropsychiatry 2006;11:285-306.
29. Loney BR, Butler MA, Lima EN, Counts CA, Eckel LA. The relation between salivary cortisol, callous-unemotional traits, and conduct problems in an adolescent non-referred sample. J Child Psychol Psychiatry 2006;47:30-6.
30. Marana E, Colicci S, Meo F, Marana R, Proietti R. Neuroendocrine stress response in gynecological laparoscopy: TIVA with propofol versus sevoflurane anesthesia. J Clin Anesth 2010;22:250-5.
31. Hall GM, Peerbhoy D, Shenkin A, Parker CJ, Salmon P. Hip and knee arthroplasty: a comparison and the endocrine, metabolic and inflammatory responses. Clin Sci (Lond) 2000;98:71-9.
32. Dolan MC, Anderson IM. The relationship between serotonergic function and the Psychopathy Checklist: Screening Version. J Psychopharmacol 2003;17:216-22.
33. Tiihonen J, Virkkunen M, Räsänen P, Pennanen S, Sainio EL, Callaway J, et al. Free L-tryptophan plasma levels in antisocial violent offenders. Psychopharmacology (Berl) 2001;157:395-400.
34. Chang FC, Tsai HY, Yu MC, Yi PL, Lin JG. The central serotonergic system mediates the analgesic effect of electroacupuncture on ZUSANLI (ST36) acupoints. J Biomed Sci 2004;11:179-5.
35. Davis KC, Anderson JL. Psychological pain: A moderating factor between personality psychopathology and self-harm. J Am Coll Health 2021;16:1-9.
36. Inanc BY, Yerlikaya EE. Re Research Oriented Theories. In: Personality Theories. Ankara: Pegem Academy, 2014: p. 245-85.
37. Kiffin-Petersen SA, Jordan CL, Soutar GN. The big five, emotional exhaustion and citizenship behaviors in service settings: the mediating role of emotional labor. Pers Individ Dif 2011;50:43-8.
38. Abdolmohamadi K, Seyed Pourmand NS, Babapour Kheyradin J. The somatization predictions derived from personal characteristics. Int J Behav Sci 2017;11:86-9.
39. Lahey BB. Public health significance of neuroticism. Am Psychol 2009;64:241-6.
40. Atay İM, Atay T, Dinçer R. Effects of psychological predictors on hospital discharge duration after total knee arthroplasty. J Exp Clin Med 2022;39:41-6.

The European Research Journal 2023;9(2):407-415 Original Article

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Investigation of the relationship between weight self-stigma, emotional eating, and diet satisfaction in obese individuals

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ABSTRACT

Objectives: Obese people are often stigmatized and discriminated against as unmotivated, incompatible, sloppy, undisciplined, and lazy because of their excess weight. The stigma associated with weight has serious adverse effects on mental health, social functioning, and physical health. It has been suggested that weight stigma can be internalized and lead to the development and maintenance of self-stigma.

Methods: Personal Information Form, Turkish Emotional Eating Scale (EES), Weight Self-Stigma Scale (WSSQ), and Diet Satisfaction Scale (DSS) were used.

Results: One hundred forty-five volunteer obese individuals who applied to the Diet Clinic and whose informed consent was obtained were included in the study. When the differences between the scale scores of the individuals according to demographic, health, and nutrition findings are examined, it is seen that the TEES scores according to the gender and income status of the obese individuals, the WSSQ scores according to the gender and employment status of the obese individuals, and the DSS scores according to the income status of the obese individuals, the psychological diagnosis status made by the physician, It was found that there was a significant difference according to the chronic disease states and the number of daily snacks ($p < 0.05$ and $p < 0.001$, respectively).

Conclusions: When the relationship between the scales was examined, a significant correlation was found between the WSSQ scores of obese individuals and their EES scores. It was found that there was a statistically significant effect of WSSQ scores on EES scores. It is thought that the findings of this study will contribute significantly to the relevant literature.

Keywords: Diet satisfaction, emotional eating, weight self-stigma, obesity

Obesity has been called various names throughout history, and being overweight in the past; While it was perceived as a symbol of power, fertility, and abundance, the importance of physical appearance in the workforce increased with the development of industry and technology, and overweight and obese individuals in the society were perceived as bulky, problematic, slow and unhealthy individuals. Today, however; Obesity is perceived as a chronic, progressive disease that cannot be prevented, affecting indi-

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viduals both psychologically and physiologically, due to the additional diseases and social problems it imposes on the person [1].

Emotional eating was first discussed within the scope of Bulimia Nervosa and seen as a trigger for it, and then it was considered as a concept on its own [2]. Emotional eating was first defined in 1950 and it has been defined as the behavior of eating more than normal given to negative and disturbing emotions [3]. There are many explanations for emotional eating. Emotional eating is a tendency to overeat in response to negative emotions [4]. Eating behavior that takes place to cope with uncomfortable emotions without feeling physical hunger has been defined as emotional eating [5]. In addition, the behavior of eating to escape from the disturbing feeling of the person despite not being physically hungry can be defined as emotional eating [6]. According to another definition, emotional eating is a behavior that occurs in response to the emotions felt by the person [7].

The concept of stigma is generally used in the sense of "black mark", which comes from the Greek word "stigma". In psychology, the exclusion of the individual from society, being seen as a shame or a black mark as it is used in general, can be considered as a reflection of my dislike by other people [8]. This concept, which was used for the first time in the scientific field by Goffman, is “less valued; less desirable and almost not perceived as human” and the stigmatized individual is defined as “not accepted in society, excluded” [9]. The concept of stigma shows a multidimensional structure feature that is defined, and measured in many ways, causing hesitations to prove it. It is stated that there are many aspects defined according to the state of experience [10], and it has consequences that affect not only the mental health of the individual but also the physical health [11].

When we look at the effects of self-stigma on the individual, it can be stated that the levels of self-esteem and self-efficacy decrease in the individual, and as a result, they exhibit negative attitudes and behaviors such as the inability to participate in society [12]. And this situation results in isolation, unemployment, and insufficient economic earnings. As a result, people who stigmatize themselves often feel and think that they are bad, weak, inferior, and incapable of accessing resources that are accessible to others [13, 14].

When the literature is examined, no research has been found on obese individuals in Turkey on weight self-stigma and emotional eating and diet satisfaction. This study aimed to investigate the relationship between weight and self-stigma, emotional eating, and diet satisfaction in obese individuals on diet.

METHODS

This cross-sectional study was conducted with an obese individual who applied to a diet clinic between August and September 2022 and continued diet counseling for at least 1 month. 145 obese individuals were included in the study. Before the study was conducted, approval was obtained from the Istanbul Okan University Ethics Committee (decision date: August 24, 2022, decision number: 2022-14). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Individuals between the ages of 18-65, who had been on a diet for at least 1 month and were obese, were included in the study voluntarily. Data collection was done face-to-face by the researcher. The personal information form was prepared by the researcher to measure the socio-demographic characteristics of individuals. While preparing the form questions, a conscious approach was adopted by considering the current literature information on the subject. In this form, There were 15 questions about the participant's age, gender, educational status, marital status, socioeconomic status, employment status, height, weight, psychiatric diagnosis status, presence of psychiatric medication used, presence of chronic disease, cigarette consumption, alcohol consumption status, nutritional status, and weight perceptions.

The Turkish Emotional Eating Scale (EES) used in the study was developed by Sinem Bilgen and its validity and reliability study was carried out with 96 people [15]. The internal consistency of the EES was found to be Cronbach's Alpha r = 0.960 at the first stage, r = 0.962 at the second stage, and r = 0.959 at the third stage. It was carried out methodologically to examine the reliability and validity of the Weight Self-stigma Questionnaire (WSSQ)for Turkish society. It has been determined that the Turkish version of the WSSQ includes the same sub-dimensions as the original 12-item form, and its validity-reliability results are at an acceptable level [16]. The scale, originally called “Diet Satisfaction Score”, was developed by

Jospe *et al*. [17]. The scale includes 5-point Likert-type response options ranging from 1 (strongly disagree) to 5 (strongly agree) and consists of 10 items. Turkish validity and reliability of the scale were done by Eskici and Karahan-Yılmaz [18]. The Cronbach Alpha coefficient of the nine-item scale was found to be 0.902. Body weight of each participant was measured using a calibrated electronic scale with an accuracy of 0.1 kg (TANITA) and body height was measured by a researcher using a tape measure. BMI was calculated using the formula [weight (kg)/height(m^2)].

Statistical Analysis

Descriptive statistics for categorical variables (demographic characteristics) are presented as frequency and percentage. The conformity of the numerical variables to the normal distribution was checked with the "Shapiro-Wilk Test". The descriptive statistics of numerical variables were given as mean ± standard deviation for data showing normal distribution, and median (min-max) values for data not showing normal distribution. The "Mann-Whitney U Test" was used for the comparison of two independent groups that did not have a normal distribution, and the "Kruskal-Wallis H Test" was used for the comparison of more than two groups. The results of the multiple comparison tests are expressed as letters next to the medians. Examination of the relationships between the scales was determined by "Spearman's Rank Differences Correlation Coefficient". In the interpretation of the correlation coefficient, "very weak correlation if < 0.2", "weak correlation between 0.2-0.4", "moderate correlation between 0.4-0.6" and "0.6- If it is between 0.8, high correlation" criteria, "0.8 > very high correlation" criteria were used. "Regression Analysis" was used to test the effect between variables. Regression analysis is the explanation of the relationship between two related variables, a dependent variable and an independent variable, with mathematical equivalence. "Cronbach's Alpha Coefficient" was calculated to determine the reliability level of the scales studied. In all calculations and interpretations, the statistical significance level was considered as "$p < 0.05$, $p < 0.01$, $p < 0.001$". Statistical analysis of the data was performed with the SPSS v26 statistical package program.

Table 1. Descriptive statistics of demographic findings of individuals

	Data
Gender, n (%)	
Men	56 (38.6)
Women	89 (61.4)
Age (years)	40.30 ± 12.34
Height (cm)	166.59 ± 9.72
Weight (kg)	95.68 ± 15.22
BMI (kg/m^2)	34.33 ± 3,12
Education Level, n (%)	
Primary school	11 (7.6)
Secondary school	16 (11.0)
High school	44 (30.3)
Graduate	59 (40.7)
Postgraduate	15 (10.3)
Marital Status, n (%)	
Married	99 (68.3)
Single	46 (31.7)
Employment Status, n (%)	
Employed	87 (60.0)
Not employed	58 (40.0)
TOTAL, n (%)	145 (100)

Data are shown as mean ± standard deviation or n (%). BMI = Body Mass Index

RESULTS

The demographic findings of the dieting obese individuals participating in the study are given in Table 1. When the descriptive statistics of the demographic findings of the obese individuals who participated in the study were examined, it was seen that 38.6% (n = 56) were male and 61.4% (n = 89) were women, and their mean age was 40.30 ± 12.34 years. According to their education level, 7.6% (n = 11) are primary school graduates, 11% (n = 16) are secondary school graduates, 30.3% (n = 44) are high school graduates, 40.7% (n = 59) are) are university graduates and 10.3% (n = 15) are postgraduate graduates, 68.3% (n = 9) are married and 31.7% (n = 46) are single according to their marital status, according to their income status % 13.1% (n = 19) have less than their income, 35.2% (n = 51) have income equal to their expenses and 51.7%

(n = 75) have more than their expenses, 60% (n = 87) according to their working status were working and 40% (n = 58) were not working (Table 1).

A comparison of EES scores of individuals on diet according to BMI groups and genders is given in Table 2. According to the results of the analysis, it was found that there was no statistically significant difference ($p > 0.05$) in all sub-factors and "EES Total" scores of the scale. When the results are examined, the median of women (42 [22-55]) in the "Eating in Tensions" score, compared to the men (40.5 [21-52]), the women's (41 [20-50]) in the "Eating to Cope with Negative Emotions" score] median compared to men (36 [13-50]), median for men [20 [21-30]) in "Self-Control" score, than women (18 [10-309) and women [115] in "TFLQ Total" score (115 [60-150]) was sta-

Table 2. Comparison of Turkish Emotional Eating Scale scores according to gender and BMI classification of obese individuals

	Sex	Mean ± SD	Median (min-max)	*p* value
EST	Men	38.37 ± 9.00	40.5 (21-52)	**0.038***
	Women	42.0 ± 76.97	42.0 (22-55)	
ECNE	Men	34.0 ± 49.29	36.0 (13-50)	**< 0.001*****
	Women	40.0 ± 46.67	41.0 (20-50)	
SC	Men	20.5 ± 4.21	20.0 (12-30)	**0.016***
	Women	19.0 ± 43.55	18.0 (10-30)	
CAS	Men	11.9 ± 12.29	13.0 (6-15)	0.088
	Women	12.6 ± 11.97	13.0 (6-15)	
EES-T	Men	104.8 ± 221.74	107.5 (60-146)	**0.025***
	Women	113.7 ± 615.44	115 (60-150)	
BMI Classification				
EST	Class 1	40.3 ± 8.05	42.0 (21-55)	0.107
	Class 2	42.1 ± 27.75	43.0 (26-55)	
	Class 3	36.1 ± 77.68	37.0 (25-46)	
ECNE	Class 1	37.5 ± 8.72	39.0 (13-50)	0.731
	Class 2	38.5 ± 97.34	40.0 (20-50)	
	Class 3	35.5 ± 7.74	36.0 (24-44)	
SC	Class 1	19.0 ± 93.70	18.0 (10-30)	0.066
	Class 2	20.7 ± 64.19	20.0 (13-30)	
	Class 3	20.1 ± 73.06	19.0 (18-26)	
CAS	Class 1	12.2 ± 22.29	13.0 (6-15)	0.773
	Class 2	12.5 ± 41.67	13.0 (9-15)	
	Class 3	12.9 ± 32.23	13.0 (9-15)	
EES-T	Class 1	109.1 ± 119.12	114 (60-150)	0.377
	Class 2	114.0 ± 17.13	116 (81-150)	
	Class 3	104.6 ± 718.12	105 (80-129)	

EST = Eating in Situations of Tension, ECNE = Eating to Cope with Negative Emotions, SC = Self-Control, CAS = Control Against Stimulus, EES-T = Emotional Eating Scale Total, BM = Body Mass Index, SD = Standard Deviation
Mann-Whitney U Test, *$p < 0.05$; ***$p < 0.001$

tistically higher than men (107.5 [60-146]).

Summary statistics of the individuals' EES, WSSQ, and DSS scores are given in Table 3. When the descriptive statistical values of the Turkish Emotional Eating Scale (EES) scores were examined, it was found that the "Eating in Tensions" sub-dimension had a value between the lowest 21.0 and the highest 55.0, and the average was 40.64 ± 7.99, "For Coping with Negative Emotions". The "Eating" sub-dimension had a value between 13.0-50.0 and the average was 37.72 ± 8.29, and the "Self-Control" sub-dimension had a value between 10.0-30.0 and the average was 19.61 ± 3.87. "Control Against Stimulus" sub-dimension took values between 6.0-15.0 and its average was 12.34 ± 2.12. On the other hand, in the "EES Total" score, it was found that the value was between the lowest 60.0 and the highest 150.0, and its average was 110.31 ± 18.58. When the descriptive statistical values of the Weight-Related Self-Stigmatization Scale (CIBS) scores were examined, the "Self-Devaluation" sub-dimension had a value between the lowest 8.0 and the highest 30.0, and the average was 22.27 ± 4.92, and the "Fear of Stigma" sub-dimension. It is seen that the value is between 8.0-30.0 and the mean is 21.03 ± 5.39. On the other hand, it was found that the value between the lowest 18.0 and the highest 60.0 in the "WSSQS Total" score was 43.30 ± 9.52. When the descriptive statistical values of the Diet Satisfaction Scale (DSS) scores were analyzed, it was found that the "DSS Total" score ranged from the smallest 13.0 to the highest 42.0, with a mean of 32.10 ± 5.57 (Table 4).

The correlation coefficients between the sub-factor and total scores of the Turkish Emotional Eating Inventory (EES), the Weight-Related Self-Stigmatization Scale (FAIS), and the Diet Satisfaction Scale (DSS) are given in Table 4. There was a significant positive correlation between the "Self-Devaulation" sub-dimension of the WSSQ and the "Eating in Situations of Tension", "Eating to Cope with Negative Emotions" and total EES scores (respectively, r = ; $p < 0.001$, r = 0.421; $p < 0.001$, r = 0.425; $p < 0.001$). When the results are analyzed, the "Self-Devaluation" scores of the EES increase, the "Eating in Stress Situations" scores of EES increase by 38.8%, "Eating to Cope with Negative Emotions" scores increase by 42.1%, and the "EES Total" scores increase by 38.8%. It was found that there was a 42.5% increase in their scores (Table 4).

There was a positive significant correlation between the "Fear of Stigma" sub-dimension of the WSSQ and the "Eating in Situations of Tension", "Eating to Cope with Negative Emotions", "Control Against Stimulus" and total EES scores (respectively, r = 0.462; $p < 0.001$, r = 0.531, $p < 0.001$, r = 0.307, $p < 0.001$, r = 0.514, $p < 0.001$). When the results are examined, the "Fear of Stigma" scores of EES increase, a 46.2% increase in the "Eating in Tension Sit-

Table 3. Summary statistics of Emotional Eating Scale, Weight Self-Stigma Questionnaire and Diet Satisfaction Scale scores

	Min	Max	Mean	SD
Emotional Eating Scale (EES)				
Eating in Situations of Tension	21.00	55.00	40.64	7.99
Eating to Cope with Negative Emotions	13.00	50.00	37.72	8.29
Self-Control	10.00	30.00	19.61	3.87
Control Against Stimulus	6.00	15.00	12.34	2.12
EES Total	60.00	150.00	110.31	18.58
Weight Self-Stigma Questionnaire (WSSQ)				
Self-Devaluation	8.00	30.00	22.27	4.92
Fear of Stigma	8.00	30.00	21.03	5.39
WSSQ Toplam	18.00	60.00	43.30	9.52
Diet Satisfaction Scale (DSS)				
DSS Total	13.00	42.00	32.10	5.57

SD =standard deviation

uations" scores, a 53.1% increase in the "Eating to Cope with Negative Emotions" scores, and "Control Against Stimulus" scores increase by 46.2%. It was found that there was an increase of 30.7% in the scores of ” and an increase of 51.4% in the scores of “Total EES” (Table 4).

There was a positive significant correlation between WSSQ total score and all sub-dimensions and total score of EES ($p < 0.05$). When the results are examined, it is seen that as the “FASS Total” scores of the EES increase, the “Eating in Tension Situations” scores increase by 45.6%, the “Eating to Cope with Negative Emotions” scores increase by 51.7%, and the “Control Against Stimulus” scores increase by 45.6%. It was found that there was an increase of 25.5% in the scores of ” and an increase of 50.4% in the scores of “Total EES” (Table 4).

A statistically significant negative weak correlation was found between DSS total score and EES subdimensions "Eating in Tension Situations" and "Eating to Cope with Negative Emotions" and "Control Against Stimulus" (respectively, (r = -0.191; $p < 0.05$). , r = -0.221; $p < 0.01$, r = 0.220; $p < 0.01$) However, there was a weak positive correlation between DSS total score and Self-Control subscale of EES (r = 0.220, $p < 0.01$). When the results are examined, as the DSS total scores increase, there is a 19.1% decrease in the "Eating in Tension Situations" scores, a 22.1% decrease in the "Eating to Cope with Negative Emotions" scores, and a 22.1% decrease in the "Eating in Stress Situations" scores. It was found that there was a 22% increase in the ” scores and a 22% decrease in the “Control Against Stimulus” scores (Table 4).

DISCUSSION

In the study, 145 dieting obese individuals were studied and the primary purpose of the study was to examine the relationship between self-stigmatization, emotional eating, and diet satisfaction in dieting obese individuals and to eliminate the deficiency in this field in the literature.

In the study, the comparison of the scores of the EES was examined and as a result, there was a statistically significant difference between the EES scores of the obese individuals on a diet according to their gender and income status, age groups, educational status, marital status, employment status. Akduman [19], in his study examining the relationship between prob-

Table 4. Examination of the relationship between the scores of the Turkish Emotional Eating Scale, the Weight Self-Stigma Scale, and the Diet Satisfaction Scale

		WSSQ-1	WSSQ-2	WSSQ-T	DSS-T
EST	**r**	0.388	0.462	0.456	-0.191
	***p* value**	**< 0.001*** **	**< 0.001*** **	**< 0.001*** **	**0.021***
ECNE	**r**	0.421	0.531	0.517	-0.221
	***p* value**	**< 0.001*** **	**< 0.001*** **	**< 0.001*** **	**0.008****
SC	**r**	0,030	-0,012	0,003	0.220
	***p* value**	0.720	0.887	0.972	**0.008****
CAS	**r**	0.158	0.307	0.255	-0.220
	***p* value**	0.058	**< 0.001*** **	**0.002****	**0.008****
EES-T	**r**	0.425	0.514	0.504	-0.141
	***p* value**	**< 0.001*** **	**< 0.001*** **	**< 0.001*** **	0.090

EST = Eating in Situations of Tension, ECNE = Eating to Cope with Negative Emotions, SC =: Self-Control, CAS = Control Against Stimulus, EES-T = Emotional Eating Scale Total, WSSQ-1 = Self-Devaluation, WSSQ-2 = Fear of Stigma, WSSQ-T = Weight Self-Stigma Questionnaire Total, DSS-T = Diet Satisfaction Scale Total, r = Spearman's Rank Differences Correlation Coefficient

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

lematic eating behaviors and childhood traumas in obese individuals, stated that there is a significant difference between the emotional eating scores of obese individuals according to their gender and employment status, but there is no significant difference between emotional eating scores according to their income status. Cihan [20], in his study examining the relationship between the feeling of stigma, eating behaviors, and depression in obese individuals, stated that there is a significant difference between the emotional eating scores of obese individuals according to their gender, but there is no significant difference according to their marital status, educational status, and BMI groups. Yaşar [21], in his study examining the relationship between self-stigma and emotional eating in obese individuals, found that there was a significant difference between the emotional eating scores of obese individuals according to their employment status and the psychiatric diagnosis received by the physician, and there was a significant difference between their emotional eating scores according to their gender, marital status, and socioeconomic status. Şen and Kabaran [22], in their study examining the effects of nutritional status and emotional eating, night eating, and sleep quality, stated that there was no significant difference between the emotional eating scores of individuals according to their gender, but there was a significant difference between emotional eating scores according to BMI groups. In this study, the comparison of the scores of the WSSQ was examined, and as a result, there was a statistically significant difference between the WSSQ scores of the obese individuals on a diet according to their gender and employment status, age groups, educational status, marital status, income status, BMI groups. It was found that there was no statistically significant difference between WSSQ scores according to psychological diagnoses, psychiatric drug use, chronic disease, smoking, alcohol consumption, daily main meals, daily snacks, and body assessment. Lillis *et al*. [16], in their study in which they examined the self-stigmatization status of individuals according to their weight, stated that there was a significant difference between self-stigmatization scores related to weight compared to individuals who were diagnosed with a psychiatric diagnosis by a physician. Hain *et al*. [23], in their study in which they examined the German version of the weight-related self-stigma scale, stated that there was no significant difference between the weight-related self-stigma scores of individuals according to BMI groups. Lin and Lee [24], in their study in which they examined the Chinese version of the weight-related self-stigma scale, stated that there was a significant difference between individuals' self-stigmatization scores regarding weight according to BMI groups and that as individuals' BMI increased, their weight-related self-stigmatization scores increased. Güzin *et al*. [25], in their study in which they examined the Turkish version of the weight-related self-stigma scale, stated that there was a significant difference between individuals' self-evaluation scores regarding weight according to BMI groups and that self-stigmatization scores related to weight increased as BMIs increased.

In this study, the comparison of the DSS scores was examined, and as a result, there was a statistically significant difference between the DSS scores of obese individuals on a diet according to their income status, psychiatric diagnosis made by the physician, chronic disease status and the number of daily snacks. It was found that there was no significant difference between DSS scores according to education status, marital status, employment status, BMI groups, psychiatric drug use status, smoking consumption status, alcohol consumption status, number of daily main meals, and body evaluation status. When the literature was examined, Hata *et al*. [26] stated that there was a significant difference between the dietary satisfaction scores of men according to age groups in their study in which they examined the composite relationship of social participation and support with personal health and diet satisfaction in men with spinal cord injury. Jospe *et al*. [17] stated that there was a significant difference between the diet satisfaction scores of individuals according to their gender and age, in their study where they developed and pre-validated the diet satisfaction score to evaluate diet satisfaction. Mioba and Ogada [27], examined the factors related to diet satisfaction in adult surgical orthopedic patients hospitalized in a teaching hospital in Lusaka, Zambia, and stated that there was no significant difference in diet satisfaction scores according to age groups, gender, education, and marital status of patients.

In this study, it was found that there was a significant positive correlation between Weight-Related Self

Stigma scores and emotional eating scores, but there was no significant correlation between diet satisfaction scores. When the literature was reviewed, Cihan [20], in his study examining the relationship between the feeling of stigma, eating behaviors, and depression in obese individuals, stated that there was a significant positive correlation between the self-stigmatization scores of obese individuals and their emotional eating scores. In his study, Yaşar [21], examined the relationship between self-stigma and emotional eating in obese individuals, it was determined that there was a significant positive correlation between the Self-Stigma Related to Weight and emotional eating scores of obese individuals. In this study, it was found that self-stigmatization scores related to weight had a positive effect on emotional eating scores, but did not have a significant effect on diet satisfaction scores.

CONCLUSION

When the results of the study conducted to examine the relationship between self-stigma in dieting obese individuals and emotional eating and dietary satisfaction were examined, it was observed that the scores of the EES were determined according to the gender and income status of the obese individuals, and WSSQ It has been found that there is a statistically significant difference between the scores of the obese individuals according to their gender and working status, and the DSS scores according to the income status of the obese individuals, their psychological diagnosis status by the physician, their chronic disease status and their daily snack consumption status. As a result of examining the effect of WSSQ scores on the EES and DSS scores, it was found that the WSSQ scores were compared to the EES scores. It was found that there was a statistically significant effect on the EES scores, but not on the DSS scores. Obese individuals should be given psychological information about self-stigmatization, the severity of emotional eating should be monitored and necessary precautions should be taken.

Authors' Contribution

Study Conception: NBD, SA; Study Design: NBD, SA; Supervision: NBD, SA; Funding: NBD, SA; Materials: NBD, SA; Data Collection and/or Processing: NBD, SA; Statistical Analysis and/or Data Interpretation: NBD, SA; Literature Review: NBD, SA; Manuscript Preparation: NBD, SA and Critical Review: NBD, SA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. Metab 2019;92:6-10.
2. Sevinçer GM, Konuk N. [Emotional eating]. J Mood Disord 2013;3:171-8. [Article in Turkish]
3. Bayles S, Ebaugh FG. Emotional factors in eating and obesity. J Am Diet Assoc 1950;26:430-4.
4. Van Strien T, Frijters JE, Van Staveren WA, Defares PB, Deurenberg P. The predictive validity of the Dutch restrained eating scale. Int J Eat Disord 1986;5:747-55.
5. Ganley RM. Emotion and eating in obesity: A review of the literature. Int J Eat Disord 1989;8:343-61.
6. Ünal SG. [Emotional eating and obesity]. Başkent Üniversitesi Sağlık Bilimleri Fakültesi Dergisi 2018;2:30-47. [Article in Turkish]
7. Bekker MH, Van De Meerendonk C, Mollerus J. Effects of negative mood induction and impulsivity on self-perceived emotional eating. Int J Eat Disord 2004;36:461-9.
8. Bostancı N. [The stigma towards to individual with mentally ill patients and practices about reducing this situation]. Dusunen Adam 2005;18:32-8. [Article in Turkish]
9. Ciftci A, Jones N, Corrigan PW. Mental health stigma in the Muslim community. J Muslim Ment Health 2013;7:17-32.
10. Wright A, Jorm AF, Mackinnon AJ. Labeling of mental disorders and stigma in young people. Soc Sci Med 2011;73:498-506.
11. Pinel EC, Bosson JK. Turning our attention to stigma: an objective self-awareness analysis of stigma and its consequences. Social Psychological Perspectives on Stigma: Routledge; 2016. p. 63-71.
12. Reynders A, Kerkhof AJ, Molenberghs G, Van Audenhove C. Attitudes and stigma in relation to help-seeking intentions for

psychological problems in low and high suicide rate regions. Soc Psychiatry Psychiatr Epidemiol 2014;49:231-9.
13. Corrigan P. How stigma interferes with mental health care. Am Psychol 2004;59:614-25.
14. Bharadwaj P, Pai MM, Suziedelyte A. Mental health stigma. Econ Lett 2017;159:57-60.
15. Bilgen Ş. Türkçe duygusal yeme ölçeği geliştirilmesi, geçerlilik ve güvenirliği çalışması. Üsküdar Üniversitesi. 2018.
16. Lillis J, Luoma JB, Levin ME, Hayes SC. Measuring weight self−stigma: the weight self-stigma questionnaire. Obes 2010;18:971-6.
17. Jospe MR, Haszard JJ, Taylor RW, Freedhoff Y. A tool for assessing the satisfaction of a diet: Development and preliminary validation of the Diet Satisfaction Score. Nutr Diet 2020;77:268-73.
18. Eskici G, Karahan-Yılmaz K. [The validity and reliability study of the Turkish form of diet satisfaction scale]. Journal of Ankara Health Sciences 2021;10:35-45. [Article in Turkish]
19. Akduman İ. Obez bireylerde problemli yeme davranışları ve çocukluk çağı travmaları ilişkisinin incelenmesi: İstanbul Gelişim Üniversitesi Sosyal Bilimler Enstitüsü; 2018.
20. Cihan G. Obez bireylerde damgalanma hissi ile yeme davranışları ve depresyon arasındaki ilişkinin incelenmesi: Başkent Üniversitesi Sağlık Bilimleri Enstitüsü; 2019.
21. Yaşar B. Obez bireylerde kendini damgalama ve duygusal yeme arasındaki ilişki: İstanbul Gelişim Üniversitesi Lisansüstü Eğitim Enstitüsü; 2020.
22. Gökçem Ş, Karaban S. [Effects of nutritional status on emotional eating, night eating and sleep quality]. Kocaeli Üniversitesi Sağlık Bilimleri Dergisi 2021;7:284-95. [Article in Turkish]
23. Hain B, Langer L, Hünnemeyer K, Rudofsky G, Zech U, Wild B. Translation and validation of the German version of the Weight Self-Stigma Questionnaire (WSSQ). Obes Surg 2015;25:750-3.
24. Lin KP, Lee ML. Validating a Chinese version of the Weight Self-stigma Questionnaire for use with obese adults. Int J Nurs Pract 2017;23:e12537.
25. Sevincer GM, Kaya A, Bozkurt S, Akin E, Kose S. Reliability, validity, and factorial structure of the Turkish version of the Weight Self-Stigma Questionnaire (Turkish WSSQ). Psychiatry Clin Psychopharmacol 2017;27:386-92.
26. Hata K, Inayama T, Matsushita M, Shinoda S. The combined associations of social participation and support with self-rated health and dietary satisfaction in men with spinal cord injury. Spinal Cord 2016;54:406-10.
27. Miyoba N, Ogada I. Diet satisfaction and associated factors among adult surgical orthopaedic inpatients at a teaching hospital in Lusaka province, Zambia; a hospital-based cross-sectional study. BMC Nutr 2019;5:1-7.

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The role of stress coping styles in the relationship between separation individualization and sexual self-schema

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ABSTRACT

Objectives: This study aimed to investigate the role of stress coping styles in the relationship between separation individualization and sexual self-schema.

Methods: Relational screening model was used in the study. In the selection of the sample group of the study, the convenience sampling method, which is one of the non-random sampling methods, was preferred. The study group of the research consists of 246 (61%) females, 157 (39.0%) males. In this study, Demographic Information Form, Separation Individuation Scale, Sexual Self Schema Scale, and Coping with Stress Scale was used.

Results: According to the results, problem-focused coping styles play a partial mediator role in the relationship between separation individuation and loving/compassionate sexual self-schema in coping with stress, and there is a relationship between separation individuation and problem-focused coping styles.

Conclusions: There was a significant relationship between separation, individuation and loving/compassionate sexual self and self-confident, optimistic approach, seeking social support. Predicts the compassionate sexual self-schema; It was determined that coping styles were a partial mediating variable in the relationship between relational problems and the loving/compassionate sexual self-schema.

Keywords: Separation individuation, sexual self-schema, styles of coping with stress

Separation and individuation are a fundamental organizing principle of human development, beginning from childhood and continuing to occur at different stages throughout our lives, with implications for life-long adaptive functioning [1]. Mahler *et al*. [2] suggested that human physiological and psychological birth are separate events and that the latter is a slow and gradual birth that occurs through physiological postnatal development.

The first stage of individuation, separation, lasts for about the first three years of life [2]. The second individuation process is adolescence period [3]. In order to reduce psychological dependence on parental introjections for standards of acceptance, self-esteem and behavior, the teenager must eliminate or transcend the internalized images of early childhood caregivers. The adolescent must learn to take on the tasks of regulating self-esteem and self-definition [4]. The third individuation process; the individual must flexibly manage the ongoing dialectic between separation and

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dependence, while avoiding the undesirable consequences of cohesion and integration on the one hand and complete separation and alienation on the other. The fourth individuation; It has been reported that middle adulthood and fifth individuation occur in late adulthood [5, 6, 7].

Studies on the self show that the self-concept is multifaceted [8, 9, 10]. Based on the idea that there may be some cognitive representation of the self that is central and likely to become active in certain life contexts, Andersen and Cyranowski [11] proposed an aspect of self-schemas that they called the sexual self-schema. Sexual self-schemas are mental generalizations about sexual aspects of the self that express an integral component of one's sexuality. The sexual self-schema structure was made functional for both women and men by using trait-adjective ratings in the Sexual Self-schema Scales [11, 12]. When sexual self-schemas are positive, sexual self-views can facilitate sexual responsiveness. However, when sexual self-views are negative, conflicting, or weak, sexual distress, difficulty, or dysfunction is likely to occur [12]. Andersen and Cyranowski, found that there were gender-related differences in sexual self-assessments. It has been observed that women have negative sexual self-schemas compared to men and these women have a high level of neuroticism, however, they report less sexual experience [13]. It is also argued that the structure of the sexual self-concept is significantly influenced by the dominant social representations of gender differences and relationships [14]. Recently, in a study to combine male and female sexual self-schema scales, sexual schemas, which were examined in 2 categories as positive and negative, were divided into loving and compassionate, sensual and provocative, and direct and outspoken [15].

Generally, coping with stress is defined as any effort to manage stress or a set of methods people use to avoid being harmed by life stresses [16]. Lazarus and Folkman [17] discussed coping styles separately as problem-oriented strategies (efforts to change the source of stress) and emotion-oriented responses (attempts to regulate emotional distress caused by the stressor). Accordingly, although emotion-oriented approaches, which are described as passive, can be active, these reactions are generally aimed at avoiding dealing with the source of stress. Problem-oriented coping strategies, on the other hand, are aimed at eliminating the negative situation. Feingold's study of coping strategies reveals that both problem-oriented and emotion-oriented coping are used in almost all stressful situations, and that the use of problem or emotion-oriented strategies differs in effectiveness among different types of stressors [18]. As a result, more use of problem-oriented methods was associated with better adjustment, while emotion-oriented responses were associated with worse outcomes [19]. This study examines the effects of separation, individuation, which is an effective concept throughout the life of individuals, and the sexual self-schema, which interacts with many psychological factors and directly affects the vital factors, by considering the concept of sexuality in a more comprehensive way and the effects of having different sexual self-schemas. It is expected to present a different perspective to mental health professionals, especially experts working in the field of sexuality, in clinical diagnosis, treatment and therapy methods.

METHODS

Study Group

In the research, it was calculated that at least 384 people should be reached at 5% sampling error with simple random sampling method. The calculations were calculated with the sampling calculation method developed by Yazıcıoğlu and Erdoğan [20]. The study group of the research consists of a total of 403 participants, 246 women (61%) and 157 men (39.0%) aged between 18 and 65. Participants were accessed through various social media platforms.

Data Collection Tools

Sociodemographic Information Form

The sociodemographic information form was prepared by the researchers. In the form, age, education level, place where they spent most of their life, romantic relationships, early caregiver(s), number of siblings, their sexual life, physical disorders and mental disorders were asked.

Separation Individuation Scale

The Separation Individuation Scale (SIS) was de-

veloped by Christenson and Wilson [21]. The scale has 39 items, 3 dimensions (difficulties in separation/differentiation, division, relationship problems). The progression of the scale's scores reveals the psychopathology that takes place during the separation-individuation process. SIS was adapted to Turkish by Göral [22]. The Cronbach's alpha number was determined as .85 for the whole scale. The study on the psychometric properties of the scale was repeated by Göral Alkan [23]. SIS was adapted to Turkish by Göral [22]. The Cronbach's alpha number was determined as .85 for the whole scale [22].

Sexual Self Schema Scale

The Sexual Self-Schema Scale created by Hill [24]. In this scale, which includes 36 adjectives, 3 dimensions, the response options range from "doesn't describe me at all" to "describes me very well" and are prepared in a 7-point likert type. Participants rate the adjectives on the scale based on how well they describe themselves in their sexual life. Koçak and Fışıloğlu [25] found the Cronbach's alpha coefficient as .85, .82, and .77 for the subscales in the adaptation study of the scale into Turkish.

Styles of Coping with Stress Scale

Originally designed by Folkman and Lazarus [26] with 66 components, this scale was condensed by ahin and Durak [27] for Turkish adaption to just 30 items. In the study of adapting this scale, which does not have a cut-off point, into Turkish, the Cronbach's alpha reliability coefficients were found to be .87, .80, .66, .75 and .71 for the sub-dimensions.

Collecting Data

Data collection was carried out between January 2022 and March 2022. After the scales in the research were transferred to the "www.googleform.com" address, online transmission was provided to the existing participants via various social media platforms and digital channels.

The application took an average of 15 minutes.

Ethical Approval

This research developed from x's thesis. The necessary permission to carry out the research was obtained from the Istanbul X University Ethics Committee Unit with the decision number 01 on 20.01.2022.

Table1. Findings of the realibity of Sexual Self-Schema Scale, Stress Coping Styles Scale, and Separation Individuation Scale

	Number of Items	Cronbach Alpha
Sexual Self-Schema Scale		
Loving/Compassionate	11	.90
Sensual/Stimulating	12	.81
Direct/Outspoken	10	.74
Stress Coping Style Scale		
Optimistic	5	.78
Self Confident	7	.88
Submissive	7	.76
Helpless	6	.60
Seeking of Social Support	4	.61
Seperation Individuation Scale	39	.93
Division	12	.82
Separation Differentiation	14	.85
Relational Problems	13	.83

Statistical Analysis

Statistical evaluations were analyzed using SPSS (Statistical Package for Social Sciences) 25.0 package program [50]. First, it was checked whether there was a normal distribution or not. For this, the skewness-Kurtosis values were checked. According to George and Mallery [28], Skewness and kurtosis values between -2 and +2 are deemed sufficient for a normal distribution. Descriptive analysis used for Sexual Self-Schema Scale, Stress Coping Styles Scale, and Separation Individuation Scale and age of participants. Descriptive statistics give information about the mean, standart deviation, minimum and maximum value. Frequency analysis used for categorical variable like Sociodemographic characteristics of participants. Pearson Correlation analysis used for between relationship of Sexual Self-Schema Scale, Stress Coping Styles Scale, and Separation Individuation Scale. PROCESS 3.5 by Andrew F. Hayes used for mediation effect (indirect effects) of Stress Coping Styles Scale on The Relationship Between Separation Individuation Scale and Sexual Self-Schema Scale. The number of questions and Cronbach Alpha values regarding the scales and their subscales are given (Table 1).

RESULTS

Descriptive Statistics Findings

Mean age of the participants (30 ± 8 years), mean years of romantic relationship (6 ± 6 years), mean number of siblings (3 ± 2), mean sibling rank (2 ± 1), mean sibling rank (2 ± 1), and age apart from parents (4 ± 3) (Table 2).

Average of loving-compassionate (50.19 ± 11.20), mean of sensual/stimulating (44.53 ± 10.26), mean of direct/outspoken (41.43 ± 8.45), mean of optimistic approach (2.64 ± 0.66), mean of self-confident approach (2.94 ± 0.65, mean of helpless approach (2.31 ± 0.62), mean of submissive approach (1.97 ± 0.51), approach to seeking social support mean (2.80 ± 0.61), separation individuation scale mean (141.30 ± 60.20), division mean (44.72 ± 20.02), separation differentiation mean (47.49 ± 23.36), relational problems mean (49.08 ± 20.75) (Table 2).

Findings Obtained Using Pearson Correlation

Loving Compassionate and Optimistic Approach ($r = .304$, $p < 0.001$) variables were moderately, positively correlated with each other, Loving Compassion-

Table 2. Descriptive values of Sexual Self-Schema Scale, Stress Coping Style Scale and Separation Individuation Scale

	n	Mininum	Maximum	Mean	Standard deviation
Sexual Self-Schema Scale					
Loving/Compassionate	403	16	66	50.19	11.20
Sensual/Stimulating	403	16	68	44.53	10.26
Direct/Outspoken	403	16	60	41.43	8.45
Stress Coping Style Scale					
Optimistic	403	1	4	2.64	0.66
Self Confident	403	1	4	2.94	0.65
Submissive	403	1	4	2.31	0.62
Helpless	403	1	4	1.97	0.51
Seeking of Social Support	403	1	4	2.80	0.61
Seperation İndividuation Scale	403	47	337	141.30	60.20
Division	403	12	110	44.72	20.02
Separation Differentiation	403	14	129	47.49	23.36
Relational Problems	403	13	113	49.08	20.75

Table 3. Findings of the relationships between Sexual Self-Schema Scale, Stress Coping Styles Scale and Separation Individuation Scale

	1	2	3	4	5	6	7	8	9	10	11	12
1-Loving/compassionate	1											
2-Sensual/stimulating	.531**	1										
3-Direct/outspoken	.432**	.600**	1									
4-Optimistic	.304**	.253**	.292**	1								
5-Self confident	.360**	.373**	.408**	.693**	1							
6-Submissive	.123*	.020	-.227**	-.216**	-.108*	1						
7-Helpless	.070	.065	-.088	.100*	.030	.474**	1					
8-Seeking of social support	.207**	.075	.104*	.069	.062	-.042	-.029	1				
9-Seperation individuation scale	-.023	.165**	-.082	-.131**	-.159**	.451**	.242**	-.133**	1			
10- Division	.077	.210**	-.015	-.126*	-.117*	.446**	.230**	-.110*	.908**	1		
11-Seperation differentiation	.075	.194**	-.052	-.025	-.075	.449**	.253**	-.108*	.931**	.828**	1	
12-Relational problems	-.210**	.045	-.150**	-.207**	-.238**	.324**	.170**	-.141**	.861**	.641**	.677**	1

**$p < 0.001$, *$p < 0.05$ Used test; Pearson Correlation

ate and Self-Confident Approach (r = .360, $p < 0.001$) variables were moderately, positively correlated with each other. Weak and positive relationship between Loving Compassionate and Helpless Approach (r = .123, $p = 0.014$) variables, weak and positive correlation between Loving Compassionate and Seeking of Social Support Approach (r = .207, $p < 0.001$) variables positive relationship, Loving Compassionate and Relational Problems (r = -.210, $p < 0.001$) variables have weak, negative relationships with each other (Table 3).

Weak and positive correlations between the Sensual Stimulating and Optimistic Approach (r = .253, $p < 0.001$) variables, moderate and positive correlations between the Sensual Stimulating and Self-Confident Approach (r = .373, $p < 0.001$) variables. Positive correlation is weak level and positive relationship between Sensual Stimulating and Separation Individuation Scale (r = .165, $p < 0.001$) variables, a weak relationship between Sensual Stimulating and Separation (r = .210, $p < 0.001$) variables there is a weak level and positive relationship between the variables of the Sensual Stimulating and the separation differentiation (r = .194, $p < 0.001$) (Table 3).

Weak and positive relationship between Direct/Outspoken and Optimistic Approach (r = .292, $p < 0.001$) variables, moderate and positive relationship between Direct/Outspoken and Self-Confident Approach (r = .408, $p < 0.001$) variables , Weak, negative relationship between Direct Ouspoken and Desperate Approach (r = -.227, $p < 0.001$) variables, eak level between Direct/Outspoken and Helpless Approach (r = .104, $p = 0.036$) variables There is a weak, negative relationship between the variables of Direct/Outspoken and Relational Problems (r = -.150, $p < 0.001$) (Table 3).

Weak and negative correlation between Optimistic Approach and Separation Individuation Scale (r = -.131, $p < 0.001$) variables, weak and negative relationship between Optimistic Approach and Division (r = -.126, $p = 0.011$) variables, Optimistic Approach and Relational Problems (r = -.207, $p < 0.001$) variables have weak, negative correlations with each other (Table 3).

Weak and negative correlations between Self-Confident Approach and Separation Individualization Scale (r = -.159, $p < 0.001$) variables, weak and negative correlations between Self-Confidence Approach and Splitting (r = -.117, $p = 0.018$) variables. Relationship, Self-Confident Approach and Relational Problems (r = -.238, $p < 0.001$) variables have weak, negative correlations with each other (Table 3).

Intermediate, positive relationship between Helpless Approach and Separation Individuation Scale (r = .451, $p < 0.001$) variables, Intermediate, positive relationship between Helpless Approach and Division (r

Table 4. Results of the findings of the mediation role of the optimistic approach in predicting the Loving-Compassionate Sexual Self-Scheme of relational problems

Model		*R*	*R^2*	*B*	*SH*	*β*	*t*	*p value*	Lower Bound	Upper Bound
	(Constant)	.29	.04	55.40	1.33		41.79	**< 0.001***	52.79	58.01
1	**Relational Problems**			-0.11	0.02	-0.21	-4.31	**< 0.001***	-0.16	-0.06
	(Constant)	.36	.11	41.87	2.71		15.45	**< 0.001***	36.54	47.20
	Relational Problems			-0.08	0.02	-0.15	-3.20	**0.001***	-0.13	-0.03
2	**OptimisticApproach**			4.59	0.81	0.27	5.66	**< 0.001***	3.00	6.19
	Undirect Total Effect (Mediator)			-	0.01				-0.01	-0.01

*$p < 0.05$, Used test: PROCESS 3.5

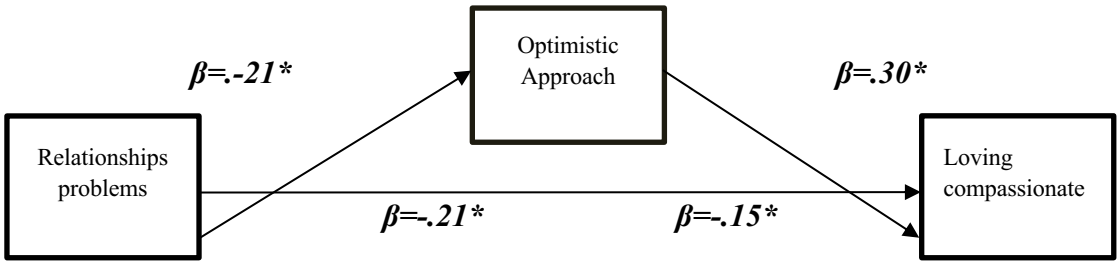
= .446, $p < 0.001$) variables, there is a moderate, positive relationship between the variables of Helpless Approach and Seperation Differentiation (r = .449, $p < 0.001$), and there is a moderate, positive relationship between the Helpless Approach and Relational Problems (r = .324, $p < 0.001$) variables (Table 3).

Weak and positive correlation between the Submissive Approach and Separation Individuation Scale (r = .242, $p < 0.001$) variables, a weak level and positive relationship between the Submissive Approach and Splitting (r = .230, $p < 0.001$) variables, Submissive Approach and Seperation Differentiation (r = .253, $p < 0.001$) variables have a weak, positive relationship with each other, Submissive Approach and Relational Problems (r = .170, $p < 0.001$) variables have a weak, positive relationship with each other (Table 3).

Weak and negative relationship between the Social Support Approach and Separation Individuation Scale (r = -.133, $p < 0.001$) variables, and the weak level between the Social Support Approach and Division (r = -.110, $p = 0.027$) variables and negative relationship, the weak and negative relationship between the Social Support Approach and the Seperation Differentiation (r = -.108, $p = 0.031$) variables and there is a weak level and negative relationship between the Social Support Approach and Relational Problems (r = -.141, $p < 0.001$) variables (Table 3).

Findings Obtained from the Hierarchical Regression Analysis

It was seen that the relational problems independent variable in the first model explained 04% of the variance in the loving-kindness sexual self-schema dependent variable score. In the established regression model, the optimistic approach independent variable was added in the second stage. It was seen that the independent variable of the optimistic approach explained 7% of the variance in the dependent variable score of the loving-compassionate sexual self-schema.



*$p < 0.05$

Fig. 1. Beta values of the Mmdiating role of the optimistic approach in predicting the Loving-Compassionate Sexual Self-Schema of relational problems

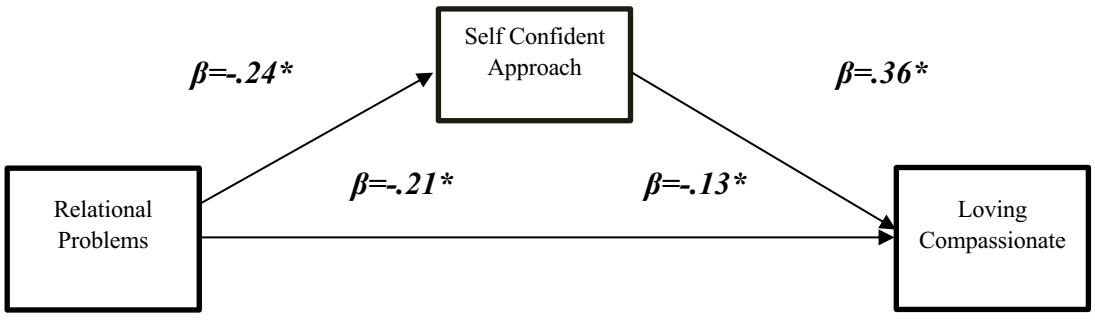
Table 5. Results of findings on the mediating role of self-confident approach in predicting the Loving-Compassionate Sexual Self-Schema of relational problems

Model		*R*	R^2	*B*	*SH*	*β*	*t*	*p value*	Lower Bound	Upper Bound
	(Constant)	.29	.04	55.40	1.33		41,79	**< 0.001***	52.79	58.01
1	**Relational Problems**			-0.11	0.02	-0.21	-4,31	**< 0.001***	-0.16	-0.06
	(Constant)	.36	.14	36.68	2.98		12,30	**< 0.001***	30.82	42.54
	Relational Problems			-0.07	0.02	-0.13	-2,78	**0.006***	-0.11	-0.02
2	**Self Confident Apporach**			5.71	0.83	0.33	6,92	**< 0.001***	4,09	7.34
	Undirect Total Effect (Mediator)			-0.04	0.01				-0.07	-0.02

*$p < 0.05$, Used test: PROCESS 3.5

Relational problems and optimistic approach independent variables were determined according to these results, which explained 11% of the variance in the score of the loving-compassionate sexual self-schema dependent variable. With the addition of the optimistic approach argument in the second stage, the beta value of the relational problems argument decreased from-.21 to-.15. After this process, the Boostrapping method was used. As the lower and upper limit values of the 95% confidence interval of the decrease in beta value of the independent variable and the indirect total effect did not include zero, the mediation effect was found to be significant. When all these results were evaluated, it was observed that there was partial mediation (Table 4) (Fig. 1).

The relational problems independent variable in the first model explained 04% of the variance in the loving-compassionate sexual self-schema dependent variable score. In the established regression model, the independent variable of self-confident approach was added in the second stage. The independent variable of the self-confident approach explained 10% of the variance in the dependent variable score of the loving-compassionate sexual self-schema. It was determined according to these results that the independent variables of relational problems and self-confident approach schema explained 14% of the variance in the score of the loving-compassionate sexual self schema dependent variable. With the addition of the self-confident approach scheme argument in the second stage, the beta value of the relational problems argument decreased from-.21 to-.13. After this process, the Boostrapping method was used. As the lower and upper limit values of the 95% confidence interval of the decrease in beta value of the independent variable and the indirect total effect did not include zero, the medi-



*$p < 0.05$

Fig. 2. Beta values of the mediating role of self-confident approach in predicting the Loving-Compassionate Sexual Self-Schema of relational problems

Table 6. Results of findings on the mediating role of seeking social support Approach in predicting the Loving-Compassionate Sexual Self-Scheme of relational problems

Model		*R*	*R^2*	*B*	*SH*	*β*	*t*	*p value*	*Lower Bound*	*Upper Bound*
	(Constant)	.29	.04	55.40	1.33		41.79	**< 0.001***	52.79	58.01
1	**Relational Problems**			-0.11	0.02	-0.21	-4.31	**< 0.001***	-0.16	-0.06
	(Constant)	.36	.07	45.48	2.96		15.36	**< 0.001***	39.66	51.30
	Relational Problems			-0.09	0.02	-0.19	-3.81	**< 0.001***	-0.14	-0.05
2	**Seeking Social Support Approach**			3.31	0.89	0.18	3.73	**< 0.001***	1.57	5.06
	Undirect Total Effect (Mediator)			-0.01	0.01				-0,03	-0.01

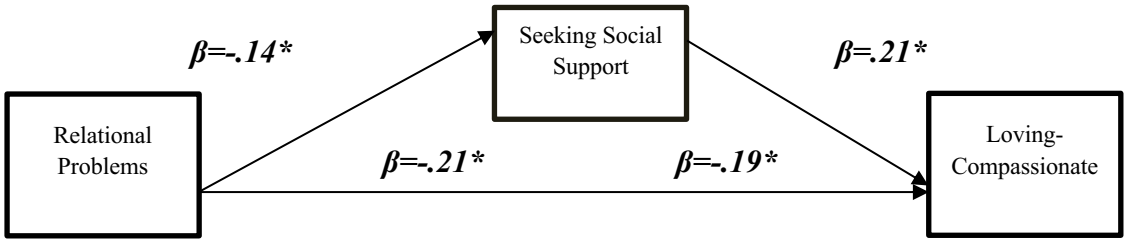
*$p < 0.05$, Used test: PROCESS 3.5

ation effect was found to be significant (Table 5) (Fig. 2).

The relational problems independent variable in the first model explained 04% of the variance in the loving-compassionate sexual self-schema dependent variable score. In the established regression model, the independent variable of approach to social support was added in the second stage. It was observed that the independent variable of approach to seeking social support explained 03% of the variance in the dependent variable score of the loving-compassionate sexual self-schema. It was determined that the independent variables of relational problems and approach to seeking social support explained 07% of the variance in the score of the loving-compassionate sexual self-schema dependent variable. In the second stage, the beta value of the relational problems independent variable decreased from-.21 to-.19 with the addition of the independent variable of approach to seeking social support. After this process, the Boostrapping method was used. As the lower and upper limit values of the 95% confidence interval of the decrease in beta value of the independent variable and the indirect total effect did not include zero, the mediation effect was found to be significant (Table 6) (Fig. 3).

DISCUSSION

In this study, the role of coping styles in the relationship between separation individuation and sexual self-schema was investigated. When the mediation analysis results were examined in this context, it was determined that problem-focused coping styles had a partial mediation role in the relationship between relational problems in separation-individuation and the loving-compassionate sexual self-schema. In the direction of the findings, in the literature review, it is seen that the relationship between the child and the caregiver in the early period and the separation-individuation process



*$p < 0.05$

Fig. 3. Beta values of the mediating role of seeking social support in predicting the Loving-Compassionate Sexual Self-Schema of relational problems

that continues throughout life are in mutual interaction. It is thought to be associated with lifelong variables such as methods of coping with stress, romantic and social relationships. Other studies in the literature [2-4, 29, 30] supports our findings. Aarestad [31] examined the relationship between attachment styles and sexual self-schema. When the results of the study were examined, it was seen that sexual self-schemas could affect the quality of the relationship and attachment styles could affect sexual function. When the literature studies and our current study are evaluated together in examining the relationship between sexual self-schemas and attachment [12, 24, 31-33], firstly, individuals with higher levels of positive sexual self-concepts reported better relationship quality; Secondly, it can be said that insecure (i.e. anxious or avoidant) attachment and separation individuation problems are associated with weaker sexual satisfaction in both genders. In addition, it can be said that there is a negative relationship between avoidant/anxious attachment and sexual satisfaction. In this direction, there is a relationship between being able to overcome the separation-individuation process in a healthy way and the preference of problem-oriented methods in coping with stress [34].

There was a significant relationship between sexual self-schemas and gender differences. Accordingly, men describe their sexual self-schemas as direct/outspoken more often than women. When the studies in the literature are examined [18, 31], gender differences between male and female sexual self-schemas were due to high anxiety problems, low level of knowledge about sexuality, introversion, negative body image perception, lack of sexual fantasy, and limited sexual experience. In accordance with the evaluation made within the framework of the literature, it can be said that in the current study, female participants were more conservative, shy and shy compared to men, and this result was influenced by the evaluation of women's sexuality in line with taboos such as chastity and gender roles. As a matter of fact, Turkish society is one of the societies in which sexuality is considered taboo and sexual issues are not openly expressed in terms of its social and cultural structure [35].

In this study, the most frequently used source for obtaining sexual information by individuals was the internet, and the least referenced source was the parents. Considering the importance of sexual life in the life of the individual, it is very important for parents to communicate with children in order to prevent them from acquiring false information about sexuality away from science, and especially to protect children in developmental age from digital risks. Obtaining inaccurate information about sexuality leads to the formation of sexual myths, which is one of the main effective factors in sexual dysfunction [36, 37]. Sexual myths are the most exaggerated, far from reality and unscientific, guilt, inadequacy, fear of failure, which are mostly exaggerated, unrealistic and unscientific, which they have recently obtained from sources of false or false information, especially from social media outlets and the internet, on issues that affect individuals' attitudes and behaviors. Belief and thought systems that cause negative emotions such as [38-40, 41].

In the findings, men use the optimistic approach more frequently in coping with stress. Gündüz [42] found that male counselor candidates had higher levels of self-confidence and optimistic approach than female counsellors. In the literature, studies proving that women adopt more emotional coping styles compared to men, similar to our current result [8, 43], while also contrasting studies [44, 45] was also observed.

In this study, individuals who do not have an emotional relationship adopt an emotion-focused (submissive) approach in coping with stress and cannot survive the separation-individuation process in a healthy way, but individuals who are in emotional relationships prefer more effective (problem-focused) coping methods in coping with stress, and healthy separation is a key part of individuation higher in individuals. Lloyd [46] found that the separation-individuation process was effective on the emotional relationships established in the later period.

In the findings, as the number of siblings increases, the use of social support decreases. It is thought that the reason for this is that individuals receive adequate social support from their siblings. In the study of Azızoğlu [47], no relationship was found between the number of siblings and seeking social support. Therefore, it can be thought that the factor that determines the need for seeking social support is related to the quality of the relationship with siblings rather than the number of siblings.

It was observed that individuals with longer duration of romantic relationship preferred problem-ori-

ented methods (self-confident approach, seeking social support) to cope with stress, and the frequency of seeking social support decreased as the duration of the emotional relationship increased. It is thought that the reason for this is that the need for social support is met with the increase in the closeness of the relationship with the partner. There is no study in the literature examining the existing variables together. In addition, according to the findings obtained in the study, it was observed that as the level of education increased, the frequency of individuals to resort to problem-oriented approaches in coping with stress increased. Similarly, in the study of Gürlek Yüksel [48], it was observed that the use of problem-focused coping styles increased with the increase in education level, the study is supported by the relevant literature.

As a result, when the sexual self-schemas are examined according to the education level, it is found that the individuals with a postgraduate degree or higher have a higher level of loving-compassionate sexual self-definition than the primary school graduates. Compared to primary and high school graduates, associate degree graduates were found to describe themselves as lascivious and provocative. In the study conducted by Uzunoğlu [49], no significant relationship was found between education level and sexual self-schemas. In the literature, different studies dealing with the relationship between these two could not be found.

CONCLUSION

The findings obtained in the study are important in terms of revealing the importance of internal models of the relational self (for example, separation-individuation) on sexual functioning and the importance of internal models of the sexual self (for example, sexual self-schema) in relationship functioning. In this study, it was found that there was a significant relationship between separation, individuation and loving/compassionate sexual self and self-confident, optimistic approach, seeking social support. Predicts the compassionate sexual self-schema; It was determined that coping styles were a partial mediating variable in the relationship between relational problems and the loving/compassionate sexual self-schema.

Authors' Contribution

Study Conception: KG, GA; Study Design: HF, GA; Supervision: HF, KG; Funding: N/A; Materials: KG, HF, GA; Data Collection and/or Processing: GA, KG; Statistical Analysis and/or Data Interpretation: KG, HF, GA; Literature Review: KG, HF, GA; Manuscript Preparation: KG, GA, and Critical Review: KG, HF.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Lapsley DK, Stey P. Separation-individuation. In: Weiner I, Craighead E, eds. Corsini's Encyclopedia of Psychology, N Y: Wiley; 2010.
2. Mahler MS, Pine F, Bergman A. Insan yavrusunun psikolojik doğumu. (Babaoğlu, Çev.) Istanbul: Metis; 2003/2012.
3. Blos P. The second individuation process of adolescence. Psychoanal Study Child 1967;22:162-86.
4. Aslan S, Güven M. [The relation between attachment and personal adjustment mediated by separation-individuation]. Eğitim ve Bilim 2010;35:181-90. [Article in Turkish]
5. Colarusso CA. The third individuation: the effect of biological parenthood on separation-individuation processes in adulthood. Psychoanal Study Child 1990;45:179-94.
6. Colarusso CA. Separation individuation processes in middle adulthood: The fourth individuation. In: Akhtar S, Kramer S, eds. The Seasons of life, New Jersey: Jason Aronson; 1997; pp. 73-94.
7. Colarusso CA. Separation-individuation phenomena in adulthood: general concepts and the fifth individuation. J Am Psychoanal Assoc 2000;48:1467-89.
8. Carver CS, Scheier MF. Attention and Self-Regulation: A Control Theory Approach to Human Behavior. New York: Springer; 1981.
9. Epstein S. The self-concept: A review and the proposal of an integrated theory of personality. In: Staub E, eds. Personality: Basic issues and current research, Englewood Cliffs, New Jersey: Prentice Hall; 1980; pp. 253-75.
10. Markus H, Wurf E. The dynamic self-concept: A social psychological perspective. Annu Rev Psychol., 1987; 38:1, 299-333.
11. Andersen BL, Cyranowski JM. Women's Sexual Self-Schema. J Pers Soc Psychol 1994;67:1079-100.
12. Andersen BL. Surviving cancer: the importance of sexual

self−concept. Med Pediatr Oncol 1999;33:15-23.
13. Andersen BL, Cyranowski JM, Espindle D. Men's sexual self-schema. J Pers Soc Psychol 1999;76:645-61.
14. Breakwell GM, Millward LJ. Sexual self-concept and sexual risk-taking. J Adolesc 1997;20:29-41.
15. Hill DB. Differences and similarities in men's and women's sexual self-schemas. J Sex Res 2007;44:135-44.
16. Cohen F, Lazarus RS. Coping with the stress of illness. In: Stone GC, Cojen F, Adler N.E. (eds). Health psychology. A handbook. Theories, applications and challenges of a psychological approach to the health care system. Jossey- Bass, San Francisco; 1979.
17. Lazarus RS, Folkman S. Stress, appraisal, and coping. Springer publishing company; 1984.
18. Feingold A. Gender differences in personality: a meta-analysis. Psychol Bull 1994;116:429-56.
19. Forsythe CJ, Compas BE. Interaction of cognitive appraisals of stressful events and coping: Testing the goodness of fit hypothesis. Clin Psychol Rev 1987;11:473-85.
20. Yazıcıoğlu Y, Erdoğan S. SPSS Uygulamalı Bilimsel Araştırma Yöntemleri. Ankara: Detay Yayıncılık; 2004.
21. Christenson RM, Wilson WP. Assessing pathology in the separation - individuation process by an inventory: a preliminary report. J Nerv Ment Dis 1985;175:561-5.
22. Göral FS. The Second Separation- Individuation Process of the Turkish Young Adults: The Relationship between the Perceived Maternal Parenting Attitudes, Second Separation- Individuation, Expanding Self and Experiences in the Romantic Relationships (Unpublished Master Thesis). Boğaziçi University, Istanbul, Turkey; 2002.
23. Göral Alkan FS. Coupling through Projective Identification: Bridging Role of Projective Identification in the Associations among Early Parenting Experiences, Personality Constructs and Couple Relationship (PhD Dissertation). Middle East Technical University, Ankara, Turkey; 2010.
24. Hazen C, Shaver P. Romantic love conceptualized as an attachment process. J Pers Soc Psychol 1987;52:511-24.
25. Koçak G, Fışıloğlu H. [A validity and reliability study of the sexual self-schema scale smong undergraduate students]. Klinik Psikiyatri 2010;13:159-69. [Article in Turkish]
26. Folkman S, Lazarus RS. An analysis of coping in a middle-aged community sample. J Health Soc Behav 1980;21:219-39.
27. Şahin NH, Durak A. Stresle başa çıkma tarzları ölçeği: Üniversite öğrencileri için uyarlanması. Türk Psikoloji Dergisi 1995;10:56-73.
28. George D, Mallery M. SPSS for Windows Step by Step: A Simple Guide and Reference, 17.0 update (10a ed.) Boston: Pearson; 2010.
29. Faraji H, Özen ZD. [The mediator role of adult separation anxiety between separation anxiety and the level of individuation in emerging adulthood]. Mavi Atlas 2022;10:47-69. [Article in Turkish]
30. Pine F. On the pathology of the separation-individuation process as manifested in later clinical work. Int J Psychoanal 1979;60:225-42.
31. Aarestad L. The role of attachment style and sexual self-schemas in romantic and sexual relationships: a study of dating couples (Doctoral dissertation, Ohio State University). OhioLINK Electronic Theses and Dissertations Center; 2000.
32. Cyranowski JM, Andersen L. Schemas, sexuality, and romantic attachment. J Pers Soc Psychol 1998;74:1364-79.
33. Villanueva C. How attachment, personality, and sexual self-schema influence sexual behavior (Master's thesis); 2012.
34. Smith AW. Separation-individuation and coping: Contributions to freshman college adjustment (Doctoral dissertation); 1994.
35. Sungur MZ. Cultural factors in sex therapy: the Turkish experience. Sex Marital Ther 1999;14:165-71.
36. Kayır A. Cinsellik Kavramı ve Cinsel Mitler. Yetkin, N., İncesu, C. (Ed.), Cinsel işlev bozuklukları monograf serisi (ss.34-9). İstanbul: Roche Müstehzarları Sanayi A.Ş; 2001.
37. Set T, Dağdeviren N, Aktürk Z. [Sexuality of adolescents]. Genel Tıp Dergisi. 2006; 16:3, 138- 40. [Article in Turkish]
38. Kukulu K, Gürsoy E, Sözer GA. Turkish University students' beliefs in sexual myths. Sex Disabil 2009;27:49-59.
39. Nobre P, Gouveia JP, Gomes FA. Sexual dysfunctional beliefs questionnaire: an instrument to assess sexual dysfunctional beliefs as vulnerability factors to sexual problems. Sex Relation Ther 2003;18:171-204.
40. Torun F, Torun SD, Özaydın AN. [Men's belief in sexual myths and factors effecting these myths]. Dusunen Adam 2011;24:24-31. [Article in Turkish]
41. Yaşan A, Gürgen F. [The ways to get sexual knowledge and the comparıson of the rate of sexual myths in nurses who have sexual partners and who do not have]. Yeni Symposium Dergisi 2004;42:72-6. [Article in Turkish]
42. Gündüz M. Psikolojik danışman adaylarının stresle başa çıkma tarzlarının çok yönlü eylemli kişilik özelliklerine, öznel iyi oluşlarına ve risk faktörlerine göre incelenmesi (Yüksek Lisans Tezi). Yıldız Teknik Üniversitesi, Sosyal Bilimler Enstitüsü (Kayıt No. 596502); 2018.
43. Kaya M, Genç M, Kaya B, Pehlivan E. [Prevalence of depressive symptoms, ways of coping, and related factors among medical school and health services higher education students]. Türk Psikiyatri Dergisi 2007;18:137-46. [Article in Turkish]
44. Çetin MÇ, Kuru E. [Investigation of physical education and sport school students' coping with the stress styles' according to some variables]. Milli Eğitim Dergisi 2010;187:193-206. . [Article in Turkish]
45. Eraslan M. [Investigation personality aspects and coping style to stress of students taking education at sport departments in terms of some variables]. Mehmet Akif Ersoy Üniversitesi Sosyal Bilimler Enstitüsü Dergisi 2015;7:65-82. [Article in Turkish]
46. Lloyd ME. Fear of intimacy in romantic relationships during emerging adulthood: the influence of past parenting and separation-individuation (Doctoral dissertation); 2011.
47. Azizoglou C. Mükemmeliyetçilik ve Stresle Başa Çıkma Yaklaşımlarının Sosyal Kaygı Üzerindeki Etkisi (Yüksek Lisans Tezi). Fatih Sultan Mehmet Vakıf Üniversitesi Lisansüstü Eğitim Enstitüsü Psikoloji Anabilim Dalı, Yayımlanmamış Yüksek Lisans Tezi, (Kayıt No. 616245), İstanbul 2020.
48. Gürlek Yüksel E. Üniversite öğrencilerinin ayrılma

bireyleşme özellikleri ve etkileyen etmenler (Yayınlanmamış Uzmanlık Tezi). Celal Bayar Üniversitesi, (Kayıt No. 193198); Manisa, 2006.
49. Uzunoğlu S. Evli kadınların cinsel benlik şemaları ile cinsel işlev düzeyleri arasındaki ilişki (Yayımlanmış Yüksek lisans tezi). İstanbul Kent Üniversi, (Kayıt No. 665438); İstanbul, 2021.
50. Corportation, IBM. IBM SPSS Statistics for Windows (Version 25.0 Armonk). IBM Corp.: Armonk, NY, USA. 2017.

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The prognostic implications of a fragmented QRS pattern in patients diagnosed with heart failure

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ABSTRACT

Objectives: The purpose of this study was to investigate the potential association between the presence of a fragmented QRS (fQRS) pattern on electrocardiogram (ECG) and prognostic outcomes in patients with heart failure (HF).

Methods: In the study, 238 patients with a left ventricular ejection fraction of less than 35% were included. The presence of fQRS pattern on ECG was analyzed in all patients. Patients were divided into two groups based on the presence or absence of fQRS on ECG. The primary clinical end points were all-cause mortality and rehospitalization.

Results: Baseline demographic characteristics were comparable between the groups. Patients with fQRS had a higher frequency of coronary artery disease and atrial fibrillation (72 (76%) vs 90 (62%), $p = 0.023$; 27 (29%) vs 25 (17%), $p = 0.038$, respectively). Additionally, patients with fQRS had a higher rate of mortality and rehospitalization during the 5-year follow-up period compared to patients without fQRS. (30 (32%) / 28 (19%), $p = 0.028$; 56 (60%) / 50 (35%), $p < 0.001$, respectively). Multivariable logistic regression analysis revealed that the presence of fQRS was independently associated with mortality and rehospitalization ($p = 0.02$ and $p < 0.001$, respectively).

Conclusions: The results of this study suggest that the presence of fQRS on ECG in patients with HF is independently related to a higher risk of mortality and hospitalization. Therefore, the presence of fQRS may be a useful indicator of poor prognosis in these patients.

Keywords: Fragmented QRS, heart failure, hospitalization, mortality

Heart failure (HF) is a prevalent medical condition that can result in considerable morbidity, including recurrent hospitalizations and the risk of mortality. The global prevalence of HF has risen to approximately 23 million individuals, with HF with reduced ejection fraction (HFrEF) accounting for roughly 50% of all cases [1]. Identifying the predictors that accurately determine the prognostic outcome in patients with HF has the potential to improve risk stratification and monitoring, leading to better clinical outcomes for the patients.

The 12-lead electrocardiogram (ECG) is a valu-

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able diagnostic and prognostic tool in patients with HF. In patients with organic heart disease, the presence of bundle branch blocks on the ECG often results in wide QRS complexes, which can indicate a poor prognosis. Additionally, even in the absence of a widened QRS complex, fragmentation in the QRS complex may indicate myocardial scarring, which can lead to heterogeneous ventricular activation and dyssynchronous contraction [2]. Of note, patients with HF, regardless of the origin (e.g., ischemic), display more myocardial fibrosis than healthy individuals, and this fibrosis serves as a substrate for negative remodeling and arrhythmias [3]. Therefore, the presence of fragmented QRS (fQRS), irrespective of duration, can be a valuable indicator of myocardial scarring and a potential predictor of adverse outcomes in these patients.

Some studies have recently demonstrated that fQRS is linked to myocardial fibrosis and poor prognosis in HF patients [4, 5]. The prognostic value of fQRS is not limited to HF and is also observed in other conditions, including coronary artery disease and cardiac sarcoidosis [6, 7]. However, some previous research has reported contradictory results regarding the association between fQRS and adverse cardiovascular outcomes in HF patients [8, 9]. Therefore, the objective of this study is to explore the potential connection between the presence of fQRS and the incidence of mortality and hospital readmissions among HF patients.

METHODS

Study Population and Design

This study enrolled 238 HF patients with reduced left ventricle ejection fraction (LVEF) (≤ %35) between January 2015 and October 2017. Patients were under treatment in accordance with the recommendations of current HF treatment guidelines [10]. All patients were older than 18 years and provided written informed consent. Patients were considered to be ischemic in origin if they had significant coronary artery disease, which was confirmed by coronary angiography and defined as greater than 50% stenosis in the left main stem or greater than 70% stenosis in a major coronary vessel. The New York Heart Association (NYHA) functional classification was used to describe patients' symptoms and exercise capacity. The clinical information and laboratory findings were collected from patient files retrospectively.

The study was in accordance with the Declaration of Helsinki and was approved by the institutional review board. The main exclusion criteria were: acute coronary syndrome experienced within last 3-months, acute myocarditis, clinically significant heart valvular disease, hemodynamic instability, heart electrical activity disorders (e.g., Brugada syndrome), wide QRS (> 120 ms, due to bundle branch block, pacemaker rhythm, etc.), and limited life expectancy. Patient enrollment process is depicted in Fig 1.

Echocardiography Acquisition and Analysis

Transthoracic echocardiography (TTE) was performed using a Philips iE33 echocardiography machine and X5 transducer in the left lateral decubitus position. The evaluation included M-mode, 2-dimensional, and Doppler studies, following the recommendations of the current echocardiography guidelines [11]. The left ventricular ejection fraction was quantified using modified Simpson's method [12] from apical 4-chamber views by manually tracing the end-diastolic and end-systolic endocardial borders.

Electrocardiogram Analysis

All patients underwent a 12-lead electrocardiogram (ECG) during clinical visits using a filter range of 0.5 Hz-150 Hz, an AC filter of 60 Hz, and a speed of 25 mm/s and amplitude of 10 mm/mV. Two independent, experienced cardiologists who were unaware of the study design and patients' clinical data analyzed the ECGs. The presence of fQRS was determined based on the criteria of RSR' patterns with or without Q waves in two continuous derivations (QRS time < 120 ms), the presence of an additional R wave (R' wave) or notching within the S wave, and the presence of more than 1 R waves without typical bundle branch block.

Statistical Analysis

The normality of the data was analyzed using the Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation for normally distributed data and median and interquartile range for non-normally distributed data. Comparisons between

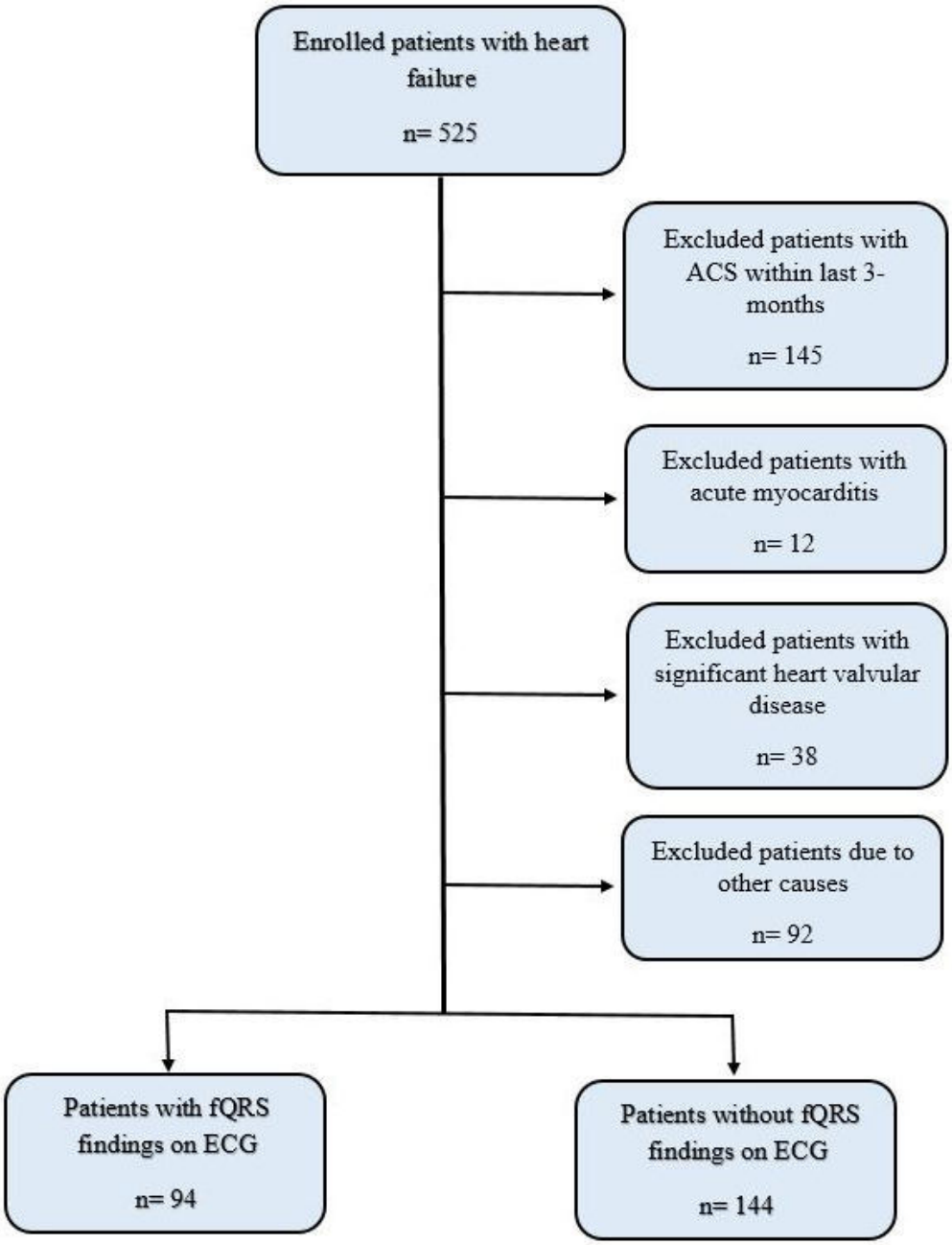

Fig. 1. Enrollment flow diagram. FQRS = fragmented QRS, ACS = acute coronary syndrome, ECG = electrocardiogram.

two groups were made with the independent samples T-test for normally distributed data and with Mann-Whitney U test for non-normally distributed data. Categorical variables were expressed as frequency and percentage. Logistic regression models were used to assess associations with all-cause mortality and rehospitalization. Multivariable regression analyses using stepwise elimination were employed. Only clinically meaningful candidate predictors were considered in the multivariable analysis. The results of multivariable regression analyses were presented as odds ratio (OR) with 95% confidence interval (CI). The two-sided significance level was set at 5%. Statistical analyses were performed using SPSS Statistics, version 25.0 (IBM Corp, Armonk, New York).

RESULTS

Study Population

Out of the 238 compensated HF patients who participated in the study, 94 patients were found to have the fQRS pattern. Table 1 summarizes the baseline demographic and clinical characteristics according to fQRS presence. Patients with and without fQRS had a similar proportion of male patients (28 (30%) vs 41 (28%), $p = 0.827$). The mean age was 64.9 ± 9.8 years in patients with fQRS and 64.4 ± 11.2 years in patients without fQRS.

No statistically significant differences were observed between the two groups in terms of smoking

Table 1. Baseline characteristics of the study population according to fQRS presence

	Patients with fQRS (n = 94)	Patients without fQRS (n = 144)	*p* value
Demographics on admission			
Age (years)	64.9 ± 9.8	64.4 ± 11.2	0.715
Male, n (%)	28 (30)	41 (28)	0.827
BMI (kg/m^2)	28.2 ± 5.3	27.5 ± 6.1	0.320
Initial physical examination			
Systolic BP (mmHg)	122.0 ± 22.6	117.8 ± 21.1	0.149
NYHA classification	3.0 ± 0.7	3.1 ± 0.7	0.490
Medical history, n (%)			
Hypertension	61 (65)	87 (60)	0.486
Diabetes mellitus	37 (39)	63 (43)	0.503
Chronic obstructive pulmonary disease	8 (8)	21 (14)	0.161
Smoking	44 (46)	76 (52)	0.368
Ischemic heart disease	72 (76)	90 (62)	0.023
Atrial fibrillation	27 (29)	25 (17)	0.038
Laboratory characteristics			
Haemoglobin (g/dL)	12.1 ± 2.0	11.6 ± 2.0	0.082
Creatinine (mg/dL)	1.20 (1.00 - 1.50)	1.29 (0.91 - 1.58)	0.785
Sodium (mEq/L)	135.6 ± 6.4	136.2 ± 5.8	0.407
Potassium (mEq/L)	4.4 ± 0.8	4.4 ± 0.7	0.819
Glucose (mg/dL)	142.5 ± 69.5	135.4± 67.9	0.435
CRP (mg/dL)	12 (5 - 55)	13 (4 - 25)	0.191
Albumine (g/dL)	3.5 ± 0.6	3.6 ± 0.6	0.116
Medication, n (%)			
Beta-blocker	69 (73)	111 (77)	0.518
ACE-I / ARB	81 (86)	106 (73)	0.021
Spironolactone	28 (29)	62 (43)	0.039
Digoxin	21 (22)	43 (30)	0.201
Furosemid	28(30)	28(19)	0.066

Data are shown as mean ± standard deviation or n (%) or mean (range). FQRS = fragmented QRS, BMI = body mass index, NYHA = New York Heart Association, CRP = C-reactive protein, ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin-receptor blocker

Table 2. Echocardiography characteristics of the study population according to fQRS presence

	Patients with fQRS (n = 94)	Patients without fQRS (n = 144)	*p* value
Ejection fraction (%)	25.5 ± 7.7	27.6 ± 6.9	0.029
Left atrial diameter (mm)	46.2 ± 6.4	47.7 ± 8.4	0.162
Mitral regurgitation level	2.0 ± 0.9	2.2 ± 0.9	0.077
LV mass index (g/m2)	151.3 ± 45.7	147.0 ± 34.7	0.410
PASP (mmHg)	49.3 ± 14.6	49.8 ± 15.4	0.819

Data are shown as mean ± standard deviation. LVEF = left ventricular, PASP = pulmonary artery systolic pressure

status, hypertension, diabetes mellitus, and chronic obstructive pulmonary disease frequency. However, patients with fQRS exhibited a higher frequency of ischemic heart disease and atrial fibrillation compared to those without fQRS (72 (76%) vs 90 (62%), p = 0.023; 27 (29%) vs 25 (17%), p = 0.038, respectively). The laboratory levels, including creatinine, hemoglobin, sodium, potassium, glucose, CRP, and albumin, did not differ significantly between the two groups. In terms of treatment for HF, no significant differences were found in the frequency of Beta-blocker, Digoxin, and Furosemide use between the two groups. However, there was a statistically significant difference in the frequency of angiotensin-converting enzyme inhibitor and angiotensin-receptor blocker (ACE-I/ARB) and Spironolactone use between the groups (81 (86%) vs 106 (73%), p = 0.021; 28 (29%) vs 62 (43%); p = 0.039, respectively).

Echocardiography Findings

Table 2 provides a detailed assessment of the echocardiography characteristics according to the groups. The group of patients with fQRS had significantly lower LVEF levels compared to the group without fQRS (25.5 ± 7.7 vs 27.6 ± 6.9, p = 0.029). There were no significant differences observed between the two groups in relation to other echocardiographic parameters, such as left atrial diameter, mitral regurgitation level, LV mass index, and PASP levels.

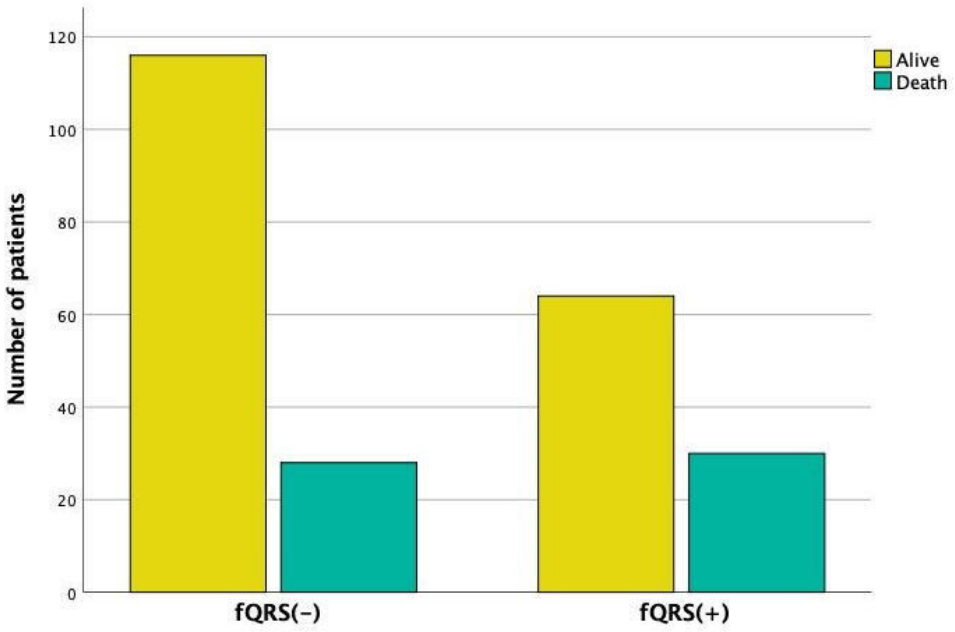

Fig. 2. Demonstration of alive/death rates for patients with and without fQRS pattern. fQRS = fragmented QRS.

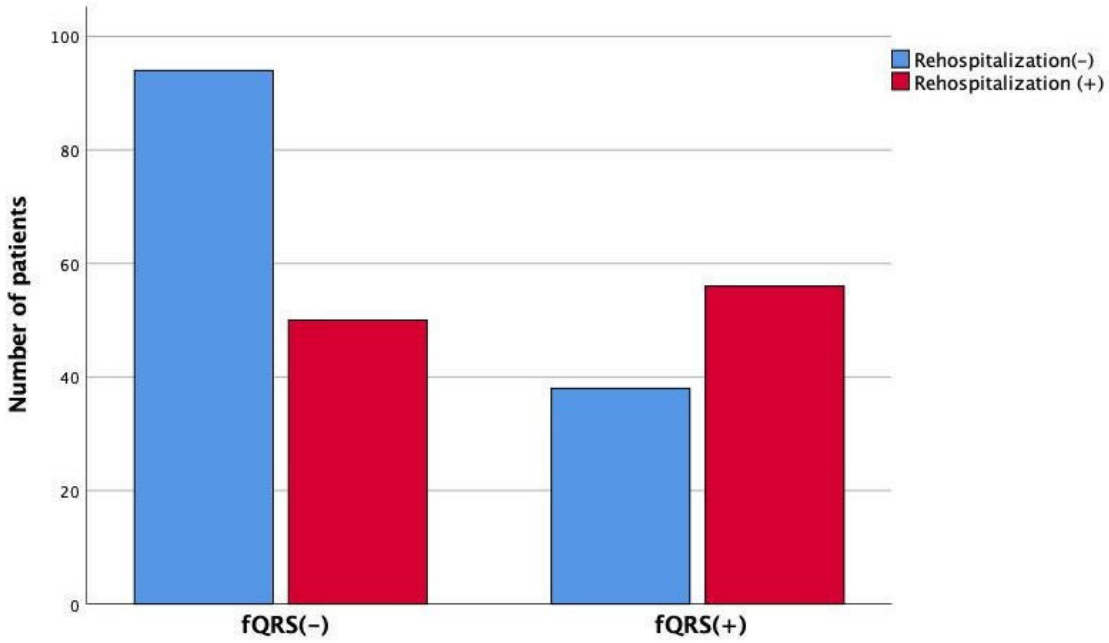

Fig. 3. Demonstration of rehospitalization rates for patients with and without fQRS pattern. fQRS = fragmented QRS.

Association with Mortality and Rehospitalization

During the 5-year follow-up period, patients with fQRS had a higher rate of mortality and rehospitalization compared to patients without fQRS (56 (60%) / 50 (35%), $p < 0.001$; 30 (32%) / 28 (19%), $p = 0.028$, respectively) (Figs. 2 and 3).

The study utilized logistic regression analysis models to evaluate the relationship between various parameters and the prognostic outcome. Table 3 presents the findings of the regression analysis models for

Table 3. Multivariable logistic regression analysis for association with mortality and rehospitalization

	Mortality			Rehospitalization		
Variables	**OR**	**95% CI**	***p* value**	**OR**	**95% CI**	***p* value**
Age (years)	1.022	0.987-1.058	0.214	1.033	1.000-1.068	0.051
Male	0.818	0.383-1.748	0.604	1.777	0.824-3.821	0.142
NYHA	3.357	2.058-5.557	**< 0.001**	5.532	3.198-9.569	**< 0.001**
LVEF	0.252	0.107-4.411	0.600	0.420	0.302-0.756	**0.048**
IHD	1.703	0.816-3.552	0.156	1.301	0.602-2.812	0.504
Hypertension	1.609	0.813-3.183	0.172	1.418	0.699-2.875	0.333
Diabetes mellitus	0.713	0.349-1.457	0.354	0.879	0.425-1.820	0.729
Sodium level	0.912	0.866-0.959	**< 0.001**	0.926	0.877-0.979	**0.006**
fQRS presence	2.192	1.130-4.248	0.020	4.128	2.028-8.388	**< 0.001**
Creatinine level	0.955	0.679-1.343	0.791	1.118	0.772-1.619	0.554
ACEİ/ARB use	1.790	0.771-4.156	0.175	1.428	0.614-3.323	0.408
Beta-blocker use	0.522	0.218-1.247	0.143	1.673	0.712-3.932	0.237

NYHA = New York Heart Association, LVEF = left ventricular ejection fraction, IHD = Ischemic heart disease, fQRS = fragmented QRS, ACE-I = angiotensin-converting enzyme inhibitor, ARB, angiotensin-receptor blocker

the association with mortality and rehospitalization. The multivariable models included the parameters of age, gender, NYHA classification, LVEF, ischemic heart disease, hypertension, diabetes mellitus, fQRS presence, sodium level, creatinine level, ACEI/ARB, and beta-blocker use. For mortality, NYHA classification, sodium level, and fQRS presence were identified as statistically significant independent predictors (NHYA classification: OR: 3.357, $p < 0.001$; sodium level: OR: 0.912, $p < 0.001$; fQRS presence: OR:2.192, $p = 0.020$). For rehospitalization, NYHA classification, LVEF, sodium level, and fQRS presence were found to be statistically significant independent predictors (NHYA classification: OR: 5.532, $p < 0.001$; LVEF: OR: 0.420, $p = 0.048$; sodium level: OR: 0.926, $p = 0.006$; fQRS presence: OR: 4.128, $p < 0.001$).

DISCUSSION

The objective of our study was to investigate how the presence of fragmented QRS (fQRS) relates to the prognosis of heart failure (HF) patients. Our study identified three key findings: firstly, patients with fQRS had a higher prevalence of ischemic heart disease and atrial fibrillation compared to those without fQRS. Secondly, over a 5-year follow-up period, patients with fQRS had a greater risk of mortality and rehospitalization. Lastly, our logistic regression analysis revealed that the presence of fQRS is independently linked to both mortality and rehospitalization in HF patients.

HF is a complex syndrome that causes shortness of breath and exercise intolerance due to impaired blood filling or ejection from the heart. It results in high rates of mortality and hospitalization, with a 1-year mortality rate of 7.2% and a 1-year hospitalization rate of 31.9% in patients with chronic HF [13]. While there are pharmacological and device-based treatments available that have demonstrated benefits for patients with reduced ejection fraction, the effectiveness of these therapies is not guaranteed and can be costly. In fact, studies have shown that only a minority of patients who receive device treatment actually receive appropriate therapy at 5 years [14]. As a result, it's critical to identify patients who are at higher risk for poor outcomes in order to improve their chances of survival and minimize the need for hospitalization. Using ECG parameters for risk stratification of HF patients may be a practical and cost-effective approach, as ECG is widely available in most healthcare settings.

The results of our study showed that HF patients who presented with fQRS had a higher prevalence of comorbidities, including coronary artery disease and atrial fibrillation. Furthermore, these patients experienced poorer clinical outcomes in terms of mortality and rehospitalization when compared to those who did not exhibit without fQRS, over a 5-year follow-up period. The presence of myocardial fibrosis is a crucial factor in the development of ventricular arrhythmias among patients with left ventricular dysfunction. Studies using cardiac magnetic resonance imaging have demonstrated that myocardial fibrosis acts as an arrhythmogenic substrate, particularly among patients with ischemic heart disease caused by a previous infarction [15]. Studies have also demonstrated that the presence of a fQRS on a 12-lead ECG is a result of myocardial conduction abnormalities due to scar tissue in patients with CAD [16]. Moreover, fQRS was found to be comparable to the epsilon wave observed in arrhythmogenic right ventricular cardiomyopathy [17]. The incidence of fQRS was found to be notably greater in patients with Brugada syndrome who experienced ventricular fibrillation (VF) compared to those who had syncope or were asymptomatic [18]. We speculate that the presence of fQRS indicates the existence of scars in the myocardium, which in turn create an environment that is contribute to the occurrence of reentry and potentially life-threatening ventricular arrhythmias, leading to poor outcomes in HF patients. Moreover, the higher incidence of atrial fibrillation in patients with fQRS may be explained by the fact that fibrosis can affect the function of the ventricles, resulting in increased left ventricular end-diastolic pressure that may also affect the left atrium, thereby increasing the risk of arrhythmia.

Recently, fQRS has been shown to predict cardiac events in various populations [19, 20], but there have also been reports of contradictory findings from some researchers [8, 9]. These discrepancies may be attributed to differences in study design, such as the inclusion of patients only in sinus rhythm or the enrollment

of predominantly male participants. In our study, we included patients with atrial fibrillation and maintained a balance between male and female participants. Further research is required to investigate the effectiveness of fQRS in risk stratification for HF.

Identifying fQRS on an ECG is a simple task for clinicians, but it can have significant implications for risk stratification and management of HF patients. The detection of fQRS can lead to more focused monitoring and follow-up care. Moreover, a greater burden of fQRS may indicate a higher risk of arrhythmias, which can be used to identify patients who may benefit from device therapy for HF. Future research could examine the potential of fQRS in other patient populations to prevent arrhythmic events.

Limitations

This study has some limitations that need to be discussed. First, the results were derived through a post-hoc analysis. Second, the sample size of the study (n = 238) is relatively small. Third, the study was conducted at a single center, which may limit the generalizability of the results to other populations. Last, the number of leads with fQRS on ECG was not examined.

CONCLUSION

In patients with heart failure, the presence of fragmented QRS on ECG was associated with a higher risk for all-cause mortality and rehospitalization.

Authors' Contribution

Study Conception: AD, AA; Study Design: AD; Supervision: AD, HAB; Funding: N/A; Materials: N/A; Data Collection and/or Processing: HAB, AA; Statistical Analysis and/or Data Interpretation: AD, AA; Literature Review: MY, ÖŞ; Manuscript Preparation: AD, AA and Critical Review: AD, AA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Murphy SP, Ibrahim NE, Januzzi JL, Jr. Heart failure with reduced ejection fraction: a review. JAMA 2020;324:488-504.
2. Xie J, Liu Y, Wei X, Ye W, Ma Z, Lu G, et al. Relationship between fragmented QRS complex and left ventricular fibrosis and function in patients with Danon disease. Front Cardiovasc Med 2022;9:790917.
3. Liu T, Song D, Dong J, Zhu P, Liu J, Liu W, et al. Current understanding of the pathophysiology of myocardial fibrosis and its quantitative assessment in heart failure. Front Physiol 2017;8:238
4. Lorgis L, Cochet A, Chevallier O, Angue M, Gudjoncik A, Lalande A, et al. Relationship between fragmented QRS and no-reflow, infarct size, and peri-infarct zone assessed using cardiac magnetic resonance in patients with myocardial infarction. Can J Cardiol 2014;30:204-10.
5. Torigoe K, Tamura A, Kawano Y, Shinozaki K, Kotoku M, Kadota J. The number of leads with fragmented QRS is independently associated with cardiac death or hospitalization for heart failure in patients with prior myocardial infarction. J Cardiol 2012;59:36-41.
6. Gong B, Li Z. Total mortality, major adverse cardiac events, and echocardiographic-derived cardiac parameters with fragmented QRS complex. Ann Noninvasive Electrocardiol 2016;21:404-12.
7. Ogura S, Nakamura K, Morita H, Nakagawa K, Nishii N, Akagi S, et al. Fragmented QRS as a predictor of cardiac events in patients with cardiac sarcoidosis. J Cardiol 2022;79:446-52.
8. Cheema A, Khalid A, Wimmer A, Bartone C, Chow T, Spertus JA, et al. Fragmented QRS and mortality risk in patients with left ventricular dysfunction. Circ Arrhythm Electrophysiol 2010;3:339-44.
9. Das MK, Maskoun W, Shen C, Michael MA, Suradi H, Desai M, et al. Fragmented QRS on twelve-lead electrocardiogram predicts arrhythmic events in patients with ischemic and nonischemic cardiomyopathy. Heart Rhythm 2010;7:74-80.
10. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2021;42:3599-726.
11. Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, et al. Guidelines for Performing a Comprehensive Transthoracic Echocardiographic Examination in Adults: Recommendations from the American Society of Echocardiography. J Am Soc Echocardiogr 2019;32:1-64.
12. Schiller NB, Acquatella H, Ports TA, Drew D, Goerke J, Ringertz H, et al. Left ventricular volume from paired biplane two-dimensional echocardiography. Circulation 1979;60:547-55.
13. Maggioni AP, Dahlström U, Filippatos G, Chioncel O, Leiro MC, Drozdz J, et al. EURObservational Research Programme: regional differences and 1-year follow-up results of the Heart Failure Pilot Survey (ESC-HF Pilot). Eur J Heart Fail 2013;15:808-17.
14. Poole JE, Johnson GW, Hellkamp AS, Anderson J, Callans DJ, Raitt MH, et al. Prognostic importance of defibrillator shocks in patients with heart failure. N Engl J Med 2008;359:1009-17.

15. Bello D, Fieno DS, Kim RJ, Pereles FS, Passman R, Song G, et al. Infarct morphology identifies patients with substrate for sustained ventricular tachycardia. J Am Coll Cardiol 2005;45:1104-8.
16. Mahenthiran J, Khan BR, Sawada SG, Das MK. Fragmented QRS complexes not typical of a bundle branch block: a marker of greater myocardial perfusion tomography abnormalities in coronary artery disease. J Nucl Cardiol 2007;14:347-53.
17. Peters S, Trümmel M, Koehler B. QRS fragmentation in standard ECG as a diagnostic marker of arrhythmogenic right ventricular dysplasia–cardiomyopathy. Heart Rhythm 2008;5:1417-21.
18. Morita H, Kusano KF, Miura D, Nagase S, Nakamura K, Morita AT, et al. Fragmented QRS as a marker of conduction abnormality and a predictor of prognosis of Brugada syndrome. Circulation 2008;118:1697-704.
19. Kanitsoraphan C, Rattanawong P, Mekraksakit P, Chongsathidkiet P, Riangwiwat T, Kanjanahattakij N, et al. Baseline fragmented QRS is associated with increased all-cause mortality in heart failure with reduced ejection fraction: A systematic review and meta-analysis. Ann Noninvasive Electrocardiol 2019;24:e12597.
20. Korhonen P, Husa T, Konttila T, Tierala U, Makijarvi M, Vaananen H, et al. Fragmented QRS in prediction of cardiac deaths and heart failure hospitalizations after myocardial infarction. Ann Noninvasive Electrocardiol 2010;15:130-7.

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Pulmonary vessel volume can help to differentiate fibrotic lung diseases

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ABSTRACT

Objectives: Idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP), and chronic hypersensitivity pneumonitis (CHP) are diffuse fibrosing lung diseases that are sometimes difficult to differentiate by only visual evaluation of CT images. We aimed to find if pulmonary vessel volume (PVV), a new quantitative CT measure, can help to differentiate these diseases at the time of diagnosis.

Methods: We retrospectively measured PVV values of IPF, NSIP, and CHP patients diagnosed within the last five years in our institution, by using their CT images at the time of diagnosis. We used CALIPER-technology (Computer-Aided Lung Informatics for Pathology Evaluation and Rating) for the quantification of CT images. We compared the PVV values of disease groups by the Kruskal-Wallis test and performed ROC curve analysis to evaluate the ability of PVV to differentiate these diseases.

Results: We measured the PVV values of 152 patients, 113 of them were diagnosed with IPF, 16 with NSIP, and 23 with CHP. The PVV value of the NSIP group was significantly lower than that of both IPF ($p = 0.028$) and CHP ($p = 0.013$) groups. However, there was no significant difference between IPF and CHP groups ($p = 0.924$). Selected cut-off values of PVV were found to differentiate NSIP from IPF with a specificity of 88%, and NSIP from CHP with a specificity of 91%.

Conclusions: PVV measured by CALIPER at the time of diagnosis can help to differentiate NSIP from both IPF and CHP.

Keywords: Hypersensitivity pneumonitis, idiopathic pulmonary fibrosis, nonspecific interstitial pneumonia, pulmonary vessel volume, quantitative computed tomography

Idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP), and chronic hypersensitivity pneumonitis (CHP) are diffuse fibrosing lung diseases that may show some overlapping features on computed tomography (CT) images which may sometimes make it very difficult to differentiate them [1]. Visual evaluation of CT features is prone to subjectivity and sometimes a considerable inter-observer variation is seen between the opinions of even expert thoracic radiologists [2,3]. Automated computer-based quantification of parenchymal CT findings of these diseases may help to differentiate them [4].

Pulmonary vessel volume (PVV), a novel quantitative computed tomographic (QCT) parameter that is measured by using volumetric CT data, is the total CT volume of intraparenchymal arteries and veins, including their walls. It excludes extraparenchymal (hilar and mediastinal) portions of the vessels. PVV is a

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purely computer based parameter that cannot be evaluated by the human eye. CT scans obtained without intravenous contrast administration are used and intraparenchymal vessels, that are visible and tractable as connected tubular structures on CT slices, are automatically detected by a computer and then the total volume of these tubular structures is calculated [3]. Instead of the "PVV" term, it is sometimes alternatively mentioned as "volume of pulmonary vessel-related (or vascular-related) structures".

PVV was reported to be a good measure of disease severity and a strong predictor of mortality in some interstitial lung diseases, in addition to its significant correlations with pulmonary function indices [4-13]. It was shown that increased PVV is strongly linked to the extent of IPF and PVV value is a very good independent CT-derived parameter that predicts pulmonary function tests, and also it is a very powerful predictor of mortality [5, 10]. In systemic sclerosis patients, PVV was reported to increase progressively in follow-up chest CT scans [7]. It was found that PVV is an independent predictor of mortality in patients with connective tissue disease-related interstitial lung disease [8]. In CHP patients, a higher PVV value was reported to indicate a more aggressive disease and an IPF-like outcome [9].

It was also reported that PVV values can be used to differentiate some of the major forms of interstitial lung diseases such as IPF, interstitial pneumonia with autoimmune features, connective tissue disease-related interstitial lung disease, and CHP [4].

In this study, we aimed to find if we can use PVV value to differentiate IPF, NSIP, and CHP at the time of diagnosis.

METHODS

In this retrospective study, we investigated all consecutive IPF, NSIP, and CHP patients diagnosed by our Institutional Council of Interstitial Lung Diseases from 2017 to 2021. We extracted a total of 242 patients from the hospital records with final diagnoses of 169 IPF, 48 CHP, and 25 NSIP. Those patients diagnosed without referral to the Council were not included. This multidisciplinary council consisted of a radiologist, a pathologist, a thoracic surgeon, and at least three respiratory clinicians, reviewed the clinical and chest CT findings of all patients that were referred to the council because of either a diagnostic difficulty and/or a request of biopsy-decision of the council that was mandatory in our institution for surgical lung biopsies of suspected interstitial lung disease patients. For all three diseases, the council established a final diagnosis after a multidisciplinary evaluation of all clinical and CT findings of patients, and pathological findings if existed. The American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association (ATS/ERS/JRS/ALAT) guideline criteria were used by the council to establish IPF diagnoses.

To get rid of any diagnostic uncertainties, we excluded those patients without a surgical lung biopsy. Therefore, 17 patients were excluded from the CHP and 9 from the NSIP group. But, since the ATS/ERS/JRS/ALAT Guideline, used by the Council, stated that, in IPF diagnosis, "surgical lung biopsy is not required for patients with a CT pattern consistent with usual interstitial pneumonia (UIP)", non-biopsied patients were not excluded from the IPF group.

For quantification of the lung parenchyma, we included only those CT scans performed in our institution within a period of three months before the diagnosis, obtained without intravenous contrast administration and by volumetric technique with a slice-thickness of 1 mm. Since they do not comply with the CT criteria mentioned above, 49 patients were excluded from the IPF, and 5 from the CHP group.

All CT scans were performed by using the same acquisition parameters (by using Philips Ingenuity 128 slice CT scanner, with a tube voltage of 120 kV, a pitch of 1, a rotation time of 0.4 seconds, and a reconstruction thickness of 1 mm).

From the axial image series of patients, only those with a relatively "soft" reconstruction filter (kernel B) were used for quantification. CT images with this kernel are recommended to be used in quantification because this kind of so-called "soft" or "neutral" kernels have relatively low signal-noise ratios, provide the most accurate CT attenuation values, and hence they are better than other kernels for quantification purposes [14, 15].

PVV measurements were performed by using a software called Lung Texture Analysis (Imbio, Minneapolis, Minnesota, USA) (this is an 'investigational use only' software in the USA). This software is based

on the CALIPER-technology (Computer-Aided Lung Informatics for Pathology Evaluation and Rating) developed by the Mayo Clinic. For quantification purposes, this software extracted (segmented) only the parenchymal areas of both lungs automatically. Occasionally this segmentation process has been terminated by the software with a "segmentation fault" message, mainly as a result of motion artifacts or gastric/colonic air content just beneath the diaphragm that confuses the computer to segment the air as the lung parenchyma. The software reported segmentation fault for 6 of the patients in the IPF and 2 of the patients in the CHP group and these patients were excluded from the study.

For each patient, following the CALIPER's segmentation sessions, all segmented axial images were reviewed by a 20-year experienced chest radiologist to ensure that the entire lung parenchyma was segmented correctly. In two of the patients (one in the IFP and another one in the CHP group), the radiologist detected that only one of the two lungs was segmented by the software, and these two patients were excluded from the study.

After segmenting the lungs, the software then segmented the intrapulmonary vessels by using a mathematical method of an optimized multi-scale tubular structure enhancement filter that determines the likelihood of a voxel belonging to a vessel as a dense tubular structure [3]. Then the absolute intrapulmonary vessel volume is measured by the computer. To make more reliable comparisons between patients with different body size parameters (such as height, weight, and body surface area), we divided this absolute vessel volume by the total lung volume (CALIPER-derived) and obtained a "normalized" PVV value as a percentage of the total lung volume.

This study was approved by our Institutional Review Board and written informed consent was waived because of its retrospective nature.

Statistical Analysis

SPSS Statistics software (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY) was used to perform a non-parametric Kruskal-Wallis test to make PVV and age comparisons between disease groups. This non-parametric test was preferred because of the small numbers of patients in two of the three disease groups (CHP and NSIP), and the lack of normal distribution of PVV values in the CHP group and age values in IPF and NSIP groups. The normality of distributions were evaluated by using histograms and Q-Q plots. All significance values have been adjusted by the Bonferroni correction and a p-value less than 0.05 was considered to be statistically significant. Fisher's exact test was used to compare the gender proportions of the groups. The diagnostic performance of PVV in distinguishing IPF, NSIP, and CHP was assessed by using Receiver Operating Characteristic (ROC) curve analysis and optimal cut-off points were determined by using the Youden index.

RESULTS

The PVV measurements were performed on a total number of 152 patients in this study; 113 of them were diagnosed with IPF, 23 with CHP, and 16 with NSIP.

The demographic data of the patients are summarized in Table 1. The median age of the IPF group was higher than that of both CHP ($p = 0.005$) and NSIP groups ($p = 0.002$) and there was no significant differ-

Table 1. Demographic data of the studied patients

Disease	Number	Median age (IQR)	Male, n (%)	Female, n (%)
IPF	113	61 (57-69)	92 (81.4)	21 (18.6)
CHP	23	52 (32-62)	10 (43.5)	13 (56.5)
NSIP	16	51 (34-62)	11 (68.8)	5 (31.2)
Total	152	61 (51-67)	113 (74.3)	39 (25.7)

IPF = idiopathic pulmonary fibrosis, CHP = chronic hypersensitivity pneumonitis, NSIP = non-specific interstitial pneumonia, IQR = interquartile range

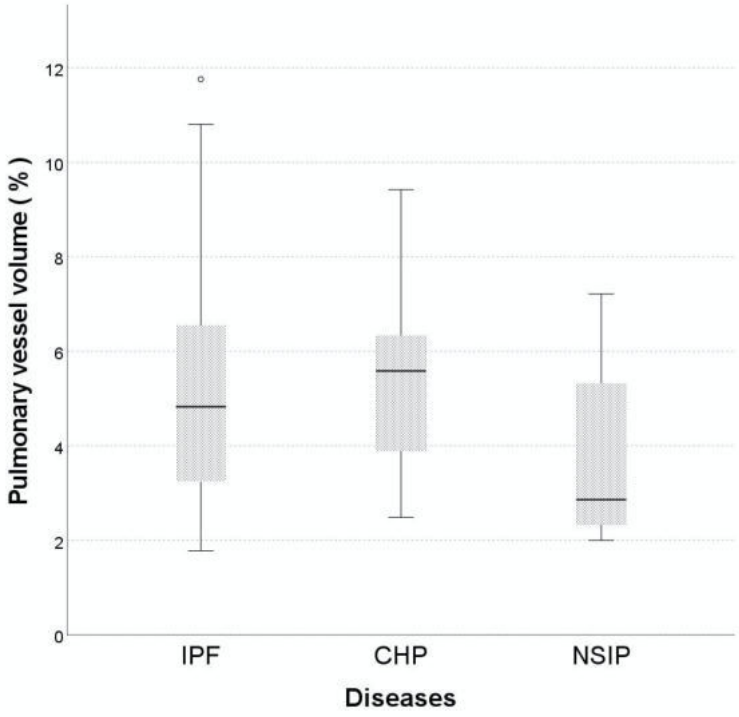

Fig. 1. The box-and-whisker chart showing the PVV distributions in the IPF, CHP, and NSIP patient groups with the boxes representing the interquartile ranges, the horizontal lines in the boxes representing the median PVV values, and the whiskers representing the minimum-maximum ranges. PVV = pulmonary vessel volume, IPF = idiopathic pulmonary fibrosis, CHP = chronic hypersensitivity pneumonitis, NSIP = non-specific interstitial pneumonia.

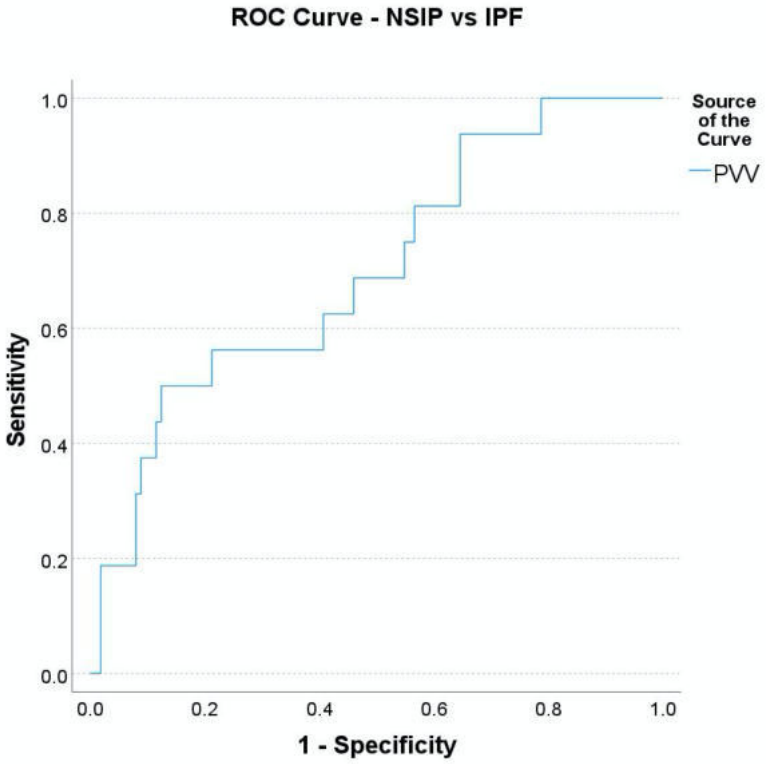

Fig. 2. ROC curve showing the ability of PVV to differentiate NSIP from IPF. AUC is 0.70 (95% confidence interval: lower bound 0.56- upper bound 0.84). PVV = pulmonary vessel volume, AUC = area under the curve, ROC = receiver operating characteristic, NSIP = non-specific interstitial pneumonia, IPF = idiopathic pulmonary fibrosis.

ence between the ages of the CHP and NSIP groups (p = 1). Regarding the gender distribution, there was no significant difference between the NSIP group and the other two groups. But the gender proportions were significantly different in the IPF and CHP groups (p < 0.001) with male dominance in the IPF group.

The PVV values of the three disease groups, expressed as median (1st quartile - 3rd quartile), were as follows: 4.83% (3.24-6.55) in the IPF, 5.58% (3.81-6.41) in the CHP, and 2.86% (2.32-5.38) in the NSIP group (Fig. 1). There was no significant difference between the PVV values of the IPF and CHP groups (p = 0.924). However, the mean PVV value of the NSIP group was significantly lower than that of both IPF (p = 0.028) and CHP (p = 0.013) groups.

To evaluate the ability of PVV to distinguish NSIP from IPF and CHP we obtained Receiver Operating Characteristic (ROC) curves. The area under curve (AUC) value for the ROC curve showing the ability of PVV to differentiate NSIP from IPF (Fig. 2) was 0.70, and NSIP from CHP (Fig. 3) was 0.78. We determined optimal cut-off points by using the maximum Youden index. A PVV cut-off value of 2.7% was found to differentiate NSIP from IPF with a specificity of 88%, with a sensitivity of 50%. A PVV cut-off value of 3.1% was found to differentiate NSIP from CHP with a specificity of 91%, with a sensitivity of 56%.

DISCUSSION

IPF is the most common and lethal form of idiopathic interstitial pneumonia. CHP and NSIP are two of the most common, and also usually the most difficult to differentiate, mimics of IPF [16]. QCT measurements can detect quantitative differences between some diffuse parenchymal lung diseases and hence may help to differentiate them [4, 17]. To our knowledge, this is the first study comparing PVV values of IPF, NSIP, and CHP patient groups in a single study. We found that the PVV is significantly higher in the IPF and CHP patients compared to the NSIP. But there was no significant difference between the IPF and CHP groups regarding the PVV values.

PVV is a relatively new QCT entity. PVV term is used to denote the total volume of intraparenchymal arteries and veins of lungs, including their walls and

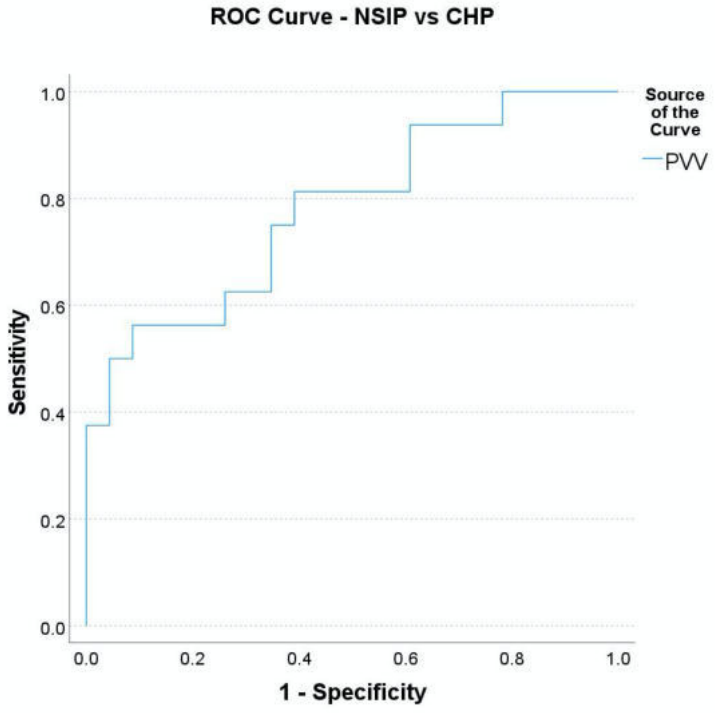

Fig. 3. ROC curve that is showing the ability of PVV to differentiate NSIP from CHP. AUC is 0.78 (95% confidence interval: lower bound 0.63- upper bound 0.93). PVV = pulmonary vessel volume, AUC = area under the curve, ROC = receiver operating characteristic, NSIP = non-specific interstitial pneumonia, CHP = chronic hypersensitivity pneumonitis

lumens, and it excludes the hilar and mediastinal portions of these vessels. Quantitative evaluation of PVV on CT images by radiologists' eyes without using quantifying software seems to be impossible. Hence, PVV is a "QCT-only" entity.

PVV value is usually expressed as a percentage of total pulmonary volume and therefore it is sometimes mentioned as "normalized pulmonary vessel volume".

In IPF patients PVV is correlated with the severity of fibrosis, it is a strong predictor of survival, its increase indicates poor prognosis and it shows a significant difference between treated and untreated patients with antifibrotics [6, 10, 11, 18, 19]. It was shown that the extent of lung fibrosis (fibrotic score) and PVV score were associated with the diffusing capacity of the lungs for carbon monoxide (DLCO) in IPF patients [20]. PVV is reported to be the most predictive of IPF progression and mortality which is independent of spirometric parameters [6, 11, 21]. PVV was also reported to predict mortality better than physiological indices and visual CT scores in patients with connective tissue disease-related interstitial lung disease [8]. Jacob *et al*. [22] demonstrated that CHP patients with a higher CALIPER-PVV had the more aggressive disease and worse prognosis. CHP patients with a PVV value above 6.5% were reported to have a similar clinical course and survival to IPF [9].

Jacob *et al*. [10] postulated that increased PVV in IPF patients can be related to the following reasons : i) Diversion of the blood flow from fibrotic areas to relatively spared lung regions, resulting in dilation of vessels and therefore an increased PVV, ii) Increased negative pressure during inspiration, due to increased lung stiffness in IPF patients, that result in the dilation effect on blood vessels, iii) Increased pleuro-parenchymal and bronchial-pulmonary arterial anastomosis that was previously described in histological lung specimens of IPF patients [23, 24].

In addition to the above explanations, Puxeddu *et al*. [19] stated one more explanation that vascular alterations might be the first pathological changes in the IPF lung on which fibrosis might build up later on, by an unclear mechanism.

Jee *et al*. [25] stated that strong correlation of PVV with the extent of interstitial lung disease but not with right ventricular systolic pressure was suggesting that PVV may reflect interstitial damage rather than pulmonary hypertension severity, and hence might provide an additional measure of disease severity not quantifiable on visual assessment.

Chung *et al*. [17] reported that vessel-related structures detected by CALIPER can differentiate pathological UIP cases from others in those patients with a non-IPF diagnosis CT category.

Crews *et al*. [4] compared CALIPER's PVV values between 58 IPF, 67 interstitial pneumonia with autoimmune features (IPAF), 42 connective tissue disease (CTD), and 58 CHP patients, and reported that pulmonary vessel-related structure volumes in IPF and IPAF were greater than those of CTD and CHP. In contrast to their results, a comparison of the PVV values between the IPF and CHP groups yielded no significant difference in our study. This incompatibility between our results and theirs may be just because these two studies compared different entities. They used the absolute PVV values measured by the CALIPER and did not normalize these values by the total lung volume, and as they pointed out in their manuscript since the absolute value could be affected by the body size, it was not quite appropriate for comparison of different patient groups. We divided the total intrapulmonary vessel volume by the total lung volume and obtained the PVV value as a percentage of the lung volume. In other words, we "normalized" our PVV value by the lung size, and hence, minimize the effect of the body size of the patient. Another difference between our study and theirs is that they included CT scans performed within one year of diagnosis, whereas we included only CT scans taken within three months of diagnosis, which can better reflect the PVV value at the time of diagnosis.

We found that a PVV cut-off value of 2.7% could differentiate NSIP from IPF with a specificity of 88%, and similarly a PVV cut-off value of 3.1% could differentiate NSIP from CHP with a specificity of 91%. Therefore, we think that, when it is difficult to differentiate NSIP from IPF or CHP by visual evaluation of parenchymal CT findings, PVV may help to differentiate them at the time of diagnosis. Crews *et al*. similarly reported that increased PVV seems to be associated with a diagnosis of some interstitial lung diseases [4].

Limitations

Our study has some limitations: i) There were relatively small numbers of patients in NSIP and CHP

groups. ii) Our institution is a tertiary referral center for lung diseases, and hence, our results may not generalize to the community setting. iii) Only those "difficult to diagnose" patients referred to our Institutional Council of Interstitial Lung Diseases were included in this study and hence our results may not be equally valid for more "typical" cases. iv) Not only a limitation of our study, but also a limitation of quantitative CT measurements is that, since quantitation algorithms as well as scanning and reconstruction parameters, such as slice thickness, reconstruction kernel, pixel size, and CT scanner used, can make radiomics features differ significantly [26], mean PVV values may differ in different institutions using different CT scanning parameters, and our institution's threshold PVV values may not be valid for others.

We think that further studies should be done with larger series to show the role of pulmonary vessel volume and other quantitative CT parameters in differential diagnosis of interstitial lung diseases. As Weatherley *et al*. [27] emphasized, machine learning or deep learning techniques may help to find some features that are not perceptible nor reproducibly assessed by humans.

CONCLUSION

In challenging cases, PVV measured by the CALIPER may help to differentiate NSIP from both IPF and CHP, in both of which PVV values at the time of diagnosis are greater than that in NSIP. More research is needed to obtain institution independent thresholds for PVV measurements in interstitial lung diseases.

Authors' Contribution

Study Conception: AG; Study Design: AG; Supervision: AG; Funding: N/A; Materials: AG; Data Collection and/or Processing: AG; Statistical Analysis and/or Data Interpretation: AG; Literature Review: AG; Manuscript Preparation: AG and Critical Review: AG.

Conflict *of* interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Horst C, Gholipour B, Nair A, Jacob J. Differential diagnoses of fibrosing lung diseases. BJR Open 2019;1:20190009.
2. Meyer KC. Diagnosis and management of interstitial lung disease. Transl Respir Med 2014;2:4.
3. Bartholmai BJ, Raghunath S, Karwoski RA, Moua T, Rajagopalan S, Maldonado F, et al. Quantitative CT imaging of interstitial lung diseases. J Thorac Imaging 2013;28:298-307.
4. Crews MS, Bartholmai BJ, Adegunsoye A, Oldham JM, Montner SM, Karwoski RA, et al. Automated CT analysis of major forms of interstitial lung disease. J Clin Med 2020;9:3776.
5. Jacob J, Bartholmai BJ, Rajagopalan S, Kokosi M, Nair A, Karwoski R, et al. Automated quantitative computed tomography versus visual computed tomography scoring in idiopathic pulmonary fibrosis: validation against pulmonary function. J Thorac Imaging 2016;31:304-11.
6. Jacob J, Pienn M, Payer C, Urschler M, Kokosi M, Devaraj A, et al. Quantitative CT-derived vessel metrics in idiopathic pulmonary fibrosis: a structure-function study. Respirology 2019;24:445-52.
7. Occhipinti M, Bruni C, Camiciottoli G, Bartolucci M, Bellando-Randone S, Bassetto A, et al. Quantitative analysis of pulmonary vasculature in systemic sclerosis at spirometry-gated chest CT. Ann Rheum Dis 2020;79:1210-7.
8. Jacob J, Bartholmai BJ, Rajagopalan S, Brun AL, Egashira R, Karwoski R, et al. Evaluation of computer-based computer tomography stratification against outcome models in connective tissue disease-related interstitial lung disease: a patient outcome study. BMC Med 2016;14:190.
9. Jacob J, Bartholmai BJ, Egashira R, Brun AL, Rajagopalan S, Karwoski R, et al. Chronic hypersensitivity pneumonitis: identification of key prognostic determinants using automated CT analysis. BMC Pulm Med 2017;17:81.
10. Jacob J, Bartholmai BJ, Rajagopalan S, Kokosi M, Nair A, Karwoski R, et al. Mortality prediction in idiopathic pulmonary fibrosis: evaluation of computer-based CT analysis with conventional severity measures. Eur Respir J 2017;49:1601011.

11. Jacob J, Pienn M, Payer C, Urschler M, Kokosi M, Devaraj A, et al. Normalized vessel volume from quantitative computed tomography predicts survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2019;199:A1040.
12. Sverzellati N, Silva M, Seletti V, Galeone C, Palmucci S, Piciucchi S, et al. Stratification of long-term outcome in stable idiopathic pulmonary fibrosis by combining longitudinal computed tomography and forced vital capacity. Eur Radiol 2020;30:2669-79.
13. Jacob J, Hirani N, van Moorsel CHM, Rajagopalan S, Murchison JT, van Es HW, et al. Predicting outcomes in rheumatoid arthritis related interstitial lung disease. Eur Respir J 2019;53:1800869.
14. Newell JD Jr, Sieren J, Hoffman EA. Development of quantitative computed tomography lung protocols. J Thorac Imaging 2013;28:266-71.
15. Wu X, Kim GH, Salisbury ML, Barber D, Bartholmai BJ, Brown KK, et al. Computed Tomographic Biomarkers in Idiopathic Pulmonary Fibrosis. The Future of Quantitative Analysis. Am J Respir Crit Care Med 2019;199:12-21.
16. Wuyts WA, Cavazza A, Rossi G, Bonella F, Sverzellati N, Spagnolo P. Differential diagnosis of usual interstitial pneumonia: when is it truly idiopathic? Eur Respir Rev 2014;23:308-19.
17. Chung JH, Adegunsoye A, Oldham JM, Vij R, Husain A, Montner SM, et al. Vessel-related structures predict UIP pathology in those with a non-IPF pattern on CT. Eur Radiol 2021;31:7295-302.
18. Romei C, Tavanti LM, Taliani A, De Liperi A, Karwoski R, Celi A, et al. Automated Computed Tomography analysis in the assessment of Idiopathic Pulmonary Fibrosis severity and progression. Eur J Radiol 2020;124:108852.
19. Puxeddu E, Cavalli F, Pezzuto G, Teodori E, Rogliani P. Impact of pulmonary vascular volume on mortality in IPF: is it time to reconsider the role of vasculature in disease pathogenesis and progression? Eur Respir J 2017;49:1602345.
20. Wu WJ, Huang WM, Liang CH, Yun CH. Pulmonary vascular volume is associated with DLCO and fibrotic score in idiopathic pulmonary fibrosis: an observational study. BMC Med Imaging 2022;22:76.
21. Jacob J, Bartholmai BJ, Rajagopalan S, van Moorsel CHM, van Es HW, van Beek FT, et al. Predicting outcomes in idiopathic pulmonary fibrosis using automated computed tomographic analysis. Am J Respir Crit Care Med 2018;198:767-76.
22. Jacob J, Bartholmai BJ, Brun AL, Egashira R, Rajagopalan S, Karwoski R, et al. Evaluation of visual and computer-based CT analysis for the identification of functional patterns of obstruction and restriction in hypersensitivity pneumonitis. Respirology 2017;22:1585-91.
23. Turner-Warwick M. Precapillary systemic-pulmonary anastomoses. Thorax 1963;18:225-37.
24. Miller WC, Heard JG, Unger KM, Suich DM. Anatomical lung shunting in pulmonary fibrosis. Thorax 1986;41:208-9.
25. Jee AS, Jo HE, Corte TJ. Hypersensitivity pneumonitis: A protean and challenging disease. Respirology 2017;22:1489-90.
26. Chen A, Karwoski RA, Gierada DS, Bartholmai BJ, Koo CW. Quantitative CT analysis of diffuse lung disease. Radiographics 2020;40:28-43.
27. Weatherley ND, Eaden JA, Stewart NJ, Bartholmai BJ, Swift AJ, Bianchi SM, et al. Experimental and quantitative imaging techniques in interstitial lung disease. Thorax 2019;74:611-9.

The European Research Journal 2023;9(2):445-453 Original Article

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Investigation of the relationship between prolonged ventilation and the Glasgow Prognostic Score after elective isolated coronary bypass surgeries in advanced-age patients

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ABSTRACT

Objectives: Coronary artery bypass graft (CABG) surgeries are the most beneficial treatment method for atherosclerotic heart disease. Prolonged ventilation is a significant condition that poses risks such as infection, renal failure, prolongs hospital stays, and increases treatment costs. In this current study, we aimed to reveal the relationship between prolonged mechanical ventilation and Glasgow Prognostic Score (GPS), in patients aged 65 years and older who underwent isolated CABG.

Methods: Patients aged 65 years and over who underwent elective isolated coronary bypass surgery with cardiopulmonary bypass in our clinic, between June 2017 and June 2022, were included in the study retrospectively. The patients who were extubated within eight hours of the surgeries were determined as Group 1, whereas those who were extubated after 8 hours were determined as Group 2.

Results: Prolonged ventilation occurred in 101 (20.7%) patients (Group 2). The median age of the 385 patients included in Group 1 and 101 patients in Group 2 was 68 (66 to 91) and 71 (66 to 88) years, respectively (p = 0.216). The two groups were similar in regards to gender, hypertension, smoking, diabetes mellitus, body mass index, history of cerebrovascular accident and left ventricular ejection fraction rates. In the multivariate analysis, left ventricular ejection fraction < 35% (OR: 1.136, 95% CI: 1.065-1.652, p = 0.029), total perfusion time (OR: 1.190, 95% CI: 1.040-1.659, p = 0.012), and GPS = 2 (OR: 1.479, 95% CI: 1.130-2.169, p = 0.004) were determined as independent predictors for prolonged ventilation.

Conclusions: GPS value calculated preoperatively in elderly patients is a parameter that indicates the increased ventilation need. With the GPS score evaluation, patient groups at risk for prolonged ventilation may be identified and necessary precautions may be undertaken.

Keywords: Glasgow Prognostic Score, coronary artery bypass graft, morbidity, postoperative term, prolonged ventilation

Coronary artery bypass graft (CABG) surgeries are the most beneficial treatment method for atherosclerotic heart disease and at present, they are carried out with high success rates [1]. However, despite the

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advances in surgical techniques, various undesirable difficulties may occur after these operations [2]. One of the most important of these is the inability to end ventilation at the scheduled time for patients operated on stably, under elective conditions.

Currently, most patients are removed from ventilation within the first 6 to 8 hours after these operations. Extubation failure is one of the most common complications in the first 24 hours in these patients. In general, not being able to wean from ventilation within the first 8 hours is considered prolonged ventilation, though some studies have established this threshold at 6 hours [3]. The need for ventilation for more than 24 hours is seen with a frequency of 5 to 10% after isolated CABG operations [4]. It is significant that poses risks such as infection, and renal failure, prolongs hospital stays, and increases treatment costs [4, 5]. The risk is increased in elderly patients and those with lung disease [5]. For this reason, in many cardiovascular centers, attention is paid to extubating patients as early as possible with appropriate anesthesia applications [6].

In various studies, low albumin and high C-reactive protein (CRP) levels have been shown in cardiovascular diseases and undesirable events after CABG surgeries [7-9]. In another study, low albumin and high C-reactive protein were shown to be associated with prolonged mechanical ventilation times in patients intubated in the intensive care unit [10]. The Glasgow Prognostic Score (GPS) is a parameter determined by albumin and CRP values, and it is associated with poor postoperative clinical outcomes after transcatheter aortic valve replacement [11]. In this current study, we aimed to reveal the relationship between prolonged mechanical ventilation and GPS, in patients aged 65 years and older who underwent isolated CABG.

METHODS

Patients aged 65 years and over who underwent elective isolated coronary bypass surgery with cardiopulmonary bypass (CPB) in our clinic, between June 2017 and June 2022, were included in the study retrospectively. A total of 908 CABG operations were performed in patients aged 65 years and over within the study dates. All operations were performed by the same surgical team, and the inten-sive care management of the patients was also carried out by the same team. Intubated emergency surgeries or those performed under oxygen support, patients with critical preoperative condition (positive inotropic support or intra-aortic balloon support), patients with intraoperative mortality, patients undergoing combined surgeries, those with renal or hepatic insufficiency, those with pulmonary infection in the last month, as well as patients who were re-explored within the first 8 hours after the operation, were all excluded from the study. Following the application of the exclusion criteria, 486 consecutive patients were included in the study. The patients were divided into two groups according to the duration of postoperative ventilation. Accordingly, the patients who were extubated in the cardiovas-cular surgery intensive care unit within eight hours of the surgeries were determined as group 1, whereas those who were extubated after 8 hours were determined as Group 2. There were 385 patients in group 1, and 101 patients in Group 2 (Fig. 1).The demographic characteristics of the patients, preoperative hemogram (white blood cell, hematocrit, thrombocyte), biochemistry (urea, creatinine, C-reactive protein), echocardiography (left ventricular ejection fraction), intraoperative perfusion times, post-operative blood product transfusions, positive inotropic needs and ventilation times were recorded.

Glasgow Prognostic Score (GPS)

In the GPS evaluation, the C reactive protein value is above 10 mg/L and the albumin value is below 35 g/L. If both of these conditions are present the score is 2, if only one of them is present, the score is 1 and in the absence of both conditions, the score is evaluated as 0 [11].

Anesthesia Technique and Surgical Procedure

General anesthesia was used during all procedures. Following intravenous midazolam administration (0.05–0.1 mg/kg; Zolamid®; Defarma, Turkey), invasive catheterization of the radial artery was carried out. Patients were then observed, thereafter fentanyl (Talinat®, Vem, Turkey) and pentothal (Pental® Sodium, Turkey) were used to induce anesthesia in all patients. Rocuronium bromide (Curon®, Mustafa Nevzat, Turkey) was administered to aid endotracheal intubation. To maintain anesthesia, sevoflurane (Sevorane®, Abbvie, Turkey) was combined with a mixture

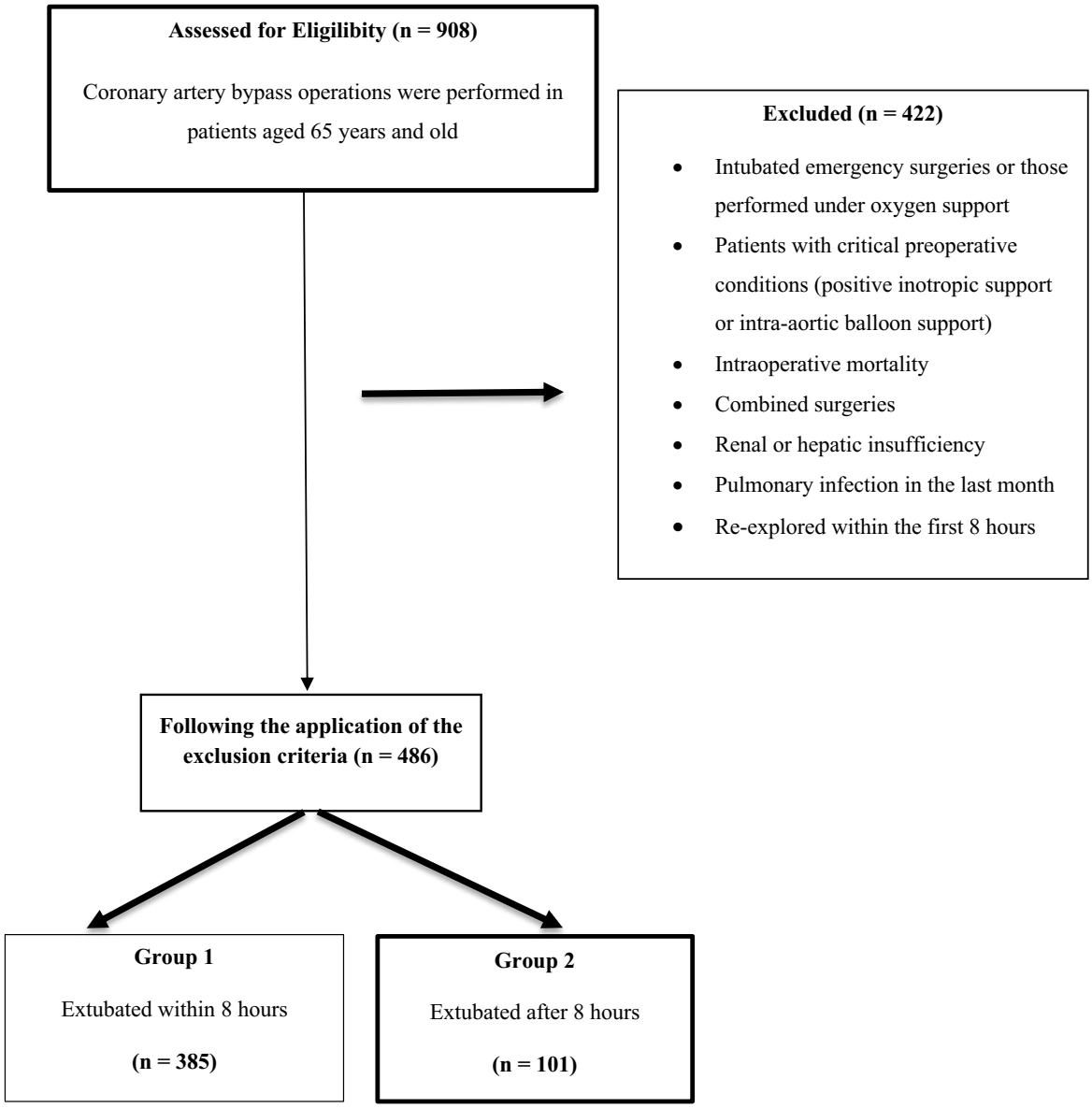

Fig. 1. Flow chart of the study.

of 50% oxygen and 50% air. For intraoperative mechanical ventilation, a Primus® anesthetic machine (Draeger Medical, Germany) was employed. Surgery commenced after an internal jugular vein catheterization. The following parameters were tracked throughout the procedure: temperature, urine output, pulse oximetry, end-tidal carbon dioxide, arterial blood pressure, and electrocardiography. Before the sternotomy, intravenous fentanyl (3-5 g/kg) was added. Before cannulation, heparin (300-400 units/kg) was given to obtain a clotting time of at least 480 seconds. During the entire CPB, midazolam, fentanyl and rocuronium were used to maintain anesthesia. Early extubation was planned in conjunction with avoiding unnecessary sedation and opioid use throughout the operation.

When doing standard CPB, moderate hypothermia (32°C) was used. Aorto-venous two-stage cannulation was used to accomplish CPB after median sternotomy and heparinization. In order to achieve cardiac arrest, the ascending aorta was clamped, and cold antegrade cardioplegia with high potassium was administered (PLEGISOL® | Pfizer). Blood cardioplegia was administered every 15 to 20 minutes to maintain cardiac arrest. CPB was established with a roller pump equipped with a membrane oxygenator and arterial line filter (Maquet, Getinge Group, Germany). The pump's flow

rates were 2-2.4 L/min/m^2. Arterial blood gas was evaluated every 20 to 30 minutes; immediately prior to removing the cross-clamp, 500 milliliters of hot blood cardioplegia were administered.

Postoperative Management

The patients were transferred to the cardiovascular surgery intensive care unit when the surgery was completed and all patients received the usual postoperative care. Patients were evaluated hourly for eligibility for extubation. The provision of hemodynamic stabilization (without the need for high-dose vasoactive inotropic support; urine output > 0.5 mL/kg/hours; without severe arrhythmia) was followed by extubation as soon as it was practicable [12]. Prolonged ventilation was accepted as 8 hours since all operations were performed by avoiding unnecessary use of anesthetic agents in elective and hemodynamically stable patients.

Statistical Analysis

For analysis, IBM SPSS version 21.0 (IBM Corp., Armonk, NY, USA) was utilized. The mean, SD, median (min-max), number, and frequency of the variables were used to express them. To analyze the normality of numerical data, the Kolmogorov-Smirnov and Shapiro-Wilk tests were applied. The Mann-Whitney U test was used to evaluate non-normally distributed variables, whereas the Student's t-test was used to study variables with a normal distribution. Comparing categorical variables was done using the chi-square test. One-Way ANOVA test was used to compare mean mechanical ventilation time according to GPS groups (0, 1, and 2) and subgroup analyzes were performed with Tukey's test (Fig. 2). To examine the predictors of PV, a multivariate logistic regression analysis was used. Statistical significance was defined as a *p* - value of 0.05 or lower.

RESULTS

Prolonged ventilation occurred in 101 (20.7%) patients (Group 2). The median age of the 385 patients included in Group 1 and 101 patients in Group 2 was 68 (66 to 91) and 71 (66 to 88) years, respectively ($p = 0.216$). The two groups were similar in regards to gender, hypertension, smoking, diabetes mellitus, body mass index, history of cerebrovascular events, and left ventricular ejection fraction rates. White blood cells, hemoglobin, platelets, creatinine, urea, and C-reactive protein levels did not significantly differ across the groups. In Group 2, albumin values were significantly lower ($p = 0.014$), whereas the EuroSCORE thereof was significantly higher ($p < 0.001$). Additionally, the GPS classifications between the two groups differed significantly (Table 1). Additionally, in patients with a GPS of 2 mean ventilation time was significantly higher than in patients with a GPS of 0 and 1 (Fig. 2).

The operative features and postoperative complication rates of the patients are presented in Table 2.

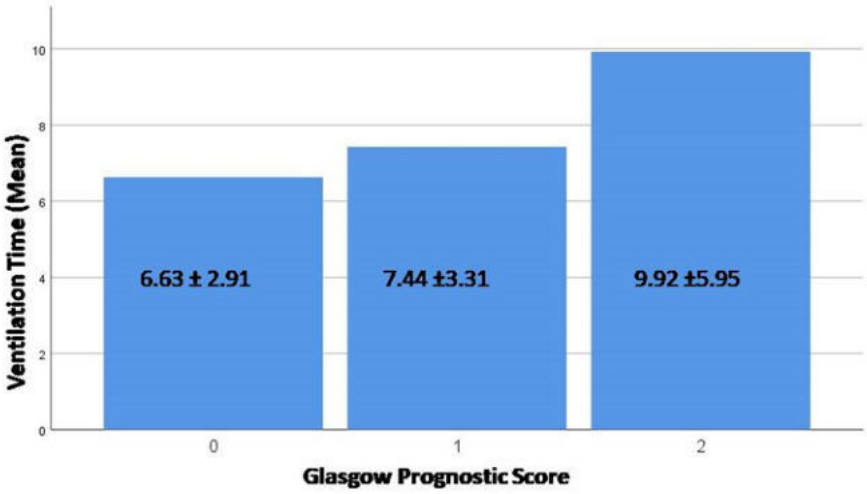

Fig. 2. Mean ventilation times according to Glasgow Prognostic Score (GPS 0 vs GPS1; *P* = 0.096, GPS 0 vs GPS 2; *P* < 0.001, GPS 1 vs GPS 2; *P* < 0.001).

Table 1. Demographic and preoperative features of the patients

Variables	Group 1 (n = 385)	Group 2 (n = 101)	*p* value
Age (years)	68 (66-91)	71 (66-88)	0.216‡
Gender, female, n (%)	107 (27.8)	30 (29.7)	0.704*
Hypertension, n (%)	232 (60.3)	66 (65.3)	0.350*
Diabetes mellitus, n (%)	88 (22.9)	29 (28.7)	0.221*
Current smoker, n (%)	149 (38.7)	44 (43.6)	0.374*
COPD, n (%)	75 (19.5)	26 (25.7)	0.167*
Previous CVA	33 (8.6)	12 (11.9)	0.307*
BMI (kg/m^2)	26.8 (24.3-38.9)	27.3 (24-39.1)	0.325‡
LVEF (%)	50 (25-65)	45 (25-65)	0.116‡
White blood cell (10^3/µL)	6.9 (5.2-11.9)	7.3 (4.8-9.8)	0.447‡
Platelet (10^3/µL)	215 (138-396)	221(128-350)	0.194‡
Hematocrit (%)	38.4 (34.1-50)	39.1 (33.3-47)	0.241‡
Creatinine (mg/dL)	0.96 (0.7-2)	0.98 (0.7-1.96)	0.416‡
Urea (mg/dL)	18 (15-48)	20 (16-42)	0.192‡
C Reactive protein (mg/dL)	8.3 (0.9-28)	8.8 (0.7-26)	0.212‡
Albumin (mg/dL)	38 (30-48)	36 (30-50)	**0.014**‡
EuroSCORE II	3.2 (0.8-6.4)	3.7 (0.8-10.1)	**< 0.001**
GPS = 0/1/2, n	231/115/39	43/30/28	**< 0.001***

Data are shown as median (minimum-maximum). BMI = Body mass index, CVA = Cerebrovascular accident, COPD = Chronic obstructive pulmonary disease, EuroSCORE = European System for Cardiac Operative Risk Evaluation, GPS = Glasgow Prognostic Score, LVEF = Left ventricular ejection fraction

*Chi-square test, ‡Mann Whitney U test

Cross-clamp times, the number of distal anastomoses, packed blood products used, intra-aortic balloon pump need rates and intensive care unit stay-days, were all similar between the two groups. Total perfusion time and postoperative atrial fibrillation rates were statistically significantly higher in Group 2 ($p < 0.001$ and $p = 0.021$, respectively), whereas renal injury, pneumonia, reintubation, and mortality rates were similar between the two groups.

In the univariate analysis, prolonged ventilation development was found to significantly correlate with age > 75 (odds ratio [OR]: 0.796, 95% confidence interval [CI]: 0.554-0.916, $p = 0.021$), ejection fraction < 35% (OR: 1.190, 95% CI: 1.050-1.894, $p = 0.008$), total perfusion time (OR: 1.240, 95% CI: 1.110-1.842, $p < 0.001$), need of inotropic support (OR: 1.370, 95% CI: 1.150-2.248, $p = 0.007$), low albumin value (OR: 0.872, 95% CI: 0.665-0.938, $p = 0.021$), and GPS = 2 (OR: 2.350, 95% CI: 1.696-3.550, $p < 0.001$). In the multivariate analysis, LVEEF < 35% (OR: 1.136, 95% CI: 1.065-1.652, $p = 0.029$), total perfusion time (OR: 1.190, 95% CI: 1.040-1.659, $p = 0.012$), and GPS = 2 (OR: 1.479, 95% CI: 1.130-2.169, $p = 0.004$) were determined as independent predictors for prolonged ventilation (Table 3).

DISCUSSION

Coronary artery bypass graft surgeries, in which CPB systems are mostly used, are performed under general anesthesia. At present, when these operations are planned electively, it is devised that the patients will be extubated as soon as possible during the inten-sive care follow-up. Various studies have been conducted investigating the reasons for longer extubation. In the

Table 2. Operative and postoperative features of the patients

Variables	Group 1 (n = 385)	Group 2 (n = 101)	*p* value
Total perfusion time (min)	83 (57-165)	88 (60-188)	**< 0.001**‡
Cross-clamp time (min)	52 (36-92)	55 (32-88)	0.294‡
Number of distal anastomoses, n (range)	3 (2-6)	3 (2-6)	0.387‡
Packed blood products (units)	4 (2- 6)	4 (2-8)	0.328‡
Inotropic support, n (%)	155 (40.3)	57 (56.4%)	**0.004***
IABP support, n (%)	45 (11.7)	14 (13.9%)	0.552*
Total ICU stay (days)	2 (2- 7)	2 (2- 10)	0.134‡
PoAF, n (%)	90 (23.4)	35 (34.7)	**0.021***
Renal injury, n (%)	97 (25.2)	32 (31.7)	0.189*
Pneumonia, n (%)	39 (10.1)	15 (14.9)	0.179*
Reintubation, n (%)	6 (1.5)	2 (2)	0.767*
Mortality, n (%)	5 (1.3)	2 (1.9)	0.609*

Data are shown as mean ± standard deviation or n (%). IABP = Intra-aortic balloon pump, ICU = Intensive care unit, PoAF = Postoperative atrial fibrillation, Inotropic support = Defined as needs of dopamine, and/or norepinephrine (> 5 mg/kg/min)
*Chi-square test, ‡Mann- Whitney U test

current research, we established that GPS value can be used to predict prolonged ventilation risk in addition to known risk factors.

Albumin is an important protein that makes up more than half of body serum proteins. In addition to its anti-inflammatory and antioxidant properties, it also has physiological properties such as anticoagulant and inhibition of platelet aggregation. In addition to all these, it also plays an important role in transmembrane fluid passages in the body [13]. Various studies have shown that cardiovascular disease development and progression are influenced by decreased albumin levels. While hypoalbuminemia has been reported at a rate of 25% in patients with chronic heart failure,

Table 3. Logistic regression analysis to identify factors affecting development of prolonged ventilation

Variables	Univariate analysis			Multivariate analysis		
	p value	Exp(B) Odds Ratio	95% CI Lower-Upper	*p value*	Exp(B) Odds Ratio	95% CI Lower-Upper
Age > 75	**0.021**	0.796	0.554-0.916	0.240	0.884	0.791-1.117
LVEF < 35%	**0.008**	1.190	1.050-1.894	**0.029**	1.136	1.065-1.652
COPD	0.144	1.214	0.849-1.453	--	--	--
C-reactive protein	0.192	0.940	0.781-1.438	--	--	--
Total perfusion time	**< 0.001**	1.240	1.110-1.842	**0.012**	1.190	1.040-1.659
Inotropic support	**0.007**	1.370	1.150-2.248	0.124	0.985	0.785-1.132
Albumin	**0.021**	0.872	0.665-0.938	--	--	--
GPS = 2	**< 0.001**	2.350	1.696-3.550	**0.004**	1.479	1.130-2.169

LVEF = Left ventricular ejection fraction, COPD = Chronic obstructive pulmonary disease, GPS = Glasgow prognostic score
The goodness of fit of the multivariate model was confirmed by a *p*-value of 0.784 in the Hosmer-Lemeshow test.

this rate has been reported up to 90% in the elderly and patients with acute heart failure [14].

The importance of albumin was investigated in a prospective study of 734 clinically stable patients with known coronary artery disease. In this study, patients were followed for 18 months and low albumin was demonstrated as an independent predictor of all-cause mortality and cardiovascular mortality [15]. In a prospective study including 588 patients, low albumin levels were found to be a reliable indicator of long-term death after CABG procedures [16]. Because of these properties, albumin has been included in various evaluation parameters used as prognostic value (the Essential Frailty Toolset, the Prognostic nutritional index, GPS, etc). In one study, the relationship between a low prognostic nutritional index and postoperative atrial fibrillation was revealed [17]. In another study conducted by Solomon *et al*. [18], patients aged 60 years and older who underwent CABG were included. Low albumin levels have been linked to all-cause postoperative mortality in this research of 500 patients with a mean age of 71.4 ± 6.4 years.

An essential acute phase reactant is the C-reactive protein. It belongs to the pentraxin protein family and activates classical and alternative complement pathways. It also induces cytokine release by activating leukocyte. In addition, CRP deposits were found in atherosclerotic foci taken from human arteries in various histological studies [19]. Studies have investigated the prognostic importance of preoperative CRP values after CABG operations. In one of these, high CRP levels before surgery have been linked to myocardial injury and low cardiac output after CABG operations [20]. In another study by Nam *et al*. [21], the prognostic role of preoperative CRP value was investigated in patients who underwent off-pump CABG operation. The authors revealed a significant relationship between postoperative mortality and high CRP values.

The GPS value is a parameter created by considering CRP and albumin values. Studies examining the connection between GPS and various cancers have been conducted in the literature, and the importance of GPS value has been demonstrated [22, 23]. Recent studies have also investigated the relationship between cardiovascular diseases and GPS. In a study by Xu *et al*. [24], the relationship between the development of major adverse events and GPS in patients with acute coronary syndrome was investigated. The authors found that the risk of developing major adverse events increased more than five times in patients with a GPS value of 2, compared to patients with a GPS value of 0 [24]. Cho *et al*. [25], on the other hand, investigated the prognostic value of GPS value in their study, which included 443 patients with stable heart failure. The authors demonstrated in this study that there is a significant relationship between high GPS values and the development of adverse events [25]. In the study conducted by Altay *et al*. [26], the relative importance of the GPS value in predicting the prognosis was investigated in some 1 004 patients requiring cardiovascular intensive care. As a result of the multivariate analysis, it was concluded that in-hospital mortality was seven times higher in patients with a GPS value of 2 compared to patients with a GPS value of 0 [26]. In a recent study, the importance of GPS value was investigated in patients who underwent transcatheter aortic valve implantation. The endpoints in this study were in-hospital mortality, cardiac rehospitalization and one-year death. At the end of the study, GPS value was shown as an independent predictor of forecasting endpoints [11].

In a study by Hessels *et al*. [27], factors affecting prolonged ventilation time after cardiac surgery were investigated. The median age of the patient group in this study was 67 years and 11% of the patients had more than 24 hours of ventilation. In this study, low ejection fraction, combined surgery, urgency of surgery, use of IABP, increased blood product use and prolonged CPB times, were found to be related to prolonged ventilation [27]. However, in this study, explanatory information about the anesthesia management of the patients was not provided. Our study, unlike the latter, was planned for patients aged 65 and over who underwent elective isolated CABG surgery. Our anesthesia management was performed in such a way as to allow the earliest extubation of all patients. For this reason, the prolonged ventilation duration was determined as eight hours. In our study, similar to Hessels *et al*., we found low LVEF, increased blood product use and long CPB times to be associated with prolonged ventilation times. In a study by Gumus *et al*. [28], the risk factors of prolonged ventilation after CABG operations were investigated

and with similar to ours, a significant relationship was found between long CPB times and prolonged ventilation.

Limitations

Although we uncovered valuable information in our study to contribute to the literature, there are some limitations. The first is that ours was a single-center retrospective study, therefore the number of patients was also limited. In our study, LVEF < 35% and the need for inotropic support were significantly correlated with prolonged ventilation. Although not statistically significant, the rates of postoperative atrial fibrillation and renal injury were also higher in patients with prolonged ventilation. Increased use of inotropic agents may also have increased these complication rates.

CONCLUSION

It is crucial to perform extubation as early as possible after CABG operations in patients with stable preoperative hemodynamics under elective conditions. In this study, we revealed that the GPS value calculated preoperatively in elderly patients is a parameter that indicates the prolonged ventilation. With the GPS score evaluation, patient groups at risk for prolonged ventilation may be identified and necessary precautions may be undertaken.

Institutional Review Board Statement

The study was approved by Bursa Yuksek Ihtisas Training and Research Hospital's clinical research ethics committee (2011-KAEK-25 2022/10- 03).

Informed Consent Statement

Written informed consent was obtained from the patients before their interventions.

Authors' Contribution

Study Conception: OG, ME; Study Design: OG; Supervision: OG, ME, ŞY; Funding: OG, ME; Materials: OG, ME, IIK; Data Collection and/or Processing: OG, ME; Statistical Analysis and/or Data Interpretation: OG, IIK; Literature Review: OG, ME, IIK, ŞY; Manuscript Preparation: OG, ME and Critical Review: OG, ME, IIK, ŞY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. As AK, Engin M, Türk T. Early-term results of early coronary artery bypass graft surgery in patients undergoing primary percutaneous coronary intervention due to acute coronary syndrome. Eur Res J 2020;7:59-65.
2. Abanoz M, Engin M. The effect of the relationship between post-cardiotomy neutrophil/lymphocyte ratio and platelet counts on early major adverse events after isolated coronary artery bypass grafting. Turk Gogus Kalp Damar Cerrahisi Derg 2021;29:36-44.
3. Doering LV, Imperial-Perez F, Monsein S, Esmailian F. Preoperative and postoperative predictors of early and delayed extubation after coronary artery bypass surgery. Am J Crit Care 1998;7:37-44.
4. Aksoy R, Karakoc AZ, Cevirme D, Elibol A, Yigit F, Yilmaz Ü, Rabus MB. Predictive factors of prolonged ventilation following cardiac surgery with cardiopulmonary bypass. Braz J Cardiovasc Surg 2021;36:780-7.
5. Yıldızeli SO, Eryüksel E, Arıkan H, Karakurt S, Çelikel T. [Clinical case solution: prolonged ventilatory support and weaning after coronary artery bypass graft surgery]. Yoğun Bakım Derg 2016;7:67-72. [Article in Turkish]
6. Myles PS, Daly DJ, Djaiani G, Lee A, Cheng DC. A systematic review of the safety and effectiveness of fast-track cardiac anesthesia. Anesthesiology 2003;99:982-7.
7. Abuş S. C-reactive protein to albumin ratio in atrial fibrillation. Eur Res J 2022 Sep 27. doi: 10.18621/eurj.1038532.
8. Yang J, Li C, Zheng Y, Gao J, Liu YP, Wang JJ, et al. The Association between high-sensitivity C-reactive protein/albumin ratio and cardiovascular prognosis in patients undergoing percutaneous coronary intervention. Angiology 2022;73:818-26.
9. Karabacak K, Kubat E, Akyol FB, Kadan M, Erol G, Doğancı S, et al. The C-reactive protein/albumin ratio as a new predictor for postoperative atrial fibrillation after coronary artery bypass graft surgery. J Card Surg 2020;35:2747-53.
10. Bae BK, Lee SH, Han SK, Yeom SR, Cho SJ, Park SW, et al. Serial measurements of C-reactive protein and albumin levels in prediction of prolonged mechanical ventilation in pesticide-intoxicated patients. J Korean Soc Emerg Med 2012;23:665-72.
11. Abacioglu OO, Koyunsever NY, Kilic S, Yildirim A, Kurt IH. Glasgow Prognostic Score as a marker of mortality after TAVI. Braz J Cardiovasc Surg 2021;36:796-801.
12. McCarthy C, Fletcher N. Early extubation in enhanced from cardiac surgery. Crit Care Clin. 2020;36:663-74.
13. Lyons O, Whelan B, Bennett K, O'Riordan D, Silke B. Serum

albumin as an outcome predictor in hospital emergency medical admissions. Eur J Intern Med 2010;21:17-20.
14. Arques S, Ambrosi P. Human serum albumin in the clinical syndrome of heart failure. J Card Fail 2011;17:451-8.
15. Chien SC, Chen CY, Leu HB, Su CH, Yin WH, Tseng WK, et al. Association of low serum albumin concentration and adverse cardiovascular events in stable coronary heart disease. Int J Cardiol 2017;241:1-5.
16. de la Cruz KI, Bakaeen FG, Wang XL, Huh J, LeMaire SA, Coselli JS, et al. Hypoalbuminemia and long-term survival after coronary artery bypass: a propensity score analysis. Ann Thorac Surg 2011;91:671-5.
17. Engin M, Ozsin KK, Savran M, Guvenc O, Yavuz S, Ozyazicioglu AF. Visceral adiposity index and prognostic nutritional index in predicting atrial fibrillation after on-pump coronary artery bypass operations: a prospective study. Braz J Cardiovasc Surg 2021;36:522-9.
18. Solomon J, Moss E, Morin JF, Langlois Y, Cecere R, de Varennes B, et al. The essential frailty toolset in older adults undergoing coronary artery bypass surgery. J Am Heart Assoc 2021;10:e020219.
19. Melnikov IS, Kozlov SG, Saburova OS, Avtaeva YN, Prokofieva LV, Gabbasov ZA. Current position on the role of monomeric C-reactive protein in vascular pathology and atherothrombosis. Curr Pharm Des 2020;26:37-43.
20. Balciunas M, Bagdonaite L, Samalavicius R, Griskevicius L, Vuylsteke A. Pre-operative high sensitive C-reactive protein predicts cardiovascular events after coronary artery bypass grafting surgery: a prospective observational study. Ann Card Anaesth 2009;12:127-32.
21. Nam K, Jeon Y, Kim TK, Kim KB, Hwang HY, Cho YJ. Intraoperative transfusion and an increased preoperative C-reactive protein level are associated with higher mortality after off-pump coronary artery bypass grafting. J Thorac Cardiovasc Surg 2020;159:558-65.
22. Manoglu B, Sokmen S, Bisgin T, Semiz HS, Görken IB, Ellidokuz H. Inflammation-based prognostic scores in geriatric patients with rectal cancer. Tech Coloproctol 2022 Oct 5. doi: 10.1007/s10151-022-02710-0.
23. Yeh KY, Wang CH, Ling HH, Peng CL, Chen ZS, Hsia S. Pretreatment Glasgow Prognostic Score correlated with serum histidine level and three-year mortality of patients with locally advanced head and neck squamous cell carcinoma and optimal performance status. Nutrients 2022;14:3475.
24. Xu X, Cai L, Chen T, Ding S, Zhang F, Gao B, et al. Predictive value of inflammation-based Glasgow prognostic score, platelet-lymphocyte ratio, and global registry of acute coronary events score for major cardiovascular and cerebrovascular events during hospitalization in patients with acute myocardial infarction. Aging (Albany NY). 2021;13:18274-86.
25. Cho A, Arfsten H, Goliasch G, Bartko PE, Wurm R, Strunk G, et al. The inflammation-based modified Glasgow prognostic score is associated with survival in stable heart failure patients. ESC Heart Fail 2020;7:654-62.
26. Altay S, Gürdoğan M, Keskin M, Kardaş F, Çakır B. The inflammation-based Glasgow Prognostic Score as a prognostic factor in patients with intensive Cardiovascular Care Unit. Medicina (Kaunas) 2019;55:139.
27. Hessels L, Coulson TG, Seevanayagam S, Young P, Pilcher D, Marhoon N, et al. Development and validation of a score to identify cardiac surgery patients at high risk of prolonged mechanical ventilation. J Cardiothorac Vasc Anesth 2019;33:2709-16.
28. Gumus F, Polat A, Yektas A, Totoz T, Bagci M, Erentug V, et al. Prolonged mechanical ventilation after CABG: risk factor analysis. J Cardiothorac Vasc Anesth 2015;29:52-8.