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ii. Walker M, Hull A. Preterm labor and birth. In: Taeusch HW, Ballard RA, eds. Avery's Diseases of the Newborn. Philadelphia: WB Saunders, 1998: 144,153.

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Original Articles

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Original Article

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Immobilization after pediatric supracondylar humerus fracture surgery: Cast or splint?

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ABSTRACT

Objective: While surgical treatment is the most accepted treatment method for displaced supracondylar humerus fractures in children, there is little data about immobilization method after surgery. The aim of the study is to determine whether there is any difference in preventing loss of reduction between long-arm cast and long-arm splint following pediatric supracondylar humerus fracture surgery.

Patients and Methods: We conducted a retrospective analysis of pediatric patients with supracondylar humerus fractures treated operatively between 2012 and 2019 at a university hospital. According to Skaggs criteria, early postoperative and 3rd-week follow-up X-rays were evaluated for the loss of reduction (LOR). Postoperative immobilization method; splint or cast was compared in the context of LOR.

Results: Cast immobilization was found to be superior in preventing LOR in the first three weeks postoperatively (p⍰0.05). There was no significant difference for other factors like fracture configuration, patient age and surgical technique.

Conclusion: Cast immobilization is superior to splint immobilization in preventing radiologic LOR after pediatric supracondylar humerus fracture surgery however, clinical relevance of this conclusion is yet to be proved.

Keywords: Supracondylar humerus fracture, Postoperative immobilization, Loss of reduction

1. INTRODUCTION

Supracondylar fractures of the humerus are the most common type of elbow fractures in children, accounting for more than 80% of all fractures [1, 2]. The typical mechanism of injury has been described by Farnsworth et al. as a fall on an outstretched hand, and thus, 98% of these fractures are extension-type injuries [3]. Closed or open reduction followed with percutaneous fixation with K-wires is widely accepted as optimal treatment. In previous studies, this treatment has been reported to have good and excellent results [4-6].

Loss of reduction (LOR) is reported in 2.9 to 18.2% of cases [5, 7]. Many factors affecting early LOR have been investigated. Factors about fracture itself, like coronal plane obliquity, proximal fractures, comminution, Gartland classification, or surgical technique factors like pin configuration, pin spread ratio (PSR), bicortical fixation have been questioned by many studies [8,9]. Even though, the cast is considered to be more stable than the splint, there is not enough evidence to choose either a long-arm cast or splint after pediatric supracondylar humerus fracture surgery.

Our study aims to determine whether there is any difference in the LOR between using splint or cast after pediatric supracondylar humerus fracture fixation.

2. PATIENTS and METHODS

After the approval for this study was obtained from Marmara University, School of Medicine Ethics Committee (09.2020.388), we retrospectively evaluated the medical records and radiographs of all children treated surgically for distal humerus fracture at a university hospital between 2012 and 2019.

Children operated for Gartland type II, III, IV fractures were included in the study. The Gartland classification is divided

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into 4 types: I: nondisplaced fracture, II: minimally displaced fracture, III: completely displaced fracture, posterior cortex intact, IV: fully displaced fracture, posterior cortex separated [8]. Patients with intraarticular extension and associating arm fracture on the ipsilateral side were excluded from the study. Patients who underwent early revision surgery due to unacceptable reduction were excluded from the study. Patients who did not have regular follow-up, proper X-rays for radiologic measurements – as positional changes can cause a difference in measuring Baumann angle – or missing data in their files were also excluded from the study.

All preoperative medical records were checked for patient's age, neurologic compromise and ipsilateral upper extremity fracture. Perioperative X-rays were evaluated for fracture type, according to Modified Gartland Classification [8]; and presence of medial or lateral comminution, the existence of more than 20˚ obliquity on the coronal plane, or proximal fracture to the olecranon fossa as defined in Johns Hopkins Classification [8, 9]. Fractures were not classified as flexion and extension types due to the high number of patients referred from different hospitals with a splint. However, flexion type fractures are proven to be more "difficult" fractures. This data was considered not accurate enough and left out of the statistical analysis [10].

Data about whether a closed or open reduction was performed and which pin configuration was chosen for fixation were collected from the operation files. The mean delay of operation after fracture was also noted. Six different surgeons with similar years of experience in paediatric orthopaedic traumatology performed the operations. The preferance for postoperative immobilization with either casting or splinting was chosen according to these surgeons habits. Because of the retrospective nature of our cohort, cast and splint group were not randomized.

Early postoperative X-rays were evaluated for PSR (as a percentage), bicortical fixation, Baumann angle, Anterior humeral line crossing capitellum, and Gordon rotation percentage [4]. PSR for patients with more than two pins was calculated as a percentage of pins' highest distance to the bone diameter at the fracture site.

Third week control X-rays measurements for Baumann angle, anterior humeral line, and Gordon rotation percentage were repeated to determine LOR. Baumann angle and anterior humeral line were measured, but the Gordon rotation percentage was excluded because of the data's inconsistencies due to bone union.

Loss of reduction was taken according to Skaggs criteria. Skaggs criteria is more than 6 degrees change in Baumann angle or a change in the anterior humeral line crossing the capitellum (Figure 1) [11].

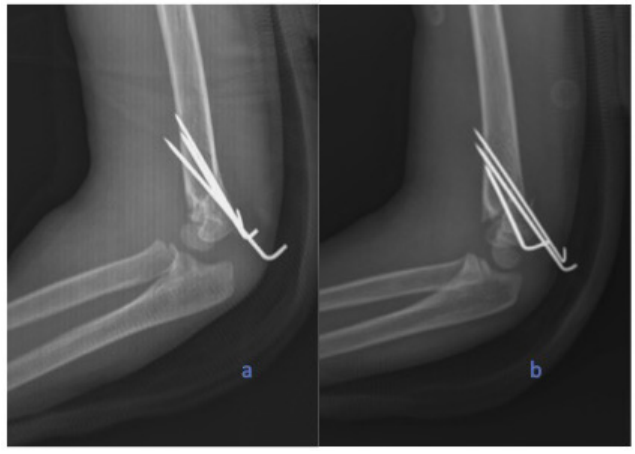

Figure 1-a. *Early postoperative lateral X-ray, showing that the anterior humeral line is crossing the capitellum,* ***1-b.*** *3rd-week X-ray of this patient, distal fragment is displaced posteriorly. Although, both X-rays are not taken exactly from the same angles, it is obvious that the anterior humeral line is not crossing the capitellum.*

Patient's ages were classified into two groups as younger than five years and older than five years. Pin configuration were classified into 3 groups: lateral configuration (2 lateral pins or 3 lateral pins), medial+ lateral (1 medial pin+ 1 lateral pin) and medial+ multiple lateral (1 medial pin + 2 lateral pins or 1 medial + 3 lateral pins) (Figure 2).

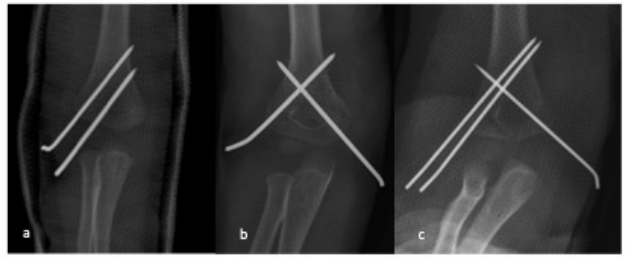

Figure 2. *Radiographic images demonstrating pin configuration: (a) Only lateral entry, multiple pinnings; (b) 1 lateral pin and 1 medial pin; (c) 2 lateral pins and 1 medial pin.*

Patients with and without LOR within three weeks were compared for postoperative immobilization methods and other risk factors.

Patients with postoperative cast and splint were compared statistically for risk factors (age, Gartland classification, presence of comminution or proximal fracture, ipsilateral upper extremity fracture, neurovascular compromise, time between fracture and operation, pin configuration, PSR at the fracture site, presence of bicortical fixation).

Postoperatively elbows were immobilized on 90 (±5) degrees flexion with standard casting or simple posterior fiberglass splint (Figure 3). Pin site infection, pin removal time, and cast

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removal time data were kept out of the analysis because they were not a part of this cohort.

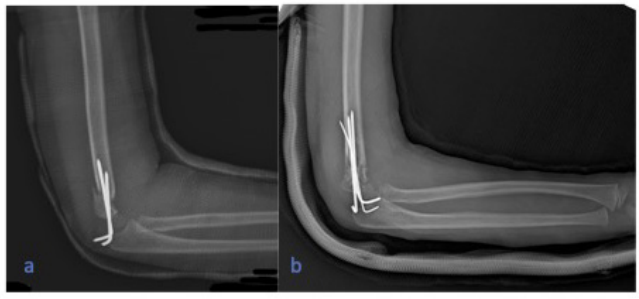

Figure 3-a/b. *Two examples for standard long-arm cast and splint made at 90 degrees of elbow flexion.*

Fig 4-a/b/c/d. *Example for splint loosening at two different patients during 3 weeks follow-up period.*

Statistical Analysis

The data analysis was carried out using the Statistical Package for Social Sciences (SPSS v. 22.0 software, Chicago, USA). The study data were evaluated using descriptive statistical methods (mean, standard deviation, median, interquartile range, frequency, ratio, minimum, maximum). The Kolmogorov-Smirnov test was used to test the normality of distribution of the quantitative data. The Mann-Whitney U test was used to compare the non-normally distributed quantitative variables. Pearson's Chi-square test, Fisher's Exact test and Fisher-Freeman-Halton test for an agreement were used in order to compare the qualitative variables. A p-value <0.05 was considered statistically significant.

3. RESULTS

Complete medical records and true anteroposterior radiographs of the distal part of the humerus and lateral radiographs of the elbow made perioperatively and at the time of fracture-healing were available for 232 patients who were treated for surgically displaced Gartland type II, III, IV fractures at the institution.

The mean age of patients was 5.59 ± 2.45. On the initial evaluation, there were 30 patients with comminution on either of the cortices, 20 patients with coronal obliquity on the fracture line, and 16 patients with proximal fracture. According to modified Gartland supracondylar humerus fracture classification, the number of patients with Type II, III and IV fractures was 59, 144, and 29, respectively. Eight patients had ipsilateral distal radius fractures.

The mean delay of operation after fracture was 0.91 days, while most patients were operated on the same or the next day after admission. The most prolonged delay was four days for two patients because of associated medical conditions.

During surgical intervention 33 patients required open reduction, bicortical bone fixation were achieved on all except 8 patients. Pin configurations were; 2 lateral pins 26 (11.2%), 3 lateral pins 11 (4.7%), 1 medial pin + 1 lateral pin 64 (27.6%), 1 medial + 2 lateral pins 119 (51.3%), 1 medial + 3 lateral 4 (1.7%), 2 medial + 2 lateral 8 (3.4%). To make a better statistical subgroup analysis, we combined groups as isolated lateral pin configuration 37 (15.9%), medial + 1 lateral pin configuration 64 (27.6%); medial + multiple lateral pin configuration 131 (56.5%). Mean Perceived State Rating: (PSR) was 37 (28-48%).

After surgical intervention, 132 patients were immobilized with a long-arm cast, while 100 patients had a long-arm splint for immobilization.

At the third week follow-up, a total of 17 patients had LOR according to Skaggs criteria (Figure 1). There was no surgical revision after the third week. Eight patients had postoperative ulnar nerve palsy.

Splint and cast immobilization groups were homogenous for other factors except for open or closed surgery. Patients with open reduction and fixation were immobilized mostly with a splint ($p<0.001$). Patients with postoperative splint immobilization had a higher value for median PSR, which was not significant ($p=0.053$) (Table I).

All parameters were compared between patients with and without LOR. The only statistically significant parameter was the choice of postoperative immobilization. Patients with splint immobilization tend to have more LOR rate compared to cast immobilization group ($p=0.017$). Patients requiring open reduction had a remarkably higher LOR rate than patients with closed reduction, but this difference was not significant statistically ($p=0.075$) (Table II).

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Table I. *Statistical analysis of risk factors between patients who had cast or splint for immobilization.*

Parameter		Cast	Splint	p
LOR	+	5	12	0.017[a]
	-	127	88	
Age (years)	?5	69	48	0.519[a]
	?5	63	52	
Surgery	open	8	25	?0.001[a]
	closed	124	75	
Pin configuration	Only lateral	23	14	0.223[c]
	1 medial, 1 lateral	41	23	
	1 medial, multiple lateral	68	63	
Bicortical pin fixation	+	126	98	0.471[b]
	-	6	2	
Ipsilateral fracture	+	129	95	0.295[b]
	-	3	5	
Preoperative nerve damage	+	8	6	0.985[a]
	-	124	94	
Modified Gartland Classification	2	36	23	0.701[c]
	3	81	63	
	4	15	14	
Proximal fracture	+	6	10	0.104[a]
	-	126	90	
Coronal obliquity	+	9	11	0.261[a]
	-	123	88	
Comminution	+	18	12	0.713[a]
	-	114	88	
Pin spread ratio Median (IQR)		0.40(0.30-0.49)	0.35(0.24-0.47)	0.052[d]

[a] Pearson's chi square test, [b] Fisher's exact test, [c] Fisher Freeman Halton Test, [d] Mann-Whitney U Test, IQR: Interquartile range

Table II. *Statistical analysis of risk factors between patients with or without LOR.*

		LOR-	LOR+	p value
Age	?5 years	110	7	0.428[a]
	?5 years	105	10	
Comminution	-	188	14	0.468[b]
	+	27	3	
Proximal fracture	-	200	16	1.000[b]
	+	15	1	
Coronal obliquity	-	196	16	1.000[b]
	+	19	1	
Modified Gartland Classification	2	57	2	0.260[d]
	3	130	14	
	4	28	1	
Preoperative nerve damage	-	202	16	1.000[b]
	+	13	1	
Ipsilateral fracture	-	208	16	0.461[b]
	+	7	1	
Bicortical pin fixation	-	8	0	1.000[b]
	+	207	17	
Pin configuration	Only lateral	35	2	0.412[d]
	1 medial, 1 lateral	57	7	
	1 medial, multiple lateral	123	8	
Surgery	Closed	187	12	*0.075*[a]
	Open	28	5	
Pin Spread Ratio Median (IQR)		0.43(0.23-0.54)	0.37(0.28-0.48)	0.352[c]

[a] Pearson's chi square test, [b] Fisher's exact test, [c] Mann-Whitney U Test, [d] Fisher Freeman Halton Test, IQR: Interquartile range

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4. DISCUSSION

There are various techniques that describe immobilization after surgically treated supracondylar humerus fractures. The two most commonly used fixation methods are a simple posterior splint or a circular long-arm cast. Our results suggest that overall, long-arm cast is superior in protecting reduction than simple long-arm splint in the first three weeks postoperatively. The difference between both groups is probably due to subtle motion and loosening of the cotton and bandage with time (Figure 4).

Surgery is accepted as the most successful treatment method for displaced pediatric supracondylar humerus fractures, but differences between immobilization methods after surgery are not studied. Many studies mention circular casting or bivalved circular casting as postoperative immobilization method. Maintaining elbow flexion under 90° is suggested to decrease compartment syndrome risk [12]. Numerous studies defined LOR's risk factors for supracondylar humerus fractures in children, but none of them took the postoperative immobilization method as a parameter [11].

Mulpuri and Wilkins investigated 44 papers in their review article in 2012, where casting was mentioned as the sole immobilization method without comparing with other methods [13]. Baratz, et al., used splinting with the elbow flexed at 60°, whereas Kim and Sponseller did not mention postoperative immobilization at all [14, 15]. McKeon et al., used an A-frame cast for postoperative immobilization to leave the antecubital fossa free of casting material and stated that this technique provided enough stability without the risk of compartment syndrome [16]. Azzolin, et al., used a posterior plaster splint and a simple sling worn for 4 weeks, and also stated this as a simple and effective method for postoperative immobilization [17]. However, they did not compare their methods with another immobilization method. All studies mentioned above were Level IV studies (a report of multiple patients with the same treatment, but no control group or comparison group), while our retrospective cohort study is Level III (a study in which patient groups are separated non-randomly by exposure or treatment, with exposure occurring before the initiation of the study).

The overall LOR rate in this study was 7.3%. Various values for LOR were reported; like Balasubramanian 18.2%, Reisoğlu, et al., 13.6%, Pennock, et al., 4.2% [4, 7, 18]. Although, our LOR rate correlated with other studies, many different criteria were taken into account in different studies. It would not be appropriate to compare all of them in this aspect. As for LOR, we used Skaggs criteria, which took a change in Baumann angle for more than 6 degrees and deviation of the intersection of the anterior humeral line with the capitellum taken into account [19]. We also excluded seven patients who required early revision (in the first three days) postoperatively due to LOR caused by K-wires' misplacement or another poor perioperative technique. As a result, we were able to provide homogeneity between splinting and casting groups. Thus, there was a slight decrease in overall LOR but still correlated with previously published results [4,7,18].

Seventeen patients were assumed to have radiologic LOR at the third-week follow-up but did not undergo any revision procedure. As the study was designed in a retrospective manner, we did not call back the patients and perform a functional scoring. The only clue about the patients' functional level was obtained from patient files and families whether or not they stated any complaints, but we do not think this data to be enough to make inferences. Our results indicated that postoperative circular cast was only more protective against LOR radiologically. We could not make any implication for the mid-and long-term functional results, a fact which could only be evaluated by performing a long-term cohort study.

The only existing parameter between using either a circular long-arm cast or a simple splint was whether an open or closed surgical method was chosen. We accepted this as a natural phenomenon due to the need for dressing change and edema observation after an open surgery where immobilization with a rigid circular cast was avoided.

Although, there was no statistically significant difference between the open and closed surgery methods in terms of LOR, the difference was still remarkable. This was an expected event as the open surgery applied cases were much more complicated. Despite this difference, the splint and circular cast applied groups were assumed to be homogenous.

We did not perform any revision surgery for patients who sustained LOR at the third-week follow-up. This data supported studies questioning follow-up radiographic examinations published by Zusman, et al., Thompson, et al., and Tuomilehto, et al. [20-22].

The mean PSR of patients with postoperative splint was remarkably higher than the mean PSR of patients with a cast, but this difference was not statistically significant. As the group with higher PSR had less LOR, this result was supporting the study published by Aarons, er al., who failed to identify an optimal amount of PSR to prevent LOR [23].

Retrospective nature is a limitation of the study which we try to overcome, with homogenos groups for splint and cast. Another limitation is that we did not have accurate data for flexion-type injuries. We also did not calculated power of our retrospective study. It was observed that the choice for postoperative immobilization was absolutely surgeon dependent as the education of these surgeons were pointing out different training centers, so they all used their personal habits. We accept that as a limitation of our study.

Our results only indicated that circular casting was much more protective against LOR. However, we do not have any objective data referring these results clinical convenience. Further long-term follow-up results are required that should mainly focus on whether these reduction losses cause any restriction of joint motion or result in cubitus varus deformity.

Compliance with Ethical Standards

Ethical Approval: Ethical approval for this study was obtained from the Ethics Committee of Marmara University, School of Medicine. (Approval number: 09.2020.388).

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Acute and subacute effects of thymoquinone on acute methanol intoxication: An assessment based on serum TBARS and BDNF levels in rat model

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ABSTRACT

Objectives: Previous studies have shown the role of oxidative stress in methanol (MeOH) neurotoxicity. In clinical practice ethanol (EtOH) is used for the treatment of MeOH intoxication. Treatment with EtOH results in depression of the central nervous system, which may occur even at therapeutic doses. It also induces oxidative stress. Antioxidant and neuroprotective effects of thymoquinone (TQ) are known in different models of neurotoxicity. There are no studies investigating the protective effect of TQ against acute MeOH intoxication. We aimed to evaluate the effect of TQ administration on serum thiobarbituric acid reactive substances (TBARS) and Brain-Derived Neurotrophic Factor (BDNF) levels in rats with experimentally-induced MeOH intoxication.

Materials and Methods: Six groups were constituted. Methotrexate (Mtx) treatment (0.3 mg/kg/day) intraperitoneally (i.p.) was given for 7 days to slow down the formate metabolism of all rats except controls in order to create a MeOH intoxication similar to that in humans. On the 8th day of the experiment, 3 g/kg MeOH was injected i.p. in MeOH, EtOH and TQ groups. Four hours after MeOH administration, 0.5 g/kg EtOH was injected i.p. in EtOH group and 30 mg/kg TQ was administered i.p. in TQ1 and TQ2 groups. In addition, a total of 5 doses of 30 mg/kg TQ was injected i.p. 24, 48, 72 and 96 hours after the first dose in TQ2 group. Saline solution was given i.p. in the other groups. Blood samples were obtained for evaluating serum TBARS and BDNF levels.

Results: The highest TBARS level was found in MeOH+MTx group and this increase was statistically significant as compared to control and Mtx groups ($p<0.001$). A statistically significant reduction was detected in serum TBARS levels in MeOH+Mtx+EtOH, MeOH+Mtx+TQ1 and MeOH+Mtx+TQ2 groups ($p<0.001$). Maximum serum BDNF level elevation was found in MeOH+Mtx group and this increase was statistically significant as compared to control and Mtx groups ($p<0.001$). Serum BDNF levels were higher in MeOH+Mtx+EtOH, MeOH+Mtx+TQ1 and MeOH+Mtx+TQ2 groups and the difference was statistically significant ($p<0.001$).

Conclusions: Thymoquinone could suppress proinflammation and lipid peroxidation in MeOH intoxication, lead to rapid toxicity adaptation, and play the role of neuroprotection more effectively than EtOH. These results may suggest that TQ could be used as an alternative treatment option in MeOH intoxication.

Keywords: Methanol, Ethanol, Thymoquinone, TBARS, BDNF

1. INTRODUCTION

Methanol (MeOH) is an alcohol that is used as a component of some industrial products such as, perfume, cologne and antifreeze. MeOH intoxication, which may be accidental or intentional as a suicide attempt, can cause severe visual impairment, cerebral infarction and death by the development of formic academia and uncompensated metabolic acidosis [1]. Although, MeOH is not very toxic, it is metabolized into quite toxic substances including formaldehyde and formic acid by alcohol dehydrogenase. Formic acid leads to a reduction in adenosine triphosphate (ATP) synthesis, an elevation in reactive oxygen species (ROS) and cell death directly or through inhibiting cytochrome oxidase in mitochondrial respiratory chain [2]. Treatment is based on the inhibition of alcohol dehydrogenase enzyme that is the first step of conversion to formic acid that is responsible for MeOH intoxication. For this purpose, ethanol (EtOH) and fomepizol are used today of which affinity to alcohol dehydrogenase enzyme is higher than that of MeOH [3].

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Optic nerve damage and basal ganglion necrosis are the well-known side effects of MeOH intoxication. Visual impairment associated with MeOH toxicity begins within 12–48 h due to relatively severe, painless, bilateral optic nerve damage, which may be transient or progressive. Besides, degeneration in cerebellar granular cell layer, an elevation in ROS, a decrease in anti-oxidant capacity, toxic effect of MeOH in rat brain and the reduction in anti-oxidant capacity were shown in autopsy examinations of the cases of MeOH intoxication [4-6].

Thymoquinone (TQ; 2-isopropyl-5 – methyl-1, 4-benzoquinone) is the prime component of the essential oil obtained from Nigella sativa seeds. TQ is a potent antioxidant molecule used as a ROS scavenger for different models of oxidative stress. Also, previous studies have shown anti-inflammatory, immunomodulatory, and neuroprotective effects of TQ in different neurodegeneration and neurotoxicity models. It is thought that TQ's recovery effect on the neural tissue occurs via promotion of neurogenesis and nerve-regeneration, in addition to prevention of neuronal degeneration due to its antioxidant activities [7-10].

Thiobarbituric acid reactive substances (TBARS) are a direct index of cell lipid peroxidation. Brain-Derived Neurotrophic Factor (BDNF) is the most widely distributed neurotrophin in the central nervous system and performs many biological functions such as neural survival, differentiation and plasticity [11, 12]. Neurotrophins, antioxidant enzymes and oxidative markers have complex and reciprocal interactions. Therefore, utilization of antioxidant and antiinflamatory defense strategies may counterbalance oxidative damage and thereby ameliorate neurotrophic imbalance and signaling to protect neuronal cell. A growing number of experimental evidence have emerged, which support the concept that TQ with that strong antioxidant and anti-inflamatory activity ameliorates apoptosis, neurotrophic factors and oxidative stress and thereby may prevent damage to the neuronal tissue [11, 12].

Compared to humans, liver folate content of rats is higher and their folate metabolism is faster. Therefore, it is difficult to detect formic acid accumulation and metabolic acidosis. Experimental studies have shown that Methotrexate (Mtx) reduces folate content in rats. Therefore, Mtx is used to slow down the formate metabolism of rats in order to create a MeOH intoxication similar to that in humans.

Current research revealed that acute MeOH intoxication leads to effects very rapidly and in a very short duration. All this information suggests that TQ will provide good results for the treatment of acute MeOH intoxication. There are no studies investigating the protective effect of TQ against acute MeOH intoxication in the literature. To the best of our knowledge, this is the first study in literature investigating acute and subacute effects of TQ and EtOH treatments following acute MeOH intoxication on neurotrophic factor and oxidative stress levels through serum TBARS and BDNF levels.

2. MATERIALS and METHODS

This study was approved by the Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Center (Approval number: 68).

Animals

A total of 52 albino Wistar Albino male rats weighing 280-320 were used in the experiments. Animals were kept and fed at normal room temperature (22°C) prior to the experiment.

Chemicals

Methotrexate (Mtx, Koçak Farma, Istanbul, Turkey) was diluted in saline. MeOH, EtOH and TQ were purchased from Sigma Chemical Co (St. Louis, MO, USA). MeOH and EtOH were diluted in saline, and administered as a 20% w/v solution. TQ was dissolved in EtOH with further dilution in 0.9 % saline.

Experimental groups

This study included six groups, each containing nine rats except control group. The control group had seven rats. The groups were control, Methotrexate (Mtx), Mtx+MeOH, Mtx+MeOH+EtOH, Mtx+MeOH+TQ1, Mtx+MeOH+TQ2. The number of animals in the groups was determined by considering the necessity of using the minimum number of animals necessary for statistical significance.

Experimental procedure

Liver folate content is higher and folate metabolism is faster in rats as compared to humans. It is therefore difficult to develop formic acid accumulation and metabolic acidosis. Mtx was shown to reduce folate content in rats in experimental studies [5]. Therefore, all rats except controls were administered Mtx (0.3 mg/kg/day) intraperitoneally (i.p.) for 7 days for developing MeOH intoxication in rats similar to humans and for slowing formate metabolism. On the 8th day of the experiment, i.p. injection of MeOH (3 g/kg) was administered in MeOH, EtOH, TQ groups. Four hours after MeOH treatment, 0.5 g/kg EtOH was injected i.p. in EtOH group; 30 mg/kg TQ i.p. in TQ1 and TQ2 groups. In addition, a total of 5 doses of 30 mg/kg TQ was injected i.p. at 24, 48, 72 and 96 hours after the first dose in TQ2 group. Saline solution was given i.p. in the other groups. Rats were sacrificed 8 hours after the administrations with 50 mg/kg ketamin HCl anesthesia. Blood samples were obtained from the animals in order to determine serum TBARS and BDNF levels.

Biochemical analysis

Venous blood samples were collected by centrifugation at 4 ^{0}C and 1000 g for 10 minutes to separate serum. Serum samples were stored at – 80 ^{0}C until the start of the experiment. Serum TBARS (Oxford Biomedical Research, Kansas City, Missouri, USA) levels were measured using Enzyme-Linked Immuno Sorbent Assay (ELISA). The results were expressed in nmol/mL. Serum BDNF (Boster Biological Technology, CA,USA) levels were measured and the results were expressed in pg/mL.

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Statistical Analysis

The Kolmogorov-Smirnov test was used for the parametric distribution of numerical parameters. All data are expressed as mean ± standard error of the mean (x ± SEM). Biochemical results were analyzed using SPSS version 20 software (SPSS, Chicago, IL, USA). Difference of variances between the groups was analyzed by ANOVA followed by post-hoc Tukey test. Pearson's correlation test was used for correlation of numerical values. Statistical significance was declared at p<0.05.

3. RESULTS

At the end of the study, all rats were evaluated without any failure. Serum TBARS and BDNF levels of the groups are presented in Table I.

Table I. *Comparison of serum BDNF and TBARS levels*

Groups		BDNF (pg/mL)	TBARS (nmol/mL)
I-Control		86.82±4.53	2.55±0.17
II-Mtx		183.23±16.64	3.54±0.22
III-MeOH+Mtx		229.56±15.20	6.08±0.67
IV-MeOH+Mtx+EtOH		150.64±12.24	5.41±0.59
V-MeOH+Mtx+TQ1		129.52±10.32	3.06±0.25
VI-MeOH+Mtx+TQ2		106.09±11.02	2.30±0.22
P values			
I-II		<0.001	<0.001
I-III		<0.001	<0.001
I-IV		<0.001	<0.001
I-V		<0.001	<0.001
I-VI		<0.001	<0.001
II-III		<0.001	<0.001
III-IV		<0.001	0.040
III-V		<0.001	<0.001
III-VI		<0.001	<0.001
IV-V		0,001	<0.001
IV-VI		<0.001	<0.001
V-VI		<0.001	<0.001

The highest TBARS level was detected in MeOH+Mtx group and this elevation was statistically significant as compared to control and Mtx groups (p<0.001). A reduction was detected in TBARS levels in treatment groups (MeOH+Mtx+EtOH, MeOH+Mtx+TQ1, MeOH+Mtx+TQ2) as compared to intoxication group (MeOH+Mtx) and the difference was statistically significant (p:0.040, p<0.001 and p<0.001, respectively). TBARS levels in groups MeOH+Mtx+TQ1 and especially MeOH+Mtx+TQ2 were found to be similar with those in control group. The reduction in TBARS levels in MeOH+Mtx+TQ2 group as compared to MeOH+Mtx+TQ1 group was statistically significant (p<0.001).

Maximum serum BDNF level elevation was found in MeOH+Mtx group and this increase was statistically significant as compared to control and Mtx groups (p<0.001). Serum BDNF levels were statistically significantly higher in MeOH+Mtx+EtOH, MeOH+Mtx+TQ1 and MeOH+Mtx+TQ2 groups as compared to control (p<0.001). A decrease was detected in serum BDNF levels in treatment groups (MeOH+Mtx+EtOH, MeOH+Mtx+TQ1, MeOH+Mtx+TQ2) as compared to intoxication group (MeOH+Mtx) and the difference was statistically significant (p<0.001). Despite the statistically significant elevation in serum BDNF levels in MeOH+Mtx+TQ1 and particularly in MeOH+Mtx+TQ2 groups (p<0.001), especially in the MeOH+MTX+TQ2 group, the serum BDNF level was closest to the control group.

Pearson's correlation analysis revealed a strong positive correlation between TBARS and BDNF (r:0.773, p:<0.001;Figure 1).

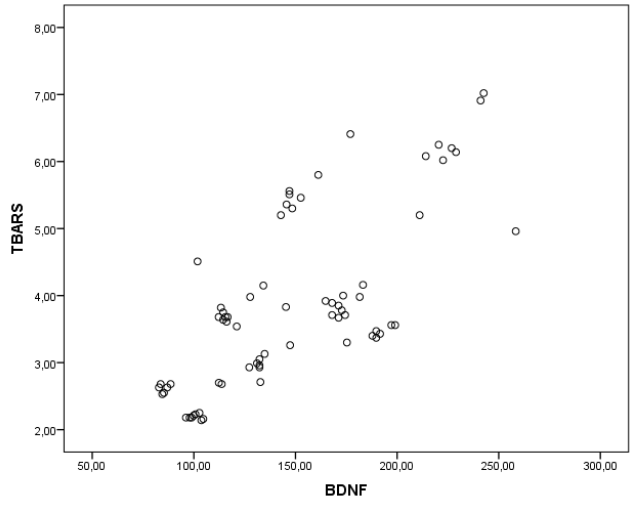

Figure 1. *Pearson's correlation coefficient (r) and p-value (p) between TBARS and BDNF (r:0.773 p:<0.001)*

4. DISCUSSION

In this experimental study, we have investigated the role of acute and subacute effects of TQ on serum TBARS and BDNF levels in rat model with acute MeOH intoxication. It has been concluded that TQ administration could significantly suppress proinflammation and lipid peroxidation that occur during MeOH intoxication, and may also lead to rapid toxicity adaptation.

Although, MeOH is not very toxic, it is metabolized into quite toxic substances including formaldehyde and formic acid by alcohol dehydrogenase. Formic acid leads to a reduction in ATP synthesis, an elevation in ROS and cell death directly or through inhibiting cytochrome oxidase in mitochondrial

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respiratory chain [2]. ROS are continuously produced during normal physiologic events and removed by antioxidant defense mechanism. The imbalance between ROS and antioxidant defense mechanisms leads to lipid peroxidation and oxidative damage in the lipid bilayers surrounding both the cell itself and membrane-bound organelles. Rajamani et al., suggested that MeOH exposure results in increased free radical generation and significant protein oxidative damage in the retina and optic nerves of the rats [5].

Thiobarbituric acid reactive substances are a direct index of cell lipid peroxidation [11]. In our study, we have detected a significant elevation in serum TBARS level that is a standard marker for lipid peroxidation, particularly in intoxication group (MeOH+Mtx). A reduction was detected in TBARS levels in treatment groups (MeOH+Mtx+EtOH, MeOH+Mtx+TQ1, MeOH+Mtx+TQ2). In the MeOH+MTX+TQ1 and especially in the MeOH+MTX+TQ2 group, TBARS levels were almost similar to the control group. In the study plan, single dose of TQ was given to MeOH+Mtx+TQ1 group and acute effects were tried to be measured. On the other hand, a total of 5 doses of TQ were given to MeOH+Mtx+TQ2 group with 24 hours of intervals and subacute effects were tried to be measured. Results of the study reveal that TQ treatment is effective in both acute and even more evident in subacute period through effectively reducing TBARS levels. The present study also reveals that TQ treatment is quite effective for preventing lipid peroxidation and this effect is more evident in MeOH+Mtx+EtOH group. Given that this effect continues even at the end of 96th hour that may be defined as subacute period, it may be suggested that TQ treatment could be a strong lipid peroxidation preventer in MeOH intoxication that may continue its toxic effect between 12 and 48 hours. Administration of TQ in subacute period has not been encountered in literature. We would like to state that the result of the study is an important stage for treatment stages of MeOH intoxication that lead to severe and irreversible damage particularly in neuronal tissue [1,5,13].

Brain-derived neurotrophic factor is the most ubiquitous and intensively studied member of the family of neurotrophins in the central nervous system (CNS). In our study, maximum serum BDNF elevation was detected in MeOH+Mtx group. Serum BDNF levels decreased in treatment groups (MeOH+Mtx+EtOH, MeOH+Mtx+TQ1, MeOH+Mtx+TQ2) and BDNF levels in MeOH+Mtx+TQ2 group were similar to those in control group. In a study, evidence was reported that the elderly women with acute low back pain exhibited higher plasma BDNF levels as compared to the subjects without pain and the researchers stated that pro-inflammation in the low back pain group could increase BDNF protein expression [14]. In another study, plasma BDNF levels of metamphetamine users were found to be significantly higher than controls and the researchers stated that this result provided evidence that BDNF played an important role for neuro-adaptation against metamphetamine neuro-toxicity [15]. In our study, the significant increase in BDNF levels in MeOH+Mtx group may suggest toxicity adaptation against acute MeOH toxicity and that acute-toxicity-induced pro-inflammation increased BDNF protein expression.

The significant decrease in BDNF levels in treatment groups (MeOH+Mtx+EtOH, MeOH+Mtx+TQ1, MeOH+Mtx+TQ2) as compared to MeOH+Mtx group may indicate that toxicity adaptation rapidly develops through EtOH and TQ treatments and also pro-inflammation is suppressed. Given the subacute course of the treatment particularly in MeOH+Mtx+TQ2 group and the similar BDNF values with control group, it may be considered that aforementioned potential mechanisms work more effectively.

Brain-derived neurotrophic factor is associated with several processes that are essential for the optimal functioning of especially neural tissues. Several studies reported altered brain and plasma BDNF levels in patients with various brain pathologies [16]. However, the pathophysiological mechanisms that underlie these changes are not yet fully understood. There is also no conclusive evidence that can discriminate whether changes in BDNF levels are a causative or the consequence of the disease onset. Neuro-inflammation is regulated by factors that are also involved in modulation of BDNF expression. Both neuro-inflammation and altered BDNF expression are common phenomena in many disorders especially neural disorders. The optic nerve, retina, and basal ganglia are the main tissues that are at risk from MeOH intoxication which may be associated with the high mitochondrial energy requirement in these tissues. Remarkably, there are only few studies that have investigated the link between BDNF and neuro-inflammation. Better understanding of the interaction between BDNF and neuro-inflammation could open new ways for therapy management and could facilitate the development of new therapeutic strategies for neurological diseases [16].

Pearson's correlation analysis revealed a strong positive correlation between TBARS and BDNF in the present study (r:0.773 p:<0.001). A positive correlation was detected between TBARS and BDNF in the previous *in vivo* and *in vitro* studies. In an *in vivo* study, elevated BDNF levels were explained with compensation mechanism of the metabolic stress and related oxidative damage [17-19]. The positive correlation between TBARS and BDNF suggest that further studies are required about the intra-cellular interactions of neurotrophins, anti-oxidant enzymes and oxidative markers.

No studies investigating the protective effect of TQ against acute MeOH intoxication were found in the literature. Studies have shown anti-inflammatory, immunomodulatory, and especially neuroprotective effects of TQ in different models of neurodegeneration and neurotoxicity. It is thought that TQ's recovery effect on the neural tissue occurs via promotion of neurogenesis and nerve-regeneration, in addition to prevention of neuronal degeneration due to its antioxidant and antinflammatory activities. In the present study, it was aimed to reveal that TQ treatment could be effective both in acute and subacute processes of MeOH intoxication through serum TBARS and BDNF levels. As a result, it was concluded that TQ administration could suppress proinflammation and lipid peroxidation occurring in acute and subacute periods of MeOH

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intoxication, lead to rapid toxicity adaptation and perform it more effectively than EtOH treatment. These results may show that TQ could be used as an alternative treatment in MeOH intoxication. Also, further studies that examine serum, tissue and histopathological data together are required in order to clearly reveal the effects of TQ treatments on MeOH metabolism.

Compliance with Ethical Standards

Ethical Approval: This study was approved by Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Center (Protocol number: 68). All methods were performed in accordance with the relevant guideline regulations.

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Effect of molecular subtypes on radiotherapy response in patients with breast cancer brain metastasis

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ABSTRACT

Objective: This study aimed to investigate survival and the response to radiotherapy (RT) among patients with molecular subtypes of breast cancer brain metastases.

Patients and Methods: We retrospectively analyzed the data of 139 breast cancer patients with brain metastases treated with whole-brain radiotherapy (WBRT) or focal brain treatment (FBT) between 2006 and 2019. Overall survival (OS) and brain metastasis progression-free survival (BMPFS) were calculated from the first RT until death or the last follow-up. Survival analyses were performed using the Kaplan–Meier method. Prognostic factors were evaluated using the Cox proportional hazard model.

Results: Twenty three (16.5%), 77 (55.4%), 14 (10.1%), and 25 (18%) patients were diagnosed with triple-negative, HER-2 (+), luminal-like A, and luminal-like B breast cancer, respectively. Of 139 patients, 66 (47.8%) underwent FBT, and 73 (52.5%) underwent WBRT. While the most preferred fraction was 10*300 Gy in WBRT, doses of 15-25 Gy in 1-5 fractions were preferred in local RT. We observed that age, Karnofsky performance scale (KPS) score, initial RT technique, extracranial disease, number of brain metastases impacted OS and BMPFS.

Conclusion: Breast cancer brain metastasis is a different, complex, and challenging disease based on the molecular subtype of the tumor, despite various local treatments. Therefore, appropriate and tailored treatment approaches should be considered for the different molecular subtypes.

Keywords: Breast cancer, Molecular subtype, Brain metastasis, Radiotherapy, Survival outcomes

1. INTRODUCTION

Metastatic breast cancer (MBC) is the second most common cause of brain metastases (BM) among solid malignancies [1, 2]. Approximately 10-30% of all breast cancer patients develop breast cancer brain metastases (BCBM) with a median survival of 14 months [3-5].

Breast cancer is divided into molecular subtypes depending on the presence or absence of the estrogen receptor (ER) and human epithelial growth factor receptor-2 (HER-2). Frequency and incidence of BCBM change based on molecular subtypes such as luminal A and luminal B type, HER-2 type, and triple-negative (TN) type; therefore treatment strategies need to be changed depending on the subtype [6, 7]. Despite recent advances in systemic treatment, HER-2 and TN subtypes still exhibit shorter survival rates than luminal subtypes [8]. In addition, while the frequency of brain metastasis development in luminal subtypes is <10%, this rate varies between 20%-30% in TN and HER-2 positive subtypes [9].

Current treatment options for patients with BCBM include surgical resection, focal brain treatment (FBT) (surgery or RT), whole-brain radiation therapy (WBRT), chemotherapy, and targeted therapy. The National Comprehensive Cancer Network (NCCN) treatment guidelines for brain metastasis are based on the number of metastases. Tumor removal, WBRT, and FBT are recommended in patients with 1 – 3 limited metastatic lesions, but WBRT or FBT are recommended for patients with more than three lesions [10-12]. The response rates to treatment in brain metastases vary according to the number of metastases, location, performance status, and subtypes [13]. To prevent systemic progression and the development of new metastases, primary systemic treatments, including hormonal therapies, targeted

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agents, and immunotherapies, are applied in combination or sequentially with versatile treatment modalities [14].

In this retrospective study, we aimed to investigate the factors affecting oncological outcomes including; overall survival (OS), brain metastasis-free progression-free survival (BMPFS), association between breast cancer subtype, and intracranial recurrence patterns in patients who developed brain metastasis at the time of admission or during follow-up after adjuvant radiotherapy for breast cancer.

2. PATIENTS and METHODS

The study included one hundred and thirty-nine breast cancer patients who developed brain metastases in their follow-up after adjuvant breast cancer RT or who had brain metastases at admission and who had undergone WBRT or FBT between 2006 and 2019. This study was approved by the the Institutional Review Board of Kartal Dr. Lütfi Kırdar City Hospital (Approval number 2018/514/122/5 on 30.01.2018). This retrospective design exempted this study from the requirement of obtaining written informed consent from the patients.

Patient Characteristics

Women older than 18 years were eligible for this study. All the patients were diagnosed with breast cancer and brain metastases. Male patients were excluded from this study. ER status, progesterone receptor (PR) status, and HER-2 expression and/or amplification status were collected for patients based on primary and/or metastatic breast cancer pathology analysis. Breast cancer subtypes were classified according to the criteria described by St. Gallen in 2015. Luminal A (ER+/HER2−, grade 1-2, Ki 67 ≤ 20% and /or low mitotic index), luminal B (ER+/ HER2+, grade 3, Ki 67 > 20% or high mitotic index), and HER-2 (HR−/HER2+) and triple-negative (TN) (ER−, PR−, HER-2 −) [15]. Demographic patient data (age, date of brain metastasis diagnosis, the number of brain metastases, Karnofsky performance scale (KPS) score at initial RT, extra-cranial disease, tumor molecular subgroup, RT type, and RT dose delivered) were collected from the electronic medical records. Patients with metastatic breast cancer treated with more than one course of brain metastasis irradiation were identified, and the clinical outcomes of re-irradiation in these patients were investigated.

Radiotherapy

Patients diagnosed with primary breast carcinoma that metastasized to the brain and treated with WBRT, FBT, or both were included. The clinical treatment volume (CTV) was determined as the brain parenchyma, and the margin was defined as the planned treatment volume (PTV). In single or oligometastatic lesions, RT was planned without margin for gross treatment volume in the post-surgical cavity or primer radiosurgery applications. Radiosurgery, (primary or postoperative cyberKnife and Gamma-Knife), and linac-based planning systems were applied as local treatments.

Statisticsal Analysis

Clinical outcomes were determined as primary endpoint OS and secondary endpoint as BMPFS. OS was defined as the time from the initial brain metastasis diagnosis to the time of death or the last follow-up. At the same time, BMPFS was defined as the time from the initial brain metastasis diagnosis to the time of BM progression. Statistical analysis was conducted using the SPSS 23 (version 23) program. Frequency distribution (number and percentage) for categorical variables and descriptive statistics (mean, standard deviation, median, minimum, and maximum) were applied for numerical variables. One-way analysis of variance (ANOVA) was used to determine whether there was a difference between more than two groups. Kaplan-Meier analysis was used to examine the differences in patient survival according to age, presence of metastasis at baseline, and subtypes. The significance level was accepted as $p<0.05$.

3. RESULTS

From 2006 to 2019, 139 patients diagnosed with breast cancer, who received RT for brain metastasis during their follow-up or admission, or who received a second course of WBRT or FBT with cranial recurrences and new lesion development during follow-up were included. Patient characteristics are summarized in Table I. The median age at diagnosis of BM was 54 years (range, 30-94). The median follow-up period from the initial breast cancer diagnosis was 83 months (range, 45.8-120). The distribution of patients according to subtypes was TN 16.5% (23), HER-2 (+) 55.4% (77), luminal-like A 10.1% (14), luminal-like B 18%. Brain metastases represented the only intracranial metastases in 82 patients (59 %), while 14 patients (10.1 %) had both brain and systemic metastases. The median KPS score before the initial RT was 90 (range, 70-100). The median elapsed time until progression after the initial RT was ten months (range, 1-116). KPS median before the second-course RT was 90 (range, 60-100). After the first RT, local progression was observed in 50 patients (38.5%), distant intracranial metastasis developed in 59 patients (45.4%). The median elapsed time until progression after the second-course RT was nine months (range, 2-24). Before the third course RT, the median KPS was 90 (range, 60-90). The median elapsed time until progression after the third course of RT was six months (range, 1-12). After the third course RT, five patients (3.5%) developed distant intracranial metastasis, and three patients (2.1%) had an intracranial progression. The median KPS before the fourth course RT was 80 (range, 70-90). In total, WBRT was administered to 97 of 139 patients, FBT to 128 lesions in 139 patients, second-course WBRT in 4 patients, and second-course FBT in 2 patients.

Treatment outcomes

The median OS was 71 months (95% CI, 46.2–95.7 months), and the median BMPFS was 14 months (95% CI, 5.3–22.6 months). The difference between overall survival and the presence of metastasis at admission according to subtype was not statistically significant ($p>0.05$). The median overall survival according to HER-2 (+), TN, luminal A and luminal B subtypes was 146, 106, 101, and 59 months, respectively (Figure 1). The BMPFS analysis showed no statistically significant difference between subtypes, age groups, and the presence of metastasis at baseline in terms of progression-free survival time after cranial metastasis ($p>0.05$).

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Table 1. Characteristics of patients

	n	(%)
Patients	139	(100)
The median age in years at brain metastasis diagnosis	54 (range, 26-94)	
Median follow-up in months	83 (range, 45.8 – 120)	
Initial T stage		
< 2 cm	14	(11.1)
2-5 cm	62	(44.1)
> 5 cm	59	(42)
Unknown	4	(2.8)
Menopause		
Premenopausal	79	(56.8)
Postmenopausal	60	(43.2)
Histology		
Invasive ductal carcinoma	124	(89.2)
Invasive lobular carcinoma	6	(4.3)
Other	9	(6.5)
Estrogen receptor status		
Positive	74	(53.2)
Negative	65	(46.8)
Progesterone receptor status		
Positive	68	(48.9)
Negative	71	(51.1)
HER-2 status		
Positive	68	(48.9)
Negative	24	(17.3)
Unknown	47	(33.8)
Subtype		
Basal	23	(16.5)
Luminal A	14	(10.1)
Luminal B	25	(18)
HER-2	77	(55.4)
Chemotherapy		
Yes	131	(94.2)
No	8	(5.8)
Targeted therapy n %		
Yes	77	(56.2)
No	34	(24.8)
Unknown	26	(19)
Extra-cranial disease		
Yes	58	(40.6)
No	43	(30.1)
Unknown	38	(27.3)
Radiation therapy		
Initial whole breast radiotherapy	72	(52.1)
Initial focal brain treatment	66	(47.8)
Number of brain metastasis		
1-4	88	(70.6)
> 4	51	(29.4)

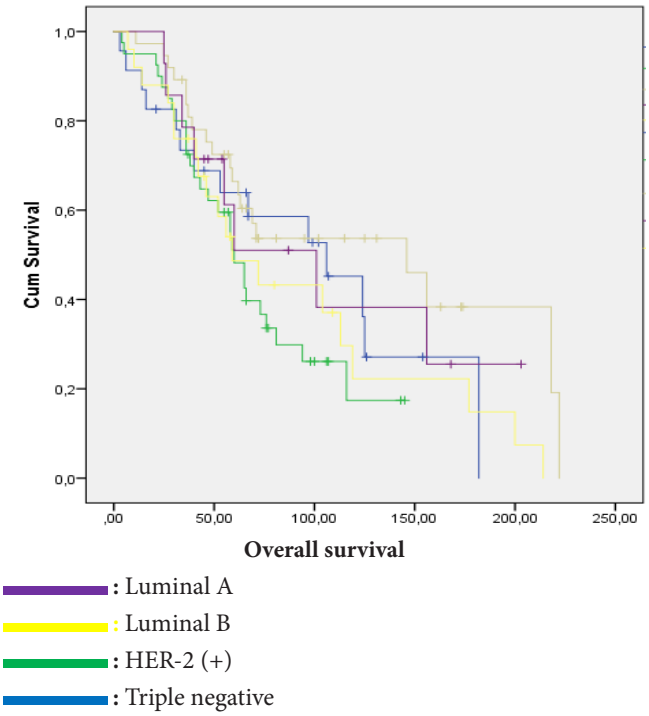

Figure 1. Overall survival among patients according to molecular subtypes

Of the 139 patients examined, 73 (52.5%) received WBRT for the brain metastasis, and 66 (47.8%) received FBT. WBRT was delivered at a median dose of 30 Gy in ten fractions. Doses of 15-25 Gy in 1-5 fractions were preferred for FBT. After the first RT, local progression was observed in 50 patients (38.5%), and distant intracranial metastasis developed in 59 (45.4%) patients. As secondary-course RT, FBT was received in 50 patients (38.5%), WBRT to 20 patients (15.3%), and second-course WBRT to 2 patients (1.5%). After the second-course RT, four patients (3%) underwent cranial metastasectomy. Therefore, WBRT was administered to 97 of 139 patients, FBT to 128 lesions in 139 patients, second-course WBRT in 4 patients, and second-course FBT to 2 patients. The change in the time elapsed until the progression after radiotherapy applications according to molecular subtypes did not show a statistically significant difference ($p>0.05$). Eighty-nine patients (64%) died due to brain metastasis during follow-up after treatment. While the highest mortality rate was observed in the HER-2 (+) subtype with 28 patients (31.8%), the lowest mortality rate was in the luminal-like A subtype with eight patients (9.1%).

4. DISCUSSION

The risk of developing brain metastasis is estimated to be as high as 25% among patients with breast cancer, with a median time of brain metastasis occurrence 2–3 years after the initial breast cancer diagnosis [16]. High tumor burden, HER-2 positivity, hormone receptor negativity, young age, and the presence of visceral organ metastasis are important

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predictive factors for the development of brain metastasis in breast cancer [17-21]. However, KPS is known to be a critical prognostic factor for demonstrating treatment effectiveness and response [21, 22]. In our study, the median KPS values before the initial RT, before the second-course RT, before the third course RT and before the fourth course RT were 90, 90, 90, and 80, respectively. Treatment of brain metastasis according to location, number of lesions, performance status, and biological subtype also makes treatment complex and challenging [23]. Although, the development and treatment of breast cancer brain metastasis involve many difficulties, FBT or WBRT, surgery, and chemotherapy are used in combination or separately with targeted agents and immunotherapy. WBRT combined with FBT showed a better survival advantage than WBRT alone. However, the superiority of salvage WBRT and FBT over FBT alone had not been demonstrated [24-29]. Although, there have been many improvements in the treatment of breast cancer patients diagnosed with brain metastasis over the years, the development and causes of brain metastasis according to molecular subtypes are still unknown [30]. In our study, the change in the time elapsed until the progression after radiotherapy applications according to molecular subtypes, did not show a statistically significant difference.

Breast cancer brain metastasis patients with luminal A and luminal B had the best OS, TN had the worst OS [7, 31-35]. In addition, the response to RT varies according to the subtype of BCBM. In two different studies according to subtypes, survival differences were stated as 7.3 months/7 months in TN subtype, 17.9 months/23 months in HER2 (+) subtype, 10 months/16 months in luminal-like A subtype, and 22.9 months/26 months in luminal-like B [31, 36]. In our study, the median OS according to the subtypes HER-2 (+), TN, luminal A and luminal B were 146, 106, 101, and 59 months respectively.

Hicks et al., stated that there is a risk of brain metastasis in the presence of visceral metastasis in the HER-2 subtype and TN subtype, independent of the stage [37]. Generally, the response to treatment in HER-2 (+) subtype were better than HER-2 negative. The median survival with local RT in HER-2 (+) brain metastasis was 31.3 months, it was 14.1 months for HER2 (-) disease [38]. The SEER database study by Wang et al., reported that patients with luminal A (HR+/HER-2 negative) subtype had a high incidence of brain metastasis and also showed that the HER-2 subtype had a more favorable cancer-specific survival rate [39-45].

Studies stated that when WBRT was used together with systemic therapy, it increased the effectiveness of the drug in brain metastasis and the response rates to treatment by 4-38% [46-49]. Although, we did not evaluate the effectiveness of specific chemotherapy together with RT in this study, 94.2% of the patients received neoadjuvant/adjuvant/palliative chemotherapy. According to a study investigating the efficacy of trastuzumab with RT, an increased level of trastuzumab (a monoclonal humanized antibody approved for the treatment of HER-2 (+) breast cancer) was observed in the cerebrospinal fluid after RT when compared with the level before RT [50-52]. Lapatinib (a dual HER-2 and epidermal growth factor receptor (EGFR) inhibitor) is another anti-HER-2 agent used in breast cancer treatment, similar to trastuzumab. However, studies have shown that a single dose of lapatinib has a higher complete response rate than local RT alone [53]. WBRT and/or FBT with targeted agents such as trastuzumab and lapatinib provide better local and distant control [54]. Although, there is no survival advantage in combined use of lapatinib with FBT over the single use of FBT, many studies have shown that it increases the median survival [54-57]. In retrospective series, studies have shown that lapatinib with FBT has a survival advantage [58].

The most significant limitation of our study is its retrospective design. In addition, HER-2 target therapy and chemotherapy information are not known in detail, and its association with RT has not been investigated. Although, the presence of metastasis on admission was evaluated in our study, visceral and other organ metastases were not assessed. However, visceral metastasis is a prognostic determinant of breast cancer brain metastasis and determines poor prognosis independent of subtype [59].

Conclusion

The response to treatment and disease-related survival vary significantly according to the molecular subtypes of breast cancer brain metastasis. Molecular subtype is an independent predictor of OS, regardless of whether the patient received any local or systemic treatment. With the contribution of RT and new agents, the survival rate of patients with HER2 (+) subtype increased when compared to that of the other subtypes. Breast cancer brain metastasis is a different, complex, and challenging disease, and tailored treatment approaches based on the molecular subtype should be considered to improve outcomes.

Compliance with Ethical Standards

Ethical approval: The study was approved by the Kartal Dr. Lütfi Kırdar City Hospital Ethical Board (Approval number: 2018/514/122/5 date: 30.01.2018).

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Conflict of Interest: The authors have no conflicts of interest to declare.

Author Contributions: AAE and MFE : Concept and design of the study, AAE : Data acquisition, MFE : Statistical analysis, AAE and MFE : Literature Review, MFE and AAE: Drafting and Writing. Both authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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Effect of different immobilization media on breakdown of whey proteins by *Streptococcus thermophilus*

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ABSTRACT

Objective: In this study, we aimed to compare the efficiency of different immobilization media to facilitate breakdown of whey proteins by *Streptococcus thermophilus (S. thermophilus)*.

Materials and Methods: *S. thermophilus* was isolated from yoghurt. High-protein whey powder was present in fermentation media and two-phase dispersion technique was used for immobilization of *S. thermophilus* in agar, agarose and κ-carrageenan. Total protein after fermentation of whey proteins with *S. thermophilus* in different media was measured. We have also performed sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis to observe changes in individual whey proteins after fermentation in different media.

Results: Total protein concentration showed a significant decrease at the end of 24 hours of fermentation in all media. SDS-PAGE results showed that the amount of both α-lactalbumin and β-lactoglobulin were reduced in all immobilization media compared to control. The effect of κ-carrageenan was considerably higher compared to other media.

Conclusion: Our results showed that immobilization in κ-carrageenan increased the breakdown of whey proteins by *S. thermophilus* and can be used to increase fermentation efficiency.

Keywords: α-lactalbumin, β-lactoglobulin, Fermentation, Immobilization, κ-carrageenan

1. INTRODUCTION

Milk is an excellent source of fat, proteins, minerals and vitamins [1]. Cow's milk consists of 90% water, 5% carbohydrates and 4–5% proteins. Milk proteins are divided into two classes (groups): soluble whey proteins and casein phosphoproteins. Soluble whey proteins comprise β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin and lactoferrin. In addition, a large number of other proteins are present at lower concentrations [2]. The main allergens in milk are caseins, β-lactoglobulin and α-lactalbumin. Reduction or elimination of milk allergens by effective methods and technologies is essential to help consumption of milk by individuals who are allergic to these ingredients.

Fermentation is a traditional food processing technology. Fermented foods exert a positive influence on human health. This is mainly due to the ability to release bioactive peptides from food proteins by microbial enzymatic hydrolysis. It has been shown that lactic acid bacteria possess a complex proteolytic system composed of proteinases, peptidases and transport proteins. During fermentation, hydrolysis of milk proteins by lactic acid bacteria may have important effects on milk digestibility and production of bioactive peptides. Moreover, proteolysis can destroy some epitopes and consequently decrease allergenicity. Dietary consumption of probiotics and fermented foods, i.e. yoghurt can alleviate some symptoms of atopy and reduce development of allergies through immune regulation [3].

Immobilized cells exhibit many advantages over floating cells in food processing [4]. Among these are maintenance of stable and active biocatalysts, reuse of biocatalysts, accelerated reaction rates, high volumetric productivity, improved process control, reduced susceptibility of cells to contamination, improved production efficiency and no possibility of cell wash-out [5]. Gel entrapment in natural polymers such as

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alginates and κ-carrageenans is favored by many workers for immobilizing living cells [6-10]. These mild, cheap and simple methods of entrapment are non-toxic and preserve the integrity of immobilized biocatalysts. The choice of the entrapment polymer depends on the conditions of the fermentation process [4]. Alginate and κ-carrageenan gels have been widely used with lactic acid bacteria [5,11-13]. In this study, we aimed to compare the efficiency of different immobilization media to facilitate breakdown of whey proteins by *S. thermophilus*.

2. MATERIALS and METHODS

S. thermophilus isolated from yoghurt was used. Home-made yoghurt was mixed with an equal amount of phosphate-buffered saline (PBS). After 72 hours of incubation on M17 agar, resulting colonies were identified as *S. thermophilus* (99%) *subsp. salivarius* (33%) [14]. High-protein whey powder (6%) was centrifuged at 3.900 x g, 4°C for 1 hour and supernatant was used in fermentation media (pH 6.5, 200 mL). Sterilization was performed using two different techniques: serial filtration was performed (5.0 µm, 0.45 µm and 0.22 µm in order) and filtered whey protein supernatant (WP-F) was kept at 4°C, whey supernatant was autoclaved at 121°C for 15 minutes to obtain whey protein (WP-A) that was also kept at 4°C.

***Immobilization*:** Two-phase dispersion technique was used to achieve immobilization in agar, agarose and κ-carrageenan [15].

Agar: 1 g agar was mixed with 50 mL ultra-pure H_2O and autoclaved at 121°C for 15 min. Polymer was cooled down to 50-55°C and then 100 µL of bacteria (1 x 10^7 cfu/mL) was added and dropped into the oil-phase serially (1 mL). Beads were left at oil-phase for 2.5 min for spherical shaping to be completed, washed with 0.01% Triton-X and put into the aqua-phase (ultra-pure water) and left for 5 min. Finally, beads were mixed with whey medium (5 mL).

Agarose: 1 g agarose was mixed with 50 mL ultra-pure H_2O and autoclaved at 121°C for 15 min. Polymer was cooled down to 40-45°C and then 100 µL of bacteria (1 x 10^7 cfu/mL) was added and dropped into the oil-phase serially (1 mL). Beads were left at oil-phase for 2.5 min for spherical shaping to be completed, washed with 0.01% Triton-X and put into the aqua-phase (ultra-pure water) and left for 5 min. Finally, beads were mixed with whey medium (5 mL).

κ-carrageenan: 1.5 g κ-carrageenan was mixed with 75 mL ultra-pure H_2O and autoclaved at 121°C for 15 min. Polymer was cooled down to 50-55°C and then 100 µL of bacteria (1 x 10^7 cfu/mL) was added and dropped into the oil-phase serially (1 mL). Beads were left at oil-phase for 2.5 min for spherical shaping to be completed, washed with 0.01 Triton-X and put into the aqua-phase (0.3 M KCl) and left for 5 min. Finally beads were mixed with whey medium (5 mL).

The protocol of Nillson et al. was used for immobilization in polyacrylamide and Sephadex [15]. Polymerization of acrylamide was not possible. Increasing the concentrations of ammonium persulfate and tetramethylenediamine which are both toxic to cells would not be appropriate, therefore this method was abandoned. Polymerization of Sephadex was achieved but beads were disintegrated. This method was also not used.

***Fermentation*:** Fermentation medium for non-immobilized floating cells was obtained as follows. Five mL of WP-F was mixed with 100 µL bacteria (1x10^7 cfu/mL). Immobilized and free cells were left for fermentation at 40-42°C in an incubator for 24 and 48 hours. After completion of the fermentation, fermentation media of free cells were centrifuged at 20,000 x g and 4°C for 15 minutes. The supernatants were collected and cells were stored in 0.9% saline at – 20°C. Beads of κ-carrageenan, agar and agarose were centrifuged at 500 x g and 4°C for 5 minutes to separate cells from the immobilization medium. Supernatants were collected and centrifuged at 20,000 x g and 4°C for 15 minutes. Cells were stored at – 20°C in 0.9% saline. All experiments were conducted in duplicate.

McFarland standard (0.5, 1x10^8 cfu/mL) was used for spectrophotometric enumeration of bacteria and a cell concentration of 1 x 10^7 cfu/mL was used for fermentation media [16]. Total protein concentration of supernatants after fermentation was measured using Pierce BCA Protein Assay Kit (Thermo Scientific, USA). Sodium dodecyl sulfate polyacrylamide gel electrophoresis {SDS-PAGE] analysis of whey proteins was performed using 12% gel and Coomassie Brilliant Blue staining.

The study was approved by Marmara University Ethics Committee (Approval no. 09.2022.245).

Statistical Analysis

Statistical analysis of data was performed with Prism 7.04 (GraphPad Software Inc., USA).

3. RESULTS

Table I shows total protein concentrations after fermentation of whey proteins with *S. thermophilus* in different media. Total protein concentration of κ-carrageenan immobilization was decreased compared to other media at the end of 24 hours of fermentation. No significant change was observed between two different fermentation periods (24 and 48 hours). We have performed SDS-PAGE analysis to observe changes in individual whey proteins after fermentation in different media (Figure 1). Both α-lactalbumin and β-lactoglobulin were decreased at the end of 24 hours of fermentation in all media. The greatest reduction was in κ-carrageenan compared to other media and control. There was no significant difference in color intensity at the end of 48 hours compared to 24 hours.

Table I. *Total protein concentrations 24 hours after fermentation of whey proteins with S. thermophilus in different immobilization media*

Immobilization media	Total protein (mg/mL)
None	2.035 ± 0.078
Agar	1.115 ± 0.097
Agarose	1.180 ± 0.050
κ-carrageenan	0.771 ± 0.031

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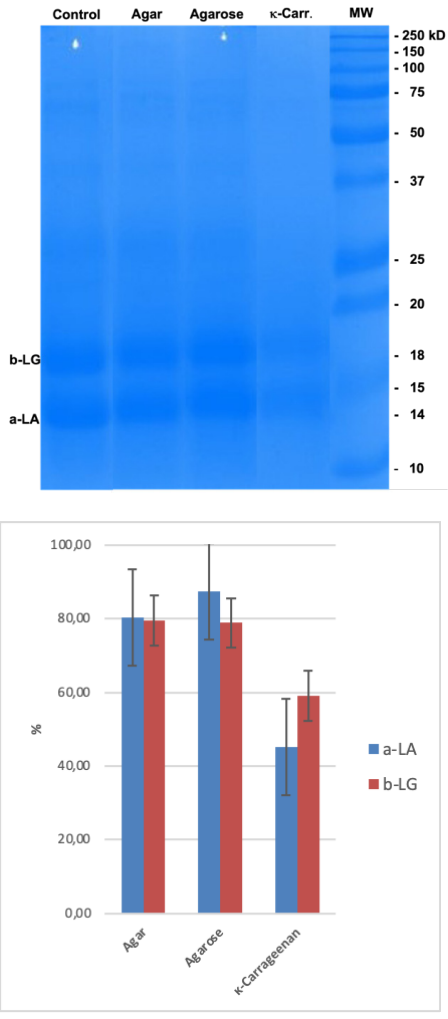

Figure 1. *SDS-PAGE analysis of whey proteins after fermentation with S. thermophilus.*
Lane 1: Control; Lane 2: Agar; Lane 3: Agarose; Lane 4: κ-Carrageenan; Lane 5: MW Markers

The graph shows the results of densitometric scanning and percentage decrease in the intensity of α-lactalbumin and β-lactoglobulin bands compared to control.

4. DISCUSSION

Food allergy is an important health problem and includes the adverse health effects in which immunological mechanisms are involved [17]. Most cases of food allergy are associated with a limited range of products. The most commonly allergenic foods are considered to be cows' milk, hens' eggs, peanuts, tree nuts, soy, wheat, shellfish and fish [18]. Fermentation of milk and milk allergens (such as α-lactalbumin, β-lactoglobulin, α-casein and β-casein), with Lactobacilli strongly reduces their allergenicity [19].

We have observed that the amount of both α-lactalbumin and β-lactoglobulin were reduced in all immobilization media used for fermentation with *S. thermophilus*. The effect was considerably higher on κ-carrageenan compared to the two other media. Immobilization refers to the prevention of free cell movement by natural or artificial means. It has been assumed that cells are distributed homogeneously in the beads that entrap them. However, in a study by Zohar-Perez et al., distribution of *E. coli* in alginate-gel beads was found to be nonhomogeneous [20]. In fact, there was a greater presence of cells on the surface of the alginate beads than in their cores. Similar effects may be responsible for the difference we have observed in different media.

Garbayo et al., hypothesize that immobilization alters cell wall-membrane due to a kind of gel-matrix-structure recognition increasing the permeability and lactic acid production [21]. Thus, use of immobilization medium seems to have potentially high value for whey processing on the commercial scale. Takata et al., reported results confirming the superiority of κ-carrageenan over other immobilization media [8]. In this study κ-carrageenan was far more efficient than agar and agarose in the destruction of β-lactoglobulin found in whey protein by *S. thermophilus*. The use of immobilization medium has the potential to reduce the allergenicity of fermented milk products. Therefore, in biotechnological processes based on the fermentation of milk and dairy products, κ-carrageenan can replace immobilization media such as agar and agarose, which have lower efficiency in reducing the components that pose a risk to health.

Compliance with Ethical Standards

Ethical Approval: The study was approved by Marmara University Ethics Committee (Approval no. 09.2022.245).

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Conflict of Interest : The authors have no conflict of interest to declare.

Author contributions: FZS, GB, and ASY: Generated the initial idea and experimental design, FZS, GB., AMY, and BA: Performed the experiments and analyzed data¸ FZS, GB, and ASY: Wrote the manuscript. All authors contributed to the critical revision and gave final approval to the submitted version.

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The role of dynamic thiol/disulfide homeostasis for the evaluation of oxidative stress in endometriosis patients

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ABSTRACT

Objective: To evaluate the role of oxidative stress in endometriosis patients by determining dynamic thiol/disulfide homeostasis and ischemia modified albumin (IMA) levels.

Patients and Methods: This prospective case-controlled study was conducted at a tertiary gynecology clinic in Istanbul, Turkey. 86 patients previously diagnosed with endometriosis and persistent endometriomas were included in the study group. 60 patients who visited the clinic during the study period for routine gynecological control were included in the control group. Thiol/disulfide parameters and IMA levels were determined from the serum samples.

Results: When the thiol/disulfide parameters were compared between the study and the control group no significant difference was observed (p=0.49). Mean disulfide level in the control group was 18.58 ± 5.73 μmol/L and in the study group was 18.61 ± 7.37 μmol/L. Levels were statistically similar in both groups (p=0.98). In addition, there were no differences between the groups in terms of IMA and albumin levels.

Conclusion: The results of this study revealed no significant difference in the dynamic thiol/disulfide homeostasis among the endometriosis patients. Although, it has been accepted as a potential oxidative stress marker in other chronic inflammatory diseases, its use in determining the systemic oxidative stress level in endometriosis patients is limited.

Keywords: Endometriosis, Endometrioma, Dynamic thiol/disulfide homeostasis, Ischemia modified albumin

1 INTRODUCTION

Endometriosis is a chronic inflammatory disease. It is characterized by the presence of endometrial-like tissue outside of the uterus elsewhere in the body [1]. It affects 10% of women of reproductive ages. Chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility are symptoms that are associated with endometriosis [1]. The presence of endometriosis in the ovaries forming a cystic tumor was first identified by Sampson and termed endometrioma [2]. The etiology of endometriosis is not entirely clear. Sampson's implantation theory, Mayer's coelomic metaplasia theory, and induction theory are among the accepted pathophysiological theories of endometriosis [3,4]. However, none of them sufficiently explain the mechanism of the disease.

It is known that oxidative stress, which occurs due to the imbalance between the production and destruction of reactive oxygen species, plays a role in the pathophysiology of endometriosis. It initiates a generalized inflammatory reaction in the peritoneum [5-8]. Reactive oxygen species are intermediary products of the

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oxygen metabolism, and they are involved in modulating cell proliferation as a part of an inflammatory response [9]. For the reduction and the inactivation of reactive oxygen species, thiol-containing molecules are essential. During this reaction, thiol groups are oxidized and reactive oxygen species become inactive [10]. Then, the oxidized thiol groups form disulfide bonds. As a result of this reversible reaction, thiol/disulfide homeostasis forms, contributing to the antioxidant mechanism of the organism. Therefore, thiol/disulfhide levels can be used as an indicator of oxidative stress [11,12].

Another potential oxidative stress marker is ischemia-modified albumin (IMA), an important marker in determining myocardial ischemia. Recently, its use in other organs has been reported [13]. Elevated levels of IMA have been observed in systemic sclerosis, diabetic ketoacidosis, acute ischemic stroke, and ectopic pregnancy. Therefore, its use as a biomarker is suitable [14-18].

The role of oxidative stress in endometriosis pathophysiology has already been established. However, data on the utilization of dynamic thiol/disulfide homeostasis and IMA as oxidative stress markers in endometriosis patients are limited.

The objective of this study was to assess the use of dynamic thiol/disulfide homeostasis and IMA levels as oxidative stress biomarkers in endometriosis by measuring the native thiol, total thiol, and IMA levels in serum samples of endometriosis patients and the control group. In addition, an evaluation was carried out to determine whether these parameters showed any changes according to the severity of endometriosis-related pelvic pain and/or endometrioma size.

2. PATIENTS and METHODS

This study was designed as a prospective case-controlled study. It was conducted at a tertiary center of obstetrics and gynecology in Istanbul between March 17 and August 1, 2020. This study was approved by the Clinical Research Ethical Committee of Bakirkoy Dr. Sadi Konuk Training and Research Hospital (approval number: 2019/490), and all subjects gave their written informed consent.

A total of 146 women who visited the outpatient gynecological clinic between March 17 2020 and August 1, 2020 were enrolled in the study. Out of these 146 patients, 86 endometriosis patients who were aged between 18 and 45 years and who had endometriomas were included in the study group. All patients had received their endometriosis diagnosis previously either by laparoscopy or by ultrasonography. The control group consisted of 60 patients who visited the outpatient clinic for routine gynecological control. Exclusion criteria were previous ovarian surgeries; endocrinological, rheumatologic and/or metabolic comorbidities; intake of hormonal medications for the previous three months; current antioxidant treatment; smoking; and alcohol abuse.

Following a thorough anamnesis, all patients underwent gynecological examination with transvaginal ultrasound (TVUS). An endometrioma was diagnosed by the presence of a unilocular ovarian cyst with homogeneous ground-glass echogenicity either with TVUS or with magnetic resonance imaging [19]. The volume of the endometrioma volume was calculated using the Orsini formula (length x width x diameter x 0.5235) [20]. TVUS was performed by the same gynecologist using an 8.5-MHz transvaginal transducer (ATL 5000 HDI, Philips, Netherlands). Endometriosis-related pelvic pain severity was self-evaluated by patients with a visual analogue scale (VAS); a score of 0 being no pain and 9 being extreme pain.

Biochemical Analysis

Venous blood samples from the patients were collected before they received any medical treatment. Serum was separated by 1200 x g centrifugation for 10 min. Dynamic thiol/disulfide parameters were measured using an automated methodology with a Cobas c501 chemical analyzer (Roche Diagnostics, Mannheim, Germany) [11]. All results were given in μmol/L. To summarize the methodology, sodium borohydride was first used to reduce disulfide bonds to thiol groups. Then, formaldehyde was added to the reaction to remove excess sodium borohydride. After reacting with 5,5'-dithiobis, reduced total thiol and native thiol groups were measured, and disulfide levels were calculated as described in the methodology. IMA levels were also measured from the same serum samples using the cobalt binding test defined by Bar-Or et al. [13]. IMA levels were recorded as absorbance units (ABSU).

Statistical Analysis

Patients' demographic data were evaluated using descriptive statistics. Comparative statistics was performed using the Mann-Whitney *U* test or Student's t-test depending on the distribution of the variables. Spearman or Pearson correlation analysis was used to evaluate the association between variables. A p-value of <0.05 was accepted as statistically significant. All statistical analyses were done using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA).

3. RESULTS

Patients were compared based on their age, endometrioma volumes, VAS scores, thiol/disulfide parameters, and IMA and albumin levels (Table I). There were no significant age differences between the groups (p= 0.507). The mean endometrioma volume of the study group was calculated to be 7.2 ± 6.05 cm^3. When groups were compared based on pelvic pain severity, a significant difference based on the VAS scores was observed (p< 0.001).

When the thiol/disulfide parameters were compared, the mean native thiol level in the control group was 313.92 ± 70.02 μmol/L and in the study group was 322.52 ± 78.31 μmol/L. No significant difference was observed (p= 0.49). Similarly, the comparison of total thiol levels did not reveal any significant difference (p= 0.48) (control group = 351.11 ± 67.49 μmol/L, study group = 359.71 ± 75.37 μmol/L). The mean disulfide level in the control group was 18.58 ± 5.73 μmol/L and in the study group was 18.61 ± 7.37 μmol/L. The difference was statistically insignificant.

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Also, there were no significant differences between IMA and albumin levels (p= 0.11 and p= 0.62, respectively).

The relationships between thiol/disulfide levels and age, endometrioma volume, and VAS scores in the study group were analyzed with a correlation analysis (Table II). Although, a negative correlation between age and native thiol (r = – 0.07, p= 0.38), total thiol (r = – 0.06, p = 0.44), and IMA levels (r = – 0.01, p = 0.81) and a positive correlation between disulfide (r = 0.11, p = 0.17) and albumin (r = 0.01, p = 0.88) levels were observed, none of these correlations were statistically significant. In the correlation analysis of endometrioma volume, the correlation coefficient with native thiol was r = 0.12, total thiol was r = 0.09, disulfide was r = – 0.06, IMA was r = – 0.015 and albumin r = 0.07. None of these were statistically significant. Similarly, a comparison of VAS scores and thiol/disulfide parameters did not reveal a significant association (Table II).

Table I. *Comparison of clinical, dynamic thiol/disulfide parameters, albumin and IMA levels between the control and the study group*

	Control group (n=60)	Study group (n=86)	p-*value*
Age (years)	32.24±4.56	32.84±4.97	0.507
Endometrioma volume (cm^3)	0	7.2± 6.05	**<0.001**
VAS score	3(1-4)	6(4-9)	**<0.001**
Native thiol (µmol/L)	313.92 ± 70.02	322.52 ± 78.31	0.49
Total thiol (µmol/L)	351.11±67.49	359.71±75.37	0.48
Disulfide (µmol/L)	18.58±5.73	18.61±7.37	0.98
IMA (ABSU)	1.06±0.24	0.98±0.33	0.11
Albumin (g/dl)	2.44±0.79	2.51±0.8	0.62

VAS: visual analogue scale; IMA: ischemia-modified albumin; ABSU: absorbance units

Table II. *Correlation analysis between dynamic thiol/disulfide parameters, IMA, albumin and mean age, mean endometrioma volume and mean VAS score of the study group*

	Age		Endometrioma volume		VAS score	
	r	p	r	p	r	p
Native thiol	-0.07	0.38	0.12	0.146	0.04	0.56
Total thiol	-0.06	0.44	0.09	0.27	0.03	0.71
Disulfide	0.11	0.17	-0.06	0.45	0.03	0.66
IMA	-0.01	0.81	-0.15	0.06	-0.11	0.17
Albumin	0.01	0.88	0.07	0.34	0.03	0.71

IMA: ischemia-modified albumin, VAS: visual analogue scale

4. DISCUSSION

Oxidative stress plays an important role in the mechanism of cell migration and proliferation. This mechanism is also an essential part of endometriosis pathophysiology [21-23]. In this study, we evaluated dynamic thiol/disulfide homeostasis and IMA as potential biomarkers of oxidative stress in endometriosis patients. Since, the levels of these markers were determined in serum samples, oxidative stress was assessed in a systemic manner rather than local. According to our results, a significant difference among the endometriosis patients in terms of dynamic thiol/disulfide homeostasis and IMA was not observed.

Oxidative stress associated with endometriosis, which is observed in the peritoneal tissue or the abdomen, occurs due to the inflammatory response to the ectopic endometrial cells. Furthermore, reactive oxygen species are formed as intermediary products of the metabolic reactions in the erythrocytes, macrophages and apoptotic endometrial tissue accumulating in ectopic implants [24]. In addition, elevated levels of oxidatively modified low-density lipoproteins and nitric oxide radicals, which are also oxidative stress indicators, were reported in the peritoneal fluid of endometriosis patients [25,26]. However, in most studies, oxidative stress has been evaluated in local tissue samples or peritoneal fluid. Systemic evaluation of oxidative stress in endometriosis is limited [27]. In our study, we evaluated oxidative stress by determining the thiol and disulfide levels in serum samples of endometriosis patients. Since, these parameters were measured from serum samples, our results reflected systemic oxidative stress levels of the patients. Although, the data in the literature have pointed out the role of oxidative stress in the pathophysiology of endometriosis, our results did not show any significance. This could be because either oxidative stress observed in endometriosis patients could only be detected at the local ectopic endometriotic loci without altering the systemic oxidative homeostasis of the individuals or thiol/disulfide as biomarkers might not be appropriate in detecting endometriosis-related oxidative stress.

Among the most important nonenzymatic antioxidant molecules that play an essential role in the oxidative stress defense mechanism are glutathione, carotenoids, tocopherols and ascorbates. Glutathione, which contains thiol groups, is an important intracellular antioxidant [28]. When the oxidative-antioxidative balance is disrupted, thiol groups form disulfide bonds in a reversible reaction. The resultant increase in disulfide bonds indicates that the equilibrium in the body has shifted to the side of oxidative stress. Furthermore, the association between the severity of endometriosis and glutathione peroxidase, superoxide dismutase, and lipid peroxidase, which are a part of the enzymatic antioxidative mechanism, has been reported [29]. However, to the best of our knowledge the role of dynamic thiol/disulfide homeostasis in endometriosis has not yet been evaluated.

The prospective design of this study and the evaluation of all the recruits by a single gynecologist who is specialized in endometriosis are among the strengths of this study. Although, the statistically insignificant nature of the results could be seen as a limitation, they could be of importance when designing future studies. As mentioned above, oxidative stress was evaluated at a systemic level in this study. Therefore, the status of local dynamic thiol/disulfide homeostasis in ectopic endometrial implants is still unclear. Therefore, these results can be considered as preliminary outcomes when designing future studies on related topics.

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Conclusion

In this study, the change in the parameters of dynamic thiol/disulfide homeostasis in endometriosis patients was not significant. Although, dynamic thiol/disulfide homeostasis has been shown as a potential oxidative stress marker in several other chronic inflammatory conditions, it does not seem to be suited for evaluating oxidative stress in endometriosis patients on a systemic level. However, local changes in dynamic thiol/disulfide homeostasis should still be evaluated. Therefore, future studies using peritoneal fluid or ectopic endometrial tissue can be meaningful.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Clinical Research Ethical Committee of Bakirkoy Dr. Sadi Konuk Training and Research Hospital (approval number: 2019/490), and all subjects gave their written informed consent.

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Rethinking large group lectures – how far in this format?

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ABSTRACT

Objective: The aim of this study is to determine the perceptions, attitudes, and behaviour of medical students and lecturers regarding the lectures and their effects on students' learning behaviour.

Materials and Methods: This was a qualitative study including multi-methods. Researchers observed lecture ambiance and activities in two courses. Lectures were observed and slide-presentations were evaluated. Additionally, in-depth and focus group interviews were conducted.

Results: Two researchers attended and observed 75 lectures. The average number of attendees was 51.21. Eighty percent of lecturers did not introduce any activities to attract attention and prepare students for the lecture. Only 12% of lectures were taught interactively. Of the evaluated 43 (69.80%) slide-presentations, sufficient association or integration was not made between clinical and basic sciences.

Conclusion: This study revealed that the lectures created negative feelings and thoughts in students and lecturers, and led to undesirable attitudes and behaviour. It is essential to focus on giving interactive lectures which aim at developing reasoning, decision-making, and evaluation competencies. The most significant factors determining students' attendance and appraisal of the lectures were related to the preparation of the lecturers, the intensity of the content, integration between basic science and clinical science, and the presentation skills.

Keywords: Integration, Lectures, Medical education, Undergraduate, Teaching methods

1. INTRODUCTION

Lectures conducted with large groups are still one of the mainstays of teaching methods in higher education. One of the reasons for its widespread use is that it offers lecturers the opportunity to transfer a wide range of contents simultaneously to large numbers of students. Being cost-effective and requiring less preparation compared to interactive learning methods are among the other reasons [1]. It is widely believed that learning is a constructive process requiring the active involvement of learners. So, learners construct knowledge through learning experiences and by reflection on the experiences and learning environment around them [2]. However, teacher-centred instruction is a controversial method, which has been discussed in the literature. Regarding lectures in large groups, studies highlight significant problems such as the inability of students to stay focused for a long time, the lack of in-depth learning opportunities, and the effect of attendance on student performance [3,4-6].

As mentioned above, although much has been written about medical students' learning and attendance in sessions, there is a paucity of literature revealing the lecture-related beliefs, attitudes, and behaviour patterns of students and lecturers. So far, no studies were performed where lectures were evaluated in a holistic and multi-faceted manner, including the stages before, during and after the lecture. Thus, research in these areas, revealing how students experience lectures and how these experiences affect their attitudes and behaviours, would provide novel evidence to revise and change the current situation regarding the still-heavily used large audience lectures.

The aim of this study is to reveal the perceptions, attitudes, behaviours, and the effects on learning of medical students

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and lecturers regarding the lectures. In this context, research problems were determined as follows:

(a) How do medical students and lecturers experience pre-, during and post-lecture sessions? (b) What is the level of interaction experienced during the sessions among learners, between learners and lecturers, and how does the depth of this interaction affect learners' attitudes and learning behaviours?

2. MATERIALS and METHODS

This was a qualitative study, which included 4 different methods and techniques: observation during the lecture, analysis of the presentation materials, interviews with students and lecturers.

The type, population, and sample of the research

The study including pre-, during and post-lecture evaluations was carried out with lecturers and the 3rd-year undergraduate students of the Marmara University, School of Medicine (MUSM). Medical education at MUSM consists of 6 years. The 3rd year curriculum is composed of five subject committees. Researchers selected two of the five subject committees, 'Nervous System and Associated Diseases' (NSAD) and 'Growth-Development, Mental Health, and Related Disorders' (GDMR) consisting of a total of 88 lectures (116 hours in total) for the study. Within the NSAD course, a total of 38 lectures (12 basic medical science, 25 clinical sciences, and 1 public health) and within the GDMR course a total of 50 lectures (6 basic medical science, 39 clinical science and 5 public health) were selected for evaluation. Researchers chose these lectures for evaluation taking into consideration the diversity of disciplines such as physiology, pharmacology, public health, family medicine, etc.

Research methods and techniques

In-class observation and analysis of the lecture materials: With the permission of the lecturers, 75 lectures in the two courses were observed and evaluated by two researchers (SA and MS) using the "Lecture Observation Checklist" in terms of attendance, interaction, interest, presentation of the content, and the use of interactive techniques (supplement 1). Of those 75 lectures, the presentation materials of 43 lectures were randomly selected and analysed independently by three researchers (CE, BCY, MAG) in terms of various criteria by using the "Evaluation Form for Presentation Material" (supplement 2). Checklist and evaluation form were created by researchers.

Interviews: Participants were selected by convenience sampling method for interviews. Out of the 56 lecturers of the 75 lectures, in-depth interviews were conducted with 10 lecturers, who agreed to participate in face-to-face interviews, while 4 lecturers were interviewed using online interview tools. The interviews lasted between 15-25 min. They were audio-recorded and terminated when data saturation was reached by answering all the interview questions.

For the focus interviews with the students who accepted to participate in the study and gave their written consent, 3 focus groups, composed of 12 students each, were formed by systematic randomization method. Focus group interviews, between 45 and 70 min, were also audio-recorded (supplement 3).

Statistical Analysis

The process of qualitative analysis is shown in Figure 1. The contents obtained from the qualitative interviews were transcribed, and content analysis was made by two researchers (SA and MS). Descriptive statistics were used in the analysis of quantitative data.

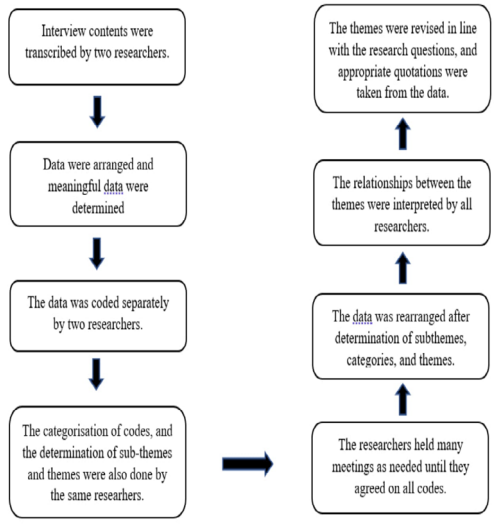

Figure 1. *The processes of qualitative analysis.*

3. RESULTS

Observation of lectures and analysis of lecture materials

Within the 218 registered students, the average number of students attending the observed lectures was 51.21 (median: 50.0, min.=12, max.=107). Eighty percent of the lecturers did not apply any warm-up activity in the beginning to prepare the students for the lecture and attract their attention. 88% straightforwardly conveyed the information. In only 12% of the lectures, the content was supported by interactive techniques (case, video, etc.). It was observed that students were distracted mostly after 10 and 20 min of a 50-min lecture. The ratio of the lecturers making a general summary about the content of the lecture at the end of the lecture was 8.0% (6 lectures). Other details regarding the data revealed in the observations of the lectures are given in Table I.

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Table I. *The data gathered from observation of lectures*

The number of students at the beginning of lectures (first 10 minutes)		Median=50 Min.=12, max.=107
The activities/behaviours of the lecturer in order to prepare, motivate and engage the students at the beginning of the lecture	Ratio (%) of all lectures	20% (n: 15)
	Duration	Mean: 2.4 min, SD=1.18
The number of students attended the class during lecture (based on 10 minutes time period)	Time period	Mean/SD (min. - max.)
	10-20 min.	1.04/2.17 (0 - 13)
	20-30 min.	0.34/0.62 (min.:0, max.: 3)
	30-40 min.	0.10/0.45 (min.:0, max.: 3)
	40-50 min.	0.04/0.19 (min.:0, max.: 1)
The number of students left the lecture before the end of the lecture (based on 10 minutes time period)	Time period	Mean/SD (min. - max.)
	10-20 min. (mean/SD)	0.02/0.16 (0 - 1)
	20-30 min. (mean/SD)	0.05/0.22 (0 - 1)
	30-40 min. (mean/SD)	0.13/0.55 (0 - 4)
	40-50 min. (mean/SD)	0.25/1.02 (0 - 8)
The number of students who did not attend the lectures (S) and The number of reasons for not attending the lectures (R) (napping, yawning, dealing with something else, talking to each other)	Time period	Mean/median/SD
	0-10 min.	S: 3.00/2.00/1.90 R: 3.00/3.00/2.27
	10-20 min.	S: 3.38/2.74 R: 2.96/2.00/2.44
	20-30 min.	S: 3.20/2.00/3.03 R: 2.53/2.00/2.21
	30-40 min.	S: 2.81/2.00/3.04 R: 2.44/2.00/2.64
	40-50 min.	S: 1.09/0.00/2.12 R: 0.85/0.00/1.57
The students attending/asking questions during lecture.		Mean/SD (min. - max.)
	Number of students	1.96/2.39 (0 - 10)
	Time of first question (minute)	14.38/14.48 (0 - 47)
	Number of questions	2.00/2.65 (0 - 10)
The questions asked by the lecturer	Number of questions	Mean/SD (min. - max.) 5.13/4.89 (0 - 20)
	To whom the question was asked (ratio)	Anonymously: 95% Directly to a student: 5%
The applications /interactive activities used during lecture (which engage the student in the teaching process actively)	The ratio	12.0% of all lectures (n=9)
	The duration (minutes) of activities (mean/SD)	7.11/2.82 (min.: 1, max.: 14)
The activities/behaviours performed by the lecturer in order to gather and stimulate the distracted attention of the class during the lecture.	The ratio	44.0% of all lectures (n=33)
	The duration of activities/behaviours per lecture	Mean/SD (min. - max.) 1.78/1.13 (1 - 4)
The activities carried out by the lecturer for summarizing the topic occasionally during the lecture	The ratio	26.6% of all lectures (n=20)
	The duration of activities for summarizing per lecture	Mean/SD (min. - max.) 1.75/0.89 (1 - 3)
The activities/behaviours of the lecturer regarding the summarization of the lecture at the end of the presentation	The ratio (%)	32.0% of all lectures (n=24)
	The duration of activities for summarizing (mean/SD)	3.71/2.17 (min: 1, max: 10)
The activities/behaviours performed by the lecturer to reveal the relationship of the subject with the professional lives of the students during and/or end of the lecture	The ratio	32.0% of all lectures (n=24)
	The number of activities/behaviours per lecture	Mean/SD (min. - max.) 3.00/1.64 (1 - 6)
What was the seating composition of the students in class?	In front: 72% (n:54) Homogeneous: 25.3% (n:19) Messy: 2.7% (n:2)	
The attention of the lecturer during the lecture	All attention is on the slide: 26.7% (n:20) Mostly towards the slide: 20% (n:15) All attention is towards the class: 53.3% (n:40)	

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The presentation materials of the total 43 lectures (79.1% clinical, 20.9% basic science lectures) were analysed, and the details regarding the data revealed in the observations of the lectures are given in Table II.

Table II. *The analysis results of the presentation materials of lectures*

		% (n)
Department of lecturers	Basic science	20.90 % (9)
	Clinical Science	79.10 % (34)
Declaration of learning outcomes with students	Yes	39.50 % (17)
	No	60.50 % (26)
The structure of slides (sentences, paragraphs, number of lines, font size *(>20)/size of material, title, color selection, using of slide area in balance, using of image appropprіately)*	Should be improved	27.90% (12)
	Acceptable	37.20% (16)
	Well structured	34.90% (15)
The content of presentations *(interdisciplinary integration, congruence of level of students, treatment/diagnosis details, etc.)*	Should be improved	27.90% (12)
	Good	41.90% (18)
	Very good	30.20% (13)
The supplementary documents/tools such as case, audio visual aids, photos, links, etc. within the presentations of lecturers	Yes	37.20% (16)
	No	62.80% (27)
The integrity and flow of the presentation	Yes	11.60% (5)
	No	88.40% (38)
Slides used to prepare students for lecture at the beginning of the presentation *(interesting/ motivating slides, etc.)*	Yes (slides are enough)	69.80% (30)
	No	30.20% (13)
	More than needed	-
Slides used to recap the lecture during presentation	Yes	93.00% (40)
	No	7.00% (3)
Slides used to summarize presentation at the end of lecture	Yes	16.30% (7)
	No	83.70% (36)
Making references between the clinical and the basic sciences within presentation	Yes	30.20 (13)
	No	69.80 (30)
Slide number		Mean/SD (min. – max.) 51.69/18.27 (25 - 97)
The characteristics of content based on visual/audio and written components in presentation of lecturers		Mean/SD/% of slide number
	Only written	32.97/16.06/66.79%
	Only visual/audio	8.93/8.69/18.10%
	Both	7.46/8.75/15.11%

Analysis of interviews with lecturers

As a result of theme analyses of the in-depth interviews with the lecturers, a total of 3 main themes emerged: "planning the lecture and evaluating student achievements", "experience and effects of the lectures", and "post-lecture review and reorganization". The details of data for themes, categories, and subthemes are presented in Figure 2 and Figure 3.

The selected quotations related with the main themes are as follows.

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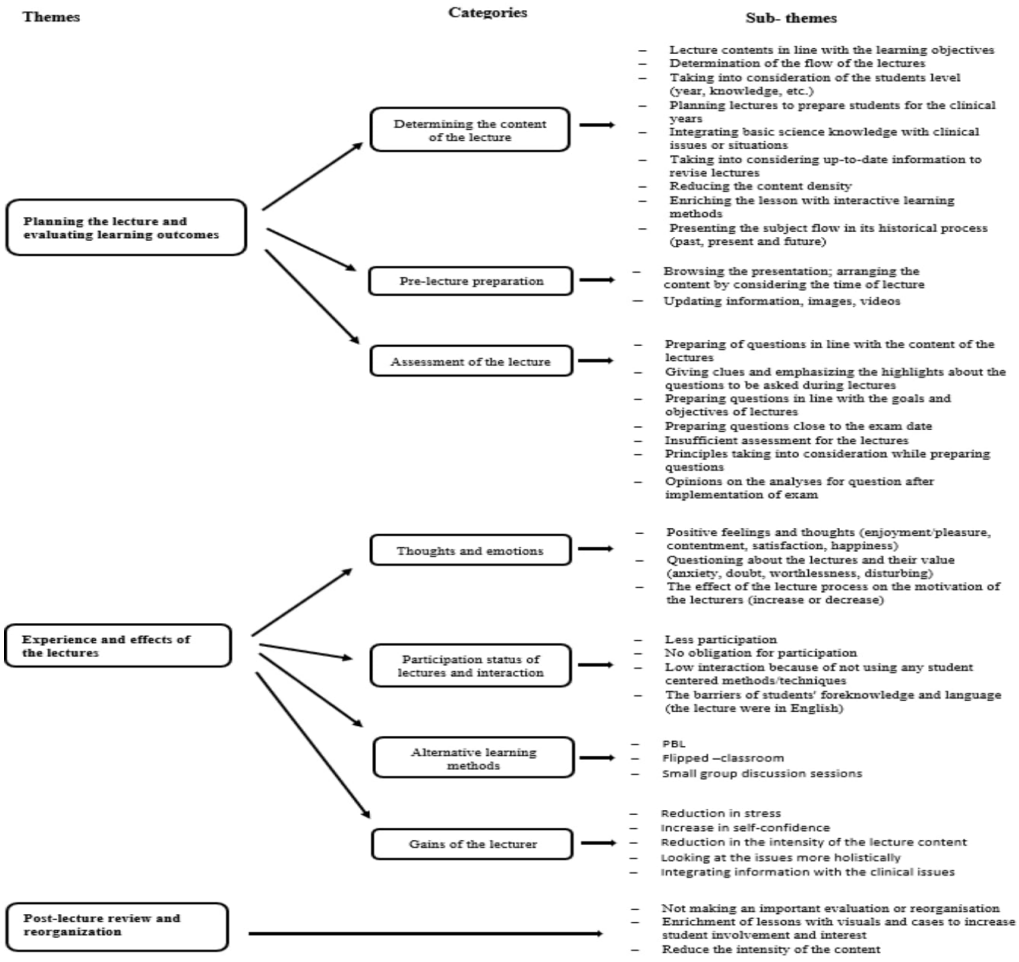

Figure 2. *Themes and sub-themes obtained from in-depth interviews with the lecturers.*

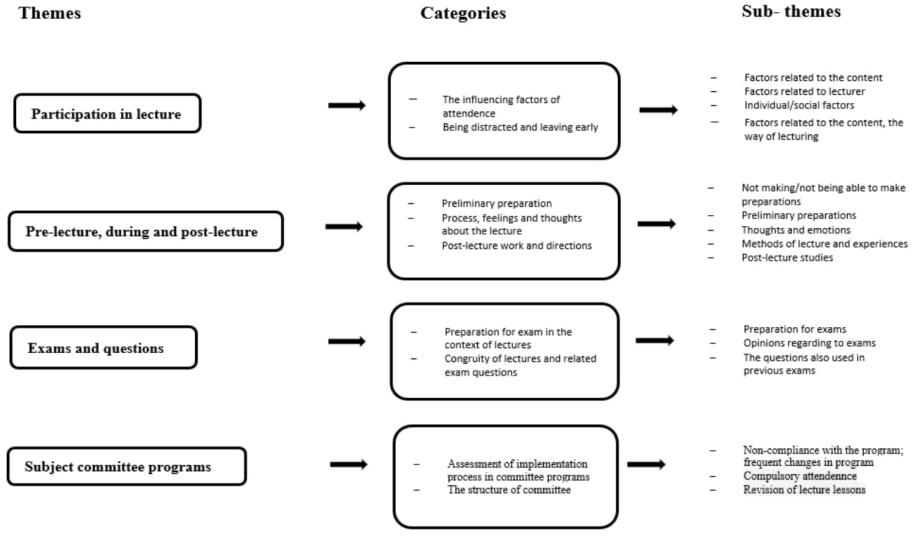

Figure 3. *Themes, categories, and sub-themes revealed from the focus group interviews with the students.*

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The first theme: "planning the lecture and evaluating student achievements"

"I try to ensure that students get basic information about the clinical situations I commonly deal with by including basic science knowledge, and I intend to provide them with basic information such as what kind of symptoms the patient will have if there is damage in various localizations… I often try to be interactive and involve the students in the lectures…

"I prepare exam questions in line with my goals and objectives. The questions are usually at a level which those who attend the lectures can understand. … I would like the questions to distinguish between those who attend and who do not."

The second theme: "experience and effects of the lectures"

"Less attendance in lectures than in previous years reduces my motivation. I do not think it is worth our effort. We are doing something wrong, but I do not know what."

"Students' interest and participation make the lecture better. However, today it is possible to access most of the materials on the internet, and there are also very good online lectures. Therefore, sometimes I think about whether it is better to give the lectures as "flipped classroom" instead of straight classroom lecture method."

The third theme: "post-lecture review and reorganization"

"Actually, I do not go through an intense evaluation process..."

"After the lecture, I always review the content and try to correct any missing points. … I reinforce the points where I feel the student's interest is diminished or remove them if not necessary. During the lecture, I make my observation on this."

Analysis of focus interviews with students

As a result of the analysis of the focus group interviews, 9 categories and 16 sub-themes emerged under 4 themes (Figure 3).

Selected quotations related with the main themes were as follows:

"Participation in lecture"

"I think the way the teacher teaches is the most important aspect. S/he does not teach the lecture directly using slides; besides, it is not very intense and boring, s/he sometimes asks questions, and an atmosphere of interaction occurs, and s/he gives examples from her/his professional life, I like these kinds of lectures more."

"The reason why I come to those classes is just to see friends, spend time, and not to stay away from the classroom."

"Pre-lecture, during and post-lecture"

"Clinical lecturers explain the contents more simply, so you feel content when you leave the class? I mean the comfort of understanding the topic when I leave the class. ... A feeling of content to attend the lecture and understand it, so you think that your time is not wasted, and it relaxes you."

"I have this feeling (uneasiness) not only in this committee but also in others because the content of the lecture is very intense."

"Exams and questions"

"Most of the questions can be answered by studying slides, so I can say that this seems to be possible even if the lectures are not given at all. Anyway, it is possible to find different presentations and videos on different websites on the internet, and we can also study by using them."

"Subject committee programs"

"Students determine the lecture they will attend depending on the lecturer, if they think it is worth attending, they do."

"I think the target of our education should be clinically focused, basic science lectures make me feel bored."

4. DISCUSSION

In our study, the most significant factors determining students' attendance, leaving the class before end of the lecture, positive and negative experiences and feelings towards the lectures were also those factors related to the preparation of the lecture, the intensity of the content, integration between basic sciences and clinical sciences, and the presentation skills. In the literature, lectures are criticized for many reasons such as differences in the lecturer's presentation skills, content intensity, inadequate interaction, inability to integrate information, etc. [7,8]. Despite severe criticism, one of the reasons why lectures continue to choose a predominantly used method in education is the insufficient research on this subject [9]. Qualitative research based on the experiences of students and lecturers is limited. This qualitative study is the only study in which the experiences of students and lecturers related to the lectures are evaluated in all aspects using four different methods, not only with the experiences during lecture but also with the experiences before and after. In practice, the experiences during the lecture halls vary a lot within themselves. For example, the presentation skills of the lecturer are very different, and the students are expected to adapt to each lecturer. In addition, students' current knowledge levels play a role in the process [10]. It is observed that the students defined the lectures they mostly benefited from and enjoyed as "interactive, interesting, and practical". The two themes that were associated mostly with positively experienced lectures were "sustaining attention" and "practicality". It is known that the attention span of students in direct instruction is generally limited to 20 min [8]. Students associated "sustained attention" during the lecture mainly with the "interactive" nature of the lecture. One aspect of the interaction is related to in-class student participation. In this context, one way to ensure interaction with students in large lecture halls is to enrich their presentations with interactive learning methods. Today, with the advances in educational technologies, using student response systems, question-answer and similar methods have become easier [11]. The second aspect of interaction is the association of the theoretical content presented in the lecture with daily life and professional life. At this point, the second theme that also

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appears to be related with sustaining attention is "practicality". Students report that such lectures motivate them more when the content of the lectures is more meaningful, useful, and applicable via integrating the content with professional life [12]. In our study, it was revealed that students are more eager to participate in and benefit more from interactive lectures. It was also observed that the experiences of lecturers who had interactive presentations, were more positive. However, the findings emphasized that positive experiences are limited, and pointed to insufficient interaction. Since, the content of the lecture was regarded as too intense for in-class interaction and forced the lecturers to apply straight lectures, the content was not deepened and reinforced with interactive techniques such as question-answer, discussion, or case use. Basic and clinical science knowledge were not integrated enough or associated enough with day to day clinical life. The feelings, attitudes and thoughts of both the students and teachers were adversely affected towards the lectures. As a result, the students chose not to attend the classes. The rate of attendance in lectures was found to be limited to one-fourth of the class size. It was observed that students' feelings about the lectures were dominated by negative emotions like "meaninglessness", "reluctance", "worthlessness", "regret", "waste of time", "anger", "boredom" and "unable to breathe" rather than "positive energy", "comfort", "relaxation and satisfaction". Lack of attendance in lectures and limited interaction cause a decrease in motivation and lead to negative thoughts and feelings in lecturers. Many lecturers reported that methods such as problem-based learning and "flipped classroom" instead of lectures could be put into practice. There are many studies in the literature regarding the positive effects of attendance in lectures on learning [13,14]. However, in recent years there has been a noticeable decrease in attendance in lectures [15]. In our study, the average attendance rate was found to be 25%. Many factors were identified regarding the non-attendance of medical students, such as prioritizing other academic activities and some personal or physical problems. For example, the transportation problem due to the distance between the campus and the place of residence was identified as an important factor affecting the attendance of the class for 70% of the students [13]. It was revealed that the morning traffic in Istanbul negatively affects their decision to attend lectures in the early hours in our study as well. Among other reasons, two factors were found to be particularly significant: the way the lecturers deliver the lectures, and having the opportunity to watch online lectures for topics covered through various online platforms. It was observed that intensive content and not using interactive techniques during lectures negatively affected attendance. On the other hand, the motivation of the lecturer and the enrichment of the presentations with visuals, relevant cases, and professional experiences/narratives positively affected the attendance. Moreover, it was observed that when participating in classes where intensive content presentation was not found meaningful, the use of different online lecture alternatives reduced participation even more. Literature supports these findings related to the lecturer factor. Studies have shown that interesting lectures, the communication of the lecturer, and the lecturers' mastery of the subject positively affect student attendance [9, 16, 17]. Another ongoing discussion regarding attendance is whether it should be compulsory or not. In a study, medical lecturers stated that compulsory attendance greatly affected student's attendance. Besides, some faculty members advise students to attend lectures to avoid problems with presentation materials shared in lecture halls and to avoid a poor performance in exams/assignments [18]. However, another relevant study did not reveal a significant relationship between medical students' attendance in lectures and their academic achievements [19]. In our study, the lecturers expressed their opinions on the necessity for compulsory attendance, but the students stated that it would not affect the efficiency of the lecture positively; on the contrary, it could negatively affect the students who really want to participate and benefit from the lectures.

In-class observation and analysis of the lecture materials revealed that the methods for increasing interaction were used in only 12.0% of the observed lectures. The number of slides for a 45-min lecture was very high, with an average of 51.7 (median:50), and the number of question-answer activities per lecture was also low. The lecture materials were content-intensive and the integration between basic and clinical sciences was not sufficient. After all these considerations, the solution lies in the improvement of the content of the lectures and the transformation of direct lecturing into interactive lectures using interactive techniques rather than making attendance compulsory. A more likely scenario would be that enhanced presentation contents and increased student interactions positively might affect participation.

Another significant factor regarding attendance in lectures is the rapid change in education and information technologies in recent years and the outcomes of this change. For example, the recording of the lectures and the opportunity for students to watch these recordings anytime decreases attendance [18,20]. Parallel to the development of video and audio recording technologies, students prefer to watch recorded content (podcasts or recorded lectures) than participate in lecture halls [19]. While, lecturers are more cautious about accepting and adapting to technological developments and the consequences of these developments, students are much more open and adapt quickly. Medical students state that following the lectures in this way is more efficient, useful, and has a better effect on their education than live lectures [18,19]. Our study results are consistent with this literature. The students in our study preferred to follow the lectures on online platforms instead of lectures that are not interactive, meaningful/useful, and involve a significant proportion of content presentation. However, in our study, some of the lecturers stated that thanks to technological developments, students can now access the course contents online and switching to student-centred interactive learning methods (problem-based learning (PBL), flipped classroom) instead of teaching in lecture halls should be considered. The transformation that has been taking place in medical education in the world for a long time is to reduce lectures and carry out education mainly by using student-centered learning techniques such as; proble-based (PBL), team-based learning (TBL),

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case-based collaborative learning (CBCL), flipped classrooms and technology-based education [21].

It is obvious that the lecturer-centred educational approach and practices based on content transfer will not be sufficient on their own for the attainment of twenty-first-century physician competencies [22]. At this point, it is essential to focus on interactive lectures that aim at the development of causation, decision-making, and evaluation competencies, rather than a straight narrative lecture that targets lower-level cognitive acquisitions and result in superficial learning. Reducing the intensity of the lectures in the curriculum and reorganizing the programs with student and interaction-centred learning activities will make learning more in-depth, integrated, and meaningful [23]. Besides, distance education provides flexibility in the existing programs [24].

According to many medical lecturers, after the pandemic there will be no return to the old system in medical education and permanent changes related to distance education will be implemented in education. It is also reported that in the future, changes in medical education will need to be closely monitored and put into practice by accreditation institutions [25]. In this context, the COVID-19 pandemic can be seen as an important opportunity to reconsider lectures. Moreover, the COVID-19 pandemic has made lecturers around the world a little keener on the use of educational technologies and online education. Lecturers are more convinced of the efficiency of online education training tools. In this process, medical education institutions have experienced the online presentation of some of the curriculum content [26,27]. The COVID-19 pandemic has been an opportunity for many faculties to implement innovative online education methods that they previously had not dared or found the opportunity to put into use [28].

This study revealed that the lectures are experienced negatively by both students and lecturers. It has been observed that these negative experiences create negative feelings and thoughts in students and lecturers, and thus lead to the development of undesired attitudes and behaviours towards lectures. Experiences related to the lectures should be handled and comprehended from all aspects, like current beliefs, acceptance, attitudes and behaviour patterns, habits, emotional, sociocultural, technical, and functional learning. Gaining a deeper insight into this issue will make the changes made in this direction more applicable. In addition, lecturer-oriented presentations should also become more effective, meaningful, and useful by the use of interactive techniques, affirmative and supportive classroom environments. However, such a transformation can only start when educational institutions ask themselves the following question and take necessary initiatives in this direction: "How far can we go with lectures?"

The main identified limitation regarding findings and conclusions that can be drawn in this study is as follow: the results obtained from this qualitative study conducted in a medical school are limited only to the culture and educational experiences of that country and the medical school. Therefore, it is pivotal to diversify such studies in different cultures and medical schools employing different qualitative research designs, methods, and tools. The outcomes and effects of the experiences related to the lectures should be revealed in a deep and holistic manner through further qualitative studies based on different research questions. It is to be expected that when based on studies which attempt to interpret and understand current experiences in different cultures around the world, the process of change and transformation in this direction will be realized more effectively.

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Compliance with the Ethical Standards

Ethical Approval: Ethical approval was obtained from the Ethical Committee of Marmara University Clinical Studies dated 7.12.2018 with protocol no 09.2018.826. All participant gave written informed consent.

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Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Author Contributions: MAG, BY, SA: Study conception, design, MAG, SA, MS, CE, BY: Data gathering, MAG, SA, MS, CE, BY: Data analysis, NB: Data analysis, statistical analysis, and design, MAG, SA, MS: Interpretation of results, MAG, SA: draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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Supplementary material 1. *Checklist for observation of lecture*

The number of students at the beginning of lectures (first 10 minutes)?		
The activities/behaviours of the lecturer in order to prepare, motivate and engage the students at the beginning of the lecture	Number	
	Duration	min.
The number of students attended the class during lecture (based on 10 minutes time period)?	10-20 min.	
	20-30 min.	
	30-40 min.	
	40-50 min.	
	50-60 min.	
	60-70 min.	
The number of students left the lecture before the end of the lecture (based on 10 minutes time period)?	10-20 min.	
	20-30 min.	
	30-40 min.	
	40-50 min.	
	50-60 min.	
	60-70 min.	
The number of students who did not attend the lectures (S) and the number of reasons for not attending the lectures (R) (napping, yawning, dealing with something else, talking to each other)	0-10 min.	S:….. R:……
	10-20 min.	S:….. R:……
	20-30 min.	S:….. R:……
	30-40 min.	S:….. R:……
	40-50 min.	S:….. R:……
	50-60 min.	S:….. R:……
	60-70 min.	S:….. R:……
Area used by the lecturer during the lecture	□ Narrow □ Medium □ Wide	
The students attending/asking questions during lecture	Number of students	
	Time of first question	… min.
	Number of questions	
The questions asked by the lecturer (A: Anonymously, D: Directly to a student)	Number of questions	
	To whom the question was asked	A: ….. S:……..
	What was the feedback to the answer?	
The applications /interactive activities used in the lesson (which engage the student in the teaching process actively).	The number of	
	The ratio to the total time	…min.
The activities/behaviours performed by the lecturer in order to gather and stimulate the distracted attention of the class during the lecture.	The number of	…min.
	The ratio to the total time	…min.
The activities carried out by the lecturer for summarizing the topic occasionally during the lecture.	The number of	…min.
	The ratio to the total time	…min.
The activities/behaviours of the lecturer regarding the summarization of the lecture at the end of the presentation.	The number of	
	The ratio to the total time	…min.
The activities/behaviours performed by the lecturer to reveal the relationship of the subject with the professional lives of the students during and/or end of the course.	The number of	
	The ratio to the total time	…min.
What was the seating composition of the students in class?	□ In front □ In back □ Homogeneous □ Messy	
The attention of the lecturer during the lecture	□ All attention is on the slide □ Mostly towards the slide □ All attention is on the class □ Mostly towards the class	

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Supplementary materials 2. *Evaluation Form for lecture presentation material*

Department of Lecturers	▯ Basic science ▯ Clinical Science
Declaration of learning outcomes with students	▯ Yes ▯ No
Slide number	
The characteristics of content based on visual/audio and written components in presentation of lecturers	▯ Only written ▯ Only visual ▯ Both
The structure of slides (sentences, paragraphs, number of lines, font size (>20)/size of material, title, color selection, using of slide area in balance, using of image appropppriately)	▯ Be developed ▯ Acceptable ▯ Adequate
The content of presentations (interdisciplinary integration, congruence of level of students, treatment/diagnosis details, etc.)	▯ Be developed ▯ Good ▯ Very good
The supplementary documents/tools such as case, audio visual aids, photos, links, etc. within presentations of lecturers	▯ Include ▯ No
The integrity and flow of the presentation	▯ Include ▯ No
Slides used to prepare students for lecture at the beginning of the presentation (interesting/ motivating slides, etc.)	▯ Include ▯ No ▯ More than needed
Slides used to recap the lecture during presentation	▯ Include ▯ No
Slides used to summarize presentation at the end of lecture	▯ Include ▯ No
Making references between the clinical and the basic sciences within presentation	▯ Include ▯ No

Supplementary materials 3. *Questions for in-depth interview*

Questions of in-depth interview with lecturers:

1. How did you prepare your lecture content? Did you review your lecture/ presentation material before the lecture? What is your main framework and what parameters do you take into account for your lectures preparation?

2. What are your feelings and thoughts about the lectures you teach? What kind of educational experience do you think you had in this committee? How has this experience affected your motivation for teaching and your thoughts on lectures?

3. Have you needed to revise your lecture(s) after implementation? Could you please give more details?

4. When did you prepare the exam questions? What did you consider while preparing those questions?

5. How would you evaluate the analyses of exams you gave, and the performance of the students in these exams?

Questions for group interview with students:

1. How did your experience with lectures affect your learning/ study motivation?

2. Were you given an assignment after the lectures? Did you make a plan and study for additional learning/completion of your aim? Which factors to affect or do not affect your plans for studying?

3. How did you prepare for the committee exam?

4. When you think about the scope of the lectures, how would you evaluate the questions in the committee exam? How did these questions affect your motivation and depth of learning?

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Clinical, demographic and genetic features of patients with congenital heart disease : A single center experience

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ABSTRACT

Objective: We aimed to evaluate the demographic and clinical characteristics of children with congenital heart disease (CHD) in a private pediatric cardiovascular genetics clinic in Istanbul from January 2016 to July 2018 and increase the awareness and emphasize the importance of genetic counseling in CHD.

Patients and Methods: One hundred and seventeen patients (50 female, 67 male) from 3 days of age to 25 years of age in 17 months period (January 2016 to July 2018) were retrospectively analyzed. Data included age, sex, echocardiography results, extracardiac features, genetic test results, consanguinity and any family member with heart disease. Pearson's chi-squared test with 1 degree of freedom and 5% significance was used for correlations.

Results: Consanguinity rate was 23.9%. Most common diagnosis was Tetralogy of Fallot (TOF) followed by atrial septal defect (ASD) and ventricular septal defect (VSD) equally. 30 patients had genetic testing which revealed a diagnosis in 36.6 % of the patients. 6 patients had DiGeorge, one had Renpenning,one had Kabuki syndrome. We had one NODAL, one MYH7 and one MYH6 variant.

Conclusion: Genetic testing in CHD has a high diagnostic yield. Genetic counseling can help diagnostic, prognostic, and therapeutic and family planning decision making.

Keywords: Congenital heart disease, Genetics, Genetic counseling

1. INTRODUCTION

The spectrum of pediatric cardiology clinic referrals in children ranges from common complaints of chest pain, palpitations, syncope, shortness of breath, to more serious congenital heart diseases (CHDs).

Congenital heart disease is one of the most common type of birth defect and occurs in 7/1000 among live births. It is the leading cause of infant and perinatal mortality from a birth defect and the most common reason for pediatric cardiology consultations [1].

Over 400 CHD genes have been discovered so far and nearly 90% of CHD cases have a suspected genetic contribution [2-4].

Congenital heart disease can occur as an isolated finding or as part of a syndrome or as a result of a teratogenic exposure. Genetic testing can help in accurately diagnosing and counseling these patients. Also, patients with CHD now reach adulthood and have an increased risk of having infants with CHD [5]. Therefore, genetic counseling can have an impact on diagnostic, prognostic, and therapeutic decision making [6]. It is also important for family planning.

In this study, we aimed to evaluate the demographic and clinical characteristics of children with CHD seen in a private pediatric cardiovascular genetics clinic in Istanbul from January 2016

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to July 2018 and increase the awareness and emphasize the importance of genetic counseling in CHD.

2. PATIENTS and METHODS

After ethical approval by Demiroglu Bilim University Ethics Commitee (approval number: 22.12.2020/ 2020-24-04) we retrospectively analyzed the clinical data of 117 patients from 3 days of age to 25 years of age in 17 months period (January 2016 to July 2018) who were referred to a private pediatric cardiovascular genetics clinic in Istanbul, Turkey for CHD. Patients records included age,sex, reason for referral, consanguinity, any previous genetic evaluation, diagnosis, family history of heart disease and other coexisting health problems. Evaluations were done by the same pediatric cardiologist and pediatric geneticist. Pediatric cardiology exam included family history and echocardiography. Pediatric genetics exam included dysmorphology exam, family history and pedigree.

Statistical Analysis

We did Pearson's chi-squared test with 1 degree of freedom and 5% significance to determine any statistically significant correlation between genetic disease prevalence determined through genetic testing and sex, consanguinity and presence of family members with additional cardiac diseases.

3. RESULTS

Among the 117 patients in our study, 28 patients (23.9%) were children of consanguinious marriages. All were first cousin marriages. None of these 117 patients had a genetic diagnosis nor had a genetic consultation before. We excluded seven patients with no congenital heart disease from the study (these patients were referred because of a murmur heard on physical exam and thought to have a CHD by their pediatricians. Echocardiography showed no structural and functional changes) and remaining 110 patients were composed of 46 female and 64 male with 10:7 ratio. The most common heart defect in our patients was Tetralogy of Fallot (TOF) followed by atrial septal defect (ASD) and ventricular septal defect (VSD) equally. Clinical and demographic characteristics of these patients are given in Table I. We offered genetic counseling to all patients with CHD and 37 of them agreed to have genetic consultation. (33.6%). Main reasons for families who did not want to have genetic consultation were; they did not believe that genetic information would add any benefit to the disease management, they are already dealing with a chronic health problem and do not want to spend time in another clinic, and they did not want to spend money on genetic tests. Attitudes of families toward genetic counseling is another research topic.

We offered karyotype analysis to a patient with aort coarctation to look for Turner syndrome and we offered fluorescence in situ hybridization (FISH) (to look for 22q11.2 deletion) to patients with conotruncal lesions with or without extra cardiac features first and if the results are normal, we offered microarray analysis. For patients with consanguineous parents, we first offered microarray analysis and for patients who could not do microarray analysis and for patients with more complex heart diseases like dextrocardia, hypoplastic left heart we offered whole exome sequencing (WES). We could only do one WES in an accredited genetic diagnosis laboratory in Istanbul and for the rest, we sent the samples to a research laboratory at Yale University Prof.Gunel laboratory. We did not have CHD genetics panel testing at our hospital, so we could not offer this test to patients before WES. Also WES has a higher yield than the panel and cost is similar, so we prefer WES over CHD panel when possible.

Table I. *Characteristics of patient population*

Condition Name	# of Patients	Sex	Consanguinity
Dilated cardiomyopathy	1	1M	1N
Brugada	1	1M	1Y
Dextrocardia	5	4M/1F	2Y/2N/1NA
Ventricular septal defect	19	11M/8F	3Y/16N
Atrial septal defect	19	9M/10F	2Y/16N/1NA
Tetralogy of Fallot	20	13M/7F	8Y/10N/2NA
Aort Coarctation	6	4M/2F	6N
Double Arcus Aorta	1	1M	1Y
Truncus Arteriosus	3	3M	3N
Interrupted aortic arch	1	1M	1N
Hypoplastic left heart	4	3M/1F	3Y/1N
Pulmonary Atresia	4	3M/1F	4N
Tricuspid anomaly	1	1F	1Y
Tricuspid atresia	1	1F	1Y
Total anomolus pulmonary venous return	1	1M	1N
Transposition of great arteries	1	1M	1N
Bicuspid Aortic Valve	4	4M	4N
Biscuspid aorta	1	1F	1N
Hypertension	2	1M/1F	2N
Noncompaction Cardiomyopathy	1	1M	1N
Transposition of great arteries	4	1M/3F	2Y/2N
Double Inlet Single Ventricle	1	1M	1N
Pulmonary stenosis	2	1M/1F	2N
Aort Regurgitation	1	1F	1N
Aort stenosis	2	1M/1F	2N
Hypoplastic right heart	1	1M	1N
Double outlet right ventricle	1	1F	1N
Atrioventricular septal defect	1	1M	1N
Right ventricle outflow tract obstruction	1	1F	1NA
Patent ductus arteriosus	2	2F	2N
Anomalous left coronary artery from the pulmonary artery	1	1M	1N
Left ventricle outflow tract obstruction	1	1F	1N
Ascending aorta hypoplasia	1	1F	1N

F: Female, M: Male, N: No, Y: Yes

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Out of 37 patients who had genetic consultations, 30 patients (24 M, 6 F) had either one or two of the following genetic tests; WES, FISH, and karyotype. 14 patients had WES (3 of these patients had negative FISH test for DiGeorge syndrome (OMIM# 188400), 18 patients had FISH for DiGeorge syndrome, and one patient had karyotype analysis. Among patients who had genetic testing, 11 of them had positive genetic test result (36.6%). Most common diagnosis was DiGeorge syndrome (22q11.2 deletion syndrome) with 6 patients affected. In addition, genetic tests revealed one patient with Renpenning syndrome (OMIM#309500), one patient with Kabuki syndrome (OMIM: 147920) with a p.Gln2004Ter stopcodon variant in *KMT2D* gene. Also exome sequencing of probands have revealed a novel stop codon variant p.Arg237X in *NODAL* gene in one patient, a missense variant p.(Thr70Ser) with uncertain significance in *MYH7* gene and a novel splice variant variant (c.1474-2A>C) in *NEXN* gene in one patient, and a missense variant p.(Met90Thr) in *MYH6* gene in another patient. (Table II).

Table II. *Characteristics of patients with genetic diagnoses*

Heart Disease	Gene	Mutation	Novelty/ClinVar Accession number	Syndrome	Age	Sex	Extra cardiac manifestation	Consanguinity	Performed Genetic Test
TGA	Nodal	p.Arg237Ter	Novel		2 years	M	N	N	WES
Hypoplastic left heart	*KMT2D*	p.Gln2004Ter	Reported (VCV000692015.2)	Kabuki	1 week	M	Anal atresia	Y	WES
TOF with pulmonary atresia	*PQBP1*	p.Arg143fs	Novel	Renpenning	17 years	M	ID, short stature, scoliosis, microcephaly	Y	WES
Hypertrophic cardiomyopathy	*MYH7*	p.Thr70Ser	Reported (VCV000042877)		9 years	M	N	Y	WES
ASD	*MYH6*	MYH6 c.1962+1G>A	Novel		17 years	M	N	Y	WES
Aort coarctation		22q11.2 deletion	Reported	DiGeorge	2 month	M	N	N	FISH
TOF		22q11.2 deletion	Reported	DiGeorge	2 weeks	M	N	Y	FISH
ASD/VSD		22q11.2 deletion	Reported	DiGeorge	4 years	M	N	N	FISH
Truncus arteriosus		22q11.2 deletion	Reported	DiGeorge	1 month	M	Anal atresia	N	FISH
TOF		22q11.2 deletion	Reported	DiGeorge	12 years	M	ID, growth delay	Y	FISH
TOF		22q11.2 deletion		DiGeorge	1 year	M	N	Y	FISH

ASD: Atrial septal defect, ID: Intellectual deficiency, M: Male, N: No, TOF: Tetralogy of Fallot, TGA: Transposition of great arteries VSD: Ventricular septal defect , Y: Yes, WES: whole exome sequencing, FISH: Fluorescence in situ hybridization

As a result of the Pearson's chi-squared tests conducted, the only statistically significant correlation observed was the prevalence of any positive genetic test result and sex with a p-value of 0.03767 where 0.05 is the threshold, nevertheless, with diseases independent from each other, this result was found to be obsolete and potentially a result of sex bias. Additionally, as the samples were too small, a conclusive statistical result cannot be obtained with high reliability.

Congenital heart diseases were tabulated against the presence of any genetic condition. However, our sample is small to merit any statistical analysis of correlation, and prevents us from reaching any statistically-significant conclusions about the genetic condition and CHD occurring simultaneously. The only exception was the occurrence of TOF and DiGeorge Syndrome simultaneously in 3 individuals, whose occurrences also revealed a statistically significant correlation of the two diseases occurring simultaneously when tested through Pearson's chi-squared test.

The heart disease occurrences were also tabulated against consanguinity. However, to be able to reach to statistical significance, the number of patients with heart disease were low in our cohort. Furthermore, 6 patients did not know and/or did not want to talk about consanguinity, reducing the data available for an already small sample, so that no further statistical analysis was conducted. It is noteworthy, however, that out of 19 individuals with ASD, 16 had no consanguinity and similarly, out of 19 individuals with VSD, 16 had no consanguinity. For

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TOF, the yes/no ratio for consanguinity was much closer to 1, as was for dextrocardia.

Extra cardiac manifestations were reported only in 15 patients. (Table II) Among those, only 8 had genetic testing and 4 had genetic diagnosis; 2 by FISH and 2 by WES. These were one patient with Renpenning syndrome, one patient with Kabuki syndrome and 2 patients with DiGeorge syndrome.

4. DISCUSSION

The consanguinity rate in Turkey is 23.5 % according to a study conducted by Hacettepe University [7]. In our cohort, consanguinity rate was similar; 23.9%. We could have found more patients with recessively inhereted variants, but unfortunately we could not do WES analysis nor CHD genetics panel to most of our patients with consanguineous parents and complex CHD because of budget restraints and negative attitude of patients towards genetic testing. In our cohort, we did not have patients with more common syndromes like Down syndrome, Turner syndrome nor Williams syndrome. The fact that we did not see any patients with more common genetic syndromes with CHD like Down, Turner or Williams syndrome may be because these patients already had a genetic diagnosis and preferred to be followed up at a public hospital pediatric cardiology clinic.

The best first line genetic assessment for most patients with CHD is a microarray analysis [8]. Next step is WES. One study showed that involving a geneticist increased the diagnosis rate of infants with CHD by 7–13%, after excluding Down syndrome [6]. TOF is the most common cyanotic CHD, that accounts for 7-10% of all CHD. In our cohort 17 % of patients had TOF.

KMT2D related Kabuki syndrome is an autosomal dominant disorder, and most cases occur de novo. It is characterized by typical facial features, infantile hypotonia, developmental delay and/or intellectual deficiency (ID) and congenital heart defects. The majority of these defects are isolated shunt lesions, conotruncal abnormalities, or various forms of arch obstruction. Hypoplastic left heart defects are seen less commonly [9] and this was the case in our patient. As targeted therapies for Kabuki syndrome are being developed, it is important to be able to make the correct diagnosis.

MYH6 can cause an autosomal dominant form of ASD and variants of the same gene in patients with hypertrophic and dilated cardiomyopathy are also reported [10]. We report a missense p.(Met90Thr) variant in a 17 year old patient with ASD. In our case patient's father had ASD and carried the same variant. If the father had a genetic diagnosis before, the family would have a genetic counseling and a more informed family planning in terms of prenatal genetic diagnosis would be made.

Our patient with hypertrophic cardiomyopathy (HC) carried a missense variant p.(Thr70Ser) with uncertain significance in *MYH7* gene and a novel splice variant variant (c.1474-2A>C) in *NEXN* gene. His father had died due to HC and never had a genetic test. HC is frequently described as a disease of the sarcomere and pathogenic variants are detected in almost all sarcomeric proteins, which are responsible for generating the molecular force of myocyte contraction. 70 % of identified variants are encoded by *MYBPC3* and *MYH7* genes [11]. *NEXN* is a filamentous actin binding protein and important in early heart development and differentiation of cardiomyocytes, and expression of contractile elements [12].

Variants in *NODAL* gene and its signaling pathways have been implicated to play a role in the pathogenetis of laterality defects. Our patient carried a heterozygous missense *NODAL* variant p.Arg237X and had transposition of great arteries (TGA) [13].

DiGeorge Syndrome also known as 22q11.2 deletion syndrome and velocardiofascial syndrome has a prevelance of 1 in 3-6000 live births [14]. Phenotype varies widely and more than 100 phenotypic features have been recorded so far. Also, most of these features may not be apparent in the neonatal period. Approximately 60-80% of patients have a cardiac malformation most commonly conotruncal defects (TOF, truncus arteriosus, interrupted aortic arch type B), conoventricular and/or ASD, and aortic arch anomalies [15]. New guidelines suggest screening for a 22q11.2 deletion in the patient with TOF, truncus arteriosus, interrupted aortic arch type B, conoventricular septal defects as well as those with an isolated aortic arch anomaly [16]. We had 3 patients with TOF out of 6. One patient had aort ic coarctation, one patient had ASD/VSD and one patient had truncus arteriosus. Early identification of a 22q11.2 deletion in the neonate or infant can be difficult when other syndromic features may not be apparent [17]. In our cohort, only 2 patients had extra cardiac features (Table III).

We had several limitations in our study. First one is this is a retrospective study and second is the small number of patients in our research. Fewer patients with congenital anomalies are treated at private hospitals than public hospitals since they require multi specialty clinics and most private hospitals have limited number of specialty clinics. Nevertheless, to the best of our knowledge, this is the first descriptive research in a private pediatric cardiovascular genetics clinic. Again, to the best of our knowledge this is the only pediatric cardiovascular genetics clinic in Turkey where pediatric cardiologist and pediatric geneticist see patients at the same time. Third limitation is that we could not do more genetic tests, especially we could not do microarray analysis which is now considered the first tier genetic testing in CHD since the patients did not want to pay for this test and most of them thought that genetic diagnosis was not necessary.

Many individuals with CHD are now of reproductive age and are at increased risk of having children with CHD and would benefit from genetic evaluation for family planning. Patients with certain types of syndromes have different survival outcomes after surgery and this is important for planning the right treatment plan as part of personalized medicine [18]. They also have different neurodevelopmental outcomes that require early and different interventions [19].

We hope that our study increases the awareness and shows the importance of genetic testing in CHD among pediatric cardiologists. Our next step is to increase our sample size by making a multicenter study and find a funding for genetic tests.

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Compliance with the Ethical Standards

Ethical Approval: Ethical approval was obtained from the Ethical Committee of Demiroglu Bilim University dated 22.12.2020 with protocol no 2020-24-04. All participant gave written informed consent.

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Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Author Contributions: WD and AGES: Did genetic analysis, reviewed the manuscipt, EN and LY: Performed data and statistical analysis, YY: Examined the patients, reviewed the manuscript, HK: Designed, supervised the article, examined the patients, took the consents, did literature review and wrote the manuscript.

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Original Article

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The role of procalcitonin as a biomarker for acute pulmonary exacerbation in subjects with cystic fibrosis and non-cystic fibrosis bronchiectasis

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ABSTRACT

Objective: Patients with cystic fibrosis (CF) and non-CF bronchiectasis are prone to exacerbations of pulmonary infections. C-reactive protein (CRP) and procalcitonin (PCT) are inflammatory markers. The aim of this study is to evaluate the role of CRP and PCT on exacerbations of CF and non-CF bronchiectasis.

Patients and Methods: The medical records of 18 CF (52 hospitalizations) and 20 non-CF bronchiectasis patients (51 hospitalizations) were reviewed retrospectively. CRP, PCT levels and, white blood cell (WBC) counts on admission and follow-up were evaluated.

Results: C-reactive protein levels correlated with PCT levels on admission in all patients. Baseline PCT levels were markedly higher (>0.5μg/L) in 12% of CF and 10% of non-CF bronchiectasis patients, however, baseline CRP values were markedly higher (>5mg/L) in 96% of CF and non-CF bronchiectasis patients (p=0.760 and p=0.100, respectively). Baseline CRP and PCT levels were positively correlated with hospitalization length (r=0.501, p=0.001 and r=0.289, p=0.04, respectively) in CF patients, but not in non-CF bronchiectasis.

Conclusion: Our study shows the potential utility of these biomarkers to determine the severity of the exacerbation particularly predicting hospitalization length in CF patients. Both biomarkers could be able to guide antibiotic treatment of infective exacerbations in CF and non-CF bronchiectasis patients.

Keywords: Cystic fibrosis, Bronchiectasis, Exacerbation, Infection, Procalcitonin, C-reactive protein

1. INTRODUCTION

Cystic fibrosis (CF) is a multisystem disorder caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene. Patients with CF have periodic flare-ups or exacerbations associated with increased mortality and morbidity [1, 2]. Most patients develop pulmonary symptoms that worsen following exacerbations and 25% of cases do not return to baseline pulmonary function test values despite treatment [2]. Most clinicians use clinical signs and symptoms with microbiology and imaging modalities. Antibiotic therapy is recommended for bacterial exacerbations, and treatment response can be seen even in cases of infection with pan-resistant strains [3].

Non-CF bronchiectasis is a chronic lung disease characterized by chronic and recurrent lower respiratory tract infection. Although, management and follow-up strategies for non-CF bronchiectasis are usually extrapolated from CF; patients with non-CF bronchiectasis have different distribution, rate of progression, degree of inflammation of the area of bronchiectasis without multiorgan disease and the data about acute exacerbation in this group is scarce. Physicians prefer to rely on biomarkers in addition to clinical features and radiologic changes to define exacerbations. The specific diagnostic marker and optimal duration of treatment for CF and non-CF bronchiectasis exacerbations are currently unknown. Unlike sputum, blood sampling is simple and feasible to monitor the severity of inflammation in entire lung. The ideal biomarker in blood should be clinically relevant to demonstrate high sensitivity and specificity for diagnosis and treatment effects, also it should predict the prognosis of exacerbation process.

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Nonspecific markers of inflammation, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and elevated white blood cell (WBC) count are well established and widely used in clinical practice to diagnose inflammation including bacterial infection. It has been reported that CRP is correlated with high resolution computerized tomography (HRCT) scores in stable non-CF bronchiectasis patients [4]. In a prospective observational 2-year cohort study, CRP crossed the upper normal limits at onset of acute exacerbation and related with symptoms in 32 outpatients with non-CF bronchiectasis [5]. Although, there is no gold standard marker for diagnosis of exacerbations in CF and non-CF bronchiectasis patients, one of the most studied biomarkers for CF pulmonary exacerbation is CRP that is increased during CF exacerbations and decreased with antibiotic treatment [6, 7]. However, it is a fact that new biomarkers are necessary for diagnosis, treatment and follow-up of CF and non-CF bronchiectasis patients because of limited specificity for bronchiectasis.

Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin which also is a newly identified marker for infection. It can be detected at high levels in bacterial diseases [8]. In the absence of infection in the body, PCT is synthesized by thyroidal C-cells and stored in secretory glands in the form of calcitonin. In the presence of bacterial infections, calcitonin gene expression is upregulated via a process that may be mediated by microbial toxins and cytokines, leading to an increased release of PCT from non-thyroid tissues into the bloodstream. Contrarily, down-regulation of calcitonin by secreted cytokines during viral infections increases PCT specificity in bacterial infections [8]. A meta-analysis has shown that PCT is more sensitive (88% vs 75%) and specific (81% vs 76%) in bacterial infections than CRP. PCT also has shorter durations for reaching peak levels than CRP (8 h vs 36 h) [9]. Apart from diagnosis, clinical assessment and routine blood tests may be insufficient and serial PCT measurements would be recommended to determine the duration of antibiotic use. Therefore, PCT guidance protocols inform the decision to start and stop the antibiotics in lower respiratory tract infections with a shorter duration of antibiotic use. [10, 11]. However, as a reliable marker to diagnose and guide the antibiotic treatment in patients with CF and non-CF bronchiectasis, the data about PCT is lacking. Louw et al., found that baseline PCT was not statistically elevated in pediatric CF acute pulmonary exacerbation group [12]. Similarly, Roderfeld et al., found significant change of CRP but not PCT in CF exacerbation period in adult patients [13]. It is a fact that, based on current international guidelines, there are no recommendations for PCT use in managing acute exacerbations of chronic lung diseases including bronchiectasis [14]. In a study assessing non-CF bronchiectasis patients, it has been reported that PCT levels in outpatient bronchiectasis patients were lower than hospitalized patients who needed intravenous antibiotics and PCT was significantly correlated with CRP. High PCT levels were related with increased likelihood antibiotic prescription and authors commented that PCT seems likely to be able to guide the treatment of an exacerbation in non-CF bronchiectasis patients [15].

We suggest that biomarkers could be helpful to define antibiotic treatment response and the length of hospitalization in severely acute exacerbated CF and non-CF bronchiectasis patients. This study aimed to determine whether CRP and PCT levels may play a role in the clinical care of CF and non-CF bronchiectasis patients with acute pulmonary exacerbations.

2. PATIENTS and METHODS

This was a retrospective study including patients aged ≥18 years with acute exacerbation of CF and non-CF bronchiectasis that were followed by Marmara University Hospital Adult Pulmonology Clinic between May 2013 and December 2016. The research project was approved by Marmara University School of Medicine Clinical Research Ethics Committee with the approval number 09.2016.515.

Patient Selection and Data Collection

A total of 102 non-CF bronchiectasis and 76 CF patients were screened, and 119 hospitalizations were reviewed. However, 4 hospitalized patients were excluded because of transfer to other intensive care units, and 12 were excluded since we could not reach all data. Inclusion criteria; age ≥18years with exacerbation requiring hospitalization as determined by the treating physician, exclusion criteria; antibiotics initiated more than 48 hours prior to admission (other than chronic azithromycin use) and pregnancy. Finally, a total of 52 hospitalizations of 18 CF patients and 51 hospitalizations of 20 non-CF bronchiectasis patients were included in the study. Medical records were reviewed by questioning patients' age, gender, body mass index (BMI), smoking history, duration of disease, comorbidities, medications, colonization status, pulmonary function tests performed during last stabilization period, exacerbation and hospitalization rate within last year. Antibiotics used during hospitalization, microbiological analyses of sputum, white blood cell (WBC) counts, CRP, and PCT levels at enrollment day and 3, 7, 10 days after starting antibiotics were recorded.

Diagnosis of CF and non-CF Bronchiectasis and Acute Exacerbations

Cystic fibrosis was diagnosed based on pilocarpine iontophoresis-induced sweat chloride levels ≥60 mEq/L or genetic analyses revealing two CF-related mutations and two CF-consistent clinical findings. Pulmonary CF exacerbation was defined according to the Clinical Practice Guidelines Signs and Symptoms of Pulmonary Exacerbation. Pulmonary exacerbation was defined as the emergence of 4 of 12 signs or symptoms, prompting changes in therapy and initiation of antibiotics (modified from Fuchs' criteria). These criteria included: change in sinus congestion, sputum, or hemoptysis; increased cough, dyspnea, malaise, fatigue or lethargy; fever; hypoxia or weight loss; change in chest physical exam; or forced expiratory volume in the first second (FEV_1) decrease >10% from a previous value [16].

Non-CF bronchiectasis patients were defined by excluding CF but confirming bronchiectasis by HRCT. Exacerbation was defined as a patient with non-CF bronchiectasis with a deterioration in three or more of the following key symptoms for at least 48 hours; cough, sputum volume and/or consistency; sputum purulence, breathlessness and/or exercise intolerance,

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fatigue and/or malaise, hemoptysis and a clinician determined change in bronchiectasis treatment was required [17].

Procalcitonin was measured in duplicates using 50 µL serum by the time-resolved amplified cryptate emission technology (Brahms, England), whereas CRP was measured using 25µL serum by an automated analyzer (Abbott Architect, Illinois, USA). Cut-off values of 0.5 µg/mL for PCT and 5 mg/L for CRP were accepted as higher than normal and WBC counts >10000/mm3 indicated leukocytosis.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences, version 23.0 for Windows® system (SPSS® Inc., Chicago, Illinois, USA). Normal distribution of variables was examined using histogram graphs and the Kolmogorov–Smirnov test. The mean ± standard deviation, median and minimum–maximum values were used when descriptive analyses were presented, and 2 × 2 tables were used to compare variables with the Pearson's chi-squared and Fisher's exact tests. When normally distributed (parametric) variables were evaluated, the Student's *t*-test was used for independent groups; when non-normally distributed variables were evaluated between groups, the Mann–Whitney U Test was used. Pearson's correlation analysis for normally distributed and Spearman correlation test for non-normally distributed data were used. A p value of <0.05 was considered statistically significant.

3. RESULTS

Demographics

In the present study, 11/18 (61%) adult CF patients and 11/20 (55%) adult non-CF bronchiectasis patients were females. The mean age of CF and non-CF bronchiectasis patients was 23.9 ± 4.8 and 47.2 ± 15.3 years, respectively (p<0.001). The characteristics of the patients are summarized in Table I. Compared with other comorbidities, pancreatic insufficiency was significantly more common in CF patients. Chronic obstructive pulmonary disease (COPD) was more frequently detected in non-CF bronchiectasis patients when compared with CF patients (p<0.011) (Table I).

Table I. *Patient Characteristics*

	Cystic Fibrosis (n:18)	Non-Cystic Fibrosis Bronchiectasis (n:20)	p value
Age, years (mean ± SD)	23.9 ± 4.8	47.2 ± 15.3	0.001*
Female, n (%)	11 (61)	11 (55)	0.703
BMI, kg/m² (mean ± SD)	20.6 ± 2.5	23.7 ± 6.2	0.075
Current or ex-smoker, n (%)	1 (6)	6 (30)	0.052
Asthma, n (%)	6 (33)	8 (40)	0.671
COPD, n (%)	0 (0)	6 (30)	0.011*
Pancreatic Insufficiency, n (%)	15 (83)	0 (0)	0.001*
Hypertension, n (%)	0 (0)	3 (15)	0.087
DM, n (%)	4 (22)	5 (25)	0.841
CRF, n (%)	0 (0)	1(5)	0.336
Pulmonary Embolism, n (%)	1 (6)	1 (5)	0.939
Colonization, n (%)	14 (78)	6 (30)	0.004*
Pulmonary function tests			
FEV_1/FVC (% predicted)	64.0±10.7	59.0±13.1	0.210
FVC (% predicted)	57.6±25.7	56.0±24.5	0.869
FEV_1 (% predicted)	44.7±25.6	43.7±25.6	0.947
Attack numbers	n=52	n=51	
CRP ** (mg/L)	55.3 (3.44-321.0)	53.5 (3.44-296.0)	0.864
PCT ** (µg/L)	0.10 (0.02-13.51)	0.10 (0.02-3.72)	0.236
WBC ** (/mm3)	11.000 (5600-33100)	11.200 (2500-31600)	0.877
Hospitalization length, days	10.7±2.5	10.0±1.9	0.191

BMI: Body Mass Index, COPD: Chronic Obstructive Pulmonary Disease, DM: Diabetes Mellitus, CRF: Chronic Renal Failure, CRP: C-Reactive Protein, PCT: Procalcitonin, WBC: White blood cell.
**p < 0.05 is statistically significant, **Measurements on enrollment day, Data are given as mean ±SD and median (min-max).*

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Pulmonary Function Testing

There was no statistically difference between pulmonary function tests of CF and non-CF bronchiectasis patients. (Table I). Ten patients of CF group (56%) and thirteen (65%) of non-CF bronchiectasis patients had severe pulmonary obstruction (FEV_1 < 50% of predicted value).

Bacterial Colonization

Sputum samples from patients with CF were colonized with bacteria at a significantly higher rate compared with non-CF bronchiectasis cases (78% vs 30%, respectively, p<0.004) (Table I). Upon examination of bacterial colonization in both groups, *Pseudomonas aeruginosa* was observed to be the most commonly colonized bacteria in both groups (67 % of CF and 30 % of non-CF bronchiectasis patients)

Signs and Symptoms

Upon evaluation of signs and symptoms; such as fever, increased sputum volume, sputum thickening, increased breathlessness, and hemoptysis during admission to the ward; there was no relation between high levels of CRP, PCT, WBC counts and these signs and symptoms.

Sputum Culture

Examination of sputum cultures obtained from patients during admission to the clinic revealed that 42 of 52 attacks (83%) of CF patients and 16 of 51 attacks (31%) of non-CF bronchiectasis patients demonstrated culture growth (Table II). *Pseudomonas aeruginosa* was observed to be the most commonly cultured bacteria (56 % of CF patients vs 16 % of non-CF bronchiectasis patients).

Table II. *Microbiological analysis of sputum samples at enrollment day in patients with CF and non-CF bronchiectasis*

Sputum Culture	Cystic Fibrosis n=52 (%)	Non – cystic fibrosis bronchiectasis n=51(%)
Pseudomonas aeruginosa	29 (56%)	8 (16%)
Achromabacter	6 (12%)	
MSSA	2 (4%)	2 (4%)
Acinetobacter baumannii	2 (4%)	
Hemophilus influenza	1 (2%)	1 (2%)
MRSA	1 (2%)	4 (8%)
Stenotrophomonas maltophilia	1 (2%)	
Serratia	1 (2%)	
Streptoccoccus pneumonia		1 (2%)
Total	43 (83%)	16 (31%)

MSSA: Methicillin-sensitive staphylococcus aureus, MRSA: Methicillin-resistant staphylococcus aureus

Length of Hospitalization

The mean duration of hospitalization was 10.7 ± 2.5 days and 10.0 ± 1.9 days for CF and non-CF bronchiectasis patients, respectively. BMI, FEV_1, PCT and CRP levels and WBC counts were examined as factors possibly affecting the duration of hospitalization. In both groups, there was no relation between BMI, FEV_1% predicted, forced vital capacity (FVC) % predicted, FEV_1/FVC%, WBC counts at the enrollment day and duration of hospitalization, whereas the length of hospitalization was significantly longer in all patients with high CRP and PCT levels at the enrollment day (r=0.315, p<0.001 and r=0.289, p<0.003, respectively). Moreover, when we analyzed the length of hospitalization in the participants who had CF and non-CF bronchiectasis separately, these linear correlations remained statistically significant for CRP levels in CF patients but not in non-CF bronchiectasis patients (r=0.501, p<0.0001 and r=0.100, p<0.479, respectively). Similarly, when we correlated the length of hospitalization with PCT levels at the admission, there was positive correlation in patients with CF (r=0.289, p<0.040) but not in non-CF bronchiectasis patients (r=0.223, p<0.112) (Figures 1 and 2). In either group, no significant difference was found between the patients colonized with *Pseudomonas aeruginosa* and the other bacteriological pathogens in terms of length of hospitalization.

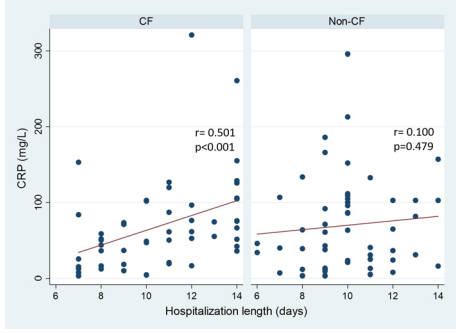

Figure 1. *Correlation between hospitalization length and CRP level in patients with CF and non-CF bronchiectasis*

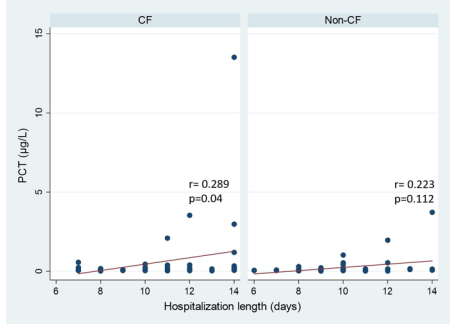

Figure 2. *Correlation between hospitalization length and PCT level in patients with CF and non-CF bronchiectasis*

Inflammatory Biomarkers

Upon examination of all attacks, median CRP level at the enrollment day was 55.3 (min-max: 3.44-321.0) mg/L in patients with CF and 53.5 (min-max: 3.44-296.0) mg/L in non-CF

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bronchiectasis (p<0.864). Median PCT level was 0.10 (min-max: 0.02-13.51) µg/L in patients with CF and 0.10 (0.02-3.72) µg/L in patients with non-CF bronchiectasis (p<0.236) (Table I). We correlated the CRP with PCT levels at the time of enrollment in all patients and found a significant linear correlation between CRP and PCT levels (r=0.573, p<0.0001). Of the 51 attacks, CRP was high (>5 mg/L) in 49 (96%) in patients with CF and 50/52 (96%) of attacks in patients with non-CF bronchiectasis (p<1.000). PCT was high (>0.5 µg/L) in 6/51 (12%) of attacks in patients with CF and 5/52 (10%) of attacks in patients with non-CF bronchiectasis (p<0.760). There was no statistically significant difference between the CRP and PCT positivity rates when we analyzed the patients with CF and non-CF bronchiectasis separately (p<1.000 and p<1.000, respectively). There was no correlation between WBC count and CRP and PCT at the admission. The median WBC, CRP and PCT levels at the enrollment day in these two bronchiectasis groups with and without positive sputum cultures did not result in any significant difference (Table III). Likewise, neither the existence of colonization nor the colonizing species did not affect CRP, PCT levels and WBC counts at the enrollment day in patients with CF and non-CF bronchiectasis.

Table III. *Relationship between sputum cultures and acute phase reactants at the enrollment day*

	Cystic Fibrosis			Non-Cystic Fibrosis		
Sputum culture	**Positive**	**Negative**	**p value**	**Positive**	**Negative**	**p value**
	n=42	**n=9**		**n=16**	**n=36**	
WBC * (10^3/mm3)	12.6 (5.6-14.4)	10.9 (5.6-33.1)	0.776	11.95 (3.0 – 31.6)	10.3 (2.5 – 20.0)	0.211
CRP * (mg/L)	60.1 (3.44–321.0)	48.9 (7.53-102)	0.266	72.9 (5.18 – 296.0)	40.3 (3.44 – 213.0)	0.234
PCT * (µg/L)	0.1 (0.03-13.51)	0.1 (0.02-0.57)	0.435	0.11 (0.03 – 1.97)	0.08 (0.02 – 3.72)	0.110

WBC: White Blood Cell, CRP: C-Reactive Protein, PCT: Procalcitonin
*Data are given as median (min-max), * Measurements on enrollment day*

In all participants, we recorded PCT and CRP levels at admission as well as after 10 days of treatment. Most PCT and CRP levels did decrease, however observation of the decreasing effect of inflammatory markers with antibiotic response at 24 h and 72 h revealed that CRP and PCT levels at 24 h did not show any significant change from baseline in CF and non-CF bronchiectasis patients (p<0.053 and p<0.479, respectively). At 72 h, there were significant decreases in both inflammatory markers, particularly in CRP in both CF and non-CF bronchiectasis patients (48% in CRP and 30% in PCT, p<0.039 vs 69% in CRP and 45% in PCT, p<0.006, respectively). When patients' CRP and PCT levels were analyzed under antibiotic treatment during the first 10 days after admission, the decrease in CRP levels was linear and the decrease in PCT levels was biphasic (Figures 3 and 4).

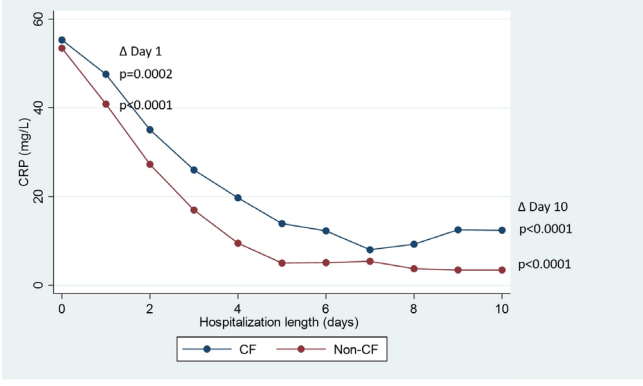

Figure 3. *10-day treatment response in CRP level in patients with CF and non-CF bronchiectasis*

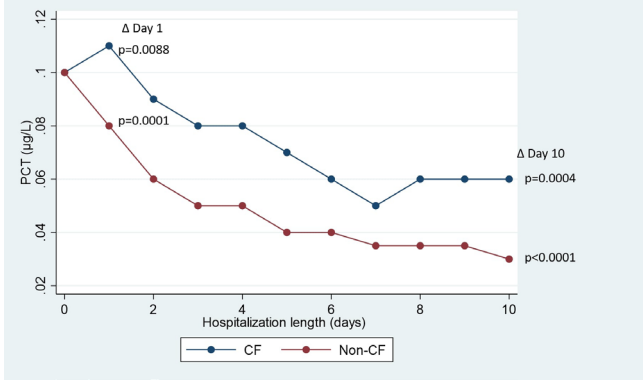

Figure 4. *10-day treatment response in PCT level in patients with CF and non-CF bronchiectasis*

4. DISCUSSION

Despite the importance of managing pulmonary acute exacerbations in the clinical course of adult CF and non-CF bronchiectasis patients, there is limited data related to the role of inflammatory biomarkers during follow-up. A serum biomarker that was able to accurately predict an acute infective exacerbation requiring antibiotic therapy and the length of hospitalization would be a great benefit for management of these patients. Our study is the first to examine the utility of PCT levels in the acute exacerbations in patients with CF and non-CF bronchiectasis requiring hospitalization. Unlike prior

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studies, we also determined the response of CRP and PCT values of CF and non-CF bronchiectasis patients in severe acute exacerbations who needed intravenous antibiotic treatment during a 10-day hospitalization. In this study, no significant differences in baseline PCT and CRP levels were noted in patients with CF and non-CF bronchiectasis.

The precise contribution of bacterial infection is difficult to define because of chronically colonized airways in these diseases. The role of acquisition of new bacteria or reactivation of colonized bacteria remains as an important factor in the pathogenesis of acute exacerbation [18]. In our study, only 12 % of exacerbated patients with CF and 10 % of non-CF bronchiectasis had PCT levels >0.5 μg/L as an evidence of bacterial infection. Thus, the contribution of bacterial infection in CF and non-CF bronchiectasis has been markedly overestimated or PCT values do not differentiate localized bacterial infection in bronchial wall from other noninfectious etiologies. It might be suggested that slight elevations of PCT may be reflective of low grade invasive bacterial infections on the mucosal surface with limited inflammation thus, low PCT levels may indicate bacterial colonization, while higher levels of PCT indicate more invasive infections. The traditional cut-off to diagnose systemic bacterial infection with PCT has been interpreted as serum PCT level<0.1 μg/L antibiotics strongly discarded, <0.25 μg/L discouraged, >0.25 μg/L encouraged, >0.5 μg/L strongly encouraged in pneumonia and COPD exacerbation [19, 20]. Our study suggested PCT and CRP as useful outcome predictors of acute pulmonary exacerbations in CF and non-CF bronchiectasis patients. PCT was significantly correlated with CRP. Although, we found detectable PCT (>0.5 μg/L) levels in 12% of attacks in CF patients and 10% of attacks in non-CF bronchiectasis patients while detectable CRP (>0.5 mg/L) levels in 96% of attacks in both groups; duration of hospitalization was longer with higher CRP and PCT levels. However, considering all patients, these correlations were noted in only CF patients but not in non-CF bronchiectasis. Furthermore, the positive sputum culture and colonization in sputum did not affect the inflammatory marker response in acute exacerbations of both groups. In case of response to antibiotic therapy, we showed that CRP and PCT levels decreased statistically significantly at 72 h in both groups and maintained during the 10-day follow-up of hospitalization.

One of the most studied biomarkers for CF and non-CF bronchiectasis acute exacerbation is CRP. CRP is an acute phase reactant and increased during acute exacerbation and decreased with antibiotic treatment [7]. In a meta-analysis, it has been reported that CRP consistently increased in exacerbation state in 5 of 6 studies and decreased statistically significantly after treatment in 18 of 20 studies [21]. CRP has previously been shown to be increased in CF pulmonary exacerbations [22]. In agreement with previous studies, PCT was found to be a less promising marker than CRP in CF patients since detectable levels of PCT was found in a small number of patients in this study. In an earlier study, Louw et al., found that PCT values did not rise significantly at the onset of a respiratory exacerbation of pediatric CF patients while CRP increased significantly [12]. Likewise, in adult CF patients, PCT did not increase however, CRP significantly increased during exacerbation, additionally higher levels of CRP predicted frequent exacerbations within a year [23]. Furthermore, in a study comparing the biomarkers of adult CF patients at an acute attack with those of stable state, CRP significantly increased however, PCT did not increase at the admission [13]. In a recent study by Bailey et al., evaluated adult CF patients with acute exacerbation. Of 40 patients, 23 had detectable levels of PCT ≥0.05 μg/L that was lower than accepted level in our study therefore numbers of patients with detectable PCT were higher than ours. There was no correlation between WBC and PCT levels but there was linear correlation between PCT and CRP at the time of enrollment as similar to our results. Moreover, PCT levels were significantly associated with pulmonary exacerbation scores and decrease in FEV_1. Those who had worsening PCT during treatment or detectable PCT at admission were more likely to be readmitted to the hospital sooner [24].

This is the first study assessing the PCT use in the acute exacerbations of adult patients with non-CF bronchiectasis. We found small number of patients with non-CF bronchiectasis have detectable PCT levels however, CRP levels increased in 96% of patients. Loebinger et al., compared CRP and PCT levels of inpatient and outpatient groups with non-CF bronchiectasis and they found that PCT levels were generally lower in outpatients than those of inpatients. PCT was significantly correlated with CRP levels. Higher PCT levels were associated with increased likelihood of high levels of symptoms and increased inpatient antibiotic prescription. The big confounding factor in that study is that they did not exclude patients who had taken antibiotics before admission. In that study, the other interesting point is the lack of relationship between the original PCT level and the likelihood of re-exacerbating over the next 6 months in patients with non-CF bronchiectasis [15].

Although, there was a linear correlation between PCT and CRP on admission in CF patients, only PCT showed correlation with admission symptoms and the authors emphasized that similar relationship could not be demonstrated with conventional CRP [24]. Similarly, Sequeiros et al., evaluated 168 acute attacks of 58 adult patients with CF who had worse symptom score did not have higher CRP level at the beginning of treatment [25].

In most adult CF patients, the lungs are chronically infected with *Pseudomonas aeruginosa* and colonization has been shown to be related to progression of disease. Furthermore, Pseudomonas colonization means that many of patients will not have an oral antibiotic option for acute exacerbation, this population is likely to have inpatient management with at least two intravenous antibiotics. In our study, *Pseudomonas aeruginosa* is the first among the microorganisms grown in sputum culture in both groups that is compatible with similar publications [15]. Upon examination of initial and follow-up PCT values, no relation was observed between sputum cultures, colonization and detectable PCT levels in patients with CF and non-CF bronchiectasis.

For the first time, we have described that high CRP and PCT levels were related to prolonged admission time in CF patients but not non-CF bronchiectasis patients. In a similar study

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predicting duration of exacerbation, baseline CRP levels were studied however, it was found not to be predictive of the duration required to control CF acute exacerbation [25]. We suggest that elevated PCT and CRP values correlated with longer hospitalization period suggesting the possibility of severe illness at admission. Thus, high PCT and CRP alert clinicians to the unresponsiveness of the antibiotics and consequently the need of longer hospitalization.

There is limited literature about the role of PCT in monitoring the response against antibiotics regarding infective exacerbations of adult CF and non-CF bronchiectasis patients. While most reports evaluating the guidance of PCT on antibiotic therapy revealed that PCT follow-up reduces the duration of antibiotic use in lower respiratory tract infections, there are some reports that are contradicting. Ito et al., also suggested that high PCT and CRP levels in diagnosis and follow-up of community-acquired pneumonia were useful in predicting early mortality [26]. Our study adds to the growing body of literature which questions the utility of PCT levels to guide antibiotic treatment in CF and non-CF bronchiectasis patients. When CRP and PCT levels in response to antibiotic therapy were evaluated at admission in the present study, it was observed that evaluation of the antibiotic response showed a statistically significant decrease in 72-h CRP and PCT levels in CF and non-CF bronchiectasis patients. In addition, PCT tends to decrease biphasically, whereas CRP tends to decrease linearly and superiority to each other was not detected. However, in another study, it was found no statistically significant change of PCT level after 10 days of antibiotic treatment in CF patients [24]. In contrast, Roderfeld et al., followed only 7 CF inpatients during antibiotic treatment and did obtain serum samples after completion of antibiotic therapy, however neither CRP nor PCT were significantly changed after antibiotic therapy for pulmonary exacerbation [13]. In a study evaluating non-CF patients, Loebinger et al., monitored acute exacerbation of non-CF bronchiectasis patients after starting antibiotic treatment and there was no significant difference in PCT concentrations between the measurements at days 0, 5, and 10 in contrast to our results [15].

Considering the shortcomings of this study, only a limited number of patients could be included because of lack of data due to the retrospective nature of the study. Also, long-term outcomes of patients were not monitored. We have not clinical scoring data for grading acute exacerbation. Additionally, sampling from a single center limits the generalizability of these principal findings.

This is the first study assessing the use of CRP and PCT in patients with CF and non-CF bronchiectasis together. We showed that PCT may be a useful biomarker in CF exacerbations to determine the outcome. Furthermore, high PCT values at the time of diagnosis of acute infective exacerbations requiring hospitalization in patients with CF but not non-CF bronchiectasis, were shown to be related to prolonged admission time. Follow-up of response to treatment with a trend of superiority were anticipated because of statistically significant decrease starting from 3rd day of antibiotic treatment. We conclude that PCT and CRP should be located in the follow-up of infective acute exacerbations of adult CF and non-CF bronchiectasis patients to determine its potential in guiding management decisions. Further studies are needed to determine PCT cut-off values and to assess PCT efficacy in acute bacterial exacerbations of CF and non-CF bronchiectasis patients.

Compliance with Ethical Standards

Ethical Approval: This study was approved by Marmara University School of Medicine Clinical Research Ethics Committee with the approval number 09.2016.515.

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Conflict of Interest: There are no conflicting interests.

Author Contributions: FM: Drafting of the work, data acquisation, critical revision, SOY, DK, EE and BC: Drafting of the work, concept and design of the study,critical revision, HA: Concept and design of the study, statistical analysis, critical revision, CC: Data acquisation, critical revision. All authors approved the final version of the article.

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Late presentations among HIV-infected patients

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ABSTRACT

Objective: The late diagnosis of human immunodeficiency virus (HIV) infection and, therefore, initiation of antiretroviral therapy (ART) in the advanced stages of the disease is still a significant problem in many countries. This study aimed to define the prevalence of late presentation and advanced HIV disease among newly diagnosed HIV infected patients.

Patients and Methods: The medical records of HIV-infected patients aged 18 and over, diagnosed between January 2011 and December 2019, were analysed retrospectively. The patients were grouped into three-year periods according to the date of diagnosis.

Results: Of the 280 patients with HIV infection, 44 (15.7%) were female and 236 (84.3%) were male. It was found that 50.7% of the patients were late presenters, and 28.5% had advanced diseases. There was no statistical difference in the prevalence of late presentation and advanced HIV disease between the three-year periods. While there was no relationship between gender and late presentation, a statistically significant relationship was found between advanced age and late presentation.

Conclusion: Despite advances in the diagnosis and treatment of HIV infection, the frequency of late presentation is still quite high, in Turkey. This situation highlights the importance of broad-based HIV screening programs to control the disease more effectively.

Keywords: HIV, Acquired Immunodeficiency Syndrome, Late presentation, Turkey

1. INTRODUCTION

In 2018, 141.552 newly diagnosed human immunodeficiency virus (HIV) infections were reported from 50 of the 53 countries in the World Health Organization (WHO) European Region. Of these newly diagnosed patients, 79% were diagnosed in Eastern Europe, 16% in Western Europe, and 5% in Central Europe. Turkey is among the fifteen Central European countries, namely Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Hungary, North Macedonia, Montenegro, Poland, Romania, Serbia, Slovakia and Slovenia. HIV infection prevalence remains relatively low in Central Europe, but the number of new diagnoses is growing faster than in other regions. A total of 6519 patients were newly diagnosed with HIV infection in the central region; 3800 of them were diagnosed in Turkey in 2018 [1].

During the natural course of HIV infection, there is a progressive loss of the CD4 T lymphocyte count, leading to a severe weakening of the infected patients' immune system. Antiretroviral therapy (ART) alters the natural course of HIV infection and significantly reduces morbidity and mortality [2,3]. However, the late diagnosis of HIV infection and ART initiation in the advanced stages of the disease is still a significant problem in many countries. Late diagnosis of HIV infection has significant consequences both for individuals and society. Morbidity and mortality rates are ten times higher in patients with the late diagnosis than in patients diagnosed in the first year after infection [4]. Besides, long-term outcomes of late diagnosis include an increased risk of neurocognitive impairment and permanent impairment in the restoration of CD4 T lymphocyte count [5]. Also, unawareness of the HIV infection causes an increased risk of transmission in the community and is a critical factor in spreading the disease [6].

This study aimed to define the prevalence of late presentation and advanced HIV disease among newly diagnosed HIV infected patients for a three-year period for nine years.

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2. PATIENTS and METHODS

The medical records of HIV infected patients who are eighteen years and older diagnosed between January 2011 and December 2019 at the university hospital were retrospectively analysed for late presentation and advanced HIV disease. According to the diagnosis date, the patients were divided into three groups of three years, including the 2011-2013, 2014-2016, and 2017-2019 periods. Patients whose CD4 T lymphocyte count was not studied before ART initiation were excluded.

Late presentation and advanced HIV disease definitions were based on the European Late Presenter Consensus working group report. Late presentation was defined as a presentation with a CD4 T lymphocyte count below 350 cells/mm3 or acquired immunodeficiency syndrome (AIDS), defining illness, regardless of CD4 T lymphocyte count. Presentation with advanced HIV disease is defined as a CD4 T lymphocyte count below 200 cells / mm^3 or with an AIDS-defining illness, regardless of CD4 T lymphocyte count [7]. In this present study, patients were evaluated on initial CD4 T lymphocyte count irrespective of their AIDS-defining illnesses.

This study was approved by the university's local ethics committee. (Approval number: B.30.2.ODM.0.20.08/407-475)

Statistical Analysis

The analyses were performed using the IBM SPSS Statistics 23 software (SPSS; Chicago, IL, USA). Continuous variables between groups were compared with the Mann-Whitney U test or Kruskal-Wallis H test when appropriate. In contrast, categorical variables were compared with Chi-square or Fisher Exact test, when applicable. The statistical significance was defined as $p<0.05$.

3. RESULTS

Between January 2011 and December 2019, a total of 280 patients, 44 (15.7%) females and 236 (84.3%) males, were diagnosed with HIV infection.

The mean ages of the newly diagnosed patients in the 2011-2013, in the 2014-2016, and in the 2017-2019 were 41.4 ± 11.0 years, 36.0 ± 11.6 years, and 38.2 ± 11.4 years. The overall mean age of patients was 38.2 ± 11.5 years. There was a significant difference in mean ages between the periods (p: 0.033), and the mean age in the 2011-2013 period was significantly higher (p: 0.048).

The CD4 T lymphocyte mean counts at the time of diagnosis were 426.9 ± 309.7 /mm^3 in the 2011-2013 period, 364.1 ± 206.0 / mm^3 in 2014-2016 period, 358.5 ± 248.1 /mm^3 in 2017-2019 period, and the overall mean was 373.8 ± 251.0/ mm^3. There was no statistically significant difference between the mean CD4 T lymphocyte counts of the periods (p: 0.465).

When comparing late and advanced HIV disease presentation prevalence between periods, no statistical difference was detected (p: 0.545 and p: 0.252, respectively).

There was no statistically significant difference between the patients who applied with late presentation and those with CD4 T lymphocyte count> 350 cells / mm^3 in terms of gender (p: 0.091).

The mean age of the late presenters (38.8 ± 11.4 years) was higher than the mean age of patients with CD4 T lymphocyte >350/ mm^3 (36.5 ± 11.9 years), and the difference was statistically significant (p: 0.015) (Table I).

Table 1. *Demographic and laboratory characteristics of patients according to three-year periods*

Periods	2011-2013	2014-2016	2017-2019	2011-2019
Number of patients (n)	56	83	141	280
Mean age± SD (years)	41.1±11.0	36.0±11.6	38.2±11.4	38.1±11.5
Mean CD4 T lymphocyte count± SD (mm^3)	426.9±309.7	364.1±206.0	358.5±248.1	373.8±251.0
Prevalence of late presenters	44.6%	49.3%	53.1%	50.7%
Prevalence of advanced HIV disease	26.7%	21.6%	31.9%	28.5%

SD: standard deviation

4. DISCUSSION

From 1985 until June 2019, 20.202 HIV infected patients and 1786 AIDS cases were identified in Turkey. Of them, 79.98% were male, and 49.7% of these patients were in the 25-39 years age group [8]. In a study performed at five training hospitals in Istanbul, which the most populous city in Turkey, 829 naive HIV infected patients participated, 84.4% of the patients were male, and the mean age was 37 years [9]. In a study conducted in Ankara, the second-most populous city in Turkey, 255 HIV-infected patients were evaluated. It was reported that 75.6% of the patients were male, and the mean age was 38 years [10]. The gender distribution under male dominance and mean age of HIV-infected patients in our study are comparable to national data.

The mean age of patients diagnosed in the 2011-2013 period in our study was significantly higher than in the later periods, which is thought to be a good indicator of early diagnosis in the last years in terms of early diagnosis. However, it was found that there was no statistical difference in the immunological status of the patients in the following periods.

A recently published study from Istanbul, Turkey, including 1,673 newly diagnosed (86% males, median age: 35 years) HIV/AIDS patients, reported that half of the patients (49.4%) were late presenters and a quarter (25.5%) of the patients presented with advanced HIV disease [11].

High late presentation and advanced HIV disease prevalence have also been reported in studies from European countries. In Greece, among 107 HIV infected patients diagnosed between

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2010 and 2018, 49.5% were reported to be late presenters and 33.6% with advanced HIV disease. [12]. More than half (57.6%) of the 3972 HIV-infected patients who followed up in Polish HIV treatment centers between 2000 and 2015 were late presenters, while 35.6% had advanced HIV disease [13]. An Italian cohort study including 19,391 antiretroviral-naive adults reported that of total patients, 54 % were late presenters, and 37.6 % were with advanced HIV disease [14]. Among the 20,496 patients, 53.9% were late presenters, and 31.2% had advanced HIV disease in a French cohort [15]. European Centre for Disease Prevention and Control (ECDC) 2018 HIV/AIDS surveillance reported that among 15 of the WHO central European Region, including Turkey, 55% of the patients were late presenters and 32% with advanced HIV disease [1]. Although, there were differences in prevalence, our data also supported that generally, at least half of HIV-infected patients were late presenters, and half of them were with advanced HIV disease across Europe and Turkey.

Our study also determined that there was no statistically significant difference in CD4 T lymphocyte mean count in late presentation and advanced HIV disease prevalence between all three-year periods. According to data from a total of 30454 HIV infected patients from 34 European countries, there was no change in late presenters and advanced HIV disease prevalence between 2010 and 2013 [16]. In a study conducted in Belgium in which 687 HIV patients participated between January 2006 and July 2017, 44% of patients found to be late presenters and 24% with advanced HIV disease, and no decrease observed in these prevalences during the study period [17]. In a study conducted in Poland between January 2009 and December 2016, the prevalence of late admission was 62.86%, and admission with advanced HIV disease was 43.20%. The percentage of late presenters remained constant over the period 2009–2015 [18]. In a study of 2,507 newly diagnosed HIV infected patients in Barcelona, Spain, the late presentation prevalences were 62.7% in 2001-2003, 51.9% in 2004-2005, 52.6% in 2006-2007 and 52% in 2008-2009. The study reported that only a decrease was observed between 2001-2003 and 2004-2005, but this ratio remained constant later [19]. Despite all development efforts related to diagnosis and treatment, it is worrisome that European-based studies do not report a significant decrease in late presentation prevalences over the years.

In our study, the mean age of late presenters was higher than the mean age (36.5 ± 11.9) of patients with CD4 T lymphocyte count> 350 cells / mm^3, and the difference was statistically significant. However, there was no statistically significant difference between these two groups regarding gender. In a review where publications between 1999 and 2010 from fifteen countries in Europe, eight countries in the Asia / Pacific region, eleven countries in North America, and two countries in South America, were evaluated in terms of predictors of late presentation, in all of the studies, advanced age determined as a risk factor for late application [20]. Among HIV-infected patients followed up between 2000 and 2015 in Poland, both the late presentation and advanced HIV disease were significantly associated with older age [13]. Older individuals may be considered to be at low risk for HIV infection by healthcare service providers, or a lower level of knowledge or risk perceptions about the disease by older patients may be the reason for their diagnosis in the later stages of the disease.

Conclusion

The findings of our study indicate that, despite advances in the diagnosis and medical treatment of HIV infection, late presentations are quite common and still a significant problem, in Turkey. This situation highlights the importance of broad-based HIV screening programs to control the disease more effectively.

Compliance with Ethical Standards

Ethical approval: This study was approved by the University's Ethics Committee (approval number B.30.2.ODM.0.20.08/407-475). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

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Author Contribution AD and **ET:** Planned and supervised the work, **HCB and AD:** Performed the analysis, and designed the figures, **HCB and AD:** Interpreted the results and wrote the article. All authors discussed the results and commented on the manuscript

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Major health concerns of refugees in an emergency department: A single-center experience in the country hosting greatest number of refugees

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ABSTRACT

Objective: Turkey hosts a large number of refugees. The extensive use of the emergency services (ED) created a high burden on the health care system. This study was conducted to determine ED utilization by refugees at a tertiary care center.

Patients and Methods: Pediatric patients presenting with trauma and all adult patients were included in the study. Demographic information, country of origin, date of admission and triage category, complaint on admission, hospital resource utilization frequency, final diagnosis, and outcome of patients were recorded.

Results: A total number of 762 patients (1266 cases) were included. The median age was 30 (range 23-39) and 317 (41.6%) were male. 627 (82.3%) of the patients were from Syria. Considering hospital resource utilization of 1266 cases, radiologic imaging was performed on 28.1% and laboratory testings were performed on 50.2% of cases. 1128 (89.1 %) of all cases were discharged, while 119/1266 (9.4 %) cases were admitted to wards and 19/1266 (1.5 %) cases to intensive care units.

Conclusion: There is not enough data regarding in which health care areas refugees need care. Access to primary care specific to refugees may reduce ED utilization. Especially, routine gynecologic primary care and follow-up centers are needed for refugees in Turkey.

Keywords: Refugee, Emergency service, Syrians

1. INTRODUCTION

Since the emergence of the civil war in Syria in 2011, many Syrians have tried to seek asylum in various countries. Turkey has received the greatest share of these asylum seekers. According to a report published by the Turkish migration administration on January 6, 2021, Turkey hosts 817.898 refugees, of whom 371.820 reside in Istanbul. The report also specifies that 115.234 of refugees are from Iraq, while over 83.495 are Syrian nationals [1].

The increasing number of refugees is becoming a global social and healthcare problem. In recent years, emergency departments (EDs) have been faced with rising numbers of patients from Syria, Iraq, and Afghanistan. Immigration causes physical, mental, and social health-related challenges [2].

Researchers should study refugees from many angles, as their situation is extraordinary and different from that of the native population. Among the possible topics, healthcare issues are of paramount importance. Most refugees live in rural and suburban areas under poor conditions, which renders them susceptible to disease and limits their access to healthcare facilities. Managing refugees can be troublesome because of difficulties with communication and a lack of previous medical data on diseases, medications, and surgical procedures, for example.

Since the first days of the refugee crisis, the Turkish government has worked to establish a healthcare system for refugees that covers all acute, chronic, and emergent health problems. Healthcare services and medicine are provided free of charge to all registered refugees in Turkey [3]. However, the vast number of refugees and their extensive use of emergency services has imposed a heavy burden on the healthcare system. Data on the scale of this burden are limited; thus, to guide future planning

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and organisation, this study examines ED utilisation at a tertiary care centre among refugees residing in Istanbul.

2. PATIENTS and METHODS

This study was designed retrospectively to evaluate the data of foreign patients who were admitted to the ED of the Marmara Research and Training Hospital between the 1st of January 2018 and the 31st of December 2019. Our hospital is a tertiary referral hospital and ED has patient load of 500.000 annually, and all patients are treated under supervision of emergency medicine specialists.

Marmara University Ethics Committee approved the study (approval number: 09.2019.870) and Istanbul Provincial Health Directorate permitted us to use the database. Informed consent was not necessary as the design of the study was retrospective. Patient admission and follow-up forms were reviewed retrospectively and the data of patients were recorded.

Any foreigner who resides in Turkey for more than six months or who has a work permit is given a foreigner ID number of eleven digits like Turkish citizen ID numbers, except it begins with '99'. This ID given by the Turkish Government allows those foreigners to benefit from the Turkish health care system just like any Turkish citizen.

Foreign patients admitted to ED during the given period were selected from hospital database by their ID numbers. Pediatric patients (<18 years old) presented with trauma and all adult (>18 years old) patients were included in the study. Demographic information including age, gender, country of origin, date of admission were recorded. Triage category, complaint on admission, hospital resource utilization frequency, final diagnosis and outcome of patients were also recorded.

In hospital records patients were categorized into five groups according to the Australasian triage scale [4]. We regrouped them into three: category 1-2 as red (emergent), 3-4 as yellow (urgent) and 5 as green (non-urgent).

Complaint on admission was grouped into ten categories mainly based on major symptoms such as trauma, muskuloskeletal pain related, neurologic (headache, dizziness, loss of consciousness, etc.), respiratory tract (sore throat, runny nose, dypsnea, cough, etc.), gastrointestinal (nausea, vomitting, abdominal pain, etc.), cardiovascular (chest pain, palpitation, etc.), genitourinary (pregnancy, vaginal discharge, dysuria, flank pain, etc.), ear, nose and throat, ophtalmologic (otalgia, red eye, etc.), soft tissue and dermatologic (swelling, itching, rash, etc.) and other (fever,intoxication, syncope, etc.) symptoms.

Considering hospital resource utilization, we recorded whether any laboratory and radiology testings (computed tomography (CT), magnetic rezonance imaging (MRI), ultrasound (USG)) were made, and the frequency of consultations. The outcome of the patient was grouped into three; discharge, admission to ward or intensive care unit (ICU). The international Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) was used to define patients'final diagnosis [5]. ICD-10 codes were organized into 17 broad categories of clinical diagnoses.

Each admission of a patient with multiple admissions over 24 hours, either with the same or different complaint were recorded as a separate case.

Statistical Analysis

Data analysis was performed using SPSS Statistics V22.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as median values and interquartile ranges (IQRs), whereas categorical variables were presented as frequencies and percentages.

3. RESULTS

A total number of 762 patients and 1266 cases were included in the study, as some of the patients had multiple admissions. 317 (41.6%) of the patients were male while 445 (58.4%) were female.

The median age of all patients was 30 (range 23-39). The age distribution of the patients is seen on Table I. 571 (74.9%) of all patients were between the age of 18-44. The median age of 71 pediatric (<18 years old) patients was 7 (range 4-12) and the median age of adults (>18 years old) was 32 (range25-40).

While 499 of (65.5%) of all patients had single admission, 153 (20.1%) of them had 2, 63 (8.3%) had 3 and 47 (6.2%) had more than 3 admissions.

Table I. *The age distribution of the patients*

		Frequency	Percent
Age	<18	71	9.3
	18-44	571	74.9
	45-65	102	13.4
	>65	18	2.4
	Total	762	100.0

Considering the country of origin 627 (82.3%) of the patients were from Syria, 79 (10.4%) were from other Turkic Republics like Azerbaijan, Turkmenistan, 16 (2.1%) were from the European union (EU) and 40 (5.2%) were from various countries like Indonesia, Tunisia, Chinese.

Of the 1266 cases, 809 (63.9%) were admitted during 2018 and 457 (36.1%) were admitted during 2019. Seasonal distribution of admissions was as follows: 300 (23.7%) during winter, 365 (28.9%) during spring, 322 (25.4%) during summer and 279 (22 %) during autumn periods. 606 (47.9%) of all cases were admitted during day time (08:00-17:00) and 660 (52.1%) were admitted between 17:00 – 08:00. While 505 (39.9%) of cases were admitted within working hours (when out-patient clinics are on services), 761 (60.1%) were admitted during evening and weekends when only ER sevices are available to admit.

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Complaint on admission was grouped into ten categories mainly based on major symptoms. Among them genitourinary symptoms like pregnancy, vaginal discharge, dysuria, flank pain were the most prominant, accounting for 18.5% of all cases. Trauma was the second most common complaint on admission with 18% of cases. Of these 233 trauma patients 71 were in the pediatric age group. Distribution of all complaint groups are listed on Table II.

***Table II**. Distribution of all complaint groups*

	Frequency	Percent
Trauma related symptoms	233	18.4
Musculoskeletal pain	123	9.7
Neurological symptoms	81	6.4
Respiratory system symptoms	142	11.2
Gastrointestinal symptoms	224	17.7
Cardiovascular symptoms	60	4.7
Genitourinary symptoms	243	18.5
Eye, ear, nose, and throat symptoms	61	4.8
Soft tissue and dermatologic symptoms	57	4.5
Other (fever, intoxication, syncope, etc.)	51	4.0
Total	1266	100.0

We regrouped triage scale into three as red, yellow and green. 716 (56.6%) of all cases were defined as green, 217 (17.1%) as yellow and 333 (26.3%) as red triage group.

Considering hospital resource utilization, radiologic imagings were performed on 356/1266 (28.1%) cases. CT was performed on 167/1266 (13.2%) cases, diffusion MRI on 10/1266 (0.8%), USG on 12/1266 (0.9%) and plain X-ray on 201/1266 (15.9%) of all cases. Laboratory testings were performed on 636 (50.2%) cases.

Attending ER specialist concluded 847/1266 (66.9%) cases without any need for consultation. 419/1266 (33.1%) cases were referred to other clinics. Of these referrals 212/419 (50.6%) were to obstetrics and gynecology, 75/419 (17.9%) to orthopedics, 28/419 (6.7%) to ophthalmology, 19/419 (4.5%) to internal medicine, 15/419 (3.6%) to cardiology. In 23/419 (5.5%) cases, consulting more than one clinic was needed.

Outcome of patient was grouped into three; 1128 (89.1%) of all cases were discharged, while 119/1266 (9.4%) cases admitted to wards and 19/1266 (1.5%) cases to ICU. Of the cases admitted to wards, 85/119 were to obstetrics and gynecology (68 for delivery) and 20/119 were to internal medicine clinics.

Table III summarizes the distributions of patients according to their final diagnosis. 219 (17%) of all 1266 cases were discharged as non-emergent cases with inconclusive or non-specific diagnosis. 237 (18.7%) of cases were diagnosed as trauma related. Of these, 29 (2.3%) suffered from facture, dislocation or amputation, 52 (4.1%) had lacerations to be sutured, 156 (12.3%) had none of these and were defined as soft tissue trauma. 4 (0.3%) cases were diagnosed as acute renal failure, while 2 (0.2%) as cardiac arrythmia, 7 (0.6%) as acute coronary syndrome, 6 (0.5%) as decompensated heart failure, 4 (0.3%) as acute abdomen, 9 (0.7%) as gynochologic emergency, 4 (0.3%) as gastrointestinal hemorrage, 3 (0.2%) as burn, 2 (0.2%) as sepsis, 2 (0.2%) as hemoptysis, 8 (0.6%) as intoxication.

***Table III**. The distributions of patients according to their final diagnosis.*

	Frequency	Percent
No definitive diagnosis	219	17.3
Trauma	237	18.7
Soft tissue diseases	45	3.6
Acute gastroenteritis	32	2.5
Upper respiratory tract infection	133	10.5
Asthma, COPD*	12	0.9
Lumbalgia	32	2.5
Dispepsia, gastritis	13	1.1
Urolithiasis	28	2.2
Labor	73	5.8
CNS** pathologies (Epilepsy, intracranial neoplasm, CVD, vertigo)	13	1.0
Pregnancy	124	9.8
Eye pathologies (conjunctivitis, corneal foreign body..)	39	3.1
Pneumonia, pulmonary embolism, pneumothorax	14	1.1
Myalgia	106	8.4
Urinary tract infection	46	3.6
Other (like burn, hyperglysemia, intoxication)	100	7.9
Total	1266	100.0

COPD: chronic obstructive pulmonary disease , CNS**: Central nervous system*

4. DISCUSSION

Turkey provides free emergency service care to all registered refugees. However, there is insufficient data regarding the healthcare areas in which refugees need care and the emergent conditions that lead to their hospital admissions. Because refugees encounter many obstacles, such as language barriers, financial problems, and a lack of health insurance, they tend to prefer the use of emergency services to address their health problems [6,7]. This study aims to evaluate the demographic and medical features of refugee patients who present to the ED.

We had a total number of 762 patients and 1266 cases due to repeated admittions. 8.4% of patients were women. This is same as in previous studies. Guess et all found 53% female, Baykan found 57.7% in their refugee ED studies [8,9].

The median age of adult patients was 32 whereas the median age of 71 pediatric (<18 years old) patients was 7 in our study. In User et al., study, infants and pre-school-aged children constitute 66% of their refugee patient group in the prediatric surgery department [10]. This is the same age group as in our study and the same pediatric group of trauma patients. In another pediatric group refugee study Yurtseven et al., found

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mean age as 3.4 ± 4.1 in all pediatric ED patients [6]. In the adult group, Pfortmueller et al., found the median age as 34, Guess et al., found 28.4, the same as in our study, the relatively young median age is similar [8,11].

Of the patients in our study, 65.5% had a single admission, 20.1% had two admissions, 8.3% had three admissions, and 6.2% had four or more admissions to the ED. Previous studies have reported that refugees tend to visit the ED at a higher frequency compared to the general population [8,12]. In addition, Kiss et al., have found that 12% of refugee patients visited the ED more than four times [13]. Because of the many issues that affect refugees, including language barriers, financial problems, and a lack of health insurance, they usually prefer to use emergency services. The Turkish government has covered all health expenditures for refugees treating acute and chronic diseases in primary, secondary, and tertiary health centres and even in private clinics and hospitals [14]; however, emergency service applications for Syrian refugees in the country exceed 10% of the total applications, and the proportion is especially high in cities on the Syrian border [6].

In recent years, ED's in the EU have been faced with a rising number of patients from Syria, Afghanistan, and Iraq seeking asylum [15]. The largest proportion of refugees was from Africa (48%) in Kiss et al., study from Canada [13]. In our study we found that 82.3% of all refugee patients were from Syria, 10.4% were from other Turkic Republics, 2.1% were from EU and 5.2% were from various countries. In Turkey, there are approximately 3.6 million Syrian citizens and approximately 330000 registered refugees and asylum seekers from other nations [15].

Our study identified no significant seasonal distribution variance, but 28.9% of patients were admitted in the spring months. In a study by Sariaydin et al., the majority of patients presented in the winter months [17]. We found that 60.1% of refugees were admitted in the evening or weekend, when only ED services are available. Meanwhile, Yurtseven et al., have reported that 47% of Syrian patients were admitted to the ED between 8:00 and 17:00 [6].

The most prominent complaints on admission were genitourinary symptoms, such as pregnancy related issues, vaginal discharge, dysuria, and flank pain, which accounted for 18.5% of all cases. Trauma was the second most common complaint. In Kiss et al.'s study, 27% of refugees presented with an injury complaint, and the predominant difference between refugee and non-refugee patients was in the percentage attending for pregnancy, childbirth, and postpartum-related conditions (ED visits: 6% for refugees vs. 3% for non-refugees) [13]. In a study by Sarıaydın et al., the most frequent complaint was an upper respiratory tract infection (URTI), which was reported by 22% of patients, followed by a soft tissue injury [17]. The hospital also had another gynaecology-specific ED, to which 29.5% of female patients were admitted for pregnancy. The researchers further observed that the most common complaint among paediatric patients was a URTI. However, only children with traumatic problems were part of our study.

Our 56.6% of all cases were defined as green (Level 5) according to triage criteria, 17.1% as yellow (Level 3-4), 26.3% as red triage (Level 1) group. Kiss et al., revealed that less urgent overall (Levels 4 and 5) was 41% in their study, too [13]. Systematic review by Hoot et al., showed that the use of emergency services by non-urgent cases caused crowding, increased mortality, treatment delays, staff shortages, and financial losses in the ED [18]. In a study conducted in Turkey, authors also found that ED use by Syrian patients was higher than that of local patients, and a high proportion of these patients were subject to only outpatient care [14]. The most likely causes of this tendency can be explained with the easy access to the ED, less language problem due to fewer formal procedures in the ED and 24/7 access to ED unlike the outpatient clinics. Overcrowded conditions in EDs may result in prolonged pain and suffering, ambulance diversions, decreased physician productivity, violence associated with prolonged wait times, and miscommunication because of increased patient volume.

Radiologic imagings were performed on 28.1% cases in our study. In Yurtseven's study, the rate of Syrian patients requesting tests is 50% [6]. We referred 33.1% cases to other clinics. Of these referrals, 50.6% were to obstetrics and gynecology. We found that 71.40% of ward admissions were to obstetrics and gynecology. Dikmen et al., revealed that Syrian refugee women still wished to have children despite the difficulties added by immigration. The immigration had no negative effects on women's fertility, and they kept on giving birth in higher numbers [19]. It was also found that female refugees utilized the emergency department twice more than non-refugees for pregnancy related issues [13].

Outcome of patients was grouped into three as discharged (89.1%), admitted to wards(9.4%) and ICU (1.5%). Baykan and Aslaner reported that 91.3% of all Syrian patients admitted to hospital were discharged while 6.9% were admitted to hospital [9].

We neither made a specific diagnosis nor found an urgent situation among 17% of the patients in our study. Pfortmueller et al., have similarly noted that a group of young asylum seeker patients suffered from unspecified somatic symptoms [11]. These symptoms may be part of bodily distress syndrome; however, we did not specifically screen this population for this diagnosis. In our population, a soft tissue injury was present in 3.6% of cases, whereas 2.5% involved gastroenteritis, and 10.5% concerned a URTI. In a study by Gülaçtı et al., the most prevalent disease was a URTI followed by myalgia and a urinary tract infection, respectively [20]. Sarıaydın has suggested that the generally crowded and unsanitary conditions in which refugees live may contribute to the spread of respiratory, skin, gastrointestinal, and genital system infections [17]. This argument is likely applicable to our population as well.

Our research had certain limitations. Since this study was conducted in a single centre, the findings cannot be easily generalised. Moreover, because of the retrospective design of this study, some older medical conditions may not have been detected. Finally, no standardised general and systemic medical history was taken; only hospital records from our institution were analysed in this study.

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The findings of our research support specific recommendations regarding the important challenge of healthcare for refugees. Greater access to primary care specific to refugees may reduce ED utilisation. In addition, routine gynaecologic primary care and follow-up are especially necessary for refugees in Turkey.

Compliance with the Ethical Standards

Ethical Approval: Marmara University Ethics Committee approved the study (approval number: 09.2019.870) and Istanbul Provincial Health Directorate permitted us to use the database.

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Which radiotherapy technique is better for neoadjuvant treatment of rectal cancer: A dosimetric comparison

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ABSTRACT

Objective: Our aim was to compare helical tomotherapy (HT) and volumetric modulated arc therapy (VMAT) plans with 3-dimensional conformal radiotherapy (3D-CRT) considering the planning target volume (PTV) and organs at risk (OARs) in rectal cancer patients treated with neoadjuvant radiotherapy.

Patients and Methods: Thirty patients, previously treated with intensity modulated radiotherapy (IMRT) or 3D-CRT from January 2014 to February 2020 were selected and 3 plans were generated for each patient using VMAT, HT and 3D-CRT. Dosimetric comparisons were made for each plan regarding PTV and OARs. Integral dose (ID) was calculated and beam on times were analyzed.

Results: The homogeneity index (HI) was significantly better in HT plans compared with VMAT and 3D-CRT plans ($p<0.001$), conformity index (CI) was better in VMAT plans. For small bowel, high doses were higher in 3D-CRT plans ($p <0.001$). HT produced lower doses for the bladder as compared to VMAT and 3D-CRT ($p<0.005$). The mean and maximum doses of bilateral femoral heads were higher in 3D-CRT plans. Beam on times were longer and IDs were higher in HT plans ($p<0.001$).

Conclusion: Both VMAT and HT improved target homogeneity and conformity and decreased OAR doses compared to 3D-CRT. Although, VMAT was the best method to decrease ID, HT produced better bladder sparing.

Keywords: Rectum cancer, Volumetric modulated arc therapy, Helical tomotherapy, 3-dimensional conformal radiotherapy, Plan comparison

1 INTRODUCTION

Colorectal tumors are the third most common tumors among men and women, not only in Europe but also in Turkey [1]. Rectal tumors account for approximately 20 % of all colorectal tumors. Preoperative chemoradiotherapy (CRT) is the standard neoadjuvant treatment in patients with locally advanced rectal cancer (LARC); T3-T4 and/or N+ since 2004 [2,3]. Conventionally fractionated radiotherapy (50.4 Gy/28 fx/6 weeks) is the most widely accepted regimen. Pathologic complete response (pCR) after CRT is associated with improved local and distant control, overall survival (OS) and disease free survival (DFS) [4]. However relatively low rate of PCR (13% to 20%) and high rate of distant metastases have led to re-evaluation of the role of treatment intensification by intensifying chemotherapy (CT) regimens or radiotherapy (RT) dose (>50 Gy). So far, six randomized trials comparing fluoropyrimidine CRT with or without oxaliplatin reported [5]. However, this treatment not only did not improve the outcome but also showed an increase in grade 3-4 toxicity. In a meta-analysis investigating the effect of radiotherapy boost on pathologic response rate it was shown that dose escalation above 60 Gy for LARC, results in high pCR-rates and grade 3 early toxicity ranges between 10%-42.6% [6]. Please note that none of the studies used IMRT. A recent randomized study about intensification of CRT by either radiotherapy dose escalation or multidrug CT also showed improved pathologic

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response rates in the escalated dose arm [7]. The standard 45-50.4 Gy RT dose may change in the future albeit at the expense of increased toxicity, and there will be an increased interest in the safe and tolerable administration of preoperative high dose CRT. In the majority of the studies reported, the most common RT technique is 3D-CRT with either AP-PA opposing fields or a four-field-box technique. Because of the concave shape of the planning target volume (PTV) to cover lymphatics, sparing of the normal tissue (bladder, small bowel, and femoral heads) is rather limited with this technique. The most common Grade 3 or 4 toxicity is mainly gastrointestinal, and the volume of small bowel receiving at least 15 Gy (V15) was found to be strongly associated with the degree of toxicity [8]. To reduce the toxicity, modern RT techniques, such as IMRT, VMAT, and HT have increasingly been used for pelvic radiotherapy. However, dosimetric studies comparing VMAT, HT, and 3D-CRT techniques concerning target volume coverage and (OAR) are lacking.

In this study, we compared the dosimetric parameters between VMAT, HT and 3D-CRT techniques in a relatively large number of rectal cancer cases who received preoperative radiotherapy.

2. PATIENTS and METHODS

Thirty patients with pathologically proven and previously treated with pelvic radiotherapy for locally advanced rectal cancer from January 2014 to February 2020 were randomly selected for this study. The research protocol was reviewed and approved by Ethics Committee of Kocaeli University School of Medicine (15.04.2021). All patients were simulated in supine position with full bladder. Computed tomography (CT) simulation scanning was done using the Siemens Definition AS (Siemens Healthcare, Erlangen, Germany) CT machine with 3-mm slice thickness through the L1 vertebral body to 2 cm below the perineum.

Treatment planning

Target volumes were defined according to the recommendations of the international commission on radiation units and measurements report No.62 [9]. The clinical target volume (CTV) included the gross tumor volume-tumor (GTV-T), the mesorectum, pre-sacral nodes, the common and internal iliac lymph nodes. PTV was generated with a 1-cm symmetrical expansion around the CTV. The bowel bag, bladder and femur neck were delineated as OAR and OARs dose constrains were determined based on the RTOG 0822 Study [10]. To avoid possible inconsistencies for the CTVs, the same physician created a new contouring task for each patient. Three sets of plans for 3D-CRT, HT and VMAT were generated for each patient for the dosimetric comparisons. The prescribed dose to planning target volume (PTV) was 50.4 Gy in 28 fractions. For 3D-CRT and VMAT planning Eclipse Planning System V13.6 (Varian Medical Systems. Palo Alto, CA), and for HT planning TomoTherapy Planning Station V5.1.1.6 (Accuray) was used. The tomotherapy plans were all helical IMRT plans with field widths of 2,5 cm, 0.287 pitch value and 2.00 planning modulation factor. 3D-CRT technique was planned with four field technique, using beam angles 0, 90, 180, 270 with 15 MV photon energy. VMAT technique was planned using 2 full arcs. Arc rotations were 181°-179° clockwise and 179°-181°counterclock wise. In order to minimize leaf leakage in the created arc areas, 30 degrees and 330 degrees collimator angles were used for each arc. All of the plans were normalized to cover 100% of the PTVs with ≥95% of the prescribed dose. No planning objective was created for OARs.

Plan Evaluation

Dose Volume Histogram (DVH) was used for PTV and OAR dose comparison. PTV D98% (Dose received by 98% of the PTV), PTV D2% (Dose received by 2% of the PTV), PTV D50% (Dose received by 50% of the PTV), HI {(PTV D2% – PTV D98%) /PTV D50%}and, CI {PTVvol / IRvol 95% (Irradiated volume enclosed by the 95% of isodose line)} were evaluated for target coverage. While a greater HI value indicates poorer uniformity of the dose distribution, the value of CI varies between 0 and 1, with a value closer to 1 indicates better conformity of the dose to the PTV. OAR (small bowel, bladder, femoral head) avoidance was evaluated using the following parameters: Dmean, Dmax, VnGy (volume receiving radiation dose ≥ n Gy). ID formula (Eintegral=Vb * pb* Db; V:Volume of body, p: density of body, D:Mean dose of body) was used to calculate the total dose delivered to the whole patient body [11-13].

Statistical Analysis

Statistical analyses were performed using the SPSS soft-ware version 20. The paired, two-tailed Wilcoxon signed-rank test was applied for statistical analysis. All p values reported were two-sided, and p value <0.05 was considered statistically significant.

3. RESULTS

Target coverage, conformality, dose homogeneity and beam on time

The average maximum doses for PTV (represented by D2) were significantly higher in 3D-CRT plans than VMAT and HT plans (p<0,001). Minimum PTV doses (represented by D98) were significantly lower in VMAT plans compared with 3D-CRT and HT plans. Although, HI was significantly better in HT plans than VMAT and 3D-CRT plans (p<0,001), CI was better in VMAT plans. The average CI of the VMAT plans was 0.9, and the average CI of 3DCRT and HT plans were 0.6 and 0.8, respectively, (p <0.001). The dosimetric parameters for target volumes and beam on times are summarized in Table I and Figure 1 shows the axial, sagittal and coronal CT slides of a patient representing the isodose distributions for the three modalities.

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Organs at risk doses, whole body integral dose

All details of the organ at risk doses are given in Tables II – IV.

Small Bowel

High doses (V50.4, V45, V40 and V30) were statistically higher in 3D-CRT plans than VMAT and HT plans (p <0.001), while there was no difference between VMAT and HT plans. V20 was lowest in VMAT plans; however V10 values were comparable between groups.

Bladder

HT produced significantly lower V50.4Gy, V45Gy, V40Gy, V30Gy, V20Gy, V10Gy and mean values for the bladder as compared to the VMAT and 3D-CRT (p<0.005). V10 value as representing the low dose volume did not differ between VMAT and 3D-CRT (p=0.593).

Right-left femoral head

The mean and maximum doses of both femoral heads were higher in 3D-CRT plans compared to VMAT and HT plans. There was no statistical difference between HT and VMAT plans regarding V50.4 values, while V45, V40 and V30 values were significantly lower in VMAT plans; however V20 values were comparable between VMAT and HT plans (p<0.005).

Whole body integral dose

ID was lowest in VMAT plans, and HT produced the highest as shown in Table I.

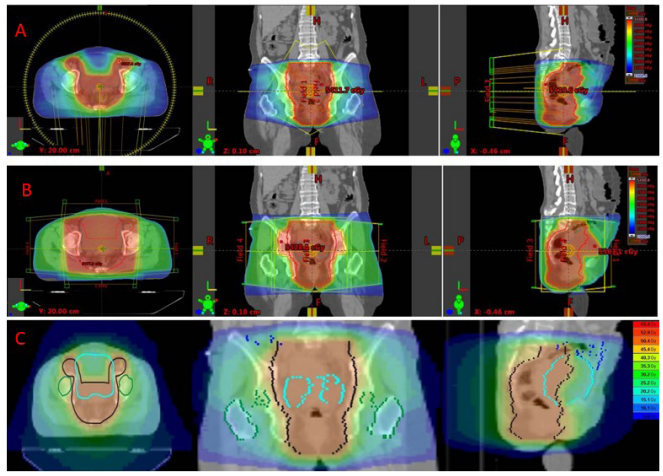

Figure 1. *Isodose distributions of a patient in the axial, coronal and sagittal plan for VMAT (A), 3D-CRT(B) and HT(C).*
VMAT: volumated modulated arc therapy, 3D-CRT: 3-dimensional conformal radiotherapy (3D-CRT), HT: helical tomotherapy

Table I. *Dose volume histogram parameters for PTV comparing 3D-CRT, HT, and VMAT Techniques (Values are presented as mean ± standard deviation)*

Parameters	VMAT	HT	3D-CRT	p-value (HT vs 3D-CRT	p-value (VMAT vs 3D-CRT	p-value (VMAT vs HT)
D2%(Gy)	53.24±6.38	51.63±0.36	54.22±7.02	0.00	0.00	0.00
D50%(Gy)	51.95±3.93	50.92±0.25	52.48±4.82	0.00	0.00	0.00
D98%(Gy)	49.54±1.86	49.64±0.32	49.68±1.84	0.00	0.003	0.00
HI	0.07±0.01	0.03±0.01	0.08±0.01	0.00	0.00	0.00
CI	0.92±0.02	0.87±0.03	0.6±0.06	0.00	0.00	0.00
Beam On Time(minute)	0.85±0.08	5.69±0.79	0.36±0.01	0.00	0.00	0.00
Body ID	375.53±112.49	410.06±91.52	404.06±92.56	0.012	0.001	0.001

PTV: planning target volume; CRT: conformal radiotherapy, HT: helical tomotherapy, VMAT: volumated modulated arc therapy, HI: homogeneity index, CI: conformity index, ID: integral dose

Table II. *Comparison of small bowell dose parameters between 3D-CRT, HT, and VMAT (Values are presented as mean ± standard deviation)*

Values (%)	VMAT	HT	3D-CRT	p – value (HT vs 3D-CRT	p-value (VMAT vs 3D-CRT	p – value (VMAT vs HT)
V50.4 Gy	6.46±8.5	6.01±7.84	20.4±15.68	0.00	0.00	0.216
V45 Gy	14.52±12.19	14.15±10.93	26.91±17.24	0.00	0.00	0.718
V40	19.76±14.28	21.53±13.74	30.34±18.14	0.002	0.00	0.07
V30	36.14±19.1	41.01±18.22	47.07±20.65	0.046	0.00	0.013
V20	56.70±21.91	72.02±23.14	71.58±17.81	0.889	0.016	0.012
V10	82.77±17.09	81.63±31.58	81.64±16.72	1	0.144	0.655
Mean	25.78±6.9	28.38±6.47	29.78±8.21	0.14	0.00	0.00

Vn Gy: percentage of the volume receiving radiation ≥ n Gy, CRT: conformal radiotherapy, HT: helical tomotherapy, VMAT: volumated modulated arc therapy

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***Table III**. Comparison of bladder dose parameters between 3D-CRT, HT, and VMAT (Values are presented as mean ± standard deviation)*

Values (%)	VMAT	HT	3D-CRT	p-value (HT vs 3D-CRT	p-value (VMAT vs 3D-CRT	p-value (VMAT vs HT)
V50.4	20.01±19.32	16.27±14.79	68.28±16.45	0.00	0.00	0.111
V45	44.29±23.30	32.66±19.26	81.08±16.07	0.00	0.00	0.024
V40	54.49±24.22	41.07±20.87	84.69±15.48	0.00	0.00	0.017
V30	70.28±22.29	59.53±25.16	95.51±9.87	0.00	0.00	0.00
V20	89.29±17.7	55.18±35.93	99.91±0.49	0.00	0.02	0.00
V10	99.85±0.69	70±46.60	99.99±0.03	0.04	0.593	0.004
Mean	38.72±7.3	3441±7.36	48.39±3.44	0.00	0.00	0.00

Vn Gy: percentage of the volume receiving radiation ≥ n Gy, CRT: conformal radiotherapy, HT: helical tomotherapy, VMAT: volumated modulated arc therapy

***Table IV**. Comparison of femoral head dose parameters between 3D-CRT, HT, and VMAT (mean ± standard deviation)*

Values(%)	VMAT	HT	3D-CRT	p-value (HT vs 3D-CRT	p-value (VMAT vs 3D-CRT	p-value (VMAT vs HT)
Right Femur						
V50.4	0.02±0.09	0.06±0.24	2.08±2.71	0.00	0.00	0.176
V45	0.58±1.19	1.64±2.44	4.37±3.71	0.01	0.00	0.02
V40	2.06±2.59	5.59±4.48	6.18±4.44	0.254	0.00	0.00
V30	13.12±7.02	20.53±8.45	38.24±19.93	0.00	0.00	0.00
V20	42.06±15.75	44.38±15.85	74.77±19.65	0.00	0.00	0.877
V10	75.59±21.76	81.63±21.67	82.39±17.09	0.495	0.00	0.14
Mean	18.19±4.55	20.59±3.98	25.30±5.44	0.00	0.00	0.00
Max	46.53±4.4	48.94±2.48	51.56±2.57	0.00	0.00	0.00
Left Femur						
V50.4	0.0±0.03	0.02±0.7	2.10±3.49	0.00	0.00	0.214
V45	0.58±1.55	1.83±2.25	4.35±4.57	0.01	0.00	0.00
V40	1.96±2.87	5.88±4.42	6.20±5.32	0.94	0.00	0.00
V30	13.18±7.2	22.81±13.05	36.52±16.43	0.001	0.00	0.00
V20	45.54±20.47	45.79±15.62	75.04±19.5	0.00	0.00	0.727
V10	76.17±22.64	86.71±15.66	83.45±16.10	0.00	0.001	0.00
Mean	18.41±4.9	21.49±4.71	25.39±5.21	0.00	0.00	0.00
Max	47.05±4.13	49.27±2.23	51.23±32.7	0.00	0.00	0.00

Vn Gy: percentage of the volume receiving radiation ≥ n Gy, CRT: conformal radiotherapy, HT: helical tomotherapy, VMAT: volumated modulated arc therapy

4. DISCUSSION

In our dosimetric study we compared the standard and traditionally used 3D-CRT technique with VMAT and HT for patients with locally advanced rectal cancer treated neoadjuvantly. In the literature, there are studies comparing different IMRT techniques with 3D-CRT, as well as with each other but no study has been published comparing 3D-CRT, VMAT, and HT techniques at the same time [14-19]. Traditionally, 3D-CRT has been used for LARC targeting the primary tumor and mesorectum as well as lymph nodes. Gastrointestinal complications are the most common toxicity in 3D-CRT, leading to a decrease in treatment compliance. In the German Rectal Cancer Study Group study, there was 27% acute and 14% late grade ≥ 3 toxicity [2]. Braendengen et al., reported a study where acute and late toxicity rates were 28% and 17% respectively [20]. The 3D-CRT was used in both studies. There are notable number of studies that have shown dose-volume relationship between the irradiated small bowel volume and the severity of diarrheal toxicity at different dose levels [21,22]. A recent meta-analysis stated that V10Gy, V30Gy, V35Gy and V40Gy were found to be significantly predictive of the toxicity incidence in a univariate logistic regression model. In our study we showed that high doses (V50.4, V45, V40 and V30) were statistically higher in 3D-CRT plans than VMAT and HT plans ($p < 0.001$), while there was no difference between VMAT and HT plans . But V20 was also lowest in VMAT plans making

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VMAT one of the best choice for small bowel protection. This meta-analysis also reported that this dose-volume relationship and risk of toxicity is continuous, without a threshold below which the risk is unchanged, and hence the priority of all clinicians should be to ensure that normal tissue receives the lowest dose as possible [23]. In a dosimetric study comparing VMAT, 5F-IMRT and 3D-CRT, VMAT was found to be not only superior in normal tissue sparing, but also V35-V45 of small bowel were significantly less than in 5F-IMRT and 3D-CRT [17].

With the introduction of different IMRT planning techniques into clinical practice, reduction of OAR doses and better target dose conformity and homogeneity were obtained [14-15]. Yu et al., showed that the dose conformality of Tomotherapy was better than that of four-box field CRT [15]. Furthermore, the irradiated mean dose of the normal organs was found to be two-thirds of the 3-dimensional RT. A study reported by Arbea et al. comparing IMRT and 3D-CRT in LARC, showed that IMRT improves target conformity at the expense of target heterogeneity [14]. They also reported that HI was lower with HT compared to 3D-CRT. In our study both HI and CI were both better in VMAT and HT than 3D-CRT. HI was also significantly better in HT plans compared with VMAT and 3D-CRT plans ($p<0.001$), although, CI was better in VMAT plans.

Bladder should have been the second and femoral heads the third importance as an OAR that should be protected but often neglected in pelvic radiotherapy. In the study carried out by Temelli et al, the bladder was best protected by HT compared to IMRT and VMAT [24]. Similarly in our study HT produced significantly lower V50.4Gy, V45Gy, V40Gy, V30Gy, V20Gy, V10Gy and mean values for the bladder compared to VMAT and 3D-CRT ($p<0.005$) while femoral head doses mean and V45, V40 and V30 values were significantly lower in VMAT plans. Hip fracture after RT of pelvic tumors has been reported to be rare after mean doses of <40 Gy to the femoral neck, but a recent prostate cancer study suggest an increased risk of hip joint arthropathy [25-26].

It is generally accepted that as the total body ID increase, secondary malignancy risk increases. In our study ID was significantly lower in VMAT hugely beneficial for the protection of healthy tissue.

Conclusion

Future strategies in the preoperative treatment of rectal cancer will be based mainly on intensification of treatment with RT where toxicity will be an important issue. In National Comprehensive Cancer Network guidelines, IMRT is still advised only in the setting of a clinical trial or in unique clinical situations such as re-irradiation. This study shows that this statement should be changed since VMAT and HT provide better OAR sparing, and high dose conformity compared to 3D-CRT. Further investigation is required in the use of VMAT and HT techniques in the neoadjuvant treatment of rectal cancer.

Compliance with the Ethical Standards

Ethical Approval: The study was approved by the Ethics Committee of Kocaeli University, School of Medicine (15.04.2021).

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Effectiveness of ADC histogram analysis in the diagnosis of focal liver lesions; is a contrast agent necessary?*

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ABSTRACT

Objective: The diagnostic success of apparent diffusion coefficient (ADC) histogram analysis in focal liver lesions, and the effects of quantitative data added to contrast-enhanced abdominal magnetic resonance imaging (MRI) on the diagnostic accuracy were investigated.

Materials and Methods: The conventional MRI findings of 524 lesions in total were retrospectively examined. Contrast enhancement kinetics and ADC values for each lesion were found through an image analysis software.

Results: Three hundred and fifty (67%) of the lesions were diagnosed as benign and 174 (33%) as malignant. Statistically significant difference was found between the benign and malignant lesion groups in terms of the minimum, maximum and mean ADC values ($p<0.001$). When optimal thresholds for minimum, maximum and mean ADC were taken as 1.47x10-3mm2/s; 1.85x10-3mm2/s; 1.72x10-3mm2/s respectively, sensitivity was found to be 97%; 83%; 95%, specificity was 100%; 98%; 99%, NPV was 100%; 99%; 99%, and PPV was 93%; 74%; 90%. ADC values added to MRI increased the diagnostic success for metastases (92%→96%), HCC (63%→73%), hemangioma (90%→99%) and FNH (56%→75%).

Conclusion: ADC measurement could not show reasonable success in the diagnosis of specific lesions while being successful in the differentiation of benign and malignant lesions. Minimum ADC is more successful than mean and maximum ADC. A non-contrast-enhanced MRI protocol based on the ADC measurement applicable to the selected patient group may be helpful.

Keywords: Contrast agent, Liver lesions, Diffusion weighted imaging (DWI), Apparent diffusion coefficient (ADC)

1. INTRODUCTION

The liver is the largest organ located in the abdomen having a rich vascular structure and a complex histopathological basis, in which a wide variety of lesions can be encountered. Radiological evaluations are performed using ultrasonography (US), computerized tomography (CT) and magnetic resonance imaging (MRI). MRI is superior to US and CT for the assessment of a broad spectrum of hepatic diseases due to its advantages such as high contrast resolution, ability to provide images at three planes and not requiring ionizing radiation. Being a state-of-the art technology product and having a high diagnostic value, MRI is used today as a problem-solving, even as a first-line diagnostic method [1, 2]. However, findings may overlap even if all data obtained for lesion characterization are combined. Intravenous gadolinium contrast agents used in MRI are known to improve diagnostic quality; however frequent use has disadvantages due to their side effects. Nephrogenic systemic fibrosis, which is a serious side effect recently identified in patients with renal failure [3-5] brought out the need for development of new MR techniques that will contribute to diagnosis without requiring the use of gadolinium. To meet this need, diffusion-weighted imaging (DWI)/apparent diffusion coefficient (ADC) without contrast agent requirement have been the subject of investigations during the recent years.

* This article was extracted from the first author's doctorate dissertation entitled "The role of diffusion weighted imaging in diagnosis and follow-up of liver mases alongside conventional MRI findings".

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The primary objective of this study was to determine the effectiveness of ADC histogram analysis in the diagnosis of focal liver lesions. The secondary objective was to investigate the effect of ADC values added to MRI on the diagnostic accuracy and the necessity of contrast agents.

2. MATERIALS and METHODS

Study Population

Approval was received from Adnan Menderes University, School of Medicine Ethics Committee for this retrospective study (approval number: 2017/1267). The intravenous contrast-enhanced upper abdominal MRI scans of 2212 patients aged 18 or above that had been obtained between December 2014 and December 2017 were retrospectively examined. The scans of 948 patients for which the MRI reports mentioned focal lesions in the liver were selected. The images of the selected scans were examined, and 254 that were not of optimal diagnostic quality for various reasons, could not be localized in the ADC map and/or have size of lesion smaller than 10 mm were excluded from the study. The remaining 694 scans were evaluated for eligibility using the following inclusion criteria.

Inclusion Criteria

1. Primary malignant liver tumor (hepatocellular carcinoma (HCC), intrahepatic cholangiocellular carcinoma (CCC): histopathological confirmation (needle biopsy and/or surgical specimen) is required.

2. Secondary malignant liver tumor (metastasis): histopathological confirmation and/or non-liver proven primary malignancy is required.

3. Malignant liver lesion: without any treatment.

4. Benign liver lesion: if no tissue diagnosis is available, presence of typical MR imaging findings as well as confirmation with a follow-up MRI obtained at our institution at least 6 months later and/or with other imaging modalities (US, CT) are required.

5. The number of lesions in the group is required to be greater than 10.

One hundred and two scans that did not meet the 1st and 2nd criteria above, 22 that did not meet the 3rd criterion, 30 that did not meet the 4th criterion, and 16 that did not meet the 5th criterion (6 hepatic abscesses, 5 hydatid cysts, 3 lipomas, 2 angiosarcomas) were excluded from the study. The remaining 524 scans were included in the analysis. Only 1 lesion was evaluated in each scan. If there were more than one similar lesion in the liver, the largest and/or most diagnostic (without artifacts) one were included, and if there were lesions of different structures, the malignant one was included in case of coexistence of malignant and benign lesions, and the rarer one was included in case that there were more than one benign lesion.

Magnetic Resonance Imaging

Scans were performed on a 1.5-T Achieva system (Philips Healthcare, Best, The Netherlands) in conjunction with an 8-element body coil array.

The liver image was acquired in the axial plane in all patients both before and after administration of gadoxetic acid at a dose of 0.15 mL/kg. The contrast agent was automatically administered intravenously at a rate of 3 mL/s with a power injector, followed by a 25-mL saline flush.

Our institutional abdominal MRI protocol for imaging the liver included a respiration-triggered axial T1-weighted turbo field-echo in-phase sequence [repetition time/echo time (TR/TE), 10/4.6; flip angle (FA), 15°; matrix size (MS), 252x151; section thickness (ST), 7 mm] and out-of-phase sequence (TR/TE, 11/6.9; FA, 15°; MS, 252x151; ST, 7 mm), T2 weighted-turbo spin echo-high resolution (TR/TE, 484/80; FA, 90°; MS, 252x173; ST, 7 mm), axial T2 weighted-spectral attenuated inversion recovery (TR/TE, 424/80; FA, 90°; MS, 236x165; ST, 7 mm), axial balanced turbo field-echo sequence (TR/TE, 3.4/16; FA, 10°; MS, 288x226; ST, 7 mm) with a 1 – to 2-mm intersection gap, and a field of view (FOV) of 30–38 cm.

For gadoxetic acid-enhanced MRI, arterial phase (20-35 s), portal phase (60 s), 3-min late phase, and 10-min delayed hepatobiliary phase images were obtained using a T1-weighted high-resolution isotropic volume examination; T1-weighted high-resolution isotropic volume excitation (THRIVE) (TR/TE, 4.2/1.98; FA, 10°; MS, 188x148) with a 2-mm section thickness and a field of view (FOV) of 30-38 cm.

Diffusion images were obtained using a free-breathing multislice spin-echo echo-planar imaging (EPI) sequence; (TR/TE, 1410/69; FA, 90°; MS, 124x99) with a 5-mm section thickness and a FOV of 30-38 cm. Three motion probing gradients with b-values of 0, 600 and 1000 s/mm^2 were applied in three orthogonal directions and trace images were synthesized for each b-value using the mean of three orthogonal directions. ADC maps were calculated on a pixel-by-pixel basis using a monoexponential fit, and b=0 was excluded from the calculation in order to eliminate perfusion effects.

Image Analysis

All examinations were evaluated together by two radiologists with 4 and 15 years of experience. The number of lesions for each examination was divided into three groups: single, two-five, more than five-multiple. Then the location (segment), size (the longest transvers diameter on the axial plane), margin [regular (or macrolobulated) or irregularity (microlobulated or speculated)], borders (well-defined or poorly defined) and T1, T2 signal [hypointense-hyperintense (pure or heterogeneous), isointense] of the selected lesion were recorded.

Apparent diffusion coefficient map was generated automatically by using the licensed imaging analysis software (Myrian; Intrasense, France) according to the algorithm of the equation ADC mm^2/s=1/b1 x ln [IS (b0) / IS (b1)]. ADC measurements were performed on the map generated based on the values b=0 and b=1000. Measurements were performed by means of a manual region of interest (ROI) drawn on a single section on the axial plane to include an area as large as possible, avoiding peripheral borders. For malignant lesions, cystic/necrotic components, if any, were excluded from the measurement area (Figure 1). The minimum, maximum and mean ADC values calculated using histogram analysis were recorded. The contrast-enhancing kinetics of the lesions (type 1, persistently increasing contrast-enhancement; type 2, plateau; type 3, wash-out) were found simultaneously with the same ROI drawn.

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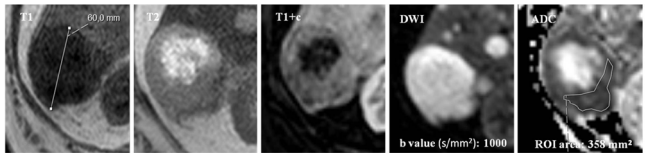

Figure 1. *A 70-year-old/male/lung cancer-liver metastasis*

T1 heterogeneous hypointense, T2 heterogeneous hyperintense mass in the segment 6 of the liver with lobulated contours, well-defined borders, and a necrotic degeneration area at the center, and ADC measurement method; the mean ADC 0.9x10^{-3}mm^2/s, minimum ADC: 0.5 x10^{-3}mm^2/s, maximum ADC: 1.1 x10^{-3}mm^2/s, contrast enhancement pattern type 1.

Statistical Analysis

The statistical analysis was conducted using the SPSS (version 21.0; SPSS Inc., Chicago, IL, USA) and MedCalc (version 18; Ostend, Belgium).

Conformance of the data to the normalized distribution was investigated using the Kolmogorov-Smirnov test. ADC values were expressed as "median (25th-75th percentile)", and age and size were expressed as "mean (±standard deviation/minimum-maximum)". ADC values were compared between the benign and malignant groups using the Mann-Whitney U test. For these two groups, the optimal threshold, specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) were found using the MedCalc software by applying the ROC receiver operating characteristic (ROC) analytical test. The Kruskal Wallis H test was used for the multiple group comparisons; and Post-Hoc tests were used to determine which group means were significantly different. Results with a p<0.05 value were considered statistically significant.

3. RESULTS

For the 524 (242 male, 282 female) patients, the mean age was 60 (±12/21-88) years. Among the lesions found by MRI, 350 (67%) were classified as benign and 174 (33%) as malignant tumors. Of the 350 benign lesions, 171 (49%) were diagnosed as cyst, 152 (43%) as hemangioma, 16 (5%) as focal nodular hyperplasia (FNH) and 11 (3) as hepatic adenoma (HA), while of the 174 malignant lesions, 121 (70%) were diagnosed as metastasis, 41 (24%) as hepatocellular carcinoma and 12 (6%) as cholangiocellular carcinoma.

Table I. *Demographic information, MRI findings and ADC values in benign lesions*

Benign Lesions			Cyst	Hemangioma	FNH	HA	Total
Sample Size (n)			171	152	16	11	350
Age[a]			56 (±12/25-84)	53 (±12/21-84)	48 (±18/43-65)	56 (±17/25-69)	54 (±14/21-84)
Male–Female			63–108	60–92	4–12	7–4	134–216
Number of lesions	Single		105 (61%)	95 (62%)	13 (81%)	9 (82%)	222 (63%)
	Two–Five		62 (36%)	53 (35%)	3 (19%)	2 (18%)	120 (34%)
	>Five–Multiple		4 (3%)	4 (3%)	-	-	8 (3%)
Selected Lesion	Dimension (mm)[b]		31 (±21/10-125)	27 (±17/10-120)	35 (±19/15-84)	40 (±23/20-85)	30 (±20/10-125)
	Segments		7 (35%)	7 (38%)	6 (38%)	5 (27%)	7 (37%)
	Shape	Regular	171 (100%)	150 (99%)	13 (81%)	11 (100%)	345 (99%)
		Irregular	-	2 (1%)	3 (19%)	-	5 (1%)
	Border	Well-defined	171 (100%)	152 (100%)	16 (100%)	11 (100%)	350 (100%)
		Poorly defined	-	-	-	-	
T1	Hypointense		171 (100%)	152 (100%)	16 (100%)	7 (64%)	346 (99%)
	Hyperintense		-	-	-	4 (36%)	4 (1%)
	Isointense		-	-	-	-	
T2	Hypointense		–	-	-	-	
	Hyperintense		171 (100%)	152 (100%)	10 (63%)	2 (18%)	335 (96%)
	Isointense		-	-	6 (37%)	9 (82%)	15 (4%)
Contrast Kinetics	No enhancement		171 (100%)	4 (3%)	-	-	175 (50%)
	Type 1		-	123 (81%)	5 (31%)	1 (8%)	129 (37%)
	Type 2		-	12 (8%)	11 (69%)	5 (46%)	28 (8%)
	Type 3		-	13 (8%)	-	5 (46%)	18 (5%)
ADC[c] (x10^{-3}mm^2/s)	Minimum		1.77 (1.70-1.94)	1.22 (1.11-1.35)	0.94 (0.81-1.05)	0.88 (0.78-1.06)	1.56 (1.17-1.77)
	Maximum		2.35 (2.19-2.47)	1.78 (1.64-1.96)	1.67 (1.53-1.84)	1.36 (1.35-1.56)	1.96 (1.75-2.37)
	Mean		2.05 (1.93-2.16)	1.49 (1.32-1.66)	1.26 (1.24-1.35)	1.06 (0.96-1.20)	1.77 (1.45-2.12)

FNH: focal nodular hyperplasia, HA: hepatic adenoma
a, b*: Age and dimension are presented as mean (± standard deviation / minimum-maximum)*
C*: ADC values are presented as median (25th-75th percentile)*

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For each subgroup within the benign and malignant lesion groups, the demographic data including age and sex distribution as well as the conventional MR findings, contrast-enhancement kinetics and ADC histogram analysis results were shown in Table I and Table II.

Of the 350 (age: 54±14/21-84 years, sex: 134 male/216 female) patients with benign lesions, 222 (%63) had single lesion. The mean size was 30 (±20/10-125) mm, and the area where such lesions were most frequently seen was the 7th segment (37%). Almost all lesions had regular margin (99%), all had well-defined borders, almost all were hypointense (99%) in T1, and hyperintense (96%) in T2. No contrast enhancement was seen in 50% of all benign lesions due to predominance of simple cysts, and the second most frequent (37%) finding was type 1 contrast enhancement (Table I).

Table II. *Demographic information, MRI findings and ADC values in malignant lesions*

Malignant Lesions			Metastasis	HCC	CCC	Total
Sample Size			121	41	12	174
Age[a]			64 (±11/34-89)	66 (±9/38-90)	65 (±10/52-78)	65 (±10/42-90)
Male–Female			71/50	31/10	6/6	108/66
Number of Lesions	Single		34 (28%)	19 (46%)	5 (42%)	58 (33%)
	Two–Five		36 (30%)	8 (20%)	4 (33%)	48 (28%)
	>Five–Multiple		51 (42%)	14 (34%)	3 (25%)	68 (39%)
Selected Lesion	Dimension (mm)[b]		41 (±32/10-200)	70 (±45/16-195)	92 (±40/60-170)	52 (±40/10-200)
	Segments		6 (51%)	6 (46%)	6 (50%)	6 (49%)
	Shape	Regular	62 (51%)	14 (34%)	6 (50%)	82 (47%)
		Irregular	59 (49%)	27 (66%)	6 (50%)	92 (53%)
	Border	Well-defined	103 (82%)	34 (83%)	8 (67%)	145 (83%)
		Poorly defined	18 (14%)	7 (17%)	4 (33%)	29 (17%)
T1	Hypointense		121 (100%)	32 (77%)	12 (100%)	165 (95%)
	Hyperintense		-	9 (23%)	-	9 (5%)
	Isointense		-	-	-	-
T2	Hypointense		7 (6%)	-	-	7 (%4)
	Hyperintense		112 (92%)	32 (87%)	10 (84%)	154 (89%)
	Isointense		2 (2%)	9 (13%)	2 (16%)	13 (7%)
Contrast Kinetics	No enhancement		1 (1%)	-	-	1 (1%)
	Type 1		10 (8%)	8 (19%)	-	18 (10%)
	Type 2		26 (22%)	22 (54%)	4 (33%)	52 (30%)
	Type 3		84 (69%)	11 (27%)	8 (67%)	103 (59%)
ADC[c] ($x10^{-3} mm^2/s$)	Minimum		0.51 (0.45-0.60)	0.70 (0.56-0.84)	0.53 (0.52-0.55)	0.53 (0.45-0.64)
	Maximum		1.05 (0.91-1.23)	1.20 (1-1.39)	1.22 (1.02-1.39)	1.06 (0.93-1.27)
	Mean		0.82 (0.74-0.98)	0.91 (0.75-1.10)	0.85 (0.77-0.86)	0.84 (0.74-0.99)

HCC: hepatocellular carcinoma, CCC: cholangiocellular carcinoma
a, b*: Age and dimension are presented as mean (± standard deviation / minimum-maximum)*
c*: ADC values are presented as median (25th-75th percentile)*

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Of the 174 (age: 65±10/42-90, sex: 108 male/66 female) patients with malignant lesions, 58 (33%) had single, 48 (28%) had two to five, 68 (39%) had more than five-multiple masses. For the selected lesions, the mean size was 52 (±40/10-200) mm, and the area where such lesions were most frequently seen was the 6th segment (49%). 53% of the lesions had irregular margin, 83% had well-defined borders, almost all were hypointense (95%) in T1, and hyperintense (89%) in T2. In the dynamic contrast-enhanced series, 59% had type 3, 30% had type 2, and 10% had type 1 contrast-enhancement (Table II).

For all benign lesions, the minimum, maximum and mean ADC values were as follows; 1.56 (1.17-1.77)x10^{-3}mm^2/s, the, 1.96 (1.75-2.37)x10^{-3}mm^2/s, and was 1.77 (1.45-2.12)x10^{-3}mm^2/s, respectively. For all malignant lesions, the minimum, maximum and mean ADC values were as follows 0.53 (0.45-0.64)x10^{-3}mm^2/s, 1.06 (0.93-1.27) x10^{-3}mm^2/s, and 0.84 (0.74-0.99)x10^{-3}mm^2/s, respectively. A marked statistically significant difference in terms of ADC values was found between the benign and malignant lesion groups (p<0.001). When optimal thresholds for minimum, maximum and mean ADC were taken as 1.47x10^{-3}mm^2/s; 1.85x10^{-3}mm^2/s; 1.72x10^{-3}mm^2/s respectively, sensitivity was found to be 97%; 83%; 95%, specificity was 100%; 98%; 99%, NPV was 100%; 99%; 99%, and PPV was 93%; 74%; 90% (Table III). The box plots indicating the distribution of the ADC values in these two groups are shown in Figure 2.

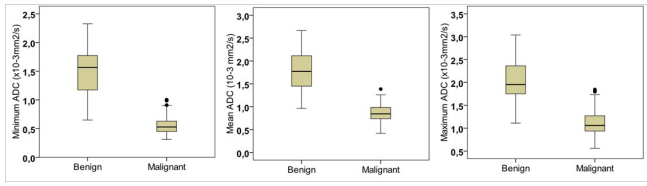

Figure 2. *Box plots graphs of minimum, mean, maximum ADC values for benign and malignant lesions*

When the ADC values of the lesions were compared; differences were found between cysts and other lesions and between hemangiomas and malignant lesions (p<0.001). While there was no difference for the minimum ADC values between focal nodular hyperplasia (FNH) and metastases, differences were found for the maximum and mean ADC values (p<0.001). No differences were found in the ADC values within the malignant lesions, within the benign lesions except for cysts, and additionally, no ADC difference was found between the hepatic adenomas and malignant lesions (Table IV). With the addition of quantitative ADC data, the diagnostic success of MRI increased from 92% to 96% for metastasis, from 63% to 73% for HCC, from 90% to 99% for hemangioma, and from 56% to 75% for FNH. ADC measurement had no effect on diagnostic accuracy for hepatic adenoma, simple cyst and CCC (Table V).

Table III. *Effectiveness of ADC values in differentiation of benign-malignant lesion groups*

ADC (x10^{-3}mm^2/s)	Benign (n:350)	Malignant (n:174)	P value	Cut-off value	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	AUC
Minimum	1.56 (1.17-1.77)	0.53 (0.45-0.64)	<0.001	1.47	97	100	100	93	0.966
Maximum	1.96 (1.75-2.37)	1.06 (0.93-1.27)	<0.001	1.85	83	98	99	74	0.948
Mean	1.77 (1.45-2.12)	0.84 (0.74-0.99)	<0.001	1.72	95	99	99	90	0.982

AUC: area under the curve, NPV: negative predictive value, PPV: positive predictive value

Table IV. *Comparison of ADC values among focal liver lesions*

Lesion Groups	Benign				Malignant			P value
	Cyst	Hemangioma	FNH	HA	Metastasis	HCC	CCC	
Sample Size	171 (33%)	149 (28%)	16 (3%)	11 (2%)	121 (23%)	41 (8%)	12 (3%)	
ADC Minimum	1.77 (1.70-1.94)	1.22 (1.11-1.35)	0.94 (0.81-1.05)	0.88 (0.78-1.06)	0.51 (0.45-0.6)	0.70 (0.56-0.84)	0.53 (0.52-0.55)	<0.001[a]
ADC Maximum	2.35 (2.19-2.47)	1.78 (1.64-1.96)	1.67 (1.53-1.84)	1.36 (1.35-1.56)	1.05 (0.91-1.23)	1.20 (1-1.39)	1.22 (1.02-1.39)	<0.001[b]
ADC Mean	2.05 (1.93-2.16)	1.49 (1.32-1.66)	1.26 (1.24-1.35)	1.06 (0.96-1.20)	0.82 (0.74-0.98)	0.91 (0.75-1.10)	0.85 (0.77-0.86)	<0.001[c]

FNH: focal nodular hyperplasia, HA: hepatic adenoma, HCC: hepatocellular carcinoma, CCC: cholangiocellular carcinoma
ADC values are presented as median (25-75th percentile)
a*: For minimal ADC, there was a significant difference between cyst-other lesions and hemangioma-malignant tumors*
b, c*: For maximum and mean ADC, there was a significant difference between cyst-other lesions, hemangioma-malignant tumors and FNH-metastasis*

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Table V. *Effect of ADC values measured in focal liver lesions on the diagnostic success of MRI*

Final Diagnosis	n	MRI Diagnosis	n	Ratio	Between Groups ADC Difference	Diagnostic Success Rate Before ADC–After ADC
Metastasis	121	**Metastasis**	111	92%		92% → 96%
		HCC	4	3%	n.s.	
		Hemangioma	5	4%	p<0.001	
		CCC	1	1%	n.s.	
HCC	41	**HCC**	26	63%		63% → 73%
		Metastasis	9	22%	n.s.	
		Hemangioma	4	10%	p<0.001	
		KSK	2	5%	n.s.	
CCC	12	**CCC**	3	25%		25% – 25%
		Metastasis	8	67%	n.s.	
		HCC	1	8%	n.s.	
Cyst	171	**Cyst**	171	100%		100% – 100%
Hemangioma	152	**Hemangioma**	137	90%		90% → 99%
		Metastasis	14	9%	p<0.001	
		FNH	1	1%	n.s.	
FNH	16	**FNH**	9	56%		56% → 75%
		Hemangioma	4	25%	n.s.	
		Metastasis	3	19%	p<0.001*	
Hepatic Adenoma	11	**Hepatic adenoma**	7	64%		64% – 64%
		Metastasis	3	27%	n.s.	
		Hemangioma	1	3%	n.s.	

n.s.: no significant difference
FNH: focal nodular hyperplasia, HCC; hepatocellular carcinoma, CCC: cholangiocellular carcinoma
** Statistical difference was found for mean and maximum ADC*

4. DISCUSSION

Diffusion-weighted imaging exploits the regional differences in the motion of water molecules within the extracellular/extravascular compartment of tissues. In highly cellular tissues (e.g., lymphoma, carcinoma and abscess), the compact nature of the extracellular space causes increased impediment to motion of water molecules and the resultant water diffusion in such tissues is said to be "restricted". On the contrary, in tissues that are necrotic or fluid filled (e.g., cysts), there is unrestricted motion of water molecules and water diffusion in such tissues, which is said to be "free". Therefore, the diffusion properties in different tissues provide information on tissue cellularity and the integrity of cellular membranes [6, 7]. Because of the relatively short T2 relaxation time of the normal liver parenchyma, the b values used for clinical diffusion imaging are typically no higher than 1000 sec/mm^2 [6]. To generate b values larger than this would generally require the use of longer diffusion-gradient pulses with longer echo times. In this case, the image distortion associated with T2 decay and the echoplanar imaging technique itself is prone to increase further with increasing b values. Moreover, there is a tendency to loss of image signal [8]. ADC is the measurable parameter of the tissue water diffusion properties obtained from DWI.

There are studies in the literature on benign-malignant differentiation and subgroup characterization in liver lesions using ADC [Table VI, 9-13]. In these studies, different success rates were reported using various ADC thresholds showing variability most probably due to factors such as differences in the parameters used to obtain ADC maps, or the measurement technique etc. However, the common view is that ADC values are higher in benign and lower in malignant liver lesions. In our study, the mean ADC threshold was slightly higher than in the mentioned studies; however it appears to be more successful in benign-malignant differentiation.

In our study, the minimum and maximum ADC values were found by histogram analysis in addition to frequently used mean ADC. Minimum ADC represents the most proliferative area with the highest cellularity in tumors of heterogeneous structure [14]. On the other hand, maximum ADC which is the opposite of this, indicates the area with the lowest cellularity with the highest extracellular fluid concentration. There are studies demonstrating that minimum ADC is an effective parameter for benign-malignant differentiation and tumor grading in breast and brain tumors [14-17]. While we could not find a similar study for liver masses in the literature, minimum ADC was markedly more successful compared to maximum ADC while being slightly more successful compared to mean ADC. Although, the cystic/necrotic component is avoided during measurement, sometimes this may not be possible. This component becomes harder to notice with the decreased size of lesion. Removing focal necrotic areas distributed within solid areas during measurement may be technically difficult, even impossible. This aspect of ADC measurement for which standardization is being attempted may vary from person to person. The fact that minimum ADC represents the

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area with the highest cellularity in the mixed structure appears to be helpful both for differentiation and measurement standardization.

There are studies suggesting that using ADC alone for characterization of liver lesions should be avoided and this method should be used in combination with conventional MR sequences [18-20]. On the other hand, some studies reported that ADC values overlap between solid benign and malignant lesions [21, 22]. Based on the results of our study, we concluded that ADC measurement alone is not enough for specific lesion characterization beyond benign-malignant differentiation. Only simple cysts could be differentiated from other benign lesions with significantly high ADC values. However, for the diagnosis of a benign lesion such as a simple cyst that can be easily identified using conventional sequences, ADC measurement will not be practical in routine practice. But ADC measurement can provide a major contribution to the diagnostic success in case of suspect in the diagnosis of hemangioma that is also benign, commonly seen, and may be confused with malignant lesions such as metastases due to atypical staining and signal characteristics**.** It should be noted that solid benign lesions such as hemangioma, FNH and hepatic adenoma can show restricted diffusion compared to normal liver parenchyma. This restriction is substantially lower than in malignant lesions, but the difference may not be noticeable to naked eye in DWI/ADC evaluation. In their recent study, Zarghampour et al., reported that ADC is successful in HA-HCC and HA-FNH differentiation while being unsuccessful in FNH-HCC differentiation [23]. Similar to the mentioned study, our study found that ADC is unsuccessful in FNH-HCC differentiation. On the contrary, ADC could not be successful in the differentiation of hepatic adenoma from other benign and malignant tumors.

Studies were conducted to investigate the diagnostic utility of DWI compared to contrast-enhanced series especially in oncology patients with impaired renal function [24-28]. Hardie et al., reported that contrast-enhanced T1 sequence and DWI show similar success in the differentiation of metastatic and benign liver lesions, and DWI can be used as an alternative to contrast-enhanced MRI [29]. On the contrary, in a similar study Donati et al., reported that DWI alone has a diagnostic accuracy lower than contract-enhanced MRI, it cannot be used alone and may be helpful only to ensure diagnostic reliability [30]. DWI-ADC is evaluated only qualitatively in our institution. With the added quantitative ADC data, the diagnostic accuracy of MRI increased for metastasis, HCC, hemangioma and FNH. It was found that the mostly confused lesion in benign-malignant differentiation is hemangioma. It was seen that 5 (4%) of 121 metastatic lesions and 4 (10%) of 41 HCCs had been reported as hemangiomas while 14 (%9) of 152 hemangiomas had been reported as metastases. When the examinations were re-evaluated, we observed that the major cause for this is the contrast enhancement pattern. The typical contrast enhancement pattern expected in hemangioma is not always seen and is non-specific (Fig. 3). Based on our results, we can say that the most effective use of ADC is in the differentiation of hemangioma and malignant tumor. The areas with the lowest diagnostic success of MRI were CCC (25%) followed by FNH (56%), HCC (63%) and HA (64%). The contrast enhancement pattern for the diagnosis of FNH and HA was non-specific, and the expected morphological signs were insufficient. Use of a hepatocyte-specific contrast agent appears to be necessary particularly in the diagnosis and differentiation of these two lesions [31]. The diagnostic success was low for CCC and HCC because of confusion with metastases. After administration of contrast agent, CCC shows centripetal contrast enhancement following continuous thick annular peripheral opacification during the early arterial phase. During the late portal venous phase, progressive heterogeneous contrast enhancement associated with late contrast enhancement of internal fibrous tissue. However, this contrast enhancement pattern is non-specific [32]. Only 33% of the CCCs in our study showed this opacification pattern. The diagnostic effect of contrast-enhanced series cannot be ignored but may sometimes be confusing. Moreover, the toxic effect resulting from exposure to contrast agent causes benefit-harm dilemma. We think that an MRI protocol based on non-contrast/-enhanced and quantitative ADC measurement applicable to the selected patient group may be sufficient for benign-malignant differentiation which is the most important point.

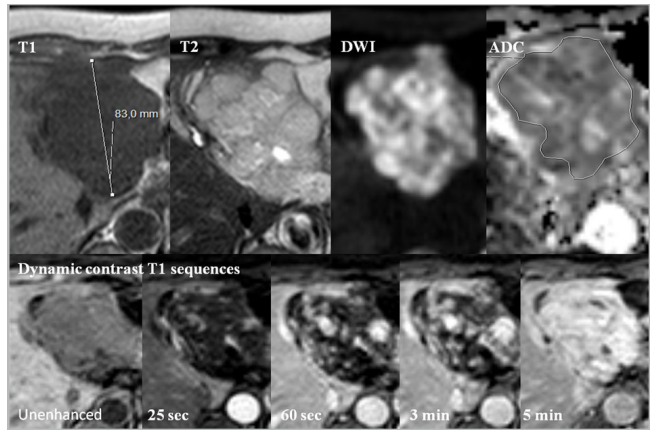

Figure 3. *A 68-year-old/female/well-differentiated HCC confused with hemangioma*

T1 hypointense, T2 heterogeneous hyperintense mass in the segment 3 of the liver with lobulated contours and well-defined borders, which becomes opacified with centripetal nodular enhancement and contrast-enhanced during the late phase; mean ADC $1.1x10^{-3}mm^2/s$, minimum ADC: $0.9\ x10^{-3}mm^2/s$, maximum ADC: $1.4\ x10^{-3}mm^2/s$, contrast enhancement pattern type 2. Even if the lesion morphology and staining pattern mimic hemangioma, malignant tumor is considered with the contribution of ADC measurement.

There are some limitations in this study. Some lesion groups were of limiter number with limited lesion types. The ADC measurement method and the b value used may be questionable. There is no consensus in the literature on this issue. The ADC measurements were performed by two radiologists together; the reproducibility of them may be questionable.

In conclusion; although, no reasonable success was found for determining the lesion subtypes of malignant cases, ADC

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measurement in focal liver lesions was found to be successful in the differentiation of benign and malignant lesions. The diagnostic success of minimum ADC was found to be higher and appears to be more suitable for measurement standardization. In the future, standardized minimum ADC data may find a place for itself as a useful biomarker in the routine practice.

Compliance with Ethical Standards

Ethical approval: Approval was received from Adnan Menderes University, School of Medicine Ethics Committee for this retrospective study (approval number: 2017/1267). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

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Conflicts of interest/competing interests: The authors have no relevant financial or non-financial interests to disclose.

Author contribution: AT: Conceived and designed the analysis, wrote the paper, MBC: Contributed data and critical analysis, OFKK:Planned the study and analyzed the data. ALL authors contributed to the final version of the manuscript.

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Could ozone therapy be used to prevent COVID-19?

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ABSTRACT

Objective: Ozone therapy has been shown to be beneficial in the prevention and treatment of various viral infections. This study aimed to investigate the beneficial effect of ozone therapy against coronavirus disease 2019 (COVID-19).
Patients and Methods: A total of 71 individuals who completed at least ten sessions of ozone therapy applied by the method of major autohemotherapy within the last six months were included and telephone call surveys were made.
Results: Two out of 71 participants were confirmed to have COVID-19. They both had mild symptoms and were discharged from the hospital in five days. None of the participants with contact or travel history was infected.
Conclusion: Ozone therapy could be preventive against COVID-19. It could be considered as an alternative, easy-to-apply, safe, and inexpensive method in the fight against COVID-19.
Keywords: Coronavirus, Ozone, Preventive Medicine

1. INTRODUCTION

The first cases of a new type of Coronavirus, later to be named coronavirus disease 2019 (COVID-19) by the World Health Organization, were reported in late December 2019, China [1,2]. Since then, it has infected 495.200.191 people and killed 6.167.271 people worldwide [3]. To date as of April 02, 2022, although, some potential drugs have been administered to the patients, there is no concrete evidence concerning the effectiveness and safety of the COVID-19 treatment [4,5]. These potential treatment options based on previous efficacy results in treating various other diseases such as malaria, rheumatoid arthritis, Middle East respiratory syndrome, severe acute respiratory syndrome, Ebola, influenza, and autoimmune diseases are included in the therapeutic guidelines [5]. Meanwhile, ozone has become a promising supportive treatment method, and clinical trials about its antiviral efficacy have already been started [4,6].

Ozone, as a triatomic oxygen molecule, is one of the most powerful oxidants. The medical ozone mixture of 1-5% ozone in 95-99% oxygen can be administrated to the body by different methods [7,8]. Ozone therapy is a safe and inexpensive therapy, and depending on the dose, its biochemical effects can last up to six months. These biochemical modulation effects are an increase in red blood cell oxygen release and endothelial nitric oxide production, improvement in mitochondrial oxygen consumption and energy generation, inflammatory response regulation, immune system modulation, and antioxidation status activation [7,9]. In addition to these well-documented in vivo effects, it has also been shown to inactivate some viruses directly by oxidizing membrane glycoproteins [7,8]. Thus, it is thought to be effective for the prevention and treatment of COVID-19 [10].

In recent years, ozone therapy has become a popular alternative method for chronic pain management of various diseases such as fibromyalgia, knee osteoarthritis, and rheumatic diseases [11-13]. As a result of this, many individuals had already received ozone therapy before the outbreak of COVID-19. This study aimed to investigate the beneficial effect of ozone therapy against COVID-19 in these individuals. It was hypothesized that

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at least ten sessions of ozone therapy received within six months decreased the prevalence and severity of COVID-19.

2. PATIENTS and METHODS

This cohort study was conducted between May 06, 2020, and May 16, 2020. Participants were selected by a retrospective analysis of the individuals who had received ozone therapy at the Pain Medicine Clinic, Sisli Hamidiye Etfal Training and Research Hospital, University of Health Sciences, Istanbul. The inclusion criteria were determined as the completion of at least ten sessions of ozone therapy applied by the method of major autohemotherapy in the last six months. Individuals who received ozone therapy locally or had fewer than ten sessions, and those who did not want to participate were excluded. Suspected and confirmed cases of COVID-19 were determined according to the seventh edition of the Diagnosis and Treatment Guidelines for COVID-19 [14]. The study was approved by the Ethics Committee of University of Health Sciences, Sisli Hamidiye Etfal Training and Research Hospital (approval number 2761), and the Republic of Turkey Ministry of Health Scientific Research Platform. The protocol was registered to ClinicalTrials.gov (NCT04400006). Patient consent was obtained from all participants.

Data collection was performed by a physiatrist with the surveys that were taken from the individuals who fulfilled the criteria. The interviews were performed by telephone calls and took approximately thirty minutes during weekdays. The survey involved questions on age, gender, height, weight, occupation, comorbidities, and concurrent medications, in addition to a detailed query concerning COVID-19 (Table I).

Table I. *The survey that was taken by all participants*

Since the beginning of March 2020 have you ever;
had a contact or travel history related to COVID-19?
☐No ☐Yes, I had a history of.....
had one or more symptoms listed below:
☐No ☐throat sore ☐fever ☐cough ☐shortness of breath ☐diarrhea ☐others.....
if yes,
How long have the symptoms lasted?
.....
Have you needed medical assistance?
☐No ☐Yes, I went to.....
Have you received radiologic imaging?
☐No ☐Yes, I received.....resulted.....
Have you tested for COVID-19?
☐No ☐Yes, The name of the test was.....resulted ☐Positive ☐Negative
diagnosed with COVID-19?
☐No ☐Yes
if yes,
Have you hospitalized?
☐No ☐Yes
Which medicines have you used?
.....
Have you needed oxygenation or intensive care support?
☐No ☐Yes.....

The major autohemotherapy procedure for all the participants was applied as follows [7]: 100mL of blood was drawn by a vacuum from the antecubital vein into a sterile glass bottle in which 12 mL of 3.13% sodium citrate solution was included as an anticoagulant (MediPac®, Germany). A corresponding volume (100mL) of gas with an ozone concentration of 10-20 µg/mL was immediately added and continuously mixed by a gentle rotating movement to avoid foaming in the blood in the bottle. Ozone was produced by a Blue-S medical ozone generator (Turkozone®, Turkey). Reinfusion was accomplished in about 15-20 minutes, and the whole procedure was carried out in approximately 30 minutes and was repeated two or three times a week.

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Statistical Analysis

Statistical analysis was performed with a statistical software package (SPSS for Windows, version 18.0, SPSS). Demographic characteristics and clinical and therapeutic features of the study population were described as the mean value and standard deviation, or as the median and range, and the number and percentage. The prevalence of COVID-19 in the study population was calculated.

3. RESULTS

A total of 74 individuals who completed at least ten sessions of ozone therapy applied by the method of major autohemotherapy within the last six months were assessed for eligibility. Of these, three of them did not want to participate; thus, 71 of them agreed to take the telephone call surveys and were included in the study. The demographic characteristics and clinical features of the participants are shown in Table II, and details of the ozone therapy administration are displayed in Table III.

Table II. *Participant characteristics and clinical features*

Age *(years)*		50.97±12.07	
	30-39	13	(18.3)
	40-49	22	(31)
	50-59	18	(25.4)
	60-69	13	(18.3)
	70-79	4	(5.6)
	80-90	1	(1.4)
Gender			
	Female	46	(64.8)
	Male	25	(35.2)
BMI *(kg/m²)*		25.4±3.94	
	Normal (18.5-24.9)	35	(49.3)
	Overweight (25-29.9)	24	(33.8)
	Obese (30-34.9)	9	(12.7)
	Extremly obese (>35)	3	(4.2)
Occupation			
	Medical professional	11	(15.5)
	Other white-collar employee	14	(19.7)
	Blue-collar employee	9	(12.7)
	Housewife	20	(28.2)
	Retiree	17	(23.9)
Comorbidity			
	None	34	(47.9)
	Hypertension	7	(9.9)
	Diabetes Mellitus	5	(7)
	Thyroid disease	4	(5.6)
	Cancer	2	(2.8)
	Chronic Respiratory disease	2	(2.8)
	Coronary artery disease	1	(1.4)
	Cerebrovascular disease	1	(1.4)
	Rheumatologic disease	1	(1.4)
	Multiple	14	(19.7)

Data= Mean±SD, n(%), BMI= Body mass index

Twenty-two of the participants had histories of either contact with a COVID-19 infected person or of international travel. Of these, 10 of them were medical professionals who actively worked during the pandemic period. Two medical professionals and two others with contact or travel history showed clinical symptoms that did not last more than a week. Their thorax computed tomography did not reveal an abnormality, and real-time polymeraz chain reaction (PCR) test for COVID-19 were negative. Eighteen participants with contact or travel history had no clinical symptoms. Forty-six of the participants did not have contact or travel history, or any clinal symptoms. Besides this, three of the participants without contact or travel history had clinical symptoms and needed medical assistance. COVID-19 was confirmed in two of them. One was a 61-year old retired male who had a history of asthma and gout, and the other one was a 41-year old obese female without any comorbidities. They were both hospitalized and received combinations of hydroxychloroquine, oseltamivir, or azithromycin. They had only mild symptoms and were discharged in five days. None of the participants other than these two had received any specific medication against COVID-19 (Table IV). None of the patients had severe symptoms, and the prevalence of COVID-19 in the study population was found to be 2.8%.

Table III. *Details of ozone therapy applied by the method of major autohemotherapy*

Indication			
Pain			
	Chronic widespread pain	28	(39.4%)
	Musculoskeletal pain	21	(29.6%)
Fatigue		10	(14.1%)
Other		12	(16.9%)
Total number of sessions		10	(10-22)
Dose of each session *(µg/mL)*		15	(10-20)
The time from the last session of ozone therapy to the survey was taken *(days)*			
	0-29	20	(28.2%)
	30-59	20	(28.2%)
	60-89	15	(21.2%)
	90-119	7	(9.9%)
	120-149	3	(4.2%)
	150-179	6	(8.5%)

Data= n(%), Median(Min-Max)

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Table IV. *The results of the survey that was taken by all participants*

Case No	Contact or travel history	Clinical manifestations	Radiological evidence	RT-PCR Test	
1	-	+	+	+	A 61-year old male who had a history of asthma and gout was administered to hospital with complaints of throat sore, myalgia, and fatigue. Bilateral multiple subpleural ground-glass densities were detected in thorax CT. He was given a combination of hydroxychloroquine and oseltamivir. He had mild symptoms, did not need oxygen supplementation.
2	-	+	+	+	A 41-year old obese housewife who referred from primary care physician had symptoms of fever, cough, and dyspnea. Atypical pneumonia findings were confirmed with thorax CT. She was given a combination of hydroxychloroquine, oseltamivir, and azithromycin. She had mild symptoms, did not need oxygen supplementation.
3 ... 10	+	-	**NA**	**NA**	Medical professionals, aged between 31 years and 58 years, actively worked against COVID-19. One of them had a malignancy history and another one had thyroid disease.
11 12	+	+	-	-	Two medical professionals actively worked against COVID-19. A 30-year old male without a history of comorbidities had fever, cough, and dyspnea symptoms for a few days. Another 34-year old female with asthma had a complaint of shortness of breath for a week.
13 ... 22	+	-	**NA**	**NA**	Individuals aged between 39 years and 60 years. Two of them had hypertension, and one had a malignancy history.
23 24	+	+	-	-	A 40-year male had fever for two days, and a 37-year old female complained about cough for several days.
25	-	+	**NA**	-	A 30-year old female complained from shortness of breath for more than a few weeks.
26 ... 71	-	-	**NA**	**NA**	Individuals aged between 36 years and 86 years

4. DISCUSSION

In this study, the surveys taken on seventy-one individuals who had completed at least ten sessions of ozone therapy within the last six months were analyzed, and it was revealed that none of the participants with contact or travel history were infected. Besides, it was shown that 45.4% of participants with contact or travel history were medical professionals who worked actively during the pandemic period, and even though two had nonspecific mild clinical symptoms, none of them were diagnosed with COVID-19. Another noteworthy finding was that the two persons in the high-risk category for COVID-19 recovered from the disease quite mildly. These results support the idea of the preventive effect of ozone therapy against COVID-19, and the hypothesis of at least ten sessions of ozone therapy received within six months decreased the severity of COVID-19.

The research and meta-analyses have emphasized that being over the age of 50 years, obesity, and comorbidities such as hypertension, diabetes mellitus, chronic respiratory disease, coronary artery disease, or cerebrovascular disease were the high-risk factors for COVID-19 [15-20]. In this study, it was discerned that 50.7% of the study population was over the age of 50 years, while 16.9% were obese or extremely obese, and 52.1% had comorbidity, 19.7% of these had multiple comorbidities. If these are considered all together, it could be easily concluded that the study population was at a higher risk than the normal population. Therefore, the prevalence of COVID-19 in the study population could be found to be higher than the general population of Turkey [3].

The possible preventive and antiviral effects of ozone therapy against COVID-19 could be through various mechanisms. The preventive effect could be the oxidative stress created by ozone in the body to stimulate the peripheral phagocytic cells, activate the antioxidant system, and restore the immune system [21]. The antiviral effect could be the peroxidation occurred by ozone to damage the viral capsule and disrupt the reproductive cycle or fusion of the virus [22]. In addition, particularly for Coronavirus, ozone therapy could disturb the virus-cell interaction by NrF2 activation and restrain the inflammatory process by NFkB inhibition [23]. Moreover, it is considered to reduce ischemia-reperfusion injury, which causes the multi-organ dysfunction

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in the advanced disease period [24]. Ozone therapy has been reported to be beneficial in various viral infections including COVID-19 [25-28]. The optimal ozone therapy dose required to provide these effects against COVID-19 remains unknown [29]. This study could only indicate that ten sessions of low-medium dose ozone therapy within the previous six months prevented COVID-19. There is still a need for future studies that investigate its usage or optimal doses for treatment.

The limitations of the study, as a consequence of not being designed before the outbreak of COVID-19, could be listed as the lack of a control group and the relatively low number of participants. Besides, the design of the study could have caused memory bias. Lastly, variational antioxidant capacities, lifestyle habits, and the COVID-19 contact duration of the participants could have confounded the findings of the study. However, drawing attention to the preventive effect of ozone therapy against COVID-19 for the first time could be one of the strengths of the study.

In conclusion, none of the individuals who completed at least ten sessions of ozone therapy within the last six months with contact or travel history was infected, and even though two of them in the high-risk category were diagnosed with COVID-19, they recovered in a short time without complications. These results support the fact that ozone therapy could be preventive against COVID-19. Ozone therapy, as an easy-to-apply, safe, and inexpensive method, should be considered to deploy worldwide in the warfare of COVID-19.

Compliance with Ethical Standards

Ethical approval: The study was approved by the Ethics Committee of University of Health Sciences, Sisli Hamidiye Etfal Training and Research Hospital (approval number 2761), and the Republic of Turkey Ministry of Health Scientific Research Platform. The protocol was registered to ClinicalTrials.gov (NCT04400006). Patient consent was obtained from all participants.

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Author Contribution: KGA**:** Concept, design, data collection, analysis, literature research, writing, critical review, TS: Concept, design, supervision, materials, data collection, writing, critical review.

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Factors associated with anxiety and post-traumatic stress symptomatology during the COVID-19 pandemic in Turkey: A comparison of youths and adults

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ABSTRACT

Objective: The aim of this study was to investigate anxiety and post-traumatic stress symptoms (PTSS) and their possible associated factors among youths, comparing to their older adult counterparts.

Patients and Methods: This cross-sectional online study assessed 1493 participants in Turkey. Beck Anxiety Inventory (BAI) and the Post-traumatic Stress Disorder (PTSD) Checklist for DSM-5 (PCL-5) were used.

Results: The Youths (15-24 years) reported to experience more anxiety and PTSS than Adults (25-59 years). There were weak correlations between BAI, PCL-5 scores and duration of recovery and isolation in Adults infected with corona virus disease 19 (COVID-19), but not in Youths. Younger age and lower economic status were common factors for severe anxiety and PTSS, additionally history of mental health needs and loss of an acquaintance due to COVID-19 were specific predictors for anxiety, and having a medical condition was predictor for PTSS in Youths. In Adults, female, lower economic status, having a medical condition, history of mental health needs, and loss of an acquaintance due to COVID-19 were common factors for worsening both PTSS and anxiety, additionally younger age for PTSS and being infected by COVID-19 for anxiety were specific predictors.

Conclusion: Pandemic might have a greater impact on mental well-being of youths than adults. Identification of risk factors can shed light on planning, prevention and intervention strategies.

Keywords: Anxiety, COVID-19, Mental health, Post-traumatic stress disorder (PTSD), Youth

1. INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak, which emerged in the last months of 2019 and spread all over the world in the first quarter of 2020, rapidly turned into a severe health crisis that dramatically changed human life. More than 100 million people become infected and more than 2 million died [1]. As of March 2020, unprecedented measures have been taken in many countries to reduce the spread of the pandemic. Like the rest of the world, Turkey also suspended face-to-face training, gone to significant work organization, and implemented curfew periods. Unemployment rates have increased, the income level has decreased, and social inequalities have deepened throughout the world [2]. During the first year of the pandemic, more than 800 million students were away from face-to-face education [3].

Since, the early stages of the pandemic which affected many aspects of daily life including social, economic, education, and health, concerns have been raised that the COVID-19 outbreak is a serious risk factor for public mental health beyond being an infectious disease [4, 5]. Studies have shown that mental health problems such as anxiety, depression, and post-traumatic stress symptoms (PTSS) varied between one third and half of the general population during the pandemic [4,6]. As the time to control the outbreak got longer, its impact on mental health and differences in individual reactions to the pandemic became more evident [7]. Some groups, such as women, children, health care providers, and those who had a pre-existing psychiatric disorder or chronic medical condition, are more vulnerable to the development of mental health problems during lockdown and social distance measures [7,8].

Despite its relation to physical well-being, young age is also considered a risk factor for poor mental health during a health

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crisis [7]. In the first months of the COVID-19 pandemic, it was indicated that young adults aged 18-29 years experienced more psychological distress symptoms than other age groups [9]. In Italy and Spain, it has been reported that 85% of children and adolescents exhibited emotional and behavioural changes during lockdown period [10]. Youths have unique challenges and milestones different from adults. Because social interaction creates the stimulus required to shape the brain during the emergence of adulthood [11]. They are in a transition period from education to work, from living under caregiver supervision to being a caregiver. Furthermore, 'becoming an adult' has been historically portrayed as a person who completes his/her puberty, works, has financial independence, has his/her own family, and becomes a parent [12]. Accordingly, adolescents and young people are more sensitive to social restriction measurements that may affect their neurological, psychosocial, and emotional development unfavourably compared to people with older ages, which make them one of the most vulnerable groups.

This study aims to provide some insight about youths' mental health problems in Turkey during the second wave of the COVID-19 pandemic. We think that the analysis made by considering the psychological and emotional stages of life, such as Erikson's life stages rather than chronological grouping, might lead to get better results [13]. From this psychosocial perspective, we used an online survey which included self-report rating scales of anxiety and PTSS of youths (aged 15 – 24) compared to their older counterparts (aged 25 – 59). We also examined the associations between anxiety, PTSS and possible risk factors such as sex, age, socioeconomic status, and COVID-19 related factors for both groups.

2. PATIENTS and METHODS

Design and study population

This study was conducted as a population-based, cross-sectional design. Data were gathered from an online survey with anonymous, self-rated questionnaires in Turkish that was distributed via mainstream social media platforms (i.e., Facebook, Twitter, Instagram). This survey took place between 15th November and 15th December 2020, during the second wave of the COVID-19 outbreak in Turkey and new national lockdown, when schools, colleges and universities were closed to most students. Participants were asked to complete a 15-minute survey which consists of four parts: basic demographic data, COVID-19-related exposures, anxiety and PTSS.

The universe of the study was the individuals who had completed the survey. Among these the ones over age of 60 years were discarded since disease-related risks increase by age which would be a confounding factor. As a final sample, we end up with individuals within an age range of 15-59 years, living in Turkey, having internet access, and volunteering in participation. To investigate differences and similarities between youths and adults, the sample was divided into two groups: the individuals between ages 15-24 years constituted "Youths", while the individuals in the older age range, i.e. 25-59 years of age, constituted "Adults" which served as a comparison group.

Initially, 1737 potential respondents completed the survey. Of these, 232 respondents with missing data were filtered out. 12 respondents were excluded from the sample because they were older than 60 years of age. A total 1493 valid surveys constituted the final study sample.

Questionnaires

Sociodemographic form: The demographic variables included were: sex, age, educational level (including primary school, secondary school, high school and university degrees), economic status, marital status, having children (yes or no), having any chronic medical conditions (yes or no), history of an admission for psychiatric care services (reason for admission and time for the first contact), employment status (employee or un employee), and being a healthcare worker or not. The economic status was measured as low-middle-high based on the participants' perception of their financial constraints and situations. It was measured with a question: 'How do you find your financial situation?' There is some evidence that the predictive value of perceived socioeconomic status is a reliable measurement in terms of health-related outcomes [14]. Those who had a prior history of health needs and looked for some form of mental health support previously were considered as those with pre-existing mental health problems.

COVID-19 related factors: Experiences of COVID-19 related factors were determined with the following questions: Have you been diagnosed with COVID-19? If you have been diagnosed with COVID-19: Did you have to take drug? Did you have to stay in the hospital? Did you have to stay in the intensive care unit? How many days did it take for you to recover from the COVID-19? How many days did your isolation period last? If you have infected with COVID-19, do you still have ongoing fatigue? Has anyone of your acquainted (family member, friend, or college i.e.) been diagnosed with COVID-19? Have you ever known someone who died because of COVID-19?

The Post-traumatic Stress Disorder (PTSD) Checklist for DSM-5 (PCL-5): PTSS was assessed with PCL-5, which is a 20-item scale to assess the symptoms of PTSD [15]. The Turkish reliability and validity of this questionnaire have been performed. A cut-off score of 48 was used in this study. This cut-off score is suggested in discriminating individuals with PTSD seems to be more reliable in community samples [16].

Beck Anxiety Inventory (BAI): It is composed of 21 items with a four-point Likert-type scale [17]. The BAI scores are classified as mild anxiety (8 to 15), moderate anxiety (16 to 25), and severe anxiety (30 to 63). The Turkish reliability and validity studies of BAI were performed [18].

Statistical Analyses

All statistical analyses were performed using SPSS 22.0 for Windows. Descriptive statistics were used to present participants data in each group. Categorical variables were defined as frequencies and percentages, and differences between the groups were assessed by the Chi-square and Fisher's Exact tests. Numerical variables were characterized by the mean,

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standard deviation (*SD*), and differences between the groups were determined by the Student's *t*-test and Mann-Whitney U test according to the Kolmogorov–Smirnov test which was used to evaluate whether the distribution of variables was normal. Comparisons of ordinal variables between groups were performed by the Mann-Whitney U test.

Correlation between PCL-5, BAI scores, and COVID-19 related factors (duration of recovery and post-infection isolation) were measured using Spearman's test among participants with history of having COVID-19 diagnosis. The strength of the correlations was evaluated according to small ≥0.10, medium ≥0.30, large ≥0.50.

In order to evaluate factors associated with the severity of anxiety and PTSS at BAI and PCL-5 (primary outcomes) as continuous variables for both group, multiple linear regression models were performed, including as independent variables: age (as a continuous variable), gender (female vs. male), education (collapsed as less than high school diploma or equivalent vs. higher high school diploma), economic status (low-middle-high), employment status (employed vs. unemployed), having a medical condition (yes vs. no), history of psychiatric admission (yes vs. no), being infected by COVID-19 (yes vs. no), acquaintance infected with COVID-19 (yes vs. no), and acquaintance died due to COVID-19 (yes vs. no). Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity (VIF < 5) and homoscedasticity.

Two-tailed p-values were used. Our results and interpretations are therefore based on a significance set at p<.05.

3. RESULTS

Participants

The total sample comprised of 1493 participants with mean age of 33.18±9.11 years (min=15, max=59). Of these, 302 (20.2%) were younger than 25 years (M = 22.3 years, min= 15, max = 24), and 1191 (79.8%) were between 25 to 59 years (M = 36 years). Only 5 were married and one had a child among Youths. Of Adults, 56.8% (n=677) were married and 51.6% (n=615) had children (p<0.001, x=295.701 and p<0.001, x=364.945). The demographic and descriptive information of the study sample were given in Table I.

Most of those who applied to psychiatric treatment before the pandemic reported that they received help for their depressive symptoms (43.8%, n=49 in Youths, 44.3%, n=212 in Adults). This was followed by anxiety symptoms (36.6%, n=41 in Youths, 32.8%, n=157 in Adults) and traumatic stress symptoms after a stressful life event (13.4%, n=15 in Youths, 15.9%, n=37 in Adults). There was no statistical difference between the groups in terms of prior or current mental health contact and their psychiatric symptoms profile. It was the first treatment contact for about one third of those who applied to mental healthcare during pandemic.

Table I. *Demographic and descriptive information of the study participants*

	Youths (≤24 years, n=302) n (%)	**Adults (>24 years, n=1191) n (%)**	**p**
Age (*M*±*SD*) *	22.03±1.72	36±7.98	p<0.001, t=-54.751
Sex			
Female	271 (89.7)	1046 (87.8)	p=0.358, x=0.845
Male	31 (10.3)	145 (12.2)	
Education Level			
Primary school	-	8 (0.7)	p=0.278, x=3.853
Secondary school	1 (0.3)	6 (0.5)	
High school	25 (8.3)	93 (7.8)	
University	276 (91.4)	1084 (91)	
Socioeconomic Status (SES) *			
Low SES	95 (31.5)	219 (18.4)	p<0.001, x=24.829
Medium SES	143 (47.4)	663(55.7)	
High SES	64 (21.2)	309 (25.9)	
Employment Status *			
Health care provider	24 (7.9)	187 (15.7)	p<0.001, x=164.211
Employee non-health care sector	52 (17.2)	597 (50.1)	
Unemployment	226 (74.8)	407 (34.2)	
Chronic medical condition *	43 (14.2)	317 (26.6)	p<0.001, x=20.172
Prior psychiatric admission before the pandemic	112 (37.2)	479 (40.2)	p=0.294, x=1.100
Psychiatric admission during the pandemic	24 (7.9)	69 (5.8)	p=0.582, x=0.303
First contact with mental healthcare during pandemic	9 (2.9%)	23 (1.9%)	
Infected with COVID-19*	90 (29.8)	276 (23.2)	p=0.017, x=5.718
Infected Acquaintances	270 (90.6)	1045 (88.3)	p=0.267, x=1.232
Deaths among Infected Acquaintances	92 (30.9)	336 (28.5)	p=0.420, x=0.651

** p<.05*

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Differences in PCL-5 and BAI scores between groups

In Youths, PCL-5 (43.93±17.82) and BAI (20.33±12.63) scores were higher than Adults (39.29±19.02 and 18.52±13.28, respectively), and the differences were statistically significant (p<0.001, t=3.831 and p=0.033, t=2.140). 42.7% of Youths and 35% of Adults had a score above the cut-off score for PTSD (p=0.013, x=6.162). Of Youths, 12.3% (n=39) reported high, 31.1% (n=94) moderate, and 56.5% (n=169) mild level anxiety symptoms. In Adults, these rates were 12.9% (n=154), 24.3% (n=290), 62.7% (n=747), respectively (p=0.047, x=6.096) (Figure 1). In both groups there was no one who had scores between 0-7.

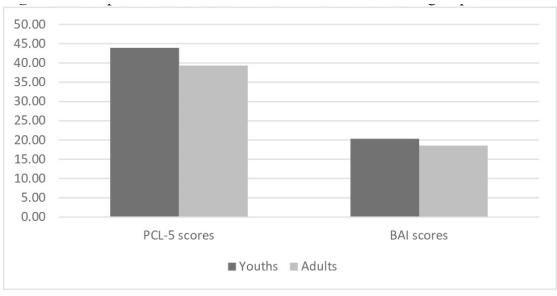

Figure 1. *Comparison of PCL-5 and BAI scores between the groups*

Differences in COVID-19 survivors between groups

When the COVID-19 survivors were examined, there were no statistical difference between the two groups in terms of gender and disease severity related parameters, such as need for treatment, type of treatment, and duration of hospitalization. The adults' recovery and the post-infection isolation periods were longer than youths. The disease-related parameters of those with COVID-19 are given in Table II.

Table II. *Comparison of variables associated with the course of COVID-19 between groups*

	Youths with COVID-19 n=90	Adults with COVID – 19 n=276	p
Female a *	85 (94.4)	227 (82.8)	p=0.005, x=8.029
Need for treatment a			p=0.089, x=6.509
No medication	32 (35.6)	66 (23.9)	
Taking prescribed medication at home	53 (58.9)	180 (65.2)	
Hospitalization	5 (5.6)	25 (9.1)	
Duration of hospitalization (days) b	7.8±6.83	6.77±2.94	p=1.000, U=65.000
Recovery (days) b *	11.90±6.5	14.79±9.16	p=0.014, U=7749.00
Post-infection isolation (days) b *	10.42±7.19	13.20±10.07	p=0.016, U=6154.00
Ongoing fatigue post-infection	61 (68.5)	198 (73.1)	p=0.410, x=0.679
Psychiatric admission within the pandemic a	8 (8.9)	35 (12.7)	p=0.327, x=0.961

*a n (%), b M±SD, *p<.05*

Correlation Analysis

The correlations between the disease related factors (recovery and post-infection isolation period) and anxiety, traumatic stress response were examined in individuals with a history of infection with COVID-19. While the duration of recovery from COVID-19 and isolation was not correlated with any of the scale scores in Youths, a statistically significant but weak correlation was found with all scale scores in Adults (Table III).

Table III. *Correlations between PCL-5, BAI scores and recovery, isolation duration among participants with infected COVID-19*

	Recovery duration (days)	Post-infection isolation (days)
PCL scores of Youths	0.076	0.057
PCL scores of Adults	0.184**	0.207**
BAI scores of Youths	0.148	-0.125
BAI scores of Adults	0.247***	0.164*

****p<.001, ** p<.01, * p<.05. PCL: The Post-traumatic Stress Disorder (PTSD) Checklist for DSM-5 (PCL-5), BAI: Beck Anxiety Inventory*

Factors associated with anxiety in Youths and Adults

In Youths, anxiety scores were statistically higher in those with poor economic status, with prior history of mental health needs, and the ones who had an acquaintance who died due to COVID-19 (Table IV). All the three variables and age were statistically significant in the multiple regression, with the economic status recording the highest beta value (*β*: – .268, %95 *C.I.*: – 6.663 – -2.768) than history of mental health needs (*β*: .150, %95 *C.I.*: .995 – 6.823), age (*β*: – .143, %95 *C.I*: – 2.030 – -.148), and deaths among infected acquaintances (*β*: – .112, %95 *C.I.*: .012 – 6.152) (Table V).

In Adults, women, those who had lower economic status, those who had a medical and/or psychiatric disease, those who were infected COVID-19, and those who lost an acquaintance due to COVID-19 had higher anxiety scores (Table IV). In the multiple regression analysis, these variables remained significant, with the prior history of mental health needs recording the highest beta value (*β*: .168, %95 *C.I*: 3.060 – 6.044) (Table V). Marital status and having children were not statistically significant for BAI scores in Adults.

Factors associated with PTSS in Youths and Adults

In Youths, PCL-5 scores were associated with economic status, and they were also significant (*β*: – .251, 95% *C.I*: – 8.765 – – 3.440) in the multiple regression analysis. Prior history of mental health needs was associated with higher PTSS (Table VI), but this did not remain significant in the multiple regression analysis. According to the regression analysis, being younger (*β*: – 2.75, 95% *C.I*: – 4.048 – – 1.537), which recorded the highest beta value, and presence of a medical condition (*β*: .133, 95% *C.I*: 1.136–13.071) were also significantly associated with worsening of PTSS (Table VI).

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Table IV. *Group comparisons of the BAI scores separately depicted with demographic and COVID-19 related variables*

		Youths M±SD	pa	Adults M±SD	pa	pb
Sex	Female	20.66±12.77	.185	19.34±13.30	<.001***	.145
	Male	17.48±11.15		12.53±11.49		.030*
Education	High school and less	24.26±13.57	.097	21.39±14.33	.019*	.356
	Bachelor's degree	19.96±12.50		18.23±13.14		.049*
Economic status	Low	24.36±13.93	<.001***	22.91±14.32	<.001***	.405
	Middle	19.47±11.58		18.12±12.83		.216
	High	16.26±11.31		16.25±12.78		.995
Employment	Healthcare provider	16.45±11.60	.293	17.98±13.78	.005**	.877
	Employee non-healthcare sectors	20.80±14.44		17.50±12.95		.082
	Unemployment	20.63±12.28		20.25±13.38		.714
Medical Condition	Yes	22.39±14.29	.249	22.30±13.31	<.001***	.966
	No	19.99±14.29		17.14±13.00		.002**
Pre-existing psychiatric disorder	Yes	23.19±11.83	.002**	21.73±13.83	<.001***	.257
	No	18.64±12.85		16.21±12.38		.017*
Infected by COVID-19	Yes	20.17±12.36	.899	20.40±14.10	.010*	.893
	No	20.40±12.78		17.95±12.98		.013
Infected Acquaintances	Yes	20.69±12.77	.096	18.70±13.45	.121	.027
	No	16.20±10.21		16.94±11.59		.773
Loss of an acquaintance due to COVID-19	Yes	22.81±13.82	.024*	20.38±13.75	.003**	.134
	No	19.21±12.06		17.84±13.03		.170

****p<.001, ** p<.01, * p<.05, pa: within group analysis, pb: comparison between groups.*

Table V. *Association between sociodemographic variables, COVID-19 related factors and BAI scores in Youths and Adults.*

	BAI							
	Youths				Adults			
	p	β	95% CI		p	β	95% CI	
Constant	.000		33.859	77.488	.000		22.498	34.659
Sex (male, ref. female)	.126	-.086	-8.681	1.073	.000*	-.152	-8.505	-3.953
Age	.024*	-.143	-2.030	-.148	.182	-.039	-.161	.031
Education level	.396	-.051	-7.871	3.121	.300	-.030	-4.200	1.295
Economic status	.000*	-.268	-6.633	-2.768	.000*	-.102	-3.176	-.911
Being an employee	1.000	.000	-3.425	3.424	.221	-.036	-2.602	.601
Having medical condition	.108	.092	-.766	7.656	.000*	.131	2.249	5.659
Having a pre-existing mental health problem	.009*	.150	.995	6.823	.000*	.168	3.060	6.044
Infected by COVID-19	.435	-.044	-4.247	1.833	.000*	.114	1.853	5.290
Infected Acquaintances	.527	.036	-3.794	7.396	.331	.028	-1.239	3.675
Loss of an acquaintance due to COVID-19	.049*	.112	.012	6.152	.009*	.074	.540	3.820
	ΔR2=0.116, p<0.001, F=4.791				ΔR2=0.115, p<0.001, F=16.162			

** p<.05, BAI: Beck Anxiety Inventory*

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Table VI. *Group comparisons of the PCL-5 scores separately depicted with demographic and COVID-19 related variables.*

		Youths M±SD	p[a]	Adults M±SD	p[a]	pb
Sex	Female	44.59±17.75	.057	40.58±18.73	.000***	.002 **
	Male	38.16±17.66		29.95±18.57		.026*
Education	High school and less	50.34±18.57	.055	40.99±20.25	.333	.034
	Bachelor's degree	43.32±17.66		39.12±18.90		.001**
Economic status	Low	48.15±19.07	.006**	45.51±18.93	<.001***	.257
	Middle	43.24±15.54		38.61±18.66		.002**
	High	39.18±19.47		36.34±18.93		.278
Employment	Healthcare provider	38.79±17.96	.339	38.14±19.18	.003**	.877
	Employee non-healthcare sectors	44.44±20.62		37.86±19.13		.018*
	Unemployment	44.35±17.10		41.90±18.56		.103
Medical Condition	Yes	47.32±20.09	.178	42.97±18.05	<.001***	.183
	No	43.36±17.39		37.95±19.20		<.001***
Pre-existing psychiatric disorder	Yes	46.88±17.44	.028*	42.47±18.44	<.001***	.022
	No	42.22±17.90		36.86±18.97		<.000***
Infected by COVID-19	Yes	42.83±18.58	.487	38.63±20.47	.532	.085
	No	44.39±17.51		39.49±18.57		<.001***
Infected Acquaintances	Yes	44.39±17.83	.123	39.48±19.15	.311	<.000***
	No	38.54±17.16		37.63±17.88		.820
Loss of an acquaintance due to COVID-19	Yes	46.94±18.04	.059	42.42±18.44	<.001***	.037*
	No	42.70±17.68		38.14±19.06		.002*

****p<.001, ** p<.01, * p<.05, pa: comparison within the groups, pb: comparison between the groups.*

Table VII. *Association between sociodemographic variables, COVID-19 related factors and PCL-5 scores in Youths and Adults*

PCL-5								
	Youths				Adults			
	p	β	95% CI		p	β	95% CI	
Constant	.000		91.827	150.333	.000		52.365	70.034
Sex (male, ref. female)	.082	-.097	-13.278	-.807	.000*	-.155	-12.406	-5.792
Age	.000*	-.275	-4.048	-1.537	.001*	-.099	-.376	-.097
Having higher education	.582	-.033	-9.940	5.595	.648	-.013	-4.920	3.064
Economic status	.000*	-.251	-8.765	-3.440	.001*	-.097	-4.426	-1.135
Being an employee	.684	.024	-3.730	5.679	.092	-.050	-4.325	.329
Having medical condition	.020*	.133	1.136	13.071	.003*	.089	1.328	6.283
Having a pre-existing mental health problem	.135	.085	-.955	7.065	.000*	.110	2.087	6.422
Infected by COVID-19	.100	-.092	-7.668	.678	.779	.008	-2.139	2.855
Infected Acquaintances	.470	.041	-5.218	11.285	.606	.015	-2.632	4.508
Loss of an acquaintance due to COVID-19	.073	.101	-.355	8.053	.004*	.083	1.130	5.896
	ΔR2=0.144, *p*<.001, *F*=5.813				ΔR2=0.084, *p*<.001, *F*=11.716			

** p<.05. PCL: The Post-traumatic Stress Disorder (PTSD) Checklist for DSM-5 (PCL-5).*

In Adults, women, poor economic status, presence of medical illness, prior history of mental health needs and loss of an acquaintance due to COVID-19 were associated with higher PCL-5 scores (Table VI). These findings were also statistically significant, with gender recording the highest beta value (*β*: – .155, %95 *C.I*: – 12.406 – -5.792) in the multiple regression analysis (Table VII). Unemployment status was associated with a higher PCL-5 score, but this did not remain significant in the multiple regression analysis. In addition, younger age (*β*: – .099, %95 *C.I*: – .376 – -.097) became a statistically significant

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predictor for PTSS in the multiple regression analysis. Infected with COVID-19, marital status and having children were not statistically significant for the PCL-5 scores in Adults.

4. DISCUSSION

Our study pointed out that, when compared to adults, anxiety and PTSS were higher in young people. This finding is consistent with findings in previous studies on the greater psychological impact of the COVID-19 outbreak on young people's mental health from many other countries [19,20]. Also, the most important finding of our study, which we conducted by reaching 1493 people, is that the factors that might be related to the burden of the pandemic on the mental well-being of Youths and Adults are not homogeneous. This means that the factors affecting anxiety and post-traumatic stress can be pretty different from each other in psychologically different age groups. While being younger and having poor economic conditions were the leading risk factors for traumatic stress and anxiety symptoms in Youths, gender and COVID-19 related parameters contributed to worsening of mental health problems in Adults. Strikingly, being infected by COVID-19 was associated with neither anxiety nor PTSS in Youths, but it was on Adults for anxiety.

About half of the Youths and one third of the Adults reported PTSS above the cut-off score of PCL-5 and high or moderate level of anxiety in our sample. These findings were higher compared to those found in Chinese sample [21,22], and similar previous studies from USA [6], Italy [23], and Turkey [24,25]. The rates of provisional PTSS and anxiety in the current study seem high, which might indicate detrimental effect of the pandemic in Turkey. On the other hand, one possible explanation might be that our sample was composed of individuals with high rates of pre-existing mental health disorders (37.2% in Youths, 40.2% in Adults) that might make them vulnerable to psychosocial stresses. Nevertheless, these findings would be alarming for mental health problems during COVID-19 outbreak.

One of the most striking findings in the current study was low economic situations as a predictor for anxiety and PTSS in both groups. There are well established links between economic losses and psychological distress. The deterioration in economic situation during pandemic may boost mental health problems in the upcoming periods as economic problems, education, and social inequalities deepen during the pandemic process [26]. Similarly, previous economic crises and outbreaks showed that youth's unemployment and school dropout rates increased during these periods, and future career and salary expectations of young people with limited financial opportunities decreased [27]. Therefore, psychosocial adversities during the pandemic might be considered to have long-term effects and may even affect future generations [27]. Accordingly, financial insecurity could be an important intervention area to buffer negative mental health consequences for vulnerable individuals, as an intervenable factor, unlike age, pre-existing psychiatric or medical diseases.

The data from our study suggests that having a COVID-19 diagnosis is not associated with traumatic stress response in either of the groups, only predicted anxiety in Adults. This might be related to the fact that our study sample, with an age range of 15-59 years, was thought to have a relatively low risk for COVID-19 mortality. The perceived stress about disease severity might determine the psychological effects of COVID-19.

We also investigated the correlation between anxiety, PTSS and the variables related to recovery process from COVID-19. The results suggested that in contrast with Youths, psychological response in Adults with COVID-19 diagnosis tend to be associated with the disease related factors. Additionally, while loss of an acquaintance due to COVID-19 was associated with both high anxiety and PTSS in Adults, it was only associated with anxiety in Youths. These findings indicated that the traumatic experiences of young people are not mainly associated with death or health outcomes in the pandemic, instead young people might perceive psychosocial challenges of the COVID-19 outbreak as more traumatic than life-threatening manifestations of the disease.

Our study confirmed that pre-existing psychiatric disorders and medical comorbidities make individuals vulnerable to experience psychosocial distress during the pandemic [19,28,29]. The findings of this study demonstrated that history of mental health needs and having a medical condition are associated with higher anxiety and traumatic stress response in Adults. However, having a medical condition predicted PTSS, while history of mental health needs predicted anxiety in Youths. Those with medical morbidity may perceive COVID-19 as a more dangerous threat to their health compared to others. In addition, their access to interrupted healthcare services can increase feelings of helplessness and loss of control, which can contribute to an increase in traumatic experiences both in youths and adults [29]. Furthermore, pre-existing psychiatric problems may impair the coping skills of the individuals and make them more vulnerable to pandemic by preventing them to attend protective daily activities such as physical activity, routines, and work.

Many of the previous studies during the pandemic suggested that women were affected more negatively [4,30]. Our study showed that both anxiety and post-traumatic stress scores of women were higher than men in Adults but not in Youths. Such a gender difference in adulthood might be due to several factors, firstly radical lifestyle changes during pandemic might have caused a greater stress for adult women. Also, it might be related to the well-known phenomenon of higher tendency for emotional problems such as anxiety and depression in women [4,30,31]. Due to the cross-sectional nature of our study, the causality relationship could not be established, but further studies are needed to understand reasons for such a gender difference in adulthood.

The results of our study should be interpreted considering some limitations. First, although it is a relatively large sample, the selection of the participants from social media networks may have increased the participation rate of those who are interested in mental health issues and seeking help. Second, as in many similar studies, individuals who completed the questionnaires are generally female and have a higher education level [6,32].

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Although, we encouraged broad participation, it affects the generalizability of our sample. Third, due to restrictive measures during the pandemic, face-to-face interviews could not be performed. Therefore, our results might be limited in reflecting mental health problems at the clinical level. Fourth, although the ratio between groups did not exceed 4:1, we should mention that the sample size between groups was different. Lastly, readers also should be aware of that this study could not determine a causal relationship between risk factors and PTSD and anxiety due to cross-sectional design of our study. Despite all these limitations, the findings of our study improve our understanding of mental health consequences during COVID-19 pandemic in the context of age-related differences.

In conclusion, our findings reflected that, amid COVID-19 global pandemic, mental health of the youths was more negatively affected than adults in Turkey, which was consistent with data from many countries all over the world [4,19, 29,30]. The youths might be more vulnerable to psychosocial distress of the pandemic rather than COVID-19 related factors. Besides age specific risk factors, poor economic status appeared as a consistent risk factor for both age groups. Intervenable risk factors and vulnerable populations should be carefully addressed by policy makers.

Compliance with Ethical Standards

Ethical approval: The study was approved by the Marmara University Medical Faculty Ethical Board (approval number: 12.2020.1356). Necessary legal permissions for studies on COVID-19 were obtained from the Ministry of Health (approval number: 2020-09-19T21.03.54).

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Conflict of Interest: The authors have no conflicts of interest to declare.

Author Contributions: OTPF, VC and NPF: Concept and design of the study, OTPF and VC: Data acquisition, OTPF: Statistical analysis, OTPF and NPF: Literature review, OTPF and VC: Drafting and writing. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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The neuroprotective and anti-inflammatory effects of Annona muricata (Graviola) on radiation-induced rat sciatic nerve injury

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ABSTRACT

Objective: This study aimed to evaluate Annona muricata's (AM) radioprotective effects on sciatic nerve injury due to ionising radiation (IR).

Materials and Methods: Thirty-two adult female Wistar albino rats were separated into four equal groups; Control (C), Annona muricata leaf extracts (AME), radiation (RAD), radiation and AME (AME+RAD). In groups AME and AME+RAD, AME was administered at a dose of 300 mg/kg for the first day and 50 mg/every day for the following one week intraperitoneally. In RAD and AME+RAD, rats were exposed to a single dose of 20 Gray (Gy) IR to their right legs. All the subjects were sacrificed at the end of the first month. Oxidative stress biochemical parameters from blood samples were analysed. In addition, right sciatic nerves were extracted and histomorphology was evaluated.

Results: Statistically significant vasculature, degenerative and necrotic changes were observed in RAD, compared to C and AME ($p<0.01$). Swelling in myelin sheath was predominantly seen in RAD. Alterations in the level of catalase ($p<0.01$), superoxide dismutase ($p<0.01$) and glutathione peroxidase ($p<0.05$) in the AME+RAD group compared to the RAD group were found to be statistically significant.

Conclusion: Our study unveiled that AM could potentially enhance biochemical and histomorphological healing in the acute period on sciatic nerve injury due to IR.

Keywords: Radiation, Sciatic nerve, Annona muricata, Neuroprotection, Oxidative stress

1. INTRODUCTION

New treatment modalities for cancer treatments or survival prolongation are widely used in clinics worldwide. Further, radiotherapy (RT) keeps itself a beneficial treatment option in many types of cancer [1]. However, serious complications may develop in patients due to the effects of both chemotherapeutic agents and RT on healthy tissues other than tumour tissues [1,2]. These complications may occur acutely, subacutely and chronically, depending on the tolerance of the tumour and the tolerance of the normal tissue to RT. Furthermore, in addition to complications such as acute radiation pneumonia, nephropathy and hepatitis, sensory and/or motor neurological damage may occur in the extremities due to the neurotoxic effect of ionising radiation (IR) [3-8]. This neurological damage may cause permanent loss in function of the extremities. Therefore, the patients face additional stress and it reduces the patients' quality of life. Due to IR, neuron damage is closely related to early biochemical and histomorphological changes and late scar formation [9,10]. The mechanism of all these changes occurs due to the process that results in cell death due to DNA damage. Nitrogen and reactive oxygen (ROS) species and free radicals that arise due to the ionization of water in the acute period are responsible for lethal DNA damage with an indirect

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mechanism rather than the damage caused by direct ionization in DNA. [11,12]. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are antioxidant enzymes that protect cells against free radical damage [10]. Since the mid-19th century, many chemical compounds, including antioxidant agents, have been used to reduce these side effects of IR [10,14-16].

Annona muricata (AM) (Graviola), the tropical tree from the Annonaceae family, consists of evergreen dark green leaves and heart-shaped fruits [13]. This fruit is widely consumed and is called a 'cancer killer' by the local people in tropical regions. They consume this fruit by boiling the leaves to treat cancer [13,17]. In addition, AM, which contains many flavonoids, alkaloids and acetogenins in its leaves, is also used to manage infections, diarrhoea, dermatitis, diabetes, fever and cardiovascular problems [18]. In support of this traditional use, various studies have shown the anticancer, antioxidant, antiviral, antihaemolytic, sedative and neuroprotective effects of AM [19,20].

Radiation-induced peripheral nerve damage (RIPND) is permanent since nerve cells do not have the regeneration ability [21,22]. Although, necessary precautions are taken to prevent the development of RIPND, there is no effective treatment, and the treatment options to restore the patients' living standards are limited [23]. The incidence of RIPND keeps increasing in correlation with aggressive RT programs. In this study, we evaluated radioprotective effect of AM on the changes in the acute period of damage to the rat sciatic nerve due to IR.

2. MATERIALS and METHODS

Animals and Experimental Protocol

All experimental procedures were carried out in the Animal Experiments Laboratory of Bülent Ecevit University Faculty of Medicine. Approval was obtained from the local ethics committee of the university (approval no: 2020/06). A total of 32 *Wistar albino* female rats, weighing 350-450 g, were randomly separated into four groups of equal numbers (n = 8, total: 32): C (Control + Vehicle), AME (Annona Muricata leaf extracts), RAD (Radiation + Vehicle), AME+RAD (Radiation + Annona Muricata leaf extracts). The sample size was determined by using G Power. The animals were kept at a constant temperature (18°C – 21°C), and adequate nutrition and photoperiod (12 hours light/dark cycle) were provided for the duration of the experiment.

For one week, rats in group C were injected intraperitoneally (IP) with 2ml/kg of normal saline per day. In the AME group, 300 mg/kg AME was administered IP on the first day and 50 mg/kg AME every day for seven days. Rats in the RAD and AME+RAD group were exposed only one single IR dose (20 Gy) to their right legs without any treatment. The rats in the AME+RAD group were given 300 mg/kg AME IP 30 minutes before IR application and 50 mg/kg AME IP every day for seven days after the application. After one month, rats in all groups were sedated with xylazine (10 mg/kg, IP; Bioveta, Ankara, Turkey) and ketamine hydrochloride (80 mg/kg, IP; Ketalar, Pfizer, Istanbul, Turkey) anaesthesia for biochemical examination was injected via artery in the ventral tail. After blood samples were taken, rats were sacrificed with high-dose anaesthetic drug (Pentobarbital 200 mg/kg, IP; Bioveta, Ankara, Turkey). Right after this procedure, the right sciatic nerves of all rats were carefully separated from the surrounding tissues by blunt dissection and removed for histomorphological examination (Figure 1).

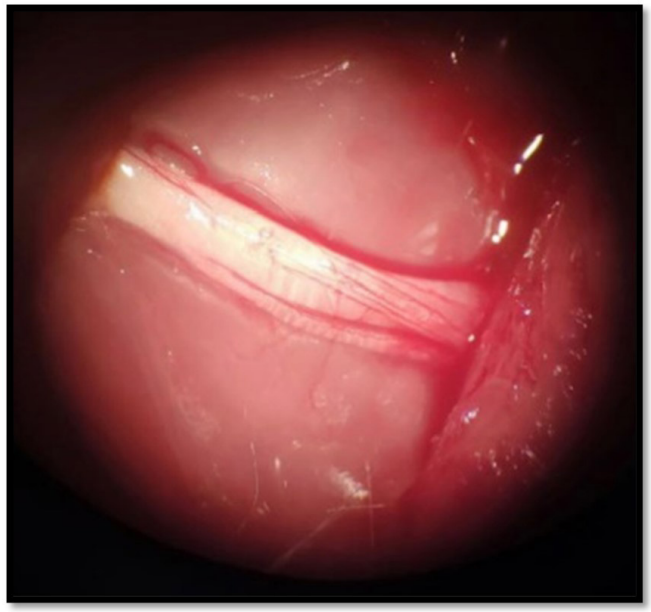

Figure 1. *Microscopically, right leg of the rat's sciatic nerve is seen (an original picture from our study).*

Irradiation

The rats in AME+RAD and RAD groups were anaesthetised using xylazine (10 mg/kg, IP; Bioveta, Ankara, Turkey) and ketamine hydrochloride (80 mg/kg, IP; Ketalar, Pfizer, Istanbul, Turkey) and placed in the prone position. Before the procedure, rat simulation was performed with a 1 mm section computed tomography scan; the dose was calculated with Eclipse treatment planning system, version 8.9 (Varian Medical Systems, Palo Alto-CA, USA) [24]. After the simulation, other parts of their bodies were protected using lead and beam collimation. A 6-mV linear accelerator (Clinac, Varian Medical Systems, Palo Alto CA, USA) was used to expose IR to the right legs of the rats in both groups with a 1.0 cm bolus source-skin (SSD) distance technique on the surface [24]. No rat was excluded due to death.

Chemical

Air-dried AM leaves were obtained from a local supplier in the Mediterranean region in Turkey. They cut the leaves into small pieces after washing with distilled water. 25 g samples

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were extracted in 70:70 ethanol for seven days with occasional shaking. The extract concentration procedure was done by removing ethanol. A rotary evaporator was used (Heidolph, Germany). After, the extract was lyophilised (Telstar – LyoQuest, Spain) overnight to prepare a dry extract, kept at – 20 °C until use.

Biochemical analysis

Superoxide Dismutase (SOD, U/ml)

Superoxide dismutase accelerates the conversion to hydrogen peroxide and molecular oxygen in the elimination steps of ROS radicals produced in oxidative reactions. This method takes place using xanthine and xanthine oxidase. Here, it is intended to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The degree of inhibition of this reaction gives information about the SOD activity (Relassay, Turkey). 1.7 ml of reagent 1(R1a.+R1b.) was added to the cuvette. Then, 50 µl of sample was added to the pathway cuvette and mixed thoroughly. Then, 250 µl of reagent 2 (Xanthine Oxidase) was immediately added to the cuvette and mixed thoroughly. The first absorbance of A1(505 nm) after 30 seconds was read and at the same time the timer was started. After 3 minutes, the final absorbance of A2 (505 nm) was read.

Catalase (CAT, U/ml)

This colourimetric assay involved two steps. The sample was incubated with certain hydrogen peroxide. Hydrogen peroxide was converted to water and oxygen by this sample. CAT concentration was proportional to this ratio. Absorbances at 405 nm were expressed in U/ml (Relassay, Turkey). 300 µl of reagent 1 was added to the pathway cuvette. Then, 17 µl of sample was added to the pathway cuvette and mixed well. After 30 seconds, OD values were measured as 480 nm. Immediately after this, 250 µl of reagent 2 was added to the cuvette, the solution was mixed well. It was incubated at 37 °C for 5 minutes. OD for absorbance was measured at 700 nm per second.

Glutathione Peroxidase (GPx, U/ml)

This method is based on the Paglia and Valentine method. GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione (GSSG), it is immediately converted to the reduced form by oxidation of NADPH to NADP. The decrease in absorbance at 340 nm is the measure (Relassay, Turkey). 250 µl of reagent 1 was added to the pathway cuvette. Then 10 µl of sample was added to the pathway cuvette and they were mixed thoroughly. Immediately after this, 40 µl of reagent 2 was added to the cuvette and they were mixed thoroughly. It was incubated at 37 °C for 5 minutes. OD for absorbance was measured at 340 nm.

Histomorphologic examination

Automated tissue processing equipment (Leica ASP300S, Wetzlar, Germany) was used for the routine processing of 32 sciatic nerve tissue samples fixed with 10% formalin. Afterwards, the tissues embedded in paraffin were cut using a Leica RM2255 rotary microtome (Wetzlar, Germany) to obtain 4 µm thick sections. Next, tissue sections stained with Hematoxylin-Eosin (HE) were evaluated under the microscope. The same procedure was performed for all samples. The camera used to observe all study materials were Nikon Digital DS-Ri2, and the microscope was Nikon Eclipse Ni-U equipped with the corresponding software. (Nikon, Tokyo, Japan).

In four separate groups, four parameters were examined for vasculature change, degenerative change, necrotic change, swelling in myelin sheath in sciatic nerve tissue. Histomorphological findings in tissues and cells were scored. Scores were between 0 and 3 for each criterion. (0=normal, 1=light, 2=medium, 3=heavy).

Statistical Analysis

SPSS version 22.0 was used to analyse the data. Descriptive statistics for quantitative variables were expressed as mean and standard deviation, and categorical variables were expressed as numbers and percentages. Normal distribution assumptions for continuous variables were checked with the help of Skewness and Kurtosis coefficients, Shapiro-Wilk test, and distributions in q-q graphs. As the data show a normal distribution, One-way analysis of variance with LSD post hoc was used to compare the differences of the groups. The differences between the groups were evaluated using Chi-Square analysis for categorical variables. Based on the p-value <0.05, the results were considered statistically significant.

3. RESULTS

Biochemical evaluation

Plasma CAT, SOD and GPx values in all the experimental groups are presented in Table I. There was a significant decrease in the activity of SOD, CAT and GPx antioxidants in the RAD group compared to the C group. ($p<0.01$, $p<0.01$ and $p<0.05$, respectively, Table I, Figure 3). It was found that the activity of CAT, SOD and GPx antioxidants in the AME+RAD group (respectively $p<0.01$, $p<0.01$ and $p<0.05$, Table I, Figure 3) increased significantly compared to the RAD group. Similarly, there was a significant difference between the AME and RAD groups CAT, SOD and GPx levels ($p<0.05$, Table I, Figure 3).

Histomorphological evaluation

Vascular system change, degenerative change, necrotic change, and swelling in the myelin sheath were evaluated for all groups. C and AME groups had a typical histomorphological structure in the sciatic nerve tissues (Figure 2A and Figure 2B). Findings were most severe in the RAD group (Figure 2C). In the AME+RAD group, the results were mild to moderate. (Figure 2D).

An increase of vasculature and degenerative changes in the RAD group were statistically significant compared to the C and AME groups ($p<0.001$, Table II, Figure 2C). In the AME+RAD group, there was a statistically significant increase

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in these changes compared to the RAD group (p<0.001, Table II, Figure 2D). While a necrotic change was not observed in the C and AME groups, it was moderate in 25% and severe in 75% of the rats in the RAD group. This change was observed less in the AME+RAD group than in the RAD group, and this difference was also statistically significant (25% mild, 75% moderate, p<0.001, Table II, Figure 2D). While swelling in the myelin sheath was severe in the RAD group, it was mild in group C. Moderate changes were observed in only 25% of the rats in the AME+RAD group, unlike the RAD group (p<0.001, Table II).

Table I. *Values of CAT, SOD and GPX in plasma*

	C	AME	RAD	AME+RAD	p
CAT	94.88 ± 28.13	100.50±26.90	48.88 ± 19.28	80.50±17.48	7.809 (< 0.01) [abc]
SOD	173 ± 17.43	175.75±29.15	99.50±19.66	154.63±45.13	11.219 (< 0.01) [abc]
GPx	696.60±115.07	750.63±304.84	450.38±102.11	663.63±165.94	3.840 (< 0.05) [abc]

[a] *Shows significant differences between Control and Radiation groups (P < 0.05).*
[b] *Shows significant differences between Radiation and Annona muricata leaf extracts groups (P < 0.05).*
[c] *Shows significant differences between Radiation and Annona muricata leaf extracts groups + Radiation groups (P < 0.05).*
C (Control), AME (Annona muricata leaf extracts), RAD (Radiation), AME+RAD (Radiation and AME)

Table II. *Comparison of histomorphologic scores between groups*

Parameters Groups	0	1	2	3	p
Vasculature change C	6 (75%)	2 (25%)	-	-	
AME	3(37.5%)	5(62.5%)	-	-	0.001*
RAD	-	-	3 (37.5%)	5 (62.5%)	
AME+RAD	-	2 (25%)	4 (50%)	2 (25%)	
Degenerative change C	6 (75%)	2 (25%)	-	-	
AME	3 (37.5%)	5 (62.5%)	-	-	0.001*
RAD	-	-	3 (37.5%)	5 (62.5%)	
AME+RAD	-	6 (75%)	2 (25%)	-	
Necrotic change C	8 (100%)	-	-	-	
AME	8 (100%)	-	-	-	0.001*
RAD	-	-	2 (25%)	6 (75%)	
AME+RAD	-	6 (75%)	2 (25%)	-	
Swelling in myelin sheath C	6 (75%)	2 (25%)	-	-	
AME	3 (37.5%)	5 (62.5%)	-	-	0.001*
RAD	-	–	2 (25%)	6 (75%)	
AME+RAD	-	6 (75%)	2 (25%)	-	

**Statistical analysis for comparison between groups with Chi-squared: P < 0.05, statistical significance. C: Control, AME Annona muricata leaf extracts, RAD: Radiation, AME+RAD: Radiation and AME*

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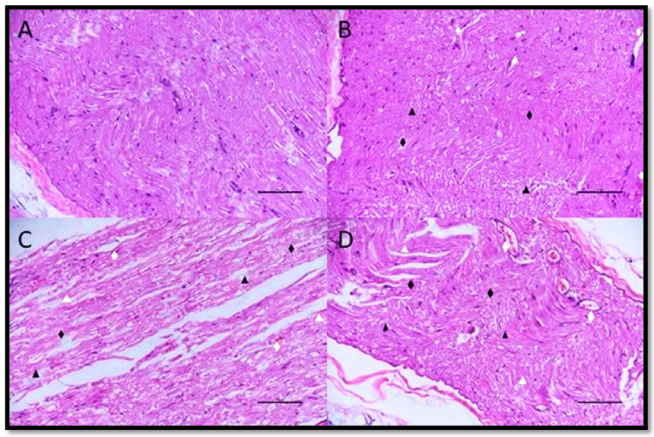

Figure 2. *(A) Histomorphologic findings of Group C. There are no vasculature changes, degenerative change, necrotic change, swelling in myelin sheath. H&E, Scale Bar: 100 µm. (B) Histopathological findings of Group AME. White diamond, light vasculature changes; black diamond light degenerative change; black triangle, light swelling in myelin sheath. H&E, Scale Bar: 100 µm. (C) Histopathological findings of Group RAD. White diamond, medium vasculature changes; black diamond heavy degenerative change; white triangle, heavy necrotic change; black triangle, heavy swelling in myelin sheath. H&E, Scale Bar: 100 µm. (D) Histopathological findings of Group AME+RAD. White diamond, medium vasculature changes; black diamond light degenerative change; white triangle, light necrotic change; black triangle, light swelling in myelin sheath. H&E, Scale Bar: 100 µm.*

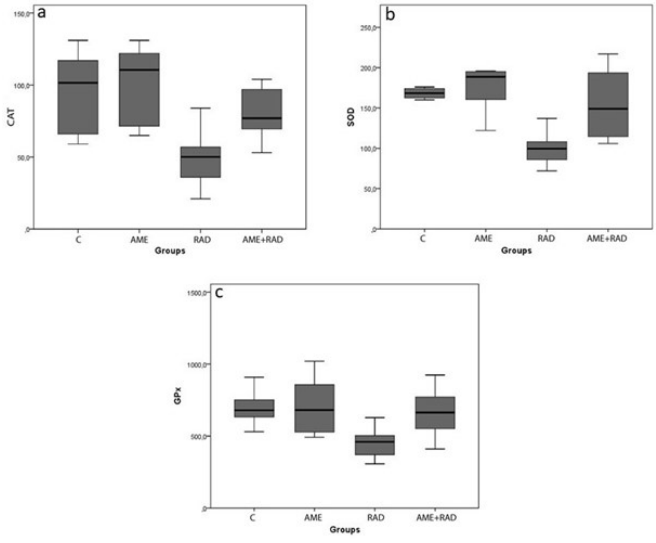

Figure 3. *Q1, Q3, median, minimum, and maximum values of CAT, SOD and GPx are resented with box plot. (a) Levels of plasma CAT (U/ml), (b) Levels of plasma SOD (U/ml), (c) Levels of plasma GPx (U/ml) in groups*

4. DISCUSSION

Due to technical advances in radiation therapy, RT has contributed significantly to cancer patients' recovery or prolonged survival [25]. RT mainly targets tumour cells; however, when irradiating the tumour with the appropriate dose, it is often impossible not to affect the surrounding tissues. RIPND is the least known and perhaps the most frightening of the late complications of RT [10]. This is because permanent damage (sensory and/or motor) may occur in the extremities both in the early stages of the disease and even years after its remission. Since, clinical results are generally irreversible in RIPND, it significantly increases morbidity. Therefore, it adversely affects the quality of life, especially in patients who have successfully fought against cancer. At this point, although, many experimental and clinical studies have been carried out from the past to the present, a radioprotective agent that can prevent RIPND has not been found. Studies on the neurotoxic effect of IR have mainly focused on the central nervous system (CNS). Egemen et al., compared the efficacy of dexamethasone and melatonin in IR induced brain and spinal cord injury [26]. As a result, they observed that the effects of melatonin to dexamethasone were equivalent and not superior to each other. Presman et al., showed that melatonin could increase sensitivity to glucocorticoids [27]. Based on the results of this study, Egemen et al. concluded that further studies are needed with the assumption that the two agents will be more effective when used together. However, these studies remained only in the experimental stage and have not been put into clinical practice. Based on our literature search, the radioprotective effects of post-radiation AM against sciatic nerve injury have not yet been evaluated in an experimental rat model before. In this study, we assessed the possible radioprotective efficacy of AM both biochemically and histopathologically.

The reason why the neurotoxic effect of IR is limited to the CNS; whether IR causes toxicity in peripheral neurons is not yet clear. It is widely believed that there are two phases. The first is the phase consisting of bioelectrical, biochemical and histochemical changes and the second consists of late fibrosis [23]. In this context, we evaluated biochemically SOD, CAT, GPx and histopathologically vascular, degenerative and necrotic changes and myelin hair changes. However, an important problem here is that the peripheral nerves are resistant to radiation [23,28]. We observed no consensus on the sciatic nerve in the literature review we did before we began our work on which will lead to the damage of the radiation intensity [10,29]. Okuhara et al., observed no significant change in sciatic nerve function for 24 weeks, even though different intensities such as 30, 50, 70 Gy were given in their preliminary evaluation before starting their studies on RIPND [29]. However, they observed no significant changes in sciatic nerve function at these doses despite histopathological changes [29]. For this reason, they increased the dose and carried out their studies with a dose of 90 Gy. They observed the desired electrophysiological and histological changes at this dose. Shabeeb et al., in their studies evaluating the radioprotective effects of melatonin, observed significant changes related to sciatic nerve damage with lower radiation (30 Gy), in contrast to this study [10]. Unlike these two studies, we observed significant changes related to nerve damage, both histomorphologically and biochemically, with 20 Gy radiation.

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AM is a member of the Annonaceae family, also known as 'Graviola', 'soursop' or 'corossol'. It is suitable for many diseases (such as fever, malaria, diabetes, rheumatism, various cancers) by local people in tropical regions; branches, bark, leaves, seeds and root parts are processed through various processes [18]. AM is known to have anticarcinogenic, anticonvulsant, anti-inflammatory, antioxidant, radioprotective (against gamma rays) and neuroprotective properties [20,30-32]. However, there are limited studies on the radioprotective effect of AM in the literature [30,33,34]. In addition, these studies were made with gamma-ray, not X-ray. El-Shahat et al., examined the biochemical changes in their studies with whole-body gamma irradiation at a dose of 2 Gy (8 Gy in total) every three days [30]. They observed that AM had an antioxidant effect against biochemical changes in the liver and kidneys. Mansour et al., showed that AM reduces radiation-induced toxicity by preventing oxidative stress and preserving antioxidant activities in lung and kidney tissues in experimental whole-body gamma irradiation (6 Gy) models [33]. We performed our study by applying a 20 Gy X-ray to the right sciatic nerves of rats. Our study is the first to evaluate the radioprotective effects of AM using X-ray. In our research, we observed that in addition to the reduction of oxidative stress caused by radiation, necrotic and degenerative changes were less in the group AME+ RAD (Figures 2 and 3).

The presence of a suitable agent with a radioprotective effect is essential to prevent the development of peripheral neuropathy and neoplasms that may occur due to IR [35]. However, neuropathic pain and motor losses due to possible nerve damage cause additional costs in terms of economy as well as negative patient psychology. Therefore, the main goal should be to protect the surrounding tissues from necrotic and/or apoptotic processes occurring in cancer cells in a patient receiving RT. Therefore, in vivo (melatonin, dexamethasone) and in vitro studies aimed to investigate whether neurotoxicity caused by ROS and DNA damage in IR-induced neurons can be prevented [26,36]. Antioxidant enzymes such as SOD, CAT and GPx play an active role in protecting against the harmful effects of oxidative stress-related ROS, hydrogen peroxide and lipid peroxidation [36]. High intracellular ROS levels can cause nerve cell death via apoptosis and/or necrosis [36,37]. The first defence mechanism against these harmful effects due to oxidative stress is provided by SOD. SOD neutralises ROS to the less toxic compound hydrogen peroxide (H_2O_2) [20]. However, excessive cellular H_2O_2 accumulation increases oxidative damage by causing the formation of reactive free hydroxyl (OH-) radicals [38-40]. The CAT enzyme reduces this damage by converting H_2O_2 to water and oxygen [41]. Another important antioxidant enzyme, GPx, reduces lipid peroxides to hydroxyl lipids and waters by converting glutathione to glutathione disulphide [41].

There are studies in the literature on the antioxidant, anti-inflammatory and neuroprotective effects of both crude extracts and phytochemical compounds of AM [30-32]. Moghadamtousi et al., examined the effects of AME on wound healing in an experimental injury model. They showed that it accelerated the stages of wound healing and increased CAT, SOD and GPx activities and reduced CO oxidative stress [31]. In addition, in another study by Moghadamtousi et al., they showed that AME also increased CAT, SOD and GPx activity in an experimental gastric injury model caused by ethanol [42]. The results of our study showed that 20 Gy radiation exposure of the sciatic nerves of rats (RAD group) caused an increase in oxidative stress in parallel with the decreased activity of antioxidant enzymes (SOD, CAT and GPx). However, there was a statistically significant increase in SOD, CAT and GPx activity in the AME+RAD group compared to the RAD group (Table I, Figure 3).

Histomorphological evaluations remain the most widely used descriptive option in studies. In this context, histomorphological evaluations in previous RIPND studies have shown that IR-induced neuronal damage followed by neuropathic changes secondary to fibrosis with necrosis [10,22,24]. Shabeeb et al., showed increased inflammatory changes in the radiation group 4 weeks after irradiation in radiation-induced sciatic nerve injury studies [10]. Normal histomorphological results were observed in the C and AME groups in our study. After IR, moderate and severe swelling of the myelin sheath and increased inflammatory changes were observed in the RAD group compared to group C (Figure 2C, Table II). However, histomorphological, vascular, degenerative and necrotic changes and swelling in the myelin sheath were significantly reduced in the AME+RAD group compared to the RAD group (Table II). In conclusion, in this study, the histomorphological results of the RAD group showed moderate sciatic nerve damage; however, AME has been observed to alleviate neurotoxic effects.

Limitations and further studies

In some studies histomorphological changes in RIPND were observed only in the 1st month, as in our study; but there are studies in which histomorphological changes were evaluated at 12, 20 weeks [10,22]. These periods are of great importance, especially in assessing the late fibrosis seen in the second phase of RIPND. In addition, unlike our study, there are studies with higher radiation intensity and follow-up periods of up to 24 weeks [29]. This provides a better assessment of sciatic nerve functional losses. In this context, evaluation of RIPND with different histomorphological and electrophysiological protocols, different intensities of radiation doses and longer sacrification times will strengthen the results obtained from the study.

Conclusion

This study showed that AME could ameliorate the histomorphological and biochemical changes of the irradiated sciatic nerve. However, despite these positive results, it should go a long way in terms of its effects on RIPND.

Compliance with the Ethical Standards

Ethics Approval: All experimental procedures were carried out in the Animal Experiments Laboratory of Bülent Ecevit University Faculty of Medicine. Approval was obtained from the local ethics committee of the university (decree no: 2020/06). All procedures performed in studies involving animals followed the institution's ethical standards or practice at which the studies

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were conducted. This article does not contain any studies with human participants performed by any authors.

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Author Contributions: EK: Concept, EK, HHKS and AG: Design, CT: Supervision, EK: Resources, EK, HHKS and AG: Data collection and/or processing, EK, CT: Analysis and/or interpretation, EK, CT and AG: Literature review, EK: Writing, CT and HHKS: Critical review.

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CT-guided lung biopsy: diagnostic accuracy and complication rates of biopsy techniques

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ABSTRACT

Objective: We aimed to evaluate the diagnostic accuracy and complication rates of computed tomography (CT)-guided core needle biopsy and fine needle aspiration biopsy (FNAB).

Materials and Methods: Patients who underwent CT-guided lung mass biopsy were included. The patients were evaluated in terms of age, gender, lesion diameter, lesion localization, depth of the mass, type of biopsy procedure (core needle biopsies and FNAB).

Results: The accuracy rate of FNAB in diagnostic material was found to be 100% in terms of benign, malignant and all lesions. The specificity and sensitivity of FNAB was found to be 100%. The diagnostic accuracy rate of core needle biopsy was found to be 70% in benign lesions, 100% in malignant lesions. The specificity of core needle biopsy was 90% and sensitivity 100%. There was no statistically significant difference between the two biopsy techniques in terms of complications.

Conclusion: In conclusion, we found that the diagnostic rates of FNAB and core needle biopsy were close in malignant lesions, the diagnostic rate of core needle biopsy was higher in benign lesions, and there was no difference in terms of complications in both biopsy techniques.

Keywords: Lung, Mass, Transthoracic biopsy, Computed tomography

1. INTRODUCTION

Transthoracic biopsy is frequently used for lesions that cannot be reached by bronchoscopy.

Compared to thoracic surgical biopsy, transthoracic biopsy is less invasive, has lower mortality and morbidity, and has lower requirement for hospitalization after biopsy [1,2].

Computed tomography (CT) has a very important role in the diagnosis of lung diseases as well as in biopsy. CT has become an indispensable modality in lung biopsies in recent years due to its advantages such as characterization of the lesion, including its relationship with neighboring structures and its distance to fissure, as well as early detection of post-biopsy complications. Transthoracic lung biopsy can be performed as core or fine needle aspiration biopsy (FNAB). Fine calibrated needles are used in FNAB, and the aspirated material is taken for cytological evaluation. Core biopsy is known as fragmentation biopsy and it is based on removing a piece of tissue from the lesion for histological diagnosis [3-6].

In this study, we aimed to evaluate the diagnosis and complication rates of both procedures in patients who underwent CT-guided trasthoracic core biopsy and FNAB.

2. MATERIALS and METHODS

Our study is a retrospective archive study, and patients who underwent CT-guided transthoracic biopsy between April 2015 and March 2020 were included in the study. This study was approved by Dicle University Ethical Committee (approval number: 88).

The patients were evaluated in terms of age, gender, mass diameter, mass localization, depth of the mass, and type of

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biopsy performed (core biopsy or FNAB). Pneumothorax and parenchymal hemorrhage were evaluated as complications in the images taken after the biopsies.

Biopsies that were not finalized due to the patient's inability to adapt to the procedure, procedures whose images were not in our system despite the biopsy having been performed, patients without pathological diagnosis after biopsy, and procedures without control images in terms of biopsy complications were excluded from the study. In addition, because of the lack of control CT images of early postoperative complications, ultrasonography (US)-guided biopsies were also excluded from the study.

Before the procedure, hemogram and bleeding parameters were checked routinely in each patient. Patients using antiaggregant and anticoagulant drugs underwent biopsy after the treatment was interrupted. Intravenous vascular access was established in the patients, and the patients were taken into the procedure, preferably following 6-8 hours of fasting. Vital functions of the patients were monitored and oxygen saturation was followed closely. Before the procedure, a CT image was taken to see the current status of the mass. The patients were placed in the supine, prone, or lateral position so that the mass was visible, no fissures were passed, and a safe entry angle was minimally passed through the parenchymal area. The most suitable angle for access to the mass was determined. Under sterile conditions, local anesthesia was performed at the entry site with an average of 8-10 cc of 2% lidocaine, keeping it away from the pleura due to the risk of pneumothorax. Then, at an angle that would not pass fissure, a semi-automatic coaxial needle was sent as a 18G needle in patients who underwent core biopsy, and a 22G Chiba needle up to the mass level in patients who underwent FNAB. The needle tip was checked with serial images. Pleural leaves were not crossed more than once due to the risk of pneumothorax. In fine needle aspiration biopsies, aspiration was performed by moving the needle with the help of an injector. In core biopsies, after entering the mass, the inner needle was removed and an 18G cutting needle was sent through the coaxial needle and at least two samples were taken. Control CT imaging was performed in terms of early complications after the biopsy and control chest radiography was performed at 2, 4, 6, and 24 hours after biopsy.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) 22.0 program was used for statistical analysis. Categorical data are shown as numbers and percentages, measurement data as mean and standard deviation, lesion diameter and needle entry length data as median (interquartile) Chi-square analysis was used to compare categorical data. While evaluating the study data, the suitability of the parameters to the normal distribution was evaluated using the Kolmogorov-Smirnov test. In comparison of paired groups in quantitative data, independent groups t-test and Mann-Whitney U test were used. Pearson's correlation analysis was used to compare the two metric data. Significance was evaluated at the $p < 0.05$ level.

3. RESULTS

The mean age of the 142 patients included in the study was found to be 64.0 ± 11.6 (39-94) years. The median of the masses in the lung was 29 mm (19.7-40.0), and the lung distance to reach the mass was 16.0 mm (5.0-29.0).The masses were most commonly located in the upper lobe of the right lung (Table I).

Fine needle aspiration biopsy was performed in 60 (42.3%) patients, and core biopsy was performed in 82 (57.7%) patients. As a result of the biopsy, 28 (19.7%) were diagnosed as benign and 95 (66.9%) as malignant, while the material taken in 19 patients (13.4%) was non-diagnostic. While the definitive diagnosis of 34 (23.9%) of the patients was benign, the definitive diagnosis of 108 (76.1%) was determined as malignant (Table I).

Table I. *Clinical and pathological features of the cases*

		Number	% (Percent)
Age (years), Average±SD		64.0±11.6	
Size (mm), Median (IQR)		29 (19.7-40.0)	
Lung distance to reach the mass (mm), Median (IQR)		16.0 (5.0-29.0)	
Location	Right	89	62.7
	Left	53	37.3
Lobar location	Right lung upper lobe	40	28.1
	Right lung middle lobe	11	7.7
	Right lung lower lobe	38	26.7
	Left lung upper lobe	21	14.7
	Left lung lingual	7	5.0
	Left lung lower lobe	25	17.6
Hemorrhage	Yes	8	5.6
	No	134	94.4
Pneumothorax	Yes	14	9.9
	No	128	90.1
Biopsy technique	FNAB	60	42.3
	Core biopsy	82	57.7
Biopsy result	Benign	28	19.7
	Malignant	95	66.9
	Non-diagnostic	19	13.4
Definitive diagnosis	Benign	34	23.9
	Malignant	108	76.1

IQR: Interquartile range

Fine needle aspiration biopsy

The material taken from 11 (18.3%) of 60 patients who underwent FNAB was non-diagnostic. Pathology reports of 8 patients (13.3%) who underwent FNAB were reported as benign. In imaging and clinical follow-up, wedge resection or histopathological examinations performed after surgery, all 8 patients were found to be benign. Histopathological examination of 41 patients (68.3%) who underwent FNAB was reported as malignant. Malignancy was found in all of these patients on histopathological examinations performed after wedge resection or surgery. When non-diagnostic biopsies were excluded, the

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accuracy rate of FNAB in diagnostic material was found to be 100% in terms of benign, malignant, and all lesions. At the same time, the specificity and sensitivity of FNAB was found to be 100%. Seven (63.6%) of the masses of the 11 non-diagnostic biopsies were benign and four (36.4%) were malignant. When all patients undergoing FNAB procedure were evaluated, 41 of 45 malignant lesions (91.1%) and 8 of 15 benign lesions (53.3%) were diagnosed correctly, and the total accuracy rate was found to be 81.6% .

Core biopsy

The material taken from 8 (9.7%) of 82 patients who underwent core biopsy was not diagnostic. Pathology reports of 20 patients (24.3%) who underwent core biopsy were reported as benign. Of these, 14 who were evaluated as benign as a result of core biopsy in imaging and clinical follow-ups, were proven to be benign after wedge resection or postoperative histopathological examinations, while 6 patients had false negative results and a diagnosis of malignancy was made. Malignancy was reported in the histopathological examination of 54 patients (65.8%) who underwent core biopsy. Malignancy was detected in all of these patients on histopathological examinations performed after wedge resection or surgery. When non-diagnostic biopsies were excluded, the diagnostic accuracy rate of core biopsy was found to be 70% in benign lesions, 100% in malignant lesions and 91.9% in terms of all lesions. At the same time, the specificity of core biopsy is 90% and sensitivity was found to be 100% (Table II). For the 8 patients for whom insufficient material for diagnosis was obtained, 5 (62.5%) were diagnosed as benign and 3 (37.5%) were diagnosed as malignant. Considering all masses with core biopsy, 54 of 61 malignant lesions (88.5%) and 16 of 21 benign lesions (76.1%) were diagnosed correctly, and the overall accuracy rate was 73.1% (Table II).

Table II. *Diagnostic values of FNAB and core biopsy techniques according to final (pathology result after resection, clinical follow-up, re-biopsy of the masses) diagnosis*

	Resection, Pathology result after Clinical follow-up (GOLD STANDARD)	
FNAB	**Malignant**	**Benign**
Malignant	41 **(91.1%)**	0
Benign	0	8 **(53.3%)**
Non-diagnostic	4	7
Total	45	15
CORE BIOPSY	**Malignant**	**Benign**
Malignant	54 **(88.5%)**	0
Benign	6	14 **(76.1%)**
Non-diagnostic	3	5
Total	61	21

Since 11 of the patients whose biopsy results were found to be benign were not followed up in our center, their files could not be accessed. Seven of the patients with benign lesions, were operated, and 16 were treated and followed up. In our system, the follow-up period of 23 patients with surgery, medical treatment, and follow-up was between 9 and 44 months, with an average of 22.6 months.

While no significant difference was found between the two techniques in terms of diagnosis rates in malignant lesions (p>0,05), core needle biopsy was found to be significantly higher in the diagnosis rate in benign lesions (p<0.05). There was no significant difference between the two techniques in terms of complications.

Final diagnosis

In patients who could not be diagnosed with fine needle aspiration biopsy and core needle biopsy, the definitive diagnosis was made by open surgical biopsy resection, clinical follow-up, or re-biopsy. All of the lesions of the patients diagnosed in clinical follow-ups were benign, and the absence of a significant increase in size in these lesions, the clinical and laboratory features of clinical and laboratory features not suspicious for malignancy, and the shrinkage or disappearance of the lesions due to medical treatment removed them from malignant features. Diagnosis was made by core needle biopsy in 6 patients, open surgical biopsy-resection in 2 patients, and clinical follow-up in 3 patients for whom fine-needle biopsy did not provide an adequate diagnosis. Open surgical biopsy-resection was performed due to the high clinical suspicion of malignancy in benign lesions.

Open surgical biopsy was performed in 5 of 8 patients who were underdiagnosed in core needle biopsy, and biopsy was repeated in 3 of them. Of the 6 patients with false negatives in core biopsy, a definitive diagnosis was made as a result of re-biopsy in 4, open surgical biopsy-resection in 2 patients. Five of these patients were diagnosed with lung cancer, and one as metastasis.

The reasons why the diagnosis could not be made in the first biopsies in patients diagnosed in biopsy repetitions; biopsy from the necrotic area, biopsy from the adjacent lung parenchyma, not from the level of the mass, and biopsy from the place where there is no involvement in terms of malignancy in positron emmision tomography (PET)-CT.

Complications

Pneumothorax and pulmonary hemorrhage were evaluated as complications, and hemorrhage was observed in 8 (5.6%) patients and pneumothorax was observed in 14 (9.9%). Hemorrhage was observed in 2 (3.3%) patients who underwent FNAB and 6 (7.3%) patients who underwent core biopsy. Pneumothorax was seen in 8 (13.3%) patients who underwent FNAB and 6 (7.3%) patients who underwent core biopsy. There was not a significant difference between the complication development of the cases according to biopsy technique. Likewise, there was not a significant difference between the technique applied and the size of the mass and the distance traveled to reach the mass (Table III).

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Table III. *Comparison of complication development status and masses of cases according to the techniques*

		FNAB	Core	p
		Number (%)	Number (%)	
Hemorrhage	Yes	2 (3.3)	6 (7.3)	0.476
	None	58 (96.7)	76 (92.7)	
Pneumothorax	Yes	8 (13.3)	6 (7.3)	0.235
	None	52 (86.7)	76 (92.7)	
Size (mm), Median (IQR)		28.5 (20.0-38.0)	30.0 (19.0-41.0)	0.667
Lung distance to reach the mass (mm), Median (IQR)		20.5 (8.5-30.0)	14.0 (5.0-26.0)	0.199

IQR: Interquartilerange

According to the development of hemorrhage or pneumothorax, no significant relationships were found between age, mass size, lung distance to reach the mass, and definitive diagnosis (p> 0.05) (Table IV).

In the biopsy results of the cases, no significant relationship was found between the diagnostic and non-diagnostic material and the size of the masses and the lung distance through which the needle passed (p> 0.05).

Table IV. *Comparison of age, size, distance and definitive diagnoses of the cases according to their complications*

	Hemorrhage		p	Pneumothorax		P
	Yes	No		Yes	No	
Age (years), Average±SD	64.1± 13.5	64.0± 11.5	0.982*	63.2± 11.6	64.1± 11.7	0.782*
Size (mm), Median (IQR)	23.5 (17-29)	30 (20-40)	0.282**	29.5 (25-32)	29 (19-40)	0.967**
Lung distance to reach the mass (mm), Median (IQR)	21 (8.5-28)	16 (5-29)	0.825**	25 (13-30)	15.5 (5-27.5)	0.238**
Benign, n (%)	1 (12.5)	33 (24.6)	0.680	3 (21.4)	31 (24.2)	0.816
Malignant, n (%)	7 (87.5)	101 (75.4)		11 (78.6)	97 (75.8)	

**t-test **Mann-Whitney U test. IQR: Interquartilerange*

4. DISCUSSION

In our study, in which we compared the diagnostic value and complication rates of FNAB and core biopsy in transthoracic lung biopsies; we found that the findings of two techniques were close to each other in malignant lesions while, core biopsy is more valuable in terms of diagnosis of benign lesions, and there was no significant difference between the two techniques in terms of complications. The diagnostic accuracy rates reported in the literature range from 65% to 96% due to changes in mean nodule size and modalities used for imaging guidance [6,7]. In their study, Yaffe et al., in core biopsies, found the accuracy rate as 94.4% in malignant lesions, 85.7% in benign lesions, and 93.6% in total [8]. In our study, the rate of patients diagnosed by biopsy was in accordance with the literature (86.6%).

It is reported that the positive diagnosis percentage of transthoracic FNAB is 70-100% in malignant lesions, while it is much lower in benign lesions, varying between 11-68%. In a study conducted by Çubuk et al., the overall diagnosis rate of FNAB was found to be 93% [9]. The sensitivity of FNAB for malignant lesions was 89%, specificity was 100%, and the accuracy rate was 95%. For benign lesions, sensitivity was 42.9%, specificity was 100%, and accuracy was 94% [8,9]. When non-diagnostic biopsies were excluded in our study the accuracy rate, the specificity and sensitivity of FNAB in diagnostic material was found to be 100% in terms of benign, malignant, and all lesions.

The percentage of diagnosis with core biopsy is higher, especially in benign lesions, and varies between 60-85%. Although, it is not much different from FNAB in malignant lesions, core biopsy often provides sufficient material for immunohistochemical studies. Both techniques can sometimes be used as complements to each other [9]. . Considering all the masses that underwent core biopsy in our study,the total accuracy rate was 73.1%. In addition, the diagnostic accuracy rate of core biopsy was higher in malignant lesions. At the same time, specificity of core biopsy was 90% and sensitivity was found to be 100%.

The most common complication in transthoracic lung biopsy procedure is pneumothorax, and its frequency has been reported to vary from 22-45% in different studies. Pulmonary hemorrhage is the second most common complication, and its frequency has been reported to vary from 5-27% in studies. A small proportion of patients who develop pneumothorax during biopsy require a thoracic tube for drainage [10-16]. Studies have reported that the development of pneumothorax depends on many factors. The most important of these are the number of pleural needle insertions, the depth of the lesion, the size of the lesion, and whether the fissure was passed during the biopsy. Although, there are studies arguing that the deep location of the lesion in the lung parenchyma has an effect on the development of pneumothorax, Yeow et al., contrary to what is known, found a higher rate of pneumothorax when lesions are located in the subpleural region [17]. They stated that the reason for this was that the distance traveled by the needle was short and the needle could easily exit the lesion. Similarly, Arıba et al., found a weak relationship between the development of pneumothorax and the depth of the lesion [18]. Massive hemoptysis, hemopneumothorax, air embolism, and seeding during the biopsy trace in malignant lesions are less common complications [12-15]. In our study, pneumothorax was observed in 10% and hemorrhage was observed in 5.6% of all the biopsy procedures. No major complications such as massive hemoptysis or air embolism were observed in any of the procedures performed.

Although, there are studies indicating that FNAB has a lower complication rate compared to core biopsy, recent studies have shown that there is no significant difference between core biopsy and FNAB in terms of complication risk. Aktaş et al., found no significant difference between FNAB and core biopsy in terms

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of the risk of pneumothorax and hemorrhage development [19]. Similarly, Chami et al., found no difference between the risk of pneumothorax and FNAB and core biopsy in their study [20]. In our study, no significant difference was found between the complication development of the cases and the applied biopsy technique. Although, there was no significant difference in complications between the two biopsy techniques, we think that the FNAB technique is much safer in lesions close to large vascular structures. Also, there was no significant relationship between the distance covered and the size of the lesion and the complication. In the literature, we did not find any studies investigating the relationship between the diagnostic and non-diagnostic biopsy specimens and the size of the lesion and the lung distance the needle passes through. In our study, no significant relationship was found between the diagnostic and non-diagnostic material after biopsy and the size of the masses and the lung distance through which the needle passed ($p>0.05$).

There are several techniques that increase the diagnostic value in percutaneous lung biopsies. These include taking a biopsy from the mass itself, not from the adjacent lung parenchyma, taking the biopsy from the walls of the cavitary masses, taking the biopsy from the area with involvement in favor of malignancy in PET-CT, and firing the biopsy gun after entering the lesion while taking biopsy from pleural-based masses [10-16]. In our study, most of the reasons of false negative core biopsies are; not being taken from the area with malignancy in PET-CT and biopsy is taken from the necrotic areas of the masses. Of six patients with false negative core biopsy results, five of them were primary lung cancer and one was metastasis. Since the characteristics on imaging of the masses, the presence of patient age group, and risk factors suggested high malignancy in this group, further investigations were performed and findings in favor of malignancy were determined. We think that one or more of the above-mentioned factors are effective in both patients with false negative core biopsy results and patients with insufficient diagnostic material.

The most important limitation of our study is that it is retrospective. In addition, the relatively low number of cases, the exclusion of US-guided biopsies in which early postoperative complication CT images could not be evaluated, the low number of patients with FNAB, and the low number of benign lesions included in the FNAB procedure are also important limitations.

In conclusion, we found that the diagnosis rates of FNAB and core biopsy were close in malignant lesions, the diagnosis rate of core biopsy was higher in benign lesions, and there was no difference in terms of complications in both biopsy techniques. We think that both methods can be used easily in lesions that are considered to be malignant, and core biopsy should be preferred primarily in lesions that are considered benign.

Compliance with the Ethical Standards

Ethical Approval: Ethical approval was obtained from the Dicle University Ethical Committee (approval number:88).

Financial Support: The authors have no relevant financial information to close.

Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Author Contributions: AKD, CC and FK: Drafting of the work, AKD and OK: Concept and design of the study, ZTD and AKD: Data acquisition, OK: Statistical analysis, AKD, CC,FK,OK,FY and ZTD: Medical practices. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

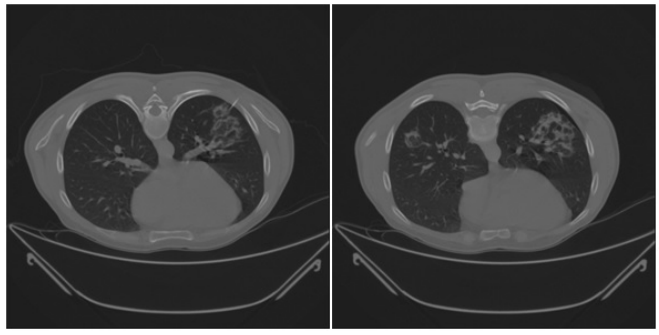

Figure 1. *A 36-year-old male patient, in the prone position for the ground glass area in the lower lobe of the left lung, (a) FNAB and (b) Minimal pneumothorax is observed in the post-procedure control image (Diagnosis: Organized pneumonia)*

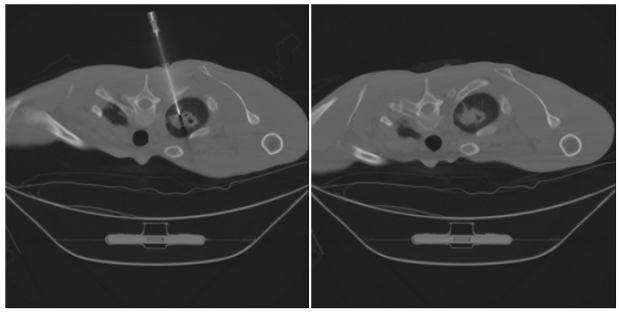

Figure 2. *A 53-year-old female patient, in the prone position for a mass lesion containing cavitation in the upper lobe apical of the left lung, (a) core biopsy procedure and (b) minimal parenchymal hemorrhage in post-procedure control image (Diagnosis: Small cell cancer)*

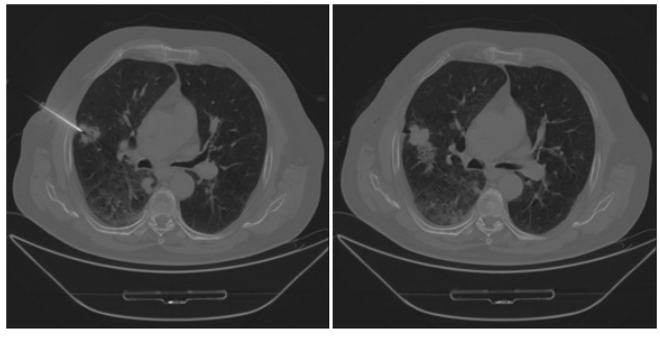

Figure 3. *A 65-year-old male patient, in the supine position for nodular lesion in the right upper lobe (a) Tru-Cut biopsy procedure and (b) parenchymal hemorrhage in the post-procedure control image (Diagnosis: Adenocarcinoma)*

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The oncological outcomes of postoperative radiotherapy in patients with stage II and III upper rectal cancer

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ABSTRACT

Objective: We assessed the oncological outcomes of postoperative radiotherapy and chemotherapy in patients with stage II or III upper rectal cancer who had undergone curative surgery.

Patients and Methods: We retrospectively investigated 133 patients who underwent primary curative resection of stage II or III upper rectal cancer. The median age was 62 years (range 30–82 years). Among these patients, 48% were stage II and 52% stage III. All received postoperative radiotherapy, and most received adjuvant 5-fluorouracil-based chemotherapy for 6 months after radiotherapy ceased. Survival curves were plotted using the Kaplan–Meier method, and survival was compared using the log-rank test.

Results: The median follow-up was 71.4 months. The 5-year local recurrence-free survival, cancer specific survival, and overall survival (OS) rates were 91.6%, 80.6%, and 75.4%, respectively. Nodal stage 2 ($p = 0.02$, $p = 0.05$) was a significant predictor of poor local recurrence-free survival and cancer specific survival rates. In the multivariate analysis, older age ($p = 0.01$) and a higher N stage ($p = 0.01$) were independent risk factors for poor OS.

Conclusion: The nodal state was predictive of all endpoints in patients with upper rectal stage II or III cancer.

Keywords: Upper rectal cancer, Postoperative radiotherapy, Outcomes

1. INTRODUCTION

Colorectal cancer is the fourth most common cancer worldwide [1]. Management of locally advanced rectal cancer is multimodal, consisting of radiotherapy (RT), chemotherapy, and total or partial mesorectal excision [2-4]. Although, management for advanced low and middle rectal cancers is now well-standardized, the optimal management for upper rectal cancer is less clear. Most studies on colorectal carcinomas do not evaluate the rectosigmoid junction alone, but together with the rectum [5-7] or colon [8]. Only a few have analyzed adenocarcinoma of the rectosigmoid junction [9-12]. Here, we present our long-term results on prognostic factors in patients with upper rectal cancers. The literature on postoperative RT is sparse.

2. PATIENTS and METHODS

Marmara University Ethics Committee approved the study (approval no. 09.2021.211). We evaluated 133 patients treated between July 1997 and December 2015. Table I summarizes their demographic and pathological characteristics. Before treatment, all patients underwent physical examination, colonoscopy, tumor biopsy, abdominal computed tomography or pelvic magnetic resonance imaging, and routine laboratory tests. Masses of 10–15, 15–20, and > 20 cm from the anal verge were considered to be in the upper rectum, rectosigmoid region, and sigmoid region, respectively. Tumor stage was classified in accordance with the seventh edition of the American Joint Committee on Cancer staging manual and handbook [13]. All patients had stage II or III disease. All received a median of 50.4 Gy (range 45–59.4 Gy) in 25–33 fractions of megavoltage external beam RT to the entire pelvis, delivered in the three-dimensional conformal mode in 82% of patients and in the intensity-modulated or volumetric arc mode in 18%. All but six patients received concurrent 5-fluorouracil (5-FU)-based

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chemotherapy, and all but five an additional 6 months of adjuvant chemotherapy.

One of the following three regimens was prescribed to all patients undergoing RT: (1) intravenous bolus of 5-FU (400 mg/m2/day) and leucovorin (20–25 mg/m2/day) during the first and last weeks of RT, (2) continuous infusion of 5-FU (225 mg/m2/day), or (3) oral capecitabine (825 mg/m2 b.i.d.) on days 1–5. All patients were examined at 3-month intervals for 2 years, at 6-month intervals for the next 2–5 years, and annually thereafter.

Overall survival (OS) was defined as the time from cancer diagnosis to the end of follow-up or the date of death from any cause. Cancer-specific survival (CSS) was defined as the time from cancer diagnosis to the end of follow-up or cancer-related death. The local control time was defined as the time from surgery to pelvic cavity relapse.

Statistical Analysis

Survival curves were obtained by the Kaplan–Meier method, and the survival curves were compared using the log-rank test. Univariate and multivariate Cox regression models were employed to estimate hazard rates (HRs) with precise 95% confidence intervals (CIs). All statistical tests were two-sided. A p-value ≤ 0.05 was considered to reflect statistical significance. SPSS ver. 22 software (IBM, Armonk, NY, USA) was used for all statistical analyses.

3. RESULTS

The median follow-up time was 71.4 months (6–274 months). Although, distant metastasis (DM) constituted the dominant failure pattern (n = 26, 19.5%), locoregional recurrence (LRR) occurred in 14 patients (10%). Most recurrent lesions developed in patients with pT3 or T4 tumors (25 with DM, 13 with LRR). LRR developed within the radiotherapy field in 12 patients (9%). Half of the DM lesions were multiorgan in nature, occurring most commonly in the liver and lungs (n = 11). The most common acute gastrointestinal side effects were not observed in 35.3% of patients, but 22.6%, 36.8%, and 5.3% of patients exhibited grade 1, 2, and 3 side effects, respectively. In terms of chronic gastrointestinal side effects, 88% of patients were not affected, whereas 6% and 4.5% of patients had grade 1 and 2 side effects, respectively; only one patient had grade 3 side effects. Grade 4–5 acute or late toxicity was not observed. The OS, CSS, and local recurrence-free survival rates at the 5-year follow-up were 75.4%, 80.6%, and 91.6%, respectively. The respective survival rates of patients with nodal stage 2 (N2) tumors were 50%, 57%, and 88%. On univariate analysis, age ≥ 64 years (p = 0.04), stage III disease (p = 0.04), and N2 (p = 0.01) were significantly associated with poor OS. N2 (p = 0.02, p = 0.049) was also significantly associated with poor CSS and local recurrence-free survival rates (Table I). On multivariate analysis, N2 [SE (Standard Error) = 0.3, HR = 2.6, 95% CI (1.2–5.6), p = 0.012] and age ≥ 64 years [SE = 0.2, HR = 1.9, 95% CI (1.1–3.5), p = 0.017] were significantly and independently predictive of poor OS (Figure 1).

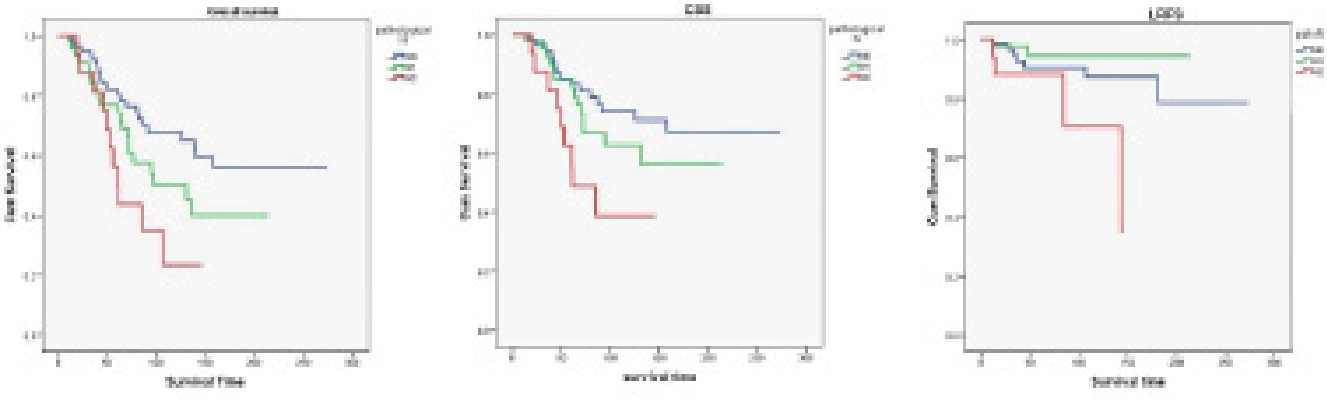

Figure 1. *Overall survival, cancer-specific survival, and local recurrence-free survival in terms of the nodal state*

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***Table I**: Demographic and pathological characteristics of the patients and univariate analysis results for overall survival, cancer-specific survival, local recurrence-free survival.*

	Number of patients (N=133) (%)	OS	CSS	LRFS
		P value	P value	P value
Age		**0.04**	0.2	0.8
Median	62 (30-82)			
<64	79 (59.4)			
≥64	54 (40.6)			
Gender		0.2	0.8	0.9
Male	74 (55.6)			
Female	59 (44.4)			
AJCC stage		**0.04**	0.06	0.8
II	64 (48.1)			
III	69 (51.9)			
AJCC T stage		0.9	0.5	0.07
T1/T2	7 (5.3)			
T3/T4	126 (94.7)			
AJCC N stage		**0.01**	**0.02**	**0.049**
N0	63 (47.5)			
N1	50 (37.5)			
N2	20 (15)			
Grade		0.6	0.1	0.2
I	10 (7.5)			
II	101 (76)			
III	16 (12)			
Unknown	6 (4.5)			
The number of lymph nodes		0.4	0.8	0.4
<11	53 (40)			
≥11	73 (55)			
Unknown	7 (5)			
Tumor size(cm)		0.2	0.3	0.6
≤4	48 (36)			
>4	76 (57)			
Unknown	9 (7)			
Surgical border		0.7	0.8	0.07
Negative	125 (94)			
Positive	8 (6)			
Localization		0.5	0.6	0.9
Upper rectum	52 (39)			
Rectosigmoid	56 (42)			
Sigmoid	25 (19)			
RT dose		0.9	0.6	0.1
<50 Gy	16 (12)			
≥50Gy	117 (88)			
RT technic		0.2	0.1	0.3
3CRT	109 (82)			
VMAT	24 (18)			

OS=Overall survival, CSS=Cancer specific survival, LRFS=Local recurrence-free survival, RT=Radiotherapy, 3CRT= 3D conformal RT, VMAT=Volumetric arc therapy

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4. DISCUSSION

In our study, N2 stage was found as a significant predictor of poor local recurrence-free survival and cancer specific survival rates. In the multivariate analysis, older age and a higher N stage were independent risk factors for poor OS.

Although, adjuvant RT has been suggested as inappropriate because the upper rectum is covered with peritoneum, postoperative chemoradiotherapy increased the local control and survival of patients with stage II and III upper rectal cancer in some studies [14,15]. Some studies found that if tumors are located ≤ 12 cm from the anal verge, lymph node involvement and pelvic recurrence are more common than DM, whereas the reverse is true for more distant tumors (45% of recurrent lesions develop in the liver) [16-20]. As locally advanced upper rectal cancer is assumed to be a systemic disease, adjuvant pelvic RT may be beneficial. Sauer et al., showed that, as was true for middle and lower rectal cancers, adjuvant chemoradiotherapy (CRT) reduced local upper rectal cancer recurrence [21]. The 5-year local recurrence rate was also low (8.4%) in our study. The DM rate was higher and independent of the upper rectal tumor stage, which also did not affect the rate of OS or local recurrence. Lymph node metastasis was an important independent risk factor for local recurrence and survival in many studies [22-25]. We found that N2 stage independently predicted poor outcomes. Vigliotti et al., suggested that postoperative adjuvant radiotherapy was useful for reducing the local recurrence of rectal and rectosigmoid adenocarcinomas. A total dose > 50 Gy to the entire target volume is often used to minimize the relapse rate (< 10%) [26]. We found that postoperative RT reduced local recurrence of upper rectal cancer. Moreover, although statistical significance was not attained, local recurrence was prevented by RT > 50 Gy (5-year local recurrence-free rate: 85.7% vs. 92.4%). Tabchouri et al., found that neoadjuvant CRT did not improve the long-term oncological outcomes of patients with locally advanced upper rectal adenocarcinoma and, in fact, increased postoperative complications [27]. Our postoperative CRT complication rates were very low. Acute gastrointestinal side effects were observed in only 5.3% of patients and grade 3 chronic gastrointestinal side effects in only one patient.

In summary, in this retrospective study, we found that local recurrence of stage II/III upper rectal cancer decreased with postoperative RT, and that N2 was negatively associated with all endpoints. A prospective randomized trial is needed to confirm our findings.

Compliance with the Ethical Standards

Ethical Approval: Marmara University Ethics Committee approved the study (approval no. 09.2021.211).

Financial Support: The authors have no relevant financial information to close.

Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Author Contribution: Both authors participated equally in the idea, concept, design, data collecting and processing, literature review, writing article, and analysis of the paper.

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Evaluation of the effectiveness of a health literacy education program during the pandemic

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ABSTRACT

Objectives: This study investigated the effectiveness of a health literacy education program on first and emergency aid students during the pandemic.

Materials and Methods: This study adopted a single group pre-test-post-test quasi-experimental design. The study population consisted of 135 students of the First and Emergency Aid Program of a private university in Konya, Turkey. No sampling was performed because we could reach the entire study population. Therefore, the sample consisted of 114 students. Participants attended a health literacy education program (intervention). Data were collected through face-to-face interviews using a personal information form and the Health Literacy Scale (HLS). Number, percentage, and Mann-Whitney U test were used to analyze the data. The study adhered to all ethical principles.

Results: Participants had a significantly higher mean post-test HLS score (117 ± 5.03) than the pre-test score (105 ± 11.50) ($p < 0.05$). Participants with a high income had a significantly higher mean post-test HLS score than those with a low income ($p < 0.05$). Participants covered by insurance had a significantly higher mean post-test HLS score than those who were not ($p < 0.05$). Participants who did regular exercise had a significantly higher mean post-test HLS score than those who did not ($p < 0.05$). Participants who knew about health literacy before the intervention had a significantly higher mean post-test HLS score than those who did not ($p < 0.05$).

Conclusion: The education program helped participants learn more about health literacy. Therefore, universities should integrate it into their curricula.

Keywords: Education, Health Literacy, Students

1. INTRODUCTION

The World Health Organization (WHO) defines health literacy as the "cognitive and social skills which determine the motivation and ability of individuals to gain access to, understand, and use information in ways which promote and maintain good health" [1]. The concept of health should be addressed from different perspectives. Health is affected by eating habits, hygiene practices, living arrangements, and diseases [2]. People with health literacy can access current health information and adopt healthy lifestyle behaviors. Health literacy is the ability to make the right decisions for health [3,4]. It promotes both our individual and sociopolitical actions and helps us achieve national and global public health goals. The term "health literacy" was first used in 1974 to describe how health information impacts education. There has been a growing body of research on health literacy since the 1990s [5,6]. Many studies have reported insufficient health literacy levels [7-9].

Literacy status, education, income, age, social status, and chronic disease history affect health literacy [7-12]. Epidemics and pandemics also affect it because people are supposed to be careful about their health during outbreaks. Adolescence is a critical period when people adopt healthy lifestyle behaviors. Young adults are more likely to adapt to the "new" normal and create a change during outbreaks. The novel coronavirus disease (COVID-19) broke out in Wuhan, China, at the end of 2019 and has taken hold of the whole world since then. Turkey shifted to online learning amidst COVID-19 from June 2020

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to April 2021. During this period, only a small number of college students could receive face-to-face education. Between June 2020 and April 2021, when the pandemic was particularly severe, most universities in Turkey continued their education and training activities, including exams and summer schools, with a hybrid education model. We should ensure that young adults have high health literacy levels to improve current health indicators, make health policies effective, and manage the pandemic effectively throughout the country. Students studying in First and Emergency Aid Programs constitute the group that will work actively in the setting of pre-hospital care while applying appropriate information in the field of health. Therefore, these students will have positive indirect effects on the health of the country. Especially in situations such as the COVID-19 pandemic, it is important that they access and apply the right information and adopt more accurate approaches in the first response to patients in the pre-hospital setting. In doing so, they can reduce the transmission pathways during a pandemic. Students of First and Emergency Aid Programs will have a tremendous impact on healthcare capacity because they are the professionals of tomorrow who will work actively in the field of healthcare. Therefore, they should be able to access and use the right information regarding health issues. First and Emergency Aid students with high health literacy levels are more likely to protect and improve public health and prevent diseases [13]. Meherali et al., argue that health literacy education can improve health outcomes and reduce health inequities in low – and middle-income countries [14]. Lin et al., maintain that health literacy education makes healthcare professionals more capable of protecting their patients from many diseases [15]. We hypothesized that an educational intervention would be effective in increasing participants' knowledge and awareness of health literacy. Therefore, this study implemented a health literacy education program (intervention) to help First and Emergency Aid Program students develop health literacy knowledge and skills. The sample consisted of 114 students. All participants attended the intervention.

2. MATERIALS and METHODS

Design

This study adopted a single-group pre-test-post-test quasi-experimental design.

Sample

This study was planned to increase the health literacy levels of students in a First and Emergency Aid Program, who have critical roles in the pre-hospital setting and will carry out the first treatment interventions in the field, in the context of the COVID-19 pandemic.The study population consisted of 135 first and second year students from the First and Emergency Aid Program of a university in Konya, central part of Turkey, in the 2020-2021 academic year. A power analysis was performed (G Power) to determine the sample size. The results showed that a sample of 100 would be large enough to detect significant differences (0.05 margin of error, 0.66 effect size, and 95% power). The sample consisted of 114 students. The inclusion criteria were (1) being a First and Emergency Aid student, (2) having received a hybrid education since the COVID-19 pandemic, and (3) agreeing to participate in the study.

Data Collection Tools

The data were collected using a personal information form and the Health Literacy Scale (HLS).

Personal Information Form

The personal information form was based on a literature review conducted by the researcher. The form consisted of ten items on demographic characteristics (age, gender, income, education, health coverage, chronic disease, regular exercise, tobacco use, regular medication use, and knowing the concept of health literacy) [7,10,11,16,17]. After the necessary form was prepared, it was finalized by obtaining expert opinions from a practitioner of emergency medicine, a lecturer from an emergency aid program, and a public health nurse, all of whom were experts in the field of first and emergency aid. Three experts checked the form intelligibility and relevance. It was revised based on expert feedback.

Health Literacy Scale

Health Literacy Scale was developed by Toçi et al. in 2013 and adapted into Turkish by Aras and Bayık Temel in 2017. The scale consists of 25 items and four subscales. The total score ranges from 25 to 125. The items are rated on a five-point Likert-type scale ("5: I have no difficulty at all, 4: I have little difficulty, 3: I have some difficulty, 2: I have very difficulty, 1: I am unable to do it / I have no talent / impossible"). Higher scores indicate higher health literacy levels [16,17].

Health Literacy Education Program

The First and Emergency Aid Program considered here is an associate degree program that provides a 2-year education. Students graduating from this program can work as emergency medicine technicians in health institutions and organizations in the public and private sectors. This group of health professionals, actively working in ambulances and emergency services, has an important impact on survival rates as they carry out the first treatment interventions in the pre-hospital setting [7,11]. The Health Literacy Education Program was based on a literature review conducted by the researchers [18-21]. The education program for health literacy created by the researchers based on the relevant literature includes four main sessions addressing areas for which health literacy and pandemic processes should be emphasized. After the education program was prepared and expert opinions were obtained from experts in the field of first and emergency aid, including a medic, a lecturer, and a public health nurse, the education program was finalized. This health literacy education program was then implemented for the students face-to-face by the researchers.

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1. Contents of the First Session:

a. Communication in health; the concept of health literacy

b. Factors affecting health literacy

c. Duties of first and emergency aid technicians in pandemics and measures to protect the public in pandemics

2. **Contents of the Second Session:**

a. Health literacy in Turkey and the world

b. The pandemic and the importance of health literacy

c. Health literacy for individuals and society

3. **Contents of the Third Session:**

a. Differences between health literacy and basic literacy

b. Role of society and state in the development of health literacy

4. **Contents of the Fourth Session:**

a. Basic principles and barriers to improving health literacy

b. Access to accurate information resources in the field of health and its importance in the pandemic process

Data Collection

Pre-test

Students were informed about the research purpose and procedure before the intervention. Written and verbal consent was obtained from those who agreed to participate in the study. Participants filled out the personal information form and HLS (pre-test). In the classroom environment, students were given a personal information form and the HLS to complete, and they were asked to fill those out individually under the supervision of the researchers. It took each participant 10-15 minutes to fill out the data collection forms.

Post-test

After the fourth session was completed, post-test data were collected from students who had attended all sessions, again under the supervision of the researchers in the classroom environment. Participants filled out the HLS (post-test) after the intervention. It took each participant ten minutes to fill out the scale.

Intervention

After the pre-test data were collected from the students included in this study, a meeting time outside of class hours was determined for the first session and the group was dispersed. The first training session started 2 days after the pre-test data were collected. The Health Literacy Education Program was implemented for the students in a face-to-face classroom environment, with two sessions two days a week, outside of class hours. Face-to-face training was thus completed in four sessions and two weeks in total. PowerPoint presentations, question-and-answer sessions, and discussion methods were used as educational techniques in these training sessions. Each session lasted about 40 minutes. Participants were taken to the lecture hall based on the number of people per square meter specified by the occupational health and safety teams. All participants and the researcher wore masks throughout the intervention. The classroom was ventilated and disinfected between the sessions.

The study was approved by the University Drug and Non-Medical Device Research Ethics Committee of Konya Karatay University (approval number: 2020/08). Permission was obtained from the institution. All students were briefed about the research purpose and procedure. They were also informed that they could withdraw from the study at any time. Written consent was obtained from those who agreed to participate in the study. The study was conducted according to the ethical principles outlined by the Declaration of Helsinki.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS, IBM v. 22.0) at a significance level of 0.05. Number, percentage, mean, and standard deviation were used for descriptive data. The Kolmogorov-Smirnov test and skewness-kurtosis were used for normality analysis. The results showed that the data were nonnormally distributed. The Mann-Whitney U test (Z) was used to compare two groups.

3. RESULTS

Table I shows all participants' sociodemographic characteristics. More than half the participants were women (60.5%). Half the participants were second-year students (50.9%). Less than a quarter of the participants had no health coverage (14.9%). Nine participants had chronic diseases (7.9%). More than half the participants did not know about health literacy (64.9%) (Table II).

Participants with a high income had a significantly higher mean post-test HLS score than those with a low income. Participants covered by insurance had a significantly higher mean posttest HLS score than those who were not. Participants who did regular exercise had a significantly higher mean posttest HLS score than those who did not. Participants who knew about health literacy before the intervention had a significantly higher mean post-test HLS score than those who did not ($p < 0.05$) (Table III).

Participants had a significantly higher mean post-test HLS score (117 ± 5.03) than the pre-test score (105 ± 11.50) ($p < 0.05$).

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Table I. *Students' sociodemographic characteristics (n:114)*

Items	n	%
Age		
X ± SS 20.0 + 1.6 (min: 15 – max: 25)	88	77.2
18 – 21 age	26	22.8
22 – 25 age		
Gender		
Famale	69	60.5
Male	45	39.5
Class		
1.st grade	56	49.1
2.nd grade	58	50.9
Income Status		
Upper income	12	10.5
Middle income	95	83.3
Low income	7	6.1
Health Insurance		
Yes	97	85.1
No	17	14.9
Chronic Disease		
Yes	9	7.9
No	105	92.1
Exercise Regularity		
Yes	30	26.3
No	84	73.7
Smoking		
Yes	34	29.8
No	80	70.2
Continuous Medication Use		
Yes	11	9.6
No	103	90.4
Know the Concept of Health Literacy		
Yes	40	35.1
No	74	64.9

X: Average/Mean, SS: Standard Deviation

Table II. *Comparison of pre-test and post-test health literacy score averages by socio-demographic characteristics*

	Pre-test		Post-test	
Items	**X±SS**	**Test value – p**	**X±SS**	**Test value – p**
Age				
18 – 21 age	105.0±11.7	Z: – 0.226	116.7±5.1	Z: – 0.382
22 – 25 age	103.0±11.2	p: 0.821	116.3±4.8	p: 0.702
Gender				
Famale	105.0±10.5	Z:-0.009	116.6±4.9	Z: – 0.458
Male	104.0±13.0	p:0.993	116.6±5.2	p: 0.647
Class				
1. class	104.5±11.7	Z:-1.058	116.6±4.9	Z: – 0.672
2. class	105.0±11.4	p: 0.290	117.2±5.0	p: 0.502
Income Status				
Upper income	108.5±4.9	Z: – 2.158	119.5±3.5	Z: – 2.207
Middle/Low income	104.0±11.8	**p: 0.031**	116.3±5.0	**p: 0.27**
Health Insurance				
Yes	106.0±10.0	Z: – 3.551	117.3±4.7	Z: – 1.536
No	88.0±14.1	**p: 0.000**	112.5±4.5	p: 0.127
Chronic Disease				
Yes	103.0±10.2	Z: – 0.168	116.2±4.6	Z: – 0.059
No	105.0±11.7	p: 0.866	116.6±5.0	p: 0.953
Exercise Regularity				
Yes	109.0±10.4	Z: – 2.122	118.3±5.0	Z: – 2.135
No	104.0±11.7	**p: 0.034**	116.0±4.9	**p: 0.033**
Smoking				
Yes	105.0±13.6	Z: – 0.533	116.3±5.4	Z: – 0.379
No	105.0±10.5	p: 0.594	116.7±4.8	p: 0.705
Continuous Medication Use				
Yes	108.0±11.0	Z: – 0.379	117.1±5.3	Z: – 0.365
No	105.0±11.6	p: 0.704	116.5±5.0	p: 0.715
Know the Concept of Health Literacy				
Yes	107.50±10.6	Z: – 2.149	117.8±4.9	Z: – 2.020
No	103.50±11.8	**p: 0.032**	115.9±4.9	**p: 0.043**

X: Average/Mean, SS: Standard Deviation, Z: Mann Whitney U test

Table III. *Comparison of students' health literacy scale scores of pre-test and post-test*

Score of Health Literacy Scale	X±SS	Q(Q1–Q3)*
Pre-Education	105.0±11.50	105.0 (94.00 – 110.25)
Post-Education	117.0±5.03	117 (112.00 – 121.00)
Test value (Z):	-9.290	
P	**0.000**	

** Quarter values are given for data that are not normally distributed. X: Average/Mean, SS: Standard Deviation, Z: Mann Whitney U test*

4. DISCUSSION

Health literacy is essential for reducing health inequities and expenditures [13]. This study was carried out to determine the effect of health literacy education on the health literacy scores of students enrolled in a First and Emergency Aid Program during the COVID-19 pandemic with the aim of increasing the health literacy levels of these students and contributing to the literature. It is important for students to understand the concept of health literacy in order to access appropriate information and apply it correctly to improve the health of the public during pandemic processes. This study investigated the effect of the Health Literacy Education Program (intervention) on First and Emergency Aid Program students' health literacy levels. Participants had a significantly higher mean post-test HLS score (117 ± 5.03) than the pre-test score (105 ± 11.50) ($p < 0.05$).

Our results indicate that universities should provide students with health literacy training programs to help them learn about health literacy and its significance on health outcomes. After the training program, the health literacy scores of the First and Emergency Aid Program students increased, and a significant difference was found between pre-test and post-test scores. In this context, in this study examining the effect of a training program on health literacy scores, the hypothesis that a health literacy education program will increase the health literacy of First and Emergency Aid Program students was confirmed.

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When the literature is examined, it is seen that Erunal et al., conducted a study with 808 nursing students and found that the overall average score was sufficient. Şahinöz et al., conducted a study with students enrolled in health departments and found that these students had sufficient health literacy, similar to our results [22,23]. In our study, similar to the literature, it was determined that levels of health literacy approached the upper value as a result of the implementation of an education program and health literacy levels were sufficient. In a study of high school and university students in Canada and Greece, it was determined that the health literacy levels of men were low [24,25]. In our study, the health literacy scores of male students were found to be lower than those of female students, but this difference was not statistically significant. We also found that as the income status of the students increased, the average health literacy score also increased, although, this was again not statistically significant. In the literature, there are studies showing no significant difference between income levels and health literacy levels, similar to our findings [26,27], although, it has been concluded that there is a positive relationship between income status and health literacy [25]. Individuals who perceive their own health positively also have higher health literacy scores [24,26,27]. Based on the limited data in the literature, it can be said that there is a significant positive relationship between positive health perceptions and health literacy. In our study, the health literacy levels of the students who exercised regularly were significantly higher. In line with these results, it is suggested that regular exercise may help students develop a positive perception of their health [28]. Cianfracca et al., investigated whether a multidisciplinary theoretical-practical training course affected Italian caregivers' health literacy levels. They found that the training course helped the caregivers develop health literacy skills and suffer less from the burden of care [29]. Doi Kanno et al., offered a health literacy training program to older Japanese-Brazilian adults and found that the participants had higher self-efficacy and life satisfaction after the training [30]. Kaper et al., determined that a health literacy consultation skills training program helped undergraduate medical students develop health literacy and self-management skills [31]. Lin et al., provided older Taiwanese adults with a community-based participatory health literacy program and found that the participants were better at controlling their body weights, doing regular exercises, and navigating health information after the training [32]. Zibellini et al., conducted a systematic review to assess the effectiveness of health literacy interventions on pregnancy outcomes and reported that the interventions helped pregnant women learn about health literacy and have better secondary outcomes, such as fetal outcomes and healthcare service utilization [33]. The researchers have drawn two conclusions. First, maternal and child health is critical for development. Second, pregnant women should be provided with health literacy training interventions to help improve health indicators [34-36].

First and Emergency Aid Program students are the professionals of tomorrow who will play a vital role in delivering healthcare services. Our results will pave the way for further research and encourage universities to integrate health literacy training programs into their curricula to help First and Emergency Aid students develop health literacy skills.

Limitations

This study had three limitations. First, it adopted a quasi-experimental design. Second, the sample was recruited from only one university. Third, the data were based on self-report

Conclusion

After attending the Health Literacy Education Program, our participants had significantly higher health literacy levels. This result suggests that Health Literacy Education Programs are affordable, user-friendly, and effective ways to teach students about health literacy. Universities should integrate Health Literacy Education Programs into their curricula and assess teaching outcomes. Researchers should recruit larger samples and adopt randomized controlled experimental research designs to better understand the effectiveness of Health Literacy Education Programs on students' knowledge and awareness of health literacy.

Compliance with Ethical Standards

Ethical Approval: This study was approved by Konya Karatay University Drug and Non-Medical Device Research Ethics Committee (approval number 2020/08). Permission was obtained from the institution. Written consent was obtained from those who agreed to participate in the study. The study was conducted according to the ethical principles outlined by the Declaration of Helsinki.

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Author Contribution: DSY, HKS and SK: Concept, DSY, HKS and SK: Design, SK: Supervision, DSY and HKS: Resources, DSY,HKS and SK: Materials, DSY and HKS: Data collection and processing, SK: Analysis and interpretation, DSY and SK: Literature search, DSY,HKS and SK: Writing the article, DYS,HKS and SK: Critical review. All author read and approved the final version of the article.

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Effect of deep and superficial endotracheal suctioning on hemodynamic parameters and pain in neurosurgical intensive care patients

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ABSTRACT

Objective: This study aimed to determine the effects of deep and superficial endotracheal suctioning on hemodynamic parameters and pain level in mechanically ventilated neurosurgical patients.

Patients and Methods: This prospective, randomized, controlled experimental study was conducted on 37 patients who underwent deep endotracheal suctioning and 37 patients who underwent superficial endotracheal suctioning using open endotracheal suctioning system. The arterial blood pressure, heart rate, body temperature, respiratory rate, oxygen saturation levels and pain status of the patient were compared before and after endotracheal suctioning at 1 min, 5 min and 30 min.

Results: There was no statistically significant difference between the effects of deep and superficial endotracheal suctioning methods ($p > 0.05$). However, there was less change in systolic and diastolic arterial blood pressure and heart rates in patients who underwent superficial endotracheal suctioning before and 30 min after endotracheal suctioning ($p > 0.05$).

Conclusion: Superficial endotracheal suctioning caused fewer changes in hemodynamic parameters and pain levels of patients compared to deep endotracheal suctioning. For this reason, nurses should first prefer the superficial endotracheal suctioning method during the suctioning practices of neurosurgery patients.

Keywords: Endotracheal suctioning, Neurosurgery, Intensive care, Hemodynamic parameters, Nurse

1. INTRODUCTION

Endotracheal suctioning is the process of taking out the respiratory system secretions using a vacuum device operating with negative pressure. Endotracheal suctioning, which is used to remove these tracheal and intraoral secretions in intensive care patients, can be applied in two ways: deep and superficial. Deep endotracheal suctioning is the insertion of a suction catheter until resistance is met in the patient's trachea and lower airway. Superficial endotracheal suctioning is cleaning process by aspirating only intratubal and intraoral spaces with a catheter without advancing it to the patient's intrathoracic cavity [1,2].

A limited number of studies in the literature investigated the effects of deep and superficial endotracheal suctioning. These studies reported that deep and superficial endotracheal suctioning protected patients from respiratory complications, besides affecting hemodynamic parameters and causing pain [3-9]. Haddad and Arabi reported that neurosurgical patients were not hemodynamically stable. They also stated that endotracheal suctioning increased intracranial pressure (ICP) and led to secondary complications. Therefore, they emphasized that endotracheal suctioning should be short and atraumatic [4]. Irajpour et al., investigated the cardiovascular effects of deep and superficial suctioning and found that both suctioning methods increased arterial blood pressure and heart rate and no statistically significant difference was found between these two groups [5]. In addition, it was emphasized in the literature that patients experienced severe pain in the 1st and 5th min after

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superficial and deep endotracheal suctioning. Although, it was stated that the intensity of pain decreases in the 30th min, the pain intensity experienced by the patients during this period was undeniable [10-13].

Endotracheal suctioning, which has important effects on the hemodynamic parameters and pain conditions of the patients, is important to be applied in the least traumatic way in neurosurgery patients whose health conditions are adversely affected, especially in the smallest changes in brain perfusion. However, although there are many studies comparing open (endotracheal suctioning method in which patients are separated from the mechanical ventilator) and closed (endotracheal suctioning method in which patients are applied without leaving the mechanical ventilator) endotracheal suctioning methods in the literature, the number of studies comparing both neurosurgery patients and deep and superficial endotracheal suctioning techniques is quite low. In the literature, the number of studies reporting the superiority of these two different endotracheal suctioning techniques over each other is quite low. In addition, there is insufficient evidence on which endotracheal suctioning method is the most appropriate in neurosurgery patients without impairing cerebral perfusion. For this reason, the study was conducted to determine the effects of deep and superficial endotracheal suctioning on arterial blood pressure, heart rate, body temperature, respiratory rate, oxygen saturation level (SpO_2), and pain in postoperative neurosurgical patients dependent on a mechanical ventilator.

2. PATIENTS and METHODS

Design and Setting

The study is a prospective, randomized controlled experimental study. Data were collected between September 1, 2015 and November 11, 2016. The patients were divided into two groups as control (deep endotracheal suction patients) and experimental group (superficial endotracheal suction patients). Patients who met the research criteria were randomized over hospital protocol numbers (simple randomization using the randomizer.org address). Afterwards, deep endotracheal suctioning was applied to 37 patients in the control group, and superficial endotracheal suctioning was applied to 37 patients in the experimental group. The study was conducted with neurosurgery patients treated in the intensive care units of a university hospital and a public hospital. Surgical patients were followed up in six intensive care units in total. An average of fifteen intensive care nurses work in each intensive care unit, and a responsible intensive care specialist or anesthesiologist of these intensive care units.

Sample

In this prospective, randomized observational study, postoperative neurosurgery patients with 74 epidural, subdural, intracranial hemorrhage and intracranial masses in intensive care unit were studied according to the sample size ($d = 6.8$, $\sigma = 10.47$, $\alpha = 0.05$, $\beta = 0.8$) [5,14].

Inclusion criteria for the study; volunteer, over 18 years old, intubated (between 2 and 7 days on the day of intubation), on mechanical ventilator, without extraventricular drainage, had not infiltrates, not sedated, not unconscious, monitored, with radial artery catheter, arterial blood pressure between 160/90 mmHg and 110/70 mmHg , heart rate between 60-100/min, respiratory rate between 16-22/min, body temperature not higher than 38° C, no cyanosis (SpO_2>86%), hourly urine output more than 30 ml, thrombolytic, not have thrombotic-lung disease and rhythm problems, did not need blood transfusion, and laboratory blood values of sodium, potassium and chlorine were within normal limits. When sodium, potassium and chlorine values are above the normal values, the patients may experience problems in cardiovascular and respiratory functions in addition to fluid-electrolyte disorders, so these values were considered to be within the normal range.

Patients who did not meet any of these criteria were not included in the study. Further, 24 patients who did not meet sampling inclusion criteria and volunteer to participate were excluded from the study.

In order to start the study, ethical committee approval was obtained (date/number: 2015-10-20/05) and implementation permission was received from the institution where the study would be conducted. Later, informed consent was obtained after the supervisor physicians and nurses in the intensive care units, patients or their relatives (for confused or lethargic patients) were informed about the study. The principles of the Helsinki Declaration were followed in the study.

Data Collection Tools

Data were collected using a data collection form, Behavioral Pain Scale (BPS) developed by Payen et al. for intensive care patients and the adaptation of the scale to Turkey was carried out by Vatansever and the Glasgow Coma Scale (GCS) developed by Teasdale and Jennett [8,15,16].

Data collection form consisted of two parts. The first part of the questionnaire comprised a total of nine questions, including eight open-ended questions and one close-ended question on patient information. The questions in the first section were related to gender, age, diagnosis, days spent in the intensive care unit, the GCS score, number of days spent by the patient under intubation, mechanical ventilator mode, hourly urine volume, and the procedure applied to the patient. The second part comprised a chart, in which the pain level measured according to the BPS and the hemodynamic parameters measured and evaluated just before and at 1 min, 5 min, and 30 min after deep and superficial endotracheal suctioning were recorded.

Behavioral Pain Scale: The behavioral pain scale was developed by Payen et al. for intensive care patients [8]. Cronbach's alpha coefficient was found to be 0.64-0.72. The adaptation of the scale to Turkish was carried out by Vatansever in 2004, and the internal discrepancy coefficient (Cronbach alpha value) was found to be 0.71-0.93 [8,15]. It consisted of three subscales, including facial expression, upper limb movements, and ventilation compliance. Each subscale had 4 sub items,

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totaling 12 items. Each subscale was scored between 1 (no pain response) and 4 (full pain response). The lowest score obtained from the scale was 3 and the highest score was 12. A score over 5 indicated that the patient experienced pain. The first items in each subscale showed the absence of pain, the second items mild pain, the third items moderate pain, and the fourth items increased pain level [15]. In a study, it is reported that the use of BPS is useful in evaluating the pain status of neurosurgery patients [17].

***Glasgow Coma Scale*:** GCS is a scale developed by Teasdale and Jennett in 1974. Patients' level of consciousness was determined according to the score taken on a scale that assessed the best eye-opening, motor, and verbal responses. The lowest score from the GCS was 3, which represented bad prognosis, and the highest score was 15, which represented good prognosis. Patients who scored 8 or below on the scale were considered to be in coma [16].

Data Collection

Patients who met the research criteria were randomized over hospital protocol numbers (simple randomization using the randomizer.org address). Afterwards, deep endotracheal suctioning was applied to 37 patients in the control group, and superficial endotracheal suctioning was applied to 37 patients in the experimental group.

The patients were rested without painful stimuli 30 min before the endotracheal suctioning. After the necessary materials were brought to the bedside and placed on a clean and easily accessible area, the procedure was performed in accordance with the superficial and deep endotracheal suctioning application procedures by open system [3]. In accordance with the suctioning application procedures, in patients; endotracheal suctioning procedures were performed in the presence of wheezing, hyperventilation, tachycardia, rhythm problems, increased blood pressure, cough, cyanosis, sweating, restlessness and secretion in the tube. Patients were hyperoxygenated for 2 min with 100% oxygen before both procedures. The patients were suctioned with a 14 Fr x 500 mm catheter (Bıçakcılar, Istanbul, Turkey) for 10 sec. In the neurosurgery patients, intracranial pressure should not exceed 20 mmHg. However, intracranial pressure may be as high as 50 mmHg during suction. At the same time, the vital parameters of neurosurgical patients can return to their original state 10 min after suction. Therefore, the depth and duration of suction and the need for a second suction are important in these patients. The suction time should not exceed 10 seconds and if possible a second suction is required until the vital parameters are restored. After suctioning, the patients were hyperoxygenated again with 100% oxygen for 1 min. Patients' arterial blood pressure, heart rate, body temperature, SpO_2 level, and respiratory rate were measured just before endotracheal suctioning and at 1, 5 and 30 min after both suction procedures. The patients' pain levels were assessed at the same time intervals according to the BPS. The previous settings of the mechanical ventilator with lowered alarm sound were restored. All applications were applied by the same researcher (Figure 1).

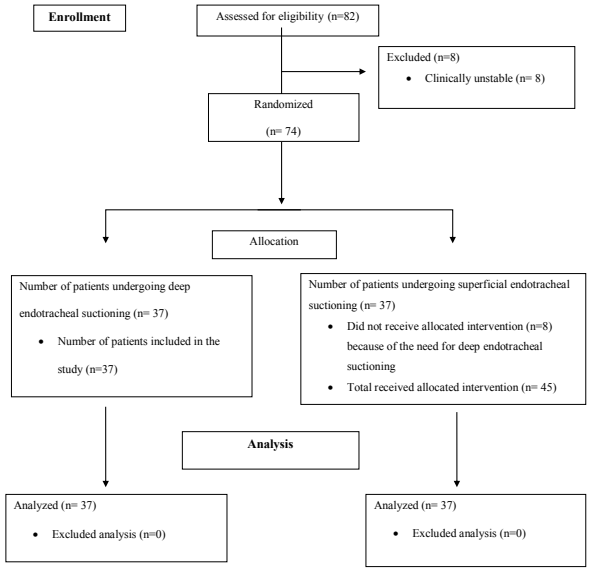

Figure 1. *Diagram showing the flow patients*

Statistical Analysis

Demographic and clinical characteristics of patients and measurement results were defined using numbers, percentages, and arithmetic mean and standard deviation tests. Chi-square and independent-sample *t* tests were used to compare the demographic and clinical characteristics of patients. Similarly, *t* test was used to compare the independent variables in deep and superficial endotracheal suctioning. Two-way analysis of variance (ANOVA) test was used to compare patients' repetitive measurements according to the endotracheal suctioning types. Greenhouse-Geisser statistical test was used in the case of a statistically significant difference in Mauchly sphericity test ($p<0.05$). A p value<0.05 was considered as statistically significant in all statistical analyses in the study.

3. RESULTS

The mean age of the patients was 62.21±1.69 years, most of whom were female (62%, n=46). Patients were in intensive care hospitalized 3.9±2.5 days. Patients had 8.72±1.17 GCS score and 89.1% of the patients had intracranial bleeding diagnosis (n=66). A comparison of the demographic and clinical characteristics of the patients who underwent deep or superficial suctioning showed that the groups were homogeneously distributed (p> 0.05) (Table I).

A comparison of hemodynamic parameters and pain scores of the patients at different measurement according to endotracheal suctioning types is shown in Table II. The mean systolic blood pressure and heart rates of the patients measured just before suctioning increased at 1 and 5 min and decreased in the 30 min after both suctioning methods. The increase was higher at 1 min after deep endotracheal suctioning. The

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mean diastolic blood pressure measured at 1 min after deep and superficial endotracheal suctioning was higher than 5th min after the suctioning. The mean diastolic blood pressure of patients who underwent deep suctioning was higher in all measures than the value before suctioning. The diastolic blood pressure values of the patients who underwent superficial endotracheal suctioning were lower than the values measured 30 min after suctioning, compared to the values measured before suctioning. The SpO_2 levels measured at 1, 5, and 30 min after both suctioning types were continuously higher when compared with values just before suctioning. The patients' pain scores showed the greatest increase in the first min after deep suctioning. In addition, the pain values that increased after deep and superficial endotracheal suctioning started to decrease after 5 min. In particular, it was determined that the values measured at 30 min after deep endotracheal suctioning fell below the pain values determined before the application (Table II). However, no statistically significant difference was found between the results of two suctioning types (p> 0.05) (Table II).

Table I. *Comparison of demographic and clinical features of the patients*

	Deep Endotracheal Suctioning	Superficial Endotracheal Suctioning	
	Number Percent (%)	Number Percent (%)	Statistical test
Ventilator mode			
SIMV	33 55.0	27 45.0	c^2=3.171, *P*=0.075
CBAP	4 28.6	10 71.4	
Sex			
Female	12 42.9	16 57.1	c^2=0.919, *P*=0.338
Male	25 54.3	21 45.7	
Diagnosis			
Intracranial bleeding	32 48.5	34 51.5	c^2=0.561, *P*=0.454
Intracranial tumor	5 62.5	3 37.5	
	X±SD	**X±SD**	
Age	65.21±15.05	59.21±18.33	*t*= –1.538 *P*=0.128
Hospitalization day	4.13±2.61	3.67±2.39	*t*= –0.788 *P*=0.433
Glasgow Coma Scale score	8.70±1.24	8.75±1.11	*t*= –0.197 *P*=0.845
Number of days with intubation	3.59±1.80	3.13±1.43	*t*= –1.213 *P*=0.229
Sodium	140.08±2.88	139.65±2.53	*t*= –0.686 *P*=0.495
Potassium	4.14±0.42	4.06±0.53	*t*= –0.674 *P*=0.502
Chloride	103.61±3.32	103.92±3.56	*t*= –0.398 *P* =0.692
Hemoglobin	12.30±1.14	11.97±0.96	*t*= –1.337 *P*=0.185
Hematocrit	38.40±2.19	37.99±2.08	*t*= –0.831 *P*=0.409

Values are presented as numbers and percentage (%). %95 CI: %95 confidence interval. SD: standard deviation; SIMV: Synchronized Intermittent Mandatory Ventilation; CPAP: Continious Positive Airway Pressure

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Table II. *Comparison of hemodynamic parameters and pain situations of patients at different measurement times according to endotracheal suctioning types*

Measurements	Type of endotracheal suctioning	Before the application	1 min after the application	5 min after the application	30 min after the application	Two-way ANOVA in repeated measurements	
		X±SD	X±SD	X±SD	X±SD	According to suctioning types	According to measurement types
Systolic blood pressure	DES	130.27±17.60	146.81±18.15	135.57±18.51	128.89±17.24	F=0.823[a]	F=64.971[a]
	SES	122.11±12.65	136.78±12.70	127.59±13.06	116.95±11.70	P=0.482	***P*=0.000**
Diastolic blood pressure	DES	74.51±10.75	83.51±11.33	78.16±10.54	75.27±13.06	F=0.520[a]	F=13.827[a]
	SES	70.91±11.30	78.54±12.32	71.54±12.20	68.10±9.17	P=0.669	***P*=0.000**
Heart rate	DES	86.75±10.25	103.19±13.18	97.00±14.86	91.94±13.82	F=1.253[a]	F=54.444[a]
	SES	83.89±11.09	96.56±12.62	94.29±13.58	86.29±14.55	P=0.291	***P*=0.000**
Body temperature	DES	36.73±0.43	36.77±0.44	36.80±0.44	36.78±0.46	F=0.450[b]	F=1.952[b]
	SES	36.65±0.43	36.68±0.44	36.68±0.45	36.70±0.47	P=0.629	P=0.148
Respiratory rate	DES	19.00±2.96	23.75±4.99	21.56±5.41	18.86±3.77	F=0.709[b]	F=54.819[b]
	SES	17.86±1.87	22.43±3.50	21.40±4.92	18.21±3.08	P=0.526	***P*=0.000**
SpO_2	DES	95.62±2.34	96.32±2.83	97.78±2.18	98.48±1.34	F=0.121[a]	F=52.096[a]
	SES	95.37±2.31	96.34±2.64	97.59±2.03	98.45±1.81	P =0.947	***P*=0.000**
Pain score	DES	3.27±0.50	5.45±1.06	3.86±0.91	3.18±0.46	F=0.991[b]	F=134.958[b]
	SES	3.21±0.58	5.24±0.89	4.05±1.10	3.24±0.64	P=0.383	***P*=0.000**

DES, Deep Endotracheal Suctioning SES, Superficial Endotracheal Suctioning
[a]*Mauchlysphericity test.* [b]*Greenhouse-Geisser test.*

4. DISCUSSION

In this current study, an increase in systolic and diastolic arterial blood pressures immediately after suctioning suggested that patients' bodies were responding to the stress experienced during endotracheal suctioning. This is because adrenaline and noradrenaline hormones released in response to stress increase the heart's contraction strength and speed by stimulating beta 1 receptors. As a result, systolic and diastolic blood pressures also increase [18].

Jongerden et al., reported that both open and closed endotracheal suctioning caused significant changes in arterial blood pressure of the patients; however, no significant differences in arterial blood pressure were observed between these two methods [18]. Also, Irajpour et al., investigated the cardiovascular effects of deep and superficial suctioning in 74 patients and reported an increase in arterial blood pressures using both methods; these changes did not show any significant difference between the groups [5]. Dastdadeh et al., to determine the effect of open and closed endotracheal suctioning system on pain and agitation, they reported significant differences in heart rate, systolic blood pressure and diastolic blood pressure variables in different time periods [7]. Christopher et al., explored the physiological effects of closed endotracheal suctioning in mechanically ventilated patients. In their study, closed endotracheal suctioning caused a significant change in the blood pressure, but this change was not clinically important [19]. The results of these previous studies were compatible with the results of the present study.

In this study, the observed increase in heart rate after suctioning compared to the rate just before endotracheal suctioning was thought to be a stress response similar to arterial blood pressures. This finding is supported by many studies [5,11,14,19,20]. These results showed that the type of endotracheal suctioning and the stress experienced did not have a statistically and clinically significant effect on the patients' bodies. On the other hand, Abbasinia et al., reported that respiratory rates of patients increased significantly during deep and superficial endotracheal suctioning [6]. However, in parallel with the results of this study, they could not find a statistically significant difference between

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endotracheal suctioning methods. Bousarri et al., reported that respiratory rates of patients increased during endotracheal suctioning and returned to normal levels after administration [21].

Hyperventilation and hypoxia can cause significant complications in neurosurgical patients [22]. Therefore, oxygenation should be at an optimal level. Rao noted that complications occurring secondary to brain trauma (such as hypoxia, hypercapnia, hypotension, and hypo-hyperglycemia) might cause an increase in the intracranial pressure and intracranial hypertension in the brain [23]. Therefore, in the study, hyperventilation with 100% oxygen for 1 min was applied to the patients 2 min before and after endotracheal suctioning to prevent the development of hypoxia. It was found that SpO_2 levels increased continuously in patients who did not develop hypoxia after deep and superficial endotracheal suctioning. This increase was statistically and clinically significant ($p<0.05$). The increase in the obtained SpO_2 levels did not show significant difference according to the suctioning type ($p> 0.05$). These findings suggested that both endotracheal suctioning types were effective in clearing secretions in airways. However, these findings were in contradiction with the results of previous studies showing that SpO_2 levels were reduced immediately after endotracheal suctioning. Özden and Görgülü determined a decrease in SpO_2 levels during and 2 min after open and closed endotracheal suctioning [11]. They reported that SpO_2 levels of patients increased in 5 and 15 min after suctioning. The SpO_2 levels were found to be significantly different in studies by Faraji et al. and Mazhari et al. investigating the effects of open and closed systems. They found that this difference was more evident in the open system suctioning [14,24].

When the effect of deep and superficial endotracheal suctioning on the pain intensity experienced by patients was evaluated, the level of pain decreased in 1 min and 5 min after both suction methods and the pain intensity reduced below the pre-procedural level at 30 min after endotracheal suctioning. However, the decrease in the 5th min of superficial endotracheal suctioning was greater than that in deep endotracheal suctioning. Statistical analysis revealed no statistically significant difference between the methods in terms of pain levels ($p> 0.05$). This result showed that both endotracheal suctioning methods were painful procedures for the patients. In addition, the superficial endotracheal suctioning was considered a less painful procedure because the pain level in 5th min of the superficial endotracheal suctioning was closer to that during the pre-procedural period. Dastdadeh et al., reported that repeated measures after suctioning showed significant difference areas of facial expression, upper limbs, and compatibility with the ventilator after open and closed suctioning [7]. Yava et al., also detected that the highest pain intensity was felt during endotracheal suctioning before and after the interventions [10]. Many previous studies also showed that endotracheal suctioning was an extremely painful procedure causing changes in the hemodynamic parameters of the patients [7,10-12].

In conclusion, the study showed that clinically and statistically significant difference was not found on comparing the effect of two endotracheal suctioning methods. As well, superficial endotracheal suctioning caused fewer changes in systolic and diastolic arterial blood pressures and heart rate in patients compared with deep endotracheal suctioning. Moreover, it had more positive effects on the oxygenation level and caused less pain in the patient. Superficial endotracheal suctioning was less traumatic compared with deep endotracheal suctioning for the patients. So, for the purpose of clearing the airway secretions of neurosurgical patients, the superficial endotracheal suctioning technique should be used first, unless deep endotracheal suctioning is indicated. We believe that this way, the quality of care of patients will increase. Also, when patients are aspirated with appropriate technique, their risks will be reduced in terms of complications.

It is important that nurses should monitor patients closely because endotracheal suctioning causes changes in the hemodynamic parameters of the patients. It is need to perform more experimental studies on the effects of deep and superficial endotracheal suctioning on patients' hemodynamic parameters and pain level.

Limitations of the Study

The study was conducted in two centers to reach the number of samples. In addition, it was long to reach the number of samples because the inclusion criteria for the patients to be studied were too high. Patients who did not meet the sampling criteria were excluded from the study, which increased the duration even longer.

Compliance with Ethical Standards

Ethical Approval: Ethical committee approval was obtained (approval number: 2015-10-20/05) and implementation permission was received from the institution where the study conducted. Later, written informed consent was obtained after the supervisor physicians and nurses in the intensive care units, patients or their relatives (for confused or lethargic patients) were informed about the study. The principles of the Helsinki Declaration were followed in the study.

Financial Support: No specific funding was received.

Conflict of Interest: There are no conflicting interests.

Author contributions: SC and SK: Planning the research, determining the method, SC obtaining all necessary permits, SK and EK Collecting the data, SC: Analyzing the data. All authors were involved in the interpretation and reporting of the research results.

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COVID-19 and related gastrointestinal symptoms: An observational study

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ABSTRACT

Objective: COVID-19 pandemic has infected the entire world and causes obsolete morbidity and mortality. While respiratory symptoms are the most frequently reported, several recent studies revealed that gastrointestinal symptoms are not uncommon. Our aim in this observational study is to reveal the gastrointestinal symptoms of COVID-19 patients.

Patients and Methods: Data of COVID-19 patients with gastrointestinal symptoms were recorded and retrospectively analyzed during their hospital follow-up period. 82 patients participated in the study and later on had a positive polymerase chain reaction (PCR) test. Gender, age, systemic and gastrointestinal complaints, medical and surgical disease history, laboratory results, thorax computer tomography (CT) findings, and characteristics of gastrointestinal system (GIS) symptoms of patients were also recorded. Data were analyzed by statistical software.

Results: The most common typical symptoms of COVID-19 patients were cough, anosmia, debility, and shortness of breath. Further, most of the gastrointestinal symptoms found in COVID-19 patients were nonspecific abdominal pain.

Lymphopenia and elevated liver transaminases were the most common findings of the tests. Patients with diarrhea indicated watery diarrhea. Two patients also had ileus, they healed spontaneously without surgical intervention. Furthermore, all patients were discharged without any problems.

Conclusion: Gastrointestinal symptoms are typical in COVID-19 patients. However, these symptoms do not seem to have a detrimental effect on the progression of the disease. In our study group, there was no need for surgical intervention, but COVID-19 patients with gastrointestinal symptoms should be treated by a multidisciplinary approach.

Keywords: COVID-19, Gastrointestinal symptom, Diarrhea, Intestinal obstruction

1. INTRODUCTION

The COVID-19 pandemic has resulted in several new challenges for the global healthcare system. Mostly recognized as a respiratory pathogen and with major symptoms thought to be fever and cough, gastrointestinal symptoms of the global pandemic were identified shortly thereafter, and emerging data revealed that the gastrointestinal system (GIS) is one of the eventual targets of the pathogenic SARS-COV-2 virus, which enters lung cells via the angiotensin-converting-enzyme (ACE-2) receptor, which is highly abundant and reproductive on GIS epithelial cells [1].

SARS-COV-2 RNA was found in the feces in 83.3% of patients, and these patients still have viral RNA particles in their stools even after elimination from the respiratory tract. So, there was concern about the likelihood of fecal to oral transmission [2, 3].

Moreover, 50% of patients experience GIS symptoms such as nausea, vomiting, diarrhea, and abdominal pain. Typically, these

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symptoms were seen after respiratory findings. The severity of the gastrointestinal symptoms is related to COVID-19 general disease severity [3]. The pathophysiology can be specifically linked to direct damage of the GIS by inflammatory response in addition to the ACE-2 receptor-related mechanism. Enterocytes might also be destroyed by viral particles, resulting in malabsorption and unbalanced intestinal secretion presented by symptoms such as diarrhea [2, 3].

Some studies revealed that patients might have GIS symptoms just before the advent of respiratory manifestations of COVID-19, meaning that patients with these symptoms at the height of the pandemic may need to be carefully assessed according to status of outpatients and inpatients [4].

In this study, COVID-19 patients were hospitalized and later had GIS symptoms. The patients were analyzed according to the characteristics of their symptoms. The possible effects of the GIS on the COVID-19 disease were observed. Our aim in this observational study is to reveal the GIS symptoms of COVID-19 patients in Kutahya, Turkey.

2. PATIENTS and METHODS

After local ethical committee approval (Kutahya Health Sciences University, Local Ethical Committee approval number: 2020/10-02), the data of COVID-19 patients with gastrointestinal symptoms were recorded and retrospectively analyzed during their hospital follow-up period between June 2020-September 2020 in Kutahya Health Sciences University, Evliya Çelebi Research and Education Hospital. Eighty-two patients who had a positive finding for polymerase chain reaction (PCR) test and were hospitalized due to COVID-19 disease and who later had gastrointestinal symptoms participated in the study.

Patients who were hospitalized for another disease and later diagnosed with COVID-19 and also those with chronic GIS symptoms were excluded from the study.

Gender, age, systemic and GIS complaints, medical and surgical disease history, thorax computer tomography (CT) findings, and characteristics of GIS symptoms of patients were also recorded.

The major GIS symptoms addressed in this study include diarrhea, dyspepsia, abdominal pain, nausea, vomiting, epigastric pain, and constipation. Characteristics of diarrhea were also recorded. These patients were hospitalized by an infectious disease clinician and regularly consulted in our general surgery unit for gastrointestinal complaints

Statistical Analysis

While evaluating the findings obtained in the study, the IBM SPSS (Statistical Package for Social Sciences) Statistics 20 software was used for statistical analysis. For descriptive data in the study, frequency tables, descriptive statistics, and pie and bar charts were used.

3. RESULTS

Forty-three patients were female and 39 were male. The median age of the patients was 52 years (20-87). Moreover, the most common symptoms linked to COVID-19 were cough, cough and debility, and cough and high temperature (24.4 %, 18.3 %, and 14.6 %, respectively). Patients' general complaints during hospitalization are shown in Table I.

Table I. *Patients' general symptoms at hospitalization*

Variables	Symptoms	Number (n)	Percentage (%)
Patient Complaints	Anosmia, Abdominal pain	**19**	**23.1**
	Fever	1	1.2
	Fever, Cough	3	3.7
	Headache, Anosmia	9	11.0
	Muscle pain	1	1.2
	Myalgia, Cough	2	2.4
	Dyspnea, Cough	**12**	**14.6**
	Cough	**20**	**24.4**
	Cough, Weakness	**15**	**18.3**
	Anosmia	1	1.2
Other system findings	Fever	3	3.7
	Fever, Cough	**23**	**28.0**
	Headache	7	8.5
	Myalgia, Cough	1	1.2
	Cough	**47**	**57.3**

Hypertension was the most common medical comorbidity, and chronic obstructive pulmonary disease (COPD) and diabetes mellitus (DM) accompanied patients' illnesses (15.9 %, 6.1 %, and 6.1 %, respectively). Cholecystectomy was the most common condition (8.5 %) among the patients' surgical experience. The medical and surgical history of patients is shown in Table II.

Laboratory investigations revealed that lymphopenia was the most common finding with 41.5 %, while normal functions had 29.3%. Further, 18.3% of patients underwent abnormal liver function examinations. Almost all patients had a thorax CT except for one patient. Bilateral typical pulmonary infiltration was observed in 69.5 %. The laboratory and radiological properties of the patients are shown in Table III.

Nausea, abdominal pain, and vomiting were the most common complaints among patients (74.4 %, 65.9 %, and 50 %). The characteristics of the gastrointestinal symptoms are shown in Table IV.

While 14 patients had diarrhea, two had constipation related to intestinal obstruction. In addition, 71.4% of the patients with diarrhea (n:10) had watery diarrhea.

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Table II. *Medical and Surgical History of COVID 19 Patients*

Characteristics		Number (n)	Percentage (%)
Medical History	Asthma	1	1.2
	Bipolar Disorder	1	1.2
	Diabetes Mellitus	**5**	**6.1**
	Gestational Diabetes	1	1.2
	Pregnancy	1	1.2
	Hypertension	**13**	**15.9**
	Hypertension, Diabetes Mellitus	5	6.1
	Congestive Heart Failure	2	2.4
	Chronic renal disease	2	2.4
	Chronic obstructive pulmonary disease	**5**	**6.1**
	Coronary Heart Disease	1	1.2
	Malignity	1	1.2
	Cerebrovascular disease	1	1.2
	Ulcerative Colitis	1	1.2
	None	42	51.2
Surgical History	Appendectomy	3	3.7
	Inguinal Hernioplasty	2	2.4
	Cholecystectomy	**7**	**8.5**
	Coronary Bypass	2	2.4
	Peripheral Arterial Disease	1	1.2
	Thyroidectomy	1	1.2
	None	66	80.5

Table III. *Radiological and laboratory results of COVID 19 Patients with gastrointestinal symptoms*

	Pathology	Number	Percentage (%)
Thorax CT findings	**Bilateral pulmonary infiltration**	**57**	**69.5**
	Not ordered	1	1.2
	Normal	7	8.5
	Unillateral pulmonary infiltration	17	20.7
Laboratory Results	High D-Dimer Level	2	2.4
	High Liver Transanimases	**15**	**18.3**
	Lymphopenia	**34**	**41.5**
	Leucopenia, lymphopenia	6	7.3
	Normal	**24**	**29.3**
	High Urea/Creatinine Levels	1	1.2

Table IV. *Gastrointestinal symptoms of COVID-19 Patients*

Categories	Patient (n)	Percentage (%)
Abdominal Pain	54	65.9
Nausea	61	74.4
Vomiting	41	50.0
Epigastric Pain	20	24.4
Diarrhea	14	17.1
Dyspepsia	19	23.2

Moreover, 69.5 % of the patients (n: 57) did not require additional GIS investigations. Also, 19.5 % of the patients (n:16) had an occult blood test, while 8.5 % (n:7) had a stool culture. Two patients also had additional abdominal CT due to intestinal obstruction (Figure 1).

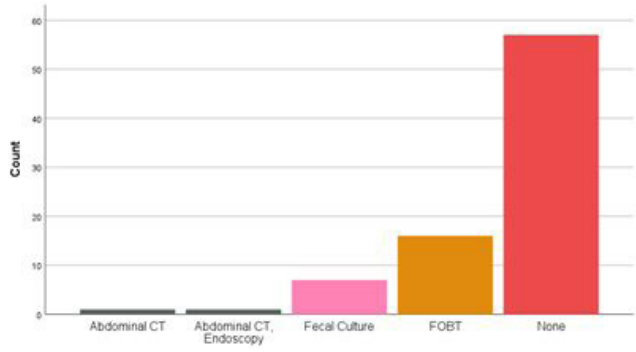

Figure 1. *Investigations for GIS symptoms*

These two patients encountered intestinal obstruction during the follow-up period. One of these patients was an 82-year-old male with prior stroke and history of upper gastrointestinal surgery with gastric outlet obstruction, which was spontaneously resolved following nasogastric decompression and supportive treatment. The other patient was a 55-year-old male, who had had inguinal hernioplasty before and had small intestinal fluids with gaseous fluids, and the ileus was also spontaneously resolved after supportive treatment and nasogastric decompression. Ranitidine was used in 85.4 % of the cases (n:70), followed by proton pump inhibitors (PPIs) (13 %). Metoclopramide, ranitidine, and PPIs were administered together to one patient with recurrent vomiting (Figure 2).

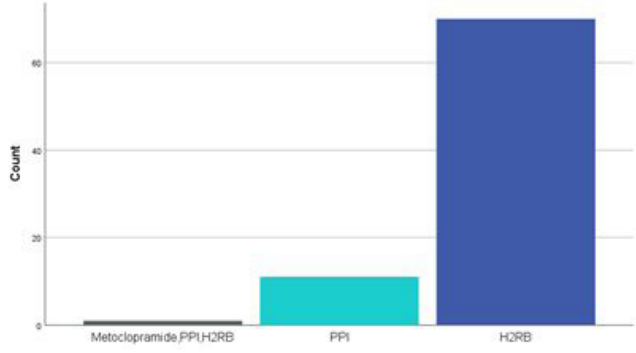

Figure 2. *GIS Treatment Agents*

4. DISCUSSSION

This prospective observational study is one of the high numbers of patient series demonstrating GIS manifestations in COVID-19 patients and Turkey's first case series.

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This study has some limitations, such as its retrospective nature and which data was obtained from the data set of the patients. Some of the information may be misleading, since symptoms may not have been assessed objectively. Patients who were added to the data were only those from the COVID-19 facilities, so patients in intensive care units were not included in the study.

In addition to respiratory tract findings, GIS symptoms are common with COVID-19 infection. As previously reported in such epidemic infections as SARS (severe acute respiratory syndrome), diarrhea, was frequently noted in 40% of patients. In those patients with gastrointestinal findings, respiratory assistance and intensive care follow-up ratios were increased. Also, some GIS symptoms were typical in patients with Middle East respiratory syndrome (MERS) [5].

In a review article, anorexia was found to be the most common GIS correlated with inflammation. Symptoms such as loss of appetite were difficult to assess and may be subjective, making diarrhea more objective [6]. In our study, we also did not ask patients about lack of appetite.

Diarrhea was seen in 17.1% (n:14) of our patients, and 71.4% of the patients with diarrhea (n:10) had watery diarrhea. Viral infection is thought to alter the intestinal permeability that causes enterocyte damage, so unbalanced intestinal activity leads to diarrhea. Lately, symptoms of diarrhea were increasingly reported in the literature [6].

One of the mechanisms linked to gastrointestinal manifestations is intestinal dysbiosis that was correlated with diminished species of *Lactobacillus* and *Bifidobacterium* [7]. Viral lung infection induces increased inflammatory response and viral translocation to the circulatory system, and sequentially altered microbiota results in increased intestinal permeability and inflammatory cytokines associated with secondary infections and multiple-organ failure [8]. Hence, the Chinese health commission recommends the use of probiotics [7]. In comparison to the literature reports, our patients had fewer GIS symptoms, but for a general assumption larger patient cohort is required.

Another proposed mechanism related to GIS is the cytokine storm, which causes severe systemic infection and multiple-organ failure, including the digestive system. Many cytokines may be dysregulated, resulting in abnormal immunity and drastic increase in disease severity. It is still unclear if the involvement of the gastrointestinal system is secondary to systemic inflammation or if primary intestinal infection may lead to disease [9]. Our patients did not need to be referred to an intensive care unit, so in mild disease condition, GIS may not contribute to disease severity.

The most common symptoms of our patients were nausea, abdominal pain, and vomiting (74.4%, 65.9%, and 50 %, respectively). In the literature, disease severity was significantly related to patients with COVID-19 who had abdominal pain, nausea, or vomiting [10]. However, there was no adverse event in our patients with GIS.

Epigastric pain and dyspepsia were also a common finding in these patients (24.4 % and 23.2 %, respectively). During our observations, these symptoms were spontaneously resolved.

In our analysis of seven patients with watery diarrhea, feces culture was requested, but no pathogens were found. For 16 patients, fecal occult blood tests were done, but none of them had positive results. In the literature, fecal-oral transmission is clearly indicated, and fecal viral shedding may continue even after respiratory tract infections have been resolved. There is still no consensus in clinical use to prevent fecal-oral transmission. However, great care must be taken against fecal-oral transmission in order to support the control of the infection resource [2].

Although, diarrhea is a prominent symptom related to unbalanced intestinal secretion, intestinal obstruction experienced by two of our patients was resolved spontaneously. These two patients underwent abdominal surgery, and possible related abdominal adhesions may lead to ileus symptoms. They did not have peritonitis during physical examination. In our study, patients had no signs of constipation. In literature, constipation is not a typical finding in COVID-19 patients; however, a recent study showed that critically ill patients with COVID-19 had higher gastrointestinal findings compared with similar patients who did not have COVID-19. High ACE-2 receptor expression might be responsible for this finding [11].

In our study, lymphopenia was the most common laboratory finding with 41.5%, while the normal range of laboratory results was 29.3%. Moreover, 18.3% of patients underwent abnormal liver function examinations. Abnormal liver function tests have been documented in a variety of studies. Persistent high levels of aspartate transferase, alanine transferase, and bilirubin were associated with serious illnesses. Several proposed mechanisms are contributing to liver injury. Direct damage to SARS-COV-2 hepatocytes, drug hepatotoxicity, systemic inflammation related to cytokine storm and hypoxic injury are the potential causes of liver injury [2, 3, 12]. In our patients, liver function tests resolved spontaneously during the follow-up period.

The management of GIS symptoms in patients with COVID-19 is supportive, so treatment must be tailored to the patients' characteristics and comorbidities. For patients with diarrhea and vomiting, adequate oral or intravenous hydration is essential [13]. ACE-2 inhibitors were associated with lower digestive symptoms by blocking potential ACE-2 receptors on the gastrointestinal system. However, its partial blockage may lead to a malfunction in the transport of amino acids in the intestine, which adversely leads to malnutrition, so its use is not widely accepted [14, 15].

In our patients, histamine-2 receptor antagonists were used in 85.4% of cases (n:70), while PPIs (13.4%) were rarely required. As recently stated in the literature, patients who are actively using PPIs are at increased risk for severe clinical outcomes related to COVID-19, so treatment for GIS gastrointestinal symptoms of COVID-19 patients must be individualized by weighing the benefits and risks [16].

Conclusion

Gastrointestinal symptoms are not rare in patients with COVID-19. In addition to taking care of respiratory symptoms

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in COVID-19 patients, gastrointestinal assessment and physical examination are essential.

Compliance with Ethical Standards

Ethical approval:

The approval was obtained from Kutahya Health Sciences University, Local Ethics Committee (approval number: 2020/10-02)

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Conflict of Interest: The authors have no conflicts of interest to declare

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Review Article

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The alterations of blood-testis barrier in experimental testicular injury models

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ABSTRACT

The blood-testis barrier is found between the Sertoli cells and divides the seminiferous tubule epithelium into basal and adluminal compartments. The germinal cell renewal, differentiation and cell cycle progression up to the preleptotene spermatocytes stage take place in the basal compartment, however, meiosis, spermiogenesis and spermiation take place in the adluminal compertment. The blood-testis barrier consists of tight junctions as well as ectoplasmic specialisations, desmosomes and gap junctions to create specific microenvironment for the completion of spermatogenesis to form spermatozoa. The blood-testis barrier is not a static ultrastructure, it undergoes extensive restructuring during the seminiferous tubule epithelial cycle of spermatogenesis to allow the transit of preleptotene spermotocytes at the blood-testis barrier from basal compartment towards the adluminal compartment. The functions of the blood-testis barrier include preventing the transport of biomolecules into the paracellular space, forming an immunological barrier, separating cellular processes during the spermatogenic epithelial cycle, and establishing the cellular polarity of the seminiferous tubule. However, various environmental conditions, chemotherapeutic agents, toxic substances and lifestyle have degenerative effects on blood-testis barrier, resulting in testicular damage, altered sperm parameters and ultimately male infertility. The alterations in morphological and molecular organization of blood-testis barrier in different experimentally induced testis injury models are reviewed in this article.

Keywords: Blood-testis barrier, Sertoli cells, Tight junction, Testicular injury models

1. INTRODUCTION

Spermatogenesis occurs within the seminiferous tubules in testis and begins after the puberty under the control of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. Seminiferous tubule epithelium contains germ cells and Sertoli cells. Spermatozoa are formed at the end of the spermatogenesis. The blood-testis barrier is found between the Sertoli cells and regulates the spermatogenesis. Leydig cells, located in the interstitium, secrete testosterone under stimulation of LH. Testosterone is essential for the maintenance of the blood-testis barrier and spermatogenesis, and assists in both the formation and breakdown of the Sertoli-germ cell association [1]. The blood-testis barrier is not a static ultrastructure, it undergoes extensive restructuring during the seminiferous tubule epithelial cycle of spermatogenesis to allow the transit of germinal cells from basal region towards the apical region of seminiferous tubules. When the blood-testis barrier function is disrupted it directly causes male infertility because the germ cells are more vulnerable to the microenvironment. However, the regulation and restructuring of the blood-testis barrier without causing "leakage" in healthy adults is not fully understood, nor is it known how disturbances in blood-testis barrier regulation contribute to testicular damage and infertility. Tight junctions between Sertoli cells are important for the integrity of this barrier. Intracellular communication between Sertoli and germ cells plays a critical role in the adult spermatogenic process. Loss of this blood-testis barrier function causes infertility [2].

This article focused on the alteration of the blood-testis barrier in different experimental testicular injury models. First, the structure and function of the blood-testis barrier and its role in spermatogenesis were discussed. Then, the alteration of the blood-testis barrier in different experimentally induced testicular injury models was reviewed.

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Formation of Blood-Testis Barrier

Sertoli cells were first described by Enrico Sertoli and first called 'Sertoli cells' by Von Ebner. The blood-testis barrier was first defined physiologically, later ultrastructurally, and basal and adluminal compartments were described [3]. Sertoli cells are polarized cells lying on the basal lamina and reaching to the tubule lumen. Germinal epithelial cells are found between the adjacent Sertoli cells (Figure 1). Spermatogenesis begins with the division of spermatogonia A to give spermatogonia B. These cells give rise to preleptotene spermatocytes, which form round spermatids after completion of meiosis. After spermiogenesis, spermatozoa are formed and released into the lumen of the seminiferous tubules. Leydig cells localising in the interstitium secrete testosterone under the stimulation of LH. Testosterone regulates the maintenance of the blood-testis barrier, spermatogenesis and fertility. Monocytes, macrophages, dentritic cells, natural killer cells and mast cells which are also present in the interstitium have roles for the maintenence of spermatogenesis [1].

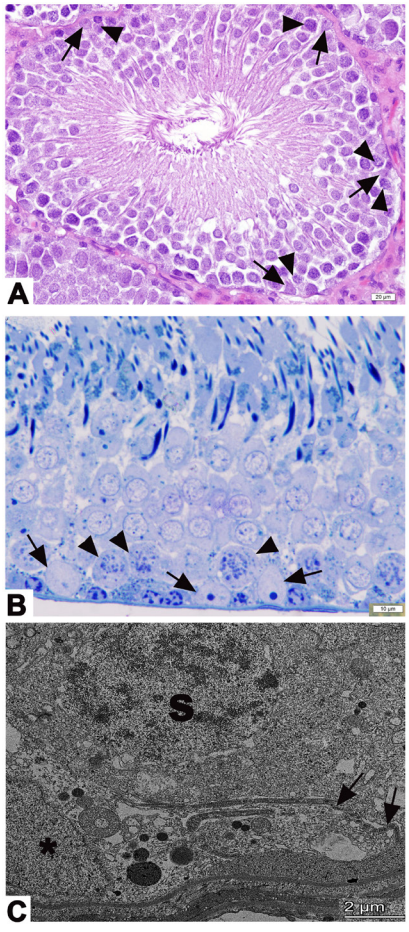

Figure 1. *Primary spermatocytes (arrowheads) and the other germ cells between the Sertoli cells (arrow) are seen in seminiferous tubule epithelium of rat testis (A and B). Tight junctions (arrow), between the Sertoli cells (*) and primary spermatocyte (s) are seen in seminiferous tubule epithelium of rat testis (C). A: Hematoxylin and eosin staining, B: Toluidine blue staining. C: Electron micrograph. Scale bar: A: 20µm, B: 10µm, C: 2µm*

The blood-testis barrier is formed by Sertoli cells close to the base of the seminiferous tubules and divides the germinal epithelium into basal and adluminal compartments. Spermatogonia and preleptotene spermatocytes are located in the basal compartment, while other primary and secondary spermatocytes, round spermatids and elongated spermatids are found in the adluminal compartment [4].

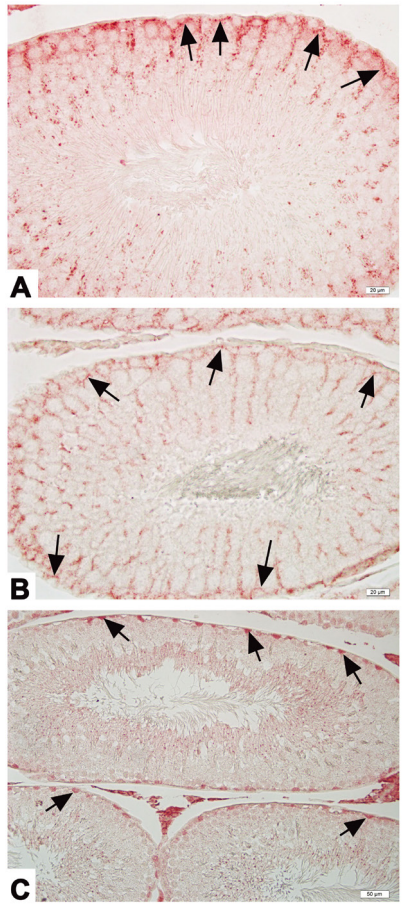

Figure 2: *The localization of ZO-1 (A), occludin (B) and connexin-43 (C) are seen in basolateral cytoplasm (arrow) of the Sertoli cells in rat testis. ZO-1 (A), occludin (B) and CX-43 (C) immunostaining. Scale bar: A and B: 20µm, C: 50µm*

Blood-testis barrier is formed by tight junctions, ectoplasmic specializations, desmosomes and gap junctions. Tight junctions are the most important component of the blood-testis barrier, they have preventive function for the passage of water, solutes and large molecules between the paracellular space, and restrictive function for the movement of proteins and lipids between the apical and basolateral cytoplasm. Ectoplasmic specializations coexist and cofunction with tight junctions. Desmosomes are cell-cell junctions that mediate vigorous adhesion. Gap junctions are cell-cell channels that permit diffusion of metabolites, ions and molecules smaller than 1 kDa. Tight junctions consist of

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integral proteins such as occludin, claudin family (claudin-1, – 3, – 5, – 11, – 12 and – 13), junctional adhesion molecule family, tricellulin, coxsackievirus and adenovirus receptor, and scaffolding proteins such as zonula occludens (ZO) [2]. ZO-1, ZO-2 and ZO-3 are well-studied adaptor proteins and are used by integral membrane tight junction proteins such as occludin and claudin for the attachment to the actin cytoskeleton. ZO-1 colocalizes with gap junction protein connexin – 43 (CX-43) and regulates gap junction communication [1, 4, 5]. Basolateral localization of ZO – 1, occludin, and CX-43 proteins using immunohistochemical technique is shown in Figure 2.

Alteration of Blood-Testis Barrier in Various Testicular Injury Models

Inflammation because of infection [6], sperm extraction procedure such as, testicular sperm extraction (TESE) [7], vasectomy operation [8], reactive oxygen/nitrogene species [9, 10], radiation and hyperthermia [11], chronic unpredictable stress [12] and various environmental contaminants, such as arsenic [13], perfluorooctane sulphonate [14] and cadmium chloride [15] affect spermatogenesis, steroidogenesis, Sertoli cells, blood-testis barrier structure and function and sperm parameters [16]. Impaired blood-testis barrier might cause disruption of spermatogenesis and finally might lead to subfertility or infertility.

It is a well-known fact that the different environmental agents negatively affect the functional development of germ cells, Sertoli cells and Leydig cells during the prenatal period. Prenatal administration of ethanol is shown to cause dilatation between tight junctions, a decrease in ZO-1 and occludin distribution in the blood-testis barrier region, an increase in athrophic tubules with a decrease in germ cells, and an increase in apoptotic cells in the rat testis [17, 18]. These results show that ethanol administration negatively affects the blood-testis barrier during the testicular development. In another study, it is shown that prenatal administration of radiation and postnatal administration of hyperthermia to the rats caused severe increase of atrophic tubules, degeneration of tight junctions with absence of ZO-1 and occludin proteins distribution. This double hit model was represented as Sertoli cell only model [11]. In another prenatal study, electromagnetic waves emitted from cell phones were shown to damage the blood-testis barrier and cause a decrease in the amount of ZO-1 protein distribution, loss of germ cells, an increase in apoptotic cells, a decrease in serum testosterone level, and an increase in oxidative stress in the testes [19, 20]. In addition, electromagnetic waves emitted by mobile phones have been shown to cause tight junction degeneration starting from intrauterine life, with a decrease in ZO-1 intensity in the urothelium, an increase in degranulated mast cells, and an increase in oxidative stress in the urinary bladder [21] and neuronal injury with oxidative stress increase in the brain [22]. It is impossible to avoid mobile phone technology, especially with the current COVID-19 pandemic where children, adults, and even pregnant women are using them for many purposes such as communication, education, etc. However, all these studies have shown that environmental factors in the prenatal period have a negative impact on the development of germ cells and the function of the blood-testis barrier, resulting in spermatogenesis disorder.

Obesity is considered as a global health problem of the 21st century. It is associated with the development of many health disorders, including cardiovascular and hepatorenal failures, type 2 diabetes, respiratory and musculoskelatal disorders as well as infertility [23]. Obesity is usually associated with excessive intake of foods high in fat and sugar, a sedentary lifestyle, genetic predisposition, or a combination of these factors [24]. Increased visceral obesity affects the reproductive function such as steroidogenesis and spermatogenesis as well as peripheral and testicular oxidative stress [25]. It has been shown that high-fat diet induced obesity causes hiperlipidemia, hiperleptinemia, alteration of hormone levels (including FSH, LH, estrogen, and testosterone) in serum, degeneration of seminiferous tubule morphology with increase in apoptosis, degeneration of blood-testis barrier structure with decrease in ZO-1, occludin, and decrease of gap junction protein CX-43, alteration of sperm parameters, increase in testicular inflammation, and oxidative stress [26-29]. However, moderate swimming exercise has been shown to improve all serum and testicular parameters including the blood-testis barrier integrity evaluated in high-fat diet induced obese rats [27, 28]. In addition, the administration of Myrtus communis L. an extract, which is rich in antioxidant components has been shown to ameliorate testicular damage and sperm parameters [26]. In experimental studies of obesity-induced male infertility, impaired blood-testis barrier has also been observed. In addition, the increasing sedentary lifestyle and consumption of high calorie foods are associated with the COVID-19 pandemic that has occurred worldwide in the last two years may cause a rapid increase in male infertility. Therefore, experimental studies are valuable in demonstrating the therapeutic role of lifestyle changes and consumption of foods high in antioxidants in improving male infertility caused by obesity.

Various methods of contraception are used in male sterilization. The most commonly used method is vasectomy. A disrupted blood-testis barrier with a decrease in occludin density and degenerated seminiferous tubules with an increase in apoptotic cells have been observed 6 months later in bilaterally vasectomized rats [8]. This has also been mentioned in the non-surgical reversible and irreversible male infertility models as male contraceptives [30]. In the study by Wong et al., synthetic occludin peptides and testosterone/estrogen implants have been shown to cause disruption of Sertoli cell junction permeability and spermatogenesis, which is reversible [31]. However, administration of the environmental toxin cadmium chloride causes irreversible degeneration of the blood-testis barrier [30] and impaired expression of gap junction protein CX-43 [32]. Moreover, the degenerative effects of thereupeutic agent cisplatin on ultrastructure of blood-testis barrier have been shown in different studies [33, 34]. These studies have indicated that reproductive surgery and various chemical agents degenerate germ cells, integrity of Sertoli cells, reduce sperm production, and thereby cause subfertility or infertility.

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In these experimental models, the degeneration of germ cells and the blood-testis barrier have been shown to be reversible or irreversible, which also give an information for the development of male contraceptives.

Conclusion

In conclusion, Sertoli cells have crucial role in the maintenance of spermatogenesis. Various experimental studies show the deleterious effects of environmental factors such as hiperthermia, radiation and electromagnetic waves, toxic substances (cadmium chloride, arsenic, etc.), chemotherapeutic agents (cisplatin, metothrexade, etc.), vasectomy operation, high calorie diet on the tight junction as well as the gap junction and ectoplasmic specialisations. Damage to the junctional complexes may also play a role in the development of male infertility. Demonstrating how the blood testicular barrier as well as other junctional complexes are affected in various experimental testicular injury models shows that it may give an information in both the prevention of male infertility and the development of male contraceptive methods.

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Case Report

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A rare cause similar to inguinal hernia in pregnancy: Two cases of round ligament varicosity

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ABSTRACT

Round ligament varicosities (RLVs) which are a very rare cause of inguinal swelling can mimic an inguinal hernia. RLVs should be considered in the differential diagnosis of inguinal swelling in a female, especially during pregnancy. The diagnosis of RLV can be established on Gray-scale with color Doppler sonography, and, if diagnosed correctly, unnecessary intervention may be prevented. We report two cases of round ligament varicosities in a 30-year-old woman at 30-week gestation and 34-year-old woman at 24-week gestation; these patients were diagnosed using ultrasonography and spontaneously resolved after delivery.
Keywords: Round ligament, Pregnancy, Inguinal hernia, Ultrasonography

1. INTRODUCTION

Inguinal region masses are not common in the human female and have a lot of differential diagnoses including round ligament varicosities, inguinal hernia, mesothelial cysts, lymphadenopathy, endometriosis, subcutaneous lipoma, cyst of Nuck (persistent embryonic remnants of the process vaginalis with cyst formation), vascular aneurysms, soft tissue malignancies, abscess, and cystic lymphangiomas [1,2].

Round ligament varicosities (RLVs) are rarely seen during pregnancy. They can cause pain and can easily be mistaken for an inguinal hernia because clinically, symptoms and signs are similar. Gray-scale and Doppler ultrasonography (US) can precisely diagnose RLVs. Distinguishing between varicosities and hernias is important in order to avoid unnecessary interventions. Here we report two pregnant women with RLV. These patients were diagnosed using ultrasonography and spontaneously resolved after delivery. By increasing the awareness of round ligament varicosities as part of the differential diagnosis and by highlighting the importance of ultrasound examination, unnecessary surgery can be avoided.

CASE REPORTS

Case 1

A 30-year-old primipara woman presented at 30-week gestation with a palpable and painless mass in her right inguinal region. After an examination by the obstetrician, the patient was referred to our radiology department for US examination with the suspicion of an inguinal hernia. Gray-scale US showed a mass in the right inguinal region composed of multiple serpentine tubular cystic structures which became more prominent during the Valsalva maneuver (Figure 1). Color Doppler US confirmed that a venous flow was detected in the tubular structures. Expansion and flow increase was detected in the venous structures with the Valsalva maneouver (Figure 2). There was no sonographic evidence of a herniated bowel, thrombus, or adenopathy. Because of these findings, the patient was finally diagnosed to have RLV. After two weeks postpartum the RLV spontaneously regressed and the symptoms completely resolved.

Case 2

A 34-year-old multipara woman presented at 24-week gestation with a palpable and painful mass in the left inguinal region.

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Gray-scale US showed a mass in the left region composed of multiple serpentine tubular cystic structures. Color Doppler US revealed hypervascularity and venous flow within the lesion (Figure 3). There was no evidence of a hernia or lymphadenopathy on US. Omentum, bowel, or thrombus was not identified in the lesion. Based on the US results, she was diagnosed as having round ligament varicosity, and the symptoms spontaneously resolved after two weeks postpartum.

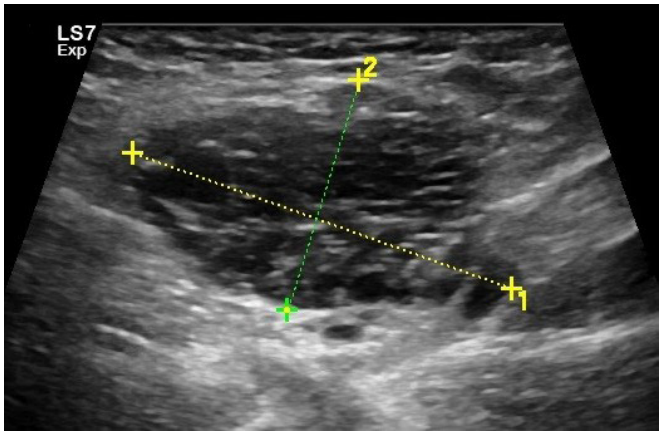

Figure 1. *A 30-year-old woman with round ligament varicosities. Gray-scale US imaging shows tubular, tortuous anechoic cyctic structures in the right inguinal region (Case 1).*

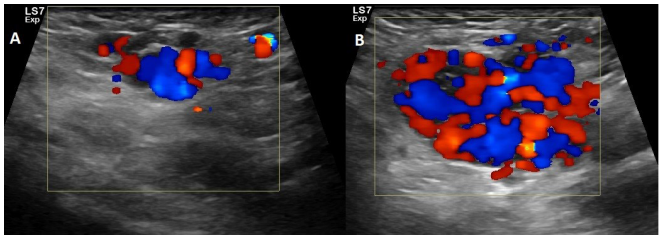

Figure 2. *(A) Color Doppler imaging shows multiple dilated veins with blood flow and (B) the mass shows dilatation and increased flow during the Valsalva maneuver (Case 1).*

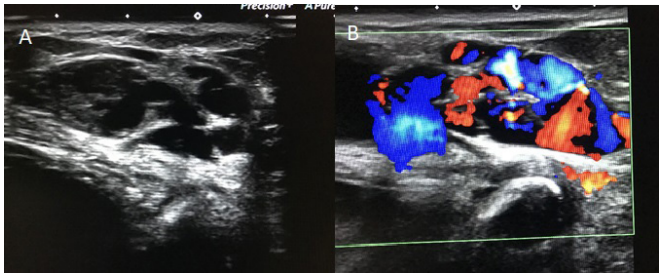

Figure 3. *A 34-year-old woman at the 24th week of pregnancy with round ligament varicosities. Gray-scale (A) and color Doppler imagings (B) show a mass in the left inguinal region composed of multiple serpentine tubular cystic structures filled with color on Doppler imaging (Case 2).*

2. DISCUSSION

Anatomically, the round ligament is a structure that extends from the lateral part of the uterus and courses through the internal abdominal ring and inguinal canal to the labia majora. The round ligament contains veins, arteries, lymphatics, and nerves. RLVs arise from the veins draining the round ligament and inguinal canal into the inferior epigastric vein [3].

During pregnancy, an increase in blood volume and venous return and an increased level of progesterone cause venous dilation along with muscle relaxation. An additional cause of RLVs in pregnancy is the increased pressure on the pelvic veins caused by the gravid uterus. These reasons make RLV a diagnosis almost exclusively in pregnant women [4]. RLV is usually unilateral, more on the right side; one third of the cases in the literature are bilateral [5]. It presented on the right side in our first case and on the left side in the second case. The coexistence of lower limb and labial varicosities might be a clue to the diagnosis of RLVs [1]. In our cases, there was no varicosity anywhere else. Like our study, in the 26-case study of Ryu et al., all the cases except one were treated conservatively and no specific complication were observed [5]. The studies of Garcia-Paredes et al., and Cicilet et al., are similar to our study [3,4].

Clinically, inguinal hernias and RLVs are not distinguishable because of similar presentations which include swelling with or without pain at the second or third trimester of pregnancy [6]. It can be easily misdiagnosed as an inguinal hernia; however, an inguinal hernia that appears for the first time during pregnancy is rare because most of the intra-abdominal structures that could potentially fill the hernia sac will be pushed aside by the growing uterus. The enlarged uterus pushes the intestines away from the inguinal canal and thus blocks the internal inguinal ring [6]. RLVs are more common than inguinal hernias, and so US imaging is important for making the diagnosis. US and Doppler US are used for a definitive diagnosis. On a Gray-scale US, a "bag of worms' appearance associated with multiple dilated drainage veins and on color Doppler US, the existence of venous flow in dilated veins and increasing venous flow with the Valsalva maneuver confirm the diagnosis. The Valsalva maneuver is important in this examination because the venous flow may be subtle at rest [4]. RLVs are often managed conservatively and most of them resolve spontaneously postpartum [7]. The other differential diagnoses of RLVs include lymphadenopathy, cystic lymphangioma, endometriosis, vascular aneurysm, soft tissue malignancies, abscess formation, and subcutaneous lipoma, all of which have characteristic findings on US imaging [1,8]. In inguinal hernias, a herniated bowel can be recognized by the presence of peristalsis, mucosal blood flow, or mesenteric fat by US and Doppler US imagings [9]. Lymph nodes may show a hypoechoic reniform appearance with an echogenic central hilum that demonstrates flow on Doppler imaging. The US findings of the lymphangioma or endometrioma are nonspecific but may usually appear as a well-defined, unilocular, or multilocular cystic mass containing diffuse hypoechoic homogeneous material. RLVs are not an emergent condition and most of them resolve spontaneously postpartum. If RLVs

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are diagnosed correctly, unnecessary intervention may be prevented [5, 8].

Conclusion

Round ligament varicosities are a very rare cause of inguinal swelling and may be mistaken for an inguinal hernia. US imaging with color Doppler US, especially in pregnant women with inguinal swelling, are diagnostic imaging methods for the diagnosis of RLV. A correct diagnosis is also of great importance in order to avoid unnecessary surgical operations.

Patient Consent: Both patients gave their consent for images and other clinical information relating to their cases to be reported in a medical publication.

Conflict of interest: The author has no conflicts of interest to declare.

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