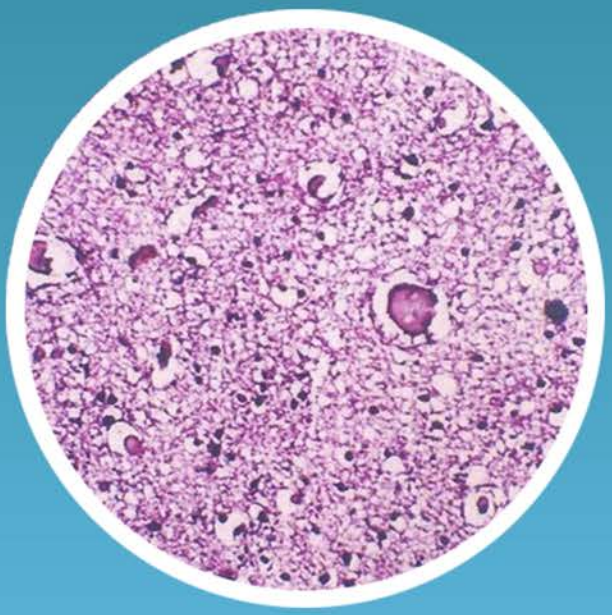
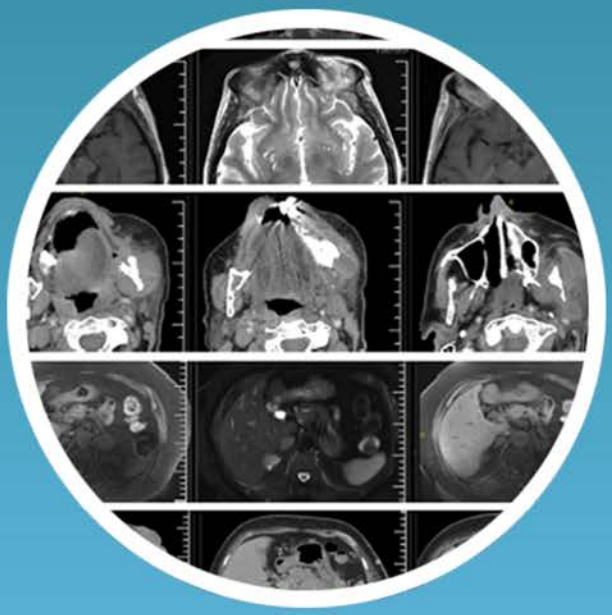
Volume 9 · Issue 6 · November 2023
e-ISSN: 2149-3189

The European Research Journal





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Abstracting and Indexing

The journal is abstracted and indexed with the following: ULAKBIM TR Index (ULAKBİM TR DİZİN), NLM Catalog (NLM ID: 101685727), Google Scholar (h-index: 12), Index Copernicus (ICV 2022: 100), EMBASE, ProQuest Central, EBSCO Academic Search Ultimate, ROAD, SciLit, MIAR (ICDS 2021: 3.8), J-Gate, SHERPA/RoMEO, BASE, EZB, CrossRef, JournalTOCs, WorldCat, TURK MEDLINE, Turkish Citation Index, EuroPub, OpenAIRE, ResearhGate, SOBIAD, Advanced Science Index, ScienceGate, OUCI, Publons, (Clarivate Web of Science)

Publisher

The European Research Journal (EuRJ)
Prusa Medical Publishing
Konak Mh. Kudret Sk. Şenyurt İş Mrk. Blok No:6 İç kapı no: 3
Nilüfer/Bursa-Turkey
info@prusamp.com

www.dergipark.org.tr/eurj/
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e-ISSN: 2149-3189

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The European Research Journal 2023;9(6):1277-1285 Original Article

DOI: 10.18621/eurj.1082965 *Rheumatology*

Interleukin-23 receptor gene polymorphisms in osteoporosis

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ABSTRACT

Objectives: Osteoporosis (OP) is a usual disease with a possible genetic predisposition. IL-23 plays a role in physiological bone remodeling and regulates the activity of cells of the bone either directly or indirectly on bone-resorbing osteoclasts as well as on bone-forming osteoblasts. Recent animal and human trials have revealed the main pro-osteoclastogenic activities for the IL-23 pathway. We examined nine single nucleotide polymorphisms (SNPs) in the interleukin-23 receptor (IL-23R) in 100 OP patients and gender- and age-matched 96 healthy volunteers. The most analyzed SNPs in the recent rheumatology literature were selected.

Methods: In addition to gene polymorphisms several laboratory parameters (osteocalcin, parathormone, vitamine D) were investigated. Independent Samples t-test and Mann-Whitney-U test were used to compare several demographic and clinical parameters between the groups. P - value < 0.05 was accepted to be statistically significant.

Results: Having the heterozygous GA genotype of IL-23R rs1004819 and the heterozygous CT genotype of Il-23R rs7530511 significantly increase the risk of developing OP (adjusted OR: 3.51, $p = 0.031$ and OR: 2.41, $p = 0.027$, respectively). The wild homozygous GG genotype of Il-23R rs11209032 had higher osteocalcin levels compared with the mutant homozygous AA genotype (18.75 ± 9.76, $p = 0.009$).

Conclusions: Our findings suggest that several IL-23R gene polymorphisms are seen more often in osteoporosis patients than in healthy volunteers. In addition, some SNPs were related to higher serum osteocalcin levels.

Keywords: Osteoporosis, interleukin 23 receptor, gene polymorphisms, osteocalcin

Osteoporosis (OP) is a widespread health problem that leads to significant morbidity and mortality, especially related to fractures. In healthy individuals, both osteoblasts and osteoclasts sustain bone homeostasis owing to controlled balanced activity including bone-forming or bone-resorbing, respectively [1]. Interleukin-23 (IL-23) is a heterodimeric proinflammatory cytokine, and includes a p40 and a p19 subunits. The p19 subunit has specific and increased attention for the interleukin-23 receptor (IL-23R). IL-23 regulates the activity of an immune system and promotes inflammation through the arrangement of IL-23R. IL-23R also acts a part in signal transduction in the IL-23/IL-17 pathway [2]. IL-23 can play a role in physiological bone remodeling and regulates the activity of cells of the bone either directly or indirectly

Received: ***March 4, 2022;*** *Accepted:* ***June13, 2022;*** *Published Online:* ***January 10, 2023***

How to cite this article: *Ulutaş F, Çetin GO, Çobankara V. Interleukin-23 receptor gene polymorphisms in osteoporosis. Eur Res J 2023;9(6):1277-1285. DOI: 10.18621/eurj.1082965*

e-ISSN: 2149-3189

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on bone-resorbing osteoclasts as well as on bone-forming osteoblasts [3]. Recent animal and human trials have revealed the main pro-osteoclastogenic activities for the IL-23 pathway. The mice who had bone marrow cells without IL-23p19-/- expression, had a lower differentiating capacity of osteoclasts, reduced osteoclast formation, and subsequently less bone resorptive activity. On the contrary, overexpression of IL-23 causes systemic bone loss in mice [4]. IL-23 has both direct and indirect effects on osteoclasts through T cells, and synovial fibroblasts in addition to osteoclast precursor cells [5]. IL-23 can induce osteoclastogenesis in a few different ways: 1) overexpression of receptor activator of nuclear factor-kB (RANK) on osteoclast precursor cells [6]; 2) overexpression of receptor activator of nuclear factor-kB ligand (RANKL) on fibroblasts or T-helper cells; 3) activation of DNAX activating protein of 12kDa-(DAP12) ITAMs through independent of RANKL; 4) stimulation of tartrate-resistant acid phosphatase (TRAP) activity of osteoclasts [7]. By supporting the whole pathophysiological mechanism, preservative effects of anti-IL-23 treatment on bone loss have been emphasized by recent studies [8].

In addition to pro-osteoclastogenic capacity, IL-23 has also pleiotropic roles on bone-forming osteoblasts. IL-23 may directly regulate osteoblast formation as its binding to the IL-23R on human mesenchymal stem cells leads to highly-expressed osteoblast-related genes. IL-23 may affect bone formation through the induction of IL-17 and IL-22. Increased expression of IL-22 leads to the new periosteal bone formation through ignal transducer and activator of transcription 3 (STAT3) activation and increased expression of genes that regulate the bone formation, including the Wnt family members. In vitro studies, IL-23R-/- mice had lower cortical bone mass related to indirect effects of IL-23R on osteoblasts [9]. Also, IL-23 can reduce osteoclastogenesis via the induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) in murine T cells, which can reduce osteoclast formation [10].

Recently, polymorphisms of the IL-23R were investigated in many inflammatory rheumatic diseases. A potential relationship with susceptibility to rheumatoid arthritis was emphasized by authors [11]. The variants of the IL-23R were related to psoriatic skin disease whereas were not related to a joint disease such as psoriatic arthritis (PsA) [12]. Also, the SNPs in the IL-23R or the IL-23 cytokine are well-known genetic factors in spondyloarthritis related to human enthesitis. So blockade of IL-23 pathway obtained therapeutic improvement in PsA with enthesitis [13]. In addition to the above-mentioned inflammatory diseases, Kasamatsu T *et al* revealed the association between the IL-23R HH genotype and plasmacytoma and bone lesions in Multiple Myeloma [14]. Also, recent studies revealed that polymorphisms of the IL-23R gene (rs1569922, rs7539625, and rs4655686) were significantly associated with an increased risk of osteonecrosis of the femoral head [15]. In the presence of all this knowledge, we aim to investigate IL-23R gene polymorphisms in OP patients, hypothesizing that it may be associated with metabolic bone diseases. The most analyzed nine single nucleotide polymorphisms (SNPs) in recent literature and in the above-mentioned studies were selected. These gene polymorphisms in the IL-23R gene were rs11805303, rs7530511, rs1004819, rs11209026, rs10489629, rs11209032, rs2201841, rs7517847 and rs10889677 subsequently.

METHODS

Patient Selection

We included 196 participants in the present study (96 participants for the control group and 100 participants for the OP group). All OP patients had no other systemic diseases. All of the patients were initially diagnosed in the rheumatology outpatient clinics between 2014-2015. Sex-, gender-, body mass index (BMI)-matched healthy individuals were enrolled voluntarily among subjects who did not have any inflammatory and/or autoinflammatory disease and/or drug use. BMI is equalized initially to form homogeneous patient groups. It was not used as a covariate factor in any of the gene examinations. BMIs of the whole study group were calculated by taking a person's weight, in kilograms, divided by their height, in meters squared [16]. This gene study was a single-center experience that was conducted in the selected regional health center where many patients have been evaluated daily from various provinces around the South Aegean region.

DNA Extraction and Sequence Analysis

All included individuals signed up consent forms for further investigation. 2 ml of venous blood from each subject was collected in ethylenediaminetetraacetic acid (EDTA) tubes for obtaining deoxyribonucleic acid (DNA) isolation, and stored at -80 °C. By using 1 microL of the tissue digest solution, a polymerase chain reaction (PCR) was used for the amplification of the target DNA. A "PCR-Restriction Fragment Length Polymorphism' (RFLP) method was applied for the examination of the IL-23R gene-related polymorphisms. Genotyping and allele identification was made after acquiring polymerase chain reaction (PCR) products using a 2.0% agarose gel electrophoresis. PCR products were restricted by specific enzymes. A total of nine candidate polymorphisms were examined. The SNP numbers of the polymorphisms and the enzymes used were as follows: rs11805303 (MnlI enzyme), rs7530511 (HphI enzyme), rs1004819 (Taal enzyme), rs11209026 (Hpy188I enzyme), rs10489629 (SspI enzyme), rs11209032 (BseMI enzyme), rs2201841 (HpyF3I enzyme), rs7517847 (BseMII enzyme) and rs10889677 (MnlI enzyme). Successfully genotyping was made for all subjects. All patients were also analyzed for serum osteocalcin (OC), parathormone (PTH), and vitamin D levels.

This study was conducted after the approval of the local Ethics Committee of our tertiary health center (Pamukkale University Ethics Committee approved by a number and date with June 206/2016), by following the Helsinki declaration.

Statistical Analysis

Initially, we determined an index study. According to the reference study results; they had a medium effect size (O.R=0.55) [17]. For this effect size, if we include 93 participants we can achieve power at %80 level with %95 confidence level. Since our study was planned to be in 2 groups, we included 196 participants in the present study (96 participants for the control group and 100 participants for the OP group). Descriptive statistics for the continuous variables were given as median, minimum, and maximum according to the distribution of the data. Categorical variables were reported as frequency and percentage. The normality of quantitative variables was checked with the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests. To compare several demographic and clinical parameters between the groups the Independent Samples t-test was used when the numerical data were normally distributed and the Mann-Whitney U test was used otherwise. To compare groups according to categorical variables, the Pearson Chi-Square test was used when the expected value for each cell was above 5 and the Fisher-Freeman-Halton test was used when the expected value was below five. The risk factors for osteoporosis were evaluated with univariate and multiple logistic regression analyses. Both clinical and statistical significance was considered for the variables which were included in the multivariate model. Statistical analyses were performed with the Jamovi project (2020), Jamovi (Version 1.8.1) [Computer Software] (Retrieved from https://www.jamovi.org), and JASP (Version 0.14.1.0) (Retrieved from https://jasp-stats.org) programs and statistical significance (p - value) was accepted to be 0.05.

RESULTS

Patient's Characteristics

The mean age was 53.0 ± 9.9 years in the OP group, and 52.6 ± 12.8 years in the healthy control group. The groups were statistically similar in terms of age, gender, and BMI. Seventy-six (76) % of the control group and 78 % of the osteoporosis group were females (p = 0.876) (Table 1). The laboratory levels of serum PTH, OC, and vitamin D were shown in Table 1. Only, osteocalcin levels were higher in OP patients than healthy controls.

Distributions of Alleles

Genotypes and allele distribution for each SNPs of 100 OP patients and 96 healthy controls are shown in Table 2. The genotypes with rs11805303(TT), rs10889677(AA), and rs7530511(CT) polymorphisms in the IL-23R gene were seen more often in OP patients than healthy controls. In addition, the TT genotype with rs2201841, GG genotype with rs11209032, AA genotype with rs10489629, and TT genotype with rs7517847 of the IL-23R gene were detected more frequently in OP patients (Tables 2 and 3).

Having the GA genotype of IL-23R rs1004819 and the CT genotype of IL-23R rs7530511 increase the risk of developing OP with a statistical significance (adjusted OR: 3.51, p = 0.031 and OR: 2.41, p = 0.027,

Table 1. Patient characteristics of the whole study group

Variables	Osteoporosis (n = 100)	Healthy controls (n = 96)	*p* value
Age	52.98 ± 9.87	52.55 ± 12.7	0.802
Gender (F/M)	78/22	73/23	0.876
BMI (kg/m2)	26.73 ± 4.46	27.18 ± 5.40	0.525
Lomber total 1-4 (T score)	-3.13 ± 1.02	-0.77 ± 1.1	**0.0001**
Femur neck (Tscore)	-1.56 ± 1.07	-0.40 ± 1.0	**0.0001**
PTH (mg/dl)	58.81 ± 30.4	52.5 ± 25.04	0.577
OCN (ng/ml)	16.32 ± 9.07	13.06 ± 11.07	**0.005**
Vitamin D (ng/ml)	24.05 ± 13.7	21.09 ± 12.62	0.321

Data are given as mean ± standard deviation. F = female, M = male, BMI = body mass index, PTH = parathormone, OCN = osteocalcin. Independent Samples t-test was used.

respectively) (Table 3).

Having the subsequent genotypes with IL-23R gene polymorphisms with rs2201841(TC, CC), rs7517847 (TG, GG), rs11209032 (GA, AA), and rs10489629 (GA, GG) had protective roles from OP with a statistical significance for each one (Table 3).

There was a statistical significance among rs11209032 different alleles for OC levels ($p = 0.009$). The GG genotype of Il-23R rs11209032 had higher OC levels than the AA genotype (18.75 ± 9.76, $p = 0.009$). No other difference was detected for PTH or Vitamin D levels (Table 4).

DISCUSSION

To the best of our knowledge, the study firstly investigates the association between IL-23R gene polymorphisms and OP in our population. Our findings suggest that several SNPs of the IL-23R gene could play a role in the predisposition to OP. The surprising conclusion was unusual high Odds ratios (ODs) for each susceptible SNPs when compared with usual genome-wide association studies. This condition may be explained by the that the study includes a relatively small patient size compared with population-based cohort studies. Also, patient selection bias could be aforementioned due to being a single-center experience. Although this conclusion needs multi-center studies including a large population to confirm the clinical significance of this association, literature involves a few trials about IL-23R gene polymorphisms in different bone diseases. As mentioned above, recently, IL-23R H3Q was investigated for susceptibility to multiple myeloma. Although the result was negative, this study showed the association between IL-23R gene polymorphism and bone disease. According to the study, having IL-23R HH genotype was related to bone lesions and poor prognosis [14].

Many trials in the literature investigate the potential effects of variable cytokine gene polymorphisms other than the IL-23R gene in OP patients. In the recent literature, the carriers of the IL-6 GG genotype had lower bone mineral density (BMD) values among postmenopausal women who were involved in the study designed to investigate OP-related cytokine gene polymorphisms including IL-1beta, IL-2, IL-6 [18]. In addition to IL-6, polymorphisms of the IL-17F gene were significantly associated with bone mineral density in a Japanese cohort [19]. On the contrary, there was no association between BMD and glutathione S-transferase (GST) and progesterone receptor gene (PROGINS) polymorphisms in OP patients [20]. This study is the first one that reveals the association between IL-23R gene polymorphism and OP.

Serum OC levels were compared between postmenopausal cases and controls in a comprehensive meta-analysis, and the analysis revealed no significant difference in serum OC levels. This condition was attributed to heterogeneous OC molecules in the circulation that can be influenced by glucose metabolism [21]. In another study, serum OC concentration was

Table 2. IL-23R gene genotype and allele distribution in OP patients and control group

IL-23R polymorphism	Osteoporosis (n = 100)	Healthy controls (n = 96)	p value
rs11805303			
(mutant) TT, n (%)	22 (22)	(-)	**< 0.001**
(heterozygous) CT, n (%)	46 (46)	61 (63.5)	
(wild) CC n, (%)	32 (32)	35 (36.5)	
C allele	110 (55)	131 (68.3)	**0.007**
T allele	90 (45)	61 (31.7)	
rs10889677			
(mutant) AA, n (%)	16 (16)	-	**< 0.001**
(heterozygous) CA, n (%)	49 (49)	60 (62.5)	
(wild) CC, n (%)	35 (35)	36 (37.5)	
C allele	119 (59.5)	132 (68.8)	0.056
A allele	81 (40.5)	60 (31.2)	
rs1004819			
(mutant) AA, n (%)	14 (14)	11 (11.5)	0.686
(heterozygous) GA, n (%)	48 (48)	43 (44.8)	
(wild) GG, n (%)	38 (38)	42 (43.8)	
G allele	124 (62)	127 (66.1)	0.393
A allele	76 (38)	65 (33.9)	
rs2201841			
(mutant) CC, n (%)	8 (8)	10 (10.4)	**< 0.001**
(heterozygous) TC, n (%)	41 (41)	82 (85.4)	
(wild) TT, n (%)	51 (51)	4 (4.2)	
T allele	143 (71.5)	90 (46.9)	**< 0.001**
C allele	57 (28.5)	102 (53.1)	
rs11209032			
(mutant) AA, n (%)	20 (20)	53 (55.2)	**< 0.001**
(heterozygous) GA, n (%)	43 (43)	37 (38.5)	
(wild) GG, n (%)	37 (37)	6 (6.3)	
G allele	117 (58.5)	49 (25.5)	**< 0.001**
A allele	83 (41.5)	143 (74.5)	
rs7530511			
(mutant) TT, n (%)	-	1 (1)	**0.046**
(heterozygous) CT, n (%)	24 (24)	11 (11.5)	
(wild) CC, n (%)	76 (76)	84 (87.5	
C allele	176 (88)	179 (93.3)	**< 0.001**
T allele	24 (12)	13 (6.7)	
rs11209026			
(mutant) AA, n (%)	-	1 (1.04)	0.066
(heterozygous) GA, n (%)	11 (11)	21 (21.8)	
(wild) GG, n (%)	89 (89)	74 (77.08)	
G allele	189 (94.5)	169 (88.1)	**0.036**
A allele	11 (5.5)	23 (11.9)	
rs10489629			
(mutant) GG, n (%)	31 (31)	45 (46.9)	**< 0.001**
(heterozygous) AG, n (%)	33 (33)	46 (47.9)	
(wild) AA, n (%)	36 (36)	5 (5.2)	
A allele	105 (52.5)	56 (29.2)	**< 0.001**
G allele	95 (47.5)	136 (70.8)	
rs7517847			
(mutant) GG, n (%)	9 (9)	16 (16.7)	**0.005**
(heterozygous) TG, n (%)	42 (42)	54 (56.3)	
(wild) TT, n (%)	49 (49)	26 (27.1)	
T allele	140 (70)	106 (55.2)	**< 0.002**
G allele	60 (30)	86 (44.8)	

IL-23R = interleukin-23 receptor

Table 3. The comparison of the control and the osteoporosis groups according to the presence of several polymorphisms

		Group			Crude		Adjusted	
	Genotype	Control (n = 96)	Osteoporosis (n = 100)	*p* value*	OR [95%CI]	*p* value	OR [95%CI]	*p* value
rs11805303 (%)	TT	0 (0.0)	22 (22.0)	**< 0.001**	46533388.5 [0-Inf]	0.983	-	-
	CC	35 (36.5)	32 (32.0)		**Reference**		-	-
	CT	61 (63.5)	46 (46.0)		0.82 [0.45-1.52]	0.538	-	-
rs10889677 (%)	AA	0 (0.0)	16 (16.0)	**< 0.001**	43760378.41 [0-Inf]	0.986		
	CC	36 (37.5)	35 (35.0)		**Reference**		-	-
	CA	60 (62.5)	49 (49.0)		0.84 [0.46-1.53]	0.568	-	-
rs1004819 (%)	AA	11 (11.5)	14 (14.0)	0.686	1.41 [0.57-3.47]	0.459	1.48 [0.36-6.1]	0.591
	GG	42 (43.8)	38 (38.0)		**Reference**		**Reference**	
	GA	43 (44.8)	48 (48.0)		1.23 [0.68-2.25]	0.494	3.51 [1.12-10.93]	**0.031**
rs2201841 (%)	CC	10 (10.4)	8 (8.0)	**< 0.001**	0.06 [0.02-0.25]	**< 0.001**	0.04 [0.01-0.25]	**< 0.001**
	TT	4 (4.2)	51 (51.0)		**Reference**		**Reference**	
	TC	82 (85.4)	41 (41.0)		0.04 [0.01-0.12]	**< 0.001**	0.03 [0.01-0.11]	**< 0.001**
rs11209032 (%)	AA	53 (55.2)	20 (20.0)	**< 0.001**	0.06 [0.02-0.17]	**< 0.001**	0.01 [0.01-0.05]	**< 0.001**
	GG	6 (6.2)	37 (37.0)		**Reference**		**Reference**	
	GA	37 (38.5)	43 (43.0)		0.19 [0.07-0.50]	**< 0.001**	0.08 [0.02-0.32]	**< 0.001**
rs7530511 (%)	TT	1 (1.0)	0 (0.0)	**0.034**	0 [0-Inf]	0.987		
	CC	84 (87.5)	76 (76.0)		**Reference**			
	CT	11 (11.5)	24 (24.0)		2.41 [1.11-5.25]	**0.027**		
rs11209026 (%)	GG	75 (78.1)	89 (89.0)	0.062	0.44 [0.20-0.97]	**0.043**	0.32 [0.08-1.28]	0.106
	GA	21 (21.9)	11 (11.0)					
rs10489629 (%)	GG	45 (46.9)	31 (31.0)	**< 0.001**	0.1 [0.03-0.27]	**< 0.001**	0.03 [0.01-0.17]	**< 0.001**
	AA	5 (5.2)	36 (36.0)		**Reference**		**Reference**	
	GA	46 (47.9)	33 (33.0)		0.1 [0.04-0.28]	**< 0.001**	0.04 [0.01-0.17]	**< 0.001**
rs7517847 (%)	GG	16 (16.7)	9 (9.0)	**0.005**	0.3 [0.12-0.77]	**0.012**	0.19 [0.04-0.89]	**0.035**
	TT	26 (27.1)	49 (49.0)		**Reference**		**Reference**	
	TG	54 (56.2)	42 (42.0)		0.41 [0.22-0.77]	**0.005**	0.21 [0.07-0.6]	**0.004**

Data are given as frequency (%). *Pearson Chi-square test or Fisher Freeman Halton test was used. OR = Odds Ratio, CI = Confidence Interval

Table 4. Laboratory values in osteoporosis patients according to polymorphisms

	PTH	*p* value	Osteocalcin	*p* value	Vitamin D	*p* value
rs11805303						
TT (n = 22)	66.45 ± 32.38	0.252**	16.78 ± 7.41	0.310**	21.88 ± 13.58	0.248**
CC (n = 67)	54.28 ± 25.74		15.29 ± 10.77		20.61 ± 12.25	
CT (n = 107)	54.30 ± 27.57		13.95 ± 10.34		23.99 ± 13.78	
rs10889677						
AA (n = 16)	60.36 ± 31.17	0.516**	18.59 ± 9.54	0.150**	21.83 ± 17.17	0.573**
CC (n = 71)	57.76 ± 33.83		15.42 ± 10.23		21.37 ± 14.28	
CA (n = 109)	53.60 ± 22.23		13.71 ± 10.20		23.51 ± 11.97	
rs1004819						
AA (n = 25)	56.23 ± 26.56	0.463**	15.88 ± 10.72	0.687**	21.91 ± 11.96	0.716**
GG (n = 80)	52.79 ± 25.05		15.10 ± 10.19		21.85 ± 12.57	
GA (n = 91)	58.02 ± 30.09		14.08 ± 10.15		23.45 ± 14.28	
rs2201841						
CC (n=18)	68.42 ± 29.76	0.093**	17.25 ± 10.33	0.152**	21.23 ± 14.81	0.362**
TT (n = 55)	57.93 ± 31.87		16.36 ± 10.03		24.85 ± 13.75	
TC (n = 123)	52.78 ± 24.80		13.62 ± 10.19		21.79 ± 12.82	
rs11209032						
AA (n = 73)	53.83 ± 29.09	0.454**	**12.67 ± 10.80**	**0.009****	22.02 ± 11.80	0.613**
GG (n = 43)	60.93 ± 32.14		**18.75 ± 9.76**		21.43 ± 12.81	
GA (n = 80)	54.49 ± 23.42		14.44 ± 9.35		23.75 ± 14.79	
rs7530511						
CC (n = 160)	56.62 ± 29.32	0.302*	14.44 ± 10.14	0.322*	22.28 ± 13.34	0.644*
CT (n = 35)	51.26 ± 18.52		16.33 ± 10.51		23.42 ± 12.80	
rs11209026						
GG (n = 164)	56.39 ± 28.96	0.404*	15.19 ± 10.43	0.146*	22.94 ± 13.59	0.413*
GA (n = 32)	51.91 ± 19.62		12.32 ± 8.73		20.83 ± 11.58	
rs10489629						
GG (n = 76)	56.28 ± 32.94	0.550**	14.73 ± 10.83	0.946**	22.53 ± 13.26	0.629**
AA (n = 41)	58.58 ± 25.44		15.12 ± 8.98		24.25 ± 13.07	
GA (n=79)	53.54 ± 22.97		14.52 ± 10.31		21.80 ± 13.50	
rs7517847						
GG (n = 25)	49.17 ± 20.75	0.248**	15.38 ± 9.86	0.477**	22.13 ± 11.40	0.920**
TT (n = 75)	54.95 ± 26.31		15.68 ± 10.55		23.09 ± 14.34	
TG (n = 96)	57.90 ± 30.10		13.81 ± 10.05		22.33 ± 12.99	

Data are given as mean ± standard deviation. *Independent Samples t-test was used. **One-Way ANOVA was used.

negatively correlated with fasting plasma glucose and predicted an increased risk for diabetes in postmenopausal women [22]. Although having comorbidities such as diabetes mellitus and insulin resistance were excluded in this study, the correlation between serum glucose and OC levels was not analyzed. Despite matching patient groups according to BMIs and absence of diabetes mellitus, OP patients had higher OC levels than healthy controls. However, it should be kept in mind, the mean BMIs of the two groups are high that may be related to insuline resistance. Recently, some authors from India investigated serum levels of OC in patients with osteopenia and osteoporosis with enzyme-linked immunosorbent assay (ELISA). They reported that having higher levels of OC upper than 14.9 ng/mL may be sensitive and cost-effective screening for low bone density. The cut-off value was higher in osteoporotic patients than in osteopenic patients [23]. In support of the previous study, high OC levels may be explained by lower bone mineral densities. In addition, other studies showed that serum levels of phosphorus and alkaline phosphatase were positively correlated with serum OC levels rather than common genetic variants of the OC gene [24]. However, the studies related to OC polymorphism have inconsistent results. The polymorphism of the OC gene did not show any effect on bone quantity in the Hungarian population [25]. However, the rs1800247 polymorphism in the OC gene was related to fracture and serum total OC levels in Chinese patients [26]. When we analyzed the laboratory values according to the different genotypes, the only significant result was detected in rs11209032 of the Il-23R gene. Having the GG genotype of rs11209032 was related to higher OC levels than having the AA genotype.

Limitations

There are a few limitations of our study. Firstly, we did not analyze the potential confounder effects of other alleles. Secondly, more homogeneous and large patient groups may be obtained by matching based on other situations as smoking, ethnic differences, age at menarche, and numbers of parity. Ethnical differences were not classified due to features of the regional health center in where many patients from various provinces around the South Aegean have been evaluated daily. Also, we did not have an opportunity to determine serum levels of sIL-23R and IL-23. We could not look for the correlation between expression levels of IL-23 and IL-23R gene polymorphisms.

CONCLUSION

Our findings suggest that several analyzed IL-23R gene polymorphisms are seen more often in OP patients than in healthy individuals. So, the IL-23/Th17 pathway can be one of the underlying pathogenetic mechanisms in OP. We need multi-center large population studies to certify the clinical significance of this relationship between gene polymorphisms and susceptibility to the OP.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1286-1292 Original Article

DOI: 10.18621/eurj.1241649 *Paediatrics*

Assessment of the knowledge and use of hypertonic saline among doctors working in paediatrics departments of tertiary institutions in the five states of South-East Nigeria

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ABSTRACT

Objectives: This study aimed to evaluate the knowledge and use of hypertonic saline among doctors in the Southeast region of Nigeria.

Methods: It was a cross-sectional study conducted amongst 182 doctors in the paediatric departments of the six tertiary institutions in South Eastern Nigeria. Data to assess knowledge and use of hypertonic saline were collected using self-administered, structured questionnaires.

Results: After aggregating the knowledge questions (definition of hypertonic saline, knowledge of available concentrations and modes of administration) and categorizing knowledge into good or poor, 148 (81.3%) had good knowledge, while 34 (18.7%) had poor knowledge. Respondents who had ever seen an infusion of hypertonic saline were 93 (51.1%), while only 62 (34.1%) had ever used it during their practice. Among those who had used it, only 33 (18.1%) obtained it from their hospital pharmacy. Most respondents (91.2%) would support advocacy for its increased availability and use in Nigeria.

Conclusions: Our study demonstrated good knowledge of hypertonic saline, however, there is low usage due to unavailability. There is a need for collaboration between paediatricians, pharmaceutical companies and other stakeholders to create demand and initiate the production of hypertonic saline.

Keywords: Hypertonic saline, knowledge, doctors, use, developing country

Hypertonic saline is a crystalloid intravenous fluid that consist of sodium chloride (NaCl) dissolved in water with a greater sodium concentration than that found in normal blood serum or physiological saline

Received: January 25, 2023; Accepted: April 15, 2023; Published Online: April 18, 2023

How to cite this article: Ndu IK, Edelu BO, Iloh KK, Nduagubam OC, Iheji CC, Ezeudu CE, et al. Assessment of the knowledge and use of hypertonic saline among doctors working in paediatrics departments of tertiary institutions in the five states of South-East Nigeria. Eur Res J 2023;9(6):1286-1292. DOI: 10.18621/eurj.1241649

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(0.9% w/v) [1]. Commonly used preparations include 2%, 3%, 5%, 7%, and 23% NaCl [2].

It works primarily by an osmotic effect, although it has other mechanisms of action.[3] Through its osmotic effect, hypertonic saline draws fluid out of edematous cerebral tissues because it has a higher concentration of sodium and a lower concentration of water than blood. These concentration differences create an osmotic gradient that promotes excess water flow from cerebral tissue to the blood via osmosis[3, 4]. Other physiological effects beneficial to cerebral injury include hemodynamic, immunomodulatory, and neurochemical changes [5].

Hypertonic saline can treat hyponatremia and is an integral component of treatment for patients with life-threatening conditions such as severe hyponatremic encephalopathy, traumatic brain injury, and cerebral edema[1, 6]. Research shows that 3% hypertonic saline decreases intracranial pressure similarly to 20% mannitol, and both fluids have similar effects on systemic hemodynamics [7]. Central venous lines were until recently the main route of administration of 3% saline, to avoid infusion-related adverse events (IRAEs) in peripheral veins; however, studies have lately reported that administration of 3% NaCl is relatively safe safe through a peripheral vein [6]. Other uses include treatment of syndrome of inappropriate antidiuretic hormone secretion (SIADH) and cerebral salt wasting (CSW) syndrome [8]. Ophthalmic preparations are used to reduce corneal swelling, and nebulized hypertonic saline can also be used to treat bronchiolitis and cystic fibrosis [9-11]. Hypertonic saline has been reported to be more effective than normal saline in alleviating the symptoms of allergic rhinitis in children [12, 13].

Comparatively, hypertonic saline is superior to mannitol in the management of raised intracranial pressure (ICP) with a more sustained effect and can improve cerebral perfusion pressure more than mannitol [14, 15]. Also, rebound cerebral edema, which is a recognized side effect of mannitol is very uncommon with hypertonic saline [16].

However, despite all these beneficial uses of hypertonic saline, it is not commonly available in the Nigerian pharmaceutical market. The Group Chairman/Chief Executive Officer, Juhel Nigeria Limited (an Indigenous conglomerate founded in 1987 as the first pharmaceutical tablet manufacturing company in old Anambra State Nigeria), stated that though hypertonic saline could be produced in Nigeria, in the absence of demand, the substantial production costs cannot be sustained by manufacturers. (Dr. Ifeanyi Okoye, Ph.D, FPSN, mni, OFR. 2022, personal communication, 11th January, 2022) Thus with significant demand increases, this situation can be reversed easily. This study aimed to evaluate the knowledge and use of hypertonic saline in our region and hopefully stimulate awareness/increase demand so drug manufacturers can commence commercial production.

METHODS

Setting

This cross-sectional study was conducted amongst doctors in the paediatric departments of the six tertiary institutions in South Eastern Nigeria namely: Enugu State University Teaching Hospital, Parklane, Enugu; University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu; Nnamdi Azikiwe University Teaching Hospital; Federal Medical Centre Owerri; Federal Medical Centre Umuahia; Alex Ekwueme Federal University Teaching Hospital, Abakiliki.

Data Collection

Self-administered, structured questionnaires were given to consultant paediatricians, paediatric residents and house-officers who consented to participate in the study between 1st – 31st March 2022. The study variables were collected into the relevant sections of the questionnaire. In the first section, predictor variables which included sociodemographic features of participants such as age, sex, years of experience, and rank of staff, were collected. The second section of the questionnaire collected information that assessed respondents' level of (a) knowledge of hypertonic saline (b) of use of hypertonic saline.

The parameters that assessed the knowledge of hypertonic saline included: (i) Whether the respondents had ever heard of hypertonic saline (categorized as yes or no), (ii) source of information about hypertonic saline (information provided by the respondent), (iii) definition of hypertonic saline (categorized as correct and incorrect) based on the definition of hypertonic saline,[1] (iv) concentrations of hypertonic saline known by the respondent (information provided by the re-

spondent), and (v) modes of administration of hypertonic saline known by the respondent (information provided by the respondent).

The parameters that assessed the level of use of hypertonic saline included: (i) Whether the respondents had ever used hypertonic saline (categorized as yes or no), (ii) conditions hypertonic saline was used for (information provided by the respondent), (iii) where hypertonic saline was obtained from (information provided by the respondent), (iv) other indications for its use (information provided by the respondent), (v) contra-indications to the use of hypertonic saline (information provided by the respondent), (vi) complications of the use of hypertonic saline (information provided by the respondent), (vii) combination of fluids to constitute 3% hypertonic saline (information provided by the respondent), (viii) willingness of the respondent to use HS if available (categorize as yes or no) (ix) anticipated challenges to its production and use (information provided by the respondent).

Table 1. Demographic characteristics of the respondents

	Frequency	Percentage
Age (years)		
20-29	19	10.4
30-39	93	51.1
40-49	51	28.1
50-59	13	7.1
60-69	6	3.3
Sex		
Male	67	36.8
Female	115	63.2
Rank		
House officer	16	8.8
Junior resident	64	35.2
Senior resident	49	26.9
Consultant	53	29.1
Years of practice		
1-5	38	20.9
6-10	56	30.8
11-15	52	28.6
16-20	18	9.9
≥ 21	18	9.9

Ethical Considerations

Ethical approval was obtained from the Ethics and Research Committee of the ESUTH, Enugu (REF NO: ESUTHP/C-MAC/RA/034/VOL.3/171). Informed consent was obtained from the consultant paediatricians, paediatric residents and house-officers.

Statistical Analysis

Data was analyzed with IBM SPSS version 26 (Chicago, IL). Descriptive statistics such as frequency, percent, mean, and standard deviation were used to summarize categorical and continuous variables. Chi-square statistical test was used to test for association between categorical variables at a 5% level of significance.

RESULTS

A total of one hundred and eighty-two doctors from different cadres responded to the self-administered questionnaire. Their ages ranged from 23 to 68 years, with a mean age of 38.8 ± 8.2 years. There were 114 (62.6%) females and 68 (37.4%) males. Table 1 shows the rank and demographics of the respondents.

Knowledge

All of the respondents had heard of hypertonic saline. Their sources of knowledge are shown in Table 2. However, only 89 (48.9%) could define hypertonic saline correctly. Fifteen (8.2%) of the respondents did not attempt the definition, while the rest (42.9%) gave incorrect definitions. In terms of concentration, 3% saline seemed to be the commonest known concentration of hypertonic saline (145 respondents, 79.7%), 24 (13.2%) respondents were aware of 2% saline, 45 respondents (24.7%) knew 5% saline, while 17 (9.3%) and 18 (9.9%) respondents had knowledge of 7% and 23% saline respectively. Respondents who had ever seen an infusion of hypertonic saline were 93 (51.1%), while only 62 (34.1%) had ever used it during their practice. Among those who had used it, only 33 (18.1%) obtained it from their hospital pharmacy, although most (96.2%) considered it essential and were

Table 2. Sources of knowledge about hypertonic saline

Source	Frequency	Percentage
Medical school	113	62.1
Hospital in-service/ Training	78	42.9
Medical journals/ Books	53	29.1
Colleagues	57	31.3
Conferences/ Seminars/ Continuing education programs	51	28.0

ready to use it if available. For the modes of administration, the intravenous route was the most known (87.9%), while the ophthalmic route was the least known (1.6%). Only 14 (7.7%) respondents could list up to five modes of administration of hypertonic saline. After aggregating the knowledge questions (definition of hypertonic saline, knowledge of available concentrations and modes of administration) and categorizing knowledge into good or poor, 148 (81.3%) had good knowledge, while 34 (18.7%) had poor knowledge.

Use of Hypertonic Saline

The uses of hypertonic saline listed by respondents are shown in Table 3. The contraindications to hypertonic saline use listed by respondents include: Hypernatraemia (37.4%), Congestive Cardiac Failure (8.2%), Acute Kidney Injury (AKI) (8.2%), Hypertension (5.5%), Metabolic acidosis (3.8%) and Addison's disease (0.5%).

The complications enumerated by the respondents include: Rebound cerebral oedema (12.1%), Seizures (12.1%), Intracranial hypertension (5.5%), Hypernatremia (19.2%), Volume overload and hypertension (11.5%), Thrombophlebitis (6.0%), central pontine myelinolysis (9.9%), AKI (1.1%), Thromboembolism (2.7%).

Twenty (11.1%) of the respondents reported how to reconstitute hypertonic saline in the absence of commercially prepared solutions, using the following combinations: NaCl + NaH_2CO_3 (35%), NaH_2CO_3 + 5% Dextrose water (5%), KCl + NaCl (5%), KCl + 5% Dextrose water (10%), NaH_2CO_3 + 10% Dextrose water (25%), NaCl+10% Dextrose water (15%), and NaCl + 5% Dextrose water (5%).

There was no significant association between rank ($x^2 = 1.002$, $p = 0.317$), years of practice ($x^2 = 0.045$, $p = 0.831$) and knowledge of hypertonic saline. Similarly, there was no significant association between rank ($x^2 = 0.168$, $p = 0.682$), years of practice ($x^2 = 0.869$, $p = 0.351$) and use of hypertonic saline. No significant association was found between knowledge and use of hypertonic saline.

Most respondents (91.2%) would support advocacy for its increased availability and use in Nigeria. The challenges anticipated by respondents towards the production and use of HS in Nigeria are enumerated in Table 4.

Table 3. Respondents knowledge of indications for the use of Hypertonic saline solution

	Frequency	Percentage
Hyponatremia	98	53.8
Raised intracranial pressure	70	38.5
Bronchiolitis	31	17.0
Traumatic brain injury (TBI)	12	6.6
Nasal congestion	10	5.5
Croup	3	1.6
Sputum induction	3	1.6
Cerebral salt wasting (CSW) syndrome	3	1.6
Adrenal crisis	1	0.5
Cystic fibrosis	1	0.5
Syndrome of inappropriate antidiuretic hormone secretion	1	0.5

Table 4. Challenges anticipated by respondents towards the production and use of hypertonic saline in Nigeria

Challenges	Frequency	Percentage
Lack of awareness	54	29.7
Affordability	48	26.4
Fake products	3	1.6
Lack of advocacy by doctors	2	1.1
Lack of hospital guidelines	3	1.6

DISCUSSION

Hypertonic saline is being increasingly used in developed countries to manage various conditions, most notably raised intracranial pressure [17]. However, despite all the beneficial uses of hypertonic saline, it seems to be unavailable in the Nigerian pharmaceutical market. This may explain the number of respondents who could not define hypertonic saline correctly. Hypertonic saline solutions are prepared in concentrations that include 2%, 3%, 5%, 7%, and 23% NaCl; however, the 3% solution is the most widely used [14, 18, 19]. This is in keeping with our study, which reported that 3% saline seemed to be the most commonly known concentration of hypertonic saline. Overall knowledge of hypertonic saline in our study was good, which is probably a reflection of the strength of our medical curriculum and theoretical knowledge of our paediatricians. This is further buttressed by the fact that majority of the participants indicated medical school as the source of their knowledge about hypertonic saline. In a Survey Report about the Emigration of Nigerian Medical Doctors most of doctors (83%) who filled out the survey were based abroad and were licensed in Nigeria, indicating that they had completed their medical education in Nigeria and were deemed competent enough to practice in developed countries [20].

Indications for using hypertonic saline include hyponatremia, traumatic brain injury, cerebral edema,

Table 5. Association between rank, years of experience and knowledge of hypertonic saline

	Knowledge, n (%)			
	Good	Poor	χ^2	*p* value
Rank				
Resident	105 (83.3)	21 (16.7)	1.002	0.317
Consultant	40 (76.9)	12 (23.1)		
Years of practice				
≤ 10 years	77 (81.9)	17 (18.1)	0.045	0.831
> 10 years	71 (80.7)	17 (19.3)		

Table 6. Association between rank, years of experience and use of hypertonic saline

	Use of hypertonic saline, n (%)			
	Good	Poor	χ^2	*p* value
Rank				
Resident	42 (33.3)	84 (66.7)	0.168	0.682
Consultant	19 (36.5)	33 (63.5)		
Years of practice				
≤ 10 years	35 (37.2)	59 (62.8)	0.869	0.351
> 10 years	27 (30.7)	61 (69.3)		

Table 7. Association between knowledge and use of hypertonic saline

	Knowledge			
Use of hypertonic saline, n (%)	Yes	No	χ^2	***p* value**
Yes	47 (31.8)	15 (45.5)	2.248	0.134
No	101 (68.2)	18 (54.5)		

CSW syndrome and SIADH [17, 21, 22]. The commonest indications for use in the present study include, hyponatremia, raised ICP, bronchiolitis, and traumatic brain injury (TBI). On the other hand, Brenkert *et al*. [20] in 2013, in the United States of America, reported raised ICP as the commonest indication followed by diabetic ketoacidosis, and hyponatremia. Cystic fibrosis, a rare condition in our environment, was not surprisingly amongst the least mentioned indications in the present study. However, it is a common indication for use in Europe and the United States of America [10, 21].

Specific contraindications to hypertonic saline are largely unknown. However, in patients with congestive heart failure or renal insufficiency, it is advised to use hypertonic saline with caution because of their already increased fluid and sodium loads [17]. Similarly, respondents in the present study reported congestive heart failure or renal insufficiency as common contraindications in addition to hypernatremia. Although one respondent listed Addison's disease as a contraindication, hypertonic saline has been used to treat severe hyponatremia in a patient with primary adrenal insufficiency [22].

Most complications of hypertonic saline are associated with more extended infusion periods, when compared to bolus administration. Possible complications include hyperchloremic metabolic acidosis, hypernatremia, central pontine myelinolysis, and rebound cerebral edema. The most common adverse effects include infection at the IV site, thrombophlebitis, extravasation, and hypervolemia. These are related to intravenous route of administration. Similar complications were reported in our study, including rebound cerebral edema. However, rebound cerebral edema in hypertonic saline occurs less compared to mannitol [11, 16].

Our study revealed a scarcity of hypertonic saline in Nigeria's entire southeast region. Only one out of the six centres in the region had 3% saline in the hospital pharmacy. However, it is pertinent to note that even this had to be imported from a foreign company because there is no local production. Consequently, doctors attempt to reconstitute 3% hypertonic saline from readily available fluids in their centers. However, only seven respondents used the combination of normal saline and sodium bicarbonate (0.9% NaCl + $NaHCO_3$) found in the relevant literature [23].

There were no significant associations between knowledge, use, and demographics (cadre and years of experience). The unavailability of hypertonic saline in the south east region of Nigeria may explain this.

Most of the conditions requiring hypertonic saline are common in our environment, and most respondents indicated their readiness to use it if available. Therefore, efforts should be made to mitigate the challenges anticipated by the respondents, such as lack of awareness, affordability, and proliferation of fake products.

CONCLUSION

Our study demonstrated good knowledge of hypertonic saline, however there is low usage due to unavailability of the product. There is need for collaboration between paediatricians, pharmaceutical companies and other stakeholders such as National Agency for Food and Drug Administration and Control (NAFDAC), to create demand and initiate production of hypertonic saline.

Authors' Contribution

Study Conception: IKN, BOE; Study Design: IKN, CCI, KKI; Supervision: OCN, OCI; Funding: ONI, JE, NNO; Materials: CEE, OMI; Data Collection and/or Processing: JE, OCI, NNO; Statistical Analysis and/or Data Interpretation: IKN, BOE, KKI, OCN,

CCI, CEE, JE, OOI, ONI, OMI, LNNO, NNO; Literature Review: OCN, CEE, LNNO; Manuscript Preparation: IKN, KKI, CCI, BOE and Critical Review: IKN, BOE, KKI, OCN, CCI, CEE, JE, OOI, ONI, OMI, LNNO, NNO.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Disclaimer

The views expressed in the submitted article are that of the authors and not an official position of Enugu State University Teaching Hospital.

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The European Research Journal 2023;9(6):1293-1302 Original Article

DOI: 10.18621/eurj.1148316 *Women's Health and Diseases*

Relationship between emotional intelligence and prenatal attachment levels of women with healthy and high-risk pregnancies

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ABSTRACT

Objectives: Emotional intelligence enables pregnant women to effectively cope with environmental effects and stressors in a dynamic and changing process. This research was planned for evaluating the relationship between emotional intelligence and prenatal attachment levels of women with healthy and high-risk pregnancies.

Methods: Based on the descriptive research design and regression analysis, the research was performed with the participation of 281 pregnant women. Research data were collected through Information Form, Emotional Intelligence Self-Evaluation Scale (EISES) and Prenatal Attachment Inventory (PAI).

Results: As per means of scores obtained from the EISES and PAI by women with healthy (133.34 ± 32.30) and high-risk (126.81 ± 35.80) pregnancies, it was found that pregnant women had high levels of emotional intelligence and medium levels of prenatal attachment. Based on research variables, there were no statistically significant differences in means of scores obtained from the Scale and the Inventory by women with healthy and high-risk pregnancies ($p > 0.05$). It was found that women who voluntarily got pregnant had higher prenatal attachment levels and this difference was statistically significant ($x^2 = 102.81$, $p < 0.001$). It was identified that there was no statistically significant difference in means of scores obtained by pregnant women from the EISES ans PAI ($r = -0.060$, $p = 0.318$).

Conclusions: It was found that there was no statistically significant difference in prenatal attachment levels of women with healthy and high-risk pregnancies and their prenatal attachment levels had no statistically significant association with their emotional intelligence levels.

Keywords: Attachment, emotional intelligence, pregnant woman, prenatal attachment

In order to continue to have a healthy pregnancy period, the women must adapt to physiological, psychological and social changes which are experienced during pregnancy [1]. In this process, the pregnant women are guided by both conscious and unconscious motives such as happiness, joy, liking, being liked and narcissism. Pregnancy is a developmental crisis which obliges the woman to cope with feelings of ambiva-

Received: ***July 25, 2022;*** *Accepted:* ***December 1, 2022;*** *Published Online:* ***March 25, 2023***

How to cite this article: *Mecdi Kaydırak M, Küçük Yüceyurt N, Ertürk Z, Oskay Ü. Relationship between emotional intelligence and prenatal attachment levels of women with healthy and high-risk pregnancies. Eur Res J 2023;9(6):1293-1302. DOI: 10.18621/eurj.1148316*

e-ISSN: 2149-3189

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lence and to adapt to the new role definition [2, 3]. The adaptation of the woman to the pregnancy period is not only affected by environmental factors but also associated with several internal dynamics such as feelings, thoughts, motives, and desires. By virtue of emotional intelligence, it is possible to adapt to changes and to cope with stressors emerging in such a dynamic process as pregnancy. Particularly in a case such as high-risk pregnancy which adversely affects the maternal and fetal health, there are several factors which the pregnant woman should cope with and adapt to [2, 3]. The concept of emotional intelligence is the amalgamation of emotion and intelligence. Goleman [4] defines the emotional intelligence as "the person's ability to be aware of his/her own and other individuals' emotions, to differentiate between emotions, to utilize emotions in his/her behaviors and thoughts". Upon the review of definitions proposed by different theorists on emotional intelligence, it is discerned that the common theme is to get to know and be aware of the self and the environment, to establish effective relations and to cope with the problem [5]. In light of the above information, emotional intelligence can be described as skills which enable the pregnant women to cope effectively with environmental impacts and stressors in such a dynamic and changing process as pregnancy and provide the pregnant women with the opportunity to develop relations in agreement with the environment inhabited by them. As per the relevant literature, it was identified that, based on the gender variable, the level of emotional intelligence differed and women obtained higher scores especially in understanding their own personal emotions, understanding others' emotions and the manipulation of emotions sub-scales of an emotional intelligence scale [1, 6, 7]. The high-level of women's emotional intelligence skills is a crucial feature which is likely to affect both the maternal and fetal health favorably [1]. The emotional connection established by the pregnant woman with the fetus, that is, the prenatal attachment does not only influence the health of fetus at that time but is essential also to the development of healthy spiritual development of the baby in postpartum period [8, 9]. Emotional intelligence is important to the enjoyment of healthy and safe feelings of attachment by individuals. It is known that having poor feelings of attachment is associated with postpartum anxiety and depression [1, 10]. In addition, pregnant women with high emotinal intelligence are more successful in discovering the emotions they feel, resolving all the fear and anxiety in them, and approaching the birth of their baby with confidence and safety [11].

This research was planned for evaluating the relationship between emotional intelligence and prenatal attachment levels of women with healthy and high-risk pregnancies. In addition, in the literature reviews, no study with the same content and purpose as this study has yet been encountered. Therefore, this study can shedding light on future studies.

Research Questions

- Is there any statistically difference in prenatal attachment levels of women with healthy and high-risk pregnancies?
- Do the socio-demographic and obstetric characteristics of women with healthy and high-risk pregnancies have statistically significant associations with their prenatal attachment levels?
- Do the socio-demographic and obstetric characteristics of women with healthy and high-risk pregnancies have statistically significant associations with their emotional intelligence levels?
- Is there any statistically significant association between pregnant women's prenatal attachment and emotional intelligence levels?

METHODS

This research is based on descriptive research design and regression analysis. It was performed at the perinatology polyclinic of two university hospitals from February to July in 2018. Research population was composed of 630 pregnant women who were admitted to two medical schools on the above dates. By virtue of the fact that the exact size of the research population was known, the size of the sample to be addressed by the research was calculated as minimum 242 pregnant women for a = 0.05, H= ± 0.05, p = 0.5, q = 0.5 [12]. Assuming that certain participants were likely to be left out of the research during the data collection process, data were collected from 281 pregnant women. No sample selection technique was employed, rather, all pregnant women who volunteered to take part in the research and complied with research criteria were included into the sample. The criteria specified for being included in the research were to be aged

above 18 years, to be in a period later than the twentieth week of the pregnancy and to have the ability to speak and understand Turkish. Research data were collected by researchers through face-to-face interviews in approximately 15-20 minutes. Research findings were evaluated by using 95% confidence interval, and statistical significance was identified if the P value was lower than 0.05 ($p < 0.05$), and also the effect size was found as 0.21.

Data Collection Tools

Data were collected through the Pregnant Women Information Form, Emotional Intelligence Self-Evaluation Scale and Prenatal Attachment Inventory.

Pregnant Women Information Form

It is a form composed of 25 questions which were created by researchers upon the literature review for identifying pregnant women's socio-demographic (age, income level, education level and so on.) and obstetric characteristics (maternal risk, fetal risk, week of pregnancy period, fetus gender and so on.) in conjunction with the research topic [1, 8].

Emotional Intelligence Self-Evaluation Scale (EISES)

The scale which was comprised of 30 items and five sub-scales was developed by Nicholas Hall (1999). Its sub-scales are Emotional Awareness, Managing One's Emotions, Self-Motivation, Empathy and Coaching Others' Emotions. The minimum and maximum scores to be obtained from this 6-point Likert-type scale are successively 30 and 180 points. A high score to be obtained from the scale demonstrates that the person has high-level emotional intelligence. The scale has certain breakpoints, that is, low (129 points and below), normal (130-154 points) and high (155 points and above). The validity and reliability test for the scale was performed in Turkish by Ergin (2000), and Cronbach's Alpha Coefficient was calculated as 0.84 [13]. Cronbach's Alpha Coefficient was calculated as 0.96 for this research.

Prenatal Attachment Inventory (PAI)

Composed of 21 items, the scale was developed by Muller (1996). The minimum and maximum scores to be obtained from this 4-point Likert-type scale are consecutively 21 and 84 points. A high score to be obtained from the scale indicates that the person has high-level prenatal attachment. The validity and reliability test for the scale was performed in Turkish by Yılmaz and Beji (2013), and Cronbach's Alpha Coefficient was computed as 0.84 [8]. Cronbach's Alpha Coefficient was computed as 0.912 for this research.

The Visual Analog Scale (VAS)

In this study, the scale used for the assessment of variations in intensity of stress and coping with stress. The scale has a total score range of minimum 0 to maximum 10 [14].

Ethical Approval

The research protocol was approved by the Social Sciences and Humanities Ethics Committee of İstanbul University (No: 3245), and informed consent was received from all participants in written format. All study procedures were performed in compliance with the Declaration of Helsinki.

Statistical Analysis

Data which were collected through the research were analyzed via IBM Statistical Package for the Social Science (SPSS) 21.0 software. Whether variables were normally distributed was evaluated on the basis of Kolmogorov-Smirnov Test and the analysis of histograms. Means, standard deviations, frequencies and percentages were used within the scope of descriptive statistical methods whereas the Student T-Test and ANOVA as parametric tests and Chi-Squared Test as a nonparametric test were utilized for the comparison of variables. Bonferroni Test and Tukey Test were employed as multiple comparison (post-hoc) tests for intergroup comparisons, and the correlation analysis was used for the assessment of the association between scales. Statistical significance was identified if the P value was lower than 0.05 ($p < 0.05$).

RESULTS

Upon the examination of the distribution of participant women with healthy and high-risk pregnancies, it was discerned that the normal distribution was the case for data across groups ($p > 0.05$). The review of socio-demographic and obstetric data of women with healthy and high-risk pregnancies indicated that there was a

statistically significant difference only on the basis of the mean age and gravida between groups ($p > 0.05$). It was ascertained that 25.3% of pregnant women (n = 71) previously had miscarriage and 3.2% of pregnant women (n = 9) thought of having abortion and there was no statistically significant difference between groups ($p > 0.05$). Of pregnant women who were informed about the sex of their babies, 47.6% (n = 134) reported that their babies would be female, 51.6% (n = 147) said that their babies would be female and 77.2% (n = 217) stated that their babies had the sex which they wished that their babies would have. In Table 1, data on socio-demographic and obstetric characteristics of participant women with healthy and high-risk pregnancies and their distribution across groups are exhibited.

On the basis of means of scores obtained from Emotional Intelligence Self-Evaluation Scale and its sub-scales and the Prenatal Attachment Inventory by women with healthy and high-risk pregnancies, it was found that they had high levels of emotional intelligence and medium levels of prenatal attachment. Upon the evaluation of stress experienced by pregnant women and the level of their success in coping with

Table 1. Socio-demographic and obstetric characteristics of participant womens' with healthy and high-risk pregnancies

Characteristics		Group 1 (n = 156)		Group 2 (n = 125)		Statistics	
		Mean	SD	Mean	SD	t	*p* value
Age (years)		28.07	4.56	32.09	6.00	6.373	**< 0.001**
Gestational week		28.96	6.89	30.28	6.14	1.682	0.094
Gravida		2.10	1.18	2.44	1.37	2.224	**0.027**
Abortous		0.38	0.76	0.17	0.50	2.404	0.170
Groups		**n**	**%**	**n**	**%**	**X^2**	***p* value**
Age groups (year)	17-30	101	64.7	47	37.6	20.510	**< 0.001**
	31-45	55	35.3	78	62.4		
Family type	Nuclear	130	83.3	103	82.4		
	Extended	26	16.7	22	17.6		
Educational status (year)	1-8	51	32.7	42	33.6	0.969	0.616
	9-17	93	59.6	77	61.6		
	18-27	12	7.7	6	4.8		
Working status	Working	57	36.5	52	41.6	0.749	0.387
	Not Working	99	63.5	73	58.4		
Income rate	Less	38	244	41	32.8	4.574	0.102
	Equal	96	61.5	61	48.8		
	Over	22	14.1	23	18.4		
Living children	Yes	95	60.9	73	58.4	0.742	0.362
	No	61	39.1	52	41.6		
Pregnancy intention	Planned	132	84.6	104	83.2	0.103	0.747
	Unplanned	24	15.4	21	16.8		
Is your baby the gender you expect	Yes	124	79.5	93	74.4	1.021	0.320
	No	32	20.5	32	25.6		

SD = Standard deviation, Group 1 = Womens' with healthy pregnancies, Group 2 = Womens' with high-risk pregnancies

stress as per VAS, it was identified that women with high-risk pregnancies had higher levels of stress than women with healthy pregnancies and this difference was statistically significant ($p < 0.001$). However, between women with healthy and high-risk pregnancies, there was no statistically significant difference in the level of success in coping with stress ($p = 0.451$). Table 2 displays means of scores obtained from the Scale and the Inventory by women with healthy and high-risk pregnancies and comparisons of means of scores.

As there was no statistically significant difference between emotional intelligence and prenatal attachment levels across groups, associations of pregnant women's socio-demographic data with their emotional intelligence and prenatal attachment levels were evaluated irrespective of whether the women had healthy or high-risk pregnancy. It was ascertained that, on the basis of the age variable, there were statistically significant differences in means of scores obtained by participant pregnant women from the overall Emotional Intelligence Self-Evaluation Scale ($p = 0.05$) and its 'self-motivation' sub-scale ($p = 0.026$). It was found that, on the basis of whether the pregnant women voluntarily got pregnant, there were statistically significant differences in emotional intelligence and prenatal attachment levels except for the 'emotional awareness' sub-scale of the EISES ($p < 0.05$). Upon the examination of the difference between two groups, it was identified that women who voluntarily got pregnant had higher emotional intelligence and prenatal attachment levels than women who involun-

Table 2. Means of scores obtained from the Emotional Intelligence Self-Evaluation Scale and the Prenatal Attachment Inventory by women with healthy and high-risk pregnancies and comparisons of means of scores.

Emotional Intelligence Self-Evaluation Scale and Sub-dimentions	Group 1 (n = 125) Mean ± SD Min-max	Group 2 (n = 156) Mean ± SD Min-max	t	*p* value
Being aware of own emotions	27.51 ± 7.30 6 - 36	26.04 ± 8.30 6 - 36	1.566	0.118
Managing emotions	24.86 ± 6.70 7 - 36	24.31 ± 7.60 6 - 36	0.640	0.523
Self motivation	27.28 ± 7.30 6 - 36	25.51 ± 7.50 6 - 36	1.987	1.987
Emphaty	27.64 ± 7.60 7 - 36	26.16 ± 8.10 6 - 36	1.557	1.557
Managing relationships	26.10 ± 7.00 9 - 36	24.77 ± 7.50 6 - 36	1.518	1.518
Total Emotional Intelligence	133.34 ± 32.30 36 -180	126.81 ± 35.80 33 -180	1.599	1.599
Prenatal Attachment Inventory	56.66 ± 15.30 21 -84	56.10 ± 13.00 27 - 84	0.322	0.748
Stress Level, VAS	6.50 ± 3.10 1 -10	4.94 ± 3.1 1- 10	4.104	**< 0.001**
Coping with Stress, VAS	6.82 ± 3.00 0 -10	6.54 ± 3.2 1 -10	0.756	0.451

SD = Standard deviation, VAS: Visual Analog Scale, Group 1 = Womens' with healthy pregnancies, Group 2 = Womens' with high-risk pregnancies

tarily got pregnant. It was discerned that, on the basis of the education level of the pregnant women (in years), there were statistically significant differences in means of scores obtained from the EISES and its sub-scales ($p < 0.05$) whereas there was no statistically significant difference in means of scores obtained from the Prenatal Attachment Inventory ($p > 0.05$). The direction of the difference is linear, and, as pregnant women's education levels (in years) go up, they have better skills in being aware of their own emotions and enhancing self-motivation. The mean of scores obtained from 'managing one's emotions' sub-scale was higher for pregnant women educated for 9-17 years (high school) than it was for those educated for 1-8 years (primary school), nevertheless, there was no statistically significant difference for other groups with different education levels ($p > 0.05$). Likewise, means of scores obtained from the overall EISES and its 'empathy' and 'coaching others' emotions' sub-scales were higher for pregnant women educated for 9-17 years (high school) and for 18-27 years (undergraduate and above) than it was for those educated for 1-8 years (primary school), and this difference was statistically significant ($p < 0.05$). However, between pregnant women who were graduates of high school and who were holders of undergraduate degree or a higher degree, there was no statistically significant difference in means of scores obtained from the overall EISES

Table 3. The relationship between sociodemographic variables and emotional intelligence and prenatal attachment level (n = 286)

Variables	Emotional awareness	Managing one's emotions	Self-motivation	Empathy	Coaching others' emotions	Total emotional intelligence	Prenatal attachment
Age							
17-30	24.96 ± 8.2	23.09 ± 6.9	24.35 ± 7.6	25.50 ± 8.4	23.94 ± 7.8	121.85 ± 35.6	56.19 ± 15.1
31-45	27.82 ± 7.2	25.53 ± 6.4	27.14 ± 6.8	27.51 ± 6.0	26.58 ± 6.3	134.60 ± 28.7	52.02 ± 14.6
Test value	F = 2.669	F = 2.285	F=3.683	F = 1.470	F = 2.271	F = 2.874	F = 2.520
p value	0.071	0.104	**0.026**	.0232	0.111	**0.050**	0.082
Pregnancy Intention							
Planned	27.07 ± 7.6	25.00 ± 7.0	26.68 ± 7.2	27.01 ± 7.5	25.83 ± 6.9	131.57 ± 30.9	57.58 ± 14.2
Not planned	24.73 ± 9.2	22.24 ± 7.8	24.26 ± 8.5	25.80 ± 9.7	22.88 ± 8.8	119.93 ± 40.6	49.88 ± 11.6
Test value	$X^2 = 40.54$	$X^2 = 55.28$	$X^2 = 44.42$	$X^2 = 51.36$	$X^2 = 72.88$	$X^2 = 140.30$	$X^2 = 102.81$
p value	0.095	**0.003**	**0.044**	**0.006**	**< 0.0001**	**0.001**	**< 0. 001**
Is your baby the gender you expect							
Yes	26.95 ± 7.5	25.03 ± 7.0	26.91 ± 7.1	27.30 ± 7.5	25.77 ± 6.9	131.94 ± 32.7	57.20 ± 14.2
No	25.82 ± 9.0	22.93 ± 7.6	24.21 ± 8.3	25.18 ± 9.1	23.98 ± 8.5	122.15 ± 39.1	53.48 ± 13.2
Test value	t = .998	t = 2.048	t = 2.560	t = 1.879	t = 1.715	t = 2.007	t = 1.939
p value	0.319	**0.041**	**0.011**	0.061	0.087	**0.046**	0.055
Education (year)							
1-8	23.82 ± 8.3	22.45 ± 7.8	23.83 ± 8.4	24.06 ± 9.3	23.08 ± 7.7	117.26 ± 38.0	55.76 ± 12.2
9-17	27.80 ± 7.5	25.51 ± 6.8	27.32 ± 6.8	28.02 ± 7.0	26.52 ± 7.0	135.17 ± 32.0	56.97 ± 15.1
18-27	31.05 ± 4.0	26.38 ± 5.0	29.33 ± 2.5	29.66 ± 2.8	26.22 ± 5.4	142.66 ± 14.2	53.61 ± 12.6
Test value	F = 11.241	F = 6.218	F = 8.569	F = 9.202	F = 6.985	F = 10.071	F = .583
p value	**< 0.001**	**0.002**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	.559

and its 'empathy' and 'coaching others' emotions' subscales ($p > 0.05$). Table 3 indicates the relationship between means of scores obtained by pregnant women from the EISES and PAI and pregnant women's socio-demographic variables.

It was found that there was no statistically significant correlation between means of scores obtained by pregnant women from the EISES and PAI ($p < 0.05$). It was only discerned that there was a weak statistically significant negative association between pregnant women's skills in managing their own emotions and their stress levels (r = -0.127, $p = 0.033$) (Table 4).

DISCUSSION

This research analyzed the relationship between emotional intelligence and prenatal attachment levels of pregnant women with healthy and high-risk pregnancies.

The maternal age below 18 years or above 35 years is accepted as a risk factor for the pregnancy [2]. Upon the review of participant pregnant women in terms of maternal age, it is an anticipated outcome that women with high-risk pregnancy would be older than women with healthy pregnancy. In a similar vein, it is likely that women with high-risk pregnancy who have higher mean age have higher gravida than women with healthy pregnancy and this difference is statistically significant. In view of the difference in mean ages of women with healthy and high-risk pregnancies (4.01 ± 0.63 years), pregnant women's socio-demographic characteristics were compared on the basis of data obtained from Turkiye Demographic and Health Survey (Turkiye DHS) 2013 and Turkiye DHS 2018. The number of pregnant women with high school education and education above high school went up in Turkiye DHS 2018 as compared to DHS 2013 whilst there was no increase in the number of pregnant women with primary and secondary school education. Even if the education level of pregnant women went up, the level of participation in labor force and income level were almost analogous both in Turkiye DHS 2013 and Turkiye DHS 2018 [15, 16]. The findings obtained alongside this current research are in parallel to Turkiye DHS report.

Upon the comparison of means of scores obtained under this current research from the overall EISES and its sub-scales to those obtained under the research by Buko and Özkan [1], it was found that means of scores obtained under this current research were higher except for 'self-motivation' sub-scale. However, it was ascertained that means of scores obtained under this current research from the overall EISES and its sub-scales were lower than those obtained under the research by Çapık *et al*. [17]. It was determined that the results of this research were similar to the emotional intelligence scores of Ozer's study [11]. It is known that there are studies suggesting that women have higher levels of emotional intelligence [18]. Also in this current research, it was identified that pregnant women had higher levels of emotional intelligence, and emotional intelligence levels of women with healthy and high-risk pregnancies were close to each other.

Even if, in previous research, there is no clear evidence suggesting that women with high-risk preg-

Table 4. Correlation of emotional intelligence scale with prenatal attachment inventory and stress level

	Emotional awareness	Managing one's emotions	Self-motivation	Empathy	Coaching others' emotions	Total emotional intelligence
Prenatal attachment	-0.085 $p = 0.157$	-0.063 $p = 0.289$	-0.068 $p = 0.258$	-0.055 $p = 0.357$	0.003 $p = 0.966$	-0.060 $p = 0.318$
Stress level. VAS	-0.072 $p = 0.227$	-0.127 $p = \mathbf{0.033}$	-0.039 $p = 0.519$	-0.081 $p = 0.173$	-0.090 $p = 0.134$	- 0.090 $p = 0.133$

VAS: Visual Analog Scale

nancy had lower prenatal attachment levels than those with healthy pregnancy, there are studies alleging that women with high-risk pregnancy had lower prenatal attachment levels [19,20]. In this current research, the prenatal attachment of women with healthy and high-risk pregnancies was found to be similar and at medium levels. Likewise, in the relevant literature, pregnant women were in general reported to have 'medium-level' prenatal attachment [10, 19-21]. As per Lazarus and Folkman's understanding [22], a prospective mother who is faced with a new situation or change suffers stress. Additionally, pregnancy complications can pave the way for increases in the perceived stress and negative emotional symptoms [22, 23]. Alongside the current research, it was identified that women with high-risk pregnancy had higher levels of perceived stress and this difference was statistically significant whereas there was no statistically significant difference in healthy pregnant women's levels of success in coping with stress. Emotional intelligence enables individuals to get to know and notice their own emotions, cope with stress more successfully and adapt to changing conditions [4, 23]. As per findings obtained within the context of this research, it is thought that women with healthy and high-risk pregnancies get psychosocially well-adapted to such a major transformation as pregnancy by virtue of having high-level emotional intelligence.

Emotional intelligence and prenatal attachment are affected by several variables such as age, socio-economic level, cultural difference, and education level, academic achievement and job performance [10, 18, 19, 23, 24]. Research results were reinforced by the fact that independent variables were homogenously distributed across groups in the current research. Emotional intelligence is a type of intelligence which can be unearthed and developed by the person [4]. According to this current research, there was a statistically significant difference in the mean of scores obtained by pregnant women only from 'self-motivation' sub-scale, and in this respect, pregnant women aged 31-45 years had higher mean of scores than other age groups. This result explains the similarity in prenatal attachment levels of women with age-related high-risk pregnancy and women with healthy pregnancy. That is because of the fact that a woman who motivates herself to have a healthful baby and healthy pregnancy period does not get dissociated from her own psychological well-being without giving up and losing courage even when pregnancy period does not progress well. Being emotionally intelligent is that the pregnant woman places her attention around an emotion by boosting her self-motivation so that she can make better decisions on her own health issues and cope with problems more successfully [1, 4]. In the relevant literature, there are researches asserting that emotional intelligence is enhanced along with the increase in age and the level of formal education [23, 24, 27]. The result of this current research is in support of the relevant literature. In this current research, it was ascertained that there was no statistically significant association between education level and prenatal attachment level of pregnant women. In the relevant literature, there are studies demonstrating that there was an inverse or no relationship between education level and prenatal attachment level [19, 20, 24, 28-31]. Watson and Clark [30] characterize emotions as organism's needs, aims and its adaptive responses which are addressed to incidents and essential to its survival and adaptation to the environment. Outer expression of emotions is closely related to the psychological well-being [30]. In the conceptual framework of this current research, it can be asserted that the prenatal attachment is the emotional response with which the pregnancy gets aligned. Whether the woman voluntarily got pregnant and whether the baby had the sex which the pregnant woman wished were found to be positively associated with whether pregnant woman had stronger ties with her baby, whether she adopted more motivating emotions and whether she established positive relations. In the relevant literature, there was no previous research to be compared to these findings of the current research. However, these results are in support of the conceptual framework of the emotional intelligence and prenatal attachment.

Along side the research, it was found that there was no statistically significant correlation between means of scores obtained by pregnant women from the EISES and its sub-scales and the PAI. However, the research by Buko and Özcan [1] which was akin to this current research in terms of its design showed that there was a statistically significant positive relationship between prenatal attachment and emotional intelligence. It is thought that this difference is likely to be related to differences in participant pregnant women's socio-demographic characteristics and cultural con-

structs and properties of cities inhabited by pregnant women. The conduct of this research in Istanbul which is the largest metropolis of Turkiye endowed the pregnant women with several advantages such as access to education and health services vis-à-vis pregnant women living in Anatolia. Another important factor is that pregnant women who participated in this current research had higher levels of emotional intelligence than those participating in the research performed by Buko and Özcan [1].

Limitations

As this research was performed in a particular hospital in a Turkish city, its results cannot be generalized to the entire population. Therefore, for the purpose of explaining the relationship between emotional intelligence and prenatal attachment, it is necessary to undertake research to be carried out on larger samples.

CONCLUSION

At the end of the research, it was ascertained that there was no statistically significant difference in prenatal attachment levels of women with healthy and high-risk pregnancies and prenatal attachment level had no statistically significant relationship with emotional intelligence level. Moreover, it was identified that pregnant women had high levels of emotional intelligence and having high-level emotional intelligence was a crucial factor for coping with stress. Furthermore, it was found that emotional intelligence level could be affected by socio-demographic variables such as age and education level in years. In this conjunction, it is recommended that studies in which concepts found to be associated as per this current study would be further examined and the extent of effective factors would be broadened should be performed on larger samples.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

We would like to thank all pregnant women who voluntarily participated in the conduct of the study

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The European Research Journal 2023;9(6):1303-1313 Original Article

DOI: 10.18621/eurj.1128240 *Psychiatry*

Do anxiety levels and attitudes towards COVID-19 vaccines among healthcare workers change after COVID-19 vaccination?

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ABSTRACT

Objectives: The COVID-19 pandemic has significant effects on the mental health of societies and individuals worldwide while especially for healthcare workers these effects pose a higher risk due to occupational exposure. COVID-19 vaccines have been a turning point in the pandemic, however concepts as vaccine hesitancy and anti-vaccination have come into question again. This study aimed to comparatively evaluate the attitudes of healthcare professionals towards the COVID-19 vaccines, their pandemic-related anxiety and phobia levels before and after vaccination.

Methods: In this cross-sectional study, the anxiety levels and attitudes towards COVID-19 vaccines of healthcare professionals working in a state hospital were evaluated by administrating Coronavirus Anxiety Scale (CAS), Coronavirus-19 Phobia Scale (CP19-S), and Attitudes Towards COVID-19 Vaccine Scale (ATV-COVID-19) to the participants both in the pre-vaccine (n = 154) and post-vaccine (n = 81) periods. All these scales were previously validates in Turkish population. 44.8% (n = 69) of pre-vaccine sample and 45.7% (n = 37) of post-vaccine sample were female and all participants were Turkish. Participants were questioned about COVID-19 symptoms during each application and kept in touch in terms of side effects.

Results: One hundres fifty-four pre-vaccine and 81 post-vaccine health workers were included in the study. After the vaccination scores of CAS and CP19-S decreased significantly compared to the pre-vaccine period ($p < 0.001$ and $p = 0.005$, respectively); while ATV-COVID-19 scores significantly increased ($p < 0.001$). Participants did not report significant adverse events in the post-vaccine period.

Conclusions: The results of the study in the form of a decrease in anxiety and fear levels and an increase in positive attitudes towards the vaccine after vaccination suggest that vaccination may have a positive effect on the mental health of healthcare workers.

Keywords: COVID-19, vaccination, healthcare workers, anxiety, phobia, vaccine hesitancy

Coronavirus Disease (COVID-19) was first identified in China in December 2019, spread rapidly all over the world and was declared as a pandemic by the World Health Organization (WHO). COVID-19 is a viral infectious disease that can cause a wide range of symptoms from mild upper respiratory tract disease

Received: ***June 9, 2022;*** *Accepted:* ***September 5, 2022;*** *Published Online:* ***March 20, 2023***

How to cite this article: *Balaban H, Demirdağ Çevikkan M, Tanyeri Kayahan S. Do anxiety levels and attitudes towards COVID-19 vaccines among healthcare workers change after COVID-19 vaccination?. Eur Res J 2023;9(6):1303-1313. DOI: 10.18621/eurj.1128042*

e-ISSN: 2149-3189

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symptoms to severe disease findings and respiratory failure, and may result in death for some of the patients [1, 2].

The COVID-19 pandemic significantly affects societies around the world in many ways, especially in mental and physical health. It has been reported in repeated studies that psychiatric symptoms are observed in the general population due to the COVID-19 pandemic, and the frequency of anxiety and depression has increased significantly [3]. It is suggested that the mental symptoms seen due to the pandemic pose a higher risk for some special groups. Health workers are among the leaders of these groups [4]. It has been reported that pandemic-related depression, anxiety and severe stress are common among healthcare workers in the early period of the pandemic [5]. However, in the later stages of the pandemic, the burden of health workers continued, the symptoms of burnout increased and the stress experience became permanent [5-7].

Vaccines developed for COVID-19 infection have set a turning point in the pandemic course. In the last months of 2020, the vaccines with proven protective effectiveness against COVID-19 approved by local authorities and WHO have been made widely available. Therefore decreased severe disease and disease-related mortality rates have become hope for all people [8]. Various studies evaluating the attitudes of healthcare professionals towards vaccination applications reported that positive attitudes are more prominent than the general population [9, 10]. However, no study has been found in the literature to date that directly investigates the effects of vaccination applications on the mental difficulties, anxiety and fear levels of healthcare workers related to COVID-19. In this study, it was aimed to measure and compare the anxiety and fear levels of healthcare workers of a public hospital before and after COVID-19 vaccination, and to investigate the changes in their attitudes towards the vaccine before and after the application.

METHODS

Study Design

This study which has a prospective and cross-sectional design, was carried out with the participation of healthcare professionals working in a state hospital. The study was approved by the University of Suleyman Demirel University Clinical Research Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. In this context, all participants who agreed to participate in the study were informed in detail about the study and their written consent was obtained on a voluntary basis.

Sampling and Data Collection

The sample of the study consisted of all participants who volunteered to participate in the study, gave informed written consent, and met the inclusion criteria. The criteria for inclusion in the sampling were determined as being 18 years old and over and being a healthcare worker for the evaluations made before the vaccination. For the evaluations made after vaccination, being vaccinated against COVID-19 was added to the previous criteria. Participants who were included in the initial assessment but were not vaccinated were excluded from the study. For the study sampling, all healthcare workers (including medical doctors, nurses, midwives, medical technicians, technical and cleaning staff and administration officers) were invited to join the assessment in the outpatient service unit of psychiatry. Data of the participants was collected with the assessment tools described below before and after COVID-19 vaccination. 154 participants were assessed pre-vaccine but only 81 of them were re-assessed for the second post-vaccine assessment.

The data collection form which includes information about sociodemographic and occupational characteristics, and the Coronavirus Anxiety Scale (CAS), the Attitudes Towards COVID-19 Vaccine Scale (ATV-COVID-19) and the Coronavirus-19 Phobia Scale (C19P-S) were applied before and after the COVID-19 vaccine administration. CAS is a 5-point Likert-type scale consisting of 5 questions and was developed by Silva *et al*. [11] in 2020 to measure the anxiety levels associated with COVID-19. Turkish validity and reliability study of the scale was conducted by Biçer *et al*. and internal consistency (Cronbach's alpha) of the scale in the sample of the study was found 0.832 [12]. ATV-COVID-19 Scale is also a 5-point Likert-type scale consisting of 9 items and examined in two sub-dimensions, positive and negative. It was developed by Geniş *et al*. [13] in 2020 and its validity and reliability study was conducted in the

same year with the internal consistency (Cronbach's alpha) 0.80 reported. C19P-S is a 5-point Likert-type scale consisting of 20 questions, was developed by Arpacı *et al*. [14] to measure the symptoms of COVID-19-related phobia and its internat consistency (Cronbach's alpha) was found 0.926 in Turkish population of study.

Before the application of each questionnaire, the participants were questioned in terms of COVID-19 symptoms (fever, weakness, cough, sore throat, inability to taste and smell, headache, myalgia) and COVID-19 contact history, then were examined if necessary. Accordingly, those who did not suspect COVID-19 were included in the study. Post-vaccine evaluation was conducted at the end of two weeks after the vaccines were applied to the participants. In addition, participants were contacted regarding post-vaccine adverse effects.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 26 was used for statistical analysis. Normality tests were performed with the Shapiro-Wilk test, normally distributed data were shown with mean and standard deviation values, and non-normally distributed data were shown with median and minimum-maximum values accordingly. While the categorical data were expressed as numbers and percentages, the evaluations of the variables before and after vaccination were made with mixed model analysis for repeated measurements. The p value for statistical significance was determined as < 0.05.

RESULTS

Sociodemographic Characteristics and COVID-19 Related Features

The study sample consisted of 154 healthcare workers in the pre-vaccine period and 81 in the post-vaccine period. Sociodemographic characteristics and COVID-19-related characteristics of the two groups are shown in Table 1 in detail. Accordingly, no statistically significant difference was found between the samples evaluated in the pre- and post-vaccination period in terms of age, gender distribution, marital status and occupational distribution. There was no significant difference between the two samples in terms of rates of COVID-19 infection, risk of COVID-19 infection, and presence of chronic disease. However, when participants were asked to subjectively evaluate their risk of contracting COVID-19 infection, similar scores were obtained in both groups ($p = 0.944$).

Opinions About COVID-19 Vaccines

In the interviews before and after vaccination, participants were asked whether they had knowledge about COVID-19 vaccines. Accordingly, in the sample evaluated after vaccination, the level of knowledge about COVID-19 vaccines increased statistically significantly ($p < 0.001$) compared to the sample evaluated before vaccination. It has been observed that the rates of concern due to not having enough information about COVID-19 vaccines and the impact of COVID-19 vaccines, concern about short-term and possible long-term side effects of COVID-19 vaccines, and opinion about the ineffectiveness of COVID-19 vaccines have decreased significantly. It was determined that the reports of indecision about the administration of COVID-19 vaccines to children decreased significantly in the post-vaccination period. Findings related to this are detailed in Table 2.

CAS, CP 19-S ve ATV-COVID-19 Scale Points Before and After Vaccination

The scores of the ATV-COVID-19, CP19-S and CAS before and after vaccination are shown in detail in Table 3. Accordingly, a statistically significant decrease was observed in CP 19-S and CAS scores in the post-vaccine period ($p = 0.005$, $p < 0.001$, respectively), while a significant increase was found in the ATV-COVID-19 score ($p < 0.001$).

CAS, CP 19-S ve ATV-COVID-19 Scale Scores in Subgroups Before and After Vaccination

In the sample of healthcare professionals, the scores of the CAS, CP 19-S and ATV-COVID-19 scales applied in the pre-vaccine and post-vaccine periods were compared due to occupational subgroups. Accordingly, the scale scores did not show a significant difference between the subgroups. However, a significant decrease was found in ATV-COVID-19 scores in each occupational group in the post-vaccine period compared to the pre-vaccine period (Table 4).

While it was determined that the scale scores did not show a significant difference in the comparison

Table 1. Distribution of sociodemographic characteristics and COVID-19 related features in pre- and post-vaccine groups

	Pre-Vaccine	Post-Vaccine	*p* value
Age (years) (Mean ± SD)	37.9 ± 10.7	38.6 ± 10.1	0.590
Gender, n (%)			
Male	85 (55.2)	44 (54.3)	0.909
Female	69 (44.8)	37 (45.7)	
Marital Status, n (%)			
Married	106 (68.8)	60 (74.1)	0.411
Single	48 (31.2)	21 (25.9)	
Occupation, n (%)			
Medical doctor	14 (9.1)	8 (9.9)	0.169
Midwife	6 (3.9)	1 (1.2)	
Nurse	49 (31.8)	30 (37.0)	
Medical technician	12 (7.8)	10 (12.3)	
IT officer	17 (11.0)	10 (12.3)	
Technical staff	4 (2.6)	5 (6.2)	
Cleaning staff	24 (15.6)	9 (11.1)	
Administration officer	12 (7.8)	4 (4.9)	
Other	16 (10.4)	4 (4.9)	
Workplace unit, n (%)			
Emergency room	45 (29.2)	12 (15.0)	0.150
Outpatient clinic	28 (18.2)	16 (19.8)	
COVID-19 (-) inpatient clinic	10 (6.5)	2 (2.5)	
COVID-19 (+) inpatient clinic	19 (12.3)	16 (19.8)	
Administrative office	19 (12.3)	9 (11.1)	
Diet Outpatient clinic	3 (1.9)	2 (2.5)	
Other	30 (19.5)	24 (29.6)	
COVID-19 infection history, n (%)			
Yes	13 (8.4)	9 (11.1)	0.516
No	141 (91.6)	72 (88.9)	
COVID-19 infection history in family, n (%)			
Yes	13 (8.4)	11 (13.6)	0.101
No	141 (91.6)	70 (86.4)	
Risk group for COVID-19 infection, n (%)			
Yes	30 (19.5)	17 (21.0)	0.415
No	100 (64.9)	56 (69.1)	
N/A	24 (15.6)	8 (9.9)	
Chronic disease, n (%)			
Yes	21 (13.6)	12 (14.8)	0.990
No	133 (86.4)	69 (85.2)	
Subjective COVID-19 Risk Assessment (%)	50 (0-100)	50 (0-100)	0.944

IT = Information Technology

Table 2. Opinions about COVID-19 vaccines before and after vaccination

	Pre-Vaccine	Post-Vaccine	*p* value
Knowledge about COVID-19 vaccines, n (%)			
Absolutely yes	18 (11.7)	18 (22.2)	**< 0.001**
Yes	44 (28.6)	33 (40.7)	
Partially	57 (37.0)	2 (2.5)	
Very little	26 (16.9)	21 (25.9)	
No	9 (5.8)	5 (6.2)	
Information source, n (%)			
News	110 (71.4)	60 (74.1)	0.657
Social media	85 (55.2)	44 (54.3)	0.899
Ministry of Health	120 (77.9)	68 (84.0)	0.756
Scientific articles	39 (25.3)	18 (22.2)	0.632
WHO declarations	35 (22.7)	26 (32.1)	0.079
"I am worried because I am not informed enough about COVID-19 vaccines", n (%)			
Yes	90 (58.4)	14 (17.3)	**< 0.001**
No	64 (41.6)	67 (82.7)	
"I am worried about the short-term side effects of COVID-19 vaccines", n (%)			
Yes	99 (64.3)	19 (23.5)	**< 0.001**
No	55 (35.7)	62 (76.5)	
"I am worried about possible long-term effects of COVID-19 vaccines", n (%)			
Yes	112 (72.7)	42 (51.9)	**0.001**
No	42 (27.3)	39 (48.1)	
"I don't think that COVID-19 vaccines will be effective", n (%)			
Yes	61 (40.1)	23 (28.4)	**0.015**
No	91 (59.9)	58 (71.6)	
"I didn't have a COVID-19 vaccine for myself or for my children due to my anti-vaccination opinions", n (%)			
Yes	7 (4.6)	3 (3.7)	0.726
No	145 (95.4)	78 (96.3)	
"I am thinking about having a COVID-19 vaccine", n (%)			
Yes	96 (62.3)	76 (93.8)	**< 0.001**
No	12 (7.8)	-	
Not decided	46 (29.9)	5 (6.2)	
"I would consider having my children vaccinated against COVID-19 if applicable", n (%)			
Yes	48 (31.2)	45 (71.4)	**< 0.001**
No	18 (11.7)	4 (6.3)	
Not decided	44 (28.6)	14 (22.2)	
No children	44 (28.6)	-	

WHO = World Health Organization

Table 3. Scores of ATV-COVID-19, CP19-S and CAS before and after vaccination

	Pre-Vaccine	Post-Vaccine	*p* value
ATV-COVID-19 (MeanPoints ± SD)	31.2 ± 7	35.5 ± 6.2	**< 0.001***
CP19-S (MeanPoints ± SD)	50.5 ± 14.8	44.9 ± 12.8	**0.005***
CAS (MedianPoint)	1 (0-18)	0 (0-8)	**< 0.001***

ATV-COVID-19 = Attitudes Towards COVID-19 Vaccine Scale, CP19-S = Coronavirus-19 Phobia Scale, CAS = Coronavirus Anxiety Scale

between the subgroups formed according to the units participants work in, the ATV-COVID-19 scores were found to decrease significantly in the post-vaccine period compared to the pre-vaccine period in the individuals working in the inpatient units and polyclinic services (Table 4).

ATV-COVID-19: Attitudes Towards COVID-19 Vaccine Scale

In the post-vaccine period compared to the pre-vaccine period, CP 19-S scale scores were statistically significantly decreased in the subgroup working in inpatient clinics serving COVID-19 negative patients ($p = 0.034$). Similarly, CP 19-S scores were found to decrease significantly in the post-vaccine period in people without a history of being infected with COVID-19 ($p = 0.012$). No significant difference was found in the intergroup evaluations made according to occupational groups and workplace units in the pre-vaccine and post-vaccination periods in CAS scores (Table 5).

DISCUSSION

The aim of this study is to determine the attitudes of healthcare workers of a public hospital who had the CoronaVac vaccine produced by Sinovac company for COVID-19 towards the COVID-19 vaccine, their anx-

Table 4. ATV-COVID-19 scores in subgroups

ATV-COVID-19 Scores	Pre-Vaccine		Post-Vaccine		*p* value	Δp
	n	Mean ± SD	n	Mean ± SD		
Occupation						
Medical doctor	14	33.9 ± 7.9	8	40.3 ± 3.2	**0.038**	0.980
Nurse/midwife	55	29.7 ± 6.5	31	34.5 ± 6.5	**0.002**	
Medical technician	12	29.2 ± 3 1	10	36.9 ± 4.4	**< 0.001**	
Cleaning staff	24	31.5 ± 6.5	9	35.8 ± 5.7	**0.045**	
Other	49	30.8 ± 7.6	23	34.7 ± 6.8	**0.040**	
Workplace unit						
Emergency room	45	29.9 ± 6.8	12	31.3 ± 6.2	0.513	0.403
Outpatient clinic	28	32.1 ± 7.8	16	37.1 ± 5.0	**0.020**	
COVID (-) inpatient clinic	10	30.1 ± 5.3	2	36.0 ± 1.4	**< 0.001**	
COVID (+) inpatient clinic	19	30.1 ± 5.7	16	35.8 ± 4.9	**0.011**	
Administrative office	19	31.6 ± 9.5	9	34.4 ± 6.3	0.928	
Other	30	32.7 ± 6.1	24	36.5 ± 7.2	0.104	
COVID-19 infection history						
Yes	13	31.5 ± 5.6	9	33 ± 4.1	0.471	0.483
No	141	31.1 ± 7.1	72	35.9 ± 6.3	0.999	

iety and fear levels towards the COVID-19 pandemic in the pre- and post-vaccination periods and whether those changed in between these periods.

When the literature on the subject was examined, no other study has been found which assessed the differences between the attitudes towards the vaccine and the pandemic, and the psychological impact among healthcare workers before and after vaccination, therefore our study is the first in this field.

During the COVID-19 pandemic, mental health problems are quite common among healthcare workers. In a recent meta-analysis related to this issue; it was stated that depression, anxiety and post-traumatic stress symptoms were reported in one out of every four healthcare workers during the COVID-19 pandemic period, while one out of every three healthcare workers had a COVID-19 phobia and nearly half had insomnia [15]. In the same study the risk factors for anxiety were examined; fear of being infected with COVID-19, poor health and presence of organic diseases, female gender, presence of frontline and high-risk contact with COVID-19, and characteristics associated with rural life were described as risk factors [15]. However, it has been reported that high levels of anxiety occurring in the early phase of the pandemic can be considered as an adaptive defense response to potentially threatening events, but chronic or disproportionate anxiety can become harmful and cause the development of various psychiatric disorders [16]. In a study investigating the reasons why mental health problems are much more common in healthcare workers during the COVID-19 pandemic; it has been reported that the ever-increasing number of confirmed and suspected cases, excessive workload, depletion of personal protective equipment, widespread media coverage of the current situation, lack of specific drugs and feelings of inadequate support were important [17]. It has been reported that mental health problems seen in healthcare professionals do not only affect themselves individually, but may be associated with a decrease in the quality of patient care and an increase in medical errors [18]. High-risk contact with patients diagnosed with COVID-19, especially in frontline and risky positions such as the emergency room and inten-

Table 5. CP19 scores in subgroups

CP19 Scores	Pre-Vaccine		Post-Vaccine		p value	Δp
	n	Mean ± SD	n	Mean ± SD		
Occupation						
Medical doctor	14	47.1 ± 9.6	8	46.4 ± 8.7	0.990	0.135
Nurse/midwife	55	47.9 ± 13.7	31	44.1 ± 15.6	0.760	
Medical technician	12	48.8 ± 16.5	10	40.6 ± 8.4	0.186	
Cleaning staff	24	58.0 ± 14.2	9	49.7 ± 8.5	0.106	
Other	49	51.3 ± 16.1	23	45.7 ± 12.8	0.138	
Workplace Unit						
Emergency room	45	48.2 ± 14.6	12	40.7 ± 13.0	0.999	0.121
Outpatient clinic	28	52.7 ± 12.6	16	49.6 ± 11.0	0.428	
COVID (-) inpatient clinic	10	58.5 ± 18.0	2	38.0 ± 17.0	0.034	
COVID (+) inpatient clinic	19	48.7 ± 15.9	16	48.3 ± 14.9	0.955	
Administrative office	19	47.5 ± 12.3	9	39.9 ± 8.2	0.085	
Other	30	52.4 ± 16.4	24	46.1 ± 11.6	0.104	
COVID-19 Infection History						
Yes	13	50.4 ± 14.5	9	41.9 ± 9.8	0.077	0.801
No	141	50.6 ± 14.8	72	45.3 ± 13.1	**0.012**	

CP19-S = Coronavirus-19 Phobia Scale

sive care unit, was associated with moderate to severe anxiety and depression rates [19]. A study conducted among healthcare professionals working in hospitals showed that healthcare professionals experience mental symptoms at a higher rate than administrative personnel [20]. In our study, persons working in each unit of a state hospital were included. In this context, a statistically significant comparison could not be made between high-risk or low-risk groups due to the number of participants.

It has been reported in studies with large samples that vaccine applications reduce anxiety and depression levels, which are seen with increased frequency in the general population [21]. Various studies evaluating the attitudes of healthcare professionals towards vaccination applications reported that positive attitudes are more prominent than the general population [9, 10]. Among the studies in which healthcare workers were evaluated in this respect, Haddaden *et al*. [22] reported that vaccination against COVID-19 resulted with increased well-being both physical and mental in their sample of 300 healthcare workers. In another study investigating the subjective feelings of healthcare workers after vaccination, it was stated that hope and positive feelings about the end of the pandemic increased after vaccination [23]. In a study that included 524 healthcare workers working in COVID-19 pandemic hospitals, it was determined that the majority of the participants had a good perception and positive attitudes towards the vaccine [24]. Turan *et al*. [25], in their study to examine the relationship between coronaphobia and attitudes towards the COVID-19 vaccine in the society, determined that the participants had moderate COVID-19 phobia and positive attitudes towards the vaccine; and as the COVID-19 phobia increased, positive attitudes towards the vaccine also increased.

In the literature, only one study was found that investigated the differences between the psychological symptoms seen in healthcare workers before and after vaccination. In this comprehensive study conducted in Turkey, it was reported that, in a sample of 475 dentists, anxiety and fear levels decreased significantly after vaccination, while the frequency of interventional dentistry applications increased [26]. Similarly, in our study, when the scales applied to health workers to examine mental symptoms in the pre- and post-vaccine period were examined, it was seen that the scores of the Attitudes towards COVID-19 Vaccine Scale significantly increased, while the CAS and C19P-S scores decreased significantly in the post-vaccine period. Considering that healthcare professionals can comprehend the questions asked in the scales more easily, due to the content of the education they received and the hospital environment, and that they can easily rate the symptoms they have or do not have, it can be thought that the confidence in the scale scores will be higher.

Vaccines are among the most effective practices in reducing the spread of COVID-19 infection and preventing disease-related deaths [27]. In addition, it is reported that attitudes such as vaccine hesitancy and vaccine rejection due to doubts about vaccine side effects and efficacy are also detected at a significant rate worldwide [28, 29]. It is stated that these attitudes pose a significant risk to global health, and the importance of informing the society in the light of scientific data and thus increasing the confidence in vaccine applications is emphasized [30]. Vaccine hesitancy, one of the barriers to vaccination, was recognized by WHO as one of the top ten threats to global health in 2019 [31]. These indecisions have been exacerbated by COVID-19. On the other hand, there is also a study showing that vaccine acceptance is higher in people with high subjective anxiety, fear and individual risk perception [32].

Attitudes such as vaccine hesitancy and vaccine rejection can also be seen in healthcare workers who are at the forefront in the fight against COVID-19 [28-30, 33]. Opponents of vaccines are very difficult to convince. However, people with vaccine hesitancy are not anti-vaccine and do not get vaccinated due to various hesitations. The most effective method in convincing people with vaccine hesitancy is to eliminate their hesitations in the light of scientific studies. Therefore, it is important to combat misinformation and create interventional educational campaigns that target populations at risk of hesitation. It has been reported that the attitudes of healthcare professionals towards the COVID-19 vaccine and vaccination recommendations will affect the vaccination decision of the public and make it easier for them to be vaccinated [28, 34]. Therefore, sharing the results obtained in our study in such educational campaigns will be beneficial in eliminating negative perceptions about the vaccine.

In a study by Akarsu *et al*. [35], "fearing the side effects of the vaccine" and "not thinking that it will be reliable because there it is a new vaccine" were reported as the most frequent reasons related to indecision and rejection for the COVID-19 vaccine. In our study, post-vaccine symptom check was also performed, but there were no reports of vaccine-related side effects, except for mild fatigue. Fewer short-term vaccine-related side effects are consistent with increased positive attitudes towards the vaccine and decreased fear and anxiety.

In a systematic review by Li *et al*. [36], it was reported that the most common concern about vaccination among healthcare professionals and the most important cause of vaccine hesitancy is mistrust of the vaccine. Concerns about vaccine safety is mostly related to especially long-term potential side effects, rapid production and administration of COVID-19 vaccines with an Emergency Use Authorization, doubts about its effectiveness, mistrust of governments and regulatory authorities, lack of information [36]. One of the reasons for the decrease in participation after vaccination compared to pre-vaccine period in our study may be that some of the participants thought of clarifying their vaccination decisions accordingly, after seeing whether there would be a negative experience related to the vaccination among volunteers.
In a systematic review by Hajure *et al*. [37], psychological factors such as fear of COVID-19, fear of transmitting the disease to relatives, presence of depressive symptoms in the last week, high risk of infection, lack of fear about vaccine safety and the government's intention to vaccinate all citizens in means of mandatory pressure have been reported as to positively affect the adoption of the vaccine among healthcare professionals. Again, among healthcare workers, it has been shown that those who have a chronic illness, who perceive themselves at risk of infection with COVID-19, who have a higher education and income level, and who directly care for COVID-19 patients are more likely to be vaccinated [38].

Limitations

Our study has some limitations. First of all, since the first vaccine to be implemented in Turkey is CoronaVac, only attitudes about this vaccine have been evaluated. It is thought that there may be different attitudes towards different vaccines, and the rates of confidence in the vaccine may change. In this context, it can be argued that with the introduction of different vaccines in the future, attitudes towards vaccines, levels of anxiety and fear can be compared, whether there is a difference between vaccines in this sense or not. However, the finding of increased positive attitudes towards the vaccine in our study may contribute to the sympathy of the vaccines that will come or be produced in our country in the future and to increase the vaccination rates. Secondly, it is seen that the number of volunteer participants in our study decreased in the post-vaccine period compared to the pre-vaccine period. It can be argued that this may be due to reasons such as vaccine hesitancy, abandonment of volunteering, the length of the questionnaires, and the inability to reach volunteers sufficiently due to flexible working practices. On the other hand, the inclusion of only healthcare workers who have not had a previous COVID-19 infection in the study sample may create a limitation in terms of generalizability. Finally, in our study, a comparison between health workers according to occupational subgroups could not be made due to the inadequacy of the number of participants within the groups. In the future, it may be recommended to conduct studies and comparisons on attitudes towards vaccines and anxiety levels in samples created by the researchers according to occupational groups among health workers. Considering that only healthcare professionals were evaluated in our study, the repetition of similar studies for other high-risk groups, such as people with chronic mental illness, may highlight the importance of extending the application of vaccination to all parts of the world and to all segments of society during the pandemic process.

CONCLUSION

The mental symptoms seen during the COVID-19 pandemic process pose a higher risk for some special groups, and healthcare workers are the leading of these groups. All of the participants in this study were selected from health professionals; the results of the study in the form of a decrease in anxiety and fear levels and an increase in positive attitudes towards the vaccine after vaccination suggest that vaccination may have a positive effect on the mental health of healthcare workers. Based on this and the results of possible

future research, practices and educational campaigns will positively affect the society's perspective on vaccines, helping to increase vaccination rates, ensure community immunity as soon as possible, and thus ensure the success of the effective fight against the pandemic.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

The authors wish to thank the study participants for their contribution to the research.

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The European Research Journal 2023;9(6):1314-1320 Original Article

DOI: 10.18621/eurj.1119037 *Nephrology*

Renal artery stenosis: a single center experience

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ABSTRACT

Objectives: Renal artery stenosis (RAS) is among the most common causes of secondary hypertension. Prevalence of RAS are seen in end-stage renal disease (ESRD) patients with hypertension between 1-10%. In our study, we evaluated the data of patients with RAS who were followed up with medical treatment and stenting.

Methods: In our study, patients who were thought to have renal artery stenosis (RAS) with renal artery doppler ultrasonography were scanned with contrast-enhanced Magnetic Resonance Angiography (MRA). Fifty-three patients (10 received medical therapy, 43 applied invasive procedure) who diagnosed with RAS evaluated.

Results: Follow-up times were 15 (12-84) months in patients who received medical therapy, and 12 (10-96) months in patients who treated with invasive therapy ($p = 0.583$). Median ages were 56 (19-74) years in medical treatment group, and 60 (15-77) years in invasive therapy group ($p = 0.955$). Compared with the beginning of treatment, diastolic hypertension was decreased of 12.5% in invasive treatment group opposite medical therapy group ($p = 0.040$), so eGFR was increased of 5.94% in invasive treatment group.

Conclusions: In recent years, several studies about survival in patients with RAS was observed that there was no significant difference between the medical and invasive treatment. Clinical, laboratory, and individual characteristics should be considered in treatment choice.

Keywords: Chronic renal failure, hypertension, renal artery stenosis

Renal artery stenosis (RAS) is the most common causes of ischemic nephropathy and secondary hypertension (renovascular hypertension-RVH). Ischemic nephropathy is one of the causes of end-stage renal disease. Instead of prevalence of RAS are seen in end-stage renal disease (ESRD) patients with hypertension between 1-10%. Atherosclerotic renal artery disease or fibromuscular dysplasia are frequently detected in the pathophysiology [1, 2]. Angiography is still the gold standard method for diagnosis. In addition, Doppler ultrasonography, computed tomography with angiography, magnetic resonance angiography (MRA), etc. are used in methods. RAS patients may present clinically with ischemic nephropathy, resistant hypertension, and unstable cardiac symptoms (such as recurrent angina attacks, pulmonary edema). Renovascular hypertension (RVH) is seen in 7% of patients over 65 years of age. The incidence of RVH in patients with coronary artery disease or aortoiliac disease is more than 50% [3, 4]. The debate about the ef-

Received: ***May 20, 2022;*** *Accepted:* ***November 22, 2022;*** *Published Online:* ***January 15, 2023***

How to cite this article: *Ayar Y, Döner B, Akgür S, Isleyen M, Ocakoglu G. Renal artery stenosis: a single center experience. Eur Res J 2023;9(5):1314-1320. DOI: 10.18621/eurj.1119037*

e-ISSN: 2149-3189

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fectiveness and superiority of medical and interventional treatments continues. In addition, which treatment option will be offered to which patient is important in terms of kidney and patient survival [5, 6].

In our study, clinical and laboratory data, kidney functions and response to treatment of patients diagnosed with RAS and receiving medical and interventional treatment evaluated, retrospectively.

METHODS

Patients

Eighty patients (25 females, 55 males) with suspected RAS, resistant hypertension (using at least three antihypertensive drugs, one diuretic), diabetes, coronary artery disease or chronic renal failure screened by renal doppler ultrasonography between 01.07.2018-30.06.2021. Because it is an easy, non-invasive method, patients with suspected RAS were first examined with renal Doppler ultrasonography for preliminary evaluation, considering their prognosis. Patients with suspected RAS [resistive index (RI) > 0.70] by renal doppler ultrasonography were scanned with contrast-enhanced MRA. Treatment, clinical and laboratory data of 53 patients (18 females, 35 males) who diagnosed with RAS by MRA evaluated retrospectively. All of our patients had atherosclerotic RAS. Interventional treatment applied in patients with renal artery stenosis of 80% or more, as in the CORAL study [7]. Other patients were followed up with medical treatment. Patients under the age of 18 and receiving renal replacement therapy were excluded from the study. Glomerular filtration rate (eGFR) calculated according to the Modification of Diet in Renal Disease (MDRD) formula [8]. Renal functions were evaluated according to serum urea and creatinine values before treatment and at the last follow-up.

Renal Doppler Ultrasonography

Renal doppler ultrasonography data performed using an angle of ≤ 60 degrees. Peak systolic flow (PSF) and end diastolic volume (EDV) were measured at 4 sites: trunk of the renal artery, hilum (renal pedicles), segmental and interlobar regions. PSF also calculated for the aorta These ultrasonic measurements made ≥ 3 times in each position and the averages of the measured values were used for the analyses. RI calculated using the formula RI = (PSF-EDV)/PSF. Patients with RI index > 0.70 evaluated with MRA. MRI angiography was performed in the second step in those with suspected RAS in the study, as it visualized vascular structures, differentiated soft tissue better, and was less nephrotoxic. Ethics committee approval obtained from our unit (09.02.2022, 2022-1 / 12).

Magnetic Resonance Angiography (MRA)

Breathing exercises were given to the patients for 20-30 seconds. A 4-hour fasting recommended to reduce the effect of bowel peristalsis. Non-contrast and contrast-enhanced series should be obtained in the same phase of respiration. The patient's arms should be elevated above the head to avoid artifact formation. MR contrast material was not used in cases with a GFR below 30 ml/min. Meglumine gadoterate preferred as a contrast agent in patients with mild to moderately impaired renal function with a GFR of 30-60 ml/min. To detect renal masses and incidental adrenal lesions that may cause hypertension, T1 axial (phase-in-phase-out) field of view (FOV) 30-35 cm, slice thickness 5-6 mm, inter-slice spacing 0.5-1.5 mm; fat suppressed T2 axial FOV 30-35 cm, slice thickness 5-6 mm, inter-slice spacing 0.5-1.5 mm; T1 coronal oblique fat-suppressed 3D gradient echo, FOV 35-50 cm, section thickness 1.6-3 mm were taken.

In standard extracellular agents, the contrast dose was 0.15-0.2 mmol/kg, at a rate of 2-3 ml/sec. followed by an injection of 20-30 ml of saline. Dynamic sections obtained from the aorta by administering 1-2 ml of contrast material followed by 20-30 ml of saline as a test dose. By placing the region of interest (ROI) on the upper abdominal aorta, 3D images obtained automatically after sufficient contrast enhancement appeared in this area. During the 3-4 seconds before the acquisition of the images, the patient was held for breath and was asked to hold his breath until the end of the examination.

The images then transferred to the workstation and processed. Processing method maximum intensity projection (MIP) algorithm was used. With MIP, images obtained in different thicknesses and planes similar to conventional angiography. T1 axial dynamic 3D contrast series also reformed. In order to evaluate the venous structures, sections obtained in the venous

phase following the arterial phase. Venous structures also evaluated with coronal 3D sequence and T1 coronal sections with late contrast. Considering the patient's clinical status, kidney functions response to medical treatment and arterial structure, balloon or stent application performed in patients with 70% or more stenosis.

Statistical Analysis

Data were expressed as median value (minimum, maximum). The percentage changes of the measurements made after the treatment compared to the baseline measurements made before the treatment calculated. Mann Whitney-U, Chi-square and Fisher's exact tests were used for comparison between groups. A p value less than 0.05 considered significant. Statistical analyzes performed using the SPSS v20 software program (SPSS, Chicago, IL, USA).

RESULTS

In our study, the data of patients diagnosed with RAS between August 31, 2018 and September 30, 2021 evaluated retrospectively. The disease detected in 66.2% (53 patients) of 80 patients who thought to have renal artery stenosis. The mean age was 56 (19-74) years in the medical treatment groups (MTG) and 60 (15-77) years in the interventional treatment groups (ITG).

In the ITG group, stent was applied to 9 patients, bypass to 2 patients, and percutaneous transluminal renal angioplasty to 32 patients. There was no difference in age distribution of both groups (p = 0.955). Sixty-three percent of MTG patients had unilateral stenosis and 37% had bilateral stenosis, 54.2% of ITG patients had unilateral and 45.8% had bilateral stenosis (p = 0.725). Gender distribution between groups was similar (p = 0.475). The mean duration of hypertension disease in those receiving medical treatment was 5 (1-20) years, and 6 (1-30) years in interventional treatment group. Use of antihypertensive drug medication was similar in the MTG and ITG [3 (3-4) and 3 (3-5), p = 0.332, respectively]. Among the antihypertensive treatments administered, the use of calcium channel blockers was intense in both groups (71.7% of all patients), but no difference observed in terms of antihypertensive treatments in both groups (p > 0.05). Duration of hypertension was similar in both groups (p = 0.583). The duration of smoking did not differ between the two groups [MTG 0 (0-120) pack/year, ITG 0 (0-80) pack/year, p = 0.920]. Pretreatment serum urea and creatinine values were higher in the ITG [serum creatinine 1.34 (0.50-10.20) mg/dL in ITG, p = 0.232; serum urea 49 (14-179) mg/dL, p = 0.317].

When the mean glomerular filtration rates (eGFR) before treatment evaluated according to MDRD, it was 54.8 (4.5-150) ml/minute/m^2 in patients with ITG and 87.05 (18.90-126.80) ml/minute/m^2 in patients with MTG. There was no significant difference in eGFR values of both groups (p = 0.312). No significant difference shew between the sizes of both kidneys measured by renal doppler ultrasonography. Right kidney sizes evaluated by MRA were smaller in ITG (p = 0.008). No difference observed between the systolic and diastolic blood pressure values of the patients, the decrease in the percent change in diastolic blood pressure values detected more significant in the post-treatment ITG group [-12.50 (-50-11.11), p = 0.040]. When the percent change in eGFR before and after treatment compared, a mean increase of 5.94 (-42.46-186.55) detected in patients with ITG after treatment (p = 0.043). None of the patients had received renal replacement therapy prior to the treatment process. During the follow-up period, progression of chronic renal failure was seen in three (11.1%) patients with MTG and six (12.5%) patients with ITG, and the patients included in the chronic dialysis program (p = 1.00) (Tables 1 and 2).

DISCUSSION

Renal artery stenosis is the most common cause of renovascular hypertension. Controlling blood pressure is the main goal in renal artery stenosis, reduces mortality and morbidity. Studies performed that renal artery stenosis was found between 5.1-6.8% in patients who underwent renal angiography. In patients with coronary artery or aortoiliac disease, the rate of RAS was found to be 50% or more [8-11]. The most common cause of RAS is atherosclerosis. It is seen between 12-45% of the cases. Fibromuscular dysplasia is the second most common cause of RAS and is detected

Table 1. Clinical and laboratory characteristics of patients undergoing medical and interventional treatment

	MTG (n = 10)	ITG (n = 43)	*p* values
Age (n)	56 (22:74)	60 (25:77)	0.955
Duration of HT (year)	5 (1:20)	6 (1:30)	0.583
Comorbidities, n (%)	10 (100%)	43 (100%)	
Hypertension	8 (80%)	29 (67.4%)	
Coronary artery disease	7 (70%)	33 (76.7%)	
Type 2 Diabetes	5 (50%)	24 (55.8%)	
Peripheral artery disease Chronic kidney disease	4 (40%)	10 (23.2%)	
Smoking (pack/year)	0 (0:120)	0 (0:80)	0.920
Renal sizes (left) (RDU) (mm)	100.50 (90:117)	98 (65:125)	0.624
Renal sizes (right) (RDU) (mm)	99 (68:129)	96 (60:127)	0.480
MRA left kidney (mm)	95 (82-114)	92 (61-120)	0.326
MRA right kidney (mm)	92 (63:123)	90 (56:120)	**0.008**
Systolic blood pressure BT (mmHg)	150 (120:180)	150 (120:260)	0.498
Systolic blood pressure_pc (AT→BT)	-19.38 (-27.78:-7.14)	-14.29 (-46.15:6.67)	0.724
Diastolic blood pressure BT (mmHg)	90 (80:100)	90 (80:150)	0.125
Diastolic blood pressure_pc (AT→BT)	-11.11 (-20:0)	-12.50 (-50:11.11)	**0.040**
Serum urea BT (mg/dL)	29.50 (21:91)	49 (14:179)	0.317
Serum urea_pc (AT→BT)	-12.25 (-64:62.79)	-10.53 (-73.17:107.14)	0.617
Serum creatinine BT (mg/dL)	0.96 (0.60:3.20)	1.34 (0.50:10.20)	0.232
Serum creatinine_pc (AT→BT)	3.94 (-15.63:14.29)	-4.88 (-59.76:61.54)	0.053
eGFR (ml/dak/1.73 m^2)	87.05 (18.90:126.80)	54.8 (4.5:150)	0.312
eGFR_pc (AT→BT)	-4.26 (-32.81:25)	5.94 (-42.46:186.55)	**0.043**
Follow-up time (months)	15 (12:84)	12 (10:96)	0.955
Gender (M/F)	5/5	30/13	0.475
Smoking (Yes), n (%)	2 (20%)	8 (18.60%)	1.00
Stenosis			
Unilateral	7 (70%)	26 (60.50%)	0.725
Bilateral	3 (30%)	17 (39.50%)	

MTG = Medical treatment groups, ITG = Interventional treatment groups, HT = Hypertension, RDU = Renal doppler ultrasonography, BT = Before treatment, AT = After treatment, MRA = Magnetic resonance angiography, PC = Percent change, eGFR = Estimated glomerular filtration

Table 2. Drug therapy in both groups

	MTG (n = 10)	ITG (n = 43)	*p* values
ACEinh_BT (Yes), n (%)	4 (40%)	17 (39.50%)	0.137
ACEinh_AT (Yes), n (%)	4 (40%)	9 (20.90%)	0.665
ARB_BT (Yes), n (%)	4 (40%)	14 (32.60%)	0.704
ARB_AT (Yes), n (%)	4 (40%)	10 (23.30%)	0.667
CCB_BT (Yes), n (%)	8 (80%)	30 (69.80%)	1.00
CCB_AT (Yes), n (%)	8 (80%)	25 (58.10%)	0.722
AB_BT (Yes), n (%)	4 (40%)	16 (37.20%)	1.00
AB_AT (Yes), n (%)	4 (40%)	16 (37.20%)	1.00
BB_BT (Yes), n (%)	5 (50%)	25 (58.10%)	0.730
BB_AT (Yes), n (%)	5 (50%)	21 (48.80%)	1.00
Diuretics_BT (Yes), n (%)	5 (50%)	16 (37.20%)	0.492
Diuretics_AT (Yes), n (%)	5 (50%)	13 (30.20%)	1.00
ALDOSANT_BT (Yes), n (%)	0	3 (7%)	1.00
ALDOSANT_AT (Yes), n (%)	0	1 (2.30%)	1.00
Progression of CKD n (%)	1 (10%)	4 (9.30%)	1.00
BT_DRUGS (n)	3 (3-4)	3 (3-5)	0.332
DRUGS (AT →BT) (n)	3 (2:3)	1 (1:3)	0.330

ACEI = Angiotensin converting enzyme inhibitors, ARB = Angiotensin receptor blockers, CCB = Calcium channel blockers, AB = Alpha blockers, BB = Beta blockers, Diuretics = Loop or thiazide, ALDOSANT = Aldosterone antagonist, CKD = Chronic kidney disease.

approximately 16% [12, 13].

In RAS patients, in general acceptance invasive procedure can be recommended after evaluating clinical and hemodynamics conditions in patients with stenosis above 70% [14]. Four-year survival was 89% in patients with < 75% stenosis, while survival was 57% in patients with stenosis greater than 75% [15]. In a study, renovascular hypertension was suspected in 38% of 459 hypertension patients, and RAS was detected in more than 70% of these patients. Bilateral stenosis was found in 37% of patients over 65 years of age [16].

Patients with renal artery stenosis have renal parenchymal changes including interstitial fibrosis, tubular atrophy, glomerulosclerosis, periglomerular fibrosis, and a variety of arteriolar abnormalities [17]. There are studies showing that invasive intervention improves or does not change kidney functions in RAS patients. In a study in which 76 RAS patients were evaluated (serum creatinine > 1.5 mg/dL, > 70% stenosis), it was found that renal values improved in 20 ± 11 months follow-up after stenting [18]. In another study, in which 20 RAS patients over 55 years (diagnosed by MR angiography, serum creatinine > 2 mg/dL) were evaluated, it was detected that the invasive procedure did not change their kidney functions in 6 months after the procedure [19]. In a study evaluating 96 patients with atherosclerotic renal disease with a creatinine value above 1.5 mg/dL, 70% of the patients preserved their kidney values after revascularization, and dialysis treatment was initiated in 17% of the patients [20].

The place of medical therapy and interventional therapy in the treatment of the disease is controversial. In the CORAL study, when 947 patients with renal artery stenosis evaluated after a mean follow-up of 43 months, there was no difference in renal and cardiovascular outcomes between the two groups that received medical treatment and interventional treatment (stent) [7]. In the STAR trial, 140 patients (64 medical treatments, 76 interventional treatments) examined for 2 years. No difference was found in renal survival in

patients who received medical and interventional treatment (stent) [21]. In a study which 806 patients with atherosclerosis-related renovascular disease followed for an average of 34 months, no difference detected between medical and interventional (stent) treatment in terms of renal event, cardiovascular disease, and death [22, 23].

Changes in GFR values in RAS patients undergoing medical and interventional treatment were found to be different in many studies. In some studies, a decrease in GFR after treatment was observed in those who underwent interventional procedures, while in some studies it was observed in those who received medical treatment [21, 24].

In our study, increase in diastolic blood pressure and decrease in glomerular filtration rate were more prominent in the interventional treatment group. There was no significant difference in renal survival between two groups, like other studies.

Limitations

Our study was retrospective. MRA used for the diagnosis of RAS, data of patients diagnosed with other methods or after normal angiography could not be used. In this respect, the number of our patients was less.

CONCLUSION

Finally; in patients with renal artery stenosis, interventional or medical treatment should be decided by evaluating clinical status and comorbid diseases. Patients should be followed closely in terms of kidney function and survival.

Ethics Committee Approval

This study was approved by the Bursa City Hospital Ethical Committee (approval number: 2022-1/12, date: 09.02.2022).

Authors' Contribution

Study Conception: YA, BD, SA; Study Design: YA, BD, Mİ; Supervision: YA, BD, SA, Mİ; Funding: N/A; Materials: YA,GO; Data Collection and/or Processing: YA, BD; Statistical Analysis and/or Data Interpretation: GO; Literature Review: YA, BD; Manuscript Preparation: YA and Critical Review: YA.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1321-1326 Original Article

DOI: 10.18621/eurj.1226077 *Emergency Medicine*

Does prolonged QTc predict pulmonary involvement in COVID-19 patients?

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ABSTRACT

Objectives: Coronavirus disease 2019 (COVID-19) is a disease with high mortality due to acute respiratory distress syndrome (ARDS) secondary to viral pneumonia. In addition to its effects on the respiratory system, coronavirus is known to have serious systemic effects on the cardiovascular system. In this study, we aimed to investigate the association between prolonged QTc duration and COVID-19 specific pulmonary involvement

Methods: Between December 2020 and February 2021, 112 patients who were diagnosed with COVID-19 in our COVID-19 outpatient clinic and met the inclusion criteria were evaluated for the association between cardiac variables (heart rate, PR width, QRS width, fragmented QRS, and corrected QT [QTc] interval), other patient characteristics and lung involvement.

Results: A significant difference was found between the QTc intervals of COVID-19 patients with and without lung involvement ($p < 0.026$). In the ROC analysis for the QTc interval, which was found to be significant in the multivariate regression analysis, the cut-off value of 419.5 ms had a sensitivity of 72% and a specificity of 51.6% in predicting pulmonary involvement.

Conclusions: Prolonged QTc duration may be useful in predicting COVID-19 pulmonary involvement in patients admitted to the emergency department.

Keywords: COVID-19, electrocardiography, prolonged QTc, pulmonary involvement

Coronavirus disease 2019 (COVID-19) is a complex disease that has affected more than 500 million patients and caused more than six million deaths since its emergence [1]. COVID-19 is typically characterized by symptoms such as shortness of breath, fever, cough, fatigue, malaise, and taste and smell impairment. Because it primarily affects the lungs, the disease can rapidly progress to interstitial pneumonia and severe respiratory failure [2]. COVID -19 disease may also have adverse effects on the cardiovascular system along with respiratory system involvement. It is known that the disease can lead to many cardiac pathologies, including myopericarditis, pericardial effusion, hypoxia, direct cytotoxic effect, and acute coronary syndrome [3, 4].

The major cause of mortality in COVID-19 is the development of acute respiratory distress syndrome (ARDS) due to viral pneumonia, and the incidence of ARDS has been reported to exceed 15% in patients hospitalized for COVID-19 [5, 6]. Although the main

Received: ***December 29, 2022;*** *Accepted:* ***January 28, 2023;*** *Published Online:* ***April 10, 2023***

How to cite this article: *Sarıhan A, Rahman ÖF, Koran S, Aytemiz F, Can Ç, Rahman F, et al. Does prolonged QTc predict pulmonary involvement in COVID-19 patients? Eur Res J 2023;9(6):1321-1326. DOI: 10.18621/eurj.1226077*

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cause of mortality and morbidity is respiratory system involvement, the effects of COVID -19 on the cardiovascular system (acute coronary syndrome, pericardial effusion, arrhythmia, etc.) have also been demonstrated [7].

Inflammation and hypoxia caused by COVID-19 pneumonia are thought to affect the QT interval. The main causes of arrhythmias in COVID-19 patients are myocardial damage, systemic and local inflammation, electrolyte imbalance, and drugs used in treatment [8, 9]. There is increasing evidence that interleukins, particularly interleukin 6 (IL-6), may prolong the corrected QT (QTc) interval by affecting the action potential through a direct action on cardiomyocyte ion channels [10].

Female gender, advanced age, electrolyte imbalances, diuretic use, and renal failure are some of the known risk factors for prolonged QTc interval [11]. Many viral infections, such as human immunodeficiency virus (HIV) and dengue fever, have been independently associated with a prolonged QT interval [12]. In an animal study, coronavirus infection was associated with a prolonged QT interval in rabbits [13]. There are publications on QTc interval prolongation in COVID -19 patients in the absence of conventional risk factors [14].

Although there are studies in the literature showing an association between COVID-19 disease and QTc interval prolongation, the complexity of this association is not yet clear [15]. Prolonged QTc interval is an ECG parameter that has been associated with malignant arrhythmias, and QTc interval assessment is an easily applicable method in the emergency department [16].

Electrocardiography (ECG) is an important diagnostic method in detecting myocardial damage or arrhythmias in COVID -19 patients and may play a role in the treatment strategies of COVID-19 patients. In this study, we aimed to investigate the association between a prolonged QTc interval and pulmonary involvement in COVID-19.

METHODS

Selection of Patients

The study was prospectively conducted with patients who presented to the COVID -19 outpatient clinic of Manisa City Hospital between December 2020 and February 2021. The study was conducted with the approval of the non-interventional ethics committee of Istanbul Medipol College (E-10840098-772.02-2485). The records of a total of 139 patients over 18 years of age and without suspected pregnancy who presented to the hospital with symptoms of COVID -19 disease were analyzed after obtaining written informed consent. Exclusion criteria were the presence of pulmonary edema, electrolyte disturbances, ECG abnormalities (ST segmental changes, atrial fibrillation, pacemaker rhythm, bundle branch block, and arrhythmia), and use of medications that prolong the QT interval (amiodarone, citalopram, clomipramine, sotalol, clarithromycin). Twenty-seven patients were excluded from the study based on these exclusion criteria, and 112 patients were evaluated. Patient data were divided into two groups: COVID-19 patients without pulmonary involvement (group 1) and COVID-19 patients with pulmonary involvement (group 2).

ECG Analysis

ECG was recorded at a rate of 25 mm/sec with a calibration of 1 mV/cm and a filter setting of 0.05-150 Hz. The parametric ECG values included HR (heart rate), PR (interval between the onset of the P wave and the beginning of the R wave), QRS (interval between the onset of the Q wave and the end of the S wave), and QT interval measurements. The heart rate corrected QT (QTc) interval was measured using the Bazett correction formula ($QTc = QT / \sqrt{RR(sec)}$).
QT was automatically calculated as the interval from the beginning of the Q wave to the end of the T wave and corrected for heart rate using the Bazett formula (QTc). All ECGs were recorded with a Philips PageWriter TC30 Cardiograph (Koninklijke Philips, Eindhoven, The Netherlands). The ECG examination was evaluated by a cardiologist. Among the selected patients, those whose chest CT scans had a high probability of pulmonary involvement with COVID-19 according to radiological reports were classified as COVID-19 pneumonia.

Statistical Analysis

SPSS 26.0 software (SPSS Inc. Chicago, IL) was used for statistical analysis. After checking the conformity of the data to the normal distribution with the

Table 1. Demographic characteristics of the patients and their distribution according to groups

	Group 1 (n = 62)	Group 2 (n = 50)	*p* value
Age (years), Mean ± SD	62.55 ± 10.20	58.94 ± 7.03	0.029*
Gender, n (%)			0.150#
Male	30 (48.4)	31 (62)	
Female	32 (51.6)	10 (38)	
Hypertension, n (%)	18 (29)	21 (42)	0.152#
Diabetes Mellitus, n (%)	11 (17.7)	10 (20)	0.761#
CAD, n (%)	10 (16.1)	16 (32)	**0.048**#
Heart Failure, n (%)	4 (6.5)	7 (14)	0.182#
COPD, n (%)	7 (11.5)	11 (22)	0.125#
CRF, n (%)	0 (0)	4 (8)	**0.037**#
PCR positivity, n (%)	26 (41)	41 (82)	**0.001**#

COPD = chronic obstruction pulmonary disease, CAD = coronary artery disease, CRF = chronic renal failure, PCR = polymerase chain reaction, SD =standard deviation

*t-test, #Chi-square test

Kolmogorov-Smirnov test, parametric tests for continuous variables with normal distribution were preferred. Data were analyzed by descriptive statistics (number, percentage, mean, standard deviation), T-test, Mann-Whitney U and Chi-square test, logistic regression, and ROC (Receiving operator characteristic) curve. The significance level accepted was $p < 0.05$.

RESULTS

Patient demographics and their distribution among groups are shown in Table 1. Coronary artery disease (CAD), chronic renal failure (CRF), and PCR test positivity were significantly higher in the group with pulmonary involvement (group II). Compared to the group without lung involvement (group I), the mean age of group II was significantly lower ($p = 0.029$). The cardiac variables (heart rate, PR, QRS, fQRS, and QTc) of the groups are shown in Table 2. The mean QTc interval of subjects in group II was significantly higher.

Univariate and multivariate logistic regression analyzes performed to determine the relationship between the variables and lung involvement are shown in Table 3. Age, PCR, and QTc variables were included in the multivariate logistic regression model. Multivariate logistic regression analysis revealed that lower age (odds ratio [OR]: 0.929; 95% CI: 0.879-0.982, $p = 0.009$), PCR positivity ([OR]: 7.28; 95%

Table 2. Distribution of cardiac variables according to groups

	Group 1 (n = 62)	Group 2 (n = 50)	*p* value
Heart rate (beat/min)	82.52 ± 15.49	85.46 ± 15.23	0.316*
PR (ms)	140.59 ± 17.86	141.74 ± 16.82	0.730*
QRS (ms)	84.51 ± 9.66	84.04 ± 11.69	0.814*
fQRS, n (%)	21 (34.4)	11 (22.4)	0.169#
QTc (ms)	421.70 ± 22.66	436.52 ± 30.72	**0.004***

Data are shown as mean±standard deviation or n (%).

f QRS = fragmented QRS, PCR = polymerase chain reaction

*t-test, #Chi-square test

Table 3. Univariate and multivariate regression analysis results of variables in predicting lung involvement

	Univariate		Multivariate	
	Odds ratio (95% CI)	***p* value**	**Odds ratio (95% CI)**	***p* value**
Age	0.955 (0.914-0.998)	**0.039**	0.929 (0.879-0.982)	**0.009**
CAD	2.44 (0.994-6.022)	0.051		
CRF	2177379136 (0.00-0.00)	0.999		
PCR positivity	6.3 (2.615-15.212)	**< 0.001**	7.28 (2.691-19.362)	**< 0.001**
QTc	1.02 (1.006-1.040)	**0.015**	1.023 (1.003-1.043)	**0.026**

CAD = Coronary artery disease, CRF = chronic renal failure

CI: 2.691-19.362, $p < 0.001$) and prolonged QTc duration ([OR]: 1.023; 95% CI: 1.003-1.043, $p = 0.026$) were significantly associated with lung involvement.

The prediction point for the QTc interval, which plays a role in predicting pulmonary involvement, was determined using ROC curve analysis (Fig. 1). In the analysis, the QTc interval with a value of 419.5 ms predicted pulmonary involvement with 72% sensitivity and 51.6% specificity (AUC:0.651, 95% CI:0.549-0.753, $p = 0.006$).

DISCUSSION

Although COVID-19 mainly targets lung tissue, it may have direct or indirect adverse effects on the heart. COVID-19-related conditions such as myopericarditis, complete AV block, acute coronary syndromes, decompensated heart failure, and pulmonary embolism have been reported in the literature [17, 18]. On the other hand, several studies have described an abnormal immune-inflammatory response to SARS-CoV-2 infection. Another study showed that the levels of interleukin (IL)-1β, IL-6, IL-8, IL-10, and soluble TNF receptor 1 (sTNFR1) were increased in patients with SARS-CoV-2 infection compared with healthy subjects [19]. In a recent meta-analysis, elevated levels of other immune-inflammatory parameters such as C-reactive protein, white blood cell count, and procalcitonin were shown to be significantly associated with disease severity [20]. Considering that inflammation can also lead to QTc interval prolongation, SARS-CoV-2 infection may prolong QTc duration through an inflammatory response [21]. Thus, prolonged QTc duration in SARS-CoV-2 infection may be a direct consequence of viral activity or may be mediated by inflammation. This helps to explain why a prolonged QTc interval is independently associated with mortality [22]. Ay *et al*. [23] reported that there may be an association between QTc interval prolongation and mortality in COVID-19 patients. Again, some studies have emphasized that the cardiac effects of COVID-19 disease increase mortality [23, 24]. QTc interval prolongation is thought to be one of the reasons for the increased mortality in COVID-19 [25]. Prolongation of the QTc interval due to hydroxychloroquine and azithromycin, which are used in the treatment of COVID-19, has also been reported in the literature

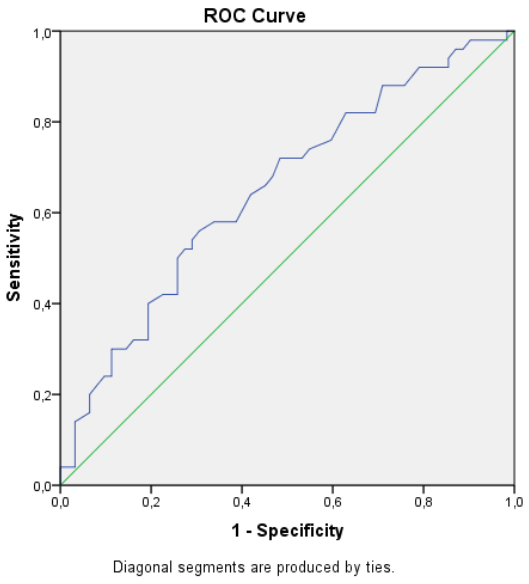

Fig 1. Evaluation of the effectiveness of the QTc interval in predicting pulmonary involvement using the ROC curve.

[26]. In a study comparing the QTC value at admission of covid-19 patients, it was found that 10% had a QTc interval and QTc prolongation was independently associated with increased mortality. This result supports our thesis that QTc can be used as a predictive factor at the time of admission [22].

Although the role of ECG in the early diagnosis of cardiovascular complications and mortality in COVID-19 is well known, the role of ECG abnormalities in predicting pulmonary involvement in COVID-19 pneumonia has not been found in the literature.

In this study, we investigated the relationship between ECG findings, patient characteristic variables, and pulmonary involvement in patients hospitalized with COVID-19 symptoms and diagnosed with COVID-19.

In our study, Group II had higher CRF and CAD rates than Group I. This suggests that COVID -19 positive patients with chronic diseases should be closely monitored because of the risk of pulmonary involvement and mortality (Table 1). The fact that the mean age of group I patients was higher than the mean age of group II patients (Table 1) suggests that younger patients were exposed to a higher viral load because of the isolation of the elderly population during the pandemic. Since CRF patients are dialysis-dependent and dialysis treatment is provided under hospital conditions, these patients have a higher COVID-19 viral load. These patients are at higher risk, not only for nosocomial opportunistic infections but also because they are transported to the hospital by public transportation.

In our study, pulmonary involvement was found to be significantly associated with QTc interval in univariate and multivariate regression analysis (Table 3) (Fig. 1). In the ROC analysis for the QTc interval, which was found to be significant in the multivariate regression analysis, the cut-off value of 419.5 ms had a sensitivity of 72% and a specificity of 51.6% in predicting pulmonary involvement. The most common ECG abnormality resulting from COVID-19-associated hypoxia is QTc interval prolongation. Significant prolongation of the QTc interval has been noted, particularly in elderly patients with right ventricular contractile defect, and a high mortality rate has been reported in these patients [21].

In our study, the relationship between pulmonary involvement and QTc was clearly demonstrated by excluding patients who were taking medications that might affect the QT interval. Because of this relationship, the use of drugs that prolong the QTc interval may worsen the clinical picture in patients with increased lobular involvement. Therefore, QTc and pulmonary involvement should be considered when prescribing these drugs, and QTc times should be monitored during treatment.

A significant increase in mortality has been observed in patients with severe COVID-19 pneumonia [27]. Considering the association between prolonged QTc interval and COVID-19 pulmonary involvement in our study, we believe that close cardiac monitoring is also important in this group of patients at risk of mortality

CONCLUSION

The presence of a prolonged QT interval on the ECG of COVID-19 patients at the time of hospital admission may be helpful in predicting pulmonary involvement. It should be kept in mind that these patients should be monitored closely, as this may lead to cardiac complications. To this end, we think that ECG, which is an inexpensive and non-invasive tool available in all healthcare facilities, as well as the use of smartwatches or devices that can perform remote cardiac monitoring, can be easily used to predict pulmonary involvement in COVID-19 pneumonia.

Authors' Contribution

Study Conception: AS, ÖFR; Study Design: SK, ÇC; Supervision: ÖFR; Funding: N/A; Materials: AS, ÇC; Data Collection and/or Processing: EB; Statistical Analysis and/or Data Interpretation: FR; Literature Review: FR, EB; Manuscript Preparation: AS, ÖFR and Critical Review: FA.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1327-1333 Original Article

DOI: 10.18621/eurj.1180149 *Cardiology*

The relationship of epicardial fat and atrial high-rate episodes in patients with permanent pacemaker

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ABSTRACT

Objectives: Atrial high-rate episodes (AHRE) can occur in patients who have permanent pacemakers (PPM). AHRE is classified as subclinical atrial fibrillation (AF). Also, AHRE is related to clinical AF. Epicardial fat tissue (EFT) thickness is linked to AF. The purpose of this study was to examine the relation between AHRE and EFT thickness in PPM patients.

Methods: Forty patients with dual-chamber PPM were enrolled. Transthoracic echocardiography was used to measure the thickness of the EFT. Patients were examined in 2 groups according to whether there was AHRE in the records: group 1 (AHRE) and group 2 (AHRE-free). A high atrial rate of more than 175 beats per minute for more than 5 minutes was defined as AHRE. The findings of the standard 2-dimensional echocardiography and the Doppler echocardiography were all recorded. A statistical relationship between EFT thickness and the development of AF was investigated.

Results: Group 1 had ten patients, while Group 2 included thirty individuals. When the demographic features of the two groups were compared, they were similar. Both groups had similar 2-D echocardiographic and Doppler results. The difference in EFT thickness between groups 1 (2.0 ± 1.1 mm) and 2 (2.9 ± 1.8 mm) was not statistically significant ($p = 0.138$).

Conclusions: In patients with PPM, AHRE may develop after implantation and may be detected asymptomatically in periodic follow-up. In this population, we did not observe a significant association between EFT thickness and the development of AF.

Keywords: Epicardial fat, atrial high rate, atrial fibrillation

In patients with permanent pacemakers (PPM) atrial fibrillation may occur with overt clinical manifestations, or it may sometimes be detected as "silent-subclinical" with an AF complication such as stroke or tachycardiomyopathy. Sometimes it can be identified even in the absence of clinical signs. For this reason, the detection and appropriate treatment of AF in these patients is a critical problem awaiting a solution. Detection of clinical AF requires documentation of a 12-lead electrocardiogram (ECG) or AF electrocardiographic features on a rhythm strip of at least 30 seconds [1]. Pacemaker telemetry can record AF episodes, enabling diagnosis and quantification of the AF burden [2]. Recent studies have investigated the

Received: ***September 26, 2022;*** *Accepted:* ***November 12, 2022;*** *Published Online:* ***April 3, 2023***

How to cite this article: *Hoşoğlu Y, Akkaya F, Kırış A. The relationship of epicardial fat and atrial high-rate episodes in patients with permanent pacemaker. Eur Res J 2023;9(6):1327-1333. DOI: 10.18621/eurj.1180149*

e-ISSN: 2149-3189

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link between AF and epicardial fat tissue (EFT) thickness, which has endocrine properties. [3, 4]. It has been shown that EFT can be found in the atrioventricular and interventricular groove, right ventricular free wall, left-right ventricular apical, around the atrium and appendage, outside the adventitia of the coronary arteries [5]. In the study in which Mazurek and his colleagues demonstrated the inflammatory activity of EFT with positron emission tomography, it was noted that EFT showed more inflammatory properties in patients with AF [6]. According to Iacobellis *et al*., EFT thickness is greater in chronic AF patients than in paroxysmal AF patients [7].

An atrial high-rate episode (AHRE) is characterized by tachycardia episodes lasting more than 5 minutes with an atrial rate greater than 175 beats per minute (b.p.m) recorded by cardiac implantable devices. The presence of AHRE is considered as a subclinical AF [8] and it increases the risk of clinical AF by 5.66 times and stroke by 2.41 times [9]. There has been significant research on the link between the thickness of the EFT and AF [10], but less is known about the link between EFT and AHRE.

Our understanding of the causes of AHRE, which is a common finding in patients with PPM and has been linked to an increased risk of stroke and systemic embolism when it occurs frequently and for prolonged periods [11], remains limited. To address this knowledge gap, we conducted a study to investigate whether there is a relationship between EFT thickness (a known risk factor for AF) and AHRE, and by extension, the development of AF in patients with PPM.

METHODS

The local Clinical Research Ethics Committee gave their approval to the study (07.04.2014/03). This study adheres to all institutional and/or national research committee and Helsinki statement of 1964 ethical standards. All subjects were informed about the research and granted their consent.

Study Population

The research was involved 40 subjects who were presented for periodic PPM follow-up, provided written consent, m*et al*l inclusion criteria, and had a conventional dual-chamber PPM (DDD-PPM) implanted but no history of permanent AF. Indications for PPM implantation were sick sinus syndrome (25%, n = 10), complete heart block (27.5%, n = 11) and other causes (47.5%, n = 19). The following were the research's exclusion criteria: Patients with > 50% stenosis of a coronary artery, hyperthyroidism, severe valvular disease (moderate-severe), hypertrophic cardiomyopathy, and systolic HF (Ejection fraction < 50%).

In the study, only patients with DDD-PPM were included. If the patient requires a pacemaker and the sinus node is intact, a DDD-mode PPM is usually implanted because it is more physiological. These pacemakers also record AHRE or other arrhythmic events. The presence of an atrial rate of > 175 b.p.m. lasting > 5 minutes was defined as AHRE [1, 8]. Since AHRE is highly correlated with AF [8], two separate groups were formed according to those with and without AF detected in the pacemaker interrogation. The group with AHRE constituted the group 1, and the group with AHRE-free patients formed group 2.

Concurrent medical conditions and medication use were recorded. The height and weight of the patients were recorded. After 5 minutes of rest, the patients' blood pressure (BP) was recorded as the average of two measures taken on the right arm while seated.

Echocardiographic Measurements

Echocardiographic measurements were performed with Vivid 7 (GE, Norway) echocardiography machine in all patients. On parasternal long axis imaging, left atrial (LA) diameter, LV end-systolic and end-diastolic diameters, interventricular septum and posterior wall thicknesses (IVS and PW), and EFT thickness were measured using 2-dimensional echocardiography. EFT thickness was measured as the echo-free space in front of the right ventricular free wall on transthoracic parasternal long-axis images according to the predefined technique [12]. The LV volumes, maximum and minimum LA volumes, right atrium and right ventricular diameters were all measured using apical four-chamber imaging. The modified Simpson's method was used to calculate left ventricular ejection fraction (EF) and LV volumes. Early diastolic wave (E) and late diastolic wave (A) were measured 1 cm distal to the mitral valve leaflets in apical four-chamber imaging with Doppler echocardiog-

raphy. In tissue Doppler examination, the sample volume was placed in the lateral mitral annulus and early (E'), late (A') diastolic and systolic (S') waves were measured. TAPSE (tricuspid annular plane systolic excursion) was measured using M-mode by placing the sample volume on the tricuspid valve's lateral annulus.

Statistical Analysis

All values were presented as the mean plus the standard deviation. Using the Kolmogorov-Smirnov test, the normality of the distribution was determined. The Student's t-test was used to evaluate continuous variables with a normal distribution, whereas the Mann-Whitney U test was used to evaluate continuous variables with a non-normal distribution. Pearson Chi-Square or Fisher Exact test was applied to categorical variables expressed as a percentage, depending on the sample size. *P* values under 0.05 were considered statistically significant. SPSS (13.0, Inc., Chicago, Illinois) software was applied for statistical analysis.

RESULTS

Forty patients with DDD PPM participated in the trial. Those with AHRE (group 1, n = 10) and those without AHRE (group 2, n = 30) were split into two groups. Sick sinus syndrome (n = 10) and third degree AV block (n = 11) were the most common indications for PPM. There was no statistical difference between the groups in terms of age. The male gender was more prevalent in both groups, but gender distribution was similar. There was no difference between the two groups regarding smoking, HT, DM and atherosclerosis history. The BP values measured at rest were similar between the two groups. While the resting heart

Table 1. Demographic characteristics of the patients

	Group 1 (AHRE) n = 10	Group 2 (AHRE-free) n = 30	*p* value
Age (year) (mean ± SD)	57.8 ± 17.0	60.5 ± 1.7	0.609
Gender, n (%)			0.858
Male	6 (60)	17 (56.7)	
Female	4 (40)	13 (43.6)	
Pacemaker indication, n (%)			0.590
Sick sinus syndrome	3 (30)	7 (23.3)	
AV complete block	3 (30)	8 (26.7)	
Other	4 (40)	15 (50)	
Hypertension, n (%)	3 (30)	17 (56.7)	0.152
Diabetes Mellitus, n (%)	1 (10)	1 (3.3)	0.415
Coronary artery disease, n (%)	2 (20)	6 (20)	0.707
Smoking, n (%)	1 (10)	3 (10)	1.0
Body Mass Index (kg/m²) (mean ± SD)	26 ± 3.8	28 ± 3.1	0.104
Aspirin, n (%)	4 (40)	10 (33.3)	0.711
Statin, n (%)	0	6 (20)	0.132
Beta blocker, n (%)	4 (40)	11 (36.7)	0.855
Calcium channel blocker, n (%)	1 (10)	7 (23.3)	0.374
ACE inhibitor/ARB, n (%)	1 (10)	11 (36.7)	0.117
Amiodarone, n (%)	1 (10)	2 (6.7)	0.737
Systolic BP (mmHg) (mean ± SD)	126 ± 16.3	119 ± 16	0.184
Diastolic BP (mmHg) (mean ± SD)	76.0 ± 9.7	73.7 ± 6.1	0.031

ACE = Angiotensin-converting enzyme, AHRE = Atrial high rate episode, ARB = Angiotensin receptor blocker, AV = Atrioventricular, BP = Blood pressure, SD = Standard deviation

rate was 74.5 ± 8.8 b.p.m. in group 1, it was 71.8 ± 14.8 b.p.m. in group 2, and there was no difference between the groups. Also, there was no difference in drug use and BMI values between the two groups. Baseline demographic characteristics of patients in group 1 and 2 are shown in Table 1. The demographic characteristics of the patients were similar in both groups. Left ventricular diameters and volumes, wall thicknesses, LVM index and LA diameter, which are the basic echocardiographic parameters, were similar between groups. Although LA minimum and maximum volumes were higher in group 1, this difference was not statistically significant. TAPSE was also similar in both groups (p = 0.44). Doppler and tissue Doppler findings were similar in both groups. Two-dimensional M-mode, Doppler and tissue Doppler findings echocardiography results are shown in Table 2. While the mean EFT thickness was 2.0 ± 1.1 mm in group 1, it was 2.9 ± 1.8 mm in group 2; this difference between the two groups was not statistically significant. There were three patients with EFT thickness ≥ 3 mm and AF. Epicardial fat thickness was < 3 mm in

Table 2. Echocardiographic 2D, M mode, Doppler findings of the study population

	Group 1 (AHRE) n = 10 Mean ± SD	Group 2 (AHRE-free) n = 30 Mean ± SD	*p* value
Left atrium			
Diameter (mm)	35.5 ± 4.5	36.1 ± 5.5	0.771
Maximum volume (ml)	52.6 ± 22.1	45.2 ± 17.3	0.282
Minimum volume (ml)	24.5 ± 12.4	18.9 ± 9.9	0.152
Left ventricle			
ESD (mm)	29.5 ± 5.1	29.9 ± 6.4	0.301
EDD (mm)	47.6 ± 3.9	48.5 ± 5.1	0.603
ESV (ml)	40.0 ± 13.5	38.4 ± 13.8	0.751
EDV (ml)	88.9 ± 30.7	87.5 ± 33.6	0.908
Ejection fraction (%)	60.6 ± 6.7	59.5 ± 5.4	0.592
IVS (mm)	12.2 ± 3.2	11.7 ± 2.5	0.591
PW (mm)	9.8 ± 1.3	10.4 ± 1.7	0.351
Right atrium diameter (mm)	40.3 ± 5.7	38.0 ± 5.3	0.245
Right ventricular diameter (mm)	33.1 ± 6.8	35.3 ± 5.2	0.283
LVM index (gr/m²)	101.5 ± 6.3	107.4 ± 5.0	0.617
E (cm/s)	58.9 ± 18.1	65.7 ± 17.2	0.333
A (cm/s)	68.2 ± 23.9	70.6 ± 19.3	0.772
E' (cm/s)	10.4 ± 4.2	9.6 ± 3.8	0.616
A' (cm/s)	11.6 ± 2.9	10.7 ± 3.2	0.772
TAPSE (mm)	21.6 ± 5.1	23.1 ± 5.2	0.449
Epicardial fat tissue (mm)	2.0 ± 1.1	2.9 ± 1.8	0.138

A = Late mitral diastolic filling velocity, A' = Mitral annular late diastolic wave, AHRE = Atrial high rate episode, E = Early mitral diastolic filling velocity, E' = Mitral annular early diastolic wave, EDD = end-diastolic diameter, EDV = end-diastolic volume, ESD = end-systolic diameter, ESV = end-systolic volume, IVS = Interventricular septum thickness, LVM = Left ventricular mass, PW = Left ventricular posterior wall thickness, S' = Mitral annular systolic wave, SD = Standard deviation , TAPSE = Systolic movement of the tricuspid annular plane

Table 3. Characteristics of patients according to EFT thickness

	EFT thickness ≥ 3 mm (n = 17)	EFT thickness < 3 mm (n = 23)	*p value*
AHRE, n (%)	3 (17.6)	7 (30)	-
Age (years) (mean ± SD	62.9 ± 12.5	57.6 ± 15.5	0.266
HT, n (%)	17 (42.5)	23 (57.5)	0.806
LA diameter (mm) (mean ± SD	35.7 ± 4.7	36.1 ± 5.7	0.672
LVM index (gr/m²) (mean ± SD	104.1 ± 25.2	107.3 ± 33.1	0.617

AHRE = Atrial high rate episode, EFT = Epicardial fat tissue, HT = Hypertension, LA = Left atrium, LVM = Left ventricular mass, SD = Standard deviation

the other seven patients with AF. While mean EFT thickness was 2.7 ± 0.8 mm in 6 patients with a BMI ≥ 30, it was 2.7 ± 1.8 mm in those with a body mass index < 30, and there was no significant difference between the two groups. Characteristics of patients according to EFT thickness are shown in Table 3. Atrial fibrillation was detected in the ECGs of three patients at the time of enrollment (Paroxysmal AF). In the other seven patients, sinus rhythm was present in the ECG, but AHRE was detected in the recordings of the pacemaker telemetry.

The lowest EFT thickness was measured as 1 mm in the whole population, and the maximum was 8 mm. Epicardial fat thickness was ≥ 3 mm in 17 (42.5%) patients and < 3 mm in 23 (57.5%) patients.

DISCUSSION

In this research article, we investigated the association between AHRE detection and EFT thickness in DDD PPM patients. AHRE was detected in 25% of patients with DDD PPM. However, no statistically significant difference was found in terms of EFT thickness between those with and without AHRE.

AHRE is not uncommon in patients with PPM. According to the literature, 10-30% AHRE has been reported in patients with PPM who were not previously known to have AF. [13]. In the earlier MOST study, 51.3% of patients with PPM were found to have an AHRE of > 220 beats/min lasting longer than 5 minutes within 27 months of follow-up. [14]. In the ASSERT study, which enrolled 2580 patients aged > 65 years, with a PPM and without a history of AF, it was determined that subclinical AHRE developed at a rate of 10.1% in a 3-month follow-up. These tachycardia episodes were associated with clinical AF, and the risk of AF development increased to 36% within 2.8 years of follow-up. In addition, subclinical AF was related with an increased risk of ischemic stroke or systemic embolism, as demonstrated by this study [15]. In our study, the incidence of AHRE in patients with PPM was 25%. Since our patients in the study population were younger than other studies, AHRE was evaluated with a one-time measurement, and comorbid conditions that could lead to AF were less in our patients, the AHRE rate may have been found to be lower.

Epicardial fat tissue functions as a paracrine organ and has been shown to cause AF via various mechanisms. A significant risk factor for AF is inflammation, which is caused by the release of inflammatory cytokines and markers in the blood from EFT. Obesity, metabolic syndrome, and atherosclerosis are also associated with EFT [7, 12, 16]. According to the findings of a cardiac tomography study, EFT is thicker in patients with persistent AF than in patients without AF [17]. Based on previous studies examining the relationship between EFT and AF, we hypothesized that EFT may also be associated with AHRE. But we found no statistically significant difference existed between the groups. Possible explanations for this finding include the relatively young age and low rates of comorbid diseases such as diabetes in group 1, as well as the slightly lower mean BMI in the AF group, although this difference was not statistically significant.

Limitations

Our study was not prospective. However, the prospective study will require a long time and increase

costs. The relatively small number of patients is also a significant limitation. However, the effect of this restriction on the results was tried to be eliminated by strictly applying exclusion criteria in patient selection and including the patient population with one type of PPM (the type of PPM most associated with AF). Finally, the evaluation of AF only with the presence of AHRE obtained from PPM records is another limiting factor of the study. Implantable loop recorders, smartwatches or smart devices also could be used to detect AF, but these technologies would also increase costs.

CONCLUSION

According to the findings of this study, the hypothesis that EFT thickness could be a predictor of AF in patients with PPM was evaluated. Still, EFT thickness was not associated with AF in this particular population. More research is needed to determine the predictors of the development of AF in patients with PPM.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1334-1342 Original Article

DOI: 10.18621/eurj.1202118 *Molecular Medicine*

Investigation of *Lactobacillus* spp. bacteria in infants consuming breast milk and formula and determination of some probiotic characters

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ABSTRACT

Objectives: It was aimed to investigate some probiotic properties of Lactic Acid Bacteria (LAB) isolated from stool samples taken from 45 healthy 0-4 month old babies, who did not take antibiotics or probiotic supplements in the last 3 months, and who did not have any health problems.

Methods: Six different species were obtained from 21 isolates selected by the method of Mass Spectrometry (MALDI-TOF MS).

Results: The most common strain was *Lactobacillus rhamnosus* with a rate of 59%, followed by *Lactobacillus paracasei* with a rate of 13.6%. Vancomycin, tetracycline, gentamicin, netilmicin, tobramycin, penicillin, ampicillin, teicoplanin and amikacin antibiotics were used to evaluate the antimicrobial activities of the strains. In our study, while all strains were resistant to the antibiotic amikacin, they were sensitive to tetracycline, penicillin, gentamicin, netilmicin, teicoplanin, vancomycin, ampicillin and tobramycin antibiotics. In the evaluation of the antagonistic activities of LAB, 6 different pathogens (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Listeria monocytogenes* ATCC 19111) were used and it was determined that the strains showed antimicrobial effects on all pathogenic microorganisms. Cholesterol assimilation abilities, T21 and T22 strains achieved the highest cholesterol assimilation rate of 39.1%.

Conclusions: It is thought that most of the isolated strains have probiotic potential, and especially *Lactobacillus gasseri* T21 and *Lactobacillus paracasei* T22 strains may be probiotic strains that can be used in the production of preparations alone or together with other *Lactobacillus strains*.

Keywords: Lactic acid bacteria, breast milk, probiotic

One of the most basic events that shape the intestinal microbiota is breastfeeding [1]. Breast milk; Milk containing 10-105 CFU of microorganisms per ml from which *Streptococcus* and *Staphylococcus* are the most, *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and short-chain fatty acid producing bacteria such as *Veilonello*, *Propionibacterium*, *Faecalibacterium* are easily isolated. Human milk plays an important

Received: ***November 10, 2022;*** *Accepted:* ***February 13, 2023;*** *Published Online:* ***April 6, 2023***

How to cite this article: *Muslu Çağal T, Kıray E, Kariptaş E. Investigation of Lactobacillus spp. bacteria in infants consuming breast milk and formula and determination of some probiotic characters. Eur Res J 2023;9(6):1334-1342. DOI: 10.18621/eurj.1202118*

e-ISSN: 2149-3189

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role in the development of the neonatal gut microbiota, as it provides a continuous supply of microorganisms to the infant gut a few weeks after birth [2].

An effective probiotic agent is known to be microorganisms that can survive the host's digestive process, colonize the gut, and produce a beneficial response in the host without pathogenic or toxic side effects [3]. Each probiotic strain is known to have its own characteristics and should be investigated in detail in this context. It is necessary to determine the types of probiotic characters to be used especially in infant formula.

For this purpose, in our study, it was aimed to determine the various probiotic characterizations of lactic acid bacteria (LAB) isolated from the stools of 0-4 month-old infants who received only breast milk and supplemented with formula in addition to breast milk.

METHODS

Isolation of Bacteria

In our study, isolated from the stools of 51 healthy babies who were admitted to Sivas Cumhuriyet University Health Services Application and Research Hospital, aged 0-4 months, born vaginally, who did not receive antibiotic and/or probiotic treatment in the last 3 months, took only breast milk or were fed with formula in addition to breast milk. The obtained LAB was used. During the collection of stool samples, the baby's gender, week of birth, birth weight, current weight, feeding style (whether he took only breast milk or infant formula in addition to breast milk) and the number of daily defecations were obtained. The ethics committee of our study was received from Kırşehir Ahi Evran University on 24.09.2019 (Decision No 2019-16/165). The samples were brought to Sivas Cumhuriyet University Faculty of Medicine, Department of Medical Microbiology, Microbiology Laboratory, under sterile conditions, and evaluated within the same day. MRS (De Man Ragosa Sharpe, Merck) broth and solid media were used in the development and activation of LAB. Each sample was incubated in MRS solid medium for 48 hours at 37°C in anaerobic conditions. Large, small, white, off-white and opaque colonies formed on the media as a result of incubation were selected and stored in TSB (Tryptic Soy Broth) medium with glycerol at -18°C until studied. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 25923, *Listeria moncytogene*s strains used to determine the antagonistic effects of LAB were obtained from Amasya University Microbiology Laboratory Culture Collection. Species of isolated strains were determined by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Biotyper 3.0 Mikroflex LT Bruker Daltonics GmbH Bremen Germany). MALDI-TOF MS detects the protein profiles of existing microorganisms and compares the obtained profiles with the microorganisms in the current library [4].

Determination of Probiotic Properties of LAB

Acid and Bile Tolerance

In order to determine the accessibility of LAB isolated from the stool of infants to the intestines by surviving in the acidic environment of the stomach, an environment similar to the gastric fluid environment was prepared. Cultures activated in broth for 18 hours were precipitated by centrifugation at 3000 × g for 15 minutes at 4^{0}C. After the precipitate was washed twice with sterile phosphate buffered saline (PBS), PBS buffer (1N NaOH and 1N HCl) was prepared in 3 different ways with pH levels of 2.0, 2.5,3.0 and the strains were incubated in low pH PBS buffers for 3 hours at 37^{0}C. has been done. PBS with a pH of 7.2 was used for the control. Serial dilutions were made by taking 1 ml from the samples that were incubated at low pH at the 0th and 3rd hours of the incubation, and they were incubated by making triple parallel cultivations on MRS agar media. At the end of the period, the colonies in the control and test groups were counted and the % viability rates were calculated. % Viability = X/X0 (X: Number of viable microorganisms in the test group, XO: Number of viable microorganisms in the control group) [5].

Bile Salt Tolerance: 0.3% by weight to determine the viability of the isolated LAB in a medium containing bile salt; MRS broths containing 0.5% and 1% bile salts (oxgall, sigma) were prepared and the other stages of the study were applied as stated above.

% Vitality = X/X0 ×100 [6].

pH Change

Each sample was inoculated into MRS broth, and after 18 hours of incubation at 37^{0}C, it was measured

with a pH meter (AZ Instrument) to determine the acidic pH values of the cultures. As a control, the pH of sterile MRS broth without inoculation was checked. Measurements were carried out in 3 repetitions [7].

Determination of Antibiotic Sensitivities

Activated cultures in MRS medium were adjusted to 0.5 McF (625 nm absorbance = 0.08-0.1) with physiological saline and spread homogeneously on sterile MRS agar with a sterile drigalski spatula. Antibiogram discs were placed in Petri dishes at appropriate intervals and incubated at 37°C for 24 hours. The diameters of the zones formed around the antibiotic discs as a result of incubation were measured in millimeters with a caliper. Measurements were evaluated as Resistant (R), Semi-Fine (I), and Sensitive (S) according to NCCLS (National Committee for Clinical Laboratory Standards) criteria. Ampicillin (AM) (10 mcg) in the study; Penicillin (P) (10 U); Teicoplanin (TEC) (30 mcg); Gentamicin (CN) (120 mcg); Tetracycline (T) (30 mcg); Netilmicin (NET) (30 mcg); Vancomycin (VA) (30 mcg); Tobramycin (TOB) (10 mcg); Amikacin (AK) (30 mcg) discs were used.

Determination of Lactic Acid Amounts

5 mL of distilled water and 250 microliters of phenolphthalein were added to each of the 5 mL samples activated for 18 hours at 37 C in MRS broth. It was titrated by adding 0.1 N NaOH dropwise. The number of drops is multiplied by 4. The lactic acid content of each sample was determined so that each 1 ml of 0.1 N NaOH consumed was equivalent to 0.009 g of lactic acid [8, 9].

Determination of Cholesterol Assimilation Capacities

In order to determine the cholesterol assimilation capacities of LAB isolated from the stool microflora of healthy infants, the total cholesterol level of serum collected from patients with serum cholesterol level of 250-300 mg/dL who applied to Amasya University Sabuncuoğlu Şerefeddin Training and Research Hospital was measured. 1 mL of cultures activated for 18 hours in MRS broth was taken and added to [0.3% (Oxgall, Sigma)] MRS broth (3 mL) containing bile salt. Each sample, 1 mL of sterile serum, was collected into 5 ml tubes by passing it through a 0.45 micro m disposable (Milipore, USA) filter with a final concentration of 100 mg/mL. After incubation at 37ºC for 24 hours, the final cholesterol values of the supernatants were determined by centrifugation at 5000 × g at 4ºC for 10 minutes. Measurements were carried out in the Biochemistry Laboratory of Amasya University Sabuncuoğlu Şerefeddin Training and Research Hospital on a Roche HITACHI cobas 8000 device. Cholesterol reduction rates were determined by comparing the cholesterol amount of the samples before incubation with the amount of cholesterol after incubation.

RESULTS

Collection of Stool Samples and Identification of LAB

Only commercial infant formula, who applied to Sivas Cumhuriyet University Hospital Pediatrics outpatient clinic for routine controls, was born vaginally, did not use antibiotics and probiotics in the last 3 months, did

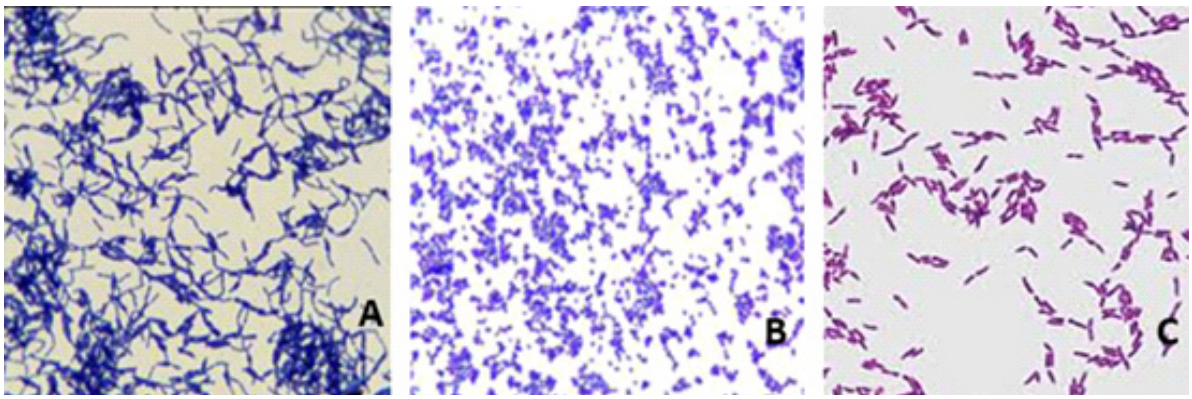

Fig 1. Gram stain images of 3 different species isolated from stool microflora, respectively (A) *L. rhamnosus*, (B) *P. acidilactici*, and (C) *L. reuteri*.

not have a history of hospitalization, took only breast milk, received and/or receives commercial infant formula in addition to breast milk. For LAB isolation, 22 gram positive, white, opaque-looking pure isolates were selected. It was observed that the babies from whom 22 selected isolates were obtained were 36 days old on average.

Gram stain images of some isolates are given in Fig. 1. Identification of isolates was performed using MALDI-TOF MS.

According to the MALDI-TOF MS method, which is based on looking at the protein profiles of microorganisms (protein, peptide, sugar) and large organic molecules after they are ionized and passed through electric and/or magnetic fields, Lactobacillus rhamnosus is the most common strain with a rate of 59%. Lactobacillus paracasei follows with 13.6%. Then there were 9% Lactobacillus reuteri, Lactobacillus gasseri and 4.5% Enterococcus faecalis, Pediococcus acidilactici strains. Gram stain images of some species are given in Fig. 1.

Determination of Probiotic Properties of LAB

Acid and Bile Tolerance of Stool LAB

Determination of resistance to acid and bile salts of LAB isolated from stool swabs was performed in vitro by exposing the strains to pH (pH 2.0, 2.5 and 3.0) adjusted PBS. The survival rates of LAB in pH

Table 1. Survival rates of LAB in a low pH environment

	pH = 2.0			pH = 2.5			pH = 3.0		
	Beginning	**End**	**%Life**	**Beginning**	**End**	**%Life**	**Beginning**	**End**	**%Life**
***L. rhamnosus* T1**	8.60	-	-	8.68	5.57	64.1	8.75	8.33	95.2
***L. rhamnosus* T2**	8.58	-	-	8.63	5.42	62.8	8.52	7.81	91.6
***L. rhamnosus* T3**	8.63	2.87	33.2	8.72	6.46	74	8.75	8.38	95.7
***L. rhamnosus* T4**	8.55	2.35	27.4	8.58	5.73	66.7	8.63	7.94	92
***L. rhamnosus* T5**	8.87	-	-	8.90	6.65	74.7	8.75	8.23	94
***L. rhamnosus* T6**	8.60	-	-	8.62	5.81	67.4	8.69	8.17	94
***L. reuteri* T7**	8.50	-	-	8.52	5.45	63.9	8.71	8.43	96.7
***L. paracasei* T8**	8.60	2.56	29.7	8.63	6.81	78.9	8.74	8.52	97.4
***P. acidilactici* T9**	8.90	-	-	8.92	6.72	75.3	8.83	8.25	93.4
***L. rhamnosus* T10**	8.89	-	-	8.75	6.38	72.9	8.72	8.28	94.9
***L. rhamnosus T*11**	8.77	-	-	8.70	6.41	73,6	8.81	8.31	94.3
***L. reuteri* T12**	8.55	-	-	8.45	5.71	67.5	8.61	8.15	94.6
***E. faecalis* T13**	8.65	2.80	32.3	8.73	6.29	72	8.77	8.01	91.3
***L. rhamnosus* T14**	8.72	-	-	8.95	6.76	75.5	8.91	8.12	91.1
***L. rhamnosus* T15**	8.90	-	-	8.98	6.72	74.8	8.87	8.18	92.2
***L. gasseri* T16**	8.65	-	-	8.83	6.61	74.8	8.75	8.02	91.6
***L. paracasei* T17**	8.75	2.51	28.6	8.80	6.56	74.5	8.86	8.09	91.3
***L. rhamnosus* T18**	8.80	-	-	8.92	6.87	77	8.72	8.35	95.7
***L. rhamnosus* T19**	8.75	-	-	8.63	5.97	69.1	8.66	8.21	94.8
***L. rhamnosus* T20**	8.80	2.29	26	8.90	6.91	77.6	8.82	8.35	94.6
***L. gasseri* T21**	8.60	-	-	8.55	5.79	67.7	8.58	7.83	91.2
***L. paracasei* T22**	8.52	-	-	8.68	5.89	67.8	8.73	8.03	91.9

Survival number (log cfu/mL) and Rate (%) of Isolates at the end of the 3rd hour in media with different pH value.

medium are given in Table 1 and the survival rates in MRS medium containing low bile salt are given in Table 2.

pH Change

After 18 hours of incubation at 37ºC in MRS broth, the mean pH values of L. rhamnosus strains were 4.36; The average pH value of L. reuteri strains was 4.04; The mean pH value of L. paracasei strains was 4.03; The average pH value of L. gasseri strains was measured as 3.9.

Determination of Antibiotic Sensitivities

In our study, while T3 and T18 strains were susceptible and semi-susceptible to ampicillin antibiotic, all other strains were resistant; T2, T12, T13, T17, T18 strains were resistant to Penicillin antibiotic, while all other strains were found to be susceptible. Although resistance to antibiotics varies according to strains, resistance to ampicillin was 91% for all strains; resistance to teicoplanin 59%; gentamicin resistance 36%; resistance to netilmicin 54%; vancomycin resistance 64%; tobramycine resistance 95%; resistance to amikacin was observed at a rate of 100%

Determination of LAB's Lactic Acid Amounts and Cholesterol Removal

The amount of rubbery acid was calculated with

Table 2. Survival rates of LAB in a low bile salt MRS medium

	%0.3			%0.5			%1		
Lactic acid bacteria	**Beginning**	**End**	**%Life**	**Beginning**	**End**	**%Life**	**Beginning**	**End**	**%Life**
***L. rhamnosus* T1**	8.92	7.74	86.7	8.73	5.46	62.5	8.56	5.21	60.8
***L. rhamnosus* T2**	8.65	-	-	8.96	-	-	9.12	6.36	69.7
***L. rhamnosus* T3**	8.81	7.85	89.1	9.25	6.21	67.1	8.87	-	-
***L. rhamnosus* T4**	8.46	7.43	87.8	8.99	6.32	70.3	8.60	5.76	66.9
***L. rhamnosus* T5**	9.23	8.56	92.7	9.17	5.52	60.1	8.79	4.24	48.2
***L. rhamnosus* T6**	8.96	5.23	58.3	8.65	5.47	63.2	9.14	-	-
***L. reuteri* T7**	9.12	6.79	74.4	9.33	6.36	68.1	8.54	4.79	56
***L. paracasei* T8**	9.17	6.84	74.5	8.87	5.94	66.9	8.98	4.93	54.8
***P. acidilactici* T9**	8.97	5.92	65.9	9.26	6.83	73.7	8.59	5.23	60.8
***L. rhamnosus* T10**	9.24	7.26	78.5	8.75	5.77	65.9	8.96	6.02	67.1
***L. rhamnosus T*11**	9.11	8.35	91.6	8.83	4.89	55.3	8.64	6.11	70.7
***L. reuteri* T12**	8.82	6.54	74.1	8.24	5.26	63.8	8.47	6.39	75.4
***E. faecalis* T13**	8.98	2.80	31.1	8.75	5.96	68.1	8.63	5.42	62.8
***L. rhamnosus* T14**	9.02	7.32	81.1	9.26	6.85	73.9	9.25	6.82	73.7
***L. rhamnosus* T15**	9.36	8.21	87.7	8.98	6.47	72	9.36	5.97	63.7
***L. gasseri* T16**	8.35	6.67	79.8	8.74	5.71	65.3	8.96	-	-
***L. paracasei* T17**	8.52	2.51	29.4	9.02	-	-	8.81	6.28	71.2
***L. rhamnosus* T18**	9.18	8.43	91.8	8.96	6.32	70.5	8.79	5.86	66.6
***L. rhamnosus* T19**	8.69	-	-	9.11	7.12	78.1	8.93	4.54	50.8
***L. rhamnosus* T20**	8.73	2.29	26.2	8.99	-	-	8.86	-	-
***L. gasseri* T21**	8.67	7.58	87.4	8.88	6.47	72.8	9.11	6.12	67.1
***L. paracasei* T22**	9.28	-	-	9.17	-	-	9.02	-	-

Survival number (log cfu/mL) and rate (%) of isolates at the end of the 3rd hour in MRS media containing different bile salts

the help of phenolphthalein and NaOH for the cultures activated in MRS broth at 37°C for 18 hours. The average amount of lactic acid produced by LAB and probiotic bacteria after being activated in MRS broth at 37°C for 18 hours is 361.2. It was observed that *L. rhamnosus* T11 strain had the highest lactic acid production capacity with 496.8. *L. paracasei* T17 strain had the lowest lactic acid production with 86.4.

Among the strains used in our study, the strains with the highest cholesterol assimilation capacity were T21 and T22 strains with a rate of 39.1%. These strains are followed by T18, T16, T17, T17, T4, T9, T13 strains. The strain with the least cholesterol assimilation capacity was found to be the T20 strain. The lactic acid amounts and cholesterol removals of LAB are shown in Table 3.

DISCUSSION

There are many factors that shape the baby's microflora immediately after birth. Some of those; The birth type of the baby, health and immunological status, whether the baby receives breast milk, the mother's diet, the GIS (Gastro-intestinal System) transition time and GIS pH are factors such as stress [10].

In another study evaluating the microbiota of infants fed with formula (only or with breast milk), the bacterial groups that colonize formula-fed infants more frequently were *Enterococcus, C. coccoides, Atopobium cluster, B. vulgatus*, *B. longum* subsp. *longum* is indicated [11].

E.coli, C. difficile, B. fragilis species are less in breastfed infants, but *Bifidobacteria*, especially *B.*

Table 3. LAB's lactic acid production and cholesterol removal rates

Lactic acid bacteria	Lactic acid amount (mg/dL)	Cholesterol ratio (mg/dL)	Cholesterol removal (%)
	74		
L. rhamnosus T1	417.6	59	20.27
L. rhamnosus T2	255.6	53	28.3
L. rhamnosus T3	147.6	54	27.02
L. rhamnosus T4	446.4	49	33.7
L. rhamnosus T5	273.6	57	22.9
L. rhamnosus T6	360	52	29.7
L. reuteri T7	453.6	50	32.4
L. paracasei T8	360	54	27.02
P. acidilactici T9	432	50	32.4
L. rhamnosus T10	432	58	21.6
L. rhamnosus T11	496.8	56	24.3
L. reuteri T12	113.4	52	29.7
E. faecalis T13	453.6	51	31.08
L. rhamnosus T14	482,6	55	25.67
L. rhamnosus T15	252	48	35.13
L. gasseri T16	468	48	35.13
L. paracasei T17	86.4	48	35.13
L. rhamnosus T18	345.6	46	37.83
L. rhamnosus T19	396	52	29.7
L. rhamnosus T20	374.4	69	6.75
L. gasseri T21	417.6	45	39.1
L. paracasei T22	482.4	46	39.1

breve and *B. longum*, are early colonizers in infants, but *B. animalis* subsp. lactis occurs only with the type of diet and is not the common infant gut microorganism [12, 13].

Similar to our study, fecal *Bifidobacterium* and *Lactobacillus/Enterococcus* counts were found to be higher in breastfed infants compared to formula-fed infants at 6 months [14]. *L. acidophilus* (20%), *L. acidophilus-3* (10%), *L. brevis* (30%), *L. casei* (15%) bacteria were isolated from colostrum. *L. brevis* (41.2%), *L. fermentum* (11.8%), *L. reuteri* (5.9%), *L. rhamnosus* (11.8%), *L. plantarum* (29.4%) were detected in stool [15]. The results of the study are similar to the results of our study. In our study, stool samples were collected from 45 healthy babies and 22 bacterial isolates were evaluated. 54.5% of the babies to whom these bacterial isolates belong are exclusively breastfed; While 31.8% are fed with commercial infant formula, 13.6% are fed with infant formula as well as breast milk. Of the strains isolated from the stools of exclusively breastfed babies, 41.6% were identified as *L. rhamnosus,* 16.6% *L. reuteri,* 25% *L. paracasei,* 12.5% *L. gasseri,* 12.5% *P. acidilactici*. Of the strains isolated from the stools of infants fed only infant formula, 62.5% *L. rhamnosus*, 12.5% *L. paracasei,* 12.5% *L. gasseri,* 12.5% *E. faecalis;* All of the strains isolated from the stools of infants who received infant formula in addition to breast milk were identified as *L. rhamnosus.* Similar to the studies, lactic acid bacteria were also found in our study. However, the stools of exclusively breastfed babies have greater microbiological diversity.

In a study of *L. rhamnosus, L.casei, L. paracase*i strains obtained from different sources (meat and dairy products, sourdough doughs, wine, beverages, vegetables and human body), survival and growth were improved after exposure to low pH values for 2 hours. 61% of strains evaluated for their restart capacity 2 hours at pH =2.5; 3.3% continued to grow after incubation at pH = 1.5. All strains survived in the presence of 1.5% bile salt after 24 hours of incubation at 37 °C [16].

The viability rates of *L. rhamnosus* 19 strain obtained from Kenya's traditional fermented food (two) at pH =2.0 decreased from 7.29 log10 cfu/ml to 4.11 log10 cfu/ml at the end of the 3rd hour. *L. rhamnosus* 19 strain was incubated at pH = 2.0 for 3 hours, and the survival rate of *L. rhamnosus* in MRS broth with added 0.3% bile salt was reported to be 3.85 log10 cfu/mL at the end of 48 hours [17].

In one study, a total of 22 *Lactobacillus strains* isolated from infant feces, low pH and resistance to bile salts, as well as 8 isolates (*L. reuteri* 3M02, 3M03; L. gasseri 4M13, 4R22, 5R01, 5R02, 5R13; *L. rhamnosus* 4B15) were evaluated.) has high tolerance to acids (99.1%) and bile salts (99.9%) [18]. It was stated that there was no significant decrease in the amount of *P. acidilactici* strain kept in acidic salt solutions (pH = 2.0) [19].

107 lactic acid bacterial isolates were isolated from 6 donor infant meconium from the Roubaix hospital in northern France. Some of the *E. faecalis* strains produced lactic acid up to 7.06g/l after 24 hours of incubation [20]. In our study, the lactic acid amount of *E. faecalis* strain obtained from a baby who received formula was 453.6 mg/dL, and lactic acid production was below this result. In another study, the average amount of lactic acid produced by *L. rhamnosus* strains obtained from vaginal swabs was 585 mg/dL; The average amount of lactic acid produced by *L. paracasei* strains was 458 mg/dL; The average lactic acid content of *P. acidilactici* strains was found to be 682 mg/dL [21]. In our study, while the lactic acid production amount of *L. rhamnosus* strains was found to be 360 mg/dL on average, it was determined that the strain with the highest lactic acid production capacity was *L. rhamnosus* T11 with 496.8.

In our study, it was determined that LAB isolated from baby feces was resistant to low pH environment and showed 100% viability especially in pH 2.5-3.0 environments. It was observed that especially *L. rhamnosus* strains survived better in pH 2.0 environment. *L. paracasei* strains isolated from Italian Castelmagno cheese after 24 hours of incubation, mean pH values of *L. paracasei* strains were found to be 3.87.30 Average pH values of *L. rhamnosus* strains isolated in our study were 4.36; The average pH value of *L. reuteri* strains was 4.04; The mean pH value of *L. paracasei* strains was 4.03; The average pH value of *L. gasseri* strains was measured as 3.9. [22].

L. rhamnosus, *L. paracasei* strains obtained from human faeces showed resistance to vancomicin, colistin sulfate, oxalinic acid, gentamicin, oxalinic acid, kanamicin under high in vitro conditions [23]. In our study, *L. rhamnosus, L. rhamnosus, L. paracasei* strains were similarly resistant to vancomicin but

highly sensitive to gentamicin. In our study, while *P. acidilactici* was resistant to ampicillin, tobramycin, amikacin, it showed sensitivity to penicillin and teicoplanin. These results are similar to other studies [24, 25]. In our study, the *E. faecalis* strain isolated from a baby who received only formula was found to be resistant to all antibiotics used in the study. Similarly, in other studies, it was determined that the *E. faecalis* strain was resistant to many antibiotics [26, 27].

L. paracasei subsp. *paracasei* (41), *L. fermentum* (24), *L. rhamnosus* (11), *L. casei* (17), *Lactobacillus* spp. (11) strains have been reported to have strong antimicrobial effects, especially on *S. aureus*. 28 *L. monocytogenes* ATCC 3512, *L. innocus* 103982, B. subtilis ATCC 6633, S. aureus ATCC 3386, *E. coli* CIPI 103982 have been reported to show antagonistic activity against pathogenic bacteria [18].

It is quite remarkable that LAB has potential cholesterol-lowering effects in recent studies [28]. High cholesterol is associated with cardiovascular diseases, a major cause of death worldwide. Current therapeutic measures, lifestyle changes, dietary interventions, pharmaceutical agents are insufficient to regulate cholesterol level. Probiotic bacteria show the potential to lower cholesterol levels through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A enzymes by different mechanisms, including bile salt hydrolase.

In the study, a total of 22 *Lactobacillus* strains isolated from infant feces were evaluated, 8 isolates (*L. reuteri* 3M02, 3M03; *L. gasseri* 4M13, 4R22, 5R01, 5R02, 5R13; *L. rhamnosus* 4B15), especially *L. rhamnosus* 4B15 and *L. gasseri* 4M13 strains have been shown to have significant cholesterol-lowering capacity compared to other strains [18].

In one study, it was stated that *L. rhamnosus* strain showed less cholesterol assimilation with a cholesterol assimilation value of 13.21% compared to *L. acidophilus, L. fermentum, B. Lactis* [29]. In our study, *L. rhamnosus, L. reuteri, L. gaseri, L. paracasei*. Among the strains, the strains with the highest cholesterol removal were *L. gasseri* (39.1%) and *L. paracasei* (39.1%) isolated from exclusively breastfed infants. The mean cholesterol assimilation percentage of the *L. paracasei* strain was 28.5%; The mean cholesterol assimilation percentage of the *L. reuteri* strain was calculated as 31.05%. With these values, previous studies showed, on average, higher cholesterol removal.

Limitations

The biggest limitation of our study is that the study is limited to Sivas province and it is necessary to study with more stool samples in order to fully reveal the microbial difference between breast milk and formula babies.

CONCLUSION

In the light of our results, it is thought that the isolated strains have strong probiotic potential, especially *L. gasseri* T21 and *L. paracasei* T22 strains, which can be used in the production of preparations alone or together with other *Lactobacillus* strains. In recent years, with the addition of prebiotics and probiotics to commercial infant formulas, the difference between infants receiving breast milk and infant formula has decreased. However, breast milk, which is a cheap, reliable, natural food that meets all the needs of the baby on its own, should always be the first choice. It is important to evaluate the use of strains as oral preparations clinically well.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

This article describes *Lactobacillus* spp. in breastfed and non-breastfed infants. It was prepared from the master's thesis named research of bacteria and determination of some probiotic characters.

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The European Research Journal 2023;9(6):1343-1349 Original Article

DOI: 10.18621/eurj.1170790 *Hematology*

Fast platelet recovery is associated with remission in primary immune thrombocytopenia

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ABSTRACT

Objectives: We aimed to reveal predictors of response and response duration to steroid therapy in first line of treatment in immune thrombocytopenia (ITP).

Methods: Fifty patients, who were diagnosed with ITP in hematology department of Suleyman Demirel University Hospital between 2005-2019, who had sufficient clinical and laboratory data, followed up for at least one year and received corticosteroid treatment in first line, were evaluated retrospectively for treatment response time, remission, prognosis on their first line treatment. The patients who maintained remission for more than 12 months was defined as group 1 and those who did not achieve remission or relapsed in less than 12 months were defined as group 2.

Results: Twenty-two (44%) patients responded in first 3 days of the treatment, 16 (32%) patients in 4 to 7 days and 4 (8%) patients responded in more than 7 days. Eighty-four percent (n = 42) of these patients had complete response to corticosteroid treatment. When the remission maintenances were examined, it was observed that 22 (44%) patients were in remission for more than 12 months, 20 (40%) patients were in remission with treatment but relapsed before 12 months and 8 (16%) patients did not respond to corticosteroid treatment. When the response time to treatment in patients with or without remission was compared, remission was significantly lower in those who responded late to treatment (p = 0.01). When the response rates to corticosteroid treatment of patients in group 1 and 2 were evaluated, it was found that the response time to treatment was not related to the maintenance of remission (p = 0.267).

Conclusions: Faster response time to treatment produced higher remission rates but, we could not find any relationship between response time to treatment and duration of remission.

Keywords: Immune thrombocytopenia, remission, fast recovery, prognosis

Primary immune thrombocytopenia (ITP) is a disease that was associated with the immune-mediated destruction of platelets. ITP is divided into three stages: newly diagnosed (covering 3 months after diagnosis), persistent (covering between 3 and 12 months after diagnosis), chronic (disease lasting more than 12 months) [1, 2]. Apart from the immune-mediated destruction caused by autoantibodies against gly-

Received: ***September 5, 2022;*** *Accepted:* ***December 30, 2022;*** *Published Online:* ***March 28, 2023***

How to cite this article: *Gür Hatip F, Özbalcı D, Alanoğlu EG, Hatip AY. Fast platelet recovery is associated with remission in primary immune thrombocytopenia. Eur Res J 2023;9(6):1343-1349. DOI: 10.18621/eurj.1170790*

e-ISSN: 2149-3189

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coprotein (GP) IIb-IIIa and Gp Ib-IX complex, decreased platelet production, which cannot keep up with this increased destruction, is thought to play a role in the pathogenesis of ITP [3-5]. Some polymorphisms in major tissue compatibility complexes (MHC), chemokines, proinflammatory cytokines, anti-inflammatory cytokines and their receptors have been observed in patients with ITP [6]. There is no specific test used to diagnose ITP. In most cases, primary ITP is a diagnosis of exclusion made by a careful history, physical examination, complete blood count, and peripheral smear examination [7, 8].

Treatment decision in adult ITP should be made according to the patient's current bleeding severity, bleeding risk, activity level, treatment side effects, and patient preference [9]. Corticosteroid therapy is preferred unless there is a contraindication to its use in the first step in treatment [1, 9, 10]. Intravenous immunoglobulin (IVIG), splenectomy, immunosuppressive agents, rituximab, thrombopoietin receptor agonists are other options in the treatment of ITP [9]. Corticosteroid therapy in first line leads to 85-9 % of responses but relapses are common [11]. There are few studies evaluating the predictors of response to corticosteroid treatment so this study aimed to reveal predictors of response and response duration to steroid therapy in first line of treatment in ITP.

METHODS

Fifty patients, above 18 years old and who were diagnosed with ITP in the hematology department of Suleyman Demirel University Hospital between 2005-2019, who had sufficient clinical and laboratory data, followed up for at least one year and received corticosteroid treatment in first line, were evaluated retrospectively. Patients who were pregnant, who had a diagnosis that can be attributed to isolated thrombocytopenia (drug induced thrombocytopenia, connective tissue disease, disseminated intravascular coagulation) were excluded from the study. Patients who were diagnosed with malignancy were also excluded from the study. The study was approved by ethics committee of Suleyman Demirel University Medical Faculty Clinical Research Ethics Committee on 05.04.2019 with protocol number 134 and detailed informed consent was obtained from all patients. The clinical and laboratory data, treatment responses and time to achieve the response were examined. Response and relapse were defined according to the immune thrombocytopenia guide of American Society of Hematology [12]. Age and gender at admission, presence of symptoms related to bleeding, presence of additional disease, presence of drugs that can affect bleeding, especially fever, hepatomegaly, splenomegaly, lymphadenopathy in physical examinations, presence of thrombocyte transfusion, laboratory tests; total blood count parameters, hepatitis serology, C-reactive protein (CRP) level, presence of antinuclear antibody (ANA) and positivity degree, reticulin fiber grade of patients who underwent bone marrow biopsy, response of patients to corticosteroid treatment (response was defined as platelet count above 30,000/mm^3), time to response, presence of remission (remission was determined as platelet count above 100,000/mm^3), ability to maintain remission for one year or longer were recorded retrospectively from medical health records. The patients who maintained remission for more than 12 months was defined as group 1 and those who did not achieve remission or relapsed in less than 12 months were defined as group 2 and these two groups were compared for response rate, response time to treatment and maintenance of response. All patients received 1 mg/kg/day methylprednisolone for twenty-one days and then slowly tapered and stopped for up to 3 months.

Statistical Analysis

IBM SPSS Statistics 21.00 statistical program was used for statistical calculations. The conformity of the quantitative data to the normal distribution was examined with the Kolmogorov Smirnov test. Student's t test and One Way Anova were used in independent groups for statistical comparisons for variables with normal distribution. Descriptive statistics were presented as standard deviation. Mann-Whitney U and Kruskall Walls tests were used for statistical comparisons for variables that were not normally distributed. Descriptive statistics were shown as mean ± standard deviation. Chi-square analysis was used for statistical comparisons for categorical variables and descriptive statistics were shown as frequency (%). $P < 0.05$ was considered statistically significant.

RESULTS

Of the 50 patients, 35 (70%) were female and 15 (30%) were male. The mean age of the patients was 49.4 ± 17. 1 years, women's mean age was 48.4 ± 17.1 years, and men's mean age was 51.7 ± 17.3 years. When all genders were compared, no statistically significant difference was found between their ages and in terms of response to corticosteroid treatment and remissions ($p > 0.05$).

At diagnosis, 92% (n = 46) of the patients had symptoms associated with ITP. Only 8% (n = 4) of the patients were asymptomatic and were diagnosed during their routine examinations. In 84.8% of symptomatic patients, the presenting symptom was mucocutaneous bleeding. For additional physical findings, hepatomegaly due to grade 3 hepatosteatosis was found in one patient, hepatosplenomegaly was found in one patient. Fever and lymphadenopathy were not detected in any patient at admission.

No additional disease was detected in 31 patients, and additional diseases that would not affect the coagulation cascade were detected in 19 patients. When the drug use of the patients was examined, 36 patients did not use any drugs, 6 patients used anticoagulant-antiaggregant drugs as additional drugs, and 8 patients used other drugs that did not affect coagulation. When the relationship between the presence of additional disease and remission was examined, no significant difference was found between the groups ($p = 0.413$). Similarly, when the additional drug use of the patients and their remission were compared, no significant difference was found between the groups ($p = 0.441$).

When the laboratory parameters of the patients were examined, the mean white blood cell (WBC) count of the group 1 was 6.88 ± 1.78 ×103/µL, while the mean WBC of group 2 was 7.93 ± 2.73 ×103/µL and, no significant difference was observed between the groups ($p = 0.257$). Also, no significant difference was found between mean leukocyte subgroups and remission groups ($p = 0.544$, $p = 0.624$ and $p = 0.567$, respectively). The mean hemoglobin was 12.9 ± 2.1 g/dL, and no statistically significant difference was found between mean haemoglobin and remission groups ($p = 0.512$, T test). Likewise, there was no significant difference between mean of MCV and hematocrit and remission groups ($p = 0.677$ and $p = 0.580$, T test; respectively). Mean platelet value was 10.4 ± 8.2 × 103/µL, the mean MPV value was 9.4 ± 1.4 fL and no statistically significant difference was observed

Table 1. Comparison of laboratory parameters of patients who maintained remission for more than 12 months and other patients

Laboratory Parameters	Group with remission lasting more than 12 months (n = 24)	Group with no remission and relapse in less than 12 months (n = 34)	Overall average	*p* value
WBC (× 10^3/µL)	6.88 ± 1.78	7.93 ± 2.73	7.45 ± 2.37	0.257
NEU*(× 10^3/µL)	7.13 ± 2.84	5.14 ± 0.45	5.84 ± 7.81	0.544
LYM (× 10^3/µL)	1.82 ± 0.45	1.99 ± 1.02	1.29 ± 0.79	0.624
MON*(× 10^3/µL)	0.60 ± 0.22	0.95 ± 0.39	0.75 ± 1.41	0.567
HGB (g/dL)	12.4 ± 2.2	13.3 ± 1.9	12.9 ± 2.1	0.512
MCV (fL)	83.5 ± 10.4	84.8 ± 4.9	84.4 ± 7.4	0.677
HCT (%)	36.7 ± 5.3	38.9 ± 5.3	38.1 ± 5.8	0.580
PLT*(× 10^3/µL)	9.1 ± 6.5	10.8 ± 8.4	10.4 ± 8.2	0.260
MPV (fL)	9.1 ± 1.6	9.5 ± 1.3	9.4 ± 1.4	0.765
CRP*(mg/L)	7.9 ± 6.4	20.6 ± 9.2	14.9 ± 3.4	0.212

Parameters with * do not comply with the regular distribution and were compared with the Mann- Whitney U test, and other parameters were compared with the t test. WBC = White Blood Count, NEU = Neutrophil, LYM = Lymphocyte, MON = Monocyte, HGB = Hemoglobin, MCV = Mean Corpuscular Volume, HCT = Hematocrit, PLT = Platelet, MPV = Mean Platelet Volume, CRP = C-reactive Protein

Table 2. Comparison of laboratory parameters of patients who went into remission and maintained for more than 12 months and relapsed before 12 months

Laboratory Parameters	Patients in remission			
	Maintenance longer than 12 months (n = 24)	Relapse before 12 months (n = 25)	Overall average (n = 49)	*p* value
WBC (× 10^3/µL)	6.88 ± 1.78	7.79 ± 2.03	7.40 ± 1.98	0.131
NEU*(× 10^3/µL)	7.12 ± 2.84	4.74 ±1.68	5.86 ± 8.42	0.668
LYM (× 10^3/µL)	1.83 ± 0.46	2.22 ± 0.82	2.01 ± 0.7	0.167
MON*(× 10^3/µL)	0.61 ± 0.21	1.12 ± 2.34	0.81 ± 1.53	0.889
HGB (g/dL)	12.4 ± 2.2	13.2 ± 2.1	12.9 ±2.2	0.618
MCV (fL)	83.5 ± 10.4	84.3 ± 4.3	84.0 ± 7.6	0.875
HCT (%)	36.7 ± 5.3	38.6 ± 5.8	38.0 ± 6.0	0.632
PLT*(× 10^3/µL)	9.1 ± 6.5	10.6 ± 8.8	10.3 ± 8.4	0.283
MPV (fL)	9.1 ±1.6	9.5 ± 1.3	9.3 ± 1.5	0.236
CRP*(mg/L)	7.9 ± 6.4	26.4 ± 12.6	16.6 ± 37.9	0.490

Parameters with * do not comply with the regular distribution and were compared with the Mann- Whitney U test, and other parameters were compared with the t test. WBC = White Blood Count, NEU = Neutrophil, LYM = Lymphocyte, MON = Monocyte, HGB = Hemoglobin, MCV = Mean Corpuscular Volume, HCT = Hematocrit, PLT = Platelet, MPV = Mean Platelet Volume, CRP = C-reactive Protein

in the comparison of both parameters between the remission groups ($p = 0.260$, Mann Whitney U and $p = 0.765$, T test; respectively). There was no significant difference in the comparison between mean CRP and remission groups ($p = 0.212$) (Table 1). When the laboratory parameters of remission groups were evaluated, no significant difference was observed in any of the parameters (Table 2).

When viral markers were examined, except for one patient whose data were missing, all patients were Anti-HIV, Anti HCV and HbsAg negative 39 patients were Anti-HBs Negative and 10 patients were positive. Eleven patients were Anti-Nuclear Antibody (ANA) positive (7 of them +; 4 of them ++). Thirty-nine patients were ANA negative. When two remission groups were evaluated for ANA positivity, there was no statistically significant difference between the remission groups (Table 3).

Bone marrow aspiration biopsy was performed in 28 patients and, 8 patients had grade 0; 8 patients grade 1; 9 patients grade 2 and 3 patients had grade 3 reticulin fiber. Reticulin fiber grade 1 and 2 was higher

Table 3. ANA status

Laboratory status	Group with remission lasting more than 12 months (n = 20)	Group with no remission and relapse in less than 12 months (n = 30)	*p* value
ANA positive	7	4	0.159
+	4	3	
++	3	1	
+++	0	0	
++++	0	0	
ANA negative	13	26	

ANA = Anti-nuclear antibody

in group 2 patients compared to group 1 however; no significant difference was found between groups (Table 4).

Thirty-four percent of the patients received platelet transfusion during treatment period. Of the patients who received platelet transfusion, 10 remained in remission for more than 12 months, and 7 either never entered remission or relapsed before 12 months of remission. Platelet transfusions did not have a statistically significant effect on remission of the patients ($p = 0.543$).

Twenty-two (44%) patients responded in first 3 days of the treatment (PLT > 30,000) and 16 (32%) patients in 4 to 7 days. It was observed that 4 (8%) patients responded in more than 7 days. 84% (n=42) of these patients had complete response (PLT > 100,000) to corticosteroid treatment. When the remission maintenances were examined, it was observed that 22 (44%) patients were in remission for more than 12 months (PLT > 100,000), 20 (40%) patients were in remission with treatment but relapsed before 12 months (PLT < 100,000) and 8 (16%) patients did not respond to corticosteroid treatment. When the response time to treatment in patients with or without remission was compared, remission was significantly lower in those who responded late to treatment ($p = 0.01$). When the response rates to corticosteroid treatment of patients in group 1 and 2 were evaluated, it was found that the response time to treatment was not related to the maintenance of remission ($p = 0.267$) (Table 5).

DISCUSSION

ITP is a chronic disease in adults, while 80-90 % of patients respond to primary treatment; most patients relapse and need additional treatment [11]. Corticosteroids are the main backbone of first line treatment; prednisolone, methylprednisolone and high dose dexamethasone was used effectively for treatment. High dose dexamethasone seemed to induce faster response and less long-term toxicity compared to prednisone [13], but overall prognosis of the disease did not significantly change in either of treatment and, at 12 months response rates were similar [14]. According to ASH guidelines for immune thrombocytopenia, both can be used as a primary treatment [12]. In another study, response was observed in 63.6% (n = 49) of the patients who were given corticosteroid treatment, and 28.6% (n = 14) of these patients relapsed over time [15]. In a study examining 43 ITP patients over 60 years of age, the responses of the patients to corticosteroid treatment at the 1st and 6th months were examined, and the response rate at 1 and 6 month was found to be 61% and 33% respectively [16]. In a study with 137 patients receiving steroid therapy, complete response and relapse were observed in 51.9% and 58.2 % of the patients during 33-month follow-up [17]. Stasi *et al*. [18] found that with prednisone treatment complete response was observed in 38.8% of these patients and only 18.7% of these patients, maintained their response at 6-month follow-up. Leung *et al*. [19] showed that, in 142 patients, complete response was

Table 5. Comparison of response rate to corticosteroid therapy and maintenance of remission

		Remission > 12 months	Relapse	Total	*p* value
Response to steroid	3 days	9	13	22	
	4-7 days	10	6	16	0.267
	More than 7 days	3	1	4	
Total		22	20	42	
		Remission (for any period of time)	Never in remission	Total	
Response to steroid	3 days	22	1	23	**0.010**
	4-7 days	16	3	19	
	More than 7 days	4	4	8	
Total		42	8	50	

47.2% and 46% of these patients maintained their response at 470-month follow-up. Chang *et al*. [11] found that, 63 % of patients with frontline steroid therapy were relapsed at median of 9.5 months. We found that, 44% of patients maintained remission for more than 12 months, compatible with literature. Response rates to corticosteroid therapy and the duration of these responses have been reported at different rates in literature. The differences for the rate of complete response to steroid treatment in the literature might be due to the distinct follow-up period, inclusion criteria, and response definitions of different studies.

Response time to treatment could be an attractive prognostic indicator in ITP, but evidence was lacking. In one study, high dose dexamethasone produced faster platelet response than prednisone (3 to 5 days respectively) but in twelve months follow-up, complete response rates were comparable (32.1 % for high dose dexamethasone and 34.1 % for prednisone) [13]. We also found that faster response time did not produce longer remissions, but faster response time produced significantly higher remission rates, and this was compatible with the literature.

Limitations

Retrospective design was the main limitation for our study. Our study evaluated 50 patients; a prospective study with more patients might show much significant results. Besides, we could not find another study directly addressing the response time to treatment and remission issue; this was the main strength of the study. It would be valuable to assess the hypotheses in a prospective study.

CONCLUSION

We showed that faster response time to treatment produced higher remission rates but, we could not find any relationship between response time to treatment and duration of remission. Prospective studies with large number of patients are needed to evaluate the issue.

Authors' Contribution

Study Conception: FGH, DÖ; Study Design: DÖ; Supervision: FGH, DÖ, EGA; Funding: N/A; Materials: FGH; Data Collection and/or Processing: FGH, AYH; Statistical Analysis and/or Data Interpretation: AYH; Literature Review: FGH, DÖ; Manuscript Preparation: FGH, DÖ, EGA and Critical Review: FGH, DÖ, EGA, AYH.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1350-1359 Original Article

DOI: 10.18621/eurj.1198712 *Radiology*

Investigation of the impact of radiotherapy on the shoulder joint by ultrasonography in breast cancer patients

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ABSTRACT

Objectives: This study aimed to investigate the impact of radiotherapy (RT) on the supraspinatus, long head of the biceps and subscapularis tendons, and shoulder subcutenous fat tissue.

Methods: Patients diagnosed with breast cancer who received RT to the anterior chest wall and axillary-supraclavicular region after breast-conserving surgery or mastectomy in our hospital. Overall, 56 (36 RT, 20 control group) patients were enrolled in this study. Pre-RT, post-RT 6-week, 3-month, and 6-month ultrasonographic and clinical assessments were performed.

Results: The mean interval between surgery and RT was 5.6 (range: 2-8) months. Ten (27.7%) patients developed ultrasonographic abnormalities during the post-RT period. However, only 2 of these patients had shoulder pain and restricted range of motion in the shoulder. The thickness of the supraspinatus, biceps and subscapularis tendons were similar between the dominant and non-dominant sides of the control and RT group patients (5.7 mm vs. 5.1 mm, 2.87 mm vs. 2.89 mm, and 4.13 mm vs. 3.97 mm; respectively, $p > 0.05$). Thirteen patients were given RT on the ipsilateral side, and 23 received RT on the contralateral side. The pre-RT supraspinatus tendon thickness was significantly higher in the ipsilateral group than in the contralateral group ($p = 0.026$). However, there was no significant difference in the post-RT period ($p = 0.408$).

Conclusions: In breast cancer patients undergoing adjuvant RT, RT may cause temporary edema in shoulder tendons. In addition, shoulder pain and restricted range of motion of the shoulder joint can be present in these patients.

Keywords: Breast cancer, radiotheraphy, shoulder, tendon, ultrasonography

In patients with lymph node metastasis or primary tumors larger than 5 cm, adjuvant RT significantly decreased the risk of local recurrence. It was also reported that adjuvant RT led to a 9% increase in survival [1-3]. These findings were also confirmed by systematic reviews [4]. Therefore, RT is accepted as a fundamental component of the therapeutic management in patients with Stage 1 or 2 breast cancer who underwent breast-conserving surgery (BCS) [5]. It was also noted that RT decreased the risk of distant metastasis [6]. Notably, the primary purpose of post-mastectomy RT is to eliminate the residual tumor cells in

Received: ***November 13, 2022;*** *Accepted:* ***December 19, 2022;*** *Published Online:* ***April 6, 2023***

How to cite this article: *Karataş S, Alıcıoğlu B. Investigation of the impact of radiotherapy on the shoulder joint by ultrasonography in breast cancer patients. Eur Res J 2023;9(6):1350-1359. DOI: 10.18621/eurj.1198712*

e-ISSN: 2149-3189

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the anterior chest wall, skin, residual breast tissue, and local lymphatics [7, 8]. Therefore, giving RT to the whole breast area has become a standard approach in patients who underwent BCS [9].

In breast cancer patients, RT is given to the anterior chest wall and peripheral lymphatic area (i.e., axillary-supraclavicular region). The total radiation dose is 50 Gy which is given in 25 fractions. For most patients, the total dose is divided into daily doses five times a week for six weeks.

Despite its benefits, RT applied to breast cancer patients has side effects correlated with the dose and treatment duration. These side effects can be listed as lymphedema, skin, breast, pulmonary, cardiac complications, cerebrovascular accidents, and the development of cancer in the contralateral breast or other organs. In addition, complications related to the shoulder, such as reduced range of motion and pain, can also occur due to the vicinity of this joint to the radiated field. Therefore, in this study, we aimed to investigate the impact of RT on the anatomical components of the shoulder, including subcutaneous tissue, subacromial bursa, supraspinatus tendon (RCTs), long head of the biceps tendon (LHBT) and subscapularis tendon. We used shoulder ultrasonography (USG) since it is a cost effective, noninvasive, and portable method for imaging shoulder pathologies [10].

METHODS

This prospective study was approved by Trakya University Medical Faculty Ethical review committee (Number: 02.2009-03/34- TUTFEK/35) and informed consent was obtained from each patients.

All patients diagnosed with breast cancer who received RT to the anterior chest wall and axillary-supraclavicular region after BCS or mastectomy in our hospital radiation oncology department. The control group was selected from the age-matched female asymptomatic volunteers who presented to the hospital for a check-up without chronic systemic disease, RT history, regular medication use, trauma, or congenital musculoskeletal anomaly.

Radiologic assessment of the acromioclavicular joint (ACJ) was done on the chest X-rays of the patients. Exclusion criteria included ACJ degeneration, patients with a history of shoulder or arm trauma, acromioclavicular joint hypertrophy, comorbidities predisposing to tendinopathies such as rheumatoid arthritis, collagen tissue disorders, and renal dysfunction.

A total radiation dose of 50 Gy was given to the patients in the RT group in 25 fractions. The total amount was divided into daily doses five times a week for six weeks. These patients were examined four times in the radiation oncology outpatient clinic, and the relevant data were collected for subsequent analysis.

All the sonographic assessments were done by a radiology resident under the supervision of a senior radiologist with four and ten years of experience, respectively. Both shoulders were examined. In addition, the dominant arm and the RT side were determined. Ultrasonographic assessment of the shoulder joint was done four times:

I. Pre-RT examination of both shoulders and determining the dominant arm (i.e., pre-RT)

II. Post-RT examination performed immediately after a 6-week RT (i.e., 6th-week assessment)

III. Follow-up examination performed three months after the first day of RT (i.e., 3rd-month assessment)

IV. Follow-up examination performed six months after the first day of RT (i.e., 6th-month assessment)

The pre-RT assessment included both shoulders, while only the radiated side was analyzed during subsequent visits. The RT group patients underwent a shoulder USG on the days of these examinations. Subsequently, they were physically examined by an orthopedic surgeon regarding the range-of-motion (ROM) of the shoulder joint and the presence or absence of shoulder pain. The radiologist and orthopedic surgeon were blinded to each other`s reports. All patients' shoulders were imaged by the Mylab 60 (Esaote, Genova, Italy) ultrasonography device with a 10 MHz linear probe and a color Doppler with high-resolution software developed explicitly for musculoskeletal ultrasound imaging. The patient sat on a swivel stool for proper positioning.

Since the tendon thickness and pathologies can be affected by the dominancy of the arm, the RT patients were classified into two subgroups called "ipsilateral" (i.e., the radiated field and the dominant arm are on the same side) or "contralateral" (i.e., the radiated area and the dominant arm are on opposite sides).

During shoulder ultrasounds, the LHBT, subscapularis tendon, and supraspinatus tendon were assessed regarding integrity, contours, thickness, and echoic features. Also, the presence or absence of potential shoulder tendon pathologies, including tears, tendinosis, tenosynovitis, subacromial bursitis (SAB), and calcific tendinitis, were investigated during these assessments.

All tendon thicknesses were measured longitudinally at locations where the tendon shows uniform thickness (Fig. 1). The results found during the pre-RT examination and three post-RT examinations were compared.

Statistical Analysis

The statistical analyses were performed using the software STATISTICA AXA 7.1 (Serial No. AXA 507C775506FAN3, StatSoft Inc, Tulsa, US). The normal distribution of the variables was tested by the Kolmogorov-Smirnov test. The normally-distributed parameters were compared by the independent groups t-test, while the non-normally distributed data were compared using the Mann-Whitney U test. The repeated measures analysis of variance (ANOVA) test and Wilcoxon two-sample test were performed to compare the time-varying covariates. The descriptive variables were given as medians, minimums, maximums, and means ± standard deviations (SDs). The *p* value was considered significant when it was lower than 0.05.

RESULTS

Overall, 56 patients were enrolled in this study. Among these patients, 36 were in the case (i.e., RT) group, while 20 were in the control group. The mean age of the RT and control groups were 50.8 (range: 33-73) years and 51 (range: 37-69) years. The mean interval between surgery and RT was 5.6 (range: 2-8) months. The demographic characteristics of the patients in the RT group are displayed in Table 1.

Among the 36 patients in the RT group, 23 (64%) did not have any ultrasonographic or clinical pathological findings. The main findings in the remaining 13 cases were peritendinous effusion of the biceps tendon (PTEBT), increased peritendinous blood flow at the biceps tendon, and intrasubstance tear in the supraspinatus tendon, peritendinous effusion of the supraspinatus tendon (i.e., subdeltoid effusion) and peritendinous effusion of the subscapularis tendon (Fig. 2).

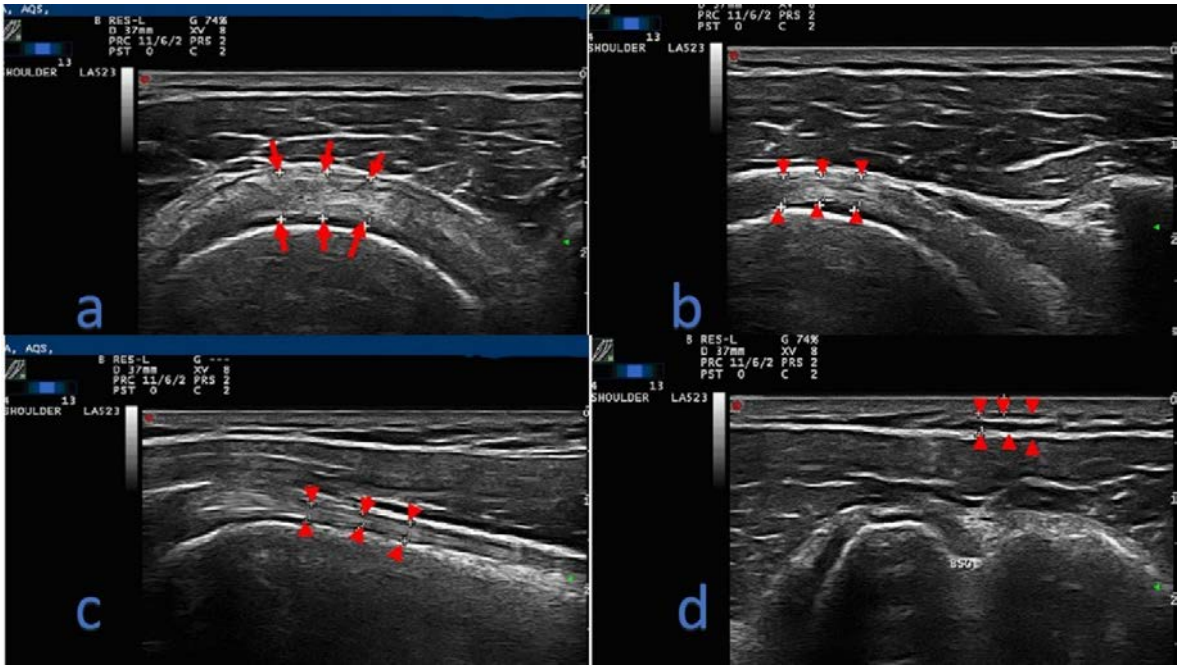

Fig. 1. Ultrasonographic measurements. (a) Supraspinatus tendon, (b) Subscapularis tendon, (c) long head of the biceps tendon, and (d) Subcutaneous fat tissue.

Table 1. Demographic data of the patients

	Initials	Age (years)	Dominant arm	Surgical side	Surgery	Surgery-radiotherapy interval (months)
1	BO	45	Right	Right	Mastectomy	**6**
2	SY	44	Right	Right	Mastectomy	**7**
3	GT	48	Right	Right	Mastectomy	**7**
4	SK	51	Right	Right	Mastectomy	**5**
5	ST	53	Right	Right	Mastectomy	**7**
6	SB	55	Right	Right	Mastectomy	**8**
7	DA	35	Left	Right	Mastectomy	**7**
8	TS	67	Right	Right	Mastectomy	**4**
9	ST	33	Right	Right	Mastectomy	**5**
10	SG	55	Right	Right	BCS	**8**
11	FÇ	59	Right	Right	BCS	**5**
12	AY	65	Right	Left	Mastectomy	**6**
13	SB	43	Right	Left	Mastectomy	**3**
14	AY	60	Right	Left	Mastectomy	**8**
15	SK	54	Right	Left	Mastectomy	**5**
16	FK	44	Right	Left	Mastectomy	**5**
17	TS	38	Right	Left	Mastectomy	**4**
18	RE	54	Right	Left	Mastectomy	**3**
19	RT	73	Right	Left	Mastectomy	**7**
20	FN	60	Right	Left	Mastectomy	**2**
21	HK	57	Right	Left	Mastectomy	**7**
22	ZK	38	Right	Left	Mastectomy	**5**
23	HG	45	Right	Left	Mastectomy	**5**
24	HG	35	Right	Left	Mastectomy	**5**
25	MÖ	62	Right	Left	Mastectomy	**7**
26	SY	53	Right	Left	Mastectomy	**5**
27	HE	57	Right	Left	Mastectomy	**7**
28	ND	33	Right	Left	Mastectomy	**5**
29	EŞ	50	Left	Left	Mastectomy	**8**
30	RB	43	Left	Left	Mastectomy	**4**
31	AY	53	Left	Left	Mastectomy	**7**
32	PI	54	Right	Left	BCS	**6**
33	LD	67	Right	Left	BCS	**7**
34	ŞE	49	Right	Left	BCS	**3**
35	AG	53	Right	Left	BCS	**6**
36	**CU**	**47**	**Right**	**Left**	**BCS**	**7**

There were 3 (8.3%) patients with abnormal ultrasonographic findings during the pre-RT assessment. These patients had peritendinous effusion of the biceps tendon, and 2 of these patients had associated pain. In 1 of these 2 cases, the effusion and shoulder pain persisted. In the other case, peritendinous effusion of

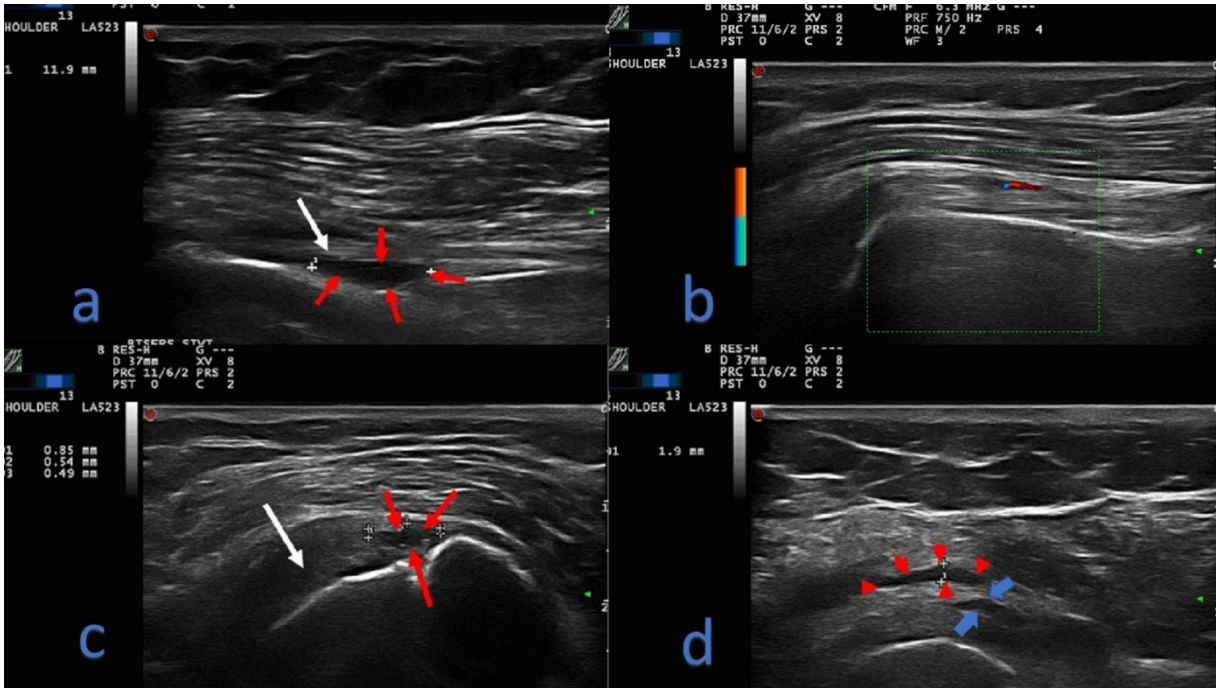

Fig. 2. Findings detected by ultrasonography. (a) effusion within the biceps long head tendon sheath, (b) Tenosynovitis of the long head of the biceps tendon, (c) Partial tear of supraspinatus tendon, and (d) Subdeltoid efusion (red arrows) and partial tear of supraspinatus tendon (blue arrows).

supraspinatus and subscapularis tendons was detected during the 6th-week and 3-month follow-up. However, this patient had peritendinous effusion of the supraspinatus tendon and pain at the post-RT 6-month visit. The third patient with pre-RT PTEBT did not have pain; this patient showed no changes during follow-up encounters.

On the other hand, 10 (27.7%) patients developed ultrasonographic abnormalities during the post-RT period (Table 2). Among these, 6 (16.6%) had PTEBT at the 6th week of the assessment without any clinical findings. Three of these 6 patients did not have any abnormal USG findings during 3-month and 6-month follow-ups. In one of the 6 cases, the peritendinous effusion persisted until it completely resorbed at the 6-month follow-up. However, this patient complained about pain and was diagnosed with restricted ROM in the shoulder joint. Another patient detected to have PTEBT at the 6-week assessment complained about pain and was determined to have restricted ROM during the 6-month visit. Finally, in one of the six patients detected to have PTEBT during the 6-week visit, peritendinous effusion of the supraspinatus tendon and intrasubstance tear were added to the picture. However, this patient did not have pain or any physical examination findings.

In 1 (2.8%) of the ten patients with post-RT abnormal findings, shoulder pain preceded the abnormal USG findings. This patient complained about pain during the 6-week assessment, but the USG was completely normal then. However, this patient was detected with PTEBT and increased peritendinous blood flow at the biceps tendon at the 6-month visit. Similarly, another patient with no pre-RT complaints or abnormal USG findings developed PTEBT and peritendinous effusion of the supraspinatus tendon three months after RT. The former finding persisted until the 6-month assessment, but this patient did not have any complaints. Finally, one (2.8%) patient developed PTEBT, while another (2.8%) had peritendinous effusion of the supraspinatus tendon at the post-RT 6-month assessment. However, these two patients had no pain or physical examination findings.

All control group patients had normal ultrasonographic findings. The thickness of the supraspinatus, biceps, subscapularis tendons and subcutaneous fat was similar between the dominant and non-dominant sides of the control group patients, and the cases (5.7

Table 2. Cases with clinical and/or radiological abnormalities

	Pre-RT			6.week			3.month					6.month				
	PBTE	PAIN	PRCTE	PBTE	PSTE	PAIN	PRCTE	PBTE	PSTE	PAIN	PRCTE	RCPT	PBTE	IPBBF	RM	PAIN
GT	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
SB	-	-	-	+	-	-	-	-	-	-	+	+	+	-	-	-
FÇ	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
AY	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+*	+
AY	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
RE	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-
HK	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
ZK	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
HG	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-
ND	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+**	+
SK	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
TS	+	+	-	+	-	+	-	+	-	+	-	-	+	-	-	+
AG	+	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-

PBTE = Peri-biceps tendon effusion, PRCTE = Peri-supraspinatus tendon effusion, PSTE = Peri-subscapularis tendon effusion, RCPT = Rotator cuff partial tear, IPBBF = Increased peri-biceps tendon blood flow, RM = Restricted motion, +* = Internal rotation+extension, +**= External rotation+abduction

mm vs. 5.1 mm, 2.87 mm vs. 2.89 mm, 4.13 mm vs. 3.97 mm, and 3.97 mm vs. 3.89 mm; respectively, $p > 0.05$).

Among 36 patients in the RT group classification into two subgroups as "ipsilateral" (i.e., the radiated field and the dominant arm are on the same side) and "contralateral" (i.e., the radiated area and the dominant arm are on opposite sides) revealed that 13 patients were given RT to the ipsilateral side and 23 received RT to the contralateral side. These groups were also compared regarding tendon and subcutaneous fat thicknesses (Table 3). This analysis showed that the pre-RT supraspinatus tendon thickness was significantly higher in the ipsilateral group than in the contralateral group ($p = 0.026$). However, the same comparison did not reveal a significant difference in the post-RT period ($p = 0.408$). As such, there was no difference between the two groups regarding the thicknesses of the biceps tendons analyzed during post-RT 3-month and 6-month assessments ($p = 0.468$). It was also the case for the thickness of the subscapularis tendon ($p = 0.385$).

The thickness of the subcutaneous fat significantly decreased in the ipsilateral group; the mean decrease was 0.6 mm ($p = 0.001$). However, this figure started to increase three months after RT and went up to its pre-RT levels before the post-RT 6-month visit.

The results of the comparative analysis of the 13 RT group patients with abnormal clinical or ultrasonographic findings with the 23 RT group who did not have any abnormal findings concerning the thickness of the subcutaneous fat are displayed in Table 4. This analysis did not reveal a significant difference.

DISCUSSION

Magnetic resonance imaging is the gold standard method for delineating the shoulder anatomy and diagnosing shoulder pathologies since it is a high-resolution multiplanar imaging tool. However, it has disadvantages as being expensive, time-consuming, and less likely to be immediately available than other cross-sectional imaging methods. On the other hand, USG can delineate the anatomy and show the pathologies of the shoulder with considerable sensitivity and specificity. Furthermore, it is portable, readily available, cheaper, and faster than MRI. Its combination

Table 3. Comparison of the tendon and subcutaneous fat thicknesses of the patients who underwent RT

Variable	Ipsilateral (n = 13) Mean ± SD Median (Min-Max)	Contralateral (n = 23) Mean ± SD Median (Min-Max)	*p value*
Pre-RT			
RCTT	6.15 ± 0.98 6.3 (4.8-8.4)	5.39 ± 0.87 5.4 (3.9-8.1)	**0.026**[*ǂ]
BTT	2.93 ± 0.29 3.1 (2.5-3.3)	2.89 ± 0.32 2.9 (2.1-3.7)	0.537*
STT	4.10 ± 0.81 3.9 (3.5-6.7)	4.24 ± 0.78 4.1 (3.2-6.2)	0.558*
SCF	5.13 ± 1.49 4.9 (1.9-7.5)	4.45 ± 1.53 4.4 (1.4-7.8)	0.204**
6. week			
RCTT	6.30 ± 0.91 6.1 (5.1-7.9)	6.04 ± 0.74 5.9 (4.5-7.5)	0.371**
BTT	3.29 ± 0.42 3.2 (2.9-4.2)	3.07 ± 0.44 3.1 (2.3-4.3)	0.296*
STT	4.15 ± 0.63 4.1 (3.3-5.6)	4.37 ± 0.72 4.3 (3.3-6.3)	0.366**
SCF	4.51 ± 1.65 4.7 (1.8-7.5)	4.39 ± 1.71 4.1 (2.0-8.4)	0.116**
3. month			
RCTT	6.25 ± 0.97 5.8 (5.1-8.2)	6.33 ± 0.77 6.2 (5.0-8.0)	0.478*
BTT	3.34 ± 0.36 3.4 (2.6-3.8)	3.32 ± 0.49 3.4 (2.3-4.1)	0.893**
STT	4.40 ± 0.72 4.1 (3.5-6.4)	4.50 ± 0.74 4.3 (3.2-5.9)	0.519*
SCF	4.83 ± 1.39 5.1 (2.0-6.5)	4.34 ± 1.63 4.2 (1.5-7.4)	0.367**
6. month			
RCTT	5.97 ± 0.94 6.1 (4.4-7.7)	6.31 ± 0.80 6.5 (5.1-7.8)	0.259**
BTT	3.32 ± 0.43 3.2 (2.5-3.9)	3.37 ± 0.61 3.4 (2.3-4.2)	0.792**
STT	4.35 ± 0.47 4.3 (3.5-5.3)	4.58 ± 0.80 4.3 (3.6-6.4)	0.729*
SCF	5.39 ± 1.51 5.9 (2.0-7.2)	4.98 ± 2.15 4.9 (1.8-9.9)	0.548**

RCTT = Supraspinatus tendon thickness, BTT = Biceps tendon thickness, STT = Subscapular tendon thickness, SCF = Subcutaneous fat thickness, RT = Radiation treatment, SD = Standard deviation

*: Mann Whitney U test **: Independent groups t test ǂ: $p < 0,05$ indicating statistical significance

Table 4. Comparison of the subcutaneous fat thickness between patients with and without clinical and/or ultrasonographic abnormalities

	Abnormality present (n = 13) Mean ± SD Median (Min-Max)	No abnormality (n = 23) Mean ± SD Median (Min-Max)	*p value*
Pre-RT	4.75 ± 0.85 4.4 (3.9-6.2)	4.64 ± 1.77 4.9 (1.4-7.8)	0.739*
6. week	4.46 ± 1.06 4.4 (3.1-6.0)	4.46 ± 1.9 4.5 (1.8-8.4)	0.724*
3. month	4.85 ± 1.05 4.7 (2.9-6.5)	4.40 ± 1.74 4.1 (1.5-7.4)	0.389*
6. month	5.52 ± 1.11 5.4 (3.9-7.2)	5.05 ± 2.24 4.9 (1.8-9.9)	0.410*

*Mann Whitney U test, RT = Radiation treatment, SD = Standard deviation

with color Doppler increases its efficacy in diagnosing the inflammatory pathologies of the muscles, tendons, and synovium. The introduction of the novel high-frequency (20 MHz) ultrasonic devices facilitated the evaluation of even the fibers of the tendons. In addition, the novel multifrequency probes rendered the synchronized imaging of the superficial and deep structures. Therefore, in recent decades, USG has become popular in imaging the musculoskeletal system [11].

The primary aim of RT is to kill the tumor cells while protecting the healthy tissue from inadvertent damage [12]. The RT-related adverse effects in patients with breast cancer are neck, shoulder, or arm pain, lymphedema in the arm, axillary paresthesia, restricted motion in the arm and shoulder, and brachial plexopathy [13, 14]. These adverse effects were reduced by the recent advances in RT techniques and modifications in the dose-fraction schemes.

Among the listed side effects, restricted shoulder motion is a common effect reducing the quality of life [15, 16]. The primary reasons for the motion restriction are pectoral muscle fibrosis, neuronal damage, lymphedema, and damage to the vascular structures. Our study did not analyze the contributions of these factors in patients with restricted ROM in the shoulder joint; however, ROM assessment was the essential assessment performed by the orthopedic surgeon. Among 36 patients in the RT group, 2 (5.6%) had restricted ROM in the shoulder joint during the post-RT 6-month visit.

There is a risk of tendon pathology in patients receiving 20 Gy or more radiation [17]. It was reported that the pathogenetic mechanisms leading to RT-related soft tissue damage involved endothelial thickening resulting from microvascular injury, degeneration, necrosis, and inflammatory reactions leading to progressive fibrosis [18]. Also, free oxygen radicals formed after an ischemia period can contribute to this process following reperfusion by causing ischemia-reperfusion injury. Recurrent hyperemia caused by radiation is also implicated in this process. Our patients in the RT group were given a total radiation dose of 50 Gy. Therefore, there was a considerable risk for shoulder tendon pathologies. In line with this assumption, all of our RT patients were detected to have minimal edema in the shoulder tendons at the 6-week assessment. The only ultrasonographic finding in 23 of 36 RT group patients was edema. However, the edema was prominent in the biceps tendon since this tendon is closer to the radiated field than the other tendons. In only 10 cases, the edema was accompanied by peritendinous effusion.

It was postulated that tenosynovitis, demonstrated by the presence of peritendinous effusion, was due to the thermal effects of the radiation and subsequent inflammation. However, in our study, PTEBT was more common than peritendinous effusion of the

supraspinatus and subscapularis tendons. This finding is in line with the fact that the long head of the biceps tendon is closer to the anterior chest wall and axillary-supraclavicular radiation fields.

It is known that one of the most common causes of tendinopathy is trauma. Nevertheless, since all of our RT group patients underwent breast surgery (BCS or mastectomy) before starting RT, it is reasonable to assume that they protected the ipsilateral shoulder and the arm from trauma. Thus, it can be suggested that all tendinopathies were due to radiation damage rather than trauma. In addition, only one of our patients had supraspinatus tendon's tear. However, more data are required to suggest that RT facilitates tendon tears in minor traumas. Our patients did not have other potential facilitators, such as a history of trauma, acromioclavicular joint hypertrophy, and comorbidities predisposing to tendinopathy.

In one of our cases, increased peritendinous blood flow at the biceps tendon and PTEBT were detected during the 6-month visit. However, this patient did not complain about shoulder pain. This finding is consistent with the reports, which noted that synovial hyperemia was not correlated with pain [19].

In all of our cases, we detected an increase in the tendon thicknesses after a 6th-week RT. However, this increase was statistically insignificant. In addition, the increased tendon thicknesses started to reduce afterward. This finding can be explained by sublethal damage repair [20, 21]. It was noted that the subcutaneous fat was susceptible to radiation, which caused a significant but temporary decrease in both the number and the size of the lipid cells [22]. Our results align with these reports since we observed a decrease and a subsequent increase in subcutaneous fat thickness during the 6th-week and 6th-month assessments.

Blomqvist *et al*. compared the patients who underwent mastectomy and RT with those who did not receive RT after mastectomy regarding shoulder motions [23]. The comparison made after a mean post-RT period of 15 months revealed that RT led to restricted mobility in the shoulder and a weakening in the strength of the shoulder muscles. The lowest impact was detected in external rotation. In our study, two patients had a restricted range of motion in the shoulder joint. One of these cases had restrictions on internal rotation and extension, while the other had restricted external rotation and abduction. These two patients were detected to have biceps tenosynovitis during the 6th-week assessment. It was postulated that radiation might cause inflammation in the synovium, and subsequent healing by a severe fibrogenic process might lead to fibrosing tenosynovitis. However, our results are insufficient to support this hypothesis, nor are they comparable to those of Blomqvist *et al*. since our six-month follow-up period was six months [23].

In our cohort, all but two cases with tenosynovitis improved before the post-RT 3-month visit. The remaining two patients healed before the 6-month assessment. Interestingly, none of the cases with tenosynovitis had pain, and the only case who complained about shoulder pain during the 6-week visit did not have any abnormal ultrasonographic findings.

It was reported that mastectomy alone could cause motion restriction in the shoulder joint, shoulder pain, and arm pain, and physiotherapy could reduce the risk of these complications [24, 25]. In our cohort, 29 of the 36 RT group patients underwent a mastectomy, and all of these patients stated that they were compliant with the post-mastectomy physiotherapy program.

Limitations

Our study has some limitations. First, the follow-up period is relatively short. Second, the sample size is relatively small. Third, the analysis does not include data regarding initial admission, including surgical parameters and complications.

CONCLUSION

In breast cancer patients undergoing adjuvant RT following BCS or mastectomy, RT causes temporary edema in shoulder tendons in the irradiated field. However, the edema is relatively more prominent in the long head of the biceps tendon. Shoulder pain and restricted range of motion can be present in these patients. Nevertheless, these complaints do not correlate with the ultrasonographic findings.

Authors' Contribution

Study Conception: BA; Study Design: SK, BA; Supervision: BA; Funding: N/A; Materials: N/A; Data Collection and/or Processing: SK; Statistical Analysis

and/or Data Interpretation: SK, BA; Literature Review: SK, BA; Manuscript Preparation: SK and Critical Review: SK, BA.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

We would like to acknowledge the contribution of Fatma Nesrin Turan, Deparment of Biostatistics - Trakya University, for the statistical analysis.

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The European Research Journal 2023;9(6):1360-1367 Original Article

DOI: 10.18621/eurj.1162806 *Rheumatology*

Connective tissue disease related interstitial lung disease: a single center experience

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ABSTRACT

Objectives: We aimed to determine the clinical features of the patients followed by our hospital with the diagnosis of connective tissue disease related interstitial lung disease (CTD-ILD).

Methods: The study included 113 patients who were followed up with the diagnosis of CTD-ILD, admitted to Kahramanmaraş Sütçü İmam University, Faculty of Medicine, Rheumatology Department between January 2019 and December 2020. Demographic characteristics, laboratory data and high-resolution computed tomography (HRCT) patterns of the patients were recorded retrospectively.

Results: Of 113 patients diagnosed with CTD-ILD; 90 were female, 23 were male. When the distribution of connective tissue disease (CTD) evaluated; 50 were rheumatoid arthritis, 41 were systemic sclerosis, 8 were sjogren's syndrome, 4 were systemic lupus erythematosus, 7 were overlap syndrome and 3 were undifferentiated connective tissue disease. There was no statistically significant difference between laboratory parameters. In the HRCT evaluation, 86 patients had nonspecific interstitial pneumonia (NSIP), 25 patients had usual interstitial pneumonia (UIP), and 2 patients had lymphocytic interstitial pneumonia (LIP) pattern. The most common pattern, including rheumatoid arthritis, was NSIP.

Conclusions: In our study, as inconsistent with the literature the most common pattern in RA patients was found to be NSIP.

Keywords: Interstitial lung disease, connective tissue disease, radiological pattern

Connective tissue diseases (CTD) are a group of systemic disorders characterized by autoimmunity and autoimmune-mediated organ damage. Although the incidence of CTD varies in many studies, the general incidence rate in the world is accepted as 1-3% [1].

The lung is a common target and all components of the respiratory system (pleura, interstitium, large and small airways, vascular structures) are at risk [1]. The pathologies that most affect mortality and morbidity are interstitial lung disease and pulmonary hypertension. The main causes of interstitial lung disease (ILD) due to CTDs are: Systemic sclerosis (SSc), Rheumatoid Arthritis (RA), Systemic Lupus Erythe-

Received: ***September 7, 2022;*** *Accepted:* ***July 3, 2023;*** *Published Online:* ***August 29, 2023***

How to cite this article: *Gezgin T, Atilla N, Yıldırım Çetin G, Akkök B, Bozkuş F, Kızıldağ B, Çulpan A, Kahraman H. Connective tissue disease related interstitial lung disease: a single center experience. Eur Res J 2023;9(6):13601367. DOI: 10.18621/eurj.1162806*

e-ISSN: 2149-3189

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matosus (SLE), Sjögren's Syndrome (SjS), Overlap syndrome (OLS), and Undifferentiated Connective Tissue Disease (UnCTD). High Resolution Computed Tomography (HRCT) is accepted as the gold standard noninvasive imaging method in the diagnosis of CTD-ILD [2, 3].

The radiologic interstitial pneumonia (IP) patterns of CTD-ILD are; nonspecific IP (NSIP), usual IP (UIP), organizing pneumonia (OP), respiratory bronchiolitis-associated ILD (RB-ILD), desquamative IP (DIP), diffuse alveolar damage (DAD), and lymphocytic IP (LIP) [2, 4].

The histopathological and radiological features of ILDs associated with CTDs are the same as their idiopathic counterparts. The histopathologic findings of CTD-ILD are follicular lymphoid hyperplasia and prominent plasma cell infiltration in interstitial inflammation suggestive of interstitial pneumonia [5].
Previous studies have clearly shown that the presence of CTD in ILD has great impact on the prognosis. Progressive fibrosing interstitial lung disease (PF-ILD) is a phenotype defined by rapid clinical progression towards respiratory failure. While idiopathic pulmonary fibrosis is the archetype of PF-ILD, CTD-ILD can also manifest as PF-ILD [6].

The treatment option is given according to the underlying CTD disease. Due to complexities in diagnosis and treatment of CTD itself and lack of evidence, current guidelines do not clearly provide strategies for evaluation and management of CTD-ILD despite its significance [7].

As a general opinion the NSIP pattern is more common in all CTD-ILDs except RA [4]. However, studies in recent years have shown that there may be epidemiological variations [8-10]. But epidemiological studies on CTD-ILD are limited. Based on this idea, in this study, we aimed to retrospectively evaluate the laboratory and radiological features of CTD-ILD patients in Kahramanmaraş city, located in the Eastern Mediterranean region of Turkey.

METHODS

The files of a total of 1589 patients, diagnosed with RA according to 2010 ACR/EULAR (American College of Rheumatology/ European Alliance of Associations for Rheumatology) classification criteria, diagnosed with systemic sclerosis according to 2013 ACR/EULAR classification criteria, Sjögren's disease according to 2016 ACR/EULAR classification criteria, diagnosed with SLE according to the 2012 SLICC (Systemic Lupus International Collaborating Clinics) classification criteria, and diagnosed with overlap syndrome and Undifferentiated Connective Tissue Disease (UnCTD) in line with EULAR and ACR recommendations followed up in the outpatient clinic of Kahramanmaraş Sütçü İmam University Faculty of Medicine, Rheumatology Department between January-2019 and January-2020 were scanned retrospectively . 113 CTD-ILD patients identified.

Radiological pattern, Complete Blood Count (CBC), Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), Lactate Dehydrogenase (LDH), Pulmonary Function Test (PFT) values of 113 CTD-ILD patients were scanned. Laboratory and spirometry data closest to the date of radiological diagnosis of ILD were noted. As a general approach in our hospital, HRCT examination for CTD-ILD is performed when respiratory system related symptoms or signs develop. Those with chronic or acute lung disease other than CTD-ILD were excluded.

The approval for the study was obtained from the Clinical Research Ethics Committee of Kahramanmaras Sutcu Imam University, Faculty of Medicine, with the number of 29.04.2021-191.

Our research is a retrospective descriptive study. Since it is a retrospective descriptive study, tables and graphs were created by giving the ratios. Statistical evaluation and p-value not studied.

RESULTS

If we look at the gender distribution, male/female ratio was 289/1281 of a total of 1589 CTD. HRCT of 113 patients was compatible with CTD-ILD and disease distribution was as follows; 50 RA, 41 SSc, 8 SjS, 4 SLE, 7 OLS and 3 UnCTD. The female/male ratio of 113 patients was 90/23. The male/female ratios of RA, SSc, OLS and UnCTD patients were 13 (26%)/37 (74%), 6 (14%)/35 (85.4%), 2 (28.5%)/5 (71.5%), 2 (66.3%)/1 (33.3%), respectively. SLE and SjS patients were all female.

The mean age of the RA patients was 64.7 ± 9.5 years, the mean age of the SSc patients was 56.6 ± 12

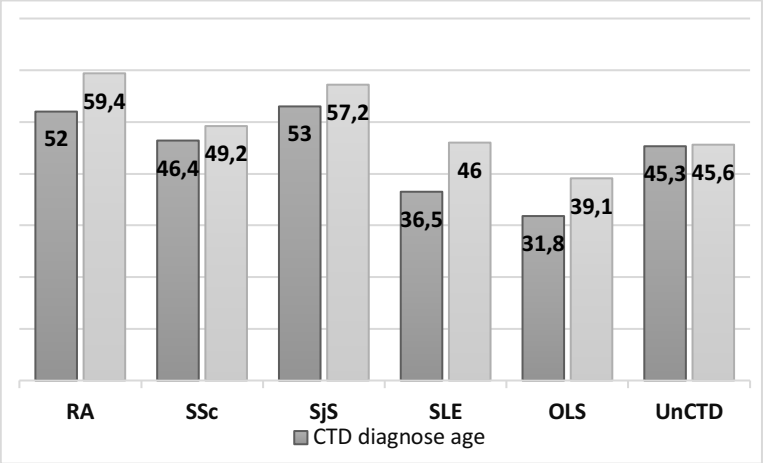

Fig. 1. The mean age of CTD diagnosis and the mean age of CTD-ILD diagnosis. RA = Rheumatoid Arthritis, SSc = Systemic Sclerosis, SjS = Sjogren's Syndrome, SLE = Systemic Lupus Erythematosus, OLS =Overlap syndrome, UnCTD = Undifferentiated Connective Tissue Disease, CTD = Connective Tissue Disease, CTD-ILD = Connective Tissue Disease related Interstitial Lung Disease

years, the mean age of the SjS patients was 61.3 ± 12.1 years, the mean age of the SLE patients was 50.2 ± 10.3 years, the mean age of the OLS patients was 45.1 ± 14.9 years and the mean age of the UnCTD patients was 52.3 ± 11.5 years.

The mean age of CTD diagnosis and the mean age of CTD-ILD diagnosis in different CTD diseases were mentioned in Fig. 1. FEV1, FVC and FEV1/FVC values of the patients were examined (Fig. 2). But we were able to access the spirometry records of only 69 of 113 patients. Laboratory parameters are summarized in Table 1. The radiological patterns of patients are summarized in Table 2.

When we analyzed ESR and CRP values between NSIP and UIP patterns; the mean ESR value for the NSIP pattern was 31.62 ± 20.73 mm/h, while it was found to be 29.04 ± 18.57 mm/h for the UIP pattern ($p > 0.05$). When the LDH values were examined, the mean LDH value was 230.46 ± 66.70 U/L in NSIP pattern and 265.08 ± 146.45 U/L in UIP pattern. No significant difference was observed between NSIP and UIP patterns in terms of LDH level ($p = 0.08$). When the mean CRP values were considered, the mean CRP in patients with NSIP pattern was 20.21 ± 33.45 mg/L, and the mean CRP value in patients with UIP pattern was 9.12 ± 6.80 mg/L. According to this result, the CRP value in patients with NSIP pattern was significantly higher than those with UIP pattern ($p = 0.005$).

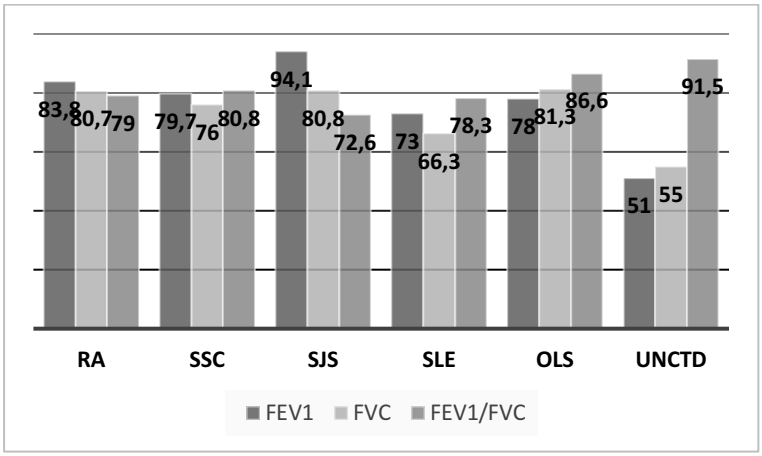

Fig. 2. Spirometry values of patients at the time of radiological diagnosis of CTD-ILD. FEV1 = Forced Expiratuar Volume in 1 second, FVC = Forced Vital Capacity, RA = Rheumatoid Arthritis, SSc = Systemic Sclerosis, SjS = Sjogren's Syndrome, SLE = Systemic Lupus Erythematosus, OLS = Overlap syndrome, UnCTD = Undifferentiated Connective Tissue Disease

Table 1. Laboratory characteristics of CTD-ILD patients

	CRP (mg/L)	ESR (mm/h)	LDH (U/L)	WBC ($\times10^9$/L)	Neutr ($\times10^9$/L)	Lymp ($\times10^9$/L)	Hb g/dL	PLT ($\times10^9$/L)
RA	27.2 ± 5.8*	32 ± 21.3	238.9 ± 91.1	8.4 ± 3.2	6.51 ± 1.05	2.28 ± 0.9	12.1 ± 2	301.6 ± 92.5
SSc	8.3 ± 1.5*	29.7 ± 17.7	260.8 ± 126	7.7 ± 3.3	6.58 ± 0.78	2.14 ± 0.73	12.4 ± 2.1	311.1 ± 109
SjS	11 ± 5.1*	47 ± 38.4	193.7 ± 47.1	8.4 ± 3.9	5.81 ± 1.14	2.33 ± 1.11	11.7 ± 4.3	326 ± 167.6
SLE	19.7 ± 16.8	39 ± 29.2	273 ± 51.3	7.8 ± 3.6	6.7 ± 1.43	2.3 ± 1.23	10.8 ± 1	298.2 ± 181
OLS	10.7 ± 7.9	28.2 ± 11.7	214.1 ± 35.4	14.6 ± 5.7	6.94 ± 0.48	1.9 ± 0.46	12.6 ± 2.1	384.5 ± 174
UnCTD	12 ± 10	26 ± 15.5*	255.6 ± 15	10.1 ± 1.3	6.33 ± 0.57	2.2 ± 0. 1	15.2 ± 1.8	258 ± 31.2

CRP = C-Reactive Protein, ESR = Erythrocyte Sedimentation Rate, LDH = Lactate Dehydrogenase, WBC = White Blood Cell, Neutr = Neutrophil, Lymp = Lymphocyte, Hb = Hemoglobin, PLT = Platelet, RA = Rheumatoid Arthritis, SSc = Systemic Sclerosis, SjS = Sjogren's Syndrome, SLE = Systemic Lupus Erythematosus, OLS=Overlap syndrome, UnCTD = Undifferentiated Connective Tissue Disease

*Standart error

DISCUSSION

Kahramanmaraş province is located in Eastern Mediterranean region, and our study is the first study to investigate the general characteristics of CTD-ILDs in this region.

In our clinic RA was the most common, and other diseases were SjS, SLE, SSc, OLS, and UnCTD in order of frequency. ILD was developed in 7.2% (n = 113) of these 1589 patients. The highest incidence of ILD development was in SSc (54%). ILD was developed in 4.4% of RA patients, and this rate was found to be quite low in other CTDs. The present findings were found to be compatible with the literature [8, 9]. When the gender distribution of all 1589 patients diagnosed with CTD with and without lung involvement was examined, the number of female patients (n = 1281) was approximately four times higher than that of men (n = 289). The gender distribution of 113 patients with lung involvement was similar to the general population, with the number of female (n = 90) nearly four times higher than that of men (n = 23). These findings were consistent with the literature [13, 14].

According to the literature data, while RA is common in female in the community, it is more likely to cause ILD in men. When we evaluated the patients with RA in our study, ILD was developed in 5.5% of male patients with RA, while this rate was 4.1% in female patients. From this point of view, the rate of ILD development was higher in male RA patients, which seemed compatible with the literature [15, 16].

When RA-ILDs were evaluated within them-

Table 2. Radiological patterns of patients

Disease, n (%)	Radiological pattern		
	UIP	**NSIP**	**LIP**
RA (n = 50)	9 (18%)	41 (82%)	-
SSc (n = 41)	14 (34.1%)	27 (65.9%)	-
SjS (n = 8)	-	6 (75%)	2 (25%)
SLE (n = 4)	-	4 (100%)	-
OLS (n = 7)	1 (14.2%)	6 (84.8%)	
UnCTD (n = 3)	1 (33.3%)	2 (66.3%)	

RA = Rheumatoid Arthritis, SSc = Systemic Sclerosis, SjS = Sjogren's Syndrome, SLE = Systemic Lupus Erythematosus, OLS =Overlap syndrome, UnCTD =Undifferentiated Connective Tissue Disease, NSIP = Nonspecific Interstitial Pneumonia, UIP = Usual Interstitial Pneumonia, LIP =Lymphocytic Interstitial Pneumonia

selves, the number of female (n:37) RA-ILD patients was three times higher than that of men (n:13). In studies evaluating the gender distribution among RA-ILD patients, there are articles that support our study, indicating that it is more common in female. For example, in the study performed by Zang *et al*. [17], 64% of RA-ILD patients were found to be female, while Jayasinghe *et al*. [18] found 52.8% to be female in both studies, it was stated that female predominance was more common in RA-ILD, contrary to the general belief. They explained this situation with epidemiological variation. However, in both studies, gender distribution was evaluated within patients with RA-ILD. Namely, these studies did not mention what percentage of male RA patients develop ILD.

In our study, RA and SjS patients had both CTD and CTD-ILD diagnosis at an older age than the other groups. Zamora *et al*. [19], in their study with 181 patients at the Mayo clinic between 1998 and 2015, found the age of diagnosis of RA as 58.5 ± 13.5 years and the age of RA lung involvement as 67.4 ± 9.9 years, similar to our study. The age ranges reported for the diagnosis of SjS-ILD in the literature are quite wide. For example, in a study including 178 patients with SjS diagnosis, Goa *et al*. [20] calculated the median age of the SjS-ILD group as 61.59 ± 11.69 advanced age, similar to our study.

The age at which the patients were diagnosed with CTD and CTD-ILD were similar for the SSc and UnCTD groups. The age at diagnosis for SLE was 36.5 ± 9.8 years, and the age of lung involvement was 46 ± 10.6, which was consistent with the literature [21].

The earliest age at diagnosis of CTD (31.8 ± 17.6) and the earliest age of lung involvement (39.1 ± 13.4) were OLS patients. More than one CTD is present in OLS. Therefore, we think that the symptoms, signs and complications of diseases may contribute to both early diagnosis and acceleration of progression by creating a synergistic effect.

The time between the diagnosis of CTD and lung involvement was the longest in SLE, and the shortest in UnCTD.

Both CRP and ESR values of the patients were above the normal range in all groups, and the CRP value was the highest in the RA-ILD group. The plasma CRP concentration above 10 mg/L CRP in healthy adults is considered high. There are studies indicating that CRP levels are persistently above 20 mg/L in RA patients [22]. However, all of these studies are randomized controlled drug studies, and retrospective and observational real-life studies argue that CRP levels may be normal even when the disease is active in the joint [23, 24].

Yang *et al*. [24] found the basal CRP level to be (29.5 ± 35.3 mg/L) in patients with RA-ILD as similar our study. CRP is a general marker of systemic inflammation and is moderately correlated with hard-to-reach tests such as IL-6 [24]. However, a threshold value that can be used to predict radiological progression has not yet been determined.

Kaduri *et al*. [25] investigated the parameters that may play a role in the early diagnosis of ILD in RA in a study that screened 52 RA-ILD patients. They argued that the high ESR level (median value: 50 mm/h [min:30, max: 77]) could guide the prediction of ILD in RA patients.

In our study, the mean ESR value was found to be higher than normal although it was not as high as in this study. We think that CRP and ESR can be a guide in predicting lung involvement in RA patients.

Spirometry is widely used in the diagnosis of ILD, and the most common restrictive pattern is seen [30]. In the restrictive pattern, FEV1 and FEV1/FVC were normal or above normal, while the FVC value decreased. In our study we were able to access the spirometry records of only 69 of 113 patients. For this reason, we did not consider it appropriate to discuss the spirometry results.

When radiological patterns are compared, between UIP and NSIP patterns; There was no statistically significant difference in gender, time between CTD and CTD-ILD development, age at developing CTD-ILD, ESR and CBC values. However, CRP was significantly higher in patients with NSIP pattern compared to those with UIP pattern ($p = 0.005$). According to literature data, UIP is the most common radiological pattern in patients with RA-ILD [32]. Zamora *et al*. [16] were examined CT scans of 63 RA-ILD patients in North America and UIP pattern was found in 26 (41%) patients, NSIP pattern was found in 19 (30%) patients, bronchiolitis was found in 11 (17.4%) patients, and OP was found in 5 (8%) patients. Kelly *et al*. [13] were examined RA-ILD types in a study in England, they found the most common pattern as UIP with a rate of 44-66%, and the NSIP pattern was observed with a

rate of 24-44%. Again, Kelly *et al*. [13] was found the patterns of RA-ILD patients as; 65% UIP, 24% NSIP, and 5% OP in a large multicenter study in the UK. Nakamura *et al*. [33], in a study with 54 patients in Japan, found the most common pattern as NSIP (30%), followed by the UIP pattern with (28%) patients. In the study with 237 RA-ILD patients in China performed by Zhang *et al*. [14], 137 patients (57.8%) were found to have NSIP, and 44 patients (18.6%) were found to have UIP. Jayasinghe *et al*. [15] found that the NSIP pattern (55.8%) was more common than the UIP pattern (34.9%) on HRCT in 44 RA-ILD patients in Sri-Lanka. In our study, 9 (18%) patients with RA-ILD had UIP, while 41 (82%) patients had NSIP pattern. According to these data, it was thought that the radiological pattern in RA-ILD showed epidemiological variation, while UIP was more common in western societies, NSIP was more common in eastern societies.

HRCT is performing to RA patients in our clinic when there are respiratory system-related symptoms, signs or worsening in pulmonary function tests compared to baseline. Li *et al*. [7] scanned 1121 RA patients with HRCT and they found that 30.12% had ILD. They reported that 39.12% of the patients with RA-ILD had respiratory symptoms. HRCT is considered as the most sensitive method in the diagnosis of ILD. From this point of view, undetected ILD is possible in our RA patients who did not undergo HRCT because they did not have respiratory system-related symptoms.

Diffuse ground glass infiltrates are often seen on HRCT in Ssc-ILD, generally consistent with NSIP. Coarse reticulation and honeycomb appearance are less common. Bourus *et al*. [34] found NSIP (commonly fibrotic NSIP) in 77% of 80 patients diagnosed with SSc-ILD, and UIP in 23%. In our study, there were 14 (34.1%) patients with UIP and 27 (65.9%) patients with NSIP pattern, and the results are consistent with the literature.

The most common radiologic pattern of SLE-ILD was found to be NSIP. Although ILD is seen at a lower rate in SLE than in other CTD, NSIP pattern was seen in all patients in our study [35].

LIP is the most common radiological pattern in SjS, and it is a benign pathology that occurs with bronchial-associated lymphoid tissue (BALT) proliferation. It is seen in 1% of SjS. In our study, NSIP pattern was observed in 6 (75%) patients, and LIP pattern was observed in 2 (25%) patients. The results were consistent with the literature [36].

These different patterns of involvement in CTD-ILD patients are also important for early treatment and the selection of the right treatment in the prevention of respiratory failure [37].

Limitations

The limitations of our study are as follows: Our study includes only the patients in Kahramanmaraş region and CTD patients without respiratory symptoms were not screened with HRCT only patients with clinically suspected ILD were screened with HRCT.

CONCLUSION

According to the literature data, while RA is common in female in the community, it is more likely to cause ILD in men. But it should be noted that when RA-ILDs were evaluated within themselves, the number of female RA-ILD patients was higher than that of men. We think that the symptoms, signs and complications of OLS and UnCTD diseases coexistence of two or more CTD disease features may contribute to both early diagnosis and acceleration of progression by creating a synergistic effect. We think that CRP and ESR height can be a guide in predicting lung involvement in RA patients. ILD patterns of CTD patients may show ethnic differences. More research is needed in this area. There is not yet a multicenter study in our country that collects demographic data on CTD-ILD and compares it with the world literature. We hope that our study, which includes single center data, will shed light on more comprehensive studies.

Authors' Contribution

Study Conception: TG, NA, GYÇ, BA, FB, BK, AÇ, HK; Study Design: TG, NA, GYÇ, BA, FB, BK, AÇ, HK; Supervision: TG, NA, GYÇ, BA, FB, BK, AÇ, HK; Funding: N/A; Materials: N/A; Data Collection and/or Processing: TG, NA, GYÇ, BA, FB, BK, AÇ, HK; Statistical Analysis and/or Data Interpretation: TG, NA, GYÇ, BA, FB, BK, AÇ, HK; Literature Review: TG, NA, GYÇ, BA, FB, BK, AÇ, HK; Manuscript Preparation: TG, NA, GYÇ and Critical Review: NA, GYÇ.

Conflict of interest

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

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1368-1379 Original Article

DOI: 10.18621/eurj.1231251 *Hematology*

Comparison of different plerixafor-based strategies for adequate hematopoietic stem cell collection in poor mobilizers

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ABSTRACT

Objectives: The main objective of the present study was to evaluate whether the use of plerixafor in combination with granulocyte colony-stimulating factor (G-CSF) or subsequent use of isolated G-CSF and then plerixafor following disease-specific chemotherapy, and whether it would allow for adequate peripheral stem cell collection in patients.

Methods: The retrospective study evaluated 54 patients with previous mobilization failure who were administered plerixafor in 2 centers. In patients without any side effects, CD 34+ cell counts, the percentage of patients who were found eligible for autologous transplantation, the engraftment kinetics of the patients who underwent transplantation, and their overall survival results were compared between the two groups where G-CSF was used with plerixafor, or where plerixafor was used after isolated G-CSF following chemotherapy.

Results: The median age of the patients was 49 years (range: 17-70), and 64.8% (n = 35) were males. It was identified that 31 (57.4%) patients underwent mobilization treatment with isolated G-CSF and plerixafor, and 23 (42.6%) patients underwent mobilization treatment with chemotherapy plus G-CSF and plerixafor. In all patients, mean hemoglobin level (11.3 ± 1.5 g/dL vs. 9.3 ± 1.3 g/dL; $p < 0.001$) and median platelet level (129.2 $\times 10^3/\mu L$ vs. 58.4 $\times 10^3/\mu L$) were found to be higher, while febrile neutropenia rate (3.3% vs. 60.9%), the percentage of replacement patients (6.7% vs. 65.2%), and median days of G-CSF (6 vs. 9) were found to be lower on the day of plerixafor administration in the isolated G-CSF and plerixafor group compared to the chemotherapy and G-CSF and plerixafor group.

Conclusions: In conclusion, our study demonstrated that administration of plerixafor is generally safe and well-tolerated. Regardless of the underlying disease, it offers an effective alternative for patients with previous failed mobilization attempts using conventional regimens, and allows stem cell collection with fewer apheresis sessions.

Keywords: Autologous stem cell transplantation, mobilization, plerixafor

Received: ***January 9, 2023;*** ***Accepted:*** ***March 22, 2023;*** ***Published Online:*** ***April 10, 2023***

How to cite this article: *Sadri S, Yönal Hindilerden İ, Mutlu YG, Tiryaki TO, Gemici Aİ, Bekoz HS, et al. Comparison of different plerixafor-based strategies for adequate hematopoietic stem cell collection in poor mobilizers. Eur Res J 2023;9(6):1368-1379. DOI: 10.18621/eurj.1231251*

e-ISSN: 2149-3189

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High dose chemotherapy followed by autologous stem cell transplantation (ASCT) remains the standard of care for transplant-eligible multiple myeloma (MM) and lymphoma patients [1-3]. Peripheral blood is the globally preferred stem cell source for ASCT [4]. However, in 5 to 40% of patients, a planned ASCT cannot be performed due to failure to mobilize sufficient number of peripheral stem cells [5]. There is evidence supporting that a minimum dose of 2×10^6 CD34 + cell/kg is required for successful hematopoietic recovery and engraftment [6-9].

It has been reported that CD34+ cell dose of $\geq 5\times10^6$/kg is associated with a shorter period of hospitalization as well as reduced need for blood transfusion and antibiotic use in transplant patients [10]. Several variables to predict mobilization failure have been identified including advanced age, bone marrow involvement, number and type of previous chemotherapies (e.g. alkylating agents, fludarabine, lenalidomide), platelet count $< 100\times10^6$/L prior to apheresis, neutropenic fever during the period of mobilization, and history of radiotherapy-particularly which targets bones that generate hematopoietic cells [5, 10-13].

The two most common mobilization strategies include using granulocyte colony-stimulating factor (G-CSF) alone or G-CSF after chemotherapy. Increment in the dose of G-CSF, administration of high-volume apheresis (processing of blood at least 3 times through a single apheresis procedure), and use of plerixafor are the options to overcome mobilization failure [14-16]. For stem cell mobilization, plerixafor (AMD3100) selectively antagonizes the chemokine receptor (CXCR-4), and reverses and inhibits the interaction with the ligand stromal cell-derived factor 1-alpha (SDF-1 alpha) [17]. Development of novel strategies with plerixafor as backbone for transplant candidates who experienced mobilization failure enables the successful implementation of ASCT.

It has been demonstrated that combination of G-CSF and plerixafor in non-Hodgkin lymphoma (NHL) and MM patients leads to a significant increase in the number of CD34 cells collected compared to G-CSF alone [18, 19].

This study evaluated the outcomes of mobilization with G-CSF in combination with plerixafor and chemotherapy plus G-CSF in combination with plerixafor. In addition, median time to neutrophil and platelet engraftment, and follow-up data after ASCT were collected. This study aimed to compare the efficacy of the use of plerixafor in combination with G-CSF or use of G-CSF and plerixafor following disease-specific chemotherapy for adequate peripheral stem cell collection. Secondary endpoints included the increase in the peripheral blood CD 34+ cell count after plerixafor administration in the different patient groups, the percentage of patients achieving to pool sufficient number of stem cells for ASCT with different mobilization regimens, the engraftment kinetics of the transplanted patients and their overall survival (OS) results. Our study demonstrated that administration of plerixafor is well-tolerated. It offers an effective alternative for patients with previous failed mobilization attempts using conventional regimens.

METHODS

This single-center, retrospective study includes 54 patients from two different transplant centers (Istanbul Medipol University and Istanbul University- Istanbul Medical Faculty) who were administered plerixafor for previous mobilization failure. This study was approved by local ethics committee of Istanbul Medipol University (study number 01.06.2021: E-10840098-772.02-2487) and performed in accordance with the principles of the Declaration of Helsinki.

Study Design

Study population consisted of adult patients diagnosed with Hodgkin lymphoma (HL), 2 NHL, MM, T-Acute Lymphoblastic leukemia (T-ALL) and 1 with testis tumor between 2015-2020 years which had mobilization failure before. All patients in the examined period were examined, no case was excluded. Medical data were obtained from patient archive files and the hospital information system.

Mobilization Failure

Mobilization failure is defined as the failure to achieve a CD 34+ cell count of < 2 × 106/kg after G-CSF or chemotherapy followed by G-CSF.

Dosing and Administration

Patients were administered either 5 mcg/kg G-CSF twice a day in combination with at least a single

dose of 0.24 mg/kg plerixafor 9 to 11 hours prior to the apheresis procedure, or chemotherapy plus G-CSF in combination with at least a single dose of 0.24 mg/kg plerixafor.

Statistical Analysis

Statistical evaluation was performed using the Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL) program. Normal distribution of data was evaluated using the Kolmogorov-Smirnov Test. Normally distributed numerical variables were indicated as mean ± standard deviation, while numerical variables not showing normal distribution were indicated as median (min-max). Categorical variables were indicated in numbers and percentages. Chi-Square, Yates Correction and Fisher's Exact Tests were used for comparison of the categorical data. Student T Test or Mann-Whitney U Test was used to compare the numeric variables between G-CSF and CT plus G-CSF groups based on the normality distribution. ANOVA Test (post-hoc: Bonferroni Test) or Kruskall Wallis H Test (post-hoc: Dunn's Test) was used to compare numerical variables based on the diagnosis groups. Although groups with a sample size of less than 5 were not included in the analysis, the relevant data distributions are shown in Tables. $P < 0.05$ (*) value was considered significant in statistical analysis.

Table 1. Clinical and demographic features of patients

Variables	HL n = 9	NHL n = 28	MM n = 14	Other n = 3	Total n = 54	*p value*
Age (years)	32 (18-60)	50.5 (20-70)	60 (41-69)	30 (30-54)	49 (18-70)	**0.001***
Gender, n (%)						
Female	4 (44.4)	10 (35.7)	5 (35.7)	-	19 (35.2)	0.924
Male	5 (55.6)	18 (64.3)	9 (64.3)	3 (100.0)	35 (64.8)	
Pre-transplant RT, n (%)						
N/A	8 (88.9)	23 (82.1)	11 (78.6)	2 (66.7)	44 (81.5)	0.999
Yes	1 (11.1)	5 (17.9)	3 (21.4)	1 (33.3)	10 (18.5)	
Number of CT lines, n (%)						
1 Line	-	6 (21.4)	3 (21.4)	2 (66.7)	11 (20.4)	0.098
2 Lines	5 (55.6)	17 (60.7)	5 (35.7)	1 (33.3)	28 (51.9)	
3 Lines	2 (22.2)	4 (14.3)	6 (42.9)	-	12 (22.2)	
4 Lines	2 (22.2)	1 (3.6)	-	-	3 (5.6)	
Previous transplantation, n (%)						
N/A	9 (100.0)	28 (100.0)	10 (71.4)	3 (100.0)	50 (92.6)	**0.004***
Yes	-	-	4 (28.6)	-	4 (7.4)	
Previous mobilization failure, n (%)						
G-CSF	7 (77.8)	15 (53.6)	8 (57.1)	1 (33.3)	31 (57.4)	0.505
CT plus G-CSF	2 (22.2)	13 (46.4)	6 (42.9)	2 (66.7)	23 (42.6)	
Cyclosphamide+etoposide	1 (11.1)	7 (25.0)	4 (28.6)	-	12 (22.2)	0.793
Cyclosphamide	-	2 (7.1)	2 (14.3)	1 (33.3)	5 (9.3)	
ICE	1 (11.1)	2 (7.1)	-	1 (33.3)	4 (7.4)	
HD MTX+ ARA-C	-	2 (7.1)	-	-	2 (3.7)	

RT = radiotherapy, CT = chemotherapy, TX = transplant, HL= Hodgkin lymphoma, NHL = Non-Hodgkin lymphoma, MM = multiple myeloma

RESULTS

Study population consisted of a total of 54 patients, including 9 (16.7%) patients diagnosed with Hodgkin lymphoma (HL), 28 (51.9%) with NHL, 14 (25.9%) with MM, 2 (11.2%) with T-Acute Lymphoblastic leukemia (T-ALL) and 1 (5.6%) with testis tumor. The median patient age was 49 years (range:17-70 years), and 64.8% (n = 35) of the patients included were males. Thirty-one (57.4%) patients underwent mobilization with G-CSF and plerixafor, and 23 (42.6%) patients with chemotherapy plus G-CSF and plerixafor. The clinical and demographic characteristics of the patients are depicted in Table 1. The median age was lower in the HL group compared to the other groups (HL: 32 years vs. NHL: 50.5 years vs. MM: 60 years; $p = 0.001$). No patients in the HL and NHL groups had previous transplant history, while 28.6% of MM patients had a history of ASCT ($p = 0.004$). Other clinical and demographic features did not differ significantly among the diagnostic groups (Table 1).

Hemoglobin (Hgb) levels, platelet, leukocyte and neutrophil counts on the day of plerixafor administration did not differ significantly among the diagnostic groups, Median number of days of G-CSF administration and the percentage of patients who were able to undergo ASCT showed no difference among the diagnostic groups (Table 2).

The rate of complete remission (CR) at 3rd month of ASCT was similar for HL and NHL patients but lower for MM patients (HL: 44.4% vs. NHL: 42.9% vs. MM: 7.1%; $p = 0.023$). Rates of partial response (PR) and progressive disease were higher in the MM patients compared to the other diagnostic groups. The rate of mortality due to infection was higher for NHL patients compared to the other diagnostic groups (HL: 11.1% vs. NHL: 21.4% vs. MM: 0%; $p = 0.023$). Previous use of lenalidomide was identified in 57.1% of MM patients (Table 3).

In the whole study group, the number of chemotherapy lines administered was higher in patients who received G-CSF and plerixafor compared to patients who received chemotherapy plus G-CSF and plerixafor ($p < 0.001$). Other clinical and demographic characteristics did not differ significantly between the groups. The comparison of the two groups with respect to clinical and demographic features in different diagnostic categories was not possible due to low sample size. Among NHL patients, the percentage of those who received 1 line of chemotherapy was higher in the chemotherapy plus G-SCF and plerixafor group compared to the G-CSF and plerixafor group, while the percentage of patients who received 2 or more lines of chemotherapy was higher in the G-CSF and plerixafor group. Among MM patients, clinical and demographic characteristics did not differ significantly for the G-CSF and plerixafor and chemotherapy plus G-CSF and plerixafor groups (Table 4).

In the whole patient cohort, mean Hgb levels (11.3 ± 1.5 1.5 g/dL vs. 9.3 ± 1.3 1.5 g/dL ; $p < 0.001$) and median platelet counts (129.2 $\times 10^3/\mu L$ vs. 58.4 $\times 10^3/\mu L$; $p < 0.001$) were higher while the rate of febrile neutropenia (3.3% vs. 60.9%; $p < 0.001$), the percentage of patients requiring transfusion support (6.7% vs. 65.2%; $p < 0.001$), median number of days of G-CSF administration (6 vs. 9; $p = 0.001$), and median CD34 + cell counts (3×10^6/kg vs. 6.8 × 106/kg; $p < 0.001$) were lower on the day of plerixafor administration in the G-CSF and plerixafor group compared to the G-CSF plus chemotherapy and plerixafor group. On the day of plerixafor administration, G-CSF and plerixafor group and G-CSF plus chemotherapy and plerixafor group showed no significant difference for the other parameters (Table 5).

G-CSF and plerixafor and chemotherapy plus G-CSF and plerixafor mobilization regimens showed no difference for median time of engraftment in the whole study cohort as well as in patients diagnosed with NHL and MM.

Of the 37 patients who underwent ASCT, 5 (13.5%), 21 (56.8%) and 9 (24.3%) and 2 (5.4%) patients were diagnosed with HL, NHL MM and other malignancies, respectively. Median age of the ASCT patients was 50 years (range: 18-70), and 67.6% (n = 25) of the ASCT patients were males. Eighteen (48.6%) patients received mobilization treatment with G-CSF and plerixafor and 19 (51.4%) patients with chemotherapy plus G-CSF and plerixafor. Clinical and demographic characteristics of the patients who underwent ASCT showed no significant difference according to the diagnostic groups.

In patients who underwent ASCT, median days to platelet engraftment (platelet count ≥ 20k) (HL:11 vs. NHL:20 vs. MM:14; $p = 0.014$) and median days to

Table 2. **Clinical findings according to Plerixafor treatment**

Variables	HL n = 9	NHL n = 28	MM n = 14	Other n = 3	Total n = 54	*p value*
Cell counts at day of plerixafor Administration						
Hgb (gr/dL)	10.3 ± 2.0	10.5 ± 1.8	10.1 ± 1.5	10.3 ± 2.7	10.4 ± 1.8	0.845
PLT ($\times 10^3/\mu L$)	99.4 (60-227)	84.5 (22-266.8)	103.6 (40-279.5)	64.3 (55-111)	96.3 (22-279.5)	0.562
WBC ($\times 10^3/\mu L$)	22.7 (7-53)	27.5 (7-98.5)	22.9 (3.6-76)	36.8 (4.7-40)	24.9 (3.6-98.5)	0.778
ANC ($\times 10^3/\mu L$)	18.3 (4.5-47)	21.3 (4-72.5)	18.9 (2.8-38.4)	26 (3.4-31.8)	19.2 (2.8-72.5)	0.656
Length of stay for mobilization (days)	12 (6-18)	13 (6-29)	16 (7-25)	35 (10-42)	14 (6-42)	0.652
Events during mobilization						
Febrile neutropenia						
No	8 (100.0)	20 (71.4)	9 (64.3)	1 (33.3)	38 (71.7)	0.163
Yes	-	8 (28.6)	5 (35.7)	2 (66.7)	15 (28.3)	
Transfusion requirement						
No	7 (77.8)	19 (67.9)	10 (71.4)	1 (33.3)	36 (67.9)	0.999
Yes	2 (25.0)	9 (32.1)	4 (28.6)	2 (66.7)	17 (32.1)	
ES, count	1 (1-1)	3 (1-5)	2 (1-3)	6 (3-9)	3 (1-9)	0.214
Platelet, count	2 (2-2)	2 (1-9)	3 (1-7)	5 (2-8)	2 (1-9)	0.932
Number of days of G-CSF used	6 (4-8)	7 (4-17)	7 (6-15)	7 (6-14)	6.5 (4-17)	0.088
Cell count, 10^6/kg	4.725 (0.2-6.8)	3.56 (0.5-7.87)	4.05 (0.8-12.4)	4.64 (3.03-8)	4 (0.2-12.4)	0.597
Autologous transplant status, n (%)						
No	4 (44.4)	7 (25.0)	5 (35.7)	1 (33.3)	17 (31.5)	0.509
Yes	5 (55.6)	21 (75.0)	9 (64.3)	2 (66.7)	37 (68.5)	

Hgb = hemoglobin, PLT = platelet, WBC = leukocyte, ANC = neutrophil, HL = Hodgkin lymphoma, NH=: Non-Hodgkin lymphoma, MM = multiple myeloma

platelet count ≥ 50k (days) (HL:16 vs. NHL:25 vs. MM:16; $p = 0.049$) were higher in NHL patients compared to the other diagnostic groups. Time to platelet engraftment did not differ significantly in the HL and MM patients ($p > 0.05$). Time to achieve neutrophil engraftment (neutrophil count ≥500) was similar for the NHL and MM patients but shorter in patients diagnosed with HL (HL:10 vs. NHL:12 vs. MM:13; $p = 0.050$), while time to achieve neutrophil engraftment count ≥ 1000 did not differ significantly among the diagnostic groups (Table 6). Two (5.4%) patients did not achieve platelet and neutrophil engraftment due to early mortality. Platelet and neutrophil engraftment failed to occur in 6 (16.2%) patients and 1 (2.7%) patient, respectively.

Response assessment at 3rd month of ASCT showed that the rate of CR was highest in HL patients, and CR rate was higher in NHL patients compared to MM patients (HL: 80% vs. NHL:57.1% vs. MM:11.1; $p = 0.002$). The PR rate was highest in MM patients, and PR rate was higher in HL patients compared to NHL patients (HL: 20% vs. NHL:9.5% vs. MM:66.7%; $p = 0.002$). Disease progression was observed only in MM patients (22.2%). Death due to infection occurred only in NHL patients (21.4%). At 3rd month of ASCT, the diagnostic groups showed no difference in OS. All HL patients were alive, while 33.3% (n = 7) of the NHL patients and 22.2% (n = 2) of the MM patients died 3 months after ASCT. History of lenalidomide administration was documentd in 55.6% of the MM patients who underwent ASCT.

Among patients who underwent ASCT, the number of chemotherapy lines administered was higher in the G-CSF and plerixafor group compared to chemotherapy plus G-CSF and plerixafor group ($p < 0.002$). Other clinical and demographic features did not differ significantly between the G-CSF and plerixafor group and the chemotherapy plus G-CSF and plerixafor group. The comparison of the two mobilization groups with respect to clinical and demographic features in different diagnostic categories was not possible due to low sample size.

In ASCT patients mobilized with G-CSF and plerixafor compared to ASCT patients mobilized with chemotherapy plus G-CSF and plerixafor, mean Hgb level (11.5 ± 1.1 g/dl vs. 9.3 ± 1.4 g/dl; $p < 0.001$), median platelet count (143 $\times 10^3/\mu L$ vs. 52 $\times 10^3/\mu L$; $p < 0.001$), median WBC count (36.8 $\times 10^3/\mu L$ vs 21.3 $\times 10^3/\mu L$; $p = 0.050$), and the percentage of patients having required ≥ 2 days of apheresis (100% vs. 52.6%; $p = 0.001$) were higher while rate of febrile neutropenia (5.6% vs. 57.9%; $p = 0.001$), the percentage of pa-

Table 3. Short-term findings after Plerixafor administration

Variables	HL n = 9	NHL n = 28	MM n = 14	Other n = 3	Total n = 54	*p value*
Response assessment after 3-months, n (%)						
CR	4 (44.4)	12 (42.9)	1 (7.1)	2 (66.7)	19 (35.2)	**0.023***
PR	1 (11.1)	2 (7.1)	6 (42.9)	-	9 (16.7)	
Progression	1 (11.1)	1 (3.6)	3 (21.4)	-	5 (9.3)	
Death due to infection	1 (11.1)	6 (21.4)	-	-	7 (13.0)	
Unknown	1 (11.1)	5 (17.9)	2 (14.3)	1 (33.3)	9 (16.7)	
Not Collected	1 (11.1)	2 (7.1)	2 (14.3)	-	5 (9.3)	
Current status						
Dead	3 (33.3)	12 (42.9)	4 (28.6)	1 (33.3)	20 (37.0)	0.679
Alive	6 (66.7)	16 (57.1)	10 (71.4)	2 (66.7)	34 (63.0)	
Number of days of stem cell collection after mobilization, n (%)	5.5 (5-16)	6 (5-21)	6.5 (5-18)	13 (6-20)	6 (5-21)	0.176
Lenalidomide, n (%)						
N/A	-	-	5 (35.7)	-	-	-
Yes	-	-	8 (57.1)	-	-	
Unknown	-	-	1 (7.1)	-	-	0.105

CR = complete response, PR = partial response, HL = Hodgkin lymphoma, NHL = Non-Hodgkin lymphoma, MM = multiple myeloma

Table 4. Distribution of clinical and demographic features in the diagnostic groups according to mobilization regimens

Variables	HL		NHL		*p value*	MM		*p value*	Other		Total		*p value*
	G-CSF n = 7	CT n = 2	G-CSF n = 15	CT n = 13		G-CSF n = 8	CT n = 6		G-CSF n = 1	CT n = 2	G-CSF n = 31	CT n = 23	
Age (years)	32 (18-42)	40.5 (21-60)	46 (20-69)	51 (41-70)	0.363	60 (41-67)	57.5 (44-69)	0.573	30	42 (30-54)	44 (18-69)	51 (21-70)	0.111
Gender, n (%)													
Female	2 (28.6)	2 (100.0)	6 (40.0)	4 (30.8)	0.910	3 (37.5)	2 (33.3)	0.999	-	-	11 (35.5)	8 (34.8)	0.999
Male	5 (71.4)	-	9 (60.0)	9 (69.2)		5 (62.5)	4 (66.7)		1 (100.0)	2 (100.0)	20 (64.5)	15 (65.2)	
Pre-transplant RT, n(%)													
N/A	6 (85.7)	2 (100.0)	13 (86.7)	10 (76.9)	0.860	7 (87.5)	4 (66.7)	0.778	1 (100.0)	1 (50.0)	27 (87.1)	17 (73.9)	0.379
Yes	1 (14.3)	-	2 (13.3)	3 (23.1)		1 (12.5)	2 (33.3)		-	1 (50.0)	4 (12.9)	6 (26.1)	
Number of CT lines, n (%)													
1 Line	-	-	-	6 (46.2)	**0.008***	-	3 (50.0)	0.116	-	2 (100.0)	-	11 (47.8)	**< 0.001***
2 Lines	4 (57.1)	1 (50.0)	12 (80.0)	5 (38.5)		4 (50.0)	1 (16.7)		1 (100.0)	-	21 (67.7)	7 (30.4)	
3 Lines	2 (28.6)	-	2 (13.3)	2 (15.4)		4 (50.0)	2 (33.3)		-	-	8 (25.8)	4 (17.4)	
4 Lines	1 (14.3)	1 (50.0)	1 (6.7)	-		-	-		-	-	2 (6.5)	1 (4.3)	
Previous TX, n (%)													
N/A	7 (100.0)	2 (100.0)	15 (100.0)	13 (100.0)	-	5 (62.5)	5 (83.3)	0.798	1 (100.0)	2 (100.0)	28 (90.3)	22 (95.7)	0.831
Yes	-	-	-	-		3 (37.5)	1 (16.7)		-	-	3 (9.7)	1 (4.3)	

RT = radiotherapy, CT = chemotherapy, TX = transplant, HL = Hodgkin lymphoma, NHL = Non-Hodgkin lymphoma, MM = multiple myeloma

tients requiring transfusions (0% vs. 57.9%; $p < 0.001$), median days of G-CSF administered (6 vs. 9; $p = 0.011$) and median count of CD34+ cells mobilized (3.7×10^6/kg vs. 6.7×10^6/kg; $p = 0.001$) were lower on the day of plerixafor administration; while other parameters did not differ significantly between G-CSF and plerixafor group compared to the chemotherapy plus G-CSF and plerixafor group.

In NHL patients who were mobilized with G-CSF and plerixafor compared to chemotherapy plus G-CSF and plerixafor, mean Hgb level (11.3 ± 1.1 g/dL vs. 9.5 ± 1.6 g/dL; $p = 0.007$) and median platelet count (136.1×10^3/µL vs. 45 ×103/µL; $p < 0.001$) and percentage of patients having required ≥ 2 days of apheresis (100% vs. 54.6%; $p = 0.008$) were higher while the percentage of patients requiring transfusions (0% vs. 54.5%; $p = 0.012$) and median count of CD34+ cells mobilized (2.8×10^6/kg vs. 6.6×10^6/kg; $p = 0.006$) were lower on the day of plerixafor administration. On the day of plerixafor administration, other parameters did not differ significantly in the G-CSF and plerixafor group compared to chemotherapy plus G-CSF and plerixafor group in the NHL patients.

In MM patients who were mobilized with G-CSF and plerixafor compared to MM patients mobilized with chemotherapy plus G-CSF and plerixafor, mean Hgb (11.3 ± 0.9 g/dL vs. 8.9 ± 1.0 g/dL; $p = 0.008$) and median platelet count (166×10^3/µL vs. 52 × 103/µL; $p = 0.016$) were higher on the day of plerixafor administration. Other parameters did not differ significantly in the G-CSF and plerixafor group compared to the chemotherapy plus G-CSF and plerixafor group for MM patients on the day of plerixafor administration.

When G-CSF and plerixafor group and chemotherapy plus G-CSF and plerixafor group were compared for engraftment findings, the whole cohort as well as NHL and MM patients showed no significant difference.

Compared to chemotherapy plus G-CSF and plerixafor, mobilization with G-CSF and plerixafor required fewer number of days of cell collection in the whole cohort as well as in NHL and MM patients (Whole cohort = G-CSF and plerixafor: 6 days vs CT plus G-CSF and plerixafor:15 days; $p < 0.001$, NHL = G-CSF and plerixafor: 6 vs CT plus G-CSF and plerixafor: 16; $p = 0.024$, MM→ G-CSF and plerixafor: 5 vs CT plus G-CSF and plerixafor: 15; $p = 0.016$). G-

Table 5. Distribution of clinical findings in the diagnostic groups according to mobilization regimens

Variables	HL		NHL		*p*	MM		*p*	Other		Total		*p*
	G-CSF n = 7	CT n = 2	G-CSF n = 15	CT n = 13		G-CSF n = 8	CT n = 6		G-CSF n = 1	CT n = 2	G-CSF n = 31	CT n = 23	
Cell counts at day of plerixafor administration													
Hgb (gr/dL)	11.1 ± 1.7	8.5 ± 1.8	11.2 ± 1.7	9.6 ± 1.5	0.013*	11.4 ± 0.8	8.9 ± 0.9	**0.001***	13.1	8.9 ± 1.7	11.3 ± 1.5	9.3 ± 1.3	< 0.001*
PLT ($\times 10^3/\mu L$)	134 (98.7-227)	62 (60-64)	129 (22-266.8)	49 (27-141)	**< 0.001***	127.5 (93-279.5)	71.5 (40-116)	**0.026***	111 (111-111)	59.7 (55-64.3)	129.2 (22-279.5)	58.4 (27-141)	**< 0.001***
WBC ($\times 10^3/\mu L$)	22.7 (7-50.2)	35.5 (18-53)	27.9 (8-98.5)	21.3 (7-70)	0.467	26.9 (20.1-76)	18.3 (3.6-32)	0.065	36.8	22.4 (4.7-40)	27.9 (7-98.5)	21 (3.6-70)	0.088
ANC ($\times 10^3/\mu L$)	18.3(4.5-45.9)	31 (15-47)	24.8 (4-72.5)	19 (4-37)	0.387	20.7 (16.9-38.4)	13.1 (2.8-29)	0.132	31.8	14.7 (3.4-26)	23.1 (4-72.5)	19 (2.8-47)	0.110
Length of stay for mobilization, days	12 (8-16)	12 (6-18)	10 (6-29)	19 (6-27)	0.142	9 (7-23)	18.5 (16-25)	0.043*	10	38.5 (35-42)	10 (6-29)	19 (6-42)	
Events during mobilization													
Febrile neutropenia													
No	6 (100.0)	2 (100.0)	14 (93.3)	6 (46.2)	**0.019***	8 (100.0)	1 (16.7)	**0.008***	1 (100.0)	-	29 (96.7)	9 (39.1)	**< 0.001***
Yes	-	-	1 (6.7)	7 (53.8)		-	5 (83.3)		-	2 (100.0)	1 (3.3)	14(60.9)	
Transfusion requirement													
Yes	5 (83.3)	1 (50.0)	14 (93.3)	5 (38.5)	**0.007***	8 (100.0)	2 (33.3)	**0.033***	1 (100.0)	-	28 (93.3)	8 (34.8)	**< 0.001***
No	1 (16.7)	1 (50.0)	1 (6.7)	8 (61.5)		-	4 (66.7)		-	2 (100.0)	2 (6.7)	15 (65.2)	
ES, count	1 (1-1)	-	2 (2-2)	3 (1-5)	0.667	-	2 (1-3)	-	-	6 (3-9)	1.5 (1-2)	3(1-9)	0.200
Platelet, count	2 (2-2)	2 (2-2)	2 (2-2)	3 (1-9)	0.999	-	3 (1-7)	-	-	5 (2-8)	2 (2-2)	2.5 (1-9)	0.700
Days of G-CSF used	6 (5-6)	6 (4-8)	6 (5-7)	11 (4-17)	**0.017***	6 (6-7)	10 (6-15)	**0.013***	7	10 (6-14)	6 (5-7)	9 (4-17)	**0.001***
Cell count, million/kg	2.4 (0.2-6.8)	6.4 (6-6.8)	2.6 (0.5-6.2)	6.8 (2-7.9)	**0.001***	3.9 (2-7)	7.5 (0.8-12.4)	0.181	4.6	5.5 (3-8)	3 (0.2-7)	6.8 (0.8-12.4)	**< 0.001***
Autologous transplant, n (%)													
No	4(57.1)	-	5 (33.3)	2 (15.4)	0.512	4 (50.0)	1 (16.7)	0.469	-	1 (50.0)	13 (41.9)	4 (17.4)	0.077
Yes	3 (42.9)	2 (100.0)	10 (66.7)	11 (84.6)		4 (50.0)	5 (83.3)		1 (100.0)	1 (50.0)	18 (58.1)	19 (82.6)	

Hgb = hemoglobin, PLT = platelet, WBC = leukocyte, ANC = neutrophil, CT = chemotherapy, HL = Hodgkin lymphoma, NHL = Non-Hodgkin lymphoma, MM = multiple myeloma

CSF and plerixafor and chemotherapy plus G-CSF and plerixafor groups showed no significant difference for other short-term findings.

DISCUSSION

A number of literature reviews describing the role of plerixafor in HSC mobilization have been published [20].This study retrospectively compared the results of mobilization with G-CSF and plerixafor and CT plus G-CSF and plerixafor in different patient groups with a history of mobilization failure. The study aimed to evaluate the increase in the number of CD 34+ cells in peripheral blood after plerixafor administration for the two mobilization regimens, the percentage of patients made eligible for ASCT, the engraftment kinetics of ASCT and their OS results.

The study by Hübel *et al*. [21] including 60 patients reported that mobilization with plerixafor provided the minimum of 2 × 106 CD34+ cells/kg required for successful mobilization [21]. In the present study, successful mobilization with plerixafor was achieved in patients. In a total study population of 56 patients, Duarte *et al*. [22] reported that ≥ 2 × 106 CD 34 cells/kg were collected using G-CSF in 42 (75%) patients collecte. In 115 patients with reported mobilization failure, Calandra *et al*. [23] reported that mobilization with G-CSF and plerixafor achieved a successful mobilization rate of 66% in their total study population - 60.3% in NHL, 71.4% in MM and 76.5% in HL. Tricot *et al*. [24] reported a rate of successful

Table 6. Engraftment data of patients who underwent ASCT

Variables	HL n = 5	NHL n = 21	MM n = 9	Other n = 2	Total n = 54	*p value*
PLT engraftment, > 20k (days)						
Early mortality, n (%)	-	2 (9.5)	-	-	2(5.4)	0.589
Poor, n (%)	1 (20.0)	3 (14.3)	-	-	4(10.8)	
Measurable, n (%)	4(80.0)	16(76.2)	9 (100.0)	2 (100.0)	31 (83.8)	
PLT engraftment	11 (11-15)	20 (12-123)	14 (10-25)	21.5 (11-32)	15 (10-123)	**0.014***
PLT engraftment, > 50k (days)						
Early mortality, n (%)	-	2 (9.5)	-	-	2 (5.4)	0.390
Poor, n (%)	1 (20.0)	5 (23.8)	-	-	6 (16.2)	
Measurable, n (%)	4 (80.0)	14 (66.7)	9 (100.0)	2 (100.0)	29 (78.4)	
PLT engraftment	16 (13-22)	25 (16-180)	16 (12-37)	27.5 (18-37)	21 (12-180)	**0.049***
Neutrophil engraftment, > 500/ x10^3/µl (days)						
Early mortality, n (%)	-	2 (9.5)	-	-	2 (5.4)	0.999
Poor, n (%)	-	1 (4.8)	-	-	1 (2.7)	
Measurable, n (%)	5 (100.0)	18 (85.7)	9 (100.0)	2 (100.0)	34 (91.9)	
Neutrophil engraftment	**10(10-12)**	12(10-34)	13(11-19)	11(11-11)	12(10-34)	0.050*
Neutrophil engraftment, > 1000 x103/µl (days)						
Early mortality, n (%)	-	2 (9.5)	-	-	2 (5.4)	0.999
Poor, n (%)	-	1 (4.8)	-	-	1 (2.7)	
Measurable, n (%)	5 (100.0)	18 (85.7)	9 (100.0)	2 (100.0)	34 (91.9)	
Neutrophil engraftment	12 (11-14)	13 (11-60)	13 (12-52)	11.5 (11-12)	13 (11-60)	0.241

PLT = platelet, HL = Hodgkin lymphoma, NHL = Non-Hodgkin lymphoma, MM = multiple myeloma

mobilization of 85% in 20 MM patients. Micallef *et al*. [25] reported a rate of successful mobilization of 63.5% in 298 NHL patients, and the reported rate of successful mobilization in 20 patients otherwise eligible for ASCT who failed previous mobilization attempts in the study by Fowler *et al*. was 85% 26. The present study in line with previous data reported a rate of successful mobilization in NHL, MM and HL.

The aforementioned results were in line with the study of Hüber *et al*. [21], who reported time to neutrophil and platelet engraftment as 12 days and 16 days, respectively, and with the study of Calandra *et al*. [23], who reported time to neutrophil and platelet engraftment as 10 days and 16 days, respectively. In the study by Dipersio *et al*. [27], time to neutrophil and platelet engraftment in MM patients mobilized with plerixafor was 11 days and 18 days, respectively while Flomenberg *et al*. [28] reported time to median neutrophil and platelet engraftment in MM patients mobilized with plerixafor containing regimen as 10.5 and 21 days, respectively. In a single-center series with similar findings in terms of safety and efficacy, notably of 33 childeren mobilized with G-CSF plus plerixafor in Moscow that included evidence of satisfactory engraftment [29]. In the present study, time to neutrophil and platelet engraftment in MM patients was 13 days and 14 days, respectively. The different diagnostic groups showed no significant difference with regards to time to engraftment.

One randomized blind placebo-controlled phase III trial including 298 NHL patients and another study including 302 MM patients demonstrated that addition of plerixafor to G-CSF results in a significantly higher yield of CD34+ cells and fewer days of apheresis for sufficient mobilization [18, 19]. In G-CSF and plerixafor group, the percentage of patients having required ≥ 2 days of apheresis was higher, while the median days of G-CSF administered and median count of CD34+ cells mobilized were lower compared to the CT plus G-CSF and plerixafor group.

Mobilization regimens including CT are associated with risk of secondary malignancy, infertility, cardiac toxicity, cytopenia and infection, which in turn lead to increase in treatment costs30-33. In our study, the rate of febrile neutropenia, the percentage of patients requiring transfusions, median days of G-CSF administered and median CD34+ cell count were significantly higher in the CT plus G-CSF and plerixafor group compared to G-CSF and plerixafor group.

Previous studies have demonstrated correlation between successful mobilization and certain factors including peripheral leukocyte count, platelet count [34-36]. Among our patients who underwent ASCT, mean Hgb level, median platelet count and median leukocyte count on the day of plerixafor administration were higher in the G-CSF and plerixafor group compared to CT plus G-CSF and plerixafor group.

Previous studies demonstrated that intensive radiotherapy and use of lenalidomide have adverse effects on stem cell mobilization [37-40]. Although recent studies recommend the use of the immunomodulator agent lenalidomide in induction treatment for MM, use of lenalidomide for MM induction treatment was shown to compromise stem cell mobilization [40, 41]. With the "just-in-time" application of plerixafor [42], in view of a low CD34+ cell count (< 10/mμ) on the anticipated first apheresis day, an adequate numbers of CD34+ cells were mobilized and collected, able to support one or two further cycles of HDC. In our study group, the mobilization outcomes showed no statistical difference for the 10 patients with previous history of radiotherapy. Moreover, in our study group, 55.6% of the MM patients who underwent transplantation had history of lenalidomide use.

Limitations

The limitation of our study is retrospective and the number of cases examined is low, disease invetstigated were too many in this manuscript. A prospective study may be useful in this area.

CONCLUSION

In conclusion, our study demonstrated that administration of plerixafor is generally safe and well-tolerated. Regardless of the underlying disease, it offers an effective alternative for patients with previous failed mobilization attempts using conventional regimens, and allows stem cell collection with fewer apheresis sessions.

Authors' Contribution

Study Conception: SS; Study Design: SS, İYH; Supervision: FDS; Funding: N/A; Materials: HSB, SKB; Data Collection and/or Processing: YGM, TOT;

Statistical Analysis and/or Data Interpretation: ÖGS, FH; Literature Review: AİG, ÖGS; Manuscript Preparation: SS and Critical Review: MN, FDS.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

The authors would like to thank the Vice Chancellor for research, Istanbul Medipol University and Istanbul University-Istanbul Medical Faculty for supporting the research.

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The European Research Journal 2023;9(6):1380-1391 Original Article

DOI: 10.18621/eurj.1238842 *Pediatric Emergency Medicine*

Differential diagnosis for multiple systemic inflammatory syndrome in children: clinical and laboratory clues

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ABSTRACT

Objectives: We aimed to identify biochemical markers and clinical findings with high sensitivity and specificity that can be used in the differential diagnosis of patients suspected of having Multisystem Inflammatory Syndrome in Children (MISC) in the pediatric emergency department (PED). Moreover, we also examined early warning signs for predicting severe MIS-C patients requiring admission to intensive care unit (ICU).

Methods: We conducted a retrospective analysis of patients presenting to the PED with suspected MIS-C. Patient records were assessed for initial complaints, physical examination findings, laboratory and ımaging test results, diagnoses, and follow-up plans. Patients diagnosed with MIS-C were categorized as the MIS-C group, while others were categorized as the non-MIS-C group. Comparisons were made between these two groups.

Results: A total of 266 patients were included, with 68 diagnosed with COVID-19-associated MIS-C, including 20 monitored in the pediatric ICU. MIS-C patients had higher mean age, hospitalization, and ICU admission rates compared to non-MIS-C. MIS-C group showed higher prevalence of respiratory symptoms, hematological involvement, and shock. We observed lymphopenia, thrombocytopenia, hyponatremia, and elevated levels of blood C-reactive protein (CRP), procalcitonin, triglycerides, troponin, Brain Natriuretic Peptide (BNP), D-dimer, and fibrinogen in the MIS-C group. ICU patients had higher procalcitonin, aspartate aminotransferase, alanine aminotransferase, triglycerides, troponin, BNP, and ferritin levels, and lower sodium levels.

Conclusions: COVID-19-associated MIS-C group had higher rates of respiratory symptoms, hematological involvement, and shock. Lymphopenia, thrombocytopenia, elevated CRP, and D-dimer can guide MIS-C differential diagnosis. Additional tests (procalcitonin, troponin, BNP, triglycerides, ferritin) are recommended for high-suspicion cases. Patients with elevated BNP levels may require ICU admission.

Keywords: Multisystem inflammatory syndrome in children (MIS-C), COVID-19-associated multisystem inflammatory syndrome, pediatrics, SARS-CoV-2, pediatric emergency medicine

Received: ***January 22, 2023;*** *Accepted:* ***July 26, 2023;*** *Published Online:* ***August 18, 2023***

How to cite this article: *Bıcılıoğlu Y, Nalbant T, Çiçek A, Ergönül E, Gökalp G, Demir G, Bardak Ş, Berksoy E. Differential diagnosis for multiple systemic inflammatory syndrome in children: clinical and laboratory clues. Eur Res J 2023;(6):1380-1391. DOI: 10.18621/eurj.1238842*

e-ISSN: 2149-3189

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Throughout the COVID-19 pandemic declared in March 2020, children and adolescents constituted 1-2% of all cases. The vast majority of reported pediatric patients experienced asymptomatic or mild manifestations of the disease [1]. In contrast, Multisystem Inflammatory Syndrome in Children (MIS-C), which was defined during the pandemic, has resulted in severe clinical presentations requiring intensive care monitoring in children. The first case was reported in the United Kingdom in April 2020 [2]. Subsequently, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) promptly established diagnostic criteria to define the clinical syndrome. The CDC's case definition criteria are shown in Table 1 [3]. MIS-C shares many similarities with various diseases, most notably Kawasaki disease (KD), as it features fever, conjunctivitis, rash, hyperemia in the oropharynx, and cardiac involvement. Clinical and laboratory findings of MIS-C also emerge in many infectious, inflammatory, and allergic/reactive diseases apart from KD. Because the treatment of diagnosed diseases can vary significantly, it is crucial to distinguish MIS-C from other potential diseases [4].

The finding that serological tests are positive in 80-90% of patients, and polymerase chain reaction (PCR) tests are positive in 20-40%, supports the hypothesis that COVID-19 associated MIS-C is an immunological phenomenon related to hyperinflammation that develops following symptomatic or asymptomatic COVID-19 infection [5]. However, some patients exhibit positivity in both serology and PCR tests. This situation poses a confusing question: Is it an active COVID-19 infection, or is it MIS-C? In other words, the differential diagnosis of active COVID-19 infection, MIS-C, KD, and other infectious, inflammatory, and allergic/reactive diseases continues to challenge physicians when evaluating patients in the pediatric emergency department (PED). Despite the emergence of new studies defining clinical and laboratory markers for differential diagnosis between COVID-19, MIS-C, and other diseases, the optimal diagnostic criteria are yet to be determined [4, 5].

In MIS-C patients, elevations have been detected in at least four inflammatory markers (C-reactive protein (CRP), neutrophil count, ferritin, procalcitonin, fibrinogen, interleukin-6, and triglycerides) in most cases. Furthermore, studies have reported thrombocytopenia (40%) and lymphopenia (30%). Elevations in cardiac biomarkers such as troponin (64-95%), Brain Natriuretic Peptide (BNP), and pro-BNP (73-95%) are prominent in patients with cardiac involvement [6].

One of the challenges faced by pediatric emergency physicians is distinguishing MIS-C, which carries the potential for serious illness requiring hospital admission, from other diseases that may not require such admission, all the while avoiding unnecessary tests. At this stage, it's crucial to identify distinctive complaints, physical examination findings, and laboratory parameters [7,8,9]. Our study aims to determine clinical features and laboratory parameters with high sensitivity and specificity that could be used in diagnosing COVID-19 associated MIS-C. Moreover, we also examined early warning signs for predicting severe MIS-C patients requiring admission to intensive care.

METHODS

The population of the study consisted of 266 patients who presented to the PED with a fever between May 2020 and February 2022 and were investigated for suspected MIS-C. Throughout the pandemic, our hospital's PED used the CDC's case definition [3] to diagnose COVID-19 related MIS-C, and a differential diagnostic approach was applied as described below to diagnose children presenting with fever. Patients with a persistent fever of 38° C or higher for over 24 hours, for whom the cause of fever could not be determined through history, physical examination, or initial laboratory tests (complete blood count, CRP, blood glucose, urea, creatinine, uric acid, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH), sodium, potassium, calcium, chloride, albumin, amylase, lipase, and full urine examination), and who had high inflammatory markers were investigated for COVID-19 infection. SARS-COV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) IGM/IGG and COVID PCR tests were performed on patients with active COVID-19 symptoms or a history of COVID-19 infection, or those who had contact with a person infected with COVID-19 in the past four weeks. Additionally, tests were conducted for procalcitonin, sedimentation, triglycerides, ferritin, troponin, BNP, Prothrombin Time (PT), Activated Par-

tial Thromboplastin Time (APTT), International Normalized Ratio (INR), and D-Dimer. An Electrocardiogram (EKG) was performed. Cardiology consultation was requested, and an Echocardiogram (ECHO) was performed on patients suspected of having cardiac involvement. Lung radiographs were taken from patients with respiratory symptoms. Following all these results, a diagnosis of MIS-C was made or ruled out considering the CDC case definition. Patients were followed up on an outpatient basis, or admitted to the ward or intensive care, depending on their clinical condition. As our study was planned as a retrospective, descriptive type, a list of patients requested for SARS-COV-2 IgM/IgG from the PED was obtained from the hospital information automation system to access patient information. The digital records of patients in the were reviewed. Presentation complaints, physical examination, laboratory results, lung radiograph, EKG, ECHO findings, diagnosis, and follow-u plans were recorded in the case report form. Patients diagnosed with MIS-C were categorized as the MIS-C group, while others were categorized as the non-MIS-C group. Comparisons were made between these two groups.

Ethical approval was received from the committee of Izmir Katip Çelebi University Non-Interventional Research Ethics Committee (approval Number/ID: 24.02.2022/0041).

Statistical Analysis

Statistical Analysis: Continuous variables such as age and laboratory results were characterized using mean, standard deviation, IQR and median values. The Mann-Whitney U test was utilized to examine the correlation between laboratory results and MIS-C. Conformity to the normal distribution was evaluated with the Shapiro-Wilk t-test. The Pearson Chi-square test was used to analyze the correlation between demographic data like gender, presenting complaints, and MIS-C. Subsequently, ROC curves were established to evaluate the prediction of MIS-C diagnosis and the need for intensive care admission based on the patient's laboratory results. Analyses were conducted on IBM SPSS Statistics version 29 (IBM Corporation, Armonk, NY), with a confidence interval of 95% and a 5% margin of error.

RESULTS

A total of 266 patients were included in the study. Out of the patients undergoing investigations for the cause of fever, 68 (34.3%) were diagnosed with COVID-19 associated MIS-C. The distribution of diagnoses among 198 patients who did not receive a MIS-C diagnosis is presented in Fig. 1.

No significant differences were observed between

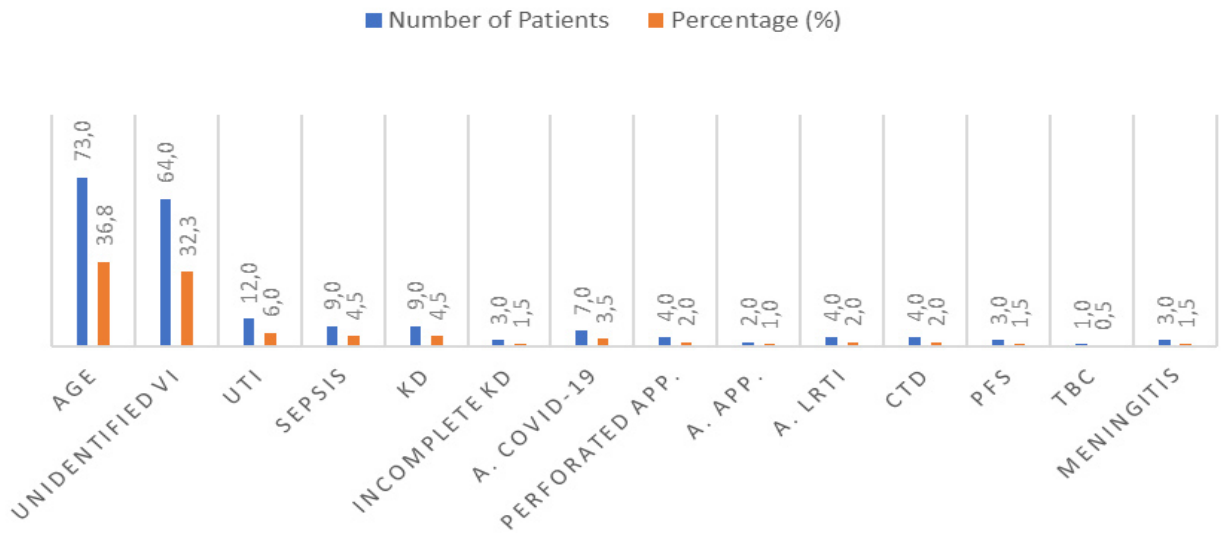

Fig. 1. The distribution of diagnoses among 198 patients in non-MIS-C group. AGE = Acute Gastroenteritis, Unidentified VI = Unidentified viral infection, UTI = Urinary tract infection, KD = Kawasaki disease, A. COVID-19 infection = Acute COVID-19 infection, Perforated App. = Perforated appendicitis, A. App. = Acute appendicitis, A. LRTI = Acute lower respiratory tract infection, CTD = Collagen tissue disease, PFS = Periodic fever syndrome, Tbc = Tuberculosis

Table 1. CDC case definition of multisystem inflammatory syndrome in children

	Criteria
1	**Age under 21 years**
2	**The patient must satisfy all of the following clinical criteria:**
	- Persistent fever of ≥ 38°C for ≥ 24 hours
	- Elevated levels of CRP, ESR, fibrinogen, procalcitonin, D-dimer, ferritin, LDH, IL-6, and presence of neutrophilia, lymphocytopenia, hypoalbuminemia
	- Multisystem involvement (2 or more organ systems) including:
	• Cardiovascular (e.g., shock, elevated troponin, elevated BNP)
	• Respiratory (e.g., pneumonia, ARDS, pulmonary embolism)
	• Renal (e.g., AKI, kidney failure)
	• Neurologic (e.g., seizure, stroke, aseptic meningitis)
	• Hematologic (e.g., coagulopathy)
	• Gastrointestinal (e.g., abdominal pain, vomiting, diarrhea, elevated liver enzymes, ileus, gastrointestinal bleeding)
	• Dermatologic (e.g., erythroderma, mucositis, other rash)
	- Severe illness requiring hospitalization
3	**No alternative plausible diagnoses**
4	**Recent or current exposure to SARS-CoV-2, indicated by any of the following:**
	- Positive SARS-CoV-2 RT-PCR test
	- Positive serology test
	- Positive antigen test
	- Exposure to COVID-19 within the 4 weeks prior to the onset of symptoms

CDC = Centers for Disease Control and Prevention, CRP = C-Reactive Protein, ESR = Erythrocyte Sedimentation Rate, LDH = Lactate Dehydrogenase, IL-6 = Interleukin 6, BNP = Brain Natriuretic Peptide, ARDS = Acute Respiratory Distress Syndrome, AKI = Acute Kidney Injury, SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2, RT-PCR = Real-Time Polymerase Chain Reaction, COVID-19 = Coronavirus Disease 2019, PT = Prothrombin Time, PTT = Partial Thromboplastin Time.

the MIS-C and non-MIS-C patients in terms of gender and nationality. However, the mean age of the MIS-C group was significantly higher compared to that of the non-MIS-C group (Mann-Whitney U test, $p = 0.02$). Among all patients, the most common presenting symptoms were diarrhea, vomiting, and abdominal pain, indicative of gastrointestinal involvement. No significant difference in these presenting symptoms was observed between the two groups (Chi-square test, $p > 0.05$). Similarly, no significant difference was noted in the duration of fever between the two groups. However, hematological involvement and hypotension were significantly more common in the MIS-C group (Mann-Whitney U test, $p < 0.001$) (Table 2).

SARS-CoV-2 IgG results were positive in 44 patients (64.7%) in the MIS-C group, and both SARS-CoV-2 IgG and IgM values were negative in 6 (8.8%) patients. Eighteen patients (26.4%) had positive results for both IgG and IgM. PCR tests of these 18 patients were negative. These results were evaluated together with clinical findings and other laboratory tests, and none of them were diagnosed with acute COVID-19 infection (Table 3).

Among the patients in the non-MIS-C group, 157 (79.2%) had negative results for both COVID-19 IgM and IgG, 28 patients (14%) had positive COVID-19 IgG results, and 13 patients (6.5%) showed positive results for both IgG and IgM. Out of these 13 patients with positive serology (IgG and IgM), 3 were confirmed to have acute COVID-19 infection through

Table 2. Comparison of demographic and clinical characteristics, and hospitalization rates between with MIS-C and Non-MIS-C group

	Total (n = 266)	MIS-C (n = 68)	Non-MIS-C (n = 198)	*p* value
Age (months), mean ± SD	88 ± 60.4	102.6 ± 53	83 ± 62	**0.02**[a]
Gender (M/F)	122/144	25/43	97/101	0.08[a]
Nationality (TC/Syria)	241/25	63/5	178/20	0.50 [a]
Diarrhea, n (%)	120 (45)	28 (41)	92 (46)	0.45[b]
Vomiting, n (%)	114 (42)	33 (48.5)	81 (40.9)	0.27[b]
Cough, n (%)	36 (13.5)	10 (14.7)	26 (13)	0.74[b]
Dermatological involvement, n (%)	60 (13.5)	20 (29.4)	40 (20)	0.11[b]
Mucous membrane involvement, n (%)	33 (12.4)	11 (16)	22 (11)	0.27[b]
Gastrointestinal system involvement, n (%)	184 (69)	50 (73.5)	134 (67.6)	0.36[b]
Respiratory system findings, n (%)	33 (12.4)	15 (22)	18 (9)	0.05[b]
Neurological system involvement, n (%)	13 (4.8)	4 (5.8)	9 (4.5)	0.43[b]
Cardiac involvement, n (%)	12 (4.5)	6 (8.8)	6 (3)	0.056[b]
Presence of hypotension, n (%)	31 (11.6)	19 (27.9)	12 (6)	**< 0.0001**[b]
Hematological system involvement, n (%)	221 (83)	20 (29.4)	6 (3)	**< 0.0001**[b]
Outpatient follow-up, n (%)	76 (28.5)	0	76 (38)	**< 0.0001**[b]
Admitted to the ward, n (%)	164 (61.6)	48 (70.5)	116 (58.5)	**< 0.0001**[b]
Admitted ICU, n (%)	26 (9.7)	20 (29.4)	6 (3)	**< 0.0001**[b]

[a]Mann-Whitney U Test, [b]Chi-Square Test

positive PCR testing (Table 3). Diagnoses for the remaining 10 patients were as follows: 5 patients with unidentified viral infections, 1 patient with tuberculosis, 1 patient with a collagen tissue disease, and 3 patients with gastroenteritis. None of the serologically tested patients had been vaccinated against COVID-19. Serological positivity was significantly higher in the group diagnosed with MIS-C compared to the group non - MIS-C (Chi-square test, $p < 0.001$).

A total of 7 patients were diagnosed with acute COVID-19. Among these, 3 patients had both serological and PCR positivity, whereas the remaining 4 patients had negative serology but tested positive on PCR.

In patients diagnosed with MIS-C, Absolute lymphocyte Count (ALC), platelet, and sodium levels were significantly lower, while triglyceride, troponin, BNP, PT, D-Dimer, and fibrinogen levels were significantly higher compared to those compared to the group non-MIS-C (Mann Whitney-U test, $p < 0.05$) (Table 4).

ROC curves were constructed for ALC, platelet count, CRP, procalcitonin, triglyceride, troponin, BNP, ferritin, PT, D-Dimer, fibrinogen, and sodium values,

Table 3. Comparison of serology test result in MIS-C and Non-MIS-C patients

Group	COVID-19 IgG (+)	COVID-19 IgM and IgG (+)	COVID-19 IgM and IgG (-)	Total patients
MIS-C	44 (64.7%)	18 (26.4%)	6 (8.8%)	68
Non-MIS-C	28 (14%)	13 (6.5%)	157 (79.2%)	198

Table 4. Comparison of laboratory parameters of patients diagnosed with MIS-C and Non-MISC group.

Laboratory Markers	Normal range	MISC (n = 68) Median (IQR)	Non-MISC (n = 198) Median (IQR)	*p* value*
WBC (×10³/uL)	4.2-10.6	10.0 (8.9) (n = 68)	11.2 (8.0) (n = 198)	0.40
ANS (×10³/uL)	2-6.9	8200 (6300) (n = 68)	7150 (7400) (n = 198)	0.13
ALC (×10³/uL)	0.6-3.4	1000 (1200) (n = 687	1800 (2350) (n = 197)	**< 0.0001**
Hemoglobin (gr/dL)	12.2-16.2	11.7 (1.8) (n = 68)	11.8 (2.2) (n = 198)	0.95
Platelets (×10³/uL)	140-400	225 (175) (n = 68)	278 (130) (n = 198)	**0.01**
CRP (mg/dL)	0-5	127 (112) (n = 68)	70.6 (104) (n = 198)	**< 0.01**
Procalcitonin (ng/mL)	0-0.1	1.28 (4.74) (n = 68)	0.39 (2.3) (n = 189)	**0.002**
ESR (mm/h)	0-20	40.5 (32.5) (n = 12)	62.5 (74.5) (n = 40)	0.192
AST (u/L)	0-35	30 (24) (n = 68)	28 (16) (n = 197)	0.40
ALT (u/L)	0-35	19 (26.5) (n = 68)	16 (11) (n = 197)	0.14
Triglycerides (mg/dL)	32-158	102 (72.7) (n = 60)	86.5 (76) (n = 130)	**0.032**
Urea (mg/dL)	10-38	25.5 (14) (n = 68)	22 (11) (n = 198)	0.48
Creatinine (mg/dL)	0.5-1.2	0.6 (0.3) (n = 68)	0.5 (0.3) (n = 198)	0.39
Troponin (ng/mL)	0.0-0.06	3.7 (33) (n = 66)	2.5 (0.0) (n = 169)	**< 0.0001**
BNP (ng/L)	2-100	180 (325) (n = 31)	18 (22.3) (n = 23)	**< 0.0001**
Ferritin (µ/L)	6-320	175 (198) (n = 58)	112 (130) (n = 157)	**0.001**
PT (sec)	10.8-15	13.6 (2.2) (n = 68)	13 (1.85) (n = 189)	**0.011**
APTT (sec)	21-36	25.3 (4.0) (n = 68)	25 (5.0) (n = 189)	0.51
INR	0.8-1.2	1.1 (0.17) (n = 65)	1.08 (0.13) (n = 192)	0.16
D-Dimer (ng/mL)	0-440	2050 (2288) (n = 66)	1240 (1990) (n = 183)	**0.005**
Fibrinogen (mg/dL)	170-420	466 (257) (n = 68)	397 (193) (n = 160)	**0.004**
Sodium (mmol/L)	35-148	133 (5) (n = 68)	135 (4) (n = 198)	**< 0.0001**
Albumin (gr/dL)	3.5-5.2	30.5 (35.5) (n = 68)	32 (35.9) (n = 195)	0.192
LDH (u/L)	110-295	294 (131) (n = 68)	292 (96) (n = 196)	0.51

WBC = White blood cell, ANC = Absolute neutrophil count, ALC = Absolute lymphocyte count, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, BNP = brain natriuretic peptide, PT = prothrombin time, PTT = partial prothrombin time, INR = International Normalized Ratio, LDH = lactate dehydrogenase
*Mann-Whitney U test.

which were significant according to the Mann Whitney U analysis. Upon examining the laboratory values with ROC curves, it was observed that the calculated AUC values for five laboratory values were statistically significant. These include Procalcitonin (AUC 0.719, $p = 0.022$), Triglyceride (AUC 0.690, $p = 0.047$), Troponin (AUC 0.698, $p = 0.039$), BNP (AUC 0.853, $p < 0.0001$), and Sodium (AUC 0.792, $p = 0.002$). However, upon examination of these laboratory parameters using a Logistic Regression model, they were not found to be significant in predicting the diagnosis of MIS-C.

Out of the 68 patients diagnosed with MIS-C, 42 (61.7%) had normal ECHO results obtained in the PED. Six patients (8.8%) were found to have heart failure, three patients (4.4%) had pericardial effusion, three patients (4.4%) exhibited mitral valve insufficiency, five patients (7.3%) had myocarditis and coronary dilation, and two patients (2.9%) showed aortic valve insufficiency. Seven patients did not receive an ECHO in the PED. All patients diagnosed with heart failure, one patient with pericardial effusion, two patients with concurrent myocarditis and coronary dilation, and one patient with mitral valve insufficiency were admitted to the pediatric ICU. All these patients had BNP values measured above 240 ng/L.

Sixty eight patients diagnosed with MIS-C were compared with 12 patients diagnosed with KD. The average age of patients diagnosed with KD was 46.3 months, whereas it was 102.6 months for those diagnosed with MIS-C, and this difference was significant (Mann-Whitney U test, $p = 0.001$). When compared in terms of gender, a significant difference was detected. All of those diagnosed with KD were male (Fisher's Exact Test, $p = 0.014$). When the physical examination findings at the first presentation were compared, tachycardia was significantly more common in the MIS-C group (Fisher's Exact Test, $p = 0.03$). Although hypotension was more frequently observed in the MIS-C group in the comparison made in terms of the presence of hypotension, the p-value was determined as 0.06. When comparing laboratory parameters; differences were found between the two groups in terms of averages for ALC, platelet count, troponin, and BNP (Mann Whitney-u test). In Kawasaki patients, ALC and platelet counts were higher than in MIS-C patients. In the MIS-C group, BNP and troponin were found to be significantly higher than in Kawasaki patients ($p = 0.014$, $p = 0.003$, $p = 0.006$, and $p = 0.014$; respectively).

All patients diagnosed with COVID-19 associated MIS-C were hospitalized. Of these patients, 20 (29.4%) were monitored in the pediatric ICU. The rates of hospitalization and intensive care need were significantly higher in patients diagnosed with MIS-C compared to those diagnosed with non-MIS-C (Chi-square test, $p < 0.0001$).

The average age of patients monitored in the ICU with a diagnosis of MIS-C was significantly higher compared to others (Mann Whitney U test, $p = 0.008$). When comparing laboratory parameters, procalcitonin, AST, ALT, triglyceride, troponin, BNP, and ferritin values were significantly higher, while sodium values were significantly lower in patients requiring intensive care monitoring (Table 5). Upon generating ROC curves, the calculated AUC values for these eight laboratory values were found to be statistically significant: Procalcitonin (AUC 0.668, $p = 0.03$), Triglycerides (AUC 0.772, $p = 0.001$), Troponin (AUC 0.709, $p = 0.07$), BNP (AUC 0.728, $p = 0.035$), Sodium (AUC 0.658, $p = 0.041$), Ferritin (AUC 0.776, $p = 0.003$), AST (AUC 0.660, $p = 0.039$), and ALT (AUC 0.694, $p = 0.012$). However, these laboratory parameters were not found to be significant in predicting intensive care admission in the logistic regression model.

DISCUSSION

Children observed for COVID-19 associated MIS-C are predominantly previously healthy individuals who have experienced a mild or asymptomatic course of COVID-19 infection. Patients most frequently present with gastrointestinal symptoms (abdominal pain, vomiting, diarrhea) and mucocutaneous inflammation signs (rashes, conjunctivitis, oromucosal changes). Upon examination, lymphopenia and elevated inflammatory markers are typically identified. However, a subset of patients develops severe illness manifestations, including hypotension/shock and cardiac involvement such as myocarditis, myocardial dysfunction, and coronary artery changes [4].

The symptoms, physical examination findings, and test results observed in MIS-C share common characteristics with many other diseases, making it

Table 5. Comparison of gender, age, and laboratory parameters of MIS-C patients admitted to the ICU and those admitted to the ward.

Parameter / Marker	Normal range	Ward admission (n = 48)	ICU admission (n = 20)	*p* value
Gender (F/M)		17/31	8/12	0.721
Age (months)		91	130	**0.008**
Fever value (°C)		38.4	38.7	0.252
WBC (×10³/uL)	4.2-10.6	11.915	11.240	0.957
ANC (×10³/uL)	2-6.9	9375	9335	0.549
ALC (×10³/uL)	0.6-3.4	1555	1055	0.178
Hemoglobin (g/dL)	12.2-16.2	11.7	12	0.835
Platelets (×10³/uL)	140-400	255	230	0.364
CRP (mg/dL)	0-5	128	146	0.270
Procalcitonin (ng/ml)	0-0.1	3.5	23.7	**0.030**
ESR (mm/h)	0-20	44.5	41	0.578
AST (u/L)	0-35	35	69	**0.039**
ALT (u/L)	0-35	29	58	**0.012**
Triglycerides (mg/dL)	32-158	107	198	**0.001**
Urea (mg/dL)	10-38	23	37	0.328
Creatinine (mg/dL)	0.5-1.2	0.59	1.0	0.182
Troponin (ng/ml)	0.0-0.06	135	3863	**0.006**
BNP (ng/L)	2-100	179	504	**0.035**
Ferritin (µ/L)	6-320	177	316	**0.003**
PT (s)	10.8-15	13.8	13	0.185
APTT (s)	21-36	25.7	25	0.479
INR	0.8-1.2	1.1	1.1	0.070
D-DIMER (ng/ml)	0-440	2940	3608	0.078
Fibrinogen (mg/dL)	170-420	477	499	0.505
Sodium (mmol/L)	35-148	133	130	0.040
Albumin (g/dL)	3.5-5.2	2.2	2.1	0.716
LDH (u/L)	110-295	290	366	0.060

WBC = White blood cell, ANS = Absolute neutrophil count, ALC = Absolute lymphocyte count, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, BNP = brain natriuretic peptide, PT = prothrombin time, PTT = partial prothrombin time, INR = International Normalized Ratio, LDH = lactate dehydrogenase

challenging to make a differential diagnosis in the PED. In the study aimed at investigating biochemical markers with high specificity and sensitivity to differentiate COVID-19 associated MIS-C from other diseases and reduce the confusion among physicians working in the PED, a total of 266 patients were evaluated. 68 of these patients were diagnosed with COVID-19 associated MIS-C, and 20 of them were

monitored in the pediatric ICU. The hospitalization and ICU admission rates of patients diagnosed with MIS-C were significantly higher compared to others.

In MIS-C patients, we detected a positivity rate of 64.7% (44 patients) for COVID-19 IgG, and 26.4% (18 patients) tested positive for both IgG and IgM. All of these patients had negative PCR results. Neutralizing antibody responses have been found in COVID-19 patients, but the relationship between SARS-CoV-2 antibody levels and disease severity is still debated. SARS-CoV-2 spike protein IgM and IgG levels are higher in severe and recovered COVID-19 patients, proportional to the time since symptom onset, reflecting a robust SARS-CoV-2-specific humoral response. SARS-CoV-2 IgG and IgM antibodies have been found at lower levels in asymptomatic SARS-CoV-2-positive individuals compared to COVID-19 patients. However, the exact timing of SARS-CoV-2 exposure in relation to MIS-C development and laboratory findings remains unclear [4]. Feldstein *et al*. [9] reported that 70% of patients had a positive RT-PCR (Real time polymerase Chain Reaction) or antibody test, with 30% being exposed to a COVID-19-positive individual in the past 4 weeks. In another study involving 52 MIS-C patients, 56% (28/50) had a positive PCR result for SARS-CoV-2 (acute infection), and 44% (22/50) tested positive for SARS-CoV-2 antibodies (post-acute) [1]. Considering these findings, although SARS-CoV-2 antibody testing is not routinely practiced in many centers, we use serological tests routinely in our center when diagnosing MIS-C to demonstrate its association with COVID-19, and we believe it can be a valuable aid in differential diagnosis.

While no significant differences were observed between the two groups in terms of gender, the mean age of the MIS-C group (102.6 months) was significantly higher. Additionally, the mean age of those in the ICU (130 months) was significantly higher compared to those admitted to the general ward for MIS-C. Our findings were consistent with the United Kingdom (UK) cohort [1] (median age 10.7 (8.3-14.1) vs. 1.6 (0.2-12.9); $p < 0.001$), Vogel *et al*. [4] (median age: 8.5 years), and Yasuhara *et al*. [10] systematic review (median age: 9.3). We believe that greater attention should be given to age criteria in the diagnosis of COVID-19 associated MIS-C. Increasing age should be considered in the clinical decision-making process when screening for MIS-C cases.

There were no significant differences in presenting complaints between the two groups; however, gastrointestinal symptoms were most commonly observed in the MIS-C group, followed by mucocutaneous involvement symptoms. Respiratory symptoms, hematological involvement (lymphopenia, thrombocytopenia, elevated D-dimer), and signs of shock (hypotensive shock) were significantly more frequent in the MIS-C group. We found hypotension in 27.9% of MIS-C patients at their initial presentation. While Roberts *et al*. [11] reported a low number of children presenting with hypotension as their initial vital sign, they identified hypotension within the first 12 hours as an indicator for MIS-C diagnosis.

While gastrointestinal symptoms tend to be predominant in the presentation of MIS-C, they are rare in traditional KD [4]. A systematic review by Panigrahy *et al*. [12], which included 875 MIS-C patients, concluded that gastrointestinal symptoms, including abdominal pain (52.8%), vomiting (44.8%), diarrhea (39.5%), and mucocutaneous symptoms (44.4%), were common. Respiratory distress (20.9%) and neurological involvement (17.5%) were less frequent. In an observational cohort study conducted in the UK, children with MIS-C had a higher rate of presenting with fatigue (51% vs. 28%); $p = 0.004$). Additionally, they had higher rates of headache (34% vs. 10%; $p < 0.001$), myalgia (34% vs. 8%; $p < 0.001$), sore throat (30% vs. 12%; $p = 0.003$), and lymphadenopathy (20% vs. 3%; $p < 0.001$) [1]. In our study, we also found that headache was the most common complaint after gastrointestinal symptoms and rash. Our observations regarding the clinical presentation of MIS-C were very similar to previously reported cases in the literature. Thus, notable differences distinguishing MIS-C from KD include older age, predominantly gastrointestinal symptoms, and a higher prevalence of shock and myocardial injury at presentation [13]. We believe that these differences, along with other factors, can be highly informative in the differential diagnosis between MIS-C and KD. However, it should be noted that in addition to the presence of fever, gastrointestinal symptoms such as vomiting, abdominal pain, and/or diarrhea, along with conjunctivitis and rashes, and headache, are not specific and can also occur in oncological diseases, non-infectious inflammatory conditions, or other infectious diseases [14]. In our

study group as well, patients other than those with MIS-C were most commonly diagnosed with invasive gastroenteritis and undetermined viral infections.

Consistent with findings reported in several MIS-C cohort studies [1, 7, 11, 15-19], we observed lymphopenia, thrombocytopenia, hyponatremia, and elevated levels of blood CRP, procalcitonin, triglycerides, troponin, BNP, D-dimer, and fibrinogen in the COVID-19 associated MIS-C group. Five laboratory (Procalcitonin, Triglyceride, Troponin, BNP, Sodium) values showed statistically significant AUC values. Although BNP had the highest significance (AUC 0.853, $p < 0.0001$), logistic regression analysis revealed that it was not significant in predicting MIS-C diagnosis. These important findings can aid in distinguishing this syndrome from other diseases, especially KD, where platelet counts typically increase. While platelet count is a discriminating feature, it was only mildly decreased in MIS-C patients. In the presence of systemic inflammation, thrombocytosis is typically observed. If mild thrombocytopenia is detected in the presence of systemic inflammation markers, and there are compatible clinical findings, MIS-C should be considered. [11]. MIS-C patients often exhibit elevated D-dimer levels (90%) [6]. Additionally, although high D-dimer levels have been associated with treatment-resistant KD, their significance in cardiac involvement in MIS-C remains uncertain [13]. Kline *et al*. [8] found that CRP > 4.5 mg/dL and ALC < 1.5 K/μL had 86% sensitivity and 91% specificity for identifying MIS-C cases. In our study, we found significant elevations in CRP, procalcitonin, triglycerides, troponin, BNP, and decreases in lymphocyte, platelet, and sodium levels, but we were unable to determine specific cutoff values.

Procalcitonin is often used as an indicator of bacterial infection and can also be elevated in severe viral infections. Elevated procalcitonin levels have also been reported in MIS-C [11]. In our study, procalcitonin levels were significantly higher in the MIS-C group compared to the non-MIS-C group. Furthermore, patients with MIS-C who were admitted to the ICU had significantly higher procalcitonin levels.

In MIS-C, elevated troponin and BNP-proBNP levels indicate cardiac involvement. Troponin and BNP are closely associated with disease activity [4]. Minocha *et al*. [20] demonstrated abnormality in at least one cardiac test in the majority of patients (78%). BNP levels have been suggested as early warning indicators for the need for inotropic/vasoactive treatment in MIS-C. Echocardiography in patients with elevated BNP levels may be useful in promptly identifying patients with cardiac dysfunction and adjusting treatment or considering alternative diagnoses [6]. We recommend using the BNP test as a screening tool in children with prolonged and unexplained fever to identify early cardiac involvement in MIS-C.

Hyponatremia, observed in a significant portion of MIS-C cases during admission, can occur secondary to dehydration, renal or cardiac failure. While hyponatremia has been identified as a predictor of poor prognosis in both COVID-19 and KD, its prognostic role in MIS-C remains uncertain [13]. In our study, we also found significantly lower sodium levels in both MIS-C patients and those requiring ICU admission. When comparing the laboratory parameters of patients admitted to the general ward and those admitted to the ICU within the MIS-C group, we observed higher procalcitonin, AST, ALT, triglyceride, troponin, BNP, and ferritin levels, as well as lower sodium levels, in the ICU group. These results were consistent with other studies in the literature [1, 10, 13, 20, 21]. However, we were unable to establish significance in predicting the need for ICU admission.

Limitations

Our study has several limitations. Firstly, it was designed retrospectively, so some laboratory tests were not performed on all patients. Specifically, due to the nighttime conditions in our PED, sedimentation rate tests were conducted on a limited number of patients, and we did not find significant elevations as observed in other studies. Additionally, although we screened patients who underwent SARS-CoV-2 IgM/IgG testing to identify those with a preliminary diagnosis of MIS-C, we may not have reached all patients through this method. Furthermore, during the screening period, there is a possibility that unnecessary tests were performed in order to avoid missing a diagnosis of MIS-C.

CONCLUSION

Children diagnosed with COVID-19-associated MIS-C were older, and gastrointestinal and mucocutaneous

involvement symptoms were predominant during presentation. Respiratory symptoms, hematological involvement (lymphopenia, thrombocytopenia, elevated D-dimer), and signs of shock were significantly more common in the MIS-C group. In the differential diagnosis of MIS-C, initial screening tests such as lymphopenia, thrombocytopenia, elevated CRP, and elevated D-dimer can be guiding factors. However, in patients with a high suspicion of MIS-C, additional tests including procalcitonin, troponin, BNP, triglycerides, and ferritin should be performed. It is important to keep in mind that patients with elevated BNP levels may require ICU admission.

Authors' Contribution

Study Conception: YB; Study Design: YB; Supervision: GG; Funding: N/A; Materials: GG; Data Collection and/or Processing: TN, AÇ; Statistical Analysis and/or Data Interpretation: EE, YB; Literature Review: ŞB, GD; Manuscript Preparation: YB and Critical Review: EB, YB.

Conflict of interest

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

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

This study was partly presented as oral presantation at 18th National Pediatric Emergency Medicine and Intensive Care Congress, November 2-5, 2022, Antalya, Turkey.

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The European Research Journal 2023;9(6):1392-1397 Original Article

DOI: 10.18621/eurj.1261344 *Pediatrics*

Evaluation of pediatric patients installed due to acute gastroenteritis

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ABSTRACT

Objectives: Acute gastroenteritis is one of the most prevalent causes of death and morbidity in children and a significant health issue in Turkey, as well as developed and developing nations. The purpose of this study is to analyze retrospectively the patients with acute gastroenteritis who sought treatment at the Kızıltepe State Hospital in Mardin.

Methods: This study was designed as a single-center retrospective study in which demographic variables were evaluated by taking fresh stool samples from the patients who applied to the Mardin Province Kızıltepe State Hospital between 01/11/2020 and 31/10/2021 with the complaint of diarrhea. The information of rotavirus, enteric adenovirus and amoeba viruses in stool samples was investigated by qualitative immunochromatographic test. The researcher assessed the research data utilizing the hospital's file archive materials and three factors set by the researcher. The researcher extracted the information about these factors from the patient files and stored it in a Microsoft Office Excel file.

Results: According to the gender variable of the research participants, Amoeba positive rates were considerably higher in males than in girls (35.8% versus 22.6%, $p = 0.046$, respectively). According to the age groups variable, rotavirus antigen positive was statistically significantly greater in the 5-24 month age group compared to other age groups ($p = 0.034$). Similarly, Amoeba positive was statistically substantially higher in the 5-24 month age group compared to other age groups ($p = 0.001$). There was no significant variation between age groups in the distribution of adenovirus. According to the seasonal variable, rotavirus antigen positivity was most prevalent in the spring, and the difference between the spring and other seasons was statistically significant ($p = 0.001$), whereas amoeba positivity was most prevalent in the summer, with no statistically significant difference between the seasons ($p = 0.003$). The frequency of undiscovered variables was greater during the spring-summer months, and a statistically significant difference ($p = 0.041$) was identified between the groups. The seasonal variation in the prevalence of Adenovirus antigen positive was not statistically significant ($p = 0.394$).

Conclusions: As a result, in poor and underdeveloped nations, it is among the top five causes of death in children under the age of five, in relation to acute gastroenteritis. Furthermore, the majority of these deaths may be minimized by preventative and prevention strategies. It is the responsibility of governments, health professionals, and families to foster a safe and healthy environment for all infants and children during their infancy

Received: ***March 7, 2023;*** *Accepted:* ***April 25, 2023;*** *Published Online:* ***May 5, 2023***

How to cite this article: *Orhan Ö, Bulut M. Evaluation of pediatric patients installed due to acute gastroenteritis. Eur Res J 2023;9(6):1392-1397. DOI: 10.18621/eurj.1261344*

e-ISSN: 2149-3189

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and development. Community-based health strategies should be undertaken to lower the morbidity and death rates of millions of people worldwide who are exposed to harmful environmental conditions and malnutrition.
Keywords: Acute gastroenteritis, kid, adenovirus, rotavirus, amoeba

Acute gastroenteritis ranks third among infectious causes of mortality worldwide. Acute gastroenteritis is one of the primary causes of baby and child morbidity and death, particularly in underdeveloped nations [1-3]. More than 80 percent of the five million under-five deaths in 2020 occurred in African and South Asian countries. The main causes of these deaths are; infectious diseases, including pneumonia, diarrhea, and malaria, complications of preterm birth, birth asphyxia, and trauma and congenital anomalies. [1, 4, 5]. In Turkey, gastroenteritis is the fourth leading cause of mortality among children aged 1 to 5 years old. Deaths from gastroenteritis are avoidable or curable if all nations have access to cost-effective health and sanitation programs. In order to reduce diarrhea-related fatalities, it is crucial to determine the underlying causes and environmental variables and to exhibit preventative measures.

Depending on its length, gastroenteritis is classed as acute, persistent, or chronic. Acute gastroenteritis is typically characterized by three or more loose, watery stools within 24 hours. Although acute gastroenteritis lasts shorter than seven days, it does not last more than fourteen days [1, 2, 6, 7]. In particular, the fact that the stool consistency is more dense than the previous consistency, rather than the decrease in the number of stools, is an important indicator of recovery from acute gastroenteritis. Although chronic gastroenteritis is defined as diarrhea lasting longer than 30 days, it is frequently seen in diseases such as metabolic diseases and malabsorption.

In the etiology of gastroenteritis, viruses, bacteria, and enteroparasites play a significant role. The incidence of acute gastroenteritis and the frequency of bacteria, viruses, and parasites in the etiology are directly connected to the patient's region's geographical and socioeconomic characteristics [8]. While enteropathogens can be transmitted by person-to-person contact, other infections, such as cholera, are often caused by food and water pollution [1, 8-11]. In the etiology of gastroenteritis, there are variations in the frequency of the pathogenic bacteria based on geography, age, and seasonality. Rotavirus is the leading cause of AGE in children across the world [1, 8, 10].

Although norovirus is the second most prevalent cause of acute gastroenteritis overall, it has been recognized as the leading cause in several studies and is predicted to rank first globally following rotavirus vaccination programs [12, 13]. Inflammatory gastroenteritis is the gastroenteritis that develops as a result of inflammation caused by the invasion of the intestinal wall and increasing intestinal motility and secretion of metabolites. Inflammatory gastroenteritis may also contain blood, mucus, and leukocytes in the stool due to inflammation. In this type of gastroenteritis, diarrhea caused by bacteria and amoeba can be given as an example. [1, 9, 10]. The aim of this study is to retrospectively examine pediatric patients admitted to Mardin Province Kızıltepe State Hospital with the diagnosis of acute gastroenteritis.

METHODS

This study was designed as a single-center retrospective study in which demographic variables were evaluated by taking fresh stool samples from the patients who applied to the Mardin Province Kızıltepe State Hospital between 01/11/2020 and 31/10/2021 with the complaint of diarrhea. The information of rotavirus, enteric adenovirus and amoeba viruses in stool samples was investigated by qualitative immunochromatographic test. The researcher assessed the research data utilizing the hospital's file archive materials and three factors set by the researcher. The researcher extracted the information about these factors from the patient files and stored it in a Microsoft Office Excel file.

The study gained permission from the Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee under the number 66597 (Date: 21.10.2022, Decision no:70891).

A total of 934 patients aged 1 month to 14 years who applied to Mardin Province Kızıltepe State Hospital between 01/11/2020 and 31/10/2021 and satisfied

the inclusion criteria for the study sample comprised the study sample.

Patient inclusion criteria for the study are the following: Patients with rotavirus, adenovirus, or amoeba as the causal agent; children between 1 month and 14 years old. Patient exclusion criteria from the study also are patients older than 14 years and those with insufficient/incomplete information in their files.

Statistical Analysis

Using the IBM SPSS 25.0 Version application, statistical analysis of the study data was conducted. Using the Chi-square Test, the link between categorical characteristics and groups was investigated. The significance level of $p < 0.05$ was acceptable in statistical analysis.

RESULTS

Distribution of demographic and causal features of the patients are shown in Table 1. Rotavirus was discovered in 297 (31.8%) of the clinical samples obtained from the patients enrolled in the study, Adenovirus in 30 (3.2%), and Amoeba in 62 (6.6%) of the clinical samples, but the agent was not detectable in 545 (58.4%) of the clinical samples. Males had greater rates of rotavirus and adenovirus positive, but there was no statistically significant difference between the two groups. Rates of amoeba positive were substantially greater in males than in girls (35.8% vs. 22.6%, p = 0.046; respectively). Rotavirus antigen positive was statistically significantly higher in the 5-24 month age group compared to all other age groups (p = 0.034). Similarly, amoeba positive was statistically substantially higher in the 5-24 month age group compared to other age groups ($p < 0.001$). There was no significant variation between age groups in the distribution of adenovirus.

During the 12-month research period, rotavirus antigen positive was most prevalent in the spring, and the seasonal variation was statistically significant ($p <$ 0.001). The frequency of amoeba positive was highest during the summer, and the difference between seasons was statistically significant ($p = 0.003$). The frequency of undiscovered variables was greater during the spring-summer months, and a statistically significant difference ($p = 0.041$) was identified between the

Table 1. Displays the demographic and etiological features of the patients who participated in the research

Features	RV (+) (n = 297)		*p* value	AV (+) (n = 30)		*p* value	Amoeba (+) (n = 62)		*p* value	Other Factors (n = 545)		*p* value	Total (n = 934)	
	n	%		n	%		n	%		n	%		n	%
Gender														
Female	136	14.6	0.065	13	1.4	0.830	27	2.9	0.727	211	22.6	**0.046**	387	41.4
Male	161	17.2		17	1.8		35	3.7		334	35.8		547	58.6
Age groups														
≤ 4 months	32	3.4	**0.034**	1	0.1	0.259	3	0.3	**< 0.001**	60	6.4	0.466	96	10.3
5-24 months	245	26.2		28	3.0		39	4.2		425	45.5		737	78.9
25-60 months	16	1.7		1	0.1		14	1.5		38	4.1		69	7.4
6-14 years	4	0.4		0	0		6	0.6		22	2.4		32	3.4
Seasons														
Autumn	35	3.7		6	0.6	0.394	15	1.6	**0.003**	84	9.0	**0.041**	140	15
Winter	69	7.4		4	0.4		6	0.6		73	7.8		152	16.3
Spring	116	12.4		7	0.7		12	1.3		198	21.2		333	35.7
Summer	77			13	1.3		29	3.1		190	20.3		309	33.1

RV = Rotavirus, AV = Adenovirus

groups. The seasonal variation in the prevalence of Adenovirus antigen positive was not statistically significant ($p = 0.394$).

DISCUSSION

One of the most prevalent causes of sickness and mortality in children is gastroenteritis [1]. Approximately 360 000 children die annually from gastroenteritis, infections, and nutrition-related reasons [5, 10, 11]. Every year, 30,000 children are lost to gastroenteritis in our nation. Approximately 80% of fatalities associated with gastroenteritis occur in infants, and the majority are caused by viruses [14]. In recent years, technical advancements have increased the pace at which viral agents may be discovered. Regarding the etiological agents, it is known that viral infections account for 30-40% of the cases [15]. Rotavirus is the most prevalent cause of diarrhea, especially in children under five years old [16]. Rotavirus is the leading global cause of serious diarrhea in newborns and young children [17]. 10-20% of infantile diarrhea in the globe is caused by rotavirus on average. In various locations of Turkey, the incidence of gastroenteritis caused by rotavirus has been estimated between 10 and 30%. In investigations done in a low socioeconomic status district of Ankara, the prevalence of rotavirus was found to be 29 and 22.7%, respectively [18]. In various parts of Turkey, rotavirus positive was found to be 39.8% in Izmir, 21% in Malatya, 25.7% in Kahramanmaraş, 29.1% in Ankara, and 32.0% in Istanbul (19, 20). In the United States, Germany, India, Pakistan, Kenya, and Saudi Arabia, the rotavirus positive rate in acute gastroenteritis ranges from 17 to 69 percent [21, 22]. In light of these findings, it may be concluded that the incidence of the disease does not change much between industrialized and developing nations. In a retrospective research done in our nation, rotavirus was detected in 37.3% of 0-18-year-old children hospitalized with acute gastroenteritis [23]. Similarly, in our study, 30% of hospitalized patients with acute gastroenteritis tested positive for rotavirus.

Although rotavirus infection can occur in all age groups, the prevalence of symptomatic infections is highest in children under the age of two [23]. The research indicates that rotavirus diarrhea is most prevalent in children aged 6 to 24 months, and between 9 and 12 months. It is said that it peaks in months [24]. We find that the rate of rotavirus antigen positivity in the 5-24 month age group is statistically higher than that in other age groups when we look at the distribution of the factor positivity rates by age groups in our study. It is possible to argue that issues related to acute gastroenteritis occur more frequently as population ages decline.

There are research indicating that there is no gender difference in the incidence of viral gastroenteritis, but there are other studies demonstrating a substantial gender difference [25]. In a research done in Istanbul, girls tested positive for rotavirus somewhat more frequently than boys [26]. In our study, while rotavirus and adenovirus positivity rates were higher in males, no statistically significant difference was found between the two groups. Rates of amoeba positive were substantially greater in boys than in girls.

In temperate countries, rotavirus outbreaks are found in a period of 4-5 months, notably in cold months (late autumn, winter, early spring), and rotaviruses are the cause of nearly 50% of pediatric diarrhea in winter months [27]. Although seasonal variability is detected in studies done in our nation according to the climatic circumstances of the location where the study was conducted, it is noticed more commonly in winter and spring seasons, as in this study (28). In the research, spring had the highest prevalence of rotavirus antigen positive, however the difference between spring and other seasons was statistically significant. While amoeba positive was reported most commonly in summer months, the difference between seasons was statistically significant.

Diarrhea remains one of the top five causes of child and newborn death, despite a decline in worldwide mortality rates over the years. Reducing diarrhea-related mortality is anticipated to result in improvements in baby and child nutrition, rotavirus vaccine programs, and treatment techniques [1, 8-10].

CONCLUSION

In conclusion, it is among the top five nations in the world in terms of the death rates among children under five caused by diarrhea. Additionally, the majority of these fatalities may be decreased by preventative and

prevention strategies. Governments, medical professionals, and families have a responsibility to establish a safe environment that will allow all children to experience infancy and childhood in a way that is both healthy and safe. In order to lower the morbidity and death rates of millions of harmful environmental conditions and malnutrition worldwide, community-based health policies should be put into place. It can be advised to improve their access to housing and community resources, to create health policies that are approachable, to give kids hygienic and healthy environments at home, school, and in their social environments, and to maintain methods of preventing diseases that will arise in this context. On the other hand, adopting prevention approaches specified by WHO are basic safety measures.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1398-1410 Original Article

DOI: 10.18621/eurj.1272648 *Nutrition and Dietetics*

Determining the level of food safety awareness by nutritional literacy in health sciences faculty students

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ABSTRACT

Objectives: The aim of the research is to determine the nutrition literacy level and food safety awareness level of students who studied at faculty of health sciences. This descriptive study also demonstrates university students' nutrition literacy statuses, food safety attitudes, nutritional statuses, and food preferences.

Methods: The research was conducted as a descriptive and cross-sectional survey study in order to determine the nutrition literacy level and food safety awareness level of the students. Two hundred and eight individuals, including 174 women and 34 men, participated in the study. The data were obtained from face-to-face and online interviews then they were analyzed in a software. The survey is consisted of three parts: socio-demographic form, Evaluation Instrument of Nutrition Literacy on Adults and the Food Safety Attitude scale.

Results: In this study, the majority of the participants studied in the department of nutrition and dietetics (55.29%) and audiology (26.92%), followed by health management (7.21%), physiology and rehabilitation (5.77%), nursing (4.33%) and social work (0.48%) department. The relationship between nutrition literacy and food safety among the students of the faculty of health sciences was significant ($p < 0.01$).

Conclusions: This study showed that the nutrition literacy level was sufficient and the food safety attitude was positive in university students. However, it is needed to prospective studies to understand the importance of nutrition literacy and food safety awareness.

Keywords: Nutrition literacy, student, food safety, health

Nutrition plays a role in the maintenance of a living organism's health as well as the healthy development of an organism. Malnutrition is associated with a decline in growth and development and a lack of immune resistance against diseases. 2022 report by the World Health Organization (WHO) emphasized that the incidence rate of non-communicable diseases such as diabetes and cardiovascular diseases and the rate of mortality from these diseases have increased since 2000 despite the fact that the incidence rate of communicable diseases has decreased, and life expectancy has extended. Nutrition depends on factors such as economic status, education, and cultural habits. The study published by the WHO found that the rate of diet-related diseases was higher in low-income countries [1]. Nutrition education is crucial to minimizing the risk of diseases. In a study conducted by Aktaç *et al*. [2] on nutrition knowledge in pregnant women be-

Received: ***March 28, 2023;*** *Accepted:* ***May 23, 2023;*** *Published Online:* ***September 2, 2023***

How to cite this article: *Sevinç AY, Demirci Ü. Determining the level of food safety awareness by nutritional literacy in health sciences faculty students. Eur Res J 2023;9(6).1398-1410. DOI: 10.18621/eurj.1272648*

e-ISSN: 2149-3189

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fore and after nutrition education, it was observed that nutrition knowledge scores of pregnant women elevated following the nutrition education, and nutrition knowledge scores prior to the nutrition education were associated with socioeconomic factors.

Following the evidence proving that nutrition education plays a role in the treatment of diseases, the term "nutrition literacy" emerged for the evaluation of individuals' levels of nutrition knowledge. Nutrition literacy is defined as the ability to obtain, process, and understand fundamental nutrition information and services that are needed to make nutrition-related decisions [3]. The objectives of nutrition literacy are to enhance the quality of nutrition education, develop a critical perspective toward nutrition information, and create awareness of problems regarding food and nutrition [4]. Nutrition literacy is associated with socioeconomic factors. A study performed with university employees showed that the nutrition literacy level was higher in university graduates than in primary school graduates, and in unmarried employees than in married employees [5]. While several scales have been designed and developed for nutrition literacy, there is not a single common scale applicable to every country and every age group. Since nutrition literacy is quite a new concept in Türkiye, there are ongoing attempts to develop a new scale to measure and evaluate it, and the number of studies on nutrition literacy has been increasing. The nutrition literacy scale that is currently in use in Türkiye is the Evaluation Instrument of Nutrition Literacy on Adults (EINLA) [6].

Food safety is of utmost importance for individuals' protection from food-related diseases and for supplying quality food. Lack of food safety causes serious issues such as difficulty in accessing quality food and a spike in food poisoning cases. Failure to provide proper and adequate conditions due to such factors as global warming, socioeconomic situation, and unstable market prices leads to the failure to ensure food safety [7]. The report published by the Food and Agriculture Organization (FAO) stated that food and agricultural policy supports across the globe have not been implemented in a just and equal manner to encourage healthy practices, and that this situation has made it difficult to access healthy food; and highlighted that individuals' access to a healthy diet will be facilitated with the development of solutions such as making public budgets cost-effective, reducing countries' trade problems, and creating healthy nutrition-focused food and agricultural policies [8]. Persistence of the food safety issue will obviously result in individuals not having access to quality and sufficient food, thereby augmenting the risk of diet-related diseases. Prevention of food safety problems depends on consumers' levels of nutrition knowledge as much as it depends on producers' knowledge of such. A study by Gözener *et al*. [9] demonstrated that most of the students participating in the study heard of the food safety concept, found the food items they consumed risky, and preferred to spend extra money on safe food items. Although there are scales developed for nutrition literacy, there is no scale for measuring the level of awareness of food safety. Thus, there is a need for studies to create a prospective scale regarding food safety awareness.

In this respect, the findings of the study conducted particularly with students at the faculty of health sciences showed that students were incompetent in terms of food literacy and food safety, they paid more attention to expiry dates and nutritional values when purchasing food and found the label information inadequate. The aim of the study is to determine the nutrition literacy level and food safety awareness level of health sciences faculty students and thus to create awareness of nutrition and food safety. This study will shed light on university students' nutrition literacy statuses, food safety attitudes, nutritional statuses, and food preferences. The data for this research were obtained through the comparison of the responses to survey questions, the Evaluation Instrument of Nutrition Literacy on Adults (EINLA), and the Food Safety Attitude scale with criteria such as participants' field of study, sex, demographic status, and nutritional status. This study will contribute to creating awareness of nutrition and food safety. The following are the hypotheses of the study:

H1: General nutrition knowledge has an impact on food safety.

H2: Reading comprehension has an impact on food safety.

H3: Knowledge of food groups has an impact on food safety.

H4: Portion size has an impact on food safety.

H5: Digital literacy and knowledge of food labeling have an impact on food safety.

METHODS

Research Methodology

The research was conducted as a descriptive and cross-sectional survey study in order to determine the nutrition literacy level and food safety awareness level of students at the faculty of health sciences.

Place, Time, and Characteristics of the Research

The research was carried out at Istanbul Aydın University and Istanbul Health and Technology University between the dates of May 2022 and July 2022. Some of the students from nutrition and dietetics, nursing, physiotherapy and rehabilitation, health management, social work, and audiology departments participated in the study through face-to-face interviews while some participated therein online. A limitation of the study is taking a limited number of university students as a sample since applying the survey to the whole society is difficult.

Research Universe and Sample

The universe of this research consisted of students at Istanbul Aydın University Faculty of Health Sciences and Istanbul Health and Technology University Faculty of Health Sciences. The formula below was used to calculate the sample of the study, and a total of 302 students were planned to be included in the research. However, the study was concluded with the participation of 208 students due to reasons such as students' unwillingness to participate in the survey and lack of time. A total of 208 individuals, 174 women and 34 men, participated in the study. The sampling formula is following:

Sample size formula based on the known number of individuals in the population:

"n = N.t2. p. q / d2. (N-1) + t2. p. q"

N: Number of Individuals in the population

t: The statistic that determines the error in the research

p: Participation status to the research

q: Non-participation status to the research

d: Standard deviation determining the sampling error in the study

n: Sample size [10].

Ethical Aspect of the Research

The research was found to be ethical with the decision no. 2022/112 and dated 04.08.2022 by Istanbul Aydın University Non-Interventional Clinical Research Ethics Committee. The faculty were informed prior to the application of the survey, and the students were given informed consent forms.

Data Collection Methodology

The data were obtained through face-to-face interviews and also online Google Forms, and the SPSS (Statistical Package for the Social Sciences) software was utilized to analyze the data. The survey is consisted of three parts: socio-demographic form including information on nutritional status and food preferences, the Evaluation Instrument of Nutrition Literacy on Adults (EINLA), and the Food Safety Attitude scale.

Socio-Demographic Form

The socio-demographic form given to the university students included questions about individuals' university and department, age, sex, income status, and satisfaction with their university and department; as part of the nutrition preferences section, questions about whether they have breakfast, and if yes, where they have it, how many meals they have in a day, if they skip a meal, and if yes, why they skip a meal, which types of foods they prefer, and their thoughts on their nutrition knowledge level and nutritional status; and as part of the food preferences section, questions about whether they have heard of the food safety concept, if label details are adequate, health risks in food items, if they get sick, why exactly they get sick because of food, and handwashing habit.

Evaluation Instrument of Nutrition Literacy on Adults (EINLA)

This is a nutrition literacy scale that is developed by Cesur (2014) in order to determine nutrition literacy status and consists of 35 questions and 5 sections. The scale was tested for validity and reliability, and it was found to be valid. The first section of the scale contains questions on general nutrition knowledge, the second reading comprehension questions, the third food groups questions, the fourth portion knowledge questions, and the fifth digital literacy and food labeling reading questions. Each correct answer is given 1 point while each unanswered or wrong answer receives 0 points. Accordingly, the nutritional literacy

level of those who score 0-11 points in total is considered "insufficient", 12-23 points "borderline", and 24-35 points "sufficient" [6].

Food Safety Attitude Scale

Developed by Memiş [11] in 2009 to determine food safety status, the scale is composed of a total of 18 questions, of which 9 are affirmative and 9 are negative. The scale was tested for validity and reliability, and it was found to be valid. It is a 3-point Likert scale and offers "Agree", "Partially Agree" and "Disagree" options. In affirmative questions, answers are given 3 points, 2 points, or 1 point respectively from "Agree" to "Disagree" while in negative questions, the scoring is reversed as 1 point, 2 points, and 3 points [11].

Statistical Analysis

SPSS Statistics 22.0 software was utilized for statistical analyses of the research findings. In the study performed with a sample size of 208 individuals, descriptive statistics of participants' answers to the survey questions were provided. The relationship between the scales was examined with correlation (Spearman's rho) and regression analyses. The normality of the scales was tested with the univariate normality test (Shapiro-Wilk). Nonparametric tests and methods were used to compare and analyze the variables that were not normally distributed according to the normality test results. The results were evaluated at 95% and 99% confidence intervals, and at $p < 0.05$ and $p < 0.01$ significance levels.

Table 1. Some socio-demographic characteristics of students by percentage

Socio-demographic characteristics		n	%
Participants' universities	Istanbul Aydın University (IAU)	94	45.19
	Istanbul Health and Technology University (ISTUN)	114	54.81
Participants' ages	18-20	104	50
	21-23	99	47.6
	24 and above	5	2.4
Participants' sexes	Male	34	16.35
	Female	174	83.65
Participants' departments	Health Management	13	7.21
	Nursing	8	4.33
	Physiotherapy and Rehabilitation	11	5.77
	Social Work	1	0.48
	Nutrition and Dietetics	110	55.29
	Audiology	65	26.92
Participants' income status	Income Lower Than Expenses	42	20.19
	Income Equal to Expenses	121	58.17
	Income Higher Than Expenses	45	21.63
Participants' satisfaction with their departments.	Yes	157	75.48
	No	13	6.25
	Partially	38	18.27
Participants' satisfaction with their universities	Yes	93	44.71
	No	33	15.87
	Partially	82	39.42

RESULTS

Descriptive Statistics and Frequency Analysis for Socio-Demographic Characteristics

Table 1 provides the socio-demographic characteristics of the study participants. Accordingly, 83.65% of the students were female, and 50% were within the age range of 18-20 years. Nutrition and Dietetics (55.29%) and Audiology (26.92%) students demonstrated a high participation rate. Of the students participating in the study, 45.19% were studying at Istanbul Aydın University while the remaining 54.81% were studying at Istanbul University of Health and Technology. It was determined that the participants were generally satisfied with the university and department they chose.

Descriptive Statistics and Frequency Analysis for Participants' Nutritional Statuses

Table 2 shows that regarding having breakfast, 55.29% of the students take care to eat breakfast, 36.06% of them have breakfast sometimes, and 8.65% do not eat breakfast. When asked about the place they have their breakfast, 61.06% of the participants said they prefer preparing breakfast at home, 12.50% purchase food from the street, 2.88% eat breakfast at the school canteen, 1.44% of them have their breakfast at a restaurant, 17.79% eat breakfast at school or dormitory dining hall, and finally, 4.33% of them said they don't eat breakfast. When it comes to the number of meals the students eat in a day, 36.54% eat 1-2 meals, 45.19% 3 meals, 12.02% 4 meals, 4.81% 5 meals, and 1.44% 6 meals. When asked if they skip a meal, 76.92% said they do while 23.08% do not skip a meal. As to the reason behind skipping a meal, 39.42% of the study participants said they don't want to prepare food, 34.13% cannot find time to prepare food, 13.94% find it difficult to prepare food, and 12.50% said they skip a meal due to other reasons. Regarding their thoughts on their nutritional statuses, 27.88% of the students believe they eat healthy and 34.62% believe otherwise, and 37.50% of them are not sure about their nutritional statuses. As for their evaluations on their nutrition knowledge level, 52.88% of the participants thought they had good nutrition knowledge, 31.73% were not sure about their nutrition knowledge level, and 15.38% did not think they had good nutrition knowledge.

Descriptive Statistics and Frequency Analysis for Food Preferences

Table 3 shows that 82.21% of the students had heard the concept of food safety before while 17.79% had not. When asked if they find the labeling information of packaged food sufficient, 38.46% of them answered yes, and the remaining 61.54% did not find the labeling information sufficient. As to food-borne health risks, the majority 51.44% of the students found food poisoning to be the most important food-borne health risk followed by cancer with 21.15% and food infections (diarrhea, vomiting, etc.) with 18.75%; finally, 6.73% of the study participants said they had no idea about this issue. In response to the question of if they have ever gotten sick because of food they ate, 53.85% of the students said yes while the remaining 46.15% answered no. Regarding the reason for getting sick due to the food they ate, 27.88% stated they had food at a restaurant, 7.21% at home, 6.73% at the school dining hall, 5.29% at the canteen, 6.25% at a kiosk, and 5.77% from a street vendor. Additionally, 40.87% of the participants said that they did not eat out. When it comes to handwashing habits of the students, it was found that 14.90% of them wash their hands when they get dirty, 0.48% after they start preparing food at home, 13.46% before they start preparing food at home, 4.33% before using the toilet, 22.60% after using the toilet, 11.06% before eating, and 2.40% after eating; on the other hand, 30.77% of the students said they practice all the options mentioned here.

Descriptive Statistics and Correlation Analysis of Variables

"Correlation analysis is a statistical method used to determine the existence of a linear relationship between two numerical measurements and the strength and direction of this relationship if any" [12].

"Interpretation of the correlation coefficient (r):

- If $0 < r < 0.19$, then very weak relationship or no correlation.
- If $0.20 < r < 0.39$, then weak correlation.
- If $0.40 < r < 0.59$, then moderate correlation.
- If $0.60 < r < 0.79$, then strong correlation.
- If $0.80 < r < 1$, then very strong correlation." [13].

Table 4 presents the results of the correlation analysis between participants' literacy sub-dimensions

Table 2. Nutritional status of students by percentage

Nutritional status		n	%
Do they have breakfast?	Yes	115	55.29
	No	18	8.65
	Sometimes	72	36.06
Where do they have breakfast?	At home	127	61.06
	I buy a toasted sandwich, etc. from the street	26	12.50
	I eat at the school canteen	6	2.88
	I have breakfast at a restaurant	3	1.44
	School or dormitory dining hall	37	17.79
	I do not eat breakfast	9	4.33
How many meals do they eat in a day?	1-2 Meals	76	36.54
	3 Meals	94	45.19
	4 Meals	25	12.02
	5 Meals	10	4.81
	6 Meals	3	1.44
Do they skip a meal?	Yes	160	76.92
	No	48	23.08
Why do they skip a meal?	It's difficult to prepare food	29	13.94
	I can not find time to prepare food	71	34.13
	I do not want to eat	82	39.42
	Other	26	12.50
Which types of foods do they prefer?	Meat dishes	63	30.29
	Vegetable dishes	33	15.87
	Fastfood	73	35.10
	Toasted sandwich (with kasar cheese, white cheese, etc.)	9	4.33
	Meatballs and similar dishes	25	12.02
	Other	3	1.44
	All except vegetable dishes	1	0.48
	I am not picky with food	1	0.48
Participants' Thoughts on Their Nutritional Status	I think I eat healthy	58	27.88
	I am not sure	78	37.50
	I do not think I eat healthy	72	34.62
Participants' Evaluation of Their Nutrition Knowledge Level	I think my nutrition knowledge is good	110	52.88
	I am not sure	66	31.73
	I don't think my nutrition knowledge is good	32	15.38

Table 3. Food Statuses of Students by Percentage

Food Status		n	%
Did they hear the food safety concept?	Yes	171	82.21
	No	37	17.79
Is the labeling information of packaged food sufficient?	Yes	80	38.46
	No	128	61.54
Food-borne Health Risks	Food infections (diarrhea, vomiting, etc.)	39	18.75
	Food poisoning	107	51.44
	Cancer	44	21.15
	I have no idea about this	14	6.73
	Other	4	1.92
Did they get sick because of the food they ate?	Yes	112	53.85
	No	96	46.15
Why did they get sick due to food?	Restaurant	58	27.88
	Home	15	7.21
	School dining hall	14	6.73
	Canteen	11	5.29
	Kiosk	13	6.25
	Street vendors	12	5.77
	I do not eat such food	85	40.87
Participants' Handwashing Habit	I wash my hands when they get dirty	31	14.90
	I wash my hands after I start preparing food at home	1	0.48
	I wash my hands before I start preparing food at home	28	13.46
	All	64	30.77
	I wash my hands before using the toilet	9	4.33
	I wash my hands after using the toilet	47	22.60
	I wash my hands before eating	23	11.06
	I wash my hands after eating	5	2.40

and food safety dimensions. According to the analysis, the relationship between the EINLA Level Score (24.80 ± 8.45) and the Food Safety Attitude Score (44.93 ± 7.14) is statistically significant "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.561)".

The relationship between the Food Safety Attitude Score (44.93 ± 7.14) and EINLA General Nutrition Knowledge Score (1. Section) (8.08 ± 2.21) was found to be statistically significant "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.470)".

Moreover, the relationship between the Food Safety Attitude Score (44.93 ± 7.14) and the EINLA Reading Comprehension (2. Section) (4.63 ± 1.54) is statistically significant "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.464)".

The relationship between the Food Safety Attitude Score (44.93 ± 7.14) and the EINLA Food Groups Knowledge Score (3. Section) (7.06 ± 3.60) was found to be statistically significant "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.480)".

Furthermore, the relationship between the Food Safety Attitude Score (44.93 ± 7.14) and EINLA Portion Size Score (4. Section) (1.75 ± 0.90) is statistically significant "($p < 0.01$)".The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.408)".

Finally, the relationship between the Food Safety Attitude Score (44.93 ± 7.14) and the EINLA Digital Literacy and Food Labeling Knowledge Score (5. Section) (3.34 ± 2.12) is statistically significant "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.408)".

When EINLA scores for different sections were analyzed, it was found that the EINLA General Nutrition Knowledge Score (1. Section) (8.08 ± 2.21) and the EINLA Reading Comprehension (2. Section) (4.63 ± 1.54) are statistically significantly correlated "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.444)".

The EINLA General Nutrition Knowledge Score (1. Section) (8.08 ± 2.21) and the EINLA Food Groups Knowledge Score (3. Section) (7.06 ± 3.60) have a statistically significant relationship "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.545)".

Moreover, the relationship between the EINLA General Nutrition Knowledge Score (1.Section) (8.08 ± 2.21) and the EINLA Portion Size Score (4. Section) (1.75 ± 0.90) is statistically significant "($p < 0.01$)". The relationship between the two variables is, therefore, a positive weak relationship "(0.20 < r < 0.39) (r = 0.317)".

The EINLA General Nutrition Knowledge Score (1. Section) (8.08 ± 2.21) and the EINLA Digital Literacy and Food Labeling Knowledge Score (5. Section) (3.34 ± 2.12) have a statistically significant relationship "(p < 0.01)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.522)".

Furthermore, the EINLA Reading Comprehension (2. Section) (4.63 ± 1.54) and the EINLA Food Groups Knowledge Score (3. Section) (7.06 ± 3.60) are statistically significantly correlated "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.535)".

The EINLA Reading Comprehension (2. Section) (4.63 ± 1.54) and the EINLA Portion Size Score (4. Section) (1.75 ± 0.90) have a statistically significant correlation "($p < 0.01$)". The relationship between the two variables is, therefore, a positive weak relationship "(0.20 < r < 0.39) (r = 0.354)".

Additionally, the EINLA Reading Comprehension (2. Section) (4.63 ± 1.54) and the EINLA Digital Literacy and Food Labeling Knowledge Score (5. Section) (3.34 ± 2.12) have a statistically significant correlation "($p < 0.01$)". The relationship between the two variables is, therefore, a positive moderate relationship "(0.40 < r < 0.59) (r = 0.470)".

The EINLA Food Groups Knowledge Score (3. Section) (7.06 ± 3.60) and the EINLA Portion Size Score (4. Section) (1.75 ± 0.90) are statistically significantly correlated "($p < 0.01$)". The relationship between the two variables is, therefore, a positive weak relationship "(0.20 < r < 0.39) (r = 0.363)".

The EINLA Food Groups Knowledge Score (3.

Table 4. Correlation analysis results between participants' literacy sub-dimensions and food safety dimensions

Variables	Mean ± SD	Spearman' s rho	EINLA Level score	Food safety attitude score	EINLA General nutrition knowledge score (1.Section)	EINLA Reading comprehension (2.Section)	EINLA Food groups knowledge score (3.Section)	EINLA Portion size score (4.Section)	EINLA Digital literacy and food labeling knowledge score (5.Section)
EINLA Level lcore	24.80 ± 8.45	r	1.000	0.561	0.753	0.665	0.835	0.512	0.836
		p value		**< 0.001**	**< 0.001**	**< 0.001****	**< 0.001**	**< 0.001**	**< 0.001**
		n		208	208	208	208	208	208
Food safety attitude Score	44.93 ± 7.14	r		1.000	0.470	0.464	0.480	0.308	0.408
		p value			**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
		n		208	208	208	208	208	208
EINLA General nutrition knowledge Score (1. Section)	8.08 ± 2.21	r			1.000	0.444	0.545	0.317	0.522
		p value				**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
		n			208	208	208	208	208
EINLA Reading comprehension (2. Section)	4.63 ± 1.54	r				1.000	0.535	0.354	0.470
		p value					**< 0.001**	**< 0.001**	**< 0.001**
		n				208	208	208	208
EINLA Food groups knowledge score (3. Section)	7.06 ± 3.60	r					1.000	0.363	0.642
		p value						**< 0.001**	**< 0.001**
		n					208	208	208
EINLA Portion size score (4. Section)	1.75 ± 0.90	r						1.000	0.310
		p value							**< 0.001**
		n						208	208
EINLA Digital literacy and food labeling knowledge Score (5. Section)	3.34 ± 2.12	r							1.000
		p value							
		n							208

Table 5. ANOVA regression results on the effect of nutrition literacy on food safety

	Sum of Squares	Standard deviation	Mean of Squares	F	*p* value
Regression	4010.055	5	802.011	24.791	**< 0.001**
Residual	6335.002	202	32.351		
Total	10545.058	207			

Section) (7.06 ± 3.60) and the EINLA Digital Literacy and Food Labeling Knowledge Score (5. Section) (3.34 ± 2.12) are statistically significantly correlated "($p < 0.01$)". The relationship between the two variables is, therefore, a positive strong relationship "(0.60 < r < 0.80) (r = 0.642)".

Lastly, the EINLA Portion Size Score (4. Section) (1.75 ± 0.90) and the EINLA Digital Literacy and Food Labeling Knowledge Score (5. Section) (3.34 ± 2.12) have a statistically significant correlation "($p < 0.01$)". The relationship between the two variables is, therefore, a positive weak relationship "(0.20 < r < 0.39) (r = 0.310)".

Regression Analysis

Table 5 shows the results of ANOVA for the effect of nutrition literacy on food safety. It is seen that there is a significant relationship between nutrition literacy and food safety (F (5.202) =24.791, p < 0.01).

The model for the effect on food safety based on Table 6 is as follows: "Food Safety = 30.871 + 0.650 × General Nutrition Knowledge + 0.873 × Reading comprehension + 0.407 × Food Groups Knowledge + 0.411 × Portion Size + 0.355 × Digital Literacy and Food Labeling Knowledge".

An examination of the t-test results for the model in Table 6 demonstrates that the effect of portion size and digital literacy and food labeling knowledge on food safety is not statistically significant ($p > 0.05$). On the other hand, general nutrition knowledge has a statistically significant effect on food safety (t = 2.607; $p < 0.05$). Reading comprehension also has a statistically significant effect on food safety (t = 2.276; $p < 0.05$). The effect of food groups knowledge on food safety is statistically significant (t = 2.310; $p < 0.05$). Furthermore, considering the result R2 = 0.380, it can be inferred that general nutrition knowledge, reading comprehension, and food groups knowledge explain

Table 6. *t*-test regression results on the effect of nutrition literacy on food safety

Model	Unstandardized Coefficients		Standardized Coefficients			Collinearity Statistics			
Food safety	**B**	**Std. Error**	**Beta**	**t**	***p* value**	**Tolerance**	**VIF**	**R^2**	**Durbin Watson**
Fixed	30.871	1.571		19.644	**< 0.001**			0.380	1.863
General nutrition knowledge	0.650	0.249	0.201	2.607	**< 0.010**	0.517	1.935		
Reading comprehension	0.873	0.383	0.189	2.276	**< 0.024**	0.447	2.238		
Food groups knowledge	0.407	0.176	0.205	2.310	**< 0.022**	0.390	2.566		
Portion size	0.411	0.506	0.052	0.813	0.417	0.746	1.341		
Digital literacy and food labeling knowledge	0.355	0.257	0.105	1.379	0.170	0.524	1.907		

food safety at a rate of 38%. Since VIF and tolerance values are less than 5, it can be deduced that there is no multicollinearity problem between the independent variables; also, the Durbin-Watson value at 1.863 which is close to two enables us to say with 95% confidence that there is no auto-correlation between the observed elements. As a result of the above-explained analyses, hypotheses H1, H2, and H3 have been confirmed while H4 and H5 have not.

DISCUSSION

In this study, university students' nutrition literacy and food safety awareness are examined as well as the relationship between the two. Besides these, the findings obtained through the socio-demographic form, the EINLA scale, and the Food Safety Attitude scale are discussed in relation to the existing literature. Nutrition and food safety surely play a big role in the maintenance of an individual's health.

Most of the students participating in this research are female students. The number of audiology department students and nutrition and dietetics department students participating in the study as well as the number of second-year undergraduates are higher compared to the others. Considering the education level of students' parents, it is indicated that mothers finished primary school while fathers completed high school; their income, on the other hand, is found to be equal to their expenses. Some studies emphasized that the literacy rate among women is low, it is thus understood that education allows women to develop an awareness of issues such as literacy and food safety [2, 14, 15].

Students are asked about their nutritional status and nutrition education. Some of the participants thought their level of nutrition knowledge is good and they eat healthy while some were not sure about their nutrition knowledge level and their eating habits. School courses are the fundamental means through which students learned about nutrition and food. The participants place higher trust and confidence in the information provided by dieticians and health personnel. It is seen that the participants of the study by Kozan [16] gave similar responses to the questions about nutrition knowledge level and nutritional status as the participants of our study. The research conducted by Uzun [17] denoted that the majority of the students received information about nutrition and food, and they did so mostly by taking courses at school and through communication sources.

As part of the food preferences section of the socio-demographic form, students are asked about whether they have heard of the food safety concept, if labeling details of packaged products are reliable, their handwashing habits, and the things they pay attention to while buying a product. The participants said they have heard of the food safety concept and they take care of hand hygiene in general. According to this study, the following are the elements they care about the most when buying a food item: price, expiry date, labeling information, storage instructions, and shelf life. Nutritional values, calories, vitamins and minerals, weight, cholesterol, sugar, and allergens are not always considered. Still, labeling information on packaged products is thought to be inadequate. Food poisoning and food infections were indicated as the biggest risks regarding food safety. A study conducted with university students in Kyrgyzstan provides similar results to our study in that the students have heard of the food safety concept and they pay attention to similar elements when buying food [18].

In this study, the average total EINLA score of the students was found to be 24.80 ± 8.45 which meant that the nutrition literacy (NL) level is "sufficient". NL level for males was "borderline" while it was "sufficient" among females. The study provided the following values and results regarding EINLA sub-dimension group scores: General Nutrition Knowledge – average score 8.08 ± 2.21 and NL level "sufficient"; Reading Comprehension – average score 4.63 ± 1.54 and NL level "borderline"; Food Groups – average score 7.06 ± 3.60 and NL level "sufficient"; Portion Size – average score 1.75 ± 0.90 and NL level "insufficient"; Digital Literacy and Food Labeling Knowledge – average score 3.34 ± 2.12 and NL level "borderline". EINLA sub-dimensions were also compared among themselves, and correlations between them were discovered. The study by Cesur found out that the participants' NL level was "sufficient" considering the total score; however, an examination of NL level based on the sub-dimensions of the developed scale showed that the NL level was "sufficient" in relation to general nutrition knowledge, reading comprehension, and food groups while it was

"insufficient" in relation to portion size and digital literacy and food labeling. This study also determined that the NL level is higher in women than men. Our research demonstrated the same result in terms of the NL level among women and men. The number of male and female participants was almost equal in Cesur's study; however, in this study, the number of female students was much higher than that of male participants, hence the higher NL level for women. Still, it should be noted that various studies highlight that nutrition literacy level is higher among women than men [2-6].

In this study, the average Food Safety Attitude score of the students was found to be 44.93 ± 7.14 which meant that the food safety attitude is "partially positive". The study by Memiş [11] presented the students' food safety attitude as "positive" among females and "partially positive" among males.

CONCLUSION

This study investigated university students' nutrition literacy statuses and food safety attitudes. Nutrition literacy influences individuals' nutritional status and eating and diet habits. Both several other studies and this study showed that the level of nutrition literacy is associated with individuals' nutrition knowledge levels. The current existing literature underlines the fact that increasing nutrition knowledge level leads to an expansion in nutrition literacy level. According to the study, the nutrition literacy level of university students is sufficient. It was found that the students generally strived to have their meals regularly, but they had to skip meals due to reasons such as lack of time and lack of food. Skipping meals did not affect nutrition literacy.

It is known that food safety is effective in protecting societies against diseases. Behaviors such as checking product labeling information and applying hygiene rules have an impact on individuals' food safety statuses. However, it is highly important that product labeling information be understandable. Students participating in this study think that product labeling information was insufficient, and they mentioned that they had food poisoning. These results show that the food safety controls of relevant facilities should be done regularly, and the labeling information should be legible and understandable. It is concluded that the food safety attitude of the students was positive, and they make conscious choices. Still, more studies should be carried out to measure societies' level of awareness of food safety.

Consequently, there is a need to improve students' awareness of nutrition literacy and food safety and conduct studies on these concepts. Listed below are the suggestions that will positively contribute to future research:

(1) Education and training programs in the field of nutrition and food safety should be organized in universities.

(2) Students' eating habits and diet may be followed, and free dietitian support can be provided when deemed necessary.

(3) A food safety scale may be prepared to determine and measure the level of food safety awareness.

(4) Product labels should be understandable to everyone. When a product contains ingredients posing health risks, such contents should be indicated on the product package in a manner recognizable by individuals.

(5) Since too many survey questions and especially too long survey time affect the number of participants and the rate of giving correct answers, survey questions in future research should be brief, easily understandable, and concise with the inclusion of really necessary questions for the field of study.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

I would like to express my gratitude to all my stu-

dents who participated in the study and shared their valuable opinions.
This study was partially presented at Istanbul Aydın University's Centennial of the Republic, Health, Education and Administrative Services Symposium from Past to Present. March 15-17, 2023, Istanbul, Türkiye.

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The European Research Journal 2023;9(6):1411-1419 Original Article

DOI: 10.18621/eurj.1245681 *Psychiatric Nursing*

Increasing self-protection skills of children staying in women's shelters with body safety training: a child sexual abuse prevention study

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ABSTRACT

Objectives: Child sexual abuse is a risk for children living in a happy family environment, as well as for children under state protection who have problems in achieving family unity.

Methods: The study was conducted with 17 children between 3-7 years of age staying at women's shelters with their mothers and aimed to improve their sexual abuse recognition and self-protection skills. 7 children identified as experimental group were provided with the Body Safety Training program and the effect of the training was evaluated with the "What If" Situation Test. The test results in the experimental group were compared with 10 children in the control group and the test was repeated three weeks later in order to determine the persistance of the training.

Results: The data obtained were evaluated by non-parametric analysis and Kruskal Wallis, Wilcoxon-signed Rank Test were used in addition to Mann Whitney U tests to determine the difference between the groups. As a result of the research, "What If" Situation Test sub-dimension mean scores showed an increase in favor of the post-test in the experimental group, but no significant increase was observed in the control group.

Conclusions: This difference observed in the experimental group shows that the Body Safety Training program is an effective program to increase self-protection skills for preventing sexual abuse in children.

Keywords: Child sexual abuse, prevention, body safety training, women's shelters

Child sexual abuse (CSA) is defined as all the actual or non-actual sexual abuse behaviors towards both girls and boys who are developmentally mature or not yet matured [1]. Child sexual abuse is still considered to be a widespread problem today and 12-18% of girls and 5-8% of boys are reported to have been exposed to abuse [2-4].

Polat [5] in 2007, states that within legal dimension, evaluations at home, school, social level and are important for prevention of child abuse and that education is the most important of these evaluations. Earlier studies have indicated that child sexual abuse is highest between the ages of 7 and 13 and that 30% of children who have been sexually abused suffer victimization before the age of 9. Thus, it is argued that educational programs for the protection of children from sexual abuse should start especially in preschool years . In a study conducted by Gibson and Leitenberg [6]

Received: ***February 1, 2023;*** *Accepted:* ***April 1, 2023;*** *Published Online:* ***April 11, 2023***

How to cite this article: *Çıtak Tunç G, Eser D, Kılınç FE. Increasing self-protection skills of children staying in women's shelters with body safety training: a child sexual abuse prevention study. Eur Res J 2023;9(6):1411-1419. DOI: 10.18621/eurj.1245681*

e-ISSN: 2149-3189

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on 825 university students, it was concluded that university students who participated in the school-based sexual abuse prevention program in their childhood were exposed to abuse by less than half compared to those who did not participate in such programs.

Child protection systems are developed in Turkey to protect children from maltreatment like abuse and violence [7]. Protective and supportive measures are implemented by several ministries and local governments for children in need of protection. Women's shelters, which are one of these units, are also designed as places where women who have been exposed to violence and are at risk can feel safe with their children (if any), receive the services they need for a specified time and receive public boarding services [8]. Sallan and Alican [9] report in their study that 39% of women in the women's shelters in Turkey stay with their children. For these reasons, services for children should not be overshadowed by the services provided to women [8, 10].

Children staying at the women's shelters with their mothers have undergone traumatic experiences of their lives. Failure to establish family unity can affect children in many ways and make them more vulnerable or distant to the environment. Since the education provided to these children is considered to be beneficial for their lives, the study focused on the sexual abuse prevention education of children staying at women's shelters.

This study aims to provide Body Safety Training to children staying with their mothers at women's shelters in Turkey, thus to increase their self-protection skills against sexual abuse, and secondly aims to determine the effect of the body safety training on children.

METHODS

Type of Research

The study aimed to teach the children staying at the women's shelters the privacy of their body at the Body Safety Training program and to improve their self-protection skills in case of possible sexual abuse. The study was designed as an experimental study with pre-post-test control group. In the study, two groups consisting of experimental and control groups, were formed and the results obtained from both groups were compared and analyzed.

Participants and Settings

The research was carried out between March 2017- May 2017 on 17 children aged 36-72 months whose mother stay in the three women's shelters under Turkey's governmental protection. The children staying at two separate shelters constituted the experimental group and the children in the third shelter constituted the control group. Because the location and name of the shelters is hid for the safety of the mothers and children, two shelters in the experimental group are coded (A) and (B), while the shelter in the control group is coded (C).

Experimental Group

Four children aged 36-72 months in the A-coded shelter, and six children in the B-coded shelter designated as the experimental group live with their mothers. The research was completed with 7 of a total of 10 children in A and B-coded women's shelters. Of the children in the experimental group, 57.1% were girls. 4 out of 7 mothers (57.1%) predict that their child will not be able to protect themselves from possible sexual abuse. According to the statements of their mothers, only 14.3% have knowledge about sexual abuse. The mothers of all children in the experimental group stated that their children have not been exposed to sexual abuse (Table 1).

Control Group

There were 17 children aged 36-72 months in the C-coded control group shelter. The age and characteristics of the children were taken into consideration as an inclusion criteria. Interviews were completed with 10 children out of 17 because seven children have left the institution for various reasons. Of the children in the control group, 50% were girls. It is foreseen by the mothers that 80% of children cannot protect themselves from possible sexual abuse. 9 mothers out of 10 in the control group stated that their children have not been exposed to sexual abuse while the mother of one child reported that her child was exposed to sexual abuse. 80% of the children witnessed their mothers being subjected to violence from their spouses (Table 1).

Materials

Demographic data were collected through a Parent/Child Information Form developed by the researcher. The children in the experimental group were provided Body Safety Training. The validity and reliability of the effect of the training was evaluated by the WIST developed by Citak Tunc *et al*. [11].

Research Tools

Parent/Child Information Form

Parent/Child Information Form, which is prepared by the researchers, consists of two different parts. In the first part, information about the child is gathered while the second part consists of questions regarding parents. The questionnaire was completed by participant children's mothers under the guidance of the researcher.

Body Safety Training (BST)

BST which is a scientific-based education program, was developed by Wurtele in 1986 and took its final form after the revision in 2007 [12]. The BST, developed for pre-school children to prevent child sexual abuse and consisting of information related to basic body safety, has been designed for the use of educators in schools, kindergartens and early childcare centers. The program consisted of 7 consecutive sessions and 30 topics in line with the expert opinions received from the Turkish adaptation studies [13]. The duration of each session is 15-20 minutes and the trainings are conducted in form of group training. The ideal number of children in each group is between 6 and 10 [12].

A training book titled "I am the Boss of My Body-Body Safety Training Program for Pre-school Children" was prepared to be used in the sessions and the stories used in the training were illustrated in this book. There is a total of seventy-two visual designs in the visual book "I am the Boss of My Body". After reading stories, feedback was received from the child by asking questions on the visuals from the book [14].

"What If" Situation Test (WIST)

WIST was developed by Wurtele, Hughes & Owens (1998) to measure the self-protection skills of pre-school children in cases of sexual abuse [15]. It was revised in 2008. The test, adapted to Turkish by Citak Tunc *et al*. [11], consists of mini stories that measure the child's ability differentiate between appropriate and inappropriate touches. The child, who listens to the stories of the "touching" sub-dimension, is expected to respond that his mother, father, nurse. baby sitter or doctor, if necessary, can touch / look at his/her private body part. In the study by Wurtele *et al*. (1998), the Cronbach's alpha coefficients of the "What If" Situation Test were found between 0.75 and 0.90. In the adaptation study to Turkish culture by Citak Tunc *et al*. [13], the Cronbach's alpha coefficients of WIST were found to be between 0.68 and 0.90.

Ethical Aspects of the Study

This study was conducted in accordance with the principles of the Helsinki Declaration. The study was approved by Social and Humanities Ethics Committee with the decision numbered 22.02.2017/05. Signed consent was obtained from volunteer parents. In order to conduct the research with children staying at women's shelters, required permissions were obtained from the relevant institution. For security reasons, permissions and institution names are not specified.

Data Collection Procedure

Before the start of the education in the A and B-coded shelters, the children were individually given the WIST at the first day; then, the seven-days of Body Safety Training was started. The training was informed to be held in the children's play rooms in the women's shelters between 09:00 and 09:30 in the morning. The actual training of the day was applied by showing children relevant pages from the book called "I am the boss of my body", which is the educational material of BST and contains colorful visuals for children. After the completion of seven sessions, the children were re-given the WIST as the post. After three weeks, the same test was repeated to check the permanence of the training. In the control group, the WIST was applied as pre-test to the children whose mothers approved the participation in the study. After the pre-test application, Body Safety Training was not provided. Then, after 7 days, the same children in control group were given the WIST as post.

RESULTS

The results of the pre-test, post-test and follow-up test as a result of Shapiro-Wilk Test at the sub-dimensions of WIST applied to the children in the experimental and control groups were found not to be normally distributed. Moreover, non-parametric statistics were used for the intra- and inter-group differences due to the sample size being less than thirty.

Table 2 shows the pre-test and post-test distribution of the WIST and mean scores of the children in the experimental and control groups across WIST's sub-dimensions. According to the results of Wilcoxon test, the difference between the pre and post-application scores related to the "Appropriate Touch", "Inappropriate Touch", "Report Skill" and "Personal Safety Questionnaire" sub-dimensions of the WIST obtained by the children in the experimental and control groups were not found to be significant. But, when the mean scores in the sub-dimensions of the children in the experimental group were examined, a significant increase was observed in favor of the post-test.

According to the Wilcoxon test results related to the sub-dimension of WIST, "Say Skill", "Do Skill", "Tell Skill"and "WIST Total Skill", a significant difference was found between pre and post-application

Table 1. Demographic characteristics of children included in the study

Characteristics	Experimental Group (n = 7)	Control Group (n = 10)
Sex, n (%)		
Girl	3 (42.9)	5 (50.0)
Boy	4 (57.1)	5 (50.0)
Age, n (%)		
36-47 months	4 (57.1)	4 (40.0)
48-60 months	1 (14.3)	1 (10.0)
61-80 months	2 (28.6)	5 (50.0)
Number of siblings, n (%)		
Single child	2 (28.6)	3 (30.0)
2 siblings	4 (57.1)	3 (30.0)
3 siblings	1 (14.3)	4 (40.0)
School attendance status, n (%)		
Continues	4 (57.1)	3 (30.0)
Does not continue	3 (42.9)	7 (70.0)
Sexual abuse information, n (%)		
Yes	1 (14.3)	3 (30.0)
No	6 (85.7)	7 (70.0)
Ability to protect yourself in the case of possible sexual sbuse, n (%)		
Can protect	3 (42.9)	2 (20.0)
Not protect	4 (57.1)	8 (80.0)
Past sexual exposure status, n (%)		
Yes	0 (0.0)	1 (10)
No	7 (100.0)	9 (90.0)
Total	**7 (100.0)**	**10 (100.0)**

Table 2. The Wilcoxon-signed rank pre-test and post-results of the children in the experimental and control groups related to the "what if" situation test sub-dimensions.

"What if" situation test		Pre-test (n = 7)		Post-test (n = 7)			
		$\bar{X}$	SD	$\bar{X}$	SD	z	*p* value
Appropriate touch	Experimental	2.42	0.97	3.00	0.00	-1.41	0.15
	Control	2.50	1.08	2.50	1.08	0.00	1.00
Inappropriate touch	Experimental	1.71	1.60	3.00	0.00	-1.73	0.08
	Control	1.30	1.15	1.70	1.25	-1.63	0.10
Say skill	Experimental	1.57	2.69	6.00	0.00	-2.33	**0.02***
	Control	1.20	2.14	1.80	2.39	-1.34	0.18
Do skill	Experimental	0.85	1.57	6.00	0.00	-2.45	0.01*
	Control	0.80	1.68	0.40	1.26	-1.00	0.31
Tell skill	Experimental	1.42	2.50	5.00	1.15	-2.20	**0.02***
	Control	0.60	0.69	1.10	1.72	-1.06	0.28
Report skill	Experimental	0.28	0.75	2.00	2.38	-1.82	0.06
	Control	0.40	1.26	0.30	0.94	-1.00	0.31
WIST total skill	Experimental	4.14	7.12	19.00	3.36	-2.36	**0.01***
	Control	3.00	4.02	3.60	3.97	-0.77	0.43
Personal safety questionnarie	Experimental	2.42	0.97	3.57	0.53	-1.80	0.07
	Control	2.00	0.94	2.40	1.07	-1.19	0.23

WIST = "What If" Situation Test

Table 3. The Wilcoxon-signed rank post-test and follow-up results of the children in the experimental group related to the "what if" situation test sub-dimensions.

"What if" situation test	Post-test n = 7		Follow-up test n = 7			
	$\bar{X}$	SD	$\bar{X}$	SD	z	*p* value
Appropriate touch	3.00	0.00	3.00	0.00	0.00	1.00
Inappropriate touch	3.00	0.00	2.83	0.40	-1.00	0.31
Say skill	6.00	0.00	5.66	0.81	-1.00	0.31
Do skill	6.00	0.00	5.66	0.81	-1.00	0.31
Tell skill	5.00	1.15	3.00	1.67	-2.12	**0.03**
Report skill	2.00	2.38	1.50	2.07	-0.44	0.65
WIST total skill	19.00	3.36	15.83	4.62	-1.72	0.08
Personal safety questionnarie	3.57	0.53	2.83	0.40	-1.63	0.10

WIST = "What If" Situation Test

scores of the experimental and control groups.

Table 3 reveals that post-test and follow-up test scores of WIST showed no difference and the mean scores were very close to each other ($p > 0.05$). In the "Tell Skill" sub-dimension ($z = -2.12$, $p < 0.05$), there was a significant difference between post-test and follow-up test scores.

According to the Table 4, the mean gain scores of the experimental and control groups related to the "What If" Situation Test "Say Skill", "Do Skill", "Tell Skill" and "Report Skill" sub-dimensions scores and total score before and after the application were found to be significantly different. WIST "Total Skill" gain score was 14.00 in the experimental group and 5.50 in the control group. There was a significant difference between the scores ($U = 00.00$, $p < 0.05$).

DISCUSSION

The Effect of Body Safety Training Program on Recognition of "Appropriate Touching"

In the study, an increase was found in the appropriate touch pre- and post-test mean scores of the experimental group, but it was found that this increase was not statistically significant (Table 2). The increase in the average of the experimental group can be explained as the effect of the training provided. In the questions measuring the appropriate touch skill, children are asked whether it is appropriate for a mother, father or a doctor to touch private body parts during an injury. Most of the children considered it normal and correct for these individuals to touch the private parts during an injury and responded accordingly during their pre-tests. The increase in the mean of the post-test after the training, but not reaching a statistically significant value suggest that children's pre-knowledge might be sufficient for appropriate touch skill.

In the study by Kenny, Wurtele and Alanso [16] no significant difference was found between the pre- and post-test mean scores of WIST's appropriate touch recognition. These results support the findings of the research. However, there are studies in the literature that mean scores of appropriate touch recognition showed an increase and were statistically significant [13, 17].

The Effect of Body Safety Training Program on Recognizing "Inappropriate Touching"

In the study, no statistically significant difference was found between the mean scores of inappropriate pre- and post-test scores in the experimental group (Table 2). In the study by Tutty [8] where "to whom can I tell?" program was applied, 117 children in the experimental group were compared with 114 children in the control group. As a result of the assessment

Table 4. U-test results of gain scores of the children in the experimental and control groups regarding the "what if" situation test sub-dimensions

Group (n = 17)	Experimental (n = 7) Gain Score Rank average	Control (n = 10) Gain Score Rank average	U	*p* value
Appropriate touch	10.43	8.00	25.00	0.36
Inappropriate touch	10.00	8.30	28.00	0.53
Say skill	12.50	6.55	10.50	**0.01***
Do skill	14.00	5.50	0.00	**< 0.01***
Tell skill	12.79	6.35	8.50	**< 0.01***
Report skill	12.07	6.85	13.50	**0.03***
WIST total skill	14.00	5.50	0.00	**< 0.01***
Personal safety questionnarie	10.71	7.80	23.00	0.27

WIST = "What If" Situation Test

using the "child abuse information" scale, no significant difference was found between the experimental and control groups in recognizing inappropriate touch. While pre- and post-test mean scores of inappropriate touch in the control group were close to each other, pre-test and post-test mean scores of inappropriate touch showed an increase in the experimental group. This difference can be explained as the effect of the Body Safety Training Program in which the children in the experimental group were involved.

The Effect of Body Safety Training Program on Gaining Self-Protection Skills

When the pre-test and post-test mean scores of the experimental group were compared, an average four-fold increase was found. The comparison of the mean gain scores of WIST Total Skill between the groups also supports the same result (Tables 2 and 4). BST program has been shown to be very effective in acquiring the skills of "Say", "Do", "Tell" and "Report" in preschool children staying with their mothers in women's shelters. The results of several studies using the BST program are similar to the findings of the present study [11, 15, 17, 19]

In the WIST's "Say Skill" sub-dimension, the score that the children will receive is in the range of 0-6. The difference between the pre and post-test mean scores of the experimental group (pre-test: 1.57, post-test: 6.00) was found to be statistically significant (Table 2). The mean scores of achievement scores between the WIST groups were found to be statistically significant (Table 4). In the BST program study conducted by Zhang *et al*. [19], post-test mean scores of "Say Skill" was found to have increased compared to the pre-test scores. In the study by Kenny *et al*. [16] the KLAS training program was applied to pre-school children of Latin origin and an increase was observed in the "Say Skill" pre-test mean score. The results show that the BST program is effective in teaching children to say "no" against possible sexual abuse cases. However, although it is very important for the child to show the ability to say "no" against a potential sexual abuse case, it is not sufficient alone [16].

The score that the children will receive from the WIST "Do Skill" sub-dimension is in the range of 0-6. In the study, the difference between the pre-test and post-test mean scores of the experimental group (pre-test: 0.85, post-test: 6.00) was found to be statistically significant and similar to the results obtained in the study by Wurtele *et al*. [15].

Studies conducted show that children exposed to sexual abuse show shyness and confidentiality in telling an adult about their experiences. Schaaf and Mccanne [20] emphasize that 5.7% of 475 university students were exposed to sexual abuse in childhood period and 33.7% of this abuse lasted for days, 31.6% for months and 34.7% for years [20]. Thus, in sexual abuse prevention programs the children are taught the skill to explain the case to a reliable adult in order to avoid the recurrence of the abuse. When the WIST "Tell Skill" sub-dimension scores were examined, a significant difference was found between the pre-test and post-test mean scores of the children in the experimental group (Table 2). In the study by Citak Tunç *et al*. [11], "Tell Skill" pre-test mean scores of the children in the experimental group were found to also have increased from 1.32 to 4.97 in the post-test. In the section where the persistence of the training was evaluated, it was determined that the persistence of the training applied only in the "Tell Skill" sub-dimension was not achieved. It is possible the children might feel the need to hide their life experiences and prefer to communicate less with their environment because of the fragmentation in the family unity, the scarcity of the father in the child's life, the psychological traumas experienced by the mother and the child's past negative experiences.

The child needs to provide some important details in communicating his experiences to the trusted adult. These are the answers to the questions "who did?" and "what did he try to do?". In "Report Skill", the child is expected to demonstrate the behavior to tell a trusted adult about the person who was requesting an inappropriate touch and what he was trying to do. In the study, no statistically significant difference was found between the "Report Skill" pre-test and post-test mean scores in the experimental group; however, a significant increase in the post-test mean score in the experimental were recorded (Table 2). The increase in favor of the post-test in the experimental group (pre-test: 0.28, post-test: 2.00) can be explained with the effect of training. During the trainings, sincere and warm relations were established with the children, and they were made to keep their attention on the subject. How-

ever, since the training was seven days, it was not possible for the trainer to integrate and share the same amount of time for each child. Moreover, because the children were required to respond in long sentences to the questions measuring the WIST "Report Skill", and identifying the abuser and explaining what they were doing required mutual interaction, all children did not choose this relationship with the trainer, some children chose to remain silent or expressed themselves in short words even if they had acquired the skills. The fact that the increase in the mean scores of WIST "Report Skill" was not statistically significant can be explained by the fact that the children were required to identify the event and the abuser in detail and that not all children preferred to engage into such an interaction with the trainer.

With the BST considered as a sub-dimension of WIST, children's personal knowledge about protection against sexual abuse and personal attitudes towards sexuality are evaluated. Related to personal safety, the pre-test and post-test mean scores of the WIST "Personal Safety Questionarie" sub-dimension of the children in the experimental group were compared and no statistically significant difference was found (Table 2). In their study conducted with pre-school children in China, Zhang *et al*. [19] found an increase in the "Personal Safety Questionarie" pre-test and post-test mean scores (pre-test: 2.04, post-test: 3.50) [19].

Of the mothers in the experimental group, 85.7% and 70% in the control group stated that their children have after did not any knowledge about sexual abuse. Positive changes that occurred as a result of the trainings are important in terms of the impact of the training.

CONCLUSION

It was concluded that the experimental and control group children included in the study had similar characteristics in terms of age and development, and the groups were homogeneous. 57.1% of the children in the experimental group included in the study were female, and 42.9% were male while 50% of the children in the control group were female and 50% were male. According to the information given by the mothers about the children, none of the children in the experimental group were exposed to sexual abuse and it was reported that one child in the control group had a history of sexual abuse. 85.7% of the mothers in the experimental group and 90% of the mothers in the control group reported that they were exposed to violence from their spouses. Of these women, 71.4% in the experimental group and 80% in the control group stated that their child witnessed this violence.

In line with aims and hypotheses the research; The BST Program is an effective educational tool to provide the children staying at women's shelters with the skills to protect themselves from sexual abuse.

As the results of the research also indicate, the majority of the children staying with their mothers at women's shelters have experienced or witnessed violence for a part of their lives. This negative process hinders their healthy development and might cause them to show problem behaviors or be vulnerable to abuse. Therefore, the measures to be taken are very important for the integration of these children to society.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgments

This study was produced from a master's thesis and was presented as an oral presentation at the 3rd International Child Protection Congress held in Ordu/Turkey on September 30-October 3, 2018.
Authors thanks to the children and their parents who participated in the study, providing the essential research data.

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The European Research Journal 2023;9(6):1420-1428 Original Article

DOI: 10.18621/eurj.1265826 *Orthopedic and Traumatology*

Proprioception analysis of patients with anterior cruciate ligament reconstruction

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ABSTRACT

Objectives: This study evaluates the effect of tibial stump mechanoreceptor preservation on proprioception, muscle strength, recovery and functional outcomes after arthroscopic anterior cruciate ligament surgery (ACLS).

Methods: Patients undergoing ACLS between January-July 2019 were evaluated by a single surgeon. The HUMAC NORM 2 device measured patients' proprioceptive sensation and muscle strength before and after surgery; KOOS and Oxford scales were used to score patients' functional results. The patients were divided into two groups: those who underwent stump-preserving surgery (SP group) and those who underwent conventional surgery (C group).

Results: Our study evaluated 27 patients, 11 in the SP and 16 in the C groups. The two groups had no statistical difference in muscle strength, proprioception, and functional scores in the first and third postoperative months. In the sixth-month evaluation, significantly better functional scores were found in the C group. Further, the athletic function was better in patients with good proprioception recovery, regardless of the group comparison.

Conclusions: Preserving the stump and mechanoreceptors on the tibial face was not determined to provide additional benefit to the patients in the first six months after surgery. Returning to sports was faster and functional scores were better in patients with good proprioception recovery.

Keywords: Anterior cruciate ligament tear, arthroscopic anterior cruciate ligament surgery, tibial remnant

The number of people engaged in recreational activities is increasing day by day. Anterior cruciate ligament (ACL) injury is most commonly seen during athletic activity after the injury to the menisci. The ACL is an essential structure in knee kinematics, and ACL injuries significantly impair pivot movements [1].

Arthroscopic anterior cruciate ligament surgery (ACLS) is one of the most common surgical procedures performed by orthopedic surgeons. Various surgical techniques (single, double bundle, transtibial, all-inside, etc.) have been devised to achieve improved mechanical stability, and studies of ideal reconstruction are ongoing. Recent studies focus on proprioception preservation, graft recovery, and mechanical

Received: ***March 15, 2023;*** *Accepted:* ***June 5, 2023;*** *Published Online:* ***September 2, 2023***

How to cite this article: *Erkurt N, Yerli M, Yüce A, Bayraktar TO, Atar S, Turan K, Çabuk H. Proprioception analysis of patients with anterior cruciate ligament reconstruction. Eur Res J 2023;9(6):1420-1428. DOI: 10.18621/eurj.1265826*

e-ISSN: 2149-3189

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stabilization [2, 3]. Animal studies have demonstrated that preserving mechanoreceptors [4] in the femoral and tibial stump region positively affects graft healing [5]. However, these studies have yet to offer definitive evidence that the stump preservation technique is superior to the standard (conventional) technique.

Stump-preserving ACL reconstruction may positively affect functional outcomes by contributing to proprioceptive recovery. Our study aims to investigate the effect of stump preservation during ACL surgery on postoperative muscle strength and proprioception recovery.

METHODS

Patients

Our study was planned as a prospective observational study, approved by the institutional review board, and performed according to the ethical standards outlined in the Declaration of Helsinki. All patients provided written informed consent before participating in the study. Twenty-seven patients who underwent ACLS between January and July 2019 and had a minimum 6-month clinical follow-up were included in the study; The same surgeon operated on all patients. The following were the criteria for inclusion in the study:

(a) Isolated ACL rupture according to clinical and radiologic findings with no additional knee lesion requiring surgical treatment,

(b) Closed physis lines,

(c) High demand and activity level, and

(d) Stage 0 or 1 knees, according to Kellgren and Lawrence (K-L) osteoarthritis radiologic staging.

The patients were separated into stump-preserving and conventional groups (Table 1). If the patients had a stump long enough to reach the lateral wall of the femur, stump-preserving surgery was performed. If the stump length was insufficient, conventional surgery was performed. The patient's muscle strength and proprioception were evaluated using the HUMAC NORM II® isokinetic dynamometer (CSMi, Stoughton, MA) described by Bayramoglu *et al*. [6]. KOOS and Oxford knee scores were used to evaluate recovery and functional outcomes [7, 8].

Surgical Technique

A single surgeon performed the surgeries on the patients included in the study. The patients' instability tests were repeated under anesthesia (Pivot shift, Lachman). Standard AM (anteromedial), AL (anterolateral), and central portals were used. In all cases, the arthroscopic examination was performed before graft harvesting. The patients were evaluated for medial, lateral meniscus or chondral damage, and isolated ACL rupture was confirmed. Patients with stumps that could extend to the lateral wall were treated with a stump-preserving surgical approach utilizing arthroscopic scissors and a shaver; in these cases, the ACL stump was released without using radiofrequency ablation to prevent possible damage to the neuronal elements in the stump (Fig. 1A). The central portal was added as the viewing portal. The suture carrier was taken out by grasping the proximal end of the stump with the Lasso loop (Fig. 1B), and a high-strength suture in the form of a loop was passed through the stump and left in the knee. One of the free feet was taken from the loop, a lasso loop technique was formed, and the stump was hanged (Fig. 1C). Both free feet were moved to the same portal. The femoral tunnel was prepared anatomically (Fig. 1D). Then, the stump was pulled anteriorly with the help of the in-

Table 1. Demographic data of study groups

	Stump-preserving surgery group (n = 11)	Conventional surgical group (n = 16)
Gender, n (%)		
Female	2 (18.2)	2 (12.5)
Male	9 (81.8)	14 (87.5)
Age (years)	28.0 ± 8.3	28.1 ± 8.6
Time elapsed until the operation (days)	363.6 ± 637.4	358.7 ± 870.8

Data are shown as mean ± standard deviation or number (percent)

serted lasso suture. A tibial drill guide was placed so that it came out of the centre of the tibial footprint. A tibial tunnel of appropriate width and length was created. The free ends of the suture on the stump were removed from the tibial tunnel and passed through the femoral EndoButton® sling of the hamstring graft used (Fig. 1E). The free ends of the stump were prepared to stay out of the tibial tunnel. While the prepared graft was pulled towards the femoral tunnel with the help of the EndoButton® system, the threads on the stump pulled the proximal end of the stump from the hanger of the EndoButton® towards the femoral tunnel. As the free ends coming out of the tibial tunnel were pulled over the EndoButton® loop, the covering of the stump was increased, and its tension was ensured. This preserved the stump and prevented it from getting stuck in the intercondylar notch.

Measurement of Proprioception Sense and Isokinetic Muscle Strength

One of the reproduction tests evaluating the perception of joint position, the passive-active method, was used in the sitting position. Proprioception was measured using the HUMAC NORM II® isokinetic dynamometer (Fig. 2). Headphones and a blindfold ensured the patients' visual and auditory senses did not affect proprioception. The isokinetic dynamometer was set to 2°/sec angular velocity in continuous passive motion mode. The target angle was set to 30°. When the device reached the target angle in flexion, it stopped and gave an audible warning for 10 seconds. The patient was asked to remember where the movement stopped, and the device was reset to the starting position. The device was operated with a constant angular velocity between 0 and 90 degrees, and the patient was asked to stop the device with the remote control when he/she felt that it had reached the target angle. The difference (MAH) between the target angle and the angle at which the patient stopped the device was recorded. The process was repeated three times with 20-second breaks. The mean measurement was calculated and defined as the sense of position [6]. The mean MAH value is inversely proportional to the proprioceptive sensitivity. Angular velocities of 60°/sec and 180°/sec were used for isokinetic muscle strength measurement. The test was started in full flexion. The submaximal strength trial tests were followed by five maximum power repetitions at 60°/sec and 15 maxi-

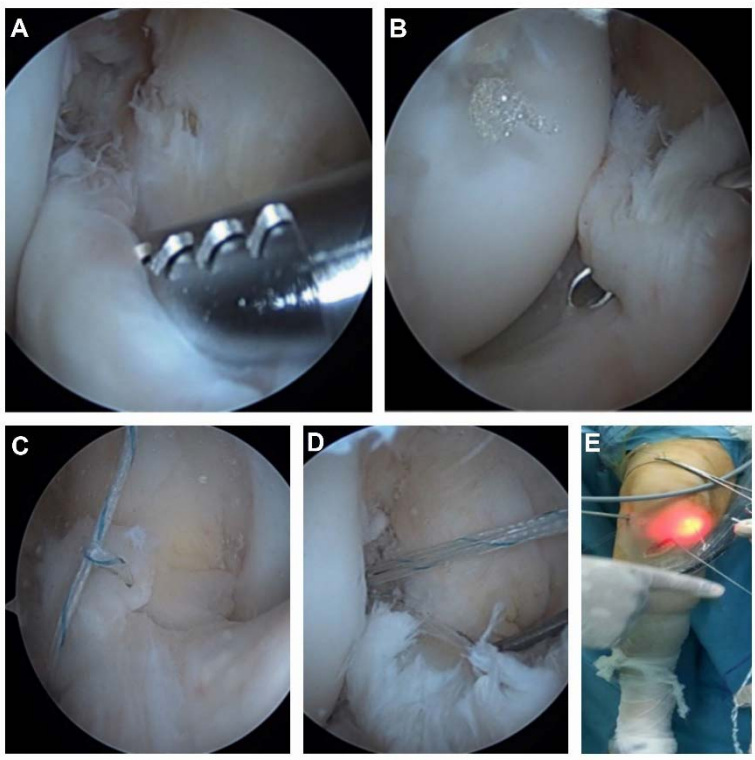

Fig. 1. (A) Release of the anterior cruciate ligament stump, (B) Suturing with a lasso suture, (C) Suturing the anterior cruciate ligament proximal stump, (D and E) Stump string threaded through endobutton sling.

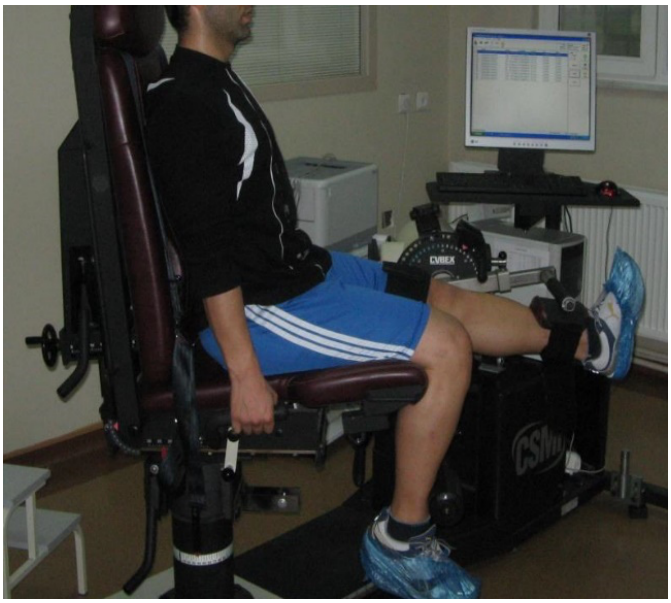

Fig. 2. Measurement of muscle strength and proprioception with the HUMAC NORM II® isokinetic dynamometer.

mum strength repetitions at 180°/sec, with 30-second breaks during speed changes. Quadriceps and hamstring peak torque values (Nm) were recorded for both speed measurements. After physical tests, functional results were evaluated with KOOS and Oxford knee scores.

Postoperative Rehabilitation Protocol

The same rehabilitation protocol was applied to all patients. Postoperative knee orthosis was not used. The patients were mobilized with full weight-bearing on the first postoperative day. Range of joint motion, isometric muscle and closed kinetic chain exercises were started on the second postoperative day. Light jogging was started in the second month, and open kinetic chain exercises were applied after the third month. Jumping, landing, bending and cutting exercises were started in the fourth month, and sprinting and other competitive exercises were started after the sixth month.

Statistical Analysis

In the preoperative period, patients' age, gender, injured side, the time elapsed until surgery, proprioception, muscle strength measurements, and Oxford knee and KOOS scores were recorded. In the postoperative first, third, and sixth months of KOOS, Oxford muscle strength and proprioception measurements were recorded. Whether the data were suitable for normal distribution was determined using the Shapiro-Wilk test. Mann-Whitney U test was used to compare the groups, and Wilcoxon sing rang test was used to compare preoperative and postoperative first, third-, and sixth-months results. Pearson correlation analysis evaluated the relationship between proprioception values and functional scores. $P < 0.05$ was accepted as statistically significant in all analyses. Data were analyzed using the SPSS version 20.0 program.

RESULTS

In the cases of stump-preserving surgery, the surgeon determined the degree of synovial coverage according to the amount of extension to the lateral wall; 87% coverage was obtained. There was no significant difference between preoperative flexion and extension muscle strengths and no significant difference in preoperative functional scores (Table 2). In the first month, functional scores decreased, and proprioceptive values increased, depending on possible pain levels, but the difference between the two groups was insignificant (Table 3). In the third and sixth months, KOOS scores were higher in pain ($p = 0.048$) and sports parameters ($p = 0.009$) in the conventional group (Table 4). In the sixth month, there was no sig-

Table 2. Comparison of study groups before surgery

	Stump-preserving surgery group	Conventional surgical group	p value[Ω]
KOOStotal⁎ (point)	51.8 (18)	58.6 (19)	0.365
Symptoms	61.3 (19.7)	62.5 (19.8)	0.883
Pain	55.3 (16.1)	63.5 (16)	0.273
Function, daily living	59.9 (22.8)	68.5 (21.1)	0.324
Function, sports and recreational activities	23.2 (20.1)	37.5 (32)	0.201
Quality of life	26.1 (18.7)	24.2 (14.7)	0.769
Oxford knee score (point)	26.0 (10.1)	30.5 (10.9)	0.297
Proprioception (degree)	6.78° (4.5)	4.95° (4)	0.281
Flexor muscle strength (Newton.meter)	107.4 (45.3)	97.1 (34.8)	0.510
Extensor muscle strength (Newton.meter)	181.2 (52.6)	143.0 (61.6)	0.106

Data are shown as median (interquartile range). ⁎KOOStotal =Knee Injury and Osteoarthritis Outcome Score-total, ranges from 0 to 100; with higher scores indicating better results.
[Ω]Mann-Whitney U test

nificant difference in flexion and extension muscle strength values; further, patients in the SP group did not regain the muscle strength of the preoperative period, though patients in the C group did. Proprioception values were better in the SP group. Although there was a significant statistical difference at the 3rd month (p = 0.010), no significant statistical difference was observed at the 6th month. When evaluated independently of the grouping, there was a significant correlation between proprioception and sports activities. Patients with low proprioception deficiency showed significantly better scores for sports activities. (r = -0.452, p = 0.018,).

DISCUSSION

Our study observed that patients who underwent ACL stump resection returned to sports activities more quickly. There was no significant relationship between stump resection and proprioception deficit at the 6th month, but we think that in reducing the number of receptors in the sensory nerve endings, stump resection positively affects pain scores, thus increasing the athletic activity scores. Better athletic scores were seen in patients with less proprioceptive deficit. We concluded that proprioception is an essential factor affecting the success of ACL reconstruction.

Although mechanical stability is achieved after reconstruction, the sense of joint position may be lost, causing poor functional results [9, 10]. After reconstruction, lost position sense and proprioception may cause degenerative changes in cartilage structures; grafts that exceed normal strength and introduce necessary tension [11] are required for a satisfactory outcome and proprioceptive sensation restoration [12]. The density of mechanoreceptors found in stump tissue may help provide this restoration [4, 13]. For mechanoreceptors to function in the remaining stump, the stump must be integrated into the graft at the appropriate tension. Although sufficient tension was provided in the stump in the six months of the study, active nerve stimulation could not be generated due to the possible incomplete neural regeneration.

Recovery of proprioceptive sense in professional athletes is essential to re-adapt to sports, recovering former prowess and preventing re-ruptures. In athletes, motor function is stimulated much faster by processing proprioceptive, visual, and auditory senses than normal individuals. Athletes who cannot achieve rapid re-adaptation may become prone to re-rupture or secondary injuries due to abnormal knee kinematics [14]. Though athletes develop keen proprioception due to intense exercise [15, 16], they experience a profound loss of proprioception when returning to sports after reconstruction [17, 18].

Table 3. Functional score, proprioception (degrees), flexion and extension muscle strength values (Newton/meter) at 1st -3rd and 6th month

	Stump-preserving surgery group	Conventional surgical group	*p* value**
KOOS* (point)			
1st month	46.4 (32.1)	59.2 (29.5)	0.730
3rd month	78.6 (26.3)	70.6 (20.5)	0.415
6th month	78.6 (28.3)	85.1 (13.3)	0.277
Oxford score (point)			
1st month	28.0 (16)	27 (15)	0.805
3rd month	40 (11)	41.5 (13)	0.941
6th month	40 (11)	42.5 (7.5)	0.294
Proprioception (º)			
1st month	4.33° (4.67)	6.83° (7.83)	0.458
3rd month	1.66° (3)	3.5° (4.3)	**0.010**
6th month	1° (2.3)	3.7° (4)	0.195
Flexion strenght (Newton.meter)			
1st month	39 (51)	51 (43.5)	0.711
3rd month	80 (26)	87.5 (50)	0.621
6th month	89 (37)	117 (62)	0.256
Extension strength (Newton.meter)			
1st month	65 (67)	53 (67)	0.729
3rd month	99 (50)	108 (61)	0.805
6th month	141 (99)	150 (107.5)	0.388

Data are shown as median (interquartile range). *KOOS =Knee Injury and Osteoarthritis Outcome Score, ranges from 0 to 100; with higher scores indicating better results.

**Mann-Whitney U test

Table 4. Statistical difference between the two groups of KOOS subunits at 3-6 months

	Stump-preserving surgery group	Conventional surgical group	*p* value$^{\Omega}$
KOOStotal⁎ (point)	74.3 (18.2)	83.2 (10.8)	**0.013**
Symptoms	69.9 (21.2)	82 (10.2)	**0.036**
Pain	75.8 (17.6)	86.6 (11)	**0.048**
Function, daily living	82.2 (16)	90.2 (10)	0.146
Function, sports and recreational activities	61.4 (29.3)	71.6 (19.1)	**0.009**
Quality of life	65.8 (23.6)	63.5 (22.5)	0.141

Data are shown as median (interquartile range) ⁎KOOStotal = Knee Injury and Osteoarthritis Outcome Score-total, ranges from 0 to 100; with higher scores indicating better results.

$^{\Omega}$Mann-Whitney U test

A proprioception deficit of 5 degrees or more adversely affects clinical outcomes [18]. Our study observed that patients with low proprioceptive deficits returned to sports activities earlier. However, there is no significant benefit of stump preservation in the first six months, and multicenter long-term results with higher patient numbers are needed to arrive at a definite conclusion.

Another measure of the success of surgical treatment is the level of muscle strength recovery. Decreased afferent nerve receptors reduce proprioception and inhibit muscle. It is thought that the loss of mechanoreceptors in the ACL impairs the ligament-muscle reflex between the ACL and the quadriceps and prevents high-threshold motor activation during voluntary quadriceps contraction [19]. However, whether more flexor or extensor muscle strength is lost is still being determined. In knees reconstructed with hamstring autograft, decreased flexion strength before and after surgery [20] and decreased quadriceps strength in reconstructions with patellar tendon seem more evident [21]. It has been generally observed that hamstring reconstructions cause weaker muscle strength and may affect knee stability in the long term [22]. Damage to the hamstring muscles during graft removal may cause more significant muscle strength loss. Hamstring autograft was used in all patients in our study, and no statistically significant difference was found between the two groups regarding knee flexor and extensor strengths over six months. The inability to reach preoperative muscle strength in the stump-preserving surgery group is due to worse pain parameters and muscle inhibition results.

Return to functional life is closely related to patient satisfaction. Despite reports of good patient outcomes in many comprehensive studies of ACL reconstruction, surgery and rehabilitation in the last 20 years, 28% of patients are still dissatisfied with the surgery regarding knee function [10]. In the literature, poor outcomes have been associated with developing flexion contracture, continued laxity and pivot shift, effusion, and wound tenderness [20]. However, proprioception defect instability may diminish function and decrease satisfaction with the surgical treatment [23]. No significant difference was observed in studies evaluating IKDC (International Knee Documentation Committee Qestionnaire) and Lysholm scores and functional status in stump-preserved and non-preserved patient groups [24]. However, in the secondary arthroscopy performed on the same patients, it was observed that the synovial coverage in the stump-preserved group was significantly higher than those in the unprotected group [24]. Similar results were seen in patients with partial tears and those undergoing selective ligament strengthening.

No significant difference was found in IKDC, Lysholm and KOOS scores evaluated at 6 and 12 months [25]. The changes in pivot shift and anterior laxity were compared in the same patient group, and there was no significant difference between the two groups [25]. In a study in which subgroups were evaluated according to the stump coverage rate in stump-preserving reconstructions with bone-tendon-bone, no significant difference was observed between the groups in IKDC and Lysholm scores [26]. However, there are results in the literature claiming the opposite. In the study of Naylor *et al*. [27], better ACL-Quality of Life (ACL-QOL) and IKDC scores were found in the stump-preserved group compared to the non-preserved group. Nonetheless, it should be remembered that patient satisfaction is an objective evaluation [28-30]. Our study found a significant difference in the third and sixth month KOOS pain, complaints, and sports parameters, which supports the literature. This difference in the early postoperative period may be due to more intra-articular debridement in the standard surgery group and excision of the receptors carrying pain sensation in the Hoffa. In addition, we think that removing the free nerve endings on the stump with debridement may contribute positively to early rehabilitation by preventing pain in the standard surgical group. The early reduction of neural impulses from these receptors ensures earlier functional recovery. However, long-term studies are needed to reveal the pros and cons for the patient definitively.

Limitations

The most important limitation of our study was the short duration of patient follow-up. Further, more than a few patients may be needed to draw firm conclusions. The HUMAC NORM II® isokinetic dynamometer enabled us to obtain targeted data from the patients. We did not use an arthrometer because we aimed to evaluate muscle strength and proprioception rather than stability.

CONCLUSION

Our study found no difference between proprioception during the 6-month follow-up period after stump-preserving surgery and conventional surgery. When all patients were evaluated independent of the surgical groupings, those with less proprioceptive sensory deficit returned to sports activities earlier and arrived at their previous performance levels more efficiently.

Authors' Contribution

Study Conception: NE, HÇ, SA; Study Design: HÇ, SA; Supervision: NE, HÇ, AY; Funding: N/A; Materials: N/A; Data Collection and/or Processing: SA, HÇ, MY; Statistical Analysis and/or Data Interpretation: TOB, AY, NE; Literature Review: NE, KT, MY; Manuscript Preparation: NE, HÇ, KT and Critical Review: TOB, AY, MY.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1429-1437 Original Article

DOI: 10.18621/eurj.1249233 *Obstetrics and Gynecology*

The effect of hypertension in pregnancy and central nervous system anomalies on fetal brain development

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ABSTRACT

Objectives: The aim of this study was to investigate whether maternal hypertension affects fetal brain maturation, and to examine whether treatment with magnesium sulfate has a protective effect on the fetal brain.

Methods: A total of 26 fetuses, including 11 dead fetuses of pregnant women who were found to have hypertension and whose pregnancy was terminated due to this reason, and 15 fetuses who did not have this risk factor but died for various reasons as the control group, were included in the study. Brain tissue samples were evaluated for the presence of morphological and histopathological changes, as well as apoptotic cells. The morphologies of the samples were examined in sections stained with hematoxylin-eosin (H&E), and apoptosis was examined with light microscopy by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method.

Results: In the control group, it was observed that the brain tissue had a morphological structure compatible with the development weeks. In the hypertension group, there were no bleeding foci and brain tissues mostly preserved morphological features similar to control patients. While edema was detected in 45.4% of the infants in the hypertension group, no edema was observed in 54.6%. In the hypertension group, Grade 1 necrosis was observed in 63.6% of the samples, Grade 2 necrosis was observed in 9.1%, and no necrosis was observed in 27.3% of the samples.

Conclusions: Based on the findings of this study, it can be concluded that maternal hypertension increases neurological maturation by causing vasodilation in the fetal brain, increasing blood flow, and decreasing cell death.

Keywords: Maternal hypertension, fetal development, brain, edema, hemorrhage, necrosis, apoptosis

Feto-placental development and functioning are vulnerable to maternal risk factors such as diabetes, obesity, and hypertension, creating an unfavorable environment for healthy fetal development [1]. Hypertensive disorders during pregnancy, such as gestational hypertension, pre-eclampsia, and eclampsia, are common medical complications affecting up to 9% of women [2]. These disorders expose the fetus to general stress and alter the intrauterine environment, leading to changes in the structural and phenotypic characteristics of the fetus [3]. As a result, changes occur in the structural or phenotypic characteristics of

Received: ***February 14, 2023;*** *Accepted:* ***March 26, 2023;*** *Published Online:* ***April 13, 2023***

How to cite this article: *Albayrak M, Köse MF, Anlar B. The effect of hypertension in pregnancy and central nervous system anomalies on fetal brain development. Eur Res J 2023;9(6):1429-1437. DOI: 10.18621/eurj.1249233*

e-ISSN: 2149-3189

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the fetus. For example, fetuses exposed to hypertension are more sensitive to auditory stimuli after birth [4].

Preeclampsia, a common cause of hypertension during pregnancy, is a vascular disease that affects 3-8% of pregnancies worldwide [5]. Its pathogenesis involves placental and trophoblastic changes, leading to a decrease in uteroplacental blood flow and fetoplacental hypoxemia. Severe preeclampsia can result in eclampsia or mortality for both the mother and fetus [6, 7]. In addition, prenatal inflammation is the most blamed factor for fetal brain injury, while vascular endothelial growth factor (VEGF) decreases in the fetal lung due to maternal hypertension [8, 9]. Maternal hypertension has been reported to increase fetal brain maturation by affecting the gyrus and sulci morphology and neurophysiological methods [10, 11]. Recent studies have shown that molecular and apoptotic mechanisms play a crucial role in fetal central nervous system development Preeclampsia. While congenital anomalies of the CNS occur in around 1/1000 cases, detailed fetal sonographic examination has made it easier to detect these malformations [12]. There is limited data available on the neurodevelopmental outcomes of fetuses of mothers whosepregnancies were complicated by hypertension. Therefore, this study aimed to investigate the impact of maternal hypertension on fetal brain maturation and the protective effects of treatment methods applied during pregnancy, especially magnesium sulfate.

METHODS

This study was conducted in Ankara Etlik Maternity and Gynecology Training and Research Hospital and Hacettepe University Faculty of Medicine, Department of Histology-Embryology. The study was approved by the local committee of our hospital, and all parents were informed about the objectives of the study and gave written informed consent.

A total of 26 fetuses were included in the study, including 11 fetuses of pregnant women who were found to have hypertension (preeclampsia and/or chronic hypertension) and whose pregnancy was terminated due to this reason, and 15 fetuses who did not have this risk factor but died for various reasons as the control group. Fetuses that had died intrauterine 12 hours before the abortion were excluded from the study due to the possibility of incorrect results as autolysis of the cells. Additionally, fetuses of parents who refused participation were also excluded.

Pregnant women's age, gravida gestational week, and infants' birth weight were recorded. Pregnancy weeks were determined precisely by taking the last menstrual period and ultrasonographic measurements of the pregnant women included in the study. Spontaneous abortion or delivery was observed during labor in the control group. In case of any obstetric problem, the pregnancy was terminated by cesarean section. Treatments given in the hypertension group (antenatal steroids, tocolysis, magnesium sulfate, and other antihypertensive treatments) were recorded.

After normal vaginal delivery, cesarean section, or abortion, fetuses were macroscopically examined and their sex, weight, and malformations were recorded. Then, an incision was made at the level of the anterior fontanelle to access the frontal lobe of the fetal brain by passing through the skin, subcutaneous tissue, and dura mater. Full-thickness tissue samples were taken from the frontal lobe, including the cortex and subcortical layers, and the ventricular wall as much as possible. All samples were taken within 12 hours at most after delivery, abortion, or death of the baby. The material taken for hematoxylin-eosin (H&E) staining was fixed in formaldehyde at room temperature. The material taken for the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was transferred to the laboratory environment at -80°C nitrogen medium. Brain tissue samples were evaluated for the presence of morphological and histopathological changes and apoptotic cells. The morphologies of the samples were examined in sections stained with H&E, and apoptosis was examined with light microscopy by the TUNEL method. Histological evaluations of H&E stained specimens were classified as follows: Grade 1: Necrosis in very few cells, Grade 2: Necrotic changes in one area and/or a small number of cells, Grade 3: Necrotic changes in multiple sites and multiple cells. Stasis was evaluated with hemorrhage foci in the cortex, medulla, and periventricular areas and dilatation of vessels. Localization of edema was detected. Edema in both cortex and medulla was evaluated as diffuse edema.

Statistical Analysis

The data obtained in this study were statistically evaluated using the SPSS (Statistical Package for Social Sciences, IBM Inc. Armonk, NY, USA) statistical software. The normality of the variables was evaluated with the Kolmogorob-Smirnov test. Since the variables were non-normally distributed, the Mann-Whitney test was used to compare continuous variables between two groups. Categorical variables were compared using the Chi-square method. Continuous variables were expressed with median, min-max values, mean ± standard deviation, while categorical variables were given as frequency and percentage. A p - value of less than 0.05 was considered statistically significant.

RESULTS

A total of 26 patients were included in the study with 11 patients being in the hypertension group and 15 patients in the control group. Demographic features and obstetric history of the groups are given in Table 1.
As seen in Table 1, gestational week and birth weight were significantly higher in the hypertension group compared with the control group (both $p < 0.001$). There were no significant differences between the two groups in terms of maternal age and gravida (both $p > 0.05$). Oligohydramnios was detected in two patients (18.2%) in the hypertension group, while it was due to membrane rupture in two (13.3%) patients in the control group. Intrauterine growth retardation (IUGR) was found in four (36.4%) infants in the hypertension group, while none of the infants in the control group had IUGR. Two of all pregnancies were terminated by C/S, and the others were terminated by vaginal delivery. While 27.8% of the infants were stillborn, 72.2% of them died shortly after birth. Magnesium sulfate was administered to six (54.5%) patients with hypertension.

Comparison of the groups in terms of edema, necrosis, and intracranial hemorrhage in the examined brain tissues is presented in Table 2.

Hematoxylin & Eosin Results

Control Group

In the examination of fetal brain samples of 15 patients in the control group, it was observed that the brain tissue had a morphological structure compatible with the development weeks. Neuron nuclei and cytoplasms were in normal histological structure. Only one 17-week-old sample of brain tissue had areas of edema in the neuropil. No edema was observed in other samples (Fig. 1).

Hypertension Group

There were no bleeding foci, and brain tissues mostly preserved morphological features similar to those of control patients. Although there was advanced dilatation of the vessels in all of the samples, it was noted that most of them did not have stasis (Fig. 2). In two of the samples with edema, the vessels were both very dilated and occluded. It was observed that necrotic cell density increased in these samples. Necrotic cells were less common in brain samples without edema (Fig. 2).

Table 1. Demographic features and obstetric history of the groups

		n	Mean	SD	Median	Min	Max	*p* value
Maternal age	**C**	15	29.5	6.7	31	18	43	0.646
	HT	11	27.5	4.8	25	21	33	
Gestational week	**C**	15	21.7	5.9	20	14	37	**< 0.001**
	HT	11	29.7	3.3	30	25	35	
Birth weight	**C**	15	598.7	658.2	400	100	2700	**< 0.001**
	HT	11	1068.2	417.3	900	600	1700	
Gravida	**C**	15	2.7	1.2	3	1	5	0.293
	HT	11	2.1	0.7	2	1	3	

HT = hypertension group, C = control group, SD: standard deviation

Table 2. Edema, necrosis and intracranial hemorrhage in the examined fetal brain tissues

		C		HT		Total	
		n	%	n	%	n	%
Edema	Yes	1	6.7	5	45.4	6	23.08
	No	14	93.3	6	54.6	20	76.9
	Total	15	100.0	11	100.0	26	100.0
Necrosis	Grade 1	4	26.7	7	63.6	11	42.31
	Grade 2	1	6.7	1	9.1	2	7.69
	Grade 3	0	0.0	0	0.0	0	0.0
	No	10	66.7	3	27.3	13	50.0
	Total	15	100.0	11	100.0	26	100.0

HT = hypertension group, C = control group

While edema was detected in 45.4% of the infants in the hypertension group, no edema was observed in 54.6%. Edema was detected in 6.7% of the control group, while there was no edema in 86.7% of the cases.

In the hypertension group, Grade 1 necrosis was observed in 63.6% of the samples, Grade 2 necrosis was observed in 9.1%, and no necrosis was observed in 27.3% of the samples. In the control group, 26.7% of the samples had Grade 1 necrosis, 6.7% had Grade 2 necrosis, while 66.7% did not have necrosis. While bleeding was not observed in the hypertension group, 20% of the control group had bleeding. Since the number of cases was insufficient, statistical significance could not be compared.

Apoptosis Findings Obtained by the TUNEL Method

A large number of diffuse apoptotic cells, especially dense in the inner cortex, were detected in 15

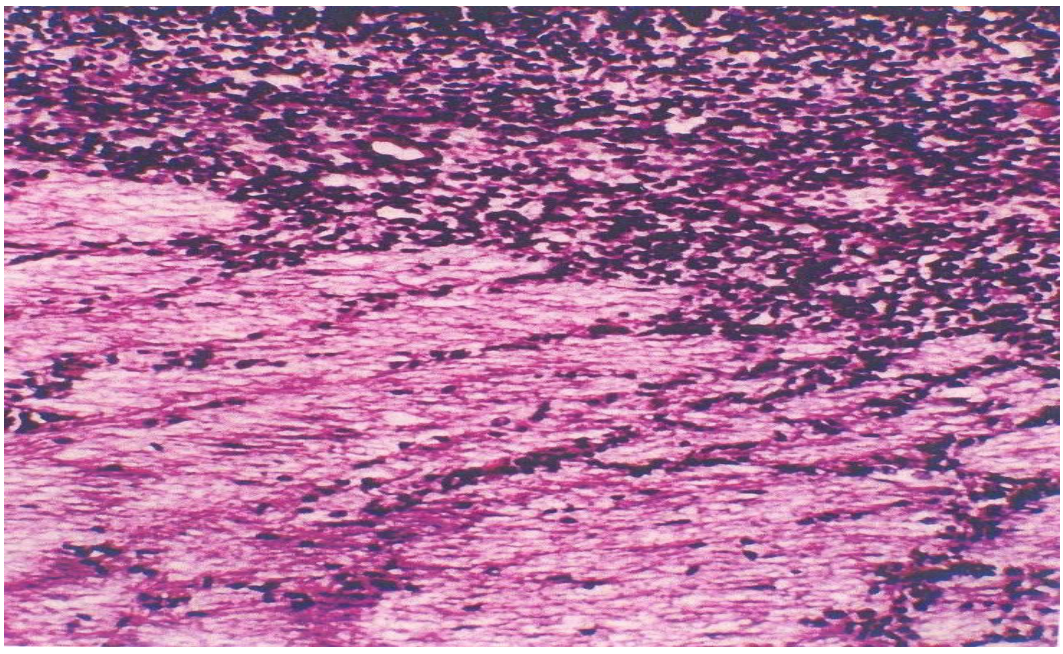

Fig. 1. Cells in the neuroepithelium have a normal histological structure in the fetal brain sample of a 20-week and 2-day infant in the control group (H&E × 20).

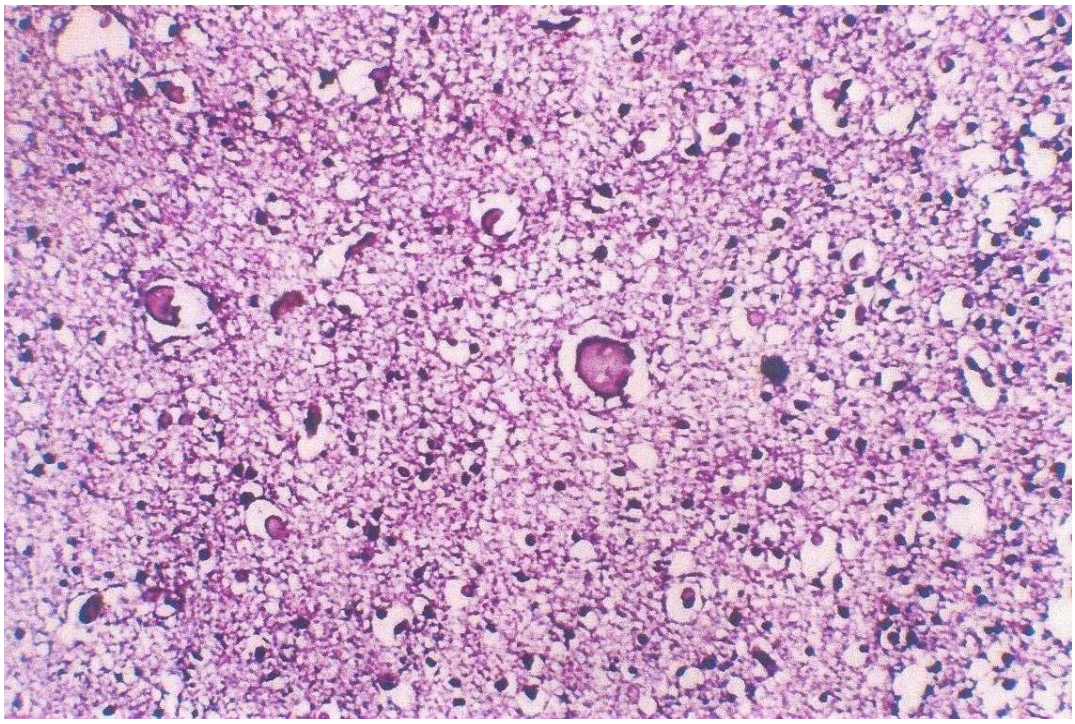

Fig. 2. Diffuse edema is noted in the medulla of the 31-week-old fetus in the hypertension group. While dilatation is observed in capillaries, there is stasis with advanced dilatation in larger vessels (H&E × 20).

samples in the control group (physiological apoptosis) (Fig 3).

In the samples from the hypertension group, although the number of apoptotic cells was less compared to the control group, it was observed that they were more concentrated in the cortex. TUNEL apoptotic cells were particularly prevalent in the outer cortex in the hypertension group (Fig. 4).

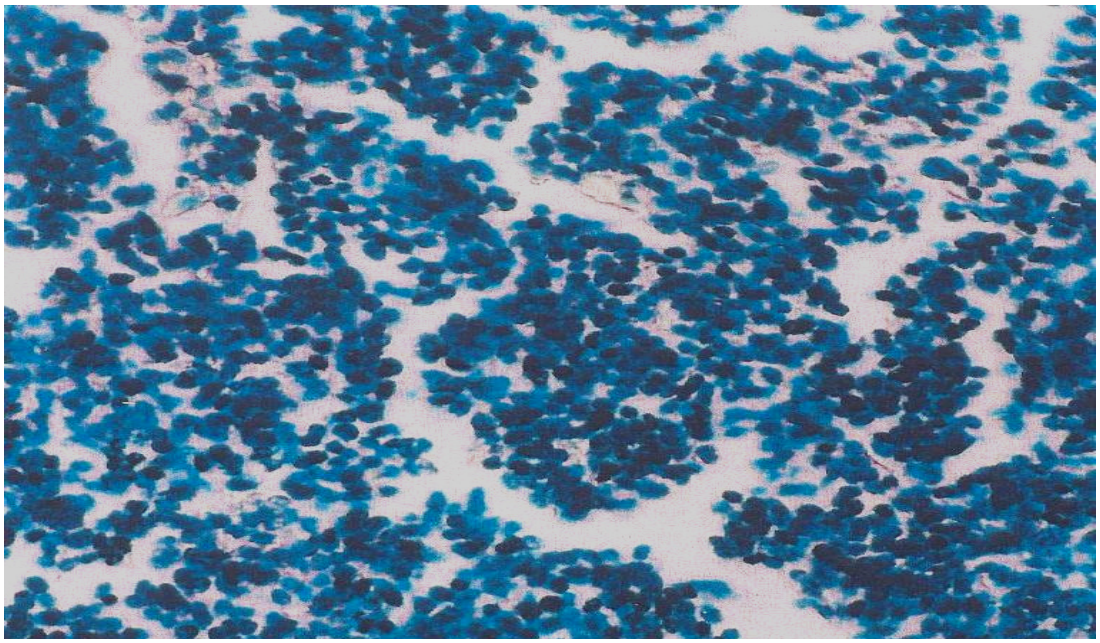

Fig. 3. Diffuse TUNEL-positive apoptotic cells in a 20-week-old fetus (TUNEL × 40).

Fig. 4. TÜNEL positive apoptotic cells (arrow) in 22-week-old hypertension group fetal brain sample (TUNNEL × 40).

DISCUSSION

In this study, before examining the effects of hypertension on the fetal brain, fetal brain samples in the control group were evaluated to get an idea about normal brain development. In the light of the results obtained with hematoxylin-eosin (H&E) staining, which was the first of the methods used for this purpose, it can be said that the neuron nuclei and cytoplasms in the normal fetal brain have a regular histological structure and tissue edema, vessel dilatation and cell necrosis are not normally encountered findings. Despite the absence of abnormal morphological findings, it has been shown that apoptotic cells detected by the TUNEL method in different parts of the fetal brain were found in all normal fetuses, which is consistent with other previous studies showing the presence of cell death in different parts of the brain at different times [13-16].

It was reported that the definition of normally occurring apoptosis may provide a basis for further studies directed at central nervous system (CNS) malformations [17]. In a study investigating apoptosis in the developing brain, Simonati *et al*. examined the fetuses between 12-23 weeks and found apoptotic cells at increasing rates towards the 21st week, especially in the subventricular and ventricular areas, and they said that apoptotic cells were found only around 21-22 weeks in the outer layers [18]. In a study by Jiang *et al*. [19], a progressive increase in the number of TUNEL-positive cells was observed in the developing cerebral cortex. In another study examining fetuses at earlier weeks, it was shown that there were sparse TUNEL positive cells in the ventricular layer of the neural tube at 4-5 weeks, and the whole brain, particularly the ventricular and subventricular areas, contained apoptotic cells at 7-8 weeks [20].

In general, it can be said that cell death occurs from the 4th week of pregnancy and it starts from the inner layers and shifts outwards after the middle of pregnancy and starts to increase from the 18th week. In a study by Toyoshima *et al*., it was suggested that proliferation, migration, and neural cell death occur during midgestation in the fetal brain [21]. In the presented study, normal fetuses between 14-37 weeks were examined and diffuse apoptotic cells were detected, especially in the intermediate area and the inner cortex, which includes the subplate. The absence of apoptotic cells in the medulla, which includes the ventricular and subventricular areas, was attributed to the advanced gestational weeks of the fetuses. In our study, the mean gestational week was found as 29.7 in the hypertension and 21.7 in the control group. Physiological apoptosis observed in the inner cortex is thought to serve for the disappearance of these regions in a short time after birth [18].

It is not always easy to determine the effects of maternal hypertension on the development of the infant. Because this development is affected by many other parameters such as IUGR and prematurity. The investigation of how the fetus is affected in the case of maternal hypertension started with the examination

of perinatal mortality rates and different results were obtained. In a study by Nakamura *et al*. [22], maternal hypertensive disorders of pregnancy was found to be associated with an increased risk for in hospital death, but it was also associated with a lower risk for mortality and adverse neurological outcomes in extremely and very preterm infants if all covariates except hypertensive disorders of pregnancy were identical. In a study by Ancel *et al*. [23], it was determined that the survival rate of preterm babies of hypertensive mothers was 96.3% and low perinatal mortality rate was associated with good perinatal care. In another study by Piper *et al*. [24], it was demonstrated that perinatal mortality in preterm infants of hypertensive mothers was decreased compared to infants of normotensive mothers, but perinatal mortality in term infants was higher in infants of hypertensive mothers, and they attributed this result to the fact that the stress created by hypertension reduces mortality due to prematurity. Since infants of hypertensive mothers are exposed to increased placental vascular resistance, it is thought that they undergo some adaptations during intrauterine life and, thanks to these adaptations, they adapt better to extrauterine life after birth. Conversely, in a study by Huang *et al*. [25], maternal hypertensive disorders of pregnancy, particularly eclampsia and severe preeclampsia, was found to be associated with increased risks of overall mortality and various cause specific mortalities in offspring from birth to young adulthood.

In a study by Härkin *et al*. [26], the infants of preeclamptic mothers exhibited an increased risk of intrauterine growth retardation; however, despite this serious complication, these infants exhibited a significant decrease in the risk for severe intraventricular hemorrhage. Epidemiological studies show that cerebral palsy rates increase in infants of pregnant women with hypertension. On the other hand, Gray *et al*. [23] showed that the rates of cerebral palsy and periventricular hemorrhage were lower in preterm infants of hypertensive mothers compared to the control group, and this low rate was found independent of magnesium sulfate treatment. It is biologically possible for magnesium sulfate, one of the drugs used in the treatment of maternal hypertension, to prevent neurological damage. In vitro and in vivo studies have shown that asphyxia, trauma, and N-methyl D-aspartate (NMDA)-mediated neuronal damage can be prevented with magnesium sulfate [27]. The magnesium ion is required for numerous cellular processes such as glycolysis, oxidative phosphorylation, protein synthesis, DNA and RNA aggregation, and maintaining plasma membrane integrity. Magnesium can reverse the effects of excitatory amino acids that initiate neuronal damage by binding to NMDA receptors, such as glutamate, by competitive inhibition with calcium . In addition, magnesium has been shown to be a cerebral vasodilator [28]. Due to these properties, magnesium sulfate can be said to be a neuroprotective agent. In our study we administered magnesium sulfate in 6/11 (54.5%) patients.

According to research, magnesium sulfate has neuroprotective properties. According to the findings of the current study, maternal hypertension promotes neurological maturation by causing vasodilation in the fetal brain, increasing blood flow, and decreasing cell death. Although these effects were observed in patients who did not receive magnesium sulfate in this study, the existence of a relationship between maternal hypertension and neurological maturation due to the small number of patients and the role of the drugs used in treatment in this relationship is an issue that requires further investigation.

In chronic fetal hypoxia due to hypertensive diseases of pregnancy, there is significant vasoconstriction in the splanchnic bed, pulmonary bed and musculoskeletal systems, while there is an increase in blood flow to the brain, heart and adrenal glands [29]. In our study, vasodilatation, which is thought to develop as a part of the protective effect on the brain, was found in the vessels of the fetal brain tissue in all babies of hypertensive mothers. This finding suggests that pregnancies with brain sparing effect should be terminated in obstetric management before an increase in the fetal cerebral arteries pulsatility index begins. While vasodilation was a finding in all patients in the hypertension group, the absence of significant cell necrosis and the absence of hemorrhage foci are consistent with studies stating that vasodilation in fetal brain vessels to protect the brain does not cause hemorrhage and ischemia in the future.

Limitations

The main limitation of this study is the small number of patients. Because of this, we could not compare edema, necrosis, hemorrhage and apoptosis statistically between the groups. However, given the limited

studies on this issue we believe that our findings could be guiding for further comprehensive studies with a larger series of patients.

CONCLUSION

In the light of the findings of this study, it can be said that maternal hypertension increases neurological maturation by causing vasodilation in the fetal brain, increasing blood flow and decreasing cell death. The effects of hypertension on neurological maturation may depend on the effects of drugs used in the treatme and the most important of these is magnesium sulfate.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1438-1443 Original Article

DOI: 10.18621/eurj.1340536 *Medical Genetics*

Next-generation sequencing panel test results in pediatric patients with progressive familial intrahepatic cholestasis: a single-center experience

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ABSTRACT

Objectives: The aim of this study is to reveal the diagnostic yield of the progressive familial intrahepatic cholestasis (PFIC) gene panel that we have used in the diagnosis of this patient group, which accounts for approximately 10% of cholestatic liver disease, and to report the clinical findings of our patients with the detected variants.

Methods: In this study, we retrospectively evaluated the results of molecular genetic analysis of pediatric patients whose PFIC gene panel contained the *ATP8B1, ABCB11, and ABCB4* genes.

Results: In 10 patients, 12 different variants were detected that could explain the PFIC clinical picture. Three of these variants were considered novel variants.

Conclusions: Our study demonstrates the usefulness of the NGS panel in diagnosing pediatric patients with PFIC findings. This diagnostic method also contributed to the variant spectrum of PFIC-related genes.

Keywords: Progressive familial intrahepatic cholestasis, novel, *ABCB11, ABCB4, ATP8B1*

Cholestasis, which may be intrahepatic or extrahepatic and caused by genetic or nongenetic multifactorial conditions, is the reduction or disruption of bile flow produced by hepatocytes and cholangiocytes [1]. insufficient bile flow due to hereditary or acquired diseases causes bile contents such as bilirubin, bile acids, and lipids to accumulate in the liver, resulting in high bilirubin and bile salt levels in the liver and blood and irregular lipid metabolism. While jaundice, itching, and clayey stools that develop due to hyperbilirubinemia are usually observed in patients, bleeding episodes in the form of intracranial hemorrhage is rare [2]. Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of genetic diseases that show signs of cholestasis and lead to liver failure. It is a rare disease with an estimated prevalence of 1-2 per 100,000 births, although the exact prevalence is unknown. In this group of diseases, which is inherited in an autosomal recessive manner, many different types have been defined, with the first three types being the best known [3]. PFIC1 and PFIC2 usually occur in infancy or early childhood. PFIC3 can occur later in infancy, childhood and even young adulthood. All three types are associated with hepatocellular transport system genes involved in bile formation. PFIC1 is caused by variants in the *ATP8B1* gene, re-

Received: ***August 10, 2023;*** *Accepted:* ***September 19, 2023;*** *Published Online:* ***September 21, 2023***

How to cite this article: *Topak A. Next-generation sequencing panel test results in pediatric patients with progressive familial intrahepatic cholestasis: a single-center experience. Eur Res J 2023;9(6):1438-1443. DOI: 10.18621/eurj.1340536*

e-ISSN: 2149-3189

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sulting in the deficiency of familial intrahepatic cholestasis 1 (FIC1) protein. *ATP8B1* protein is located on the canalicular membrane of hepatocytes. It is responsible for transporting phospholipids (phosphatidylserinne and phosphaticylethanolamine) inside the canalicular membrane. PFIC2 is caused by the variants in the *ABCB11* gene, resulting in the deficiency of bile salt export pump (BSEP). BSEP is a transporter protein, expressed at the canalicular membrane of hepatocyte. It is the major exporter of bile acids from the hepatocyte to the canaliculi against a concentration gradient. PFIC3 is caused by variants in the *ABCB4* gene, resulting in the deficiency of the multidrug resistant 3 (MDR3) protein. It is expressed in the canalicular membrane of hepatocytes and acts as a floppase responsible for the biliary secretion of phospholipids [4]. In recent years, the use of next-generation sequencing (NGS) has increased to obtain a molecular diagnosis in PFIC. NGS is a technology proposed for the molecular diagnosis of PFIC and compared to classic Sanger, NGS allows rapid sequencing with more information at lower costs [5].

In this study, the results of molecular genetic analysis of pediatric patients examined with cholestasis whose *ATP8B1, ABCB11*, and *ABCB4* genes responsible for PFIC types 1, 2, and 3 were sequenced by NGS in our laboratory were retrospectively evaluated.

METHODS

Thirty-seven patients who presented to the Medical Genetics Department Bursa Yüksek Ihtisas Training and Research Hospital with a preliminary diagnosis of PFIC between 2015 and 2019 were retrospectively evaluated. Informed consent for genetic testing was obtained from all patients or their legal guardians. The local clinical research ethics committee granted approval for the study (2011-KAEK-25, 2019/08-01).

An NGS platform (NextSeq 500, Illumina, San Diego, California, USA) was used for this study. The PFIC panel (An NGS platform (NextSeq 500, Illumina, San Diego, California, USA) was used for this study. The PFIC panel (PFIC Solution, Sophia™, Saint Sulpice, Switzerland) was studied in patients, and all steps were performed according to the original manufacturer's protocol. The raw data obtained were filtered and analyzed using the appropriate program (Sophia DDM, Saint Sulpice, Switzerland). Considering the clinical findings, and family history of the patients, variants that could be significant were determined. These significant variants, which were detected during the analysis of the PFIC panel and could be associated with any disease, were evaluated using the Human Gene Mutation Database (HGMD) [6]. This allowed us to determine whether the change had been reported in the literature and, if so, to which disease it was associated. For alterations not reported in the literature, classification by American College of Medical Genetics and Genomics (ACMG) criteria and frequency in population studies (gnomAD; Genome Aggregation Database) were determined using the Varsome Analysis Program (https://varsome.com/) [7, 8]. This panel includes PFIC types 1, 2, and 3 genes.

RESULTS

Patients who were referred with a prediagnosis of PFIC, particularly with evidence of cholestasis, were included in the study. In 10 (27%) of 37 patients whose PFIC gene panels were examined, variants were detected in the *ATP8B1, ABCB11,* and *ABCB4* genes that could be associated with the clinical picture of patients (Table 1). Nine of the patients had symptoms of cholestasis. One patient had no other findings except mild jaundice. Two patients, one with PFIC1 and other with PFIC2, underwent liver transplant. The mean age at diagnosis of patients with variants in the *ATP8B1, ABCB11* and *ABCB4* genes was 24 months. Consanguinity between parents was observed as 60%. A total of 12 different variants were detected in the patients, most of which were missense. Among these, 8 were pathogenic/likely pathogenic variants. Three variants classified as VUS have been previously reported in patients with PFIC. Three of these variants had not been previously reported in the literature and were considered novel. When the novel alterations were evaluated according to the ACMG criteria, one variant was classified as being of uncertain clinical significance and the other two as likely pathogenic and pathogenic variants. The novel frameshift variants p.Gln989Serfs*18 and p.Arg1249Serfs*39 in the

Table 1. General variant list and clinical findings

Patient No	Gender	Age	Gene (Transcript)	Variant	Variant type	Zygosity	HGMD/Novel	ACMG classification	Inherited from	Clinical findings
1	F	5 mos	*ATP8B1 (NM_005603)*	c.2854C > T (p.Arg952*)	Nonsense	Hom	CM043830	P	Parents	I, J, D, C, ALF, LT
2	F	3 mos	*ABCB11 (NM_003742)*	c.2842C > T (p.Arg948Cys)	Missense	Hom	CM081493	P	Parents	I, J, C, ALF
			ATP8B1 (NM_005603)	c.1798C > T (p.Arg600Trp)	Missense	Het	CM043820	LP	Mother	
3	M	2 yrs 2 mos	*ABCB11 (NM_003742)*	c.1160G > A (p.Arg387His)	Missense	Het	CM103533	VUS	Mother	C, H, S, GR
				c.3703C > T (p.Arg1235*)	Nonsense	Het	CM081481	P	Father	
4	M	7 yrs 7 mos	*ABCB4 (NM_000443)*	c.2858C > A (p.Ala953Asp)	Missense	Hom	CM055899	P	Parents	I, J, D, C, H
5	M	13 mos	*ABCB4 (NM_000443)*	c.2858C > A (p.Ala953Asp)	Missense	Hom	CM055899	P	Parents	I, J, C, H
6	M	5 yrs 6 mos	*ABCB11 (NM_003742)*	c.2708T>G (p.Val903Gly)	Missense	Hom	Jeyaraj *et al.* [16]	VUS	Parents	H, C
7A	F	1y	*ABCB11 (NM_003742)*	c.2636T > G (p.Ile879Arg)	Missense	Hom	Bakır *et al.* [22]	VUS	Parents	J, C, H, S, GR
7B	F	5 yrs 8 mos	*ABCB11 (NM_003742)*	c.2636T > G (p.Ile879Arg)	Missense	Hom	Bakır *et al.* [22]	VUS	Parents	J
8	F	3 mos	*ABCB4 (NM_000443)*	c.3100A > G (p.Ile1034Val)	Missense	Hom	Novel	VUS	Parents	I, J, C, H
9	M	2 mos	*ABCB11 (NM_003742)*	c.403G > A (p.Glu135Lys)	Missense	Het	CM092737	LP	Mother	I, J, C, ALF, LT
				c.2965delC (p.Gln989Serfs*18)	Frameshift	Het	Novel	P	Father	
			ABCB4 (NM_000443)	c.3768_3769delAG (p.Arg1249Serfs*39)	Frameshift	Het	Novel	LP	Father	

Variants significant for progressive familial intrahepatic cholestasis (PFIC) are listed with their clinical manifestations and characteristics of the variants. Variants reported in the literature are given with the HGMD number. Variants not reported in the literature are given by evaluating according to ACMG criteria.

F = female, M = male, HDMD = Human Gene Mutation Database, ACMG = American College of Medical Genetics and Genomics, VUS = variants of uncertain significance, LP = Likely pathogenic, P = Pathogenic, Hom = Homozygous, Het = Heterozygous, I = itching, J = jaundice, D = diarrhea, GR = growth retardation, ALF = acute liver failure, C = cholestasis, H = hepatomegaly, LT = liver transplant, S = splenomegaly.

ABCB11 gene, and p.Ile1034Val in the *ABCB4* gene were predicted as 'damaging' by the SIFT/PROVEAN and PolyPhen-2 web software.

DISCUSSION

Variants in genes encoding hepatobiliary transport proteins can cause a wide range of cholestatic liver diseases, from PFIC to milder forms with limited episodes of cholestasis. PFIC is classified into subgroups depending on clinical examination, laboratory findings, and genetic defect. This disease, classically divided into three types (1-3), is known to be caused by biallelic pathogenic variants of *ATP8B1* (encoding FIC1 protein), *ABCB11* (encoding BSEP protein), and *ABCB4* (encoding MDR3 protein) [9, 10].

PFIC is a disease diagnosed in childhood. It ranges from symptoms suggestive of cholestasis, such as pruritus and jaundice, to liver findings that can lead to cirrhosis and liver failure, vitamin K insufficiency, diarrhea, and developmental delays due to malabsorption. Extrahepatic findings such as pancreatitis and hearing loss may be detected, and severe FIC1 defects occur as early as the first year of life [11]. According to our list, we detected a homozygous *ATP8B1* nonsense variant previously reported in the literature only in a 5-month-old female patient. For the first time in the literature, the *ATP8B1* gene p.Arg952* variant was discovered as compound heterozygous in three different patients (two with PFIC and one with BRIC) by Klomp *et al*. [12]. This variant, which has not been reported in biallelic form in the literature, was homozygous in our patient and resulted in a severe clinical course [4]. Our patient, who complained of jaundice and diarrhea in the first month after birth, developed liver failure within a short time and had to undergo liver transplantation at the age of 2 years. In addition to PFIC1, she had congenital hypothyroidism and umbilical hernia.

In our study, we detected different variants associated with PFIC2 in 6 patients from 5 different families. The variant p.Gln989Serfs*18 detected in the *ABCB11* gene of patient 9 was novel. It is generally believed that PFIC2, also known as BSEP defect, shows rapid progression of hepatic fibrosis [13]. Although we do not have sufficient data to support this finding, we see patients referred to our laboratory for molecular genetic analysis within the first five years of life. Indeed, the diagnoses of patients 2 and 9 were confirmed by molecular genetic analysis within a few months of birth. Vitale *et al*. detected the variant p.Glu135Lys on the *ABCB11* gene in a 16-year-old male patient as compound heterozygous with another pathogenic frameshift variant. The patient's liver symptoms, which began as neonatal jaundice and itching, progressed to failure requiring transplantation [14]. We detected the p.Glu135Lys variant as a compound heterozygote with the p.Gln989Serfs*18 variant, which was classified as possibly pathogenic according to ACMG criteria, in our 2-month-old male patient who had similar clinical findings to this case. Liver transplantation was performed in our patient, who rapidly developed liver failure at seven months of age. Moreover, in addition to the alterations causing PFIC2, we incidentally detected a likely pathogenic heterozygous alteration in the *ABCB4* gene. It is complicated to comment on the effects of this alteration, which he inherited from his healthy father and which has not been reported in the literature with respect to the patient's clinical findings. Similarly, we detected an incidental variant in patient 2. A heterozygous *ATP8B1* variant was detected together with a homozygous *ABCB11* variant causing PFIC2. It has been reported in the literature that heterozygous variant of the *ATP8B1, ABCB11*, and *ABCB4* genes can cause mild forms of cholestatic liver disease. However, as in patients 2 and 9, there are insufficient data in the literature on the effects of other incidental heterozygous alterations detected in addition to those causing the actual PFIC clinical picture on the clinical findings of patients [15]. This situation will become more precise as the number of patients and functional studies of the detected variants increase. Patient 6 with the homozygous c.2708T > G variant had hepatomegaly and cholestasis findings. Patients with the same variant reported by Jeyaraj *et al*. [16] had acute liver failure in addition to the findings in our patient. Patients 3 and 7A also had cholestasis and hepatosplenomegaly and developmental delay. Patient 7B, the sibling of patient 7A, had no other findings except mild jaundice. It has been reported in the literature that different phenotypes are observed in siblings with the same genotype. The presence of genetic, epigenetic, or environmental modifiers could partially explain the different expression of the disease in family

members with the same homozygous gene variant. The literature reports that splicing and frameshift variants in the *ATP8B1, ABCB11,* and *ABCB4* genes are usually associated with severe disease, whereas missense variants are associated with less severe disease. In our patient cohort, there were insufficient variants to evaluate these data [17, 18].

We detected homozygous *ABCB4* variants associated with PFIC3 in three cases from different families. Although there is no association between them, we found the same *ABCB4* variants in patients 4 and 5. In patient 8, we detected a novel homozygous variant of uncertain clinical significance (according to ACMG criteria). In contrast to the literature, our patients had no other characteristics besides the findings of cholestasis. Unlike other groups, PFIC3 may show an insidious onset. Biochemically, it is associated with high GGT, and gallstones are a common USG finding. The development of hepatocellular carcinoma and cholangiocarcinoma is more common in PFIC3 patients [19].

We found a homozygous c.616A > G alteration in the *ABCB11* gene in our 13-month-old male patient who is not on the list. Although this alteration is considered benign, it has not been reported in the homozygous form in healthy individuals in population studies (gnomAD; genome aggregation database). The fact that our case with cholestasis findings was homozygous for this alteration suggested PFIC2 as a preliminary diagnosis, but her healthy 5-year-old sister was also homozygous for this alteration. This situation showed us once again the importance of segregation analysis.

It has been reported in the literature that panel-based NGS is a very useful tool for the diagnosis of cholestatic liver disease when extrahepatic causes have been excluded [20, 21]. In our study, a molecular genetic diagnosis was made in 10 of 37 patients whose PFIC gene panel was examined, representing a diagnostic yield of 27%. In a similar study recently conducted by Bakır *et al*., the diagnostic yield was found to be 40% [22]. Moreover, in a substantial number of patients with the PFIC phenotype, no variants can be identified in the *ATP8B1, ABCB11,* and *ABCB4* genes. Some of these cases are known to be associated with variants in other genes involved in the secretion of bile salts [23]. Considering that there are currently 12 subtypes of PFIC and other genetic diseases that cause cholestasis, future efforts will be made to achieve a higher diagnostic rate to determine the molecular background of cholestatic liver disease by adding new genes to the panel gene content we used.

CONCLUSION

In this study, we evaluated the results of molecular genetic analysis and clinical findings of patients who underwent NGS study with the gene panel covering the most common PFIC subtypes. Three variants considered novel have been presented in the literature. The beneficial clinical use of NGS-based genetic panels has been demonstrated in cases where PFIC is clinically suspected.

Authors' Contribution

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

Conflict of interest

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

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1444-1453 Original Article

DOI: 10.18621/eurj.1354513 *Radiology*

Unusual metastases of breast cancer: a single-center retrospective study

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ABSTRACT

Objectives: While clinical and radiological awareness of the usual, frequent metastatic sites of breast cancer is high, unexpected 'atypical' localisations may be more easily overlooked in the cancer management process. The aim of this article is to contribute to this awareness in order to facilitate and improve the diagnosis and follow-up of breast cancer.

Methods: In this study, we retrospectively evaluated breast cancer patients who were diagnosed and followed up between 2017 and 2022 in our hospital, which is an important oncology center.

Results: Of the 852 patients included in this study, 79 had an unusual metastasis localization. The most common unusual metastasis sites were mediastinal lymph nodes and brain. These sites were followed by atypical bone involvement, mesenteric lymph nodes, cervical lymph nodes, hilar lymph nodes and surrenal metastases. The most rare metastases were more common in the uterus and ovary, followed by pancreas, parotid gland, spleen, colon, pericardium-atrium, orbital soft tissues and lacrimal gland. The unusual site of metastasis was mediastinal lymph nodes in 17.7% (n = 14), brain in 17.7% (n = 14), atypical bone sites in 12.6% (n = 10), mesenteric lymph nodes in 11.3% (n = 9), cervical lymph nodes in 11.3% (n = 9), hilar lymph nodes in 5% (n = 4) and surrenal in 6.3% (n = 5).

Conclusions: Sharing knowledge and experience about unusual metastases of breast cancer will contribute to the diagnosis and treatment of metastatic diseases by increasing the awareness of this issue. For this, multicentre studies should be conducted to combine these experiences.

Keywords: Breast cancer, unusual metastases, rare metastases, atypical metastases

Breast cancer is a very common malignant tumour and metastatic disease is still the leading cause of death in breast cancer patients. Today, the rate of metastasis is increasing even in regions known to be rare. This is due to more effective treatments that prolong the overall life expectancy of breast cancer patients and the progress made in early diagnosis thanks to the development of new imaging techniques [1]. However, since it is still possible to encounter metastatic disease in breast cancer patients even after many years, the evaluation of each symptom reported by a breast cancer patient can lead us to a conclusion that may affect survival by detecting a metatastic focus early. Therefore, knowing even the rare metastatic

Received: ***September 4, 2023;*** *Accepted:* ***October 5, 2023;*** *Published Online:* ***October 8, 2023***

How to cite this article: *Özdemir Akdur P, Çiledağ N. Unusual metastases of breast cancer: a single-center retrospective study. Eur Res J 2023;9(6):1444-1453. DOI: 10.18621/eurj.1354513*

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e-ISSN: 2149-3189

sites of breast cancer will help clinically correct interpretation of new symptoms and selection of correct diagnostic techniques in patients followed up for breast cancer [2]. The aim of this article is to increase the awareness of every physician in the chain of physicians involved in the diagnosis and follow-up of breast cancer patients about the presence of unusual metastases by sharing our own experience of unusual metastases in breast cancer patients in our center.

METHODS

In this study, we retrospectively evaluated breast cancer patients who were diagnosed and followed up between 2017 and 2022 in our hospital, which is an important oncology center. Among these patients, we included 852 patients for whom we could obtain complete patient information from hospital notes and access to radiological computed tomography (CT) and magnetic resonance imaging (MRI) images from the hospital radiology archive system (PACS), and excluded other patients with missing patient information or imaging findings. Of the 852 patients included in the study, 12 were missing imaging findings, and 10 were missing information on one or more receptor subtypes.

Statistical Analysis

Analyses were performed using SPSS Version 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY). A two-tailed Kolmogorov-Smirnov test was applied to examine for whether the continuous quantitative variables follow a Gaussian distribution. Characteristics of patients, as n (percent) or median (minimum-maximum) for categorical and continuous variables, respectively, and were compared among treatment groups using chi-square or Mann-Whitney tests, as appropriate. A p value was set at < 0.05 for statistical significance.

RESULTS

Of the 852 patients included in this study, 79 had an unusual metastasis localisation. The unusual site of metastasis was mediastinal lymph nodes in 17.7% (n = 14), brain in 17.7% (n = 14), atypical bone sites in 12.6% (n = 10), mesenteric lymph nodes in 11.3% (n = 9), cervical lymph nodes in 11.3% (n = 9), hilar lymph nodes in 5% (n = 4) and surrenal in 6.3% (n = 5). In our retrospective analysis, some of the unusual metastasis sites included in our cohort; parathyroid involvement, orbital involvement, mandibular involvement, metastasis to the surrenal gland, pancreas, and ovaries are shown in Fig. 1.

The remaining 28.7% of the unusual metastases were rarer metastases and were as follows; two patients had uterus and ovary involvement, two patients had only ovarian metastatic mass, two patients had spleen metastasis, one patient had pancreas metastasis, one patient had pericardium and atrium metastasis, one patient had parotid metastasis, one patient had orbit metastasis and one patient had colon and endobronchial involvement. When we made a comparison between breast cancer groups with and without unusual metastasis in terms of age and tumour stage, 8.3% (16 patients) in Grade I and 10.3% (68 patients) in Grade II-III had unusual metastasis and there was no statistically significant difference between these two groups (Table 1).

We also divided the patients into two groups as invasive ductal carcinoma and lobular carcinoma, which are the two main histopathological subtypes, and examined the relationship between these two groups and the presence of unusual metastasis. There were 71 (10.1%) unusual metastases in the invasive ductal carcinoma group and 6 (9.8%) unusual metastases in the invasive lobular carcinoma group. Since the number of cases in some of the foci with unusual metastases was very small (in some localisations there was only one patient), we could not look at the relationship between histopathological subtypes and unusual metastases in each localisation and histopathological subtype. We further divided the patients into four receptor subgroups; group 1: Estrogen receptor (ER) and/or progesteron receptor (PR) (+), human epidermal growth factor receptor 2 (CERB) (-); group 2: ER and/or PR (+), CERB (+); group 3: ER (-), PR (-), CERB (-) and group 4: ER (-), PR (-), CERB (+). There were 42 (9.3%) patients in ER and/or PR (+), CERB (-), 30 (12.1%) patients in ER and/or PR (+), CERB (+), 11 (12.8%) patients in ER (-), PR (-), CERB (-) and 1 (15%) patient in ER (-), PR (-), CERB (+) (Table 2).

There was a statistically significant difference in

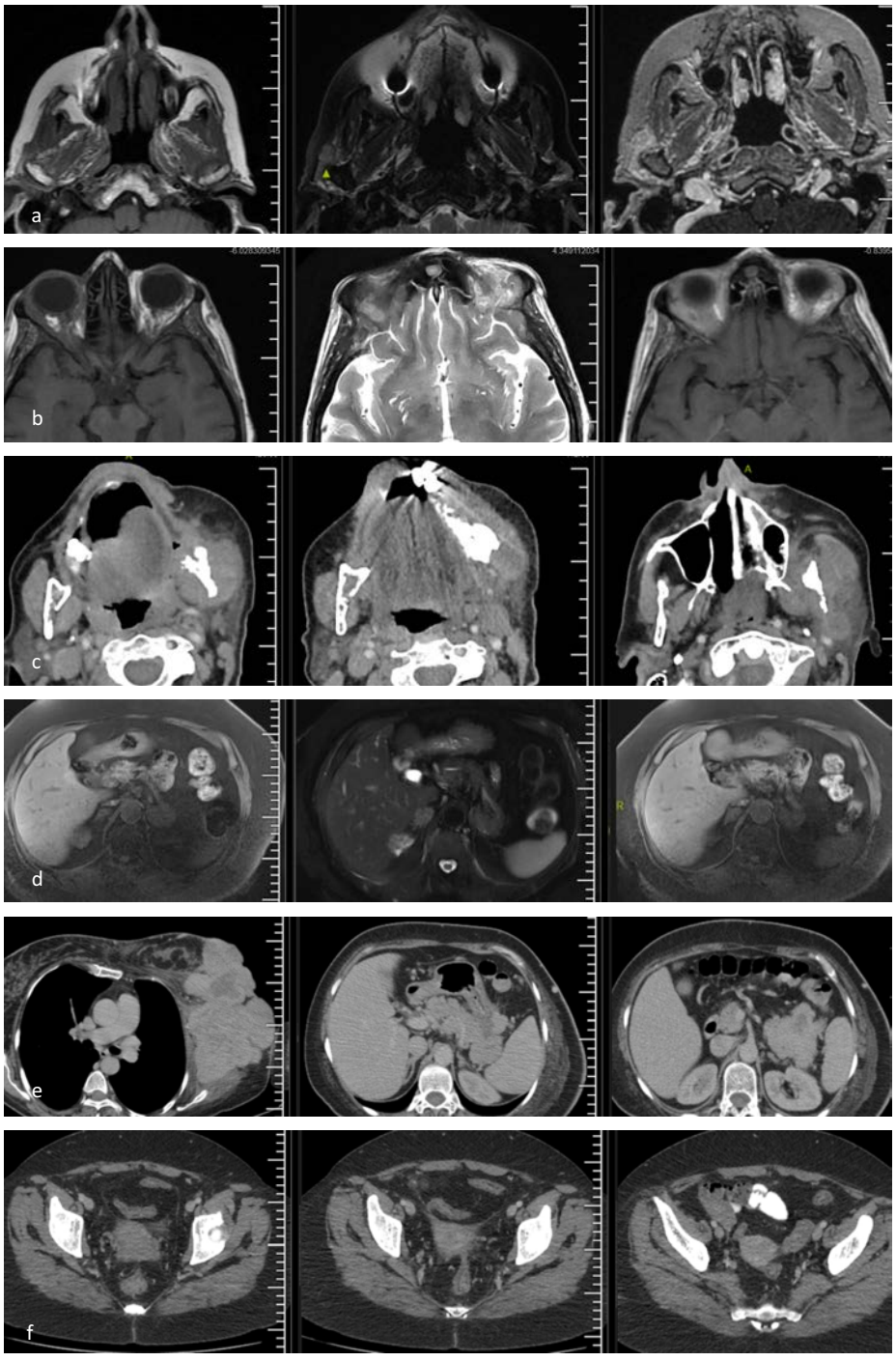

Fig. 1. (a) Anterior part of the superficial lobe of the right parotid gland showing hypointense signal on T1-weighted sequences and isointense signal with the parotid gland on T2-weighted sequences, showing peripheral weighted contrast enhancement after IVKM, nodular lesion, (b) A metastatic focus with isointense on T1-weighted sequences and increased intensity on T2-weighted sequences, with mild oedematous sinya extraconal distance and lacrimal gland and upper eyelid level on STIR sequence, extending from the right orbital apex laterally to the retrobulbar area, (c) Left mandibular corpus containing an expansile sclerotic component extending towards the mandibular angulus and causing fractures at the level of the mandibular angulus condyle in places, also metastatic focus with a soft tissue component with a thickness of approximately 10 mm extending into the masseter muscle, (d) Hyperintense in T2A series and hypointense in T1A series in the localisation of the right surrenal lobe body and left surrenal gland body, metastatic lesions with contrast enhancement in early phases, contrast release in late phases and diffusion restriction after IVKM in dynamic series, (e) Metastatic lesion with cystic-necrotic areas approximately 36×24 mm in the tail of the pancreas with unclear borders and (f) Both ovaries contain heteogenous hypodense masses with increased size.

Table 1. Distribution of unusual metastasis sites of the participants

Metastasis sites (n = 79)	n	%
Mediastinal lymph nodes	14	17.7
Brain	14	17.7
Atypical bone metastasis	10	12.6
Mesenteric lymph nodes	9	11.3
Cervical lymph nodes	9	11.3
Hilar lymph nodes	4	5.0
Surrenal	5	6.3
Others	12	28.7

terms of age between the patient groups with and without unusual metastasis (p = 0.005). In addition, there was a statistically significant correlation between metastasis groups and receptor groups (p = 0.023), while there was no significant correlation with tumor types (p = 1.000) (see Table 2).

DISCUSSION

Today, breast cancer still occupies an important place in the cancer agenda of the scientific world as a disease that is the main cause of cancer deaths in women, maintains its top position in cancer statistics with more than one million new diagnoses per year and increases cancer treatment costs. As in all cancer types, the presence of metastasis in breast cancer, its onset and course of development cannot be predicted and its determinant effect on the patient's clinic increases exponentially, the presence of metastasis remains the main challenge to be overcome in the war against breast cancer [3].

Like all other malignancies, the evolution of tumour metastasis in breast cancer is still controversial. Starting with Paget's "seed and soil" hypothesis, numerous studies have confirmed his findings and integrated the idea of a multi-stage process in which tumour cells, like a seed, are nourished and grow when they meet a fertile soil, in which both the tumour cell and the host are active. Findings that translate Paget's theory into clinical practice show that breast cancer preferentially spreads to some organs at a much higher rate, while it is less common to encounter breast cancer metastasis in some remote areas. In this study, we focussed mainly on anatomical regions where few metastasis rates have been reported in the literature [1].

According to many studies, distant metastases of

Table 2. Distribution of demographic and clinical findings according to unusual metastasis development

Variables	Unusual metastasis		
	Yes (n = 79)	No (n = 773)	*p* value
Age (years)	50 (25-85)	53 (22-88)	**0.005**
Tumor grade, n (%)			0.820
Grade I	17 (21.5)	175 (22.6)	
Grade II-III	62 (78.5)	598 (77.4)	
Receptor group, n (%)			**0.023**
ER and/or PR (+), CERB (-)	30 (38.0)	424 (54.9)	
ER and/or PR (+), CERB (+)	33 (41.8)	212 (27.4)	
ER (-), PR (-), CERB (-)	8 (10.1)	78 (10.1)	
ER (-), PR (-), CERB (+)	8 (10.1)	59 (7.6)	
Tumor type, n (%)			1.000
Invasive ductal	67 (91.8)	633 (92.0)	
Invasive lobular	6 (8.2)	55 (8.0)	

Data are shown as median (minimum-maximum) or n (%). ER = Estrogen receptor, PR = progesterone receptor, CERB =HER 2 = human epidermal growth factor receptor 2.

breast carcinoma are present in approximately 10% of patients at the time of diagnosis, while 30% of patients develop metastatic disease during and after treatment [3-5]. Although breast cancer can metastasise to a wide variety of organs, the most common metastatic sites are bone, lung, liver and skin. In addition, more and more types of breast cancer metastatic sites are reported in the literature. For these uncommon metastatic sites, there are publications that define "unusual metastasis", which is a definition that defines a frequency of ≤ 1% in each site, and according to this definition, unusual metastasis includes metastases involving the central nervous system, secretory / endocrine organs and glands, some internal organs and gynecological organs [6, 7].

Mediastinal lymph node involvement is a rare site of metastasis in breast cancer and mediastinal lymph node metastases are present in only 2% of patients with breast cancer [8]. Another risk of mediastinal lymph node involvement is that it may affect the oesophagus and there is a risk of spread of the existing involvement to the oesophagus. According to breast cancer autopsy reports, the prevalence of mediastinal metastases affecting the oesophagus is 0.4-6% [7, 8]. In our study, only 14 patients had mediastinal lymph node involvement among unusual metastasis localisations and the rate of mediastinal lymph node metastases among unusual metastases was 17.7%. In our analysis, there was no case with proven involvement of the oesophagus.

Involvement of the trachea and bronchial structures within the mediastinal structures is also rare and especially endobronchial metastasis is a very rare metastatic pattern in breast cancer [9]. In our study, one patient had a case involving the bronchial tree. Although lung metastases are common in breast cancer, pulmonary metastases with hilar masses as the primary finding are also rare in clinical practice and this involvement may present as spiculated masses with a picture including obstructive pneumonia and mediastinal lymphadenopathy [10]. In our study, hilar lymphadenopathy was present in four cases presenting with hilar lymphadenopathy, in other words, hilar lymphadenopathy was present in 5% of unusual metastases.

If we continue to look at mediastinal structures; secondary or metastatic tumours spread to the pericardium and heart more frequently than primary tumours. The most common causes of metastatic involvement are lung carcinomas, breast carcinomas, haematological malignancies and gastrointestinal carcinomas. Metastatic involvement of the heart and pericardium is usually associated with a very poor prognosis and most cases are clinically silent and not diagnosed in vivo until autopsy [11, 12]. In our study, we had one patient with metastases invading the mediastinum and spreading to the pericardium and right atrium.

Breast cancers are among the most common tumours metastasising to the head and neck, and metastases have been reported in almost every anatomical subregion of the head and neck. However, cervical metastases of breast carcinoma are still relatively rare in clinical practice, as the presence of metastases to the head and neck is rare in cancer practice [2]. The most frequently involved tissue in the head and neck region is the cervical lymph nodes and its incidence is as low as 1% [13, 14]. In our study, we found the rate of cervical lymph node metastasis among unusual metastases to be 11.3% (n = 9). The thyroid gland and salivary glands are the most rarely reported metastatic sites in the head and neck region. In our retrospective analysis, we did not detect any metastasis to the thyroid gland, but we found metastasis to the parotid gland in only one case (Fig. 1a). The parotid gland is the salivary gland with the most frequent metastatic involvement among other salivary glands. The most common malignancies that metastasise to the parotid gland are tumours originating from the head and neck, such as squamous cell carcinoma and malignant melanoma, and in fact breast cancer is among the malignancies that rarely metastasise to the parotid gland and also has a poor prognosis. To give a statistic, only 21 cases have been reported from 1982 to 2017 [15, 16]. Again anatomically, orbit is one of the rare metastatic localisations in the head and neck region. Orbital metastases from solid cancers are rare and constitute only 1-13% of orbital tumours. Breast cancer is responsible for 28-58.5% of these metastases. However, orbital involvement is exceptional in breast cancer, with only 0.7% of cases being symptomatic, and orbital metastasis is an important cause of morbidity that reduces patient quality of life [17].

Lacrimal gland tumours are also rare among or-

bital structures. The most common type of malignant lacrimal gland tumour is epithelial tumours, which are responsible for 58% of all malignant tumours in the lacrimal gland. Metastasis to the lacrimal gland is extremely rare and only sporadic cases have been previously reported [18]. In our case, cancer metastasised to the soft tissues including the upper eyelid and lacrimal gland (Fig. 1b).

The oral cavity is also an anatomical region that is rarely metastatically involved, accounting for less than 1% of all malignant neoplasms. Metastasis of breast cancer to the oral cavity is relatively less common, with 85% of metastatic tumours in the oral cavity occurring most commonly in the jaw bones, particularly the mandible in 80-90% of cases. Metastasis to the soft tissues of the oral cavity is extremely rare, accounting for approximately 0.1% of all oral malignancies. The most commonly involved metastatic sites are the tongue, gingiva and lips; less frequently involved sites are the palatal and buccal mucosa, respectively [19]. In our single case, there was a metastatic focus involving the mandibular ramus, extending the bone and invading the adjacent soft tissue (Fig. 1c).

Although the tendency of breast cancer to spread to the central nervous system is well known, the prevalence of symptomatic central nervous system metastases in breast cancer ranges from 5% to 16%, Brain metastases are an important source of morbidity and mortality in breast cancer patients as in other types of cancer. Metastatic breast cancer has become the second most common cancer causing brain metastases after lung cancer [20]. The blood-brain barrier plays a critical role in maintaining normal brain function. The blood-brain barrier, the biological structure between blood and brain parenchyma, separates the blood compartment from the brain tissue, restricting the entry of toxic molecules and protecting the parenchyma by expelling metabolites and waste products. This barrier is also one of the main barriers for extravasation and colonisation of cancer cells into the brain. However, with the development of primary or metastatic tumours in the brain, significant changes occur in this context: During tumour evolution, the blood-brain barrier is disrupted with new anarchic vascularisation and tumour cells are likely to cross the barrier [21-23].
Spinal cord metastasis as a component of the central nervous system is even rarer. The mechanism of intramedullary dissemination has not been well established. Possible routes of transmission may be lymphatic or haematological transmission, direct spread from the vertebrae or descending from the brain. In our study, there was no case with spinal cord involvement. Metastasis to the leptomeninges, another component of the central nervous system, is also rare and studies have reported the predisposition of the lobular histopathological subtype to leptomeningeal carcinomatosis [2, 24]. In our study, leptomeningeal involvement was present in 2 cases and both of them were lobular type invasive carcinoma in accordance with the literature.

Breast cancer is known to metastasise to all organs of the body. However, abdominal lymph node metastases from breast cancer are extremely rare. The mechanism of abdominal lymph node metastases from breast cancer is still unclear. Studies have shown that the metastatic patterns of breast cancer depend on the pathological type and the rate of metastatic spread to the gastrointestinal tract is higher in invasive lobular cancer type than in invasive ductal cancer type and this molecular subtype may be a determinant in metastasis preference. Based on these data, although abdominal lymph node metastasis from invasive ductal type breast cancer is rare [25], the rate of mesenteric lymph node involvement among unusual metastases was 11.3% (n = 9) and 6 of these patients had mixed pattern and 3 had invasive ductal carcinoma histopathological subtype.

As mentioned above, gastrointestinal system metastases in breast cancer are also rare and unique and the incidence in autopsy series varies between 8% and 35%. Any part of the gastrointestinal tract from the tongue to the anus may be involved. In most series, lobular carcinoma has been reported to have a higher tendency to metastasise to the gastrointestinal tract and peritoneum, but the data on this tendency are not conclusive. In a large review, the most common site of gastrointestinal tract metastasis was the stomach with 185 cases, while colon involvement was reported in 59 cases and small bowel involvement in 24 cases [2]. Although the stomach is the most common site of breast cancer metastasis, the incidence is quite low, approximately 0.2%-0.7% [26]. Colon metastases are even rarer and often pose a diagnostic dilemma and are difficult to diagnose due to their non-specific pres-

entation and similarity to other malignant or benign lesions [27]. In our breast cancer cohort, only one of the 79 patients with unusual metastases had a gastrointestinal system component, which was localised in the colon, and the patient had only nonspecific abdominal pain.

Adrenal metastases are the most common malignancy in the adrenal glands, another anatomical localisation where metastases are much less common. In order of frequency, the main cancer types metastasising to the adrenal gland are lung cancer, gastric and colon cancer, oesophageal cancer, liver/ biliary tract cancer and renal cell carcinoma. The symptoms of adrenal gland metastasis are almost always asymptomatic. However, if a large part of the adrenal cortex is damaged by a tumour or if both adrenal glands are affected, they may be associated with signs and symptoms of adrenal insufficiency [28]. The presence of adrenal metastases in breast cancer is very rare and some publications have stated that adrenal gland metastases in invasive ductal carcinoma represent an extremely low number of cases [29]. However, in our retrospective analysis, 5 of 79 unusual metastasis localisations had adrenal metastases. Contrary to the literature, none of the adrenal metastases had lobular subtype and four cases had invasive ductal carcinoma histopathological subtype and only one case was solid papillary carcinoma with neuroendocrine differentiation (Fig. 1d).

Among the anatomical sites of visceral metastasis of breast cancer, kidney, spleen and pancreas are also metastatic sites that have been reported rarely. Spleen metastasis is rarely seen as a single site without a component of other metastatic foci and this condition is very rarely reported in the literature. In a recent publication on this subject, it is mentioned that solitary spleen involvement was detected in only three cases before them [30]. In our study, two cases had splenic metastases and they were not solitary but were components of multiple foci seen in other regions. When we look at the metastases of breast cancer to the pancreas, although the prevalence of pancreatic metastases was reported to be as high as 11% in a large autopsy series, resection of pancreatic metastases from breast cancer is extremely rare and only 20 cases have been reported so far in a recent study [31]. In our study, one patient had a metastatic mass localised in the tail of the pancreas (Fig. 1e).

In rare cases, breast cancer metastases can also involve the genital organs and placenta. Symptomatic cases of uterine metastases may present as vaginal bleeding or abdominal discomfort, but most cases are diagnosed post-mortem. Studies have shown that the incidence of ovarian metastases in breast cancer patients is 13-47%, based on autopsy series. Ovarian metastases usually present as an asymptomatic ovarian mass. Metastatic lesions are known to reach the genital organs via lymphatic and blood vessels or transcoelomic spread through the peritoneal cavity, thus forming metastatic foci [2, 32]. In our cases, uterus and ovary were involved together in 2 cases, while 2 cases had metastatic foci in the ovary.

Since approximately 65-75% of patients with metastatic disease from breast cancer have bone metastases, we can talk about 'relatively rare bone metastases' in the case of breast cancer. The pattern of occurrence of atypical bone metastases and the presence of other bones with concomitant involvement also affect this 'relative rarity'. Breast cancer metastases most commonly affect the spine, ribs and pelvic bones; however, only 3.5% of breast cancer patients develop long bone metastases [33, 34]. In two of our cases, only femur was involved among the bone structures. In the category of bone metastases, skull metastases are malignant bone tumours with an increasing incidence. Cranium involvement has been reported in lung, breast and thyroid carcinoma, renal cell carcinoma, malignant melanoma in adults and neuroblastoma in children. In four of our cases, only the cranium was involved, while in one case the cranium and humerus were involved together. The occurrence of the sternum as a focus of bone metastasis in breast cancer patients is relatively rare and the reported incidences are 1.9%-5.2%, respectively [35-37]. We had metastasis to the sternum in one of our cases. One of the other involvements, which can be considered as our relatively rare metastasis experience, was isolated pelvis and isolated scapula involvement (Fig. 1f).

Breast cancer is a heterogeneous group of tumours with variable morphology, behaviour, response to treatment and molecular profiles. Invasive ductal carcinoma is the most common histological type accounting for 72-80% of all invasive breast cancers, while invasive lobular carcinoma is less common and ac-

counts for 5-15% of all invasive breast cancers. These two subgroups differ from each other in many aspects such as risk factors, histological features and molecular profile. The metastatic site tropism of these two subgroups is also different from each other. Metastatic pattern analysis studies in breast cancer have shown that lobular tumours have a higher tendency to metastasise to the peritoneum, adrenal glands, uterus and pleural surface. We discussed the histopathological subtype relationships of the unusual metastases we detected above in the text. In some respects, our study is consistent with previous studies in terms of histopathological subtype/unusual metastasis localisations, while in some cases we found different data from previous studies. Molecular profiling studies, as well as histopathological subtype, are another important issue discussed in the context of cancer and its preferred metastatic foci [38-41]. Since the number of 79 unusual metastasis localisations we detected in our retrospective analysis of our large cohort of 852 cases was insufficient on a group basis (some groups had one patient), we compared invasive ductal carcinoma and lobular carcinoma histological subtypes in terms of the presence of unusual metastasis without dividing the patients into groups, but we did not find a statistically significant difference between them. We then divided the patients into four different receptor subgroups and analysed the difference between receptor groups and the presence of unusual metastasis; however, we did not find a statistically significant relationship between receptor groups and the presence of unusual metastasis (Table 2).

Limitations

The limitations of this study are the small sample size and the single centre of the study.

CONCLUSION

Despite all the latest technological advances in the diagnosis and treatment of breast cancer, most deaths from cancer are caused by metastases that are unresponsive to conventional therapies. Scientifically, all studies that will enable us to understand the process of metastatic development at the cellular and molecular level have the potential to serve very important studies for the improvement and development of effective treatments for cancer. In clinical practice, timely diagnosis of unusual metastases provides an important chance to control the disease and prolong survival. When past studies are reviewed, we come face to face with the fact that the rates of unusual metastases detected in autopsy cases are much higher and that some symptoms and metastatic foci of patients are missed in practice. In this study, we wanted to contribute to the awareness of unusual metastases of breast cancer by sharing the archive of our hospital, which is an important oncology centre in Turkey, through our cases.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1454-1463 Original Article

DOI: 10.18621/eurj.1349473 *Cardiology*

Evaluation of the clinical value of sodium examination in spot urine in patients presenting with acute heart failure while using SGLT2i – "SPOT HF STUDY"

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ABSTRACT

Objectives: Sodium measurement in spot urine provides important information about the adequacy of the initial dose in acute heart failure (AHF) patients using intravenous diuretic (IV) and makes a guiding contribution to the titration decision. It is not clear whether spot urine sodium measurement has the same clinical value as the effect of sodium-glucose cotransporter 2 inhibitor (SGLT2i) drugs on urinary electrolytes. Our aim in our study is to investigate the clinical value of sodium examination in spot urine in AHF patients while using SGLT2i.

Methods: Our study was conducted retrospectively and single-centered. AHF patients, administered IV diuretics were included in the study. Patients who were using and were not using SGLT2i were examined in two groups. The 2nd and 6th-hour sodium values in spot urine were measured.

Results: Patients using SGLT2i (n = 46) and not using it (n = 54) were included. The mean age was 69.91 ± 11.84 years and 47% were female. The standard deviation value for the sodium in spot urine in patients using SGLT2i was clearly high and its distribution was significantly higher. A weak correlation was found between the sodium value in spot urine and the 24-hour urine volume in this group. Hospitalization history within 1 month after discharge was found to be 39% in the group using SGLT2i, and 51% in the group that did not use it, and this difference was statistically significant.

Conclusions: The measurement of sodium in spot urine does not seem to have the same clinical value in HF patients using SGLT2i. Its correlation with urine volume is also decreasing in this group.

Keywords: Acute heart failure, sodium-glucose cotransporter 2 inhibitor, spot urine

Acute heart failure (AHF) presents a scenario in which rapid response is desired in patients with diuretic treatment requiring decongestion, and numerous factors are considered for dosage adjustments. At this juncture, the titration of diuretic doses and the ability to predict treatment prognosis become crucial.

Among patients hospitalized with acute AHF, nearly 40% face mortality within the first year. A substantial number of patients are also rehospitalized shortly after discharge. In fact, 27% of patients diagnosed with acute AHF return for readmission within the first month [1]. Despite all treatments, one in four

Received: ***August 28, 2023;*** *Accepted:* ***September 29, 2023;*** *Published Online:* ***October 5, 2023***

How to cite this article: *Aslan O, Demirci EE. Evaluation of the clinical value of sodium examination in spot urine in patients presenting with acute heart failure while using SGLT2i – "SPOT HF STUDY". Eur Res J 2023;9(6):1454-1463. DOI: 10.18621/eurj.1349473*

e-ISSN: 2149-3189

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patients carries residual congestion findings during the discharge phase, which has been associated with a worse prognosis [2, 3]. Consequently, the primary treatment goal of AHF patients is to achieve safe and effective decongestion while reducing early rehospitalization and mortality rates [4, 5].

The tracking of diuretic response commonly relies on measures of input and output, which seem to exhibit a moderate level of consistency [6]. Furthermore, these measurements have limited utility in the context of acute phase treatment titration. Neurohormonal activation triggered by urinary sodium content and antinatriuretic mechanisms eliminates the surprise of diuretic resistance [7, 8]. It has also been demonstrated that effective decongestion resulting from a favorable diuretic response leads to better outcomes [8]. From another perspective, reduced sodium content in urine is associated with higher cardiovascular mortality and hospitalization due to heart failure in patients with acute AHF [9]. At this point, the measurement of spot urine sodium level, which can be utilized within the first 2 hours of treatment, has been reported to exhibit excellent correlation with both the sodium content in urine at the time of examination and the total sodium content in 24-hour urine [10]. Spot urine sodium examination reflects the urinary sodium concentration at the time the urine sample is provided. As highlighted in an important consensus paper published by the Heart Failure Association of the European Society of Cardiology (HFA-ESC), early assessment of spot urine sodium after the initiation of diuretic treatment is recommended for patients with acute heart failure [4].

Based on significant data in heart failure patients, sodium-glucose cotransporter 2 inhibitors (SGLT2i) drugs, which have been strongly recommended, are expected to be prominently featured in the medication regimen for AHF patients in the near future [5]. In this drug class with important outcomes and a range of mechanisms proposed for cardiovascular mortality and rehospitalization due to heart failure [11, 12], the addition of a new player involves mechanisms intertwined with natriuresis and effects on urinary electrolytes. Complex renal interactions and the inhibition of major reabsorption mechanisms such as SGLT2 during the course of sodium reabsorption in renal tubules, followed by compensatory reabsorption mechanisms, result in complex effects on sodium absorption. It is known that natriuresis after usage of SGLT2i results in increased renal vascular resistance, and initial reduction in GFR, due to these effects on urinary sodium content [13]. Given these effects on urinary sodium content, it is not clear whether spot urine sodium measurement, which can be used early in the treatment of AHF patients, holds the same clinical value in this patient group using SGLT2i drugs.

Therefore, the aim of this study is to investigate the clinical value of spot urine sodium examination in patients using SGLT2i drugs while receiving IV diuretic treatment due to acute heart failure.

METHODS

Our study was designed as a retrospective and single-center study, conducted by scanning hospital automation and patient records. The study was planned to include patients admitted to the emergency department between January 1, 2022, and March 1, 2023, who were diagnosed with heart failure, received inpatient care, and underwent 24-hour diuretic therapy. Two groups were formed; patients with SGLT2i and patients not using them. One hundred and five patients not using SGLT2i and 66 patients using SGLT2i were recorded for the study. Patients who have missing laboratory data (n = 29), cardiogenic shock (n = 2), incomplete clinical follow-up (n = 25), acute renal failure (n = 4), glomerular filtration rate (GFR) < 60 mL/min (n = 11) were excluded from the study. Sodium measurements were taken from spot urine samples of all patients at the 2nd and 6th hours.

The Ethics Committee approval was obtained under protocol number 2023/05-13 before the study was initiated.

Patients with a diagnosis of heart failure with reduced ejection fraction (HFrEF) were included in the study if they had been followed for at least 6 months with the diagnosis of HF and at least one month with stable symptoms using guideline-directed medical therapy. Patients diagnosed with AHF who were followed for 24 hours in the intensive care unit (ICU) due to heart failure and received intravenous diuretics were included in the study. The diagnosis of acute heart failure was based on the ESC heart failure guidelines, in patients presenting with signs and symptoms of congestion, requiring intravenous diuretic therapy [5].

Exclusion criteria for the study included in the fol-

lowing: (1) Missing laboratory data, (2) Incomplete clinical follow-up data, (3) Taking medications that could affect the proximal tubule, such as diazoxide, (4) GFR < 60 mL/min, (5) Cardiogenic shock, (6) Acute kidney injury, and (7) Sepsis.

Urinary sodium measurements were performed using the Siemens Advia chemistry XPT device (Siemens®, Germany). Echocardiographic findings of patients were recorded by a cardiology specialist.

Statistical Analysis

The data were recorded in the Statistical Package for the Social Sciences (SPSS, IBM SPSS Statistics for Windows, NY: IBM Corp) 17.0 software package. Normally distributed continuous variables were expressed as mean ± standard deviation, while non-normally distributed variables were expressed as median (minimum-maximum). Categorical variables were presented as numbers and percentages. The signifi-

Table 1. Comparison of demographic data, medical histories, medication usage, and clinical findings of patients in the group using and not using SGLT2i

	SGLT2i group (n = 46)	Non-SGLT2i group (n = 54)	*p* value
Demographic data			
Age (years)	69.76 ± 11.39	70.03 ± 12.22	0.897
Women	21 (45.65%)	26 (48.15%)	0.746
Medical history			
DM	24 (52.17%)	17 (31.48%)	0.545
HT	38 (82.61%)	41 (75.93%)	0.789
HL	13 (28.26%)	14 (25.93%)	0.467
CAD	8 (17.39%)	11 (14.81%)	0.208
COPD	7 (15.22%)	8 (14.81%)	0.387
Medications			
ACEI/ARB	28 (60.86%)	27 (50.00%)	0.771
β-blocker	41 (89.13%)	44 (81.48%)	0.907
ARNI	7 (15.22%)	9 (16.67%)	0.522
MRA	27 (58.70%)	29 (55.77%)	0.884
Furosemide	26 (56.52%)	30 (55.55%)	0.767
Torasemide	12 (26.09%)	13 (24.07%)	0.621
Vital signs			
Systolic blood pressure (mmHg)	140.93 ± 37.18	141,08 ± 35,65	0.923
Diastolic blood pressure (mmHg)	90.75 ± 17.95	89,83 ± 14,69	0.798
Saturation (%)	88.45 ± 3.21	89,11 ± 3,11	0.841
Ejection fraction	37.23 ± 6.25	38,74 ± 7,67	0.632
Intracardiac device	8 (17.39%)	9 (16.67%)	0.441
NIMV	5 (10.87%)	6 (11.11%)	0.912
Inotropic support	3 (6.52%)	2 (3.70%)	0.565

Data are shown as mean±standard deviation or n (%). DM = Diabetes mellitus, HT = Hypertension, HL = Hyperlipidemia, CAD = Coronary artery disease, COPD = Chronic obstructive pulmonary disease, ACEI = Angiotensin-converting enzyme inhibitor, ARB = Angiotensin reseptor blocker, ARNI = Angiotensin reseptor neprilysin inhibitor, MRA = Mineralocorticoid reseptor antagonist, NIMV = Noninvasive mechanical ventilation.

cance of differences between the means of groups with continuous variables was assessed using the Mann-Whitney U test for non-normally distributed groups and the Student's t-test for normally distributed groups. Spearman's correlation test was used to measure the correlation between continuous variables. Pearson's chi-squared test and, when appropriate, Fisher's exact test were used to assess the significance of differences between categorical variables. All calculations were performed as two-tailed tests. Values of $p < 0.05$ were considered statistically significant.

RESULTS

A total of 100 patients, previously diagnosed with acute heart failure (AHF), who were monitored in the intensive care unit (ICU) for 24 hours and received in-

Table 2. Comparison of laboratory data, urine volumes, and admission histories of patients in the group using SGLT2i and the group not using SGLT2i

	SGLT2i group (n = 46)	Non-SGLT2i group (n = 54)	*p* value
Laboratory			
Na (mEq/L)	135.89 ± 4.76	136,45 ± 5.16	0.724
K (mEq/L)	4.12 ± 0.87	3.98 ± 0.79	0.067
BUN (mg/dL)	32.34 ± 9.65	34.74 ± 8.19	0.365
Creatinine (mg/dL)	1.01 ± 0.34	0.98 ± 0.23	0.232
ALT (IU/L)	34.12 ± 13.21	36.33 ± 14.12	0.457
AST (IU/L)	37.78 ± 12.67	38.15 ± 13.29	0.387
WBC ($\times 10^{-3}/\mu L$)	9.34 ± 5.67	8.87 ± 6.71	0.105
Hb (g/dL)	13.25 ± 2.13	12.98 ± 2.58	0.656
Htc (%)	39.87 ± 7.12	38.91 ± 7.52	0.545
PLT ($\times 10^{-3}/\mu L$)	211.12 ± 67.32	198.59 ± 71.13	0.189
eGFR (mL/min)	62.48 ± 12.78	64.12 ± 13.32	0.754
TSH (mU/mL)	3.24 ± 1.01	3.18 ± 0.98	0.841
BNP (pg/mL)	701.22 ± 132.84	69716 ± 143.62	0.789
Spot urine sodium			
2nd hour	79.45 ± 44.12	82,13 ± 18.95	0.245
6th hour	64.54 ± 31.02	62,10 ± 11.89	0.365
Urine volume			
1st hour	209.07 ± 39.40	214.12 ± 48.64	0.089
2nd hour	181.22 ± 29.78	187.88 ± 31.11	0.105
3rd hour	170.03 ± 28.57	173.46 ± 29.97	0.242
24 hour	2734.23 ± 876.71	2813.55 ± 965.23	0.211
Diuretic dose			
Initially	85.12 ± 23.41	82.89 ± 19.87	0.221
24 hour	148.34 ± 43.47	144.73 ± 38.89	0.423
Rehospitalization in the first month	18 (39.13%)	28 (51.85%)	**< 0.001**

Data are shown as mean±standard deviation or n (%). Na= sodium, K = potassium, BUN = blood urea nitrogen, ALT = alanine transaminase, AST = aspartate transferase, WBC = white blood cell, Hb = hemoglobin, Htc = hematocrit, PLT = platelets, eGFR = estimated glomerular filtration rate, TSH = throid stimulating hormone, BNP = brain natriuretic peptide

travenous diuretic therapy, were included in the study. Among these patients, 46 were using SGLT2i and 54 were not using them. The mean age of the patients evaluated in the study was 69.91 ± 11.84 years, with an age range of 53 to 88. Among the included patients, 47% (47) were female.

When comparing patients using SGLT2i with those not using it, there were no significant differences in terms of age and gender. The most common comorbidity was hypertension (HT) in their medical history (n = 79). The numbers of patients with comorbidities like HT, diabetes mellitus (DM), hyperlipidemia (HL), coronary artery disease (CAD), and chronic obstructive pulmonary disease (COPD) were similar between the two groups. There was no significant difference between the two groups in terms of pre-admission medication use (beta-blockers, angiotensin-converting enzyme inhibitors, mineralocorticoid reseptor antagonists, angiotensin reseptor neprilysin inhibitors, furosemide, or torasemide). Vital signs at the time of admission were similar in both groups. There was no significant difference between the two groups in the use of noninvasive mechanical ventilation (NIMV) and inotropic support during acute admission. The echocardiographic findings, including ejection fraction (EF) values and intracardiac device usage, were not statistically significantly different between the two groups (Table 1).

Hematological parameters, biochemical results, and B-type natriuretic peptide (BNP) levels were similar between the SGLT2i-using and non-using groups. While sodium levels measured in spot urine at 2 hours and 6 hours were lower in the SGLT2i-using group, this difference was not statistically significant. Urine output volumes did not differ between the two groups. The diuretic doses used in both groups were similar. Within 1 month after discharge, the history of readmission was observed in 18 patients (39.13%) in the SGLT2i-using group and 28 (51.85%) patients in the non-using group, with this difference being statistically significant ($p < 0.001$) (Table 2).

Upon detailed analysis of sodium values measured in spot urine at 2 hours, it is noteworthy that the SGLT2i using group had a quite higher standard deviation value (79.45 ± 44.12). The distribution of sodium values measured in spot urine at 2 hours for the patients included in the study is graphically represented in Fig. 1 and Fig. 2. The distribution in the SGLT2i-using group appears to be significantly wider compared to the other group.

In patients using SGLT2i, both the 2nd and 6th-hour sodium values in spot urine exhibited weak correlations with the 24-hour urine volumes. In contrast, in patients not using SGLT2i, both the 2nd and 6th-hour sodium values in spot urine showed strong correlations with the 24-hour urine volumes (Table 3).

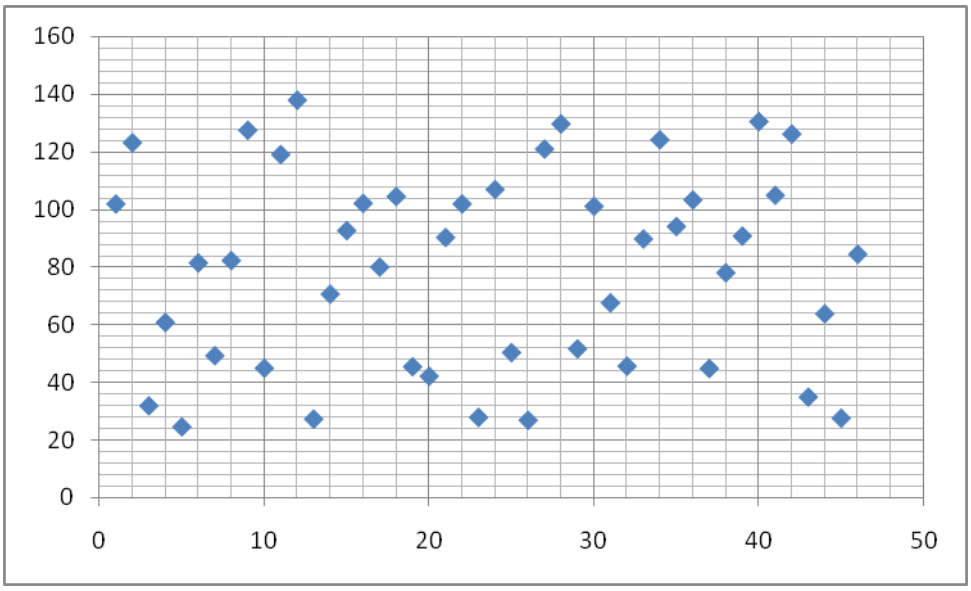

Fig. 1. Distribution of sodium values measured within the first 2 hours in patients using SGLT2 inhibitors.

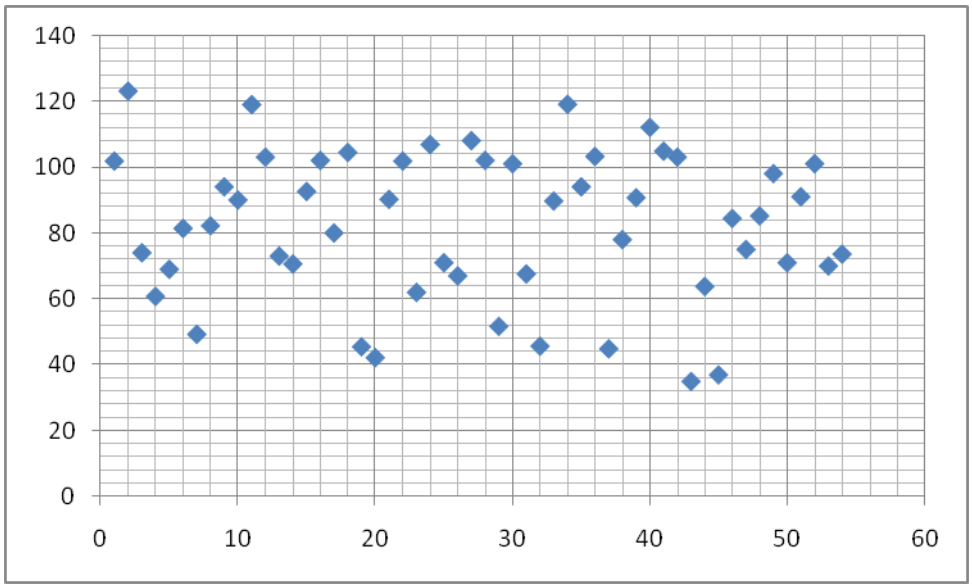

Fig. 2. Distribution of sodium values measured within the first 2 hours in patients not using SGLT2 inhibitors. .

DISCUSSION

The main finding of this study is that the clinical value of spot urine sodium measurement, an indicator of diuretic response, is significantly compromised in patients using SGLT2i and receiving IV diuretic therapy for AHF. Another important result is that the spot urine sodium value measured at 2 hours has a strong correlation with the 24-hour urine volume in patients not using SGLT2i, whereas this correlation is absent in patients using SGLT2i. Finally, patients using SGLT2i showed significantly fewer readmissions within one month.

In acute heart failure patients, urine volume and sodium content have attracted attention in terms of treatment response and prognosis. While negative fluid balance is one of the prominent monitoring tools in clinical practice, urinary sodium appears to be a cornerstone of this topic. In fact, some studies concluded that urine volume was not associated with survival but sodium excretion was [14], whereas a study showed that urine volume and sodium excretion were not correlated [15]. Yet another study suggested that the prognostic importance of urinary sodium excretion in acute heart failure patients changes with the use of mineralocorticoid receptor antagonists (MRAs), including spironolactone [16]. However, the challenges of consistent collection and measurement of urine have also been highlighted in studies [6, 17].

In light of these debates, contemporary guidelines have recognized urinary sodium excretion and its measurement through spot urine as a clear recommendation in AHF patients [5]. The advantages of being able to assess sodium in spot urine early in treatment

Table 3. Correlation between spot urinary sodium levels and 24-hour urine volume in the group using and not using SGLT2i

	24-hour urine volume	
	SGLT2i group	**Non-SGLT2i group**
2nd hour spot urine sodium	$p = 0.141$	$\mathbf{p < 0.001}$
	r:0.237	r: 0.772
6th hour spot urine sodium	$p = 0.102$	$\mathbf{p < 0.001}$
	r:0.262	r: 0.746

and its ease of measurement have strongly supported the measurement of sodium in spot urine. When introduced into the scientific discourse, a study has demonstrated that spot urine sodium measurement significantly reduces urine volume and the occurrence of rehospitalization and emergency department visits within one month [18]. The study by Testani et al. [10] showed that spot urine sodium measurement at 1 or 2 hours provides robust data for predicting subsequent poor diuretic response.

However, in none of these mentioned studies, the inclusion of a potent new player, SGLT2i, which is involved in heart failure treatment and can impact urinary sodium, has been investigated. With the added contribution of spot urine sodium measurement and the potential effect of SGLT2i, in addition to loop diuretics' impact on urinary sodium, it remains unclear whether this measurement will retain the same clinical value.

SGLT2i drugs have emerged as an important drug group that reduces hospitalization due to HF, demonstrating this effect in both heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) patients. The fact that these effects are observed in both diabetic and non-diabetic patient groups implies the introduction of an effective instrument into heart failure treatment [11, 12, 19]. While the mechanisms are not fully understood, being a diuretic or natriuretic agent is an inevitable assumption. In the proximal tubule, for every glucose molecule absorbed, one sodium ion is absorbed due to the significant effect of the SGLT2 transporter. If sodium is not absorbed here, absorption continues in the segments after the proximal tubule through SGLT1 and Na-H exchangers [20]. Since the majority of this absorption occurs through SGLT2, other mechanisms are suppressed in normal physiology. With the reduction of sodium absorption in the proximal tubule due to SGLT2i, the amount of sodium reaching the macula densa increases, leading to vasoconstriction of afferent arterioles and consequently a decrease in glomerular pressure, resulting in hyperfiltration [21]. The extent to which natriuresis can be compensated for due to these complex effects is not clear, but it is known that the activities of SGLT1 and NHE3 cannot fully compensate for this effect [22]. In a simplified perspective, expecting a clear effect towards natriuresis is reasonable. However, there are much more complex intrarenal effects and interactions with drugs such as loop diuretics that affect this region [23]. As a result, the effects on urinary sodium and many related electrolytes may diminish the decision-making ability in tests that measure these parameters.

In the study by Griffin et al. [24], empagliflozin was compared to placebo, and it significantly increased fractional sodium excretion. This effect was found to be synergistic when combined with bumetanide. As a result, empagliflozin induced effective natriuresis and potentiated this effect when combined with loop diuretics. Furthermore, in conjunction with this effect, there was no increase in neurohormonal activity, electrolyte wasting, or renal dysfunction observed. Although the natriuretic effect of SGLT2i was evaluated in this study, the study population consisted of stable HF patients. Another study demonstrated that the addition of empagliflozin to early treatment in acute HF increased cumulative diuresis after four days, leading to a reduction in HF-related events [25]. In another significant study, acute HF patients were divided into empagliflozin and placebo groups. While there was no difference in spot urine sodium levels between the two groups at admission, a significant reduction in spot urine sodium levels was observed, especially at 48 hours, in the empagliflozin group [26]. In the same study, FeNa and FeCl did not change between the two groups, but plasma osmolality increased. Urine volume increased, but urine osmolality remained unchanged. All of these findings suggest that the effect of SGLT2i treatment on electrolytes and urine, as well as the response of renal tubule physiology, is complex and multifaceted.

The confusing effect of SGLT2i on sodium is further enriched by some studies. For example, dapagliflozin has been shown to reduce tissue sodium in type 2 DM patients, in addition to inducing natriuresis and glucosuria [27]. Another study indicates significant evidence that SGLT2i agents bind to Na-H exchanger 1 (NHE) receptors in the myocardium as inhibitors (28). Indeed, these exchanger isoforms are also present in the kidney. In another study using dapagliflozin, it was demonstrated that while dermal tissue sodium decreased with treatment, serum sodium, and 24-hour urine sodium excretion did not change compared to placebo [27]. In a study with canagliflozin, it was shown that canagliflozin use increased urine sodium excretion and correlated with in-

creased urine volume on the first day [29]. However, this effect was not observed from the second day onwards, further complicating the interpretation of its effect on urine sodium. Another perspective is that in this study, the natriuretic effect of diuresis was more related to natriuresis than the osmotic effect of glucosuria. Other studies have also suggested an inverse correlation between natriuretic and glucosuric effects [24].

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The positive impact of SGLT2i use on hospitalization due to HF has been clearly demonstrated. Moreover, this effect has been shown to be independent of the presence of DM [11, 12]. Whether in the presence or absence of chronic kidney disease (CKD), the beneficial effects of SGLT2i on cardiovascular outcomes and HF-related hospitalizations have been clearly established [32-34]. In a study involving acute HF patients, the empagliflozin arm showed a significant increase in urine output compared to the placebo arm, resulting in a much stronger negative fluid balance [26]. The use of dapagliflozin, with or without diuretics, has been found to be safe and effective [19]. The impact of SGLT2i use on a more profound negative fluid balance, tissue sodium, and the interstitial space has been demonstrated [25, 35].

Considering all the impacts on HF and based on all these data, it does not seem surprising that the hospitalization duration is significantly shorter in the group using SGLT2i, as we observed in our study.

CONCLUSION

Spot urinary sodium analysis is an effective tool that can be utilized in the early stages of treatment for AHF patients. Its correlation with urine volume further reinforces its significance. In this patient group, the examination of spot urinary sodium, which will increasingly find a place in evaluating the diuretic response, becomes crucial. The results of our study are also important in demonstrating that the same spot urinary sodium analysis, which will progressively become more common in assessing diuretic response in this patient population, does not have a similar clinical impact in individuals using SGLT2 inhibitors. This outcome will significantly contribute to the clinical perspective. We believe that these results provide guiding insights for subsequent larger-scale studies that can be conducted in this patient group.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1464-1473 Original Article

DOI: 10.18621/eurj.1347626 *Neurosurgery*

Comparative retrospective analysis of patients with idiopathic normal pressure hydrocephalus and aqueductal web-related aqueductal stenosis

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ABSTRACT

Objectives: Aquaductal web (AW) is a special form of aqueductal stenosis with similar clinical presentation with idiopathic normal pressure hydrocephalus (iNPH). iNPH is indeed a communicating hydrocephalus syndrome whereas AW is a noncommunicating subtype. Here, we aimed to investigate the similarities and differences between these two different chronic hydrocephalus syndromes in terms of clinical signs and symptoms, response to shunt treatment and postoperative complications.

Methods: Forty-one patients who underwent shunt operation with the diagnosis of iNPH or AW at our clinic between January 2010-May 2019 were retrospectively analyzed. Patients were evaluated by age, gender, clinical sign and symptoms, comorbidities, intraoperative and postoperative complications, and early and late postoperative outpatient follow-up findings.

Results: Twenty-six patients were classified as iNPH group and 15 patients as AW group. Patients in the AW group were significantly younger (45.5 ± 15.6 years vs. 60.3 ± 15.4 years) than the iNPH group (p = 0.006). There was no statistical difference between the groups in terms of subdural effusion formation, need for shunt revision (p = 1.000). Chronic hydrocephalus symptoms regressed in 23 (88.5%) patients in the NPH group, and at least one of them improved. symptoms. This rate was 66.7% (n = 10) in the AW group. Both groups showed similar clinical improvement with VPS (p = 0.1169).

Conclusions: The placement of ventriculoperitoenal shunt is widely used in the treatment of iNPH. As iNPH and AW has clinical similarities despite the discrepancies between underlying pathophysiological mechanisms and both clinical entities respond similarly to shunt treatment we advocate VPS surgery in the management of AW as well.

Keywords: Normal pressure hydrocephalus, aqueductal stenosis, ventriculoperitoneal (VP) shunt

Idiopathic normal pressure hydrocephalus (iNPH) is a chronic hydrocephalus syndrome usually seen in the elderly population and typically presents with balance and gait disturbances, cognitive dysfunction, and

Received: ***August 22, 2023;*** *Accepted:* ***October 2, 2023;*** *Published Online:* ***October 9, 2023***

How to cite this article: *Türkkan A, Eser Ocak P, Altunyuva O, Sönmez B, Özpar R, Bekar A. Comparative retrospective analysis of patients with idiopathic normal pressure hydrocephalus and aqueductal web-related aqueductal stenosis. Eur Res J 2023;(6):1464-1473. DOI: 10.18621/eurj.1347626*

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urinary incontinence [1, 2]. It was first described by Hakim and Adam in 1965 [2]. It may present with varying combinations or degrees of each element of the classic clinical triad [3-5]. iNPH is not a rare clinical condition. The prevalence of iNPH in the elderly population is estimated to be approximately 0.2%-5% [6]. One of the main characteristics of iNPH is that cerebrospinal fluid (CSF) pressure is within normal ranges. Typical brain imaging shows ventriculomegaly, periventricular hyperintensities, wide opening of the sylvian fissure, narrowed subarachnoid space, and highly convex cortical sulci [7-9].

The pathophysiological processes of iNPH are poorly understood, leading to a lack of clear diagnostic criteria [10]. In addition, the clinical characteristics of iNPH can be confused with those of different neurologic extrapyramidal disorders such as Parkinson's disease, Alzheimer-type dementia, and leukoarthrosis [4, 7, 11]. This makes patient selection for surgical treatment and its differentiation from other neurodegenerative disorders difficult. Since it is the only variant of dementia disorders that can be treated surgically, it is recommended to diagnose and treat it as early as possible [1, 4, 12].

Aqueductal Web (AW) is a rare pathology that causes chronic hydrocephalus syndrome by causing stenosis in the distal part of the aqueductus cerebri. AW is a translucent membrane composed of clusters of ependymal cells and fibrillar neuroglia [13]. AW is a special form of aqueductal stenosis [13]. Very similar to iNPH, AW also clinically presents with gait disturbance, cognitive decline, and incontinence, except it often presents at younger ages. There is no globally accepted magnetic resonance imaging (MRI) protocol for evaluating patients with a prediagnosis of AW; therefore, it is often difficult to clearly define the etiology of hydrocephalus using routine MR images. Of note, 3D T2-weighted gradient-echo sequences of CSF flow MRI examination may help better assess the flow dynamics in CSF and may define a better characterization of the aqueductal opening and associated abnormalities [14-16].

Although iNPH and AW harbor clinical and radiological similarities, iNPH is indeed a communicating hydrocephalus syndrome whereas AW is a noncommunicating subtype [17]. Since both iNPH and AW can benefit from CSF diversion, it is important to differentiate them from other causes of dementia with ventriculomegaly.

Therefore, we aimed to investigate the similarities and differences between these two different chronic hydrocephalus syndromes in the present study in terms of clinical signs and symptoms, diagnosis, response to shunt treatment, and complications. In addition, we examined the results of ventriculoperitoneal shunt surgery in the treatment of both patient groups.

METHODS

This retrospective examination was approved and authorized by the hospital clinical research ethics committee with the number 2023/03 and was subsequently performed by the regulations of the Declaration of Helsinki. Forty-one adult patients who underwent shunt operation with the diagnosis of iNPH or AW at our clinic between January 2010 and May 2019 were retrospectively analyzed. The patients were evaluated by age, gender, clinical signs and symptoms, comorbidities, neurologic examination, intraoperative and postoperative complications, and early and late postoperative outpatient follow-up.

The patients who had ventriculomegaly (Evan's index > 3) on brain computed tomography (CT) or magnetic resonance imaging (MRI) either accompanied or not accompanied by the classical triad symptoms (difficulty in walking, urinary incontinence, and dementia) were further examined for a chronic hydrocephalus syndrome. Gait disturbance was assessed using the 10-m walk test, and dementia was assessed using the Mini-Mental State Examination (MMSE). In addition, urinary continence was assessed by interviewing the patients and/or their caregivers. In all patients, the surgical decisions were supported by gait assessment after a lumbar tap test including drainage of 40 ml CSF by lumbar puncture. Recovery after lumbar puncture was defined as subjective improvement reported by patients and/or their family members. This test was considered supportive, not mandatory, for shunting decisions in the patients.

For radiologic evaluation, brain MRI, CSF flow MRI (3D T2-weighted gradient-echo sequences), diffusion tensor MRI, and brain CT imaging were performed in all patients. The Evans index was calculated

in each patient by dividing the maximum width between the frontal horns of the lateral ventricles by the distance between the two inner tabulae. Patients with CSF flow MRI and brain MRI revealing a membranous structure at the aqueductal level were diagnosed with AW. Those with Evans index < 0.30 were excluded from the study. Patients with a potential underlying cause for hydrocephalus such as a history of head trauma, intracranial hemorrhage, stroke, meningitis, primary malignancy, etc. were also excluded.

Ventriculoperitoneal Shunting Protocol

All patients included in the study underwent ventriculoperitoneal shunt (VPS) placement surgery. The surgical procedure included the placement of a ventricular catheter through a burr hole in the right frontal Kocher's point and a peritoneal catheter through a midline or paraumbilical mini-laparotomy. Codman programmable valve (Johnson and Johnson, MA, USA) was used in all patients.

After VPS surgery, all patients underwent regular follow-up examinations at 3, 6, and 12 months and then annually. Lengths of hospital stay, complications, or readmissions related to the ventriculoperitoneal shunting procedure were documented.

Shunt Response Assessment

Response to VPS was assessed approximately 6 months after surgery during outpatient follow-up visits. A 20% improvement in the 10-m walk test was considered a significant improvement. In addition, an increase of 2 or more in the MMSE score was considered a significant improvement. However, due to the study's retrospective design, postoperative objective measures of gait and cognition were only available in approximately 80% of the patients. For the remaining patients, scores were obtained by interviewing the patients and/or their caregivers.

Statistical Analysis

Continuous variables were expressed in terms of mean ± standard deviation. Independent samples t-test was used for comparisons between the two groups according to normality test results. Categorical variables were presented as frequency and percentage values [n (%)] and compared using the Pearson Chi-square test. Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). A p value of <0.05 indicated statistical significance.

RESULTS

Our study included 41 patients with chronic hydrocephalus. Twenty-six patients (14 males and 12 females) which included patients with clinical, laboratory, and routine cranial MR findings suggestive of iNPH were classified as iNPH group. The mean age of the patients in this group was 60.3 ± 15.4 years. Those with a membranous structure at the aqueductal level in their CSF flow MRI and brain MRI were classified as AW group and this group included 15 (7 males and 8 females) patients. The mean age of the patients in this group was 45.5 ± 15.6 years. The AW group patients were significantly younger than the iNPH group (p = 0.006).

In the iNPH group, the main presenting complaint was dementia (n = 22, 84.6%) followed by ataxia and gait disturbance (n = 20, 76.9%) and urinary incontinence (n = 16, 61.5%). In the AW group, 11 (73.3%) patients presented with dementia, 8 (53.3%) with ataxia and gait disturbance, and 8 (53.3%) with urinary incontinence. The initial symptom developed in both groups was dementia (n = 18 vs. n=7; 69.2% vs. 46.7%, respectively), followed by ataxia and gait disturbance (n = 6, 23.1% vs. n=5, 33.3%), and urinary incontinence (n = 2, 77% vs. n = 3, 20%). Naturally, dementia was the symptom with longest duration in both the iNPH and AW groups (28.8 ± 45.5 months vs. 22.3 ± 33.1 months).

In the AW group, headache was observed in 7 (46.7%) patients and nausea/vomiting in 4 (26.7%) patients, which were the symptoms that might have potentially been of significance for increased intracranial pressure. However, in the iNPH group, 9 (34.6%) patients had headaches, and only 1 (3.8%) had nausea/vomiting. The distribution of presenting symptoms was similar between groups (p > 0.05).

Type-2 diabetes mellitus was the most common comorbidity which was seen in 14 (38.5%) patients in the iNPH group and 4 (26.7%) patients in the AW group (Table 1).

Radiologic examinations revealed ventriculomegaly without enlargement of the sulci in the iNPH

Table 1. General demographic characteristics and clinical conditions of patients with iNPH and AW

Variables	iNPH (n = 26)	AW (n = 15)	*p* value
Sex, n (%)			
Female	12 (46.1)	8 (53.3)	0.7513
Male	14 (53.8)	7 (46.7)	
Mean age (years)	60.3 ± 15.4	45.5 ± 15.7	**0.006**
Symptoms at presentation, n (%)			
Dementia	22 (84.6)	11 (73.3)	0.433
Gait disturbance	20 (76.9)	8 (53.3)	0.167
Urinary incontinence	16 (61.5)	8 (53.3)	0.744
Paraparesis	16 (61.5)	6 (40)	0.211
Headache	9 (34.6)	7 (46.7)	0.517
Dizziness	1 (3.8)	4 (26.7)	0.051
Nausea/vomiting	1 (3.8)	4 (26.7)	0.051
First symptom, n (%)			
Dementia	18 (69.2)	7 (46.7)	0.194
Gait disturbance	6 (23.1)	5 (33.3)	0.490
Urinary incontinence	2 (7.7)	3 (20)	0.336
Mean duration of symptoms (months)			
Dementia	28.8 ± 45.5	22.3 ± 33.1	0.602
Gait disturbance	17.2 ± 24.9	21.3 ± 35.5	0.697
Urinary incontinence	15.7 ± 21.6	13.7 ± 30.6	0.826
Comorbidities, n (%)			
Diabetes	10 (38.5)	4 (26.7)	0.511
Hypertension	9 (34.6)	3 (20)	0.479
Coronary artery disease	5 (19.2)	-	0.139
Thyroid goiter	3 (11.5)	1 (6.7)	1.000
Parkinson	3 (11.5)	0	0.286
Alzheimer's sisease	2 (7.7)	0	0.524
Benign prostate hypertrophy	1 (3.8)	1 (6.7)	1.000
Cerebrovascular disease	1 (3.8)	1 (6.7)	1.000
Atrial fibrillation	1 (3.8)	1 (6.7)	1.000
Schizophrenia	-	1 (6.7)	0.365

Data are shown as mean ± standard deviation or n (%). AW = Aqueductal web, iNPH = Idiopathic normal pressure hydrocephalus

group. In CSF flow measurements of all patients, a sinusoidal flow pattern symbolizing forward-backward movement was found in the aqueductus cerebri, while no-signal void (hyperdynamic CSF flow) reflecting rapid CSF flow was detected at the aqueductal level.

All patients underwent shunt surgery using a programmable VPS. In the early postoperative period, one (3.8%) patient in the iNPH group developed an intraventricular hematoma, and one patient (6.7%) in the AW group suffered from internal carotid artery (ICA) infarction. The patient who developed intraventricular hematoma was followed up in the intensive care unit for 13 days with an external ventricular drainage. The patient with ICA infarction underwent thrombectomy by interventional radiology. Both patients were discharged home without additional neurological deficits.

In 14 (53.8%) patients in the iNPH group, the shunt settings required adjustment due to subdural effusion or inadequate clinical improvement during outpatient follow-ups. In five of these cases (19.2%), shunt pressure changes were insufficient in treating subdural effusion and subdural drainage was required. Shunt revision was performed in one (4%) case. In seven (46.7%) patients in the AW group, the shunt settings were adjusted due to subdural effusion or inadequate clinical improvement. Subdural drainage was

Table 2. Overall early (postoperative first 24 hours) and late postoperative (postoperative 6 months) complications following ventriculoperitoneal shunt surgery for idiopathic normal pressure hydrocephalus and aqueductal web

	iNPH (n = 26)	AW (n = 15)	All patients (n = 41)
Early Complications, n (%)			
ICA infarct	1 (3.8)	0	1
Intraventricular hematoma	0	1 (6.7)	1
Late Complications, n (%)			
Subdural effusion/hematoma requiring drainage	5 (19.2)	3 (20)	8 (19.5)
Shunt revision	2 (7.7)	1 (6.7)	3 (7.3)
Wound discharge	0	1 (6.7)	1 (2.4)

AW = Aqueductal web, ICA = Internal carotid artery, iNPH = Idiopathic normal pressure hydrocephalus

needed in two (13.3%) of these cases, one (6.7%) of which also underwent shunt revision (Table 2).

During a mean follow-up period of 37.8 ± 27.1 months (42.4 ± 30.2 vs. 29 ± 20.4 months), 23 (88.5%) of the patients in the iNPH group showed regression of chronic hydrocephalus symptoms and improvement in at least one of the symptoms. In the AW group, this rate was 66.7% (n = 10). There was no statistical difference between the groups in terms of the occurrence of subdural effusion (p = 1.000), and the need for shunt revision (p = 1.000). Both groups showed similar clinical improvement with VPS (p = 0.1169) (Table 3).

DISCUSSION

The third edition of the iNPH guidelines published in Japan in 2021 proposes a new classification for NPH [4]. According to this newly revised classification, the iNPH category is clearly distinguished from NPH by its congenital/developmental and acquired etiologies [4]. Diagnosing acquired NPH is not difficult, which occurs after a specific etiologic event. On the other side, the differential diagnosis of iNPH is difficult due to the similarity of nonspecific symptoms with many other diseases. However, AW which causes hydrocephalus in adulthood, is defined as late-onset congenital hydrocephalus [4]. AW is actually a special form of aqueductal stenosis [13]. The web consists of a thin ependymal membranous septum, and the cause of its formation is unknown.

Although there are large series in the literature about iNPH, the literature data regarding AW mainly consists of case reports. For example, Chen *et al*. [18] reported that 21 of 2009 patients treated for obstructive hydrocephalus had a membranous structure at the aqueductal level. Matsuda *et al*. [19] presented a case

Table 3. Clinical outcome of patients who underwent ventriculoperitoneal shunt surgery for idiopathic normal pressure hydrocephalus and aqueductal web

	iNPH (n = 26)	AW (n = 15)	*p* value
Mean follow-up time (months)	42.4 ± 30.2	29 ± 20.4	0.099
Adjustment in valve pressure	14 (53.8)	7 (46.7)	0.751
Clinical improvement	23 (88.5)	10 (66.7)	0.116

Data are shown as mean ± standard deviation or n (%). AW = Aqueductal web, iNPH = Idiopathic normal pressure hydrocephalus

of late-onset aqueductal membranous occlusion hydrocephalus. Terada *et al*. [20] presented an adult case of hydrocephalus caused by a membranous structure at the aqueductal level. However, most of these studies reported cases that were diagnosed intraoperatively. Therefore, patients with adult-type AW may not have been adequately identified due to flaws in the preoperative diagnosis. In this context, our study is one of the most extensive series of patients treated for adult-type AW-related hydrocephalus who were diagnosed with the presence of an AW preoperatively.

On the other side, iNPH is highly prevalent, particularly in the elderly population. In a population-based study in West Sweden, the prevalence of iNPH was estimated to be between 0.2%-5.9% in the seventh and eighth decades [6]. The average onset for iNPH is around 70 years of age, and men and women are affected equally [6, 21]. However, AW is usually detected in childhood due to the obstructive type of hydrocephalus. As AW is extremely rare in adults, it is believed to present in these patients since childhood, and become symptomatic after years of compensated ventriculomegaly [13, 19, 20]. AW often needs clarification with iNPH. Nevertheless, the age of onset is much earlier than that of in iNPH patients. In consistence with the literature, in the current study, we found that patients diagnosed with adult-type AW were significantly younger compared to the group of patients diagnosed with iNPH.

Since iNPH and AW both cause chronic hydrocephalus, they display clinical similarities although they differ pathophysiologically [22]. iNPH is a nonobstructive hydrocephalus, and the exact underlying pathophysiological mechanisms remain unclear although several different theories have been proposed so far. According to the most accepted theory, there is an increased resistance to CSF absorption from the arachnoid villi [23].As a result, lack of adequate CSF absorption leads to ventricular enlargement. CSF leaks through the ependymal spaces into the periventricular white matter and causes a decrease in tissue metabolism, resulting in ischemia and hypoxia in white matter axons. Eventually, demyelination and neural apoptosis secondary to chronic ischemia and hypoxia occur [23]. Kuriyama *et al*. [10] reported that retrograde flow in the internal jugular vein during the Valsalva maneuver was significantly higher in patients with iNPH compared to the control group. They emphasized that this condition may cause the resultant picture with increased central venous pressure and decreased CSF absorption [10].

Contrarily, AW causes obstructive hydrocephalus due to the presence of a translucent membrane formed by clusters of ependymal cells and fibrillary neuroglial cells in the distal part of the aquaeductus cerebri [13, 24]. For its development, various previous inflammatory processes as well as congenital glial cell occlusion have been blamed [20]. At the diagnostic stage, many AW patients are mistaken for iNPH patients as both groups show findings known as the classic clinical triad of Hakim and Adams [2] which consists of a symmetrical gait disorder with slow, short, and wide-based steps, urinary incontinence, and dementia. In the literature, gait disturbance has been reported to be the most common abnormal walking pattern [4, 25, 26]. However, this gait pattern is also common in Parkinson's disease as well as other degenerative and vascular diseases affecting the CNS such as peripheral neuropathies, spinal stenosis, myelopathy, vitamin B12 deficiency, and sometimes even combination of these diseases [4, 7, 11]. Of note, less than two-thirds of patients with iNPH have been reported to display all three components of the triad [4, 5, 11].

In the present study, gait disorders were found in 76.9%, urinary incontinence in 61.5%, and cognitive impairment in 84.6% of the iNPH patients. Clinical symptoms caused by increased intracranial pressure, such as headache and nausea/vomiting were more common in patients with AW (46.7%) than in the patients with iNPH (36.6%). A similar occurrence of Hakim's triad was present in slightly smaller percentages of patients in the AW group as well with a ratio of 53.3%, 53.3%, and 73.3% for gait impairment, urinary incontinence, and cognitive impairment respectively. The most common presenting symptom and first symptom in both iNPH and AW was forgetfulness. The ratios for the presence of other symptoms and mean symptom duration were similar between groups (Table 1). In this respect, there is a high degree of clinical similarity between iNPH, a nonobstructive chronic hydrocephalus syndrome, and AW, an obstructive hydrocephalus syndrome.

Obviously, we need accurate imaging techniques to show CSF pathways in detail in patients for whom treatment is planned with the diagnosis of hydrocephalus. In fact, iNPH and AW cannot be differenti-

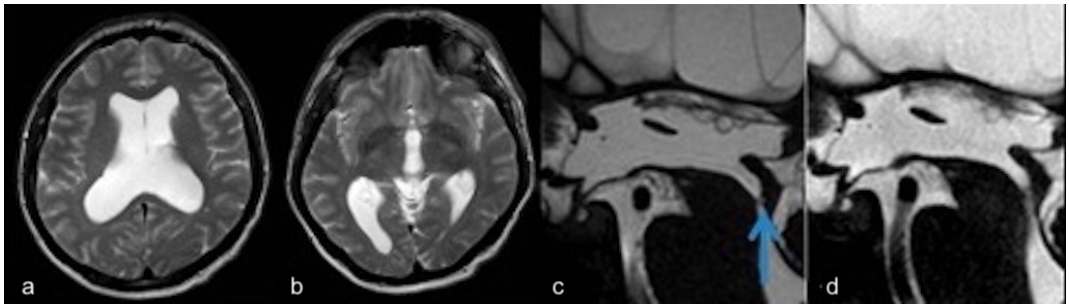

Fig. 1. CSF Flow MRI image of a patient with an aqueductal web. There is chronic hydrocephalus in the lateral ventricles (a) and third ventricle (b) on T2-weighted images. (c) In the 3D Balanced Heavy T2 image there is membrane formation distal to the aqueduct (blue arrow). (d) No expected flow void area of aqueductal CSF flow in flow-sensitive T2 DRIVE sequence.

ated from each other based on conventional radiologic examinations such as cranial CT and MRI [7, 17, 27]. Therefore, patients with AW may be overlooked. Invasive methods such as radionuclide cisternography, CT cisternography with iodinated contrast or air, and MR cisternography with gadolinium-based contrast have been described and used so far. However, these methods have not gained widespread acceptance due to their invasive nature. In examinations such as 3D T2-weighted gradient-echo sequences of CSF flow MRI, the membrane formation of the aqueductal level can be detected in patients with AW, and the expected signal void area due to CSF flow may be absent (Fig. 1). On the other hand, iNPH can be diagnosed by detecting the signal void area representing rapid CSF flow at the aqueductal level (Figs. 2 and 3). According to the current literature, CSF flow MRI supports the diagnosis of iNPH and helps diagnose AW [3, 7, 14, 27]. In the present study, all patients underwent 3D T2-weighted gradient-echo sequences of CSF flow MRI preoperatively to confirm our diagnosis. We found that 36.6% of the patients for whom we planned surgery with a prediagnosis of iNPH had indeed an AW in the radiologic examinations. This was in line with the study by Giordan *et al*. [16] who reported the presence of aqueductal stenosis in a low percentage of iNPH patients (10%) and suggested investigation with high-resolution MRI in patients with iNPH. We observed AW in 36.6% of our chronic hydrocephalus patients. We believe that further investigations, such as 3D T2-weighted gradient-echo sequences of CSF flow MRI are more sensitive to display web formation at the aqueductal level, leading to higher rates of AW diagnosis. Although there are studies on various CSF biomarkers for the diagnosis of iNPH, the relationship between AW and these biomarkers has yet to be stud-

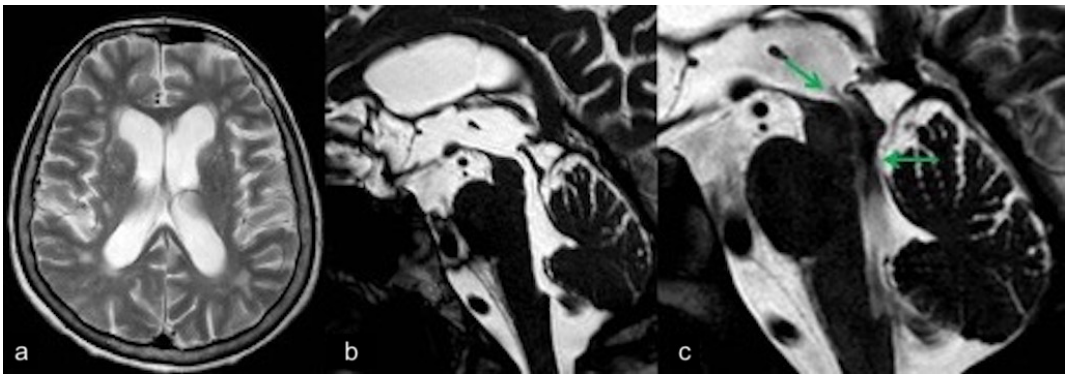

Fig. 2. CSF Flow MRI image of a patient with iNPH. (a) T2-weighted image shows chronic ventriculomegaly in both lateral ventricles. (b) No obstruction is seen at the aqueductal level in the 3D Balanced Heavy T2 image obtained for morphological evaluation. (c) Flow void reflecting rapid CSF flow at the aqueductal level (arrows) in flow-sensitive T2 DRIVE image.

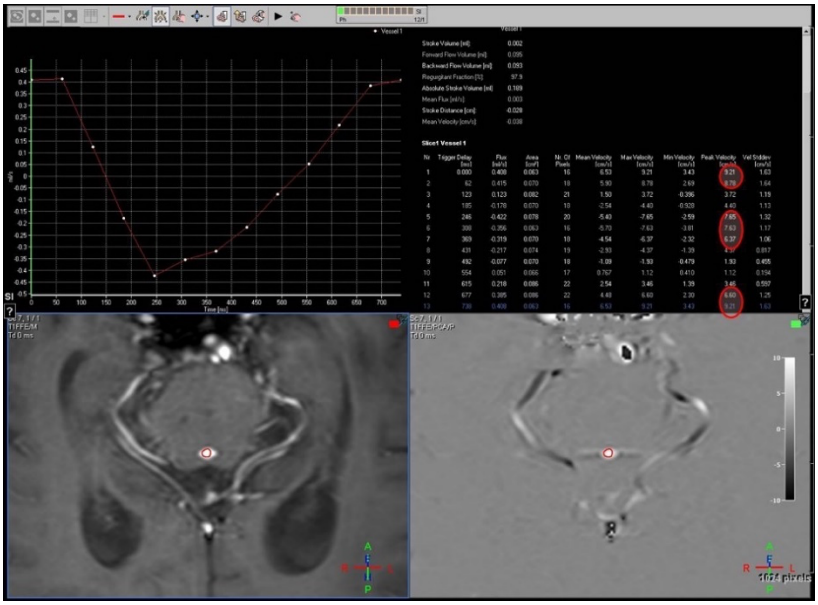

Fig. 3. Increase in expected peak systolic velocities (normally 4-6 cm/sec) in quantitative transverse phase contrast imaging obtained from the aqueductal level of a patient with iNPH (round inside).

ied [14]. Therefore, comparing patients with radiologically differentiated AW or iNPH will contribute to the literature in the future.

Systemic diseases such as hypertension, diabetes mellitus, hyperlipidemia, ischemic heart disease, atherosclerotic cerebrovascular disease, and peripheral arterial disease may cause additional morbidity in patients with iNPH. At the same time, neurodegenerative diseases including Alzheimer's and Parkinson's disease as well as cerebrovascular white matter diseases may be observed together with iNPH [4, 11, 16, 28]. These comorbid diseases may increase the severity of iNPH symptoms. The presence of concomitant neurologic disorders may also affect recovery and long-term prognosis after treatment with shunt placement as well [4].

In our study, comorbid diseases were more common in patients with iNPH, a disease of advanced age [1, 4, 8, 9] compared to the group of AW patients, albeit with a nonstatistical significance (Table 1).

Ventriculoperitoneal shunt surgery remains current in the surgical treatment of iNPH [1, 3, 4, 16, 26, 29]. In cases with AW, the membranous structure at the aqueductal level can be removed by endoscopic methods [30]. Surgical complications and recurrences may be seen in fiberoptic ventriculoscopy, endoscopic third ventriculostomy, and posterior transcranial approaches used in the treatment of AW [15, 16, 30]. In addition, endoscopic applications require good anatomical knowledge and experience. The learning process is long and technically requires neurological surgical equipment that is not available in all centers. For this reason, shunt surgery for AW can be applied as an alternative option to other surgical methods. Ventriculoperitoneal shunt surgery is technically easier to perform and more noninvasive. In addition, patients with AW are younger and usually have fewer comorbid diseases. From this point of view, a higher surgical performance can be expected in shunt surgery in patients with AW patients.

With the advancements in technology, the use of shunt systems with programmable pressure valves has proven to be successful in the treatment of hydrocephalus [3, 29]. Skalicky *et al*. [3] has reported that, compared to fixed pressure shunts, the use of programmable valves results with significantly lower rates of revision surgeries. Early and late complications following shunt surgery in the treatment of iNPH have been reported in the literature [4, 16]. In a recent meta-analysis, Giordian *et al*. [16] has reported that the development of subdural effusion requiring drainage was the most common complication followed by infections after VPS surgery for iNPH.

In our study, we used VPSs with programmable

valves in all patients with iNPH and AW. Using a ventriculoperitoneal shunt with a programmable valve gave us advantages in preventing the development of subdural effusion while closely monitoring clinical improvement. During the follow-up period for patients with iNPH and AW who underwent VPS surgery of 42.4 ± 30.2 months vs. 29 ± 20.4 months, respectively chronic hydrocephalus symptoms regressed in 88.5% of iNPH patients and 66.7% of AW patients (Table 3). Statistically, we did not observe a difference between the two groups in terms of response rate to the shunt surgery. During outpatient follow-ups, shunt settings were adjusted in almost half of the patients in both the iNPH and AW group (53.8% vs. 46.7%, respectively) due to the occurrence of subdural effusion. Yet, some patients required 19.2% vs. 13.3%) burr hole drainage as shunt pressure adjustment failed to manage the effusion. One patient per group underwent shunt revision due to malfunction in the present series. (Table 2). According to our results, these two groups of patients did not show a difference in the rate of development of subdural effusion after shunting as well as the rate of shunt revisions. In addition, the low need for revision surgery due to the use of shunts with programmable valves was consistent with the literature. The rates of infection in the present series (2.4%) were similar to the literature [1, 16].

Limitations

There are some limitations of this study. First, the study was not double-blinded. Due to the retrospective design of the study, only approximately 80% of patients had objective postoperative measures of gait and cognition available. For the remaining patients, scores were obtained by interviewing the patients and/or their caregivers. Second, the group size was small, reflecting both the relatively low incidence of NPH and the smaller subgroup of complex NPH within the overall disease spectrum. Third, this was a single-center study. Studies with better design and more participants are needed to confirm our findings.

CONCLUSION

iNPH and AW display similar clinical presentation although the underlying pathophysiological mechanisms are largely different, and they occur at different age groups. The placement of VPS is a widely accepted treatment of iNPH. Based on the similar clinical response and postoperative success rates, we advocate VPS surgery in the management of AW, which seems a more advantageous technique considering the technical difficulties of other surgical methods.

Authors' Contribution

Study Conception: AT, PEO; Study Design: AT, PEO; Supervision: AB; Funding: N/A; Materials: AT, AB; Data Collection and/or Processing: AA, OA, BS; Statistical Analysis and/or Data Interpretation: AT, PEO, RÖ; Literature Review: OA, BS, RÖ; Manuscript Preparation: AT, PEO, OA, BS, RÖ and Critical Review: PEO, AB.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgment

The abstract of this paper was presented as oral presentation at 7th Congress of the European Academy of Neurology- Virtual 2021, June 19-22.

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The European Research Journal 2023;9(6):1474-1482 Original Article

DOI: 10.18621/eurj.1354942 *Physical Medicine and Rehabilitation*

Comparison of manual lymphatic drainage massage and negative pressure massage therapy efficacy in lymphedema patients: a randomized controlled study

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ABSTRACT

Objectives: Lower extremity lymphedema due to secondary causes is a lifelong complication that can be encountered. Its treatment is essential, because it has significant impact on quality of life and daily living activities related to lower extremity involvement. This research aims to compare the effects of Manual Lymphatic Drainage Massage (MLD) and Negative Pressure Massage Therapy (NPMT) treatments in order to provide maximum benefit to patients.

Methods: This prospective, randomized study included 30 patients with lower extremity lymphedema due to secondary causes. Patients, randomized using computer software, were divided into two groups. The first group (n =15) received 45 minutes, 15 sessions of MLD, while the second group (n =15) received 45 minutes of 15 sessions of NPMT using the LymphaTouch device. Compression bandaging was applied to both groups and self-drainage training was given to all patients. The circumference of the extremity at 6 reference points were measured and their pain and discomfort assessed by the Visual Analogue Scale (VAS) were recorded before and after treatment. Changes within the groups and between the groups were compared using the SPSS statistical program.

Results: Statistically significant improvement was observed in all parameters in both treatment groups. The decrease in VAS pain and VAS discomfort scores ($p < 0.05$ and $p < 0.01$; respectively), circumference measurement of the extremity ($p < 0.01$) was statistically greater in the NPMT group compared to the MLD group. Conclusions: In conclusion, NPMT appears to be a beneficial non-invasive treatment method for reducing extremity volumes and decreasing subjective pain and discomfort in lymphedema patients.

Keywords: Lymphedema, manual lymphatic drainage, topical negative pressure therapy, compression bandages

Lymphedema is defined as the abnormal accumulation of interstitial fluid and fibroadipose tissue resulting from injury, infection, or congenital abnormalities of the lymphatic system [1]. Surgical interventions, radiotherapy, trauma, infections, tumors, chronic venous insufficiency, and pathological, congenital, and/or hereditary etiologies can lead to the development of lymphedema. The most common factors

Received: ***September 4, 2023;*** *Accepted:* ***October 6, 2023;*** *Published Online:* ***October 16, 2023***

How to cite this article: *Ersoy S, Kesiktaş N, Şirin B, Buğdaycı ND, Kibar H, Paker N. Comparison of manual lymphatic drainage massage and negative pressure massage therapy efficacy in lymphedema patients: a randomized controlled study. Eur Res J 2023;9(6):1474-1482. DOI: 10.18621/eurj.1354942*

e-ISSN: 2149-3189

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contributing to lymphedema in the lower extremities are aplastic/hypoplastic/hyperplastic lymphatic abnormalities, gynecological cancers, surgical procedures, trauma, radiotherapy, and recurrent infections [2, 3]. In patients with lymphedema, affected limb circumference increase, swelling, changes in appearance, limited range of motion, in addition to edema that does not leave a trace, can lead to various problems such as pain, discomfort, decreased quality of life, depression, and more [4]. Due to these problems, a wide range of treatments are needed.

While there is no definitive cure for lymphedema, patients often benefit from conservative treatment methods. Surgical approaches are not curative and can even exacerbate lymphedema in some cases [5]. Treatment for lymphedema falls under three main categories: medical, surgical, and conservative. Pharmacological methods have not been reported to have a consistent role in lymphedema treatment. Unlike hydrostatic forms of extracellular fluid accumulation, lymphedema may respond very little to diuretic therapy [6]. In advanced-stage lymphedema patients who do not benefit from conservative treatment and show significant morbidity, surgical procedures may be indicated. Frequent recurrent infections, significant impact on the patient's quality of life and function, and advanced psychological distress due to appearance can also be indications for surgery [7].

Physical medicine and rehabilitation specialists have been observed using physical modalities as part of a comprehensive rehabilitation program to reduce pain, increase strength, accelerate tissue healing while preventing possible malignant tumor growth in lymphedema patients [8]. Low-power lasers, electrical stimulations using transcutaneous electric currents, and modalities such as extracorporeal shock wave therapy can be used in lymphedema treatment [8].
Lymphedema treatment is challenging, but lymphedema patients often benefit from conservative treatment and can remain stable with the gains from treatment [9]. After lymphedema is diagnosed, specific physiotherapy treatment methods are needed for its treatment (10). Patient education is crucial within conservative treatment. Attitude towards trauma, maintaining the natural moisture balance of the skin, recognizing and intervening in infections can be achieved through patient education [8, 9].

Intermittent Pneumatic Compression (IPC) is used in lymphedema treatment as an adjunct to Complex Decongestive Physical Therapy (CDPT) [11]. CDPT is currently considered the gold standard in lymphedema treatment (12). CDPT consists of two phases: the intensive treatment phase (phase 1) and the maintenance phase (phase 2). The intensive treatment phase includes manual lymphatic drainage (MLD), compression bandaging, exercise, and skin care. These two phases are also referred to as combined decongestive therapy. MLD is a special massage technique applied directly to the skin with low pressure to stretch lymph capillaries and increase lymphangiomotoricity and lymphatic drainage [13]. It has been shown that manual lymph drainage stimulates lympho-lymphatic and lympho-venous anastomoses and provides symptom reduction that compression alone cannot achieve [12, 14]. The basic working principle of compression bandages includes preventing and reducing edema, increasing venous flow rate, decreasing venous diameter, increasing venous and lymphatic pump activity, and increasing arterial flow [10, 12, 15].

In our country and around the world, there have been limited studies conducted on the rehabilitation of lower extremity lymphedema. Applications using negative pressure massage therapy devices are relatively new but have shown promise as a viable option for lymphedema treatment. This study aimed to compare this treatment method with the gold standard, manual lymphatic drainage, and two treatment protocols consisting of both treatments along with compression bandages, exercises, and skin care.

METHODS

Patients with lower extremity lymphedema associated with stage 1-2 secondary causes who applied to the Lymphedema Unit of Istanbul Physical Medicine and Rehabilitation Training and Research Hospital for treatment were included in the study. All patients had completed chemotherapy and radiotherapy. The inclusion criteria for the study were being between the ages of 18-75, having a diagnosis of unilateral lower extremity lymphedema, being willing to participate in the study. The exclusion criteria were having mental and cognitive disorders, being unable to communicate and cooperate, having an active infection, having bilateral lymphedema, excluding complications such as

pulmonary edema and congestive heart failure that would restrict treatment.

Using numbers obtained with computer software, patients were divided into two groups by randomization. The first group received 45 minutes of 15 sessions of manual lymphedema massage, and the second group received 45 minutes of 15 sessions of negative pressure massage therapy (NPMT) using the LymphaTouch device. Compression bandages were applied to both groups five days a week, and self-drainage training was provided to all patients after the sessions. Before treatment, patients' extremity circumference measurements at 6 reference points and their discomfort assessed by the visual analog scale (VAS) method were recorded. After completing 15 sessions, patients were re-evaluated with measurements at 6 reference points and evaluated for discomfort using the VAS.
For this study, ethical approval was obtained from the Istanbul Kanuni Sultan Süleyman Training and Research Hospital Ethics Committee with protocol number 2022.12234. Individuals who met the study criteria and voluntarily agreed to participate were provided with written and verbal information about the study. Informed consent forms were obtained from individuals both verbally and in writing for their participation in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Measurements

VAS Pain and Discomfort

A 100-mm VAS was used to determine the severity of pain and discomfort. Patients quantified their pain and heaviness sensations on two separate VAS ratings on a scale ranging from 0 to 10, with 0 indicating no discomfort and 10 indicating the most severe discomfort. VAS satisfaction was assessed using the same 100 mm visual analog scale, with the level of satisfaction with the treatment being queried as 0 for not satisfied and 10 for very satisfied, and it was marked in the same way as VAS pain at the beginning and end of the treatment [16].

Lower Extremity Circumference Measurement

Patients' circumferences were measured at 6 levels with a flexible tape measure at 10 cm intervals starting from the metatarsophalangeal joint, with the ankle-ankle joint in a neutral position. Two measurements were taken, and their mean was used [17].

Interventions

The intervention group received treatment with the NPMT device (LymphaTouch) and the control group received MLD. The LymphaTouch is Food and Drug Administration approved as a therapeutic massage device in the United States. This handheld device administers negative pressure in the range of 80-250 mmHg under the treatment head, which gently pulls the underlying tissue into the suction cup [18]. MLD is a special massage technique applied directly to the skin with low pressure to stretch lymph capillaries and increase lymphangiomotoricity and lymphatic drainage. The basis of MLD is based on the 4 basic hand positions defined by Vodder [19].

Statistical Analysis

The power analysis method was used to determine the number of individuals to be included in the study, and it was calculated that at least 12 individuals should be included in each group, with a power ratio of 80% and an alpha risk of 0.05 accepted for each group. Considering a 10% dropout rate in both groups, 15 participants were determined per group according to

Table 1. Demographic data of patients

	NPMT (n = 15)	MLD (n = 15)	*p* value
Age (years)	57.73 ± 7.12	57.67 ± 5.95	0.569
Height (cm)	160.13 ± 4.29	159.20 ± 4.09	0.496
Weight (kg)	84.73 ± 15.07	83.27 ±1.06	0.877
BMI (kg/m^2)	31.03 ± 47.29	31.21 ± 47.18	0.833

Data are shown as mean ± standard deviation. NPMT = Negative Pressure Massage Therapy, MLD = Manual Lymphatic Drainage, BMI = Body Mass Index

the article of Corum *et al* [20].The MedCalc statistical program was used for sample size calculation. A total of 42 patients were screened for the study, and 30 participants who met the criteria were included. Descriptive statistics for categorical variables were presented as numbers and percentages, and descriptive statistics for numerical variables were presented as mean and standard deviation. Comparisons between two measurements were performed with the Wilcoxon signed-rank test. Mann-Whitney U test was used for intergroup comparisons. A significance level of $p < 0.05$ was accepted. SPSS version 10.0 statistical computer program was used for the analysis.

RESULTS

A total of 30 participants, all of whom were female, were included in the study. When comparing age, height, weight, and BMI between the groups, it was determined that there was no significant difference in weight and BMI ($p < 0.05$). Demographic data for the individuals are shown in Table 1.

Our patients were observed to be in stages 1 and 2 of lymphedema, and all of them have secondary lymphedema. It has been noted that individuals have had lymphedema for an average of 5 years (ranging from 1 to 12 years). Two-thirds of them have hypertension. When comparing the initial measurements, no statistically significant differences were found between all measurements. Initial measurements can be observed in Table 2.

When comparing the pre- and post-treatment results in both NPMT and MLD, ($p < 0.05$ and $p < 0.01$; respectively) statistically significant changes were observed (Table 3). When comparing between the groups, while some values were similar, overall, the NPMT ($p < 0.01$) group achieved more successful results (Table 3).

DISCUSSION

Lower extremity lymphedema is a chronic condition that requires lifelong treatment [21]. While treatment options are increasing with new developments, conservative treatments can still be insufficient in some cases [18]. Skin care, exercise, bandaging, and manual lymphatic drainage are standard treatment methods used in lymphedema. Options outside of these methods, which are part of the Complex Decongestive Therapy group, include pneumatic compression, medical treatment, electrotherapy, and surgery [21, 23]. Negative pressure massage therapy (NPMT) has started to be included in lymphedema treatment and its effectiveness has been investigated in recent years [24, 25]. There are few studies in the literature that investigate the effectiveness of NPMT compared to manual massage therapy in lymphedema patients. Moreover, there has been no similar research on lower

Table 2. Comparison of measurements before treatment

	NPMT (n = 15)	MLD (n = 15)	*p* value
VAS-pain	7.31 ± 3.82	7.23 ± 2.89	0.891
VAS-discomfort	6.43 ± 2.88	6.68 ± 31	0.766
MTP (cm)	25.60 ± 3.26	25.29 ± 3.18	0.801
Ankle (cm)	28.53 ± 4.01	29.01 ± 4.12	0.295
10 cm to ankle	40.40 ± 11.23	41.65 ± 9.8	0.324
20 cm to ankle	47.96 ± 8.96	46.12 ± 9.02	0.321
30 cm to ankle	47.60 ± 9.87	47.23 ± 7.76	0.801
40 cm to ankle	55.46 ± 8.40	54.35 ± 9.74	0.323

Data are shown as mean±standard deviation. NPMT = Negative Pressure Massage Therapy, MLD = Manual Lymphatic Drainage, VAS = Visual Analogue Scale, MTP = Metatarsophalangeal

Table 3. Comparison of the differences of before and treatment measurements within and between groups

	NPMT (n = 15) ΔBT-AT	*p* value	MLD (n = 15) ΔBT-AT	*p* value	Between Groups *p* value
VAS-pain	1.85 ± 1.86	**0.001**	1.97 ± 2.86	**0.001**	**< 0.05**
VAS-discomfort	3.21 ± 1.92	**0.001**	3.91 ± 2.42	**0.001**	**< 0.01**
MTP (cm)	1.20 ± 1.50	**0.008**	1.02 ± 1.04	**< 0.01**	**< 0.01**
Ankle(cm)	1.20 ± 1.37	**0.004**	1.01 ± 0.92	**< 0.01**	**< 0.05**
10 cm to ankle	3.43 ± 5.88	**0.040**	2.54 ± 6.10	**< 0.01**	**< 0.01**
20 cm to ankle	3.30 ± 4.81	**0.019**	2.14 ± 5.7	**< 0.01**	**< 0.01**
30 cm to ankle	2.23 ± 4.02	**0.019**	1.4 ± 5.7	**< 0.05**	**< 0.05**
40 cm to ankle	1.83 ± 2.16	**0.016**	1.01 ± 4.22	**< 0.05**	**< 0.01**

Data are shown as mean ± standard deviation. NPMT = Negative Pressure Massage Therapy, MLD = Manual Lymphatic Drainage, VAS = Visual Analogue Scale, MTP = Metatarsophalangeal

ΔBT-AT the difference between treatment and after treatment

extremity lymphedema patients. Therefore, our study is the first of its kind in this regard. Most of the existing studies have focused on upper extremity lymphedema patients associated with breast cancer, which is why the number of studies on lower extremity lymphedema is quite limited. With this research, we aim to determine the effectiveness of negative pressure massage therapy in lymphedema patients by evaluating changes in limb circumference measurements, pain, and discomfort and comparing it with manual massage therapy.

Manual massage therapy facilitates lymphatic drainage by creating a mild pressure gradient within the tissues [22]. While there are still some unclear points in the mechanism of negative pressure therapy, it is believed to stimulate lymphatic circulation by mobilizing and stretching the skin and subcutaneous tissue, thus exerting its effects. There are opinions in the literature that this mechanism is effective in achieving better results compared to manual massage therapy [18]. Mihara *et al*. [26] demonstrated in their study that lymphatic vessels in chronic lymphedema patients were sclerotic and in a constant state of contraction in advanced stages. It is thought that negative pressure therapy may facilitate mobilization in contracted vessels in chronic lymphedema patients. This study highlights that NPMT therapy may be a suitable option even in cases where the response to treatment is limited and the condition has become chronic.

The average age of the individuals included in our study, 57 years, is in line with the literature. Beesley *et al*. [27] mentioned in their study that individuals aged 50 and above are at a higher risk group. Deura *et al*. [28] also stated that the average age of individuals who developed lymphedema in their lower extremities after gynecological cancer was 55.

It is known that a BMI above 30 kg/m2 is an important risk factor for the development of lymphedema [29]. The average BMI of the lymphedema patients included in our study is above this value. The high average BMI in this population is consistent with the literature results regarding the relationship between lymphedema and obesity.

Measurement of volume and circumference is commonly used in the diagnosis and monitoring of lymphedema [17]. In addition to circumference and volume measurements, various other methods are also used, such as tonometry to measure the resistance of the tissue to applied compression, volumetry, bioimpedance spectroscopy, and tissue dielectric constant measurement. When we look at the literature, we see that circumference measurement is the most commonly used simple and objective criterion to measure the level of lymphedema [30]. Circumference meas-

urement is an objective assessment method that can easily be applied in repeated visits, and research has shown that it accurately identifies edema independently of volume measurement, with an accuracy rate of 84% [31]. Karges *et al*. [32] also demonstrated that circumference measurements made at 4 cm intervals in the upper extremity are a valid and reliable alternative to volume measurement. In our study, circumference measurements of the extremities were taken at the metatarsophalangeal joint, ankle level, and lateral malleolus of the ankle at 10 cm intervals, following this approach.

Devoogdt *et al*. [33] found that manual lymphatic drainage, in addition to education and exercise, was not effective in preventing lymphedema development in women who underwent unilateral axillary dissection due to breast cancer in both the short and long term. There are several studies in the literature that support the notion that MLD is insufficient in terms of preventing and treating lymphedema development [34, 35]. Lin *et al*. [36] published a meta-analysis in which they stated that MLD was effective in reducing pain in breast cancer-related lymphedema patients but did not result in a significant reduction in limb volume or improvement in quality of life. Similarly, Huang *et al*. [37] also found MLD to be insufficient in preventing and treating breast cancer-related lymphedema.
In contrast, in the study by Sitzia *et al*. [38], manual lymphatic drainage (MLD) and self-lymphatic drainage methods were compared, and volume reduction was found to be 33.8% in the MLD group, while this rate was determined as 22% in the self-lymphatic drainage group. This research emphasizes the effectiveness of MLD therapy and the importance of its application by trained therapists rather than by the patient themselves. Self-lymphatic drainage, on the other hand, maintains its value as an auxiliary method that the patient or a caregiver can use outside the hospital for the continuity of treatment. In a meta-analysis involving 457 patients, the efficacy of MLD in breast cancer-related lymphedema patients was evaluated. Although its volume-reducing effect was not significant, a significant reduction in extremity volume was recorded in applications lasting more than 2 weeks or with a total session count exceeding 20 [39]. This study demonstrates the importance of the number of sessions and the duration of MLD therapy in terms of effectiveness. In our research, we also observed a statistically significant reduction in extremity circumference measurements before and after treatment in the group receiving MLD therapy. Additionally, there was a significant decrease in the patient's pain and discomfort levels after treatment. Our total treatment duration was 3 weeks, which is consistent with the treatment duration emphasized in the study by Qiao *et al*. [39]. In a case report published by Borman *et al*. [40], a 48-year-old woman with polio sequelae who was mobilized with a wheelchair and had lymphedema and infected wounds in both lower extremities was mentioned. It was reported that significant improvements in extremity volumes and wound healing were achieved by applying a 4-week course of 20 sessions of skincare education, MLD, exercise, and bandaging treatment [40]. In another study, it was observed that a 20-session complex decongestive therapy was beneficial in reducing extremity volume and alleviating symptoms of depression and anxiety in 27 patients with unilateral lower extremity lymphedema [41]. In a study investigating the effects of manual lymphatic drainage in pediatric lymphedema patients, MLD was considered a useful non-invasive treatment method for reducing pain and achieving lymphedema decongestion [42]. Liu *et al*. [43]also found complex decongestive therapy to be effective in reducing extremity circumference measurements and improving the degree of lymphedema in patients with gynecological cancer-related lymphedema. As you can see, while there are conflicting results in the literature regarding MLD therapy, there are also many different studies that report its effectiveness (44-46).

There are only a few studies available in the literature regarding the effectiveness of negative pressure massage therapy. Campisi *et al*. [47] applied intermittent negative pressure therapy to 50 lymphedema patients and reported it as an effective treatment method. In the study by Lampinen *et al*. [18], the effectiveness of NPMT and MLD treatments was compared in patients with unilateral upper extremity lymphedema related to breast cancer. When the results were evaluated using extremity circumference measurements, it was observed that the group treated with NPMT showed a significantly greater reduction in volume [18].

In our study, similar to the mentioned study, circumferential measurements of the extremities signifi-

cantly decreased in both the MLD and NPMT groups compared to the beginning of treatment. This reduction was found to be significantly greater in the NPMT group compared to the MLD group. In the study by Vorinen and colleagues, 13 patients who had undergone mastectomy and axillary dissection due to breast cancer were divided into two treatment groups: 6 patients received MLD, and 7 patients received NPMT using the LymphaTouch® device. Patients were evaluated for upper extremity joint range of motion, grip strength, circumferential and volumetric measurements, skin elasticity, body composition analysis, and quality of life before and after treatment. While there were no significant changes in joint range of motion and grip strength measurements in both groups, NPMT treatment was found to provide better reductions in tissue stiffness and edematous muscle volume and improved quality of life [24]. However, it's important to consider the limited sample size when evaluating the study. In our study, a larger sample size has been maintained to enhance its reliability.

Weber and colleagues compared the effectiveness of manual lymphatic drainage and negative pressure therapy in patients who developed lymphedema after elbow surgery. In their results, they found that both methods led to a similar reduction in extremity circumference measurements, but the reduction in subjective pain sensation was more pronounced in the group receiving negative pressure therapy [48]. Similarly, Saul *et al*. [25] found negative pressure therapy effective in reducing upper extremity swelling after surgery.

In our study, in line with the literature, the subjective pain and discomfort caused by edema were evaluated using the VAS (Visual Analog Scale) score. We observed a significant reduction in pain and discomfort in both treatment groups compared to before treatment, with a higher decrease in the VAS score in the NPMT group ($p < 0.05$ and $p < 0.01$; respectively), which is consistent with the findings of these studies.

Limitations

However, it's important to note that there are limitations to this study, including the relatively small sample size and the lack of long-term follow-up. To further validate these findings, larger patient groups and randomized controlled trials with long-term assessments are needed.

CONCLUSION

Based on the results of this study, NPMT appears to be a beneficial non-invasive treatment method for reducing extremity volumes and decreasing subjective pain and discomfort in lymphedema patients. It is believed to be a preferred option over manual therapy methods due to its ease for therapists, reduced direct contact with the patient, lower risk of serious complications, and ease of patient compliance with the device. By preventing skin-to-skin contact, it can minimize the risk of transferring toxins or body fluids caused by chemotherapy and radiotherapy.

Authors' Contribution

Study Conception: SE; Study Design: NK; Supervision: NK; Funding: BŞ; Materials: SE; Data Collection and/or Processing: BŞ; Statistical Analysis and/or Data Interpretation: HK; Literature Review: NDB; Manuscript Preparation: SE and Critical Review: NP.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1483-1492 Original Article

DOI: 10.18621/eurj.1359362 *Hematology*

Efficacy and safety outcomes of single-agent ibrutinib therapy in chronic lymphocytic leukemia and relapsed/refractory mantle-cell lymphoma

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ABSTRACT

Objectives: We aimed to evaluate patients using ibrutinib for the treatment of chronic lymphocytic leukemia (CLL) and relapsed/refractory mantle-cell lymphoma (MCL), focusing on high-risk subgroups, predictors of efficacy, response levels, and safety profile.

Methods: This retrospective cohort study included adult patients diagnosed with CLL and relapsed/refractory MCL who were started on ibrutinib as a single-agent between May 2015 and December 2021 in Bursa Uludag University, Department of Hematology.

Results: Of the 45 patients (23 CLL, 22 MCL) started on ibrutinib, the median age was 65 (range: 48-86) years, and 66.7% were male. Del(17p) was present in 47.8% of CLL patients; there was no remarkable difference between del(17p) status and the rates of achieving CR. The median follow-up with ibrutinib treatment in CLL patients was 13.3 (range: 0.3-77.8) months. In univariate analysis, progression-free survival (PFS) and overall survival (OS) were associated with the advanced Eastern Cooperative Oncology Group (ECOG) score ($p = 0.003$ and $p = 0.004$, respectively), and > 2 lines treatment regimens before ibrutinib ($p = 0.016$ and $p = 0.050$, respectively). In multivariate analysis, the ECOG performance status remained significant for OS. The median use of ibrutinib for MCL patients was 6 (range: 1-48) months, and the proportion of patients who achieved CR was 27.3%. In the univariate analysis of MCL patients, the ECOG performance status for PFS and OS was statistically significant ($p = 0.045$ and $p = 0.016$, respectively). Patients' most common non-hematological adverse events were pneumonia and urinary tract infection.

Conclusions: Our investigation of patient outcomes treated outside clinical trials confirms ibrutinib's sufficient efficacy and safety profile in CLL and relapsed/refractory MCL.

Keywords: Ibrutinib, efficiency, safety, chronic lymphocytic leukemia, mantle-cell lymphoma

Ibrutinib is the first oral agent that inhibits Bruton's tyrosine kinase (BTK) and is used in the treatment of chronic lymphocytic leukemia (CLL), Waldenstrom macroglobulinemia and relapsed/refractory mantle-cell lymphoma (MCL) in our country. Although there are randomized controlled studies evaluating treatment selection for these diseases, duration of therapy, and disease outcomes at different treatment stages, there

Received: ***September 12, 2023;*** *Accepted:* ***October 19, 2023;*** *Published Online:* ***October 24, 2023***

How to cite this article: *Pınar İE, Özkocaman V. Efficacy and safety outcomes of single-agent ibrutinib therapy in chronic lymphocytic leukemia and relapsed/refractory mantle-cell lymphoma. Eur Res J 2023;9(6):1483-1492. DOI: 10.18621/eurj.1359362*

e-ISSN: 2149-3189

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is a lack of data in the literature to assess real-life data. Although conventional chemoimmunotherapy has improved clinical outcomes in CLL, subsequent relapse is common, and shorter remissions are related to shorter survival, independent of salvage therapy [1]. In addition, chemotherapeutic regimens are related to typical and notable toxicities in elderly individuals, who comprise the majority of patients and have comorbidities, their use is limited [2-4].

The impact of identifying genomic features on survival results in CLL patients is critical. The presence of an unmutated immunoglobulin heavy-chain variable region (IGHV) gene, 11q deletion, or del(17p)/TP53 mutations has been related to lower progression-free survival (PFS) in cases administered chemoimmunotherapy, including fludarabine, cyclophosphamide, and rituximab [4-9]. Also, 17p deletion was identified as the most significant adverse prognostic marker for PFS and overall survival (OS) [4, 9].

On the other hand, outcomes from the international, open-label, phase 2 study that provided approval for ibrutinib in relapsed/refractory MCL showed a high overall response rate of 68%, with a complete remission (CR) of 21% [10].

We evaluated CLL and MCL patients according to high-risk subgroups, efficacy markers, and response levels. Since it is a continuous oral therapy in the treatment of CLL, we also focused on safety data that expands over time.

METHODS

Adult patients diagnosed with CLL and relapsed/refractory MCL who started ibrutinib treatment as a single agent between May 2015 and December 2021 were included in the study. This retrospective cohort study was conducted in Bursa Uludag University Faculty of Medicine, Department of Hematology.

The following characteristics of the participants in the study were recorded: cytogenetic features, previous treatments and response status, OS (time, rate), PFS (time, rate), and side effects during ibrutinib treatment. Patients who had an allergic reaction to the active substance or component of the drug and whose files had missing data for analysis were excluded from the study. Additionally, patients who were administered ibrutinib treatment with B-cell lymphoma diagnoses other than MCL and who used ibrutinib for graft versus host disease after allogeneic stem cell transplantation were excluded from the study.

If no dose adjustment was required, ibrutinib was administered once daily for CLL and MCL patients, 420 mg and 560 mg, respectively. The presence of the 17p deletion was assessed at local laboratories using interphase fluorescence in situ hybridization (FISH). Quality of response, such as CR, partial response (PR), stable disease, and progressive disease (PD), was assessed by the 2008 International Workshop on CLL response criteria [11]. Side effects during ibrutinib treatment were graded by the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4 criteria [12]. PFS was defined as the time from the ibrutinib start date to the first relapse or death/last follow-up. OS was defined as the time from diagnosis to death or final follow-up. The Rai staging system for CLL is a prognostic marker using physical examination and complete blood count; It ranges from 0 (low risk), I or II (moderate risk), III or IV (high risk). The Eastern Cooperative Oncology Group (ECOG) score ranges from 0 to 5, with higher scores indicating more severe functional impairment. The MCL International Prognostic Index (MIPI) divides patients into three risk groups (low, intermediate, and high risk) based on survival status, using performance score, age, serum lactate dehydrogenase, and leukocyte count.

The study complied with good clinical practice guidelines and obtained ethics committee approval (Bursa Uludag University, date, 24 November 2021; no, 2021-17/34). The study was conducted by the 1964 Declaration of Helsinki and subsequent revisions.

Statistical Analysis

Descriptive statistics were used to identify essential characteristics. PFS and OS were evaluated according to the Kaplan-Meier method, and comparisons were made with the log-rank test. The chi-square or Fisher's exact tests were used to compare categorical data in clinical parameters. Data were expressed ade-

quately as mean ± standard deviation, median (min-max), or percentage (%). Both univariate and multivariate analyses were performed using Cox regression analysis as proper, including the following potential prognostic parameters: age (≤ 65 vs. > 65), gender, 17p deletion (negative vs. positive), Rai stage at ibrutinib onset (0-2 vs. 3-4), ECOG performance score (0-1 vs. 2-4), treatment regimens before ibrutinib (0-2 vs. > 2), response status (CR vs. Others). Statistical analysis was performed using IBM SPSS Statistics 22.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Ibrutinib therapy was started in 45 cases, 23 of whom were diagnosed with CLL and 22 with MCL, at Bursa Uludag University, Department of Hematology, between May 2015 - December 2021. The median age was 65 (range: 48-86) years, 66.7% of the participants were male, and 71.1% were classified as ECOG performance score 0-1.

Of the cases diagnosed with CLL, 78.3% were Rai stage 0-2, and del(17p) was detected in 47.8% by the

Table 1. Essential characteristics of the study cohort

		CLL		MCL		Total	
		n or median (min - max)	%	n or median (min - max)	%	n or median (min - max)	%
Age (years)		68 (48-82)		65 (49-86)		65 (48-86)	
Gender	**Male**	13	56.5	17	77.3	30	66.7
	Female	10	43.5	5	22.7	15	33.3
Bulky disease	**No**	20	87.0	18	81.8	38	84.4
	Yes	3	13.0	4	18.2	7	15.6
ECOG performance score	**0**	10	43.5	11	50.0	21	46.7
	1	7	30.4	4	18.2	11	24.4
	2	3	13.0	3	13.6	6	13.3
	3	3	13.0	4	18.2	7	15.6
	4	0	0.0	0	0.0	0	0.0
Rai classification	**Stage 0**	4	17.4				
	Stage 1	10	43.5				
	Stage 2	4	17.4				
	Stage 3	2	8.7				
	Stage 4	3	13.0				
WBC ($\times10^9$/L)		39.3 (1.54-342)		6.85 (1.93-37.22)		7.43 (1.54-342)	
Neutrophil ($\times10^9$/L)		2.81 (0.36-10.6)		3.38 (0.82-11.1)		3.33 (0.36-11.1)	
Lymphocyte ($\times10^9$/L)		33.37 (0.62-316)		1.75 (0.79-34.54)		3.41 (0.62-316)	
Hemoglobin (g/dL)		9,5 (6.5-15.3)		13.2 (8.3-16.4)		11.2 (6.5-16.4)	
Platelet ($\times10^9$/L)		103 (8.5-406)		151.4 (34.9-297.6)		127 (8.5-406)	
LDH (U/L)		228 (129-928)		217 (158-507)		220 (129-928)	
Creatine (mg/dL)		0.9 (0.6-1.2)		0.8 (0.6-2.5)		0.8 (0.6-2.5)	
17p deletion	**Negative**	12	52.2				
	Positive	11	47.8				

CLL = Chronic lymphocytic leukemia, MCL = Mantle-cell lymphoma, ECOG = Eastern Cooperative Oncology Group, WBC = White blood cell count, LDH = Lactate dehydrogenase

Table 2. Cox regression analysis outcomes for CLL patients

Variables	PFS				OS			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*p value*	HR (95% CI)	*p value*	HR (95% CI)	*p value*	HR (95% CI)	*p value*
Age								
≤ 65 years	Reference		Reference		Reference			
> 65 years	9.9 (1.2-79.1)	**0.030**	5.7 (0.6-57.5)	0.138	4.8 (0.6-38)	0.138		
ECOG performance score								
0-1	Reference		Reference		Reference		Reference	
2-3-4	7.1 (2-25.7)	**0.003**	2.4 (0.6-10.5)	0.244	6.4 (1.8-23.1)	**0.004**	4.9 (1.2-20.2)	**0.030**
Pre-ibrutinib treatment lines								
≤ 2	Reference		Reference		Reference		Reference	
> 2	5.1 (1.4-18.7)	**0.016**	3.5 (0.7-16.7)	0.115	3.6 (1-13)	**0.050**	1.9 (0.4-7.7)	0.398
Rai stage								
0-1-2	Reference				Reference			
3-4	0.8 (0.2-3.7)	0.763			1 (0.2-4.8)	0.989		
17p deletion								
Negative	Reference				Reference			
Positive	0.8 (0.2-2.9)	0.740			1 (0.3-3.6)	0.983		
Bulky disease								
No	Reference				Reference			
Yes	2.3 (0.5-11.2)	0.284			4.1 (0.8-21.5)	0.094		

CLL = Chronic lymphocytic leukemia, PFS = Progression-free survival, OS = Overall survival, HR = Hazard ratio, CI = Confidence interval, ECOG = Eastern Cooperative Oncology Group

FISH test. Of the participants diagnosed with MCL, 45.5% were at high risk according to the MIPI, 22.6% received >2 lines of treatment regimen before ibrutinib, and 27.3% had refractory disease. Particular essential characteristics of the patients are presented in Table 1.

Outcomes of Cases Diagnosed with CLL

Patients diagnosed with CLL administrated a median of 2 (range: 0-5) treatment regimens before ibrutinib. The median follow-up with ibrutinib treatment was 13.3 (range. 0.3-77.8) months. While CR rates were 28.6% in patients who received 1 or 2 lines of treatment before ibrutinib therapy, CR could not be obtained in patients who received 3 or more treatment regimens. There was no remarkable difference in the rate of achieving CR according to prognostic markers such as age, ECOG performance score, number of treatment lines before ibrutinib therapy, presence of bulky disease, del(17p) status, and Rai stage (p > 0.05).

During ibrutinib therapy, lymphocytosis was observed in 21.7% of CLL cases. In patients with lymphocytosis, the median maximum lymphocyte count was 195 (range: 94.7-688) × 109/L. The median development of lymphocytosis with ibrutinib treatment was 17 (range: 3-97) days. Lymphocytosis returned to baseline, median 86 (range: 9-272) days. Symptomatic lymphocytosis was observed in only one patient during ibrutinib treatment.

Median PFS was not reached. The mean PFS was 41.1 (95% CI: 24.8 to 57.5) months. In univariate Cox regression analysis, factors associated with shortened PFS included old age (p = 0.030), advanced ECOG score (p = 0.003), and 3 or more treatment series before ibrutinib therapy (p = 0.016). Rai stage of disease (p = 0.763), presence of 17p deletion (p = 0.740), and bulky disease status (p = 0.284) were not associated with PFS (Table 2).

Median OS was 120.4 (95% CI: 81.1-159.8) months. Based on the survival analysis, the ECOG performance score and pre-ibrutinib treatment series significantly affected both PFS and OS (Fig. 1). PD and infections were the most common reasons for

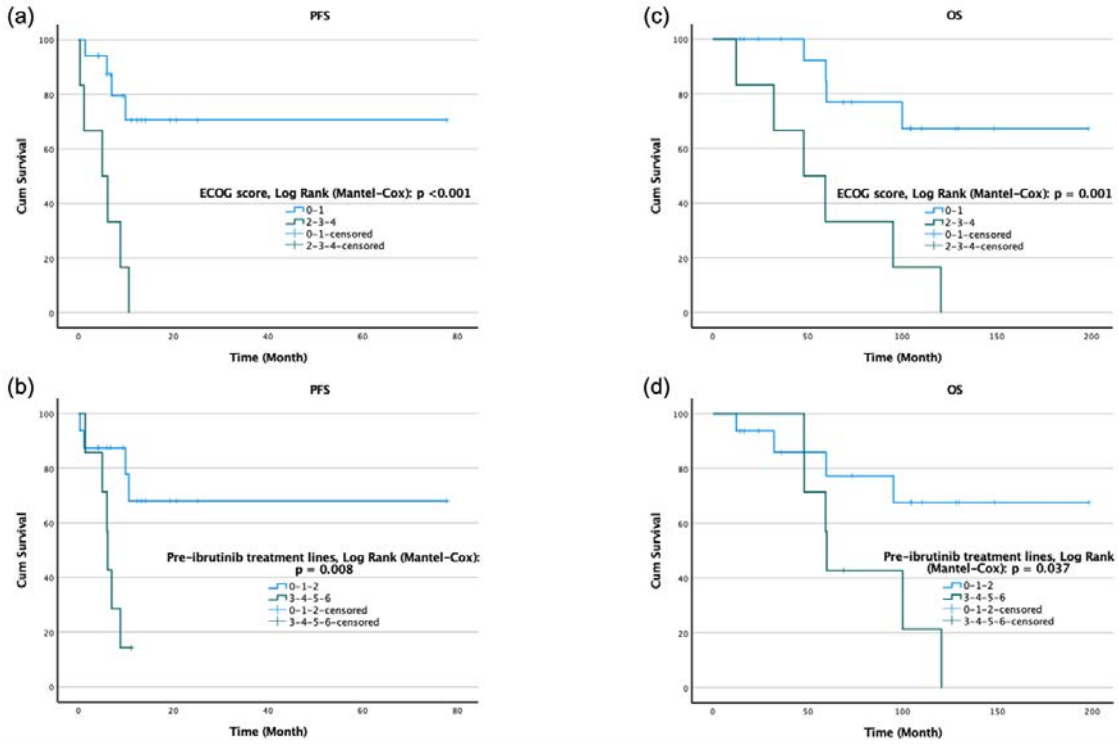

Fig. 1. PFS and OS curves for CLL patients. (a) PFS curves for ECOG performance score, (b) PFS curves for pre-ibrutinib treatment line, (c) OS curves for ECOG performance score, and (d) OS curves for pre-ibrutinib treatment line. CLL = Chronic lymphocytic leukemia, PFS = Progression-free survival, OS = Overall survival, ECOG = Eastern Cooperative Oncology Group

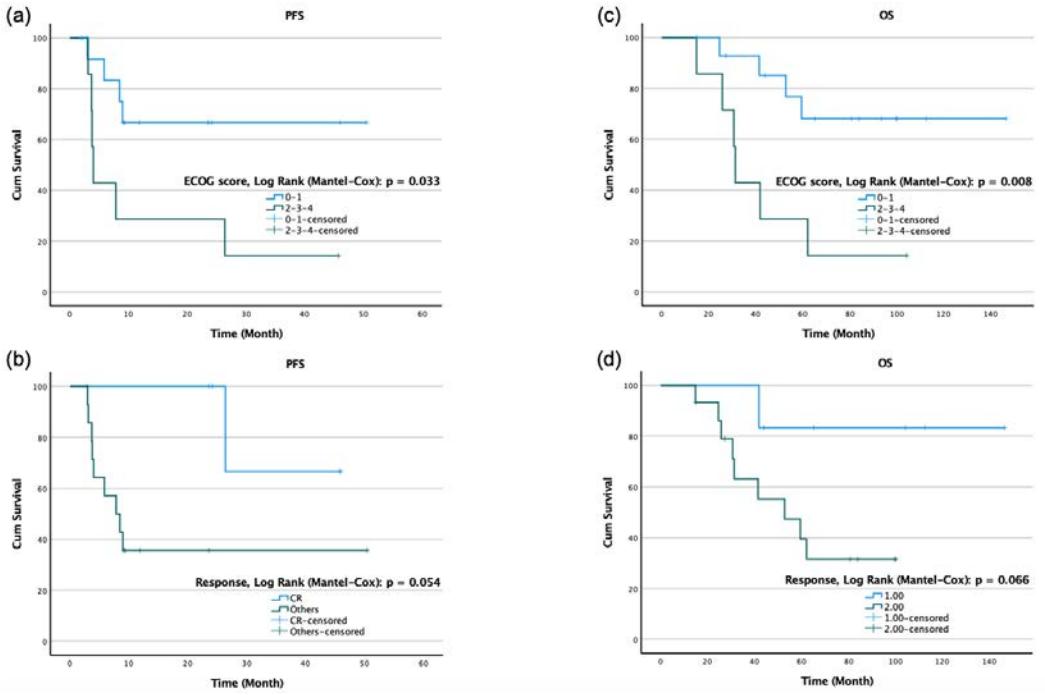

Fig. 2. PFS and OS curves for MCL patients. (a) PFS curves by ECOG performance score, (b) PFS curves by treatment response status, (c) OS curves by ECOG performance score, and (d) OS curves by treatment response status. MCL = Mantle-cell lymphoma, PFS = Progression-free survival, OS = Overall survival, ECOG = Eastern Cooperative Oncology Group

death. According to univariate Cox regression analysis, patients with high ECOG performance scores (p = 0.004) and patients who received three or more treatment lines before ibrutinib (p = 0.05) significantly reduced OS. OS results were comparable with the Rai stage (p = 0.989), age (p = 0.138), bulky disease (p = 0.094), and presence of del(17p) (p = 0.983). In multivariate analysis, the ECOG performance status remained significant for OS (Table 2).

Outcomes of Cases Diagnosed with MCL

For all MCL patients, the median duration of ibrutinib use was 6 months (1 - 48), and the proportion of patients achieving CR was 27.3%. The median OS was not reached for patients with CR, while the mean estimated OS was 129 months (95% CI: 97.8-160.2). At a median follow-up of 93.5 months for all cases diagnosed with MCL, the median PFS was 26.4 months (95% CI: 0-53.6%); the median OS was not reached, while the mean estimated OS was 92.1 months (95% CI: 68-116.3). Based on the survival analysis, the ECOG performance score significantly affected both PFS and OS. Participants with CR showed a trend for more prolonged survival for PFS and OS, although not statistically significant (Fig. 2). According to Kaplan-Meier, 12-month PFS was 52.6%, and 5-year OS was 54.7%.

In Cox regression analysis, no remarkable difference was found in PFS and OS according to age, tumor volume, and the number of treatment regimens before ibrutinib therapy. In the univariate Cox analysis, the ECOG performance score for both PFS and OS was statistically significant (p = 0.045 for PFS, p = 0.016 for OS) (Table 3). In univariate Cox analysis, patients achieving CR showed a trend for more prolonged survival was observed for both PFS and OS, although not statistically significant (p = 0.085 for PFS, 95% CI: 0.8-54.8, for OS p = 0.102, 95% CI: 0.7-44.6) (Table 3).

Safety and Adverse Event Profile in the Study Cohort

Patients' most common non-hematological side effects were pneumonia and urinary tract infection. Neutropenia was reported as the most common grade ≥ 3 hematological side effect. No hematological events led to the discontinuation of ibrutinib. The most common hematological and non-hematological side effects are listed in Table 4.

Table 3. Cox regression analysis outcomes for MCL patients

Variables	PFS Univariate Analysis		OS Univariate Analysis	
	HR (95% CI)	*p value*	HR (95% CI)	*p value*
Age				
≤ 65 years	Reference		Reference	
> 65 years	1.5 (0.4-5.5)	0.521	1.4 (0.4-4.8)	0.638
ECOG performance score				
0-1	Reference		Reference	
2-3-4	3.8 (1-13.9)	**0.045**	4.8 (1.3-17.3)	**0.016**
Pre-ibrutinib treatment lines				
≤ 2	Reference		Reference	
> 2	0.2 (0-1.9)	0.174	0.2 (0-1.9)	0.177
Bulky disease				
No	Reference		Reference	
Yes	1.8 (0-7.2)	0.377	3.2 (0.8-12.4)	0.098
Treatment response				
CR	Reference		Reference	
Others	6.5 (0.8-54.8)	0.085	5.6 (0.7-44.6)	0.102

MCL = Mantle-cell lymphoma, PFS = Progression-free survival, OS = Overall survival, HR = Hazard ratio, CI = Confidence interval, ECOG = Eastern Cooperative Oncology Group

During the study period, 60% of patients were complicated by infections; 30.4% had grade ≥ 3 conditions. Pneumonia was the most frequently reported infectious complication, followed by urinary tract infection and cellulitis. The most commonly reported grade ≥ 3 infection was pneumonia. Due to life-threatening diseases, sepsis was observed in 1 patient and septic shock in 5 patients.

Grade 5 adverse event was seen in only one patient who died due to intracranial hemorrhage. Upper gastrointestinal bleeding was detected in one patient. These results indicate that the incidence of grade ≥ 3 hemorrhagic events is low with continuous ibrutinib treatment.

Atrial fibrillation (AF) was a rare condition seen in 3 patients. AF occurred at a median of 4 months of ibrutinib treatment. Beta-blockers were used as rate-limiting drugs in the treatment of these patients; low molecular weight heparin or new-generation oral anticoagulants were preferred for anticoagulation prophylaxis. AF was transient in one of the patients. While ibrutinib treatment was discontinued in one of the participants with sustained AF, the ibrutinib dose was reduced in the other.

Ibrutinib treatment was discontinued for any reason in 22.2% of the study group. PD was the most common reason for discontinuing therapy.

DISCUSSION

Ibrutinib, the first oral agent to inhibit BTK, is indicated for the treatment of CLL and is used for many B-cell lymphomas, primarily MCL. Although previous studies focused mainly on a single disease, this research included all participants with diagnoses of CLL and MCL who were prescribed ibrutinib. Our investigation of patient outcomes treated outside clinical trials confirms ibrutinib's sufficient effectiveness and safety profile in CLL and relapsed/refractory MCL.

The one-year OS rate in our study does not significantly deviate from the outcomes of the RESONATE research (90%) [13]. However, the 10-month OS in Sweden's compassionate use program reported is

Table 4. Safety and side-effect profile during ibrutinib treatment

	Total (n)	Grade 1 (n)	Grade 2 (n)	Grade 3 (n)	Grade 4 (n)	Grade 5 (n)
Hematological Side effects						
Neutropenia	3	1		2		
Thrombocytopenia	1			1		
Anemia	1		1			
Non-Hematological side effects						
Pneumonia	9	1	2	2	4	
Urinary tract infection	4	2	1		1	
Cellulite	3	1	2			
Upper respiratory tract infection	2	2				
Urticaria	3	2		1		
Headache	1	1				
Atrial fibrillation	3	3				
Heart failure	2	2				
Nausea	1			1		
Diarrhea	2	1	1			
Upper gastrointestinal bleeding	1			1		
Intracranial hemorrhage	1					1

lower (83%) than the RESONATE study [14]. The difference in prognosis may partly be explained by the fact that cases with poor performance status, who comprise a substantial proportion of patients, were exclusion criteria for the RESONATE study. In parallel, our analysis found a higher ECOG performance score as an independent risk factor of shorter PFS and OS. In this respect, our research is similar to the Polish Adult Leukemia Group results [15]. However, our results differ from the Swedish CLL cohort data, which found no worse outcomes in patients with ECOG performance scores > 1 [14].

In our study and other supportive studies,[16] the presence of 17p deletion in CLL patients using ibrutinib did not demonstrate a significant difference in PFS and OS. A study parallel to our outcomes stated that, high-risk prognostic factors such as 17p deletion did not substantially affect treatment response rates [17]. In contrast, the Swedish study demonstrated substantially shorter PFS and OS in cases with the del(17p)/TP53 mutation, noting that these mutations remain a therapeutic challenge [14].

In our study, the superior PFS and OS results in cases who received ≤ 2 treatment lines before ibrutinib therapy appear consistent with other outcomes from real-life data [16]. In addition, another study highlights the possibility of deepening responses with continued ibrutinib treatment and better efficacy with earlier initiation of ibrutinib therapy [18].

Bortezomib and lenalidomide have been used to treat cases with relapsed/refractory MCL, and response rates were 33% with bortezomib and 28% with lenalidomide. The effectiveness and safety profile of ibrutinib in our study can be compared favorably with these approved agents. In addition, our study's rate of discontinuation of treatment for any reason was 22.2%. The discontinuation rate due to side effects was similar to the pivotal studies with bortezomib and lenalidomide (26% and 19%, respectively) [19, 20]. According to the final analysis from RESONATE, the discontinuation rate of ibrutinib treatment due to side effects was 16.4%. In parallel with our study, the main reason for therapy discontinuation at extended follow-up was PD rather than drug-related toxicity [21].

Although the risk of bleeding increases with ibrutinib treatment, it should be remembered that, the use of anticoagulants and antiplatelet drugs is not contraindicated with ibrutinib treatment. On the other hand, an appropriate risk-benefit assessment should be made when evaluating ibrutinib therapy for the patient group who needs these agents [22]. Our study observed that the incidence of hemorrhagic events was lower compared with other studies. This is most likely due to patients' or treating physicians' underreporting of minor bleeding events.

In our study, AF led to the discontinuation of ibrutinib therapy in only one patient, while appropriate anti-arrhythmic therapy allowed long-term treatment with ibrutinib therapy in other patients. Therefore, careful bleeding risk assessment and proper medical management may ensure that most patients who develop AF to continue ibrutinib therapy.

The frequency of infection is increased in non-Hodgkin lymphoma because chemotherapy suppresses the immune system, and the disease also leads to an immunocompromised state. The most frequently reported infections in this study included pneumonia and urinary tract infections; most were handled in an outpatient setting and were self-limited.

Ibrutinib may be a promising treatment option for CLL and various B-cell lymphomas with a tolerable adverse events profile and a high and favorable response rate [23].

Another essential issue is drug-drug interaction. In a pharmacokinetic study of 18 healthy volunteers, the pharmacokinetics of ibrutinib were compared with the pharmacokinetics of ibrutinib combined with ketoconazole, the potent CYP3A4 inhibitor. Dose-normalized ibrutinib maximum serum concentration and area under the curve increased 29-fold and 24-fold, respectively, when co-administered with ketoconazole [24]. In a case report, a patient with steroid-refractory graft vs. host disease was successfully treated with reduced doses of ibrutinib and itraconazole to minimize the ibrutinib dose and costs by 75% [25]. Given all these results, if the treatment of a patient using ibrutinib requires the administration of potent or moderate CYP3A inhibitors, ibrutinib therapy may need to be discontinued or the dose modified.

Limitations

The most important limitations of our study are the relatively small participant size, the retrospective design of the research, and the short follow-up period with ibrutinib therapy to evaluate the extended time-dependent effectiveness and side effects profile.

CONCLUSION

Consequently, the analysis of real-life data from CLL and MCL patients parallels the outcomes of many other studies of ibrutinib therapy. Ibrutinib is a good treatment option for patients with CLL and relapsed/refractory MCL because of its encouraging response rates and acceptable toxicity. Early treatment with ibrutinib therapy may reduce the toxicity of conventional chemoimmunotherapy and thus improve patients' quality of life.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1493-1501 Review

DOI: 10.18621/eurj.1324556 *Physical Therapyand Rehabilitation*

Assessment of chiropracticinterventioninfluence on painand life quality in cervicogenic headache afflicted office workers: A review

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ABSTRACT

Cervicogenic headache, characterized by referred pain perceived in the head but originating from the cervical spine, is a prevalent non-migraine headache impacting a significant proportion of the global population, particularly office workers. Recent studies have identified a co-occurrence of cervicogenic headaches with neck pain, often exacerbated by long hours of stationary sitting and computer use common in desk jobs. Despite the growing evidence supporting the effectiveness of chiropractic treatment in mitigating head and neck pain, a distinct gap persists in the literature concerning chiropractic interventions specifically targeting office workers suffering from cervicogenic headaches. This review aims to scrutinize the current literature on the effectiveness of chiropractic interventions on pain management and life quality improvement for office workers afflicted with cervicogenic headaches. Specifically, the review will delve into high-velocity, low-amplitude (HVLA) thrust maneuvers, a widely employed strategy in spinal manipulative therapy, an extension of chiropractic treatment. In the context of cervicogenic headaches, the link between the Rectus Capitis Posterior Minor (RCPM) muscle and the Dura Mater, and how neck strains affecting this connection can instigate headaches, will be explored. Through the comprehensive evaluation of existing literature and studies, this review seeks not only to elucidate the potential of chiropractic treatment in improving the life quality of office workers suffering from cervicogenic headaches, but also to stimulate further research in this essential yet under-explored area of study.

Keywords: Cervicogenic headache, office workers, chiropractic treatment, pain management, life quality improvement

Cervicogenic headache, a type of unilateral headache characterized by neck involvement, ranks among the most common non-migraine headaches [1,2]. These headaches present as referred pain that is perceived in any region of the head but originates from a noxious source within the musculoskeletal tissues innervated by cervical nerves. Likely sources of cervicogenic headaches include structures innervated by the C1 to C3 spinal nerves. This encompasses the upper cervical synovial joints, upper cervical muscles, the C2-3 disc, vertebral and internal carotid arteries, dura mater, and the posterior cranial fossa [3].

Among office workers, the most frequently re-

Received: ***July 8, 2023;*** *Accepted:* ***August 5, 2023;*** *Published Online:* ***August 8, 2023***

How to cite this article: *İlter SE, Gökçe A. Assessment of chiropracticinterventioninfluence on painand life quality in cervicogenic headache afflicted office workers: a review. Eur Res J 2023;(6):1493-1501. DOI: 10.18621/eurj.1324556*

e-ISSN: 2149-3189

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ported musculoskeletal complaints include neck pain, shoulder pain, and lower back pain. Moreover, within this demographic, there is a noticeable co-occurrence of cervicogenic headaches with neck pain. Numerous empirical studies have illuminated the effectiveness of chiropractic treatment in mitigating neck and head pain [4-10]. However, the literature lacks comprehensive exploration of chiropractic interventions specifically tailored towards office workers afflicted with cervicogenic headaches.

Chiropractic care, taking a holistic view of the spine, has emerged as a preferred intervention for addressing biomechanical dysfunctions. This treatment strategy involves the use of high-velocity, low-amplitude (HVLA) thrust maneuvers to restore motion in identified lesions, correct abnormalities in joint structures, and enhance overall function [11]. Spinal manipulative therapy, an integral component of chiropractic treatment, has seen widespread application in the management of head and neck pain [4-10].

Of particular interest is the observed propensity for cervicogenic headaches to occur in conjunction with neck pain [12]. Sitting posture is known to influence the incidence of neck pain, and sitting for over 5 hours daily is considered a potential risk factor for the development of neck pain [13]. According to a study conducted in Turkey, 60.1% of participants reported working for over 8 hours daily, with stationary sitting periods ranging from 4.1 to 8 hours [14]. Globally, headaches reportedly afflict approximately 47% of the population, with a higher incidence observed among office workers [15]. In a study examining the incidence of cervicogenic headache among bank office workers, it was found that 20% experience cervicogenic neck pain, primarily attributable to extended periods of upright sitting and continuous computer use [16].

Despite the robust documentation of chiropractic treatment's efficacy for cervicogenic headache [4-10], no research to date has assessed the impact of such treatment specifically on cervicogenic headache among office workers. This creates a critical knowledge gap regarding the effect of chiropractic treatment on cervicogenic headaches in this demographic, particularly in the context of Turkey.

In 1995, a seminal dissection study led by Dr. Gary Hack identified a connective tissue that links the Rectus Capitis Posterior Minor (RCPM) muscle between the cervical bones C0 (Occiput) and C1 (Atlas) to the Dura Mater via the Posterior Atlanto-occipital Membrane (PAO) in all ten cadavers examined [12]. This specific muscle-dura connection, which is sensitive to neck strains, has potential implications for the initiation of headaches.

The present study is aimed at addressing this knowledge gap by evaluating the impact of chiropractic interventions on office workers suffering from cervicogenic headaches. The ultimate goal of this research is to enrich the literature regarding the effects of chiropractic treatment on cervicogenic headaches and to provide a foundation for future investigations in this field. Given the prevalence of cervicogenic headaches among office workers, and the established efficacy of chiropractic care for the alleviation of neck and head pain, this research holds significant potential for contributing to treatment strategies and improving overall wellbeing in this demographic.

The sample selection criteria, data collection procedures, and analytical processes for this study have been meticulously designed to ensure a comprehensive and accurate assessment of this complex issue. This study not only seeks to enhance our current understanding of cervicogenic headaches among office workers and the potential therapeutic role of chiropractic care but also aims to pave the way for future research aimed at informing and refining treatment strategies for this pervasive issue. In conclusion, this study recognizes the importance of the pervasiveness of cervicogenic headaches and the potential therapeutic benefits of chiropractic care. The scarcity of studies focusing on this specific population further underscores the significance and timeliness of this research, as it adds a much-needed perspective to our understanding of chiropractic treatment's potential benefits for office workers suffering from cervicogenic headaches. This research could serve as a stepping stone for future studies, inform treatment strategies, and potentially contribute to improving the wellbeing of office workers worldwide who are grappling with this debilitating condition.

DEFINITION OF CHIROPRACTIC

Chiropractic is defined by the World Federation of Chiropractic as a health profession concerned with the

diagnosis, treatment, and prevention of mechanical disorders of the musculoskeletal system. Additionally, it considers the effects of these disorders on the function of the nervous system and overall health [17,18]. Prominent amongst chiropractic treatment methods is spinal manipulation or adjustment. Furthermore, physical therapy modalities, exercise programs, dietary advice, the use of orthotics, lifestyle modifications, and patient education can also be incorporated into the chiropractic treatment process [19].

The fundamental clinical action agreed upon by all chiropractors is spinal manipulation. Chiropractors tend to favor the term "adjustment," reflecting their belief in the therapeutic and health-promoting effect of correcting spinal joint abnormalities. There exist dozens of adjustment "techniques", and debates over their relative value constitute much of chiropractic academic discourse. This procedure, in its broadest definition, describes the application of a load (force) to specific body tissues for therapeutic purposes. Traditionally administered by hand, this load can vary in its speed, amplitude, duration, frequency, as well as its anatomical position, lever choice, and direction of force [20].

Chiropractic manipulations focus on biomechanical disorders of the muscular, skeletal, and spinal system that have not reached surgical levels and the problems these disorders cause on the nervous system. Biopsychosocial causes and consequences are significant factors in patient treatment [21].

Chiropractic Subluxation

Chiropractic subluxation refers to a lesion or dysfunction in a joint or motion segment where contact of joint surfaces is maintained but alignment, movement integrity, and/or physiological function are altered. This condition is essentially a functional disturbance that can affect biomechanical and neurological integrity. Mechanical spinal functional disturbances, defined by chiropractors as subluxation and vertebral subluxation complex, are thought to lead to significant neurophysiological consequences [21].

Chiropractic Manipulation

Chiropractic manipulative therapy stimulates the release of various neurotrophins, some of which, such as brain-derived neurotrophic factor and nerve growth factor, are needed for the treatment of depression. Harmless mechanical stimulation of the skin, for instance, induces the release of nerve growth factor in rats, supporting neuron survival and function. Thoracic spinal manipulative therapy stimulates different responses related to the sympathetic nervous system, hypothalamic-pituitary axis, and the endocrine system. The theory of the relationship between spinal manual therapy and spinal cord neuroplasticity is under investigation, and several studies have demonstrated such a connection. Chiropractic therapy acts on the nervous system, stimulating the release of various chemicals and hormones that regulate blood pressure and flow, calm the brain, and reduce inflammation [22].

Over the past century, Doctors of Chiropractic (DCs) have developed a variety of chiropractic techniques. These techniques provide options for application tailored to a specific patient or condition. For better understanding, manual articular manipulative and adjustment procedures have been divided into several categories: specific contact thrust procedures (e.g., high velocity low amplitude [HVLA] thrusts), nonspecific contact thrust procedures (e.g., mobilization), manual force mechanically assisted procedures (e.g., drop tables or flexion-distraction tables), and mechanically force manually assisted procedures (e.g., stationary or hand tools). These procedures represent the various methods in which chiropractic treatment is applied. Manipulative treatment consists of a high-velocity low-amplitude movement applied in the pathophysiological domain and performed just beyond the passive joint motion range. Spinal manipulative therapy is a commonly used treatment, involving a high-velocity and low-amplitude (thrust) joint manipulation [23].

Indications for Chiropractic Practice

Chiropractic application is indicated for conditions such as acute or chronic low back and neck pain, tendinitis, sprains, cervical, thoracic and lumbar disc herniations, chronic and acute soft tissue strains, myofascial pain syndrome, occupational and sports-related injuries, joint dysfunctions, radiculopathies, scoliosis, coccyx dislocation, flexion and extension directional sudden strains [24].

Contraindications to Chiropractic Practice

Acute fractures, Osteomyelitis, Hematoma, Unstable Odontoid, Odontoid Hypoplasia, Spinal Cord

Tumors, Meningeal Tumor, Vertebral Tumor, Neurological Deficit, Arnold Chiari Malformation, Vertebral Luxation, Aneurysmal Bone Cyst, Bone Tumors, Osteoblastoma, Osteoidosteoma, Prosthetics, Neoplastic Diseases Syringomyelia, Hydrocephalus, Cauda Equina Syndrome, Kerning or Lhermitte's Sign are contraindications [24].

CHIROPRACTIC METHODS

There exist 132 different chiropractic techniques, such as Diversified, Thompson Drop, Gonstead, and Logan Basic. These techniques typically involve high-velocity, low-intensity corrective thrust maneuvers. Practitioners use one or more techniques, depending on their training, skills, and personal perspectives [25]. In terms of the utilization of different chiropractic techniques, Pehlivanoğlu [26] provides insightful statistics. He reveals that the Diversified technique holds the majority share, being used in 95.5% of cases. Next in line is the Activator technique, which is employed in 62.8% of treatments. The Gonstead technique follows closely, with its usage standing at 58.5%. Similarly, the Cox Flexion/Distraction technique is deployed in 58% of the instances. The Thompson Drop technique's usage extends to 56% of cases. The Sacro-Occipital technique, on the other hand, is put to use in 41.3% of cases. Meanwhile, both the Palmer Upper Cervical and Logan Basic techniques hold lower shares, with their respective usages standing at 28.8% and 28%.

Diversified Technique

The most commonly used chiropractic treatment method is the manipulation and adjustment of the spine, arms, or legs. This method is also known as the Diversified Technique because it combines the best features of other manipulation techniques. It uses a low-amplitude thrust motion to adjust the spine and correct joint dysfunction, making it an effective treatment method for patients of all ages and health histories [26].

The Diversified technique is applied quickly and often accompanied by a "popping" sound when aligning the spine and joints. Only manual adjustments are used during the treatment process. The Diversified technique is unique in that it involves a small thrust motion on a specific joint. This motion results in the separation of joint surfaces and the release of trapped gas and air. The decrease in joint pressure leads to the formation of a gas bubble. When this bubble is released, a popping or clicking sound may be heard in the joint [26].

Gonstead Technique

The Gonstead Technique, named after its founder, is an adjustment method used for realigning the spine. It offered a better strategy for chiropractic with its comprehensive spinal approach, the temporary model called the Gonstead Technique (later to be replaced by the Diversified Technique). The distinctive features that make the Gonstead technique stand out include its successful outcomes, inclusion of the entire spine, use of high-velocity low-amplitude (HVLA) adjustments, the reasonable hypotheses on spinal biomechanics at that time, and the utilization of chiropractic instruments [27,28].

Thompson (Drop) Technique

Also known as the Drop technique, in this method, the chiropractor applies a thrust motion to adjust a person's spine or other extremities. A specialized table is used in this technique, which includes a mechanism that can drop with a small increase in pressure. The drop mechanism provides a greater sense of vibration during the adjustment to reduce the likelihood of pain or discomfort. Several thrusts may be required for complete spinal manipulation. To employ this technique, the chiropractor typically examines the length of a person's legs and how they relate to the spine and pelvis [28].

Activator Method Technique

The Activator Method Technique (AMT) involves the assessment of joint dysfunction believed to contribute to a wide range of health issues. These dysfunctions are part of a larger "subluxation complex," which is a component of the broader "subluxation syndrome." The AMT analysis is based on the assumption that faulty biomechanical behavior of joints is reflected in differences and changes in leg lengths. A step-by-step evaluation protocol is used, starting from the cervical spine and progressing towards the feet, including observation of leg length and application of provocative tests to assess joint function. This protocol

allows for a detailed examination by systematically evaluating the functionality of the joints. It is believed that to adequately assess more rostral structures, "clearing" of dysfunction in more caudal segments (i.e., removing or reducing the lesion through adjustment) is necessary. The protocol has both theoretical and empirical roots. Initially derived from various isolation, pressure, and stress tests related to leg checks, it has evolved significantly based on the clinical experiences of Activator practitioners. The chiropractor uses a specialized Activator adjusting instrument to perform this technique [29].

HEADACHES

A headache is a pain felt in any region of the head. Headaches are generally categorized into two main types: primary and secondary headaches. Primary headaches refer to conditions where the headache itself is the primary cause, while secondary headaches arise as a symptom of another health problem. The primary causes of headaches include migraines, tension-type headaches, and cluster headaches. These headaches typically occur spontaneously and are not associated with another health issue. Headaches are usually examined in conjunction with evaluating the symptoms, intensity, and duration of the pain. Secondary headaches, on the other hand, occur as a result of underlying health problems such as tumors, infections, or issues with brain blood vessels. In such cases, if the primary cause of the headache is not a structural change, systemic disease, or previous head trauma, it is referred to as "primary headache" and accounts for approximately 90% of all headaches. The International Headache Society's 2018 classification recognizes numerous types of headaches. Treatment methods vary depending on the cause of the headache and often involve the use of medications, stress management, relaxation techniques, and other appropriate treatment methods [30, 31].

Cervicogenic Headache

Cervicogenic headache is a chronic headache felt in one or more areas of the head and/or face, usually originating from the atlanto-occipital and upper cervical joints [32]. The International Headache Society (IHS) defines cervicogenic headache (headache originating from the neck) as a headache caused by a disorder or lesion in the cervical spine, its bones, discs, and/or soft tissue elements. It is typically accompanied by neck pain, although not always, and confirmed as a secondary type of headache presumed to originate from cervical nociception [31].

Diagnosis

Cervicogenic headache is a common underlying cause of misdiagnosed chronic headaches. Its presentation features can be complex and resemble many commonly encountered primary headache syndromes. The hallmark symptoms of cervicogenic headache include unilateral pain and a combination of ipsilateral widespread shoulder and arm pain. Reduced function of the neck joints and relief of pain with anesthesia blocks are indicative of this diagnosis. The essential characteristic for this diagnosis is the disappearance or self-resolution of the headache within one month with appropriate and adequate treatment. The International Headache Society (IHS) has specified the diagnostic criteria for cervicogenic headache as follows:

A. Any headache that fulfills criterion C.

B. Evidence of a disorder or lesion within the cervical spine or soft tissues of the neck known to cause headache, as confirmed by clinical and/or imaging evidence.

C. At least two of the following criteria providing evidence of causation:

1. Headache has developed in temporal relation to the onset of the cervical disorder or appearance of the lesion.

2. Headache has significantly improved or resolved in parallel with improvement in or resolution of the cervical disorder or lesion.

3. Cervical range of motion is reduced and headache is significantly aggravated by provocative maneuvers.

4. Headache is abolished following diagnostic blockade of a cervical structure or its nerve supply.

D. Not better accounted for by another ICHD-3 diagnosis.

Radiological findings in the upper cervical spine can be commonly observed in individuals without headaches. Although suggestive, they do not provide definitive evidence of causation [32].

Pathophysiology

The trigeminal nucleus and C1-C2 nerves in the brainstem have long been closely related. The significance of the spinal trigeminal nucleus in cervicogenic headache is demonstrated by Kerr, who showed that fibers from the trigeminal nerve and fibers from the upper cervical levels converge on the same units. Clinical evidence supporting this theory is the reduction or disappearance of pain from afferents of the trigeminal nerve with a greater occipital nerve block. The greater occipital nerve is composed of dorsal roots from C2. After branching from C2, it passes through the muscles of the neck and is particularly vulnerable to pressure as it penetrates the muscles, which can cause cervicogenic headaches. Cervicogenic headaches can be caused by structures innervated by the C1-C3 roots [33].

Clinical Findings

The clinical presentation of cervicogenic headache can be challenging to diagnose, but it typically includes the following [34-39]:

- Unilateral dominant headache (excluding those with symptoms indicative of bilateral headaches or migraines),
- Aggravation of symptoms with neck movement or posture,
- Tenderness in the upper 3 cervical spinal joints,
- Association with neck pain or dysfunction,
- Definitive diagnosis through selective nerve blockade via injection,
- Tendency for increased tension and trigger points in the upper trapezius, levator scapulae, scalenes, and suboccipital extensors in patients with cervicogenic headache compared to migraine headache and control groups,
- Weakness in deep neck flexors,
- Increased activity in superficial flexor,
- Atrophy in suboccipital extensors and consequent impairment of the deep muscular sleeve crucial for active support of cervical segments,
- Association with upper trapezius, sternocleidomastoid, scalenes, levator scapulae, pectoralis major and minor, and short suboccipital extensors.

Office Workers

Office workers are individuals responsible for the daily operations and smooth functioning of businesses in office settings. They belong to a professional group that involves prolonged static sitting positions and minimal use of muscles such as the arms, wrists, and elbows during sedentary work activities, leading to decreased mobility. As a result, their body posture may be compromised [40, 41]. These conditions have been reported to contribute to pain and reduced quality of life. It is known that sitting posture has a positive impact on the development of neck pain. Sitting for more than 5 hours per day is considered a potential risk factor for the development of neck pain, and a study conducted in Turkey found that office workers spend an average of 4.1 to 8 hours per day sitting. Research conducted in Italy reported that headache is one of the most common symptoms among office workers, with 5.7% of male office workers and 9.3% of female office workers reporting that headaches affect them weekly [42, 43].

DISCUSSION

Chiropractic practices are based on manipulative interventions primarily focused on the spine since the establishment of the chiropractic profession. Over time, the development of various techniques within the profession has led to the formation of distinct branches, necessitating the continuous dynamic nature of the profession to keep up with advancements. However, most of these practices revolve around certain core techniques. Nevertheless, it is equally important to note that the experience and professional skills of the clinician play a crucial role during the application of almost all techniques.

When scientific research is examined through literature review, Yates *et al*.[44] conducted a study in 1988 where instrument-assisted thoracic spine manipulation was applied to 21 patients. The results showed a significant decrease in systolic and diastolic blood pressure in the active treatment group, while no significant changes were observed in the placebo and control groups. Osterbauer *et al*. [45] conducted a study supported by the Activator company, demonstrating that instrument-assisted interventions had a dramatic positive effect on patients with sacroiliac joint pain. Gemmell *et al*. [46] compared the Meric technique, a traditional chiropractic practice, with the Activator technique in patients with acute low back

pain and found that both techniques were effective without a superiority of one over the other. Yurkiw *et al*. [47] compared the efficacy of traditional chiropractic methods and instrument-assisted chiropractic interventions in neck pain and concluded that both techniques were effective without a significant superiority. Wood *et al*. [48] compared the effectiveness of traditional chiropractic techniques and instrument-assisted chiropractic interventions in a group of patients with functional loss in cervical spinal segments and found that both techniques were effective without a significant superiority. DeVocht *et al*. [49] investigated the effectiveness of the Activator method in temporomandibular joint disorders and obtained statistically significant results. Shearar *et al*. [50] compared manual and mechanically assisted chiropractic techniques in sacroiliac joint disorders and found that both techniques significantly supported improvement without a significant superiority of one over the other. Similarly, a study showed that applications using the Activator device for trigger point therapy, nonspecific neck pain, and upper trapezius trigger points were more effective than myofascial band therapy and sham ultrasound [51]. Gorrell *et al*. [52] compared the effectiveness of manual and instrument-assisted manipulative interventions in mechanical neck pain and found that a single cervical manipulation provided both immediate and short-term benefits for mechanical neck pain, although different application techniques yielded different results. Schneider *et al*. [53] compared the effects of spinal manipulation methods and usual medical care in acute and subacute low back pain and found that manual thrust manipulation resulted in slightly greater reductions in self-reported disability and pain scores compared to mechanical assisted manipulation (Activator) or usual medical care in the short term (4 weeks).

As stated above, instrument-assisted chiropractic manipulations produce effects similar to manual manipulations. However, there is currently no evidence from database analysis supporting the superiority of instrument-assisted interventions over traditional methods. Nevertheless, the literature demonstrates positive outcomes for both intervention styles in different patient populations. Considering the risk factors associated with traditional interventions, instrument-assisted interventions can be considered as a preferable approach. All instrument-assisted interventions, like other chiropractic techniques, should be used as part of a multidisciplinary treatment approach. Using these interventions as standalone treatment methods would contradict the holistic perspective of modern medicine. In fact, considering the use of instrument support as a step within chiropractic interventions seems reasonable. Incorporating instrument support when deemed necessary during the application of other manual chiropractic techniques can make the practice safer and reduce the workload for clinicians.

CONCLUSION

The findings of this review suggest that chiropractic interventions, particularly HVLA thrust maneuvers, show promise in reducing pain and improving the quality of life for office workers with cervicogenic headaches. The positive outcomes observed in the included studies support the potential of chiropractic treatment as a non-pharmacological approach to managing cervicogenic headaches. However, it is important to note that the number of studies available is limited, and the sample sizes in some studies were small. Therefore, further research with larger sample sizes and rigorous study designs is needed to confirm the efficacy and generalizability of chiropractic interventions for this specific population. Moreover, the exploration of the RCPM muscle and its connection to the Dura Mater provides valuable insights into the underlying mechanisms of cervicogenic headaches. The identification of this relationship highlights the importance of targeting specific regions in the cervical spine during chiropractic interventions. The integration of chiropractic treatment with other complementary approaches, such as physical therapy and ergonomic interventions, may further enhance the outcomes for office workers with cervicogenic headaches.

Based on the current literature, chiropractic interventions, including HVLA thrust maneuvers, hold promise in reducing pain and improving the quality of life for office workers suffering from cervicogenic headaches. However, the limited number of studies and small sample sizes indicate the need for further research to confirm these findings. Future studies should employ robust methodologies and larger sample sizes to provide more definitive evidence. Furthermore, the

understanding of the relationship between the RCPM muscle and the Dura Mater provides valuable insights into the mechanisms underlying cervicogenic headaches and informs targeted treatment strategies. This review highlights the importance of continued research in this underexplored area to optimize the management and enhance the quality of life for office workers afflicted with cervicogenic headaches.

Authors' Contribution

StudyConception: SEİ; Study Design:SEİ, AG; Supervision: AG; Funding: SEİ; Materials: N/A; Data Collection and/orProcessing: SEİ; Statistical Analysis and/or Data Interpretation: SEİ, AG; LiteratureReview: SEİ, AG; ManuscriptPreparation: SEİ, AGand Critical Review: SEİ, AG.

Conflict of interest

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

Financing

Theauthordisclosedthat they did not receiveanygrantduringconductionorwriting of thisstudy.

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The European Research Journal 2023;9(6):1502-1508 Review

DOI: 10.18621/eurj.1193573 *Histology-Embryology*

The possible effects of COVID-19 on the human reproductive system

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ABSTRACT

Spike surface glycoprotein and small envelope matrix-nucleocapsid proteins, is from the Coronaviridae family and binds to host receptors via spike surface proteins. Although it shows its symptoms especially on the respiratory tract, various studies have been carried out considering that it also affects other systems in the body. For the virus to enter the host cell, it must bind to ACE2 (angiotensin converting enzyme 2). ACE2 is a key protein involved in balancing Ang I and Ang II levels. With receptors such as TMPRSS2 (transmembrane serine protease 2), the effects of the virus on the human reproductive system are much better understood. Since human germ cells and early embryos express ACE2, there is a potential risk of the Coronavirus associated with germ cells. Studies show that the coronavirus changes the amount and density of hormones in the human reproductive system. The fact that most of the partners of 35 female patients who had SARS-CoV-2 in the studies were infected individuals suggests that sexual transmission may be possible. It was determined that TMPRSS4, Cathepsin B and L, FURIN, MX1 and BSG gene expressions were high in the menstrual cycle, while ACE 2 and TMPRSS2 were moderately expressed. It has been shown that the ACE2 enzyme is most intensely expressed is the testes. Studies have shown that sperm DNA (deoxyribonucleic acid) fragmentation, changes in hormone levels and the formation of anti-sperm antibodies are an important cause of male infertility. Infected men have been found to have an impaired spermatogenesis. This rewiew; it aims to draw attention to the possible effects of the corona virus on the human reproductive system and to reveal new mechanisms for new research to be done.

Keywords: COVID-19, human reproductive system, ACE2, TMPRSS2

The first case of pneumonia occurred in Wuhan City, Hubei Province of China, on December 31, 2019, and on January 7, 2020, it was stated that the cause of this situation was a new virus that has not yet been detected in humans [1]. It has been shown to be similar to viral pneumonia in terms of clinical features. The World Health Organization (WHO) has named the disease COVID-19. The International Committee on Virus Taxonomy (ICTV) named this virus as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [2]. Coronaviruses are enveloped, positive single-stranded large RNA viruses that can infect humans and a wide variety of animals [3]. It was firstly described by Tyrell and Bynoe in 1966 in patients with a cold, and it was named in Latin (corona = crown) [4]. It has four families, alpha-, beta-, gamma-, and

Received: ***October 24, 2022;*** *Accepted:* ***December 27, 2022;*** *Published Online:* ***March 4, 2023***

How to cite this article: *Dağdeviren T, Yolcu HK. The possible effects of COVID-19 on the human reproductive system. Eur Res J 2023;9(6):1502-1508. DOI: 10.18621/eurj.1193573*

e-ISSN: 2149-3189

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delta-coronaviruses. Alpha and beta-coronaviruses originate from mammals, especially bats, while gamma and delta originate from pigs and birds [5]. Compared to SARS and Middle East Respiratory Syndrome (MERS), the COVID-19 virus has a very high contagiousness and infectivity despite its low mortality rate [6]. When the genome analysis of each was examined, the genome analysis results of the new coronavirus sequences showed that the whole genome sequence similarity rates of SARS-CoV and bat SARS coronavirus (SAR-Sr-CoV-RaTG13) were 79.5% and 96%, respectively [7]. It can be assumed that the new coronavirus disease may originate from bats. Like other viruses, SARS-CoV-2 infects lung alveolar epithelial cells using the receptor-mediated endocytosis pathway via angiotensin-converting enzyme 2 (ACE2) [5]. In our country, the first case was announced on March 11, 2020. The disease was declared as a pandemic by WHO after cases were seen in many parts of the world [8]. In this review, it is aimed to see what effects it can have on the reproductive system and coronavirus disease and what can be done.

Clinical Features of the Virus

SARS-CoV-2 infection shows different clinical symptoms. While the most common clinical symptoms of the disease were fever, cough, and severe fatigue, diarrhea and vomiting were recorded as fewer common symptoms [9]. The period from the onset of symptoms to acute respiratory distress syndrome (ARDS) was found to be only 9 days among the initial patient groups with COVID-19 infection [10]. Patients with severe illness are prone to serious complications, including acute respiratory distress syndrome, acute heart injury, and a secondary infection [7]. In general, the radiographic features of coronavirus are similar to pneumonia [11]. As a result of blood analysis, it was seen that 82.1% of the patients had lymphopenia and 36.2% had thrombocytopenia. High levels of C-reactive protein, lactate dehydrogenase (LDH), and creatinine kinase (CK) occurred in most patients. In some patients, elevated transaminase, abnormal myocardial enzyme spectrum or elevated serum creatinine were detected [9].

Angiotensin Converting Enzyme (ACE) and SARS CoV-2

Angiotensin converting enzyme (ACE) is from the zinc metallopeptidase family. It is commonly found on the surfaces of endothelial and epithelial cells. ACE converts angiotensin-I (Ang I) to angiotensin-II (Ang II), a very potent vasoconstrictor. Thus, a polypeptide network is formed. These polypeptides are the basic elements of the renin-angiotensin system (RAS) [12]. RAS controls blood volume and blood pressure in the human body. renin: It is secreted from the juxtaglomerular cells of the kidneys during water and salt loss, blood volume reduction, and activation of the sympathetic nerves. In the liver, renin is first converted to angiotensin 1, and then to Ang II by the ACE enzyme found in the lung alveolar epithelium [12]. Ang I is a powerful vasoconstrictor. By affecting the adrenal cortex, sodium and water are reabsorbed from the kidneys and aldosterone secretion occurs [13]. Ang II, together with cytokines, stimulates many growth factors and plays a role in cell proliferation [14].

Angiotensin Converting Enzyme 2 (ACE2)

ACE2 has been identified as the first human homologue of the ACE enzyme. The ACE2 enzyme is the main enzyme that is effective in cardiovascular diseases, narrowing of the vessels, and oxidative stress. The ACE2 enzyme is a transmembrane protein. SARS-CoV2 and ACE2 are receptor enzymes of each other [12]. ACE2 is the main key to cell entry for SARS-CoV-2 [15]. Highest expression level of ACE2 in human tissue; the lowest expression levels are in the spleen, blood, bone marrow, brain, and blood vessels, while the small intestine, kidneys, testis, thyroid, and heart. Consequently, ACE2 is expressed in various organs and tissues besides the lungs [16, 17]. As a result of all these data, SARS-CoV-2 poses a threat to many systems. In other words, it is a systemic virus and its main target organ is not only the lungs [18]. ACE2, Ang II, and Ang 1-7 are regulators on the reproductive system (such as folliculogenesis, oocyte maturation, ovulation and endometrial regeneration in women; testicular function regulator, sperm functions and contribution to sperm embryo quality in men) [19]. There are studies showing that SARS-CoV-2 will also infect the male reproductive system, eventually. Although it was stated that there was no virus in the semen in the studies, a report published by the journal JAMA network revealed that 38 of 6 semen samples of the COVID-19 patients (analyzes were done using polymerase chain reaction by reverse transcription

method from RNA) were positive [17]. These analyzes support the opinion that SARS-CoV-2 may have an effect on the male reproductive system. In addition, the possibility of an effect of SARS-CoV-2 on the testicles was supported by other data indicating that patients with COVID-19 infection had a decreased testosterone/LH (luteinizing hormone) ratio [20]. Another protein is transmembrane serine protease 2 (TMPRSS2). When entering a cell, the virus first uses ACE2, then facilitates viral entry following TMPRSS2-mediated proteolytic processing of the SARS-2 spike protein [21]. TMPRSS2 is also an androgen-regulated gene in prostate tissue and contributes to prostate cancer pathogenesis by aberrantly directing oncogene expression. Almost half of prostate cancers involve a fusion using TMPRSS2 together [22]. These findings indicated that the testicles were vulnerable to SARS-CoV-2 infection.

SARS-Cov-2 on Ovary and Testicle

ACE2 is a key protein involved in balancing Ang I and Ang II levels. This feature has been shown to induce steroid production in the ovaries [23]. Ovarian tissue has its own RAS system, it can respond to angiotensin II with its own receptors [24]. Ang II has also been shown to be necessary for normal endometrial functions and regular menstrual cycles. Some studies showed that ACE2 is expressed in relatively higher amounts in the ovaries. In a study by Jing et al. [20], ACE2 mRNA levels were found in both human and rat uterus. Considering the functions of Ang II on the ovary, it is effective in the progression of the corpus luteum as well as its important effects such as oocyte maturation and follicle development. Ang II also plays a role in the resumption of meiosis in oogenesis. Considering all these factors, considering that SARS-CoV-2 enters the cell with ACE2 receptors, many problems may arise in a possible reproductive system infection. The fact that most of the partners of 35 female patients who had SARS-CoV-2 in the studies were infected individuals suggests that sexual transmission may be possible [25]. In addition, there are hypotheses that mother-to-child transmission may occur by droplet route and contact. As a result, it is predicted that the coexistence of ACE2 and SARS CoV-2 in female reproductive system functions may cause damage that may result in infertility and fetal problems [25, 26]. Although the literature studies clearly show that the virus is effective on the male reproductive system with experimental studies, its possible effects on the female reproductive system remain mostly in the form of theoretical hypotheses. Some of these hypotheses will also be evaluated through the following organs.

Effects on the Female Reproductive System

Ovary and Gametogenesis

Jing et al. [20] in their studies with immature rat ovaries, ACE2 protein was found in ovarian stromal cells, oocytes and granulosa cells; showed that it is expressed in antral follicles. Ang (1-7) and ACE2, which show their effects with the G-protein-Linked MAS receptor, are also known to be present in the ovary and play an active role in granulosa lutein cell apoptosis, oocyte maturation and ovulation [10]. It is predicted that any change in the expression of Angiotensin II and Ang 1-7, which plays a role in the formation of the corpus luteum and the continuity of the corpus luteum, may negatively affect the early pregnancy processes [27, 28]. In another study, it was stated that Covid-19 causes problems such as excessive coagulation and blood flow, causing damage to the endothelium and causing deeper vein thrombosis [26]. In this sense, since ACE2 is expressed in the ovarian tissues of both reproductive and post-menopausal women, it is predicted that SARS-CoV-2 infection may pose a potential risk in the female genital tract. It is also thought that it may cause infertility as it will cause a decrease in oocyte quality [27]. In addition to these hypotheses, Mohammadi et al. [29] in their published work; reported right ovarian vein thrombosis in 26-year-old pregnant COVID-19 (+) patients who applied to their clinics and stated that COVID-19 caused endothelial damage due to complications such as excessive coagulation and blood flow stagnation, and constituted a source for deep vein thrombosis [29]. The ACE2 protein has also been identified in the uterus. Studies have shown that ACE2 expression is more overexpressed, especially in the secretory phase compared to the proliferative phase [30]. In the endometrium, angiotensin II is involved in regeneration and has important functions in the vascular bed. Spiral arteries provide vasoconstriction, especially during menstruation. The balance between Angiotensin II and Ang (1-7) is very important for endometrial self-regeneration and myometrial activity [25-30]. In addition, ang II increases the proliferation of uterine epithelium and stromal

cells, so the change that occurs here may cause endometrial fibrosis. In the case of fibrosis, it has been reported that excessive activity will be inhibited by Ang 1-7 [25]. It was brought to the literature by Henarejos-Castillo et al. [16] in a study on the possible effects of SARS-CoV-2 on the endometrium and implantation. These investigators measured the viral gene expression of SARS-CoV-2 infection-associated endometrial ACE 2, TMPRSS2 and 4, Cathepsin B and L, FURIN, MX1 and BSG to explain virus and endometrial susceptibility. As a result of their study on 112 women with normal endometrial pathology, 29 of which were proliferative, 29 were early secretory, 43 were mid-secretory, and 8 were late secretory, TMPRSS4, Cathepsin B and L, FURIN, MX1 and BSG gene expressions remained high in the menstrual cycle, ACE 2 and They revealed that TMPRSS2 was moderately expressed. Researchers also stated that the amount of gene expression was different in different periods of the cycle, and emphasized that the only stable gene throughout the entire cycle was TMPRSS2. Viral genes have the highest expression in the early and middle phases of the cycle; stated that ACE2, TMPRSS4 and Cathepsin L were expressed weakly but co-expressed in the implantation window. Researchers who stated that ACE2 and TMPRSS4 are always expressed weakly but co-expressed during menstruation; Viral gene expressions are expressed differently in different periods of the menstrual cycle, and ACE 2 gene expression increases with increasing age, thus making the tissue more susceptible to SARS-CoV-2 infection [31, 32].

Effects on the Male Reproductive System

The male reproductive system consists of two testes located in the scrotum, attached glands, ducts and penis. The testicles are responsible for the formation, storage, release of spermatozoa, and the production of testosterone. The testicular parenchyma consists of seminiferous tubular rings, where spermatogenesis occurs. Each of the seminiferous tubules is 30-70 cm in length and 150-250 µm in diameter, and sperm are produced in them. Spermatogenesis in mammals; It is a dynamic process that continues with self-renewal and cell differentiation of spermatogonial stem cells. Spermatogenesis is controlled in a special microenvironment in the testicular seminiferous tubules. Sertoli cells are the only somatic cell type found in the tubules and interact directly with spermatogenic cells to control paracrine signaling and spermatogenic cell differentiation. Interstitial Leydig cells are adjacent to the seminiferous tubules. Abnormalities in male germ cells or disorders of somatic cells that support the stages of sperm formation cause male infertility [33]. Sertoli cells are rather large support cells that sit on the basal lamina and do not divide. It has supportive, protective and nutritional properties in spermatogenesis. The formations called 'tight-junction' between Sertoli cells ensure the tight connection of the cells. This structure together with a basement membrane forms the blood-testis barrier. This barrier prevents some antigenic substances formed during the development of germ cells from entering the blood. Thus, the formation of autoantibodies is prevented. In addition, toxic substances in the blood are prevented from reaching the spermatogonia. The content of the fluid in the lumen of the seminiferous tubule is also kept different from the plasma by this barrier. It is known that viruses can infect the testicles directly, because the blood testicular barrier is not sufficiently protected to completely isolate the viruses [34]. It has been shown that the ACE2 enzyme is a potential receptor for the SARS virus, and in independent studies, it has been shown that one of the tissues in which the ACE2 enzyme is most intensely expressed is the testes [35]. The male reproductive system, and particularly the testicles, express all classical members of the RAS system (AngI, AngII, Ang1-7 and ACE2) and have been cited as both source and target tissues for active angiotensin peptides. The effects of angiotensins include the regulation of steroidogenesis (production of steroidal hormones), especially through Leydig cell inhibition by Ang II, and their effects on epididymal contraction and sperm cell function [12]. Studies have shown that the virus disrupts the structure of the blood-testis barrier, which is very important for the immunity of the male reproductive system [36]. Non-dependent studies have revealed that the testes are among the tissues with the highest concentration of ACE2 enzyme. The RNA expression profiles of the ACE2 enzyme in the mature human testis were investigated within the framework of single cell resolution, and it was revealed that this enzyme is intensely present in the spermatogonia and contributes to the development of Sertoli and Leydig cells. These studies suggest that SARS-CoV-2 may cause infection and

loss of function in the male reproductive system [12]. Studies have shown that sperm DNA (deoxyribonucleic acid) fragmentation, changes in hormone levels and the formation of anti-sperm antibodies, which occur as a result of the attack of the coronavirus on the male reproductive system, are an important cause of male infertility. The studies by Wang and Xu [36] also state that infected men have impaired spermatogenesis, which supports all these. In some studies (ribonucleic acid) using probes, taking samples from the testicles by swab method, she conducted a study on six patients and found the results negative for SARS-CoV-2. However, in another study, in testicular epithelium and Leydig cells, the results of the research conducted with electron microscopy with the same method, this time the SARS-CoV-2 test was positive [12]. In the studies and researches, it was observed that the results of the SARS-CoV-2 test in their sperm were still positive in patients who were diagnosed with COVID-19 and their conditions returned to normal. Damage to the ductus deferens-epididymis barriers in the testicles by blood supply can affect the male reproductive system in the presence of SARS-CoV-2, and even if this virus does not multiply in the male reproductive system, this privileged immunity of the testicles can provide an environment for the continuity of viruses [12, 15]. Recently, Gagliardi et al. [37] estimated that orchiepididymitis observed in a 14-year-old boy was a complication of COVID-19 . In the autopsy of all 6 patients, who were observed to be infected and killed by SARS-CoV-2, which is closely related to SARS-CoV-2, it was revealed that the testicles had orchitis. This orchitis does not only cause testicular destruction; it has also been reported to cause destruction of germ cells and seminiferous tubule necrosis [15, 36]. These data are insufficient and it is not known what kind of effect and pathology will be encountered in the long term on the tissues -ovaries and testicles- in which ACE2s are expressed intensely in young individuals who survived the COVID 19 disease [12]. Compared to women, more cases of COVID-19 are seen in men. However, it should not be forgotten that men infected at a young age have a strong protector against adverse situations. It is stated that testosterone has anti-inflammatory functions by suppressing both cellular and humoral immune systems. In fact, testosterone has been found to reduce IL-6 (interleukin 6) and TNF-alpha (tumor necrosis factor alpha) levels through inhibition of the NF-kB (nuclear factor kappa B) proinflammatory pathway, similar to estrogen. In addition, testosterone deficiency has been associated with autoimmune diseases. In a laboratory study, gonads were removed from male mice with influenza infection. Male mice with intact gonads had higher mortality rates [37]. Low testosterone levels are also associated with elevated inflammatory markers such as IL-6. This may underlie the risk of lung injury after pneumonia. Testosterone can be converted to estrogen via the aromatase enzyme, which can have an anti-inflammatory effect. Several studies have shown that testosterone therapy can benefit patients with chronic obstructive pulmonary disease. Females have been shown to have stronger immune function as a result of the extra X chromosome [37]. In some clinical situations (including death), it has been observed that the LH level is much higher than normal and the testosterone level is decreased in infected men. In addition, considering the presence of ACE2 and TMPRSS in Leydig cells, it is predicted that SARS-CoV-2 may cause deterioration in testosterone levels [15, 37]. The ACE gene encodes two isozymes. While the somatic isozyme is expressed in many tissues, including vascular endothelial cells, renal epithelial cells, and testicular Leydig cells, testicular or germinal ACE is expressed only in sperm [38]. It has been observed that the expression of ACE in sperm is important for egg fertilization and it has been observed that sperm without ACE are insufficient for transport in the oviduct and binding to the zona pellucidae [39].

CONCLUSION

Coronavirus, which has been in our lives for more than two years, has affected many tissues and organs in different ways. The infectious disease caused by the new type of coronavirus spread rapidly all over the world and caused thousands of deaths. This has affected the health system, the human socio-psychological situation and the global economy. Studies have shown that the ACE2 enzyme, which is the cellular receptor for COVID-19, is not a viral infection that only affects the respiratory tract of COVID-19. The ACE2 enzyme is widely expressed in many tissues in the human body. However, the number of studies showing the damage

caused by Covid-19 in systems other than the respiratory system is insufficient. It is not yet known exactly what kind of pathological condition will be encountered in the long-term in the ovaries and testes, which are the tissues in which the ACE2 enzyme is intensely expressed in young patients with Covid-19. Despite this, literature studies have found that the damage caused by the SARS-Cov-2 virus in the male reproductive system or ovaries is more intense than in the female reproductive system. In every study, the gender distribution of the patients must be taken into account in order to ensure that the data are more accurate. Considering the theoretical information, the close relationship of the virus with ACE2 and TMPRSS2 molecules suggests that especially the ovary and endometrium may be affected by the virus. It is thought that COVID-19 infects sperm or egg cells and the damage that may occur in this process is not fully known, which may create risky situations for embryo formation. It can cause infertility in all young male patients who have had this disease. In women of childbearing age, miscarriages may occur or may result in the birth of genetically defective children. Studies on viruses and the human reproductive system are very important for the health of the next generation in terms of new babies, male and female infertility. The mechanism of action of this virus, which is still present in our lives, and the damage that may occur in the tissues and organs it affects are still not fully understood. More studies are needed to fully understand these cellular mechanisms of action.

Authors' Contribution

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

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1509-1519 Review

DOI: 10.18621/eurj.1352743 *Occupational Health and Safety*

Integration of digitalization into occupational health and safety and its applicability: a literature review

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ABSTRACT

The primary aim of this study is to review the transformation of occupational health and safety (OHS) practices in the digital age, particularly in light of the onset of Industry 4.0. The study seeks to understand the emergence of OHS 4.0 methodologies and their implications for enhancing performance, reducing risks, and addressing workplace challenges. The overarching objective is to explore the innovations in the OHS domain influenced by digitalization and ascertain the benefits and challenges of integrating digital methodologies into OHS practices. A comprehensive literature review was conducted, scanning multiple sources to gather insights on the innovations brought about by digitalization in the OHS domain. The study further analyzed contemporary research and application areas of new technologies in occupational health and safety. Findings from the study confirm that the integration of digital technologies into the OHS domain can lead to a significant reduction in workplace accidents. However, as workplaces embrace digital processes, new types of risks emerge for employees. In adapting to digitalization, there are recognized challenges in areas like privacy, security, clarity, and responsibility. Digitalization has redefined the landscape of OHS, ushering in an era of OHS 4.0. While the digital methodologies offer significant advantages in reducing workplace accidents and enhancing performance, they also present new risks and challenges. As the workplace undergoes rapid changes due to technological advancements, there's a pressing need to develop OHS approaches that align with the demands of the modern age, ensuring that health and safety remain paramount amidst uncertainties in applicability.

Keywords: Digitalization in occupational health, industry 4.0 and safety, modern workplace risks, technological adaptation challenges, transformation in work practices

The COVID-19 pandemic has placed technology at the heart of human life; during the pandemic, technology has been used in all processes ranging from education, health, shopping, and work life, with the aim of protecting and ensuring the continuity of business processes from the outbreak [1]. In this context, digitalization has become a commonly heard concept [2].

Digitalization is one of the most significant technological advances and intervenes in every aspect of life [3]. Besides daily life, it is a phenomenon that restructures many sectors in the business world, changing various service and business models and providing convenience [4].

The usual development process of work and working conditions has gained momentum with Industry

Received: ***August 30, 2023;*** *Accepted:* ***October 2, 2023;*** *Published Online:* ***October 8, 2023***

How to cite this article: *Akyıldız C. Integration of digitalization into occupational health and safety and its applicability: a literature review. Eur Res J 2023;9(6):1509-1519. DOI: 10.18621/eurj.1352743*

e-ISSN: 2149-3189

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4.0 and the digital transformation process [1]; industrial models have changed, and the concepts of time and space have disappeared. With massive advancements in internet technologies such as the Internet of Things (IoT), augmented reality, and cloud computing, digital environments have brought the world to the brink of a profound transformation, becoming applications in today's digital world [5]. New technological terms, employment platforms, new professions, different management understandings, and various robotic applications have also entered the digital transformation process [6-8].

After the Covid-19 pandemic, digital transformation is no longer a choice but a necessity [9]. In this regard, the transformation of occupational health and safety (OHS) practices is inevitable [10]. As in many fields of work, the OHS field is affected by technological advancements [11]. Overcoming encountered challenges, reducing costs, and increasing safety require technological developments [12]. Indeed, OHS is very conducive to innovations and technology use [13]. The phrases commonly associated with OHS, such as "wear your helmet" or "wear your gloves," are now replaced by entirely different concepts, and perspectives on OHS are changing [12].

OHS covers processes of identifying risks at workplaces and taking control measures for these risks; it is a proactive and multidisciplinary field [13-15]. The primary goal in OHS is to ensure the safety of both employees and the enterprise [14, 15]. To achieve this goal, Industry 4.0 technologies are used to prevent occupational accidents [16]. However, the digitalization of OHS processes cannot be realized instantly to improve overall OHS performance. Solutions provided by digital OHS technologies, expressed as OHS 4.0, are supports that will enhance and improve the existing activities of organizations [10, 14]. In this context, the benefits of new technologies can be maximized to reduce occupational risks [17].

Despite offering many opportunities, digitalization has brought new challenges [17]. Legal gaps have started to arise in the OHS field during the process [8]. This study aims to address the integration of digitalization into the OHS field and the resulting positive and negative aspects for both work life and employees. The study includes recommendations on digital new technologies in the OHS field, the new risks they bring, the advantages and challenges of new processes, and potential solutions.

Digitalization is a renewal process that shows sociological, cultural, and economic change in parallel with technological developments [18]. In simple terms, it is the transfer of an entity or object to the digital environment. For example, it is about transferring all the information and accumulation that businesses have to the digital environment and managing it under new conditions, with new opportunities and problems [19]. Digitalization is not a new phenomenon and has existed for decades. A handwritten text being converted into digital form can be given as an example [20]. Especially with the invention and spread of the internet, digitalization has become one of the most critical concepts of our era [19]; information technologies (IT) have been a driving force in this transformation. IT has paved the way for the present situation with the technological tools it offers [21].

The essence of this change and transformation is the ability to perform tasks faster, more effectively, and cheaply thanks to the technological advancements, alongside recording information instantly, processing it very quickly, and using it in decision-making processes [7]. Today, all information and documents that cause time loss and spatial loss, archived in a primitive way in analog systems, have given way to a process that offers digital resources with the help of current technology and tools. Thus, digitalized information and documents have become more easily accessible and shareable, maximizing material and time savings [4].

Another concept that needs to be emphasized after digitalization is digital transformation [20]. Digital transformation is a rapidly evolving process that brings innovations, covering the past, present, and future, and is efficient [22]. It is continuous and dynamic [23]. Digital transformation not only applies new technologies but also changes working methods to evaluate new opportunities. With the increasing effect of digitalization in daily life and corporate life, all processes and organizations are expected to adapt to the rapidly changing structure [22]. In this context, digital transformation accelerates digitalization steps by helping organizations cope better with change and quickly adapt to developments in the ongoing process [24].

Research plays a critical role in understanding the implications and ramifications of large-scale phenomena, such as the digital transformation resulting from the COVID-19 pandemic. It provides insights into the mechanisms of such changes and offers potential solutions to anticipated challenges. In the context of this study, the research sheds light on how digital transformation impacts occupational health and safety, thus assisting stakeholders in preparing and adjusting for the evolving landscape of the work environment. The research's value extends beyond mere observation, enabling proactive strategies to maximize the benefits and mitigate the risks associated with digital transformation.

LITERATURE RESEARCH

This study is prepared in the form of a compilation and aims to identify uncertainties, challenges, and risks related to the integration and applicability of digitalization in occupational health and safety, and to provide solutions. To achieve this, articles, theses, reports, and papers published in peer-reviewed scientific journals in English or Turkish between 2018 and 2023 focusing on the topic of "Digitalization and Occupational Health and Safety" have been examined. Databases such as Google Scholar, PUBMED, and YÖK Thesis Center were scanned online within this scope. The research was conducted using keywords such as "Digitalization," "Digital Transformation," "Digital Technologies," "Industry 4.0," "Occupational Health and Safety," "New Technologies in Occupational Safety," "OHS 4.0," and "Digital Solutions in Occupational Accidents." Comprehensive literature review was conducted, titles and abstracts were considered to eliminate similar studies, all relevant sources were read, and additional studies related to the topic were identified by considering the reference lists of the accessed studies. In total, 50 studies have been included in the compilation.

RESULTS

Digital Occupational Health and Safety Technologies: OHS 4.0

OHS 4.0 is a new concept that aims to maximize the harmony between humans and machines by integrating the basic building blocks of Industry 4.0 and digital transformation processes into the field of occupational health and safety [13, 25]. OHS 4.0 technologies are utilized to prevent potential accidents and illnesses, targeting a safer workplace and employee safety. The damages caused by workplace accidents and occupational diseases have not only economic consequences but also institutional and societal impacts. With new technologies and smart devices, digital applications are being developed to focus on occupational risks, including digital task analysis, dynamic risk assessment, real-time monitoring of employees, and protection against unauthorized access [14, 26]. Additionally, digital technologies bring opportunities such as labor market access for vulnerable groups of workers [17]. The components of digital occupational health and safety technologies within the scope of OHS 4.0 are discussed below [10, 13, 25].

Internet of Things (IoT)

IoT is the way devices interact with each other over the internet, mostly wirelessly. Through IoT, various information is shared in real-time between devices and can be controlled remotely [27]. IoT enables real-time observations and events to be directly recorded in an OHS management system and online OHS records, providing instant access to information as needed [28]. In the field of OHS, IoT is used especially for personnel tracking. For instance, in coal mining, personnel tracking with IoT allows for real-time location identification during accidents, enabling rapid response and access [13]. Similarly, solutions using IoT technology have been developed for tasks such as preventing vehicle/person collisions and collecting data through inactivity sensors for lone workers [10].

IoT Components

Radiofrequency Identification (RFID)

RFID, a component of IoT technology, is used to identify living beings and objects using radio waves. It prevents unauthorized equipment usage and unauthorized actions by individuals, in addition to preventing collisions or falls that workers may encounter in the workplace [15, 29].

Real-Time Location System (RTLS)

RTLS enables continuous monitoring and identi-

fication of the locations of objects and employees in the workplace, helping to prevent risks and accidents by ensuring that equipment and workers are where they are supposed to be [14, 15].

Augmented Reality (AR)

Augmented reality combines computer-generated data such as sound and graphics with the physical environment, creating an enhanced perception that blends with the real world [30]. AR applications have ushered in an innovative era in OHS training, removing many constraints such as physical locations and equipment. AR allows for activities that are difficult to simulate theoretically, such as emergency response, case analysis, fire training, and working at heights, to be conveyed to employees in a virtual environment for improved performance [10, 13]. For example, workers can practice and visually learn the workings of machines without direct interaction, thus reducing risk levels. This way, risks can be experienced beforehand, creating a safer working environment and saving time and costs in terms of training and safety [15, 17]. Additionally, AR can provide information about hidden hazards like asbestos, electrical cables, and gas pipelines [17, 28].

Artificial Intelligence (AI)-Enhanced Security Systems

Artificial intelligence is a computer program designed to acquire information similar to human cognition [31]. The interaction among robotic systems is made possible through AI [25]. Many technologies are used in conjunction with AI algorithms and are being continuously improved. For instance, AI-powered image processing technology allows camera images to be processed based on the requested content, enabling early warnings and proactive measures. Software placed in cameras can trigger alert systems when flame images are detected, aiding in fire detection [10]. Furthermore, digital AI technologies allow real-time analysis of workers using mobile devices, wearable technologies, and personal protective equipment (PPE), both within and outside the workplace. While transparency with data is crucial when using these systems, they offer opportunities to enhance OHS audits, support evidence-based prevention, and increase audit efficiency when used correctly [17, 32]. Microsoft's AI-powered safety monitoring system tracks employees and their activities, vehicle and equipment usage in real-time, detects risks in hazardous areas, and alerts authorized personnel. This software aims to maximize workplace safety and prevent work accidents [33].

Cloud Computing

Cloud computing is a computing system that enables shared information sharing over the internet, rather than using device memory [34]. Technologies like big data and IoT are realized through cloud computing [25], and training programs can also be based on cloud computing [29]. With data from wearable devices transmitted to the cloud, all employees can be alerted in case of potential accidents, enabling instant intervention. All data is recorded to prevent future risks, and sensor data from tags is transferred to the cloud data system for storage and processing. This allows for visualization of results and presentation to authorized personnel. This system allows rapid resolution of incidents and reduction of accident rates [15].

Big Data Analysis

Big data is a technology model that can analyze complex data sets using advanced algorithms and high technology [18]. It enables data to be analyzed at an advanced level compared to traditional tools and can process and combine data from different systems, databases, or websites even if they are not compatible [35]. Big data analysis is significant in terms of OHS as it provides opportunities such as measurement, prediction, goal setting, decision support in OHS, planning the future, and determining action plans [10, 25, 28].

Smart Robot Usage

The use of robotics will be revolutionary in fields such as mining/tunneling, underwater/closed-space operations, or hazardous tasks involving human lives. Particularly, autonomous robots with programmable intelligence can communicate with each other, conduct analyses without the need for an operator, and perform various tasks [25]. Smart robots improve the quality of work and keep employees away from hazards by working in collaboration with humans, enhancing efficiency. For example, drones with camera systems can minimize the risk of falls from heights during inspections, and applications for tasks like lifting heavy

objects are expanding with autonomous robots [10].

Personal Data and Process Security System

The security of personal data, including health records, is gaining importance, bringing up new developments in the field of OHS [10]. Process safety systems are important in processes with a potential for significant industrial accidents, preventing deviations through safety barriers, and managing and controlling risks. By providing preventive measures in hazardous situations, pressure and flow sensors allow monitoring of processes [25].

Innovative OHS Software

Organizations are developing software to ensure "process safety" in line with production processes and to enhance OHS performance. In recent years, various software programs have been developed for digital work permits, digital risk assessments, and tracking machinery and equipment, among other areas. Additionally, software that processes and stores OHS data continues to develop [10]. OHS software systems allow tasks such as e-signatures and health examinations to be conducted using computers, phones, and tablets, enabling OHS professionals to manage documents digitally [34]. The IBM Maximo software, integrated with AI, IoT, and cloud technologies, allows for comprehensive management of facilities and employees. Real-time access to data from various devices (such as IoT-enabled wearable devices and mobile phones) and sensors enables the detection of violations, predictions, and monitoring using advanced analytical analyses. As a result, the system facilitates the identification and elimination of hazards [14].

Advantages and Disadvantages of Industry 4.0 Technologies in Occupational Health and Safety

In addition to the advantages of Industry 4.0 technologies, it is also necessary to mention some of their disadvantages. In this regard, based on the literature review conducted, the advantages and disadvantages of Industry 4.0 technologies are presented in Table 1.

Wearable Digital Occupational Health and Safety Technologies

Wearable Devices and Smart Personal Protective Equipment (PPE)

In jobs where exposure to hazardous substances is a concern, such as those involving hazardous materials, robotic or remote-controlled units can prevent direct exposure to these substances. However, in tasks where employees themselves need to perform actions, wearable technologies come into play. For instance, through the Internet of Things (IoT), employees can be monitored using online and wearable cables, ensuring that their health status and performance are continually monitored. In fact, by detecting brain signals, interfaces can be generated to prevent individuals from entering hazardous situations when their focus shifts [28] thereby reducing the response time in case of a potential accident. Sensors can detect movements like standing or sitting, as well as psychological states of employees [29, 36].

Embedded monitoring devices within Smart PPE offer real-time monitoring of hazards, enabling early warnings about harmful exposures. They can also provide personalized real-time recommendations to positively influence an employee's behavior, thereby enhancing their health and safety [17, 28]. The sensors within Smart PPE collect information and alerts, transmitting them to employees and authorities. When unusual behaviors are detected, all the gathered data is communicated to relevant units through the cloud [15]. These insights can aid organizations in anticipating potential OHS issues and determining areas where OHS interventions are necessary [17, 28].

Exoskeletons

Exoskeletons are auxiliary devices worn on the body designed to monitor real-time body postures and movements, reduce the load on the musculoskeletal system by performing manual lifting tasks, and support employees. They are used to assist physically impaired workers and prevent work-related musculoskeletal disorders. Digitally enabled exoskeletons can perform manual tasks from an OHS perspective, reducing the occurrence of injuries and aiding operators in improving their postures. Additionally, exoskeletons have the potential to reduce physical efforts, thereby decreasing work fatigue and increasing productivity [17, 36].

Despite the benefits, exoskeletons can introduce new risks from an OHS perspective. For instance, if exoskeletons malfunction, workers could become trapped or injured. The long-term physiological, biomechanical, and psychosocial effects of exoskeleton

Table 1. Advantages and disadvantages of industry 4.0 technologies

Component	Advantages	Disadvantages	References
IoT-based personnel and vehicle tracking systems	Reduces negative interactions with personnel and vehicles, enhances field control/efficiency. Enables person identification in emergencies, identifies and prevents inappropriate situations. Vehicle tracking systems allow tracking of rule-compliant vehicle usage, duration, location, and speed.	Privacy and security concerns; lack of protection against service continuity and integrity issues, and malicious software attacks.	[25, 29, 36]
AI-supported security measures	Reduces exposure to risks including harassment and violence, offers early warning for fatigue and stress, provides personalized real-time advice, influences employee behavior, decreases risk and workplace accidents, improves occupational health and safety audits.	Can lead to fatal consequences in situations that occur beyond human control and require initiative.	[25]
Robotic use in hazardous tasks	Mitigates risks, eliminates personal errors, removes employees from dangerous work environments (e.g., chemical, biological), reduces workplace accidents, offers healthier operations with shorter exposure.	Detrimental effects on employee mental health (performance pressure), reduces human interaction and socialization.	[17, 28]
Augmented reality applications	Provides effective and qualified personnel training, offers new perspectives and awareness in occupational health and safety, increases awareness of risks among employees, includes instructions to mitigate human errors.	Reliability depends on the continuity, quality, and timeliness of information sources.	[25, 28]
Big data analysis	Enables perpetual digital record-keeping, facilitating continuous improvement and risk reduction. Allows retrospective accident data analysis and comparison with other facilities.	Challenges in maintaining control and privacy.	[15, 25]
Cloud computing	Eliminates hardware issues, offers high accessibility, and flexible structure that does not require memory alterations.	Challenges in data management, reliability and efficiency issues due to factors like communication in the clouds.	[15, 35]
Personal data security systems	Ensures secure data provisioning and prevents data leakage, enhances employee and business partner satisfaction.	High labor requirements and costs.	[15]
Process safety systems	Eliminates human errors, minimizes risks, ensures facility/employee safety, provides risk-mitigating solutions.	-	[15]
Innovative OHS software	Enhances reporting, analysis, centralized employee management, time management, document tracking, corrective and preventive activity control, traceability, ease of access with digital storage, increased accessibility through training modules, facilitates tracking, data/archive management, process standardization, easy data sharing.	Potential privacy issues due to remote accessibility.	[15, 34]

usage are still unknown. Therefore, personal precautions are considered the last resort in the control hierarchy, similar to other measures [17].

Risks Associated with Digital Occupational Health and Safety Applications and Emerging Risks

Digitalization brings about unexpected hazards that require new solutions and approaches, while also amplifying existing hazards [37]. In this context, OHS risks will evolve. New working styles that accompany new technologies can lead to safety and ethical concerns. Monitoring technologies can induce stress and anxiety due to concerns about privacy violations. Especially in terms of OHS risks, factors such as performance pressure affecting mental health, reduced social interactions, prolonged working without breaks leading to musculoskeletal issues, and cardiovascular diseases can arise [17, 32]. Within this framework, the existing and emerging risks associated with Industry 4.0 OHS technologies are provided in Table 2.

Remote work requires significant responsibilities for OHS professionals, and it should not be forgotten that remote workers may require more frequent monitoring and support [38]. Furthermore, adjustments to regulations related to changing risk factors [13], continuous monitoring of these risk factors by employers, and seeking solutions for emerging risks are essential [34].

Effects of Digital Occupational Health and Safety Applications on Work Life and Employees

With digital technologies, the requirement for physical presence at the workplace is diminishing [34]. Flexible working arrangements are being adopted, allowing data to be archived on digital platforms, and mobile communication resources are always accessible. As a result, remote and flexible work styles such as working from home are becoming increasingly common [4]. Consequently, the number of employees in offices is expected to increase, leading to a rise in workplace accidents and occupational diseases. Thus, occupational health and safety measures need to be applied in office settings that function as workplaces. The increasing number of people working from home may also raise discussions about what kind of OHS measures employees should take in their homes [35].

Table 2. Existing and emerging risks associated with digital occupational health and safety applications

Existing Risks	Emerging Risks
Physical Risks	Privacy and Security
Chemical Risks	Work Accidents
Biological Risks	Occupational Diseases
Ergonomic Risks	Skilled Workforce Shortage
Psychosocial Risks	Risks Related to Online Work

Effects of Digital OHS Applications on Employees

Positive Effects

Digital OHS technologies' monitoring of employees is seen as a significant advancement in preventing potential accidents. For instance, augmented reality applications can provide information and experience to employees about how to act in hazardous situations, and technologies like big data and IoT can analyze information rapidly to prevent numerous potential risks and diseases [14]. Moreover, by utilizing robots and exoskeletons for manual tasks, especially older and disabled employees can continue their tasks with reduced physical effort. Additionally, diseases resulting from sedentary lifestyles and situations like traffic accidents that remote workers might face can be prevented [28].

Negative Effects

The rapid pace of technological change requires employees to learn quickly and continuously. Not only do employees need to know how to use technology, but they also need to possess the relevant skills for new work methods. Changing work models and job nature could mean workers have more responsibility for their own learning and training needs. Smart robots constantly learning might pressure employees to keep up with the pace and level of work, leading to a high level of performance pressure. Furthermore, AI-supported digital monitoring technologies can cause stress and feelings of insecurity, negatively impacting employees' mental health [17, 28, 34]. Overreliance on robots or exoskeletons for manual tasks can lead to reduced physical fitness, resulting in muscle, bone, or joint loss [28]. Additionally, prolonged screen time is believed to cause certain occupational diseases, par-

ticularly affecting the eyes (as shown in Table 2). Moreover, psychological ailments like stress, often referred to as the "disease of our time," are likely to become more widespread [34]. According to research by the European Foundation for the Improvement of Living and Working Conditions and the International Labour Organization in 2017, remote workers experience not only increased stress levels but also blurred lines between free time and work hours [38].

DISCUSSION

While digital technologies offer significant innovations, they also bring about new challenges due to changes in work processes [37]. Within this scope, based on a review of the literature, the challenges in digitalization for occupational health and safety can be listed as follows [28, 34, 38].

Inadequate regulations, as technology becomes increasingly complex, there are very few government policies available for the safe integration of technologies like robotics into workplaces.

Elimination of location and time constraints in work through new working methods, as 24/7 flexible work becomes more common, employees will become more scattered and diverse, making OHS inspections and regulations more challenging. Employers will have less control and influence over factors affecting employees.

Lack of awareness among managers about issues and inadequate risk assessment, new situations creating new risks will require new solutions. Without considering the impact of digital technology and work processes, applying old methods to new processes can create difficulties. For example, infrastructure designed for old technology might not be suitable for new technology, leading to unforeseen risks.

Excessive workload and increased mental strain with modern technology, the pressure of being accessible 24/7, intensified work processes, changing expectations of managers and customers, overtime, uncertainties, and the use of artificial intelligence or autonomous machines can cause stress among employees and hinder their engagement.

Internal collaboration problems within companies and ambiguity of responsibilities; the ambiguity of digitalization strategies, lack of clear communication to employees, unclear definition of responsibilities for outputs, errors, or consequences of new processes (such as uncertainty about those responsible for OHS) can create challenges within the company.

Lack of transparency and clarity along with inadequate data protection; transparency and clarity issues may arise regarding new working models and processes, and the increase in data may lead to gaps in data protection and security.

Those responsible for OHS must adapt to technology in line with the developments that occur. In the coming years, employers and employees will not be able to see each other, and therefore, OHS services will also be provided remotely. In this case, the issue of cyber attacks will negatively affect OHS. It is expected that in the coming years, cybersecurity experts will be included in OHS teams; however, it is observed that companies working in the technology field or using technology now employ cybersecurity experts and make significant investments in this area [34].

In situations where security is at risk, the issue of cybersecurity becomes significant regarding the potential of hackers taking control. This pertains to the control and communication of business processes and devices over the internet (such as GPS technology, IoT systems, wireless networks, central databases, etc.) [28]. In this context, a cybersecurity expert is an individual who has received training to protect the electronic information systems of the company from both external world attacks and internal attacks. Cybersecurity experts will work in coordination with OHS experts to ensure internal workplace safety [32].

The following proposed solutions can help mitigate the challenges brought about by digitalization on occupational health and safety [28, 34, 37]:

- (a) Ethical framework for proper management and behavioral rules should be established for digitalization; adjustments should be made regarding clear boundaries, transparent processes, manageable workloads, effective communication, reliable information, and feedback.
- (b) A regulatory framework should be established to clarify OHS obligations and responsibilities for new systems and working methods; responsibilities should be redefined and clearly stated.
- (c) Training for employees; two-way exchange of knowledge and experience should be established to enable employees to enhance their competencies and

creativity as needed, and communication and feedback practices should be adjusted.

- (d) Effective OHS services should be provided to all employees in the digital business world; OHS processes should be organized, taking into account features such as new types of work such as daily work, job sharing, or freelance work.
- (e) Employee participation in the design and implementation of digitalization strategies; employees should be given the freedom to make their decisions and follow their ideas, and resources should be made available.
- (f) A strong 'design for prevention' approach that combines the human factor and employee-centered design should be adopted.
- (g) Comprehensive risk assessment taking into account all factors and their interactions; processes should be evaluated, working conditions should be enhanced, and special data should be given importance.
- (h) Increasing awareness for the rethinking and reshaping of previous knowledge and processes; new approaches should be developed, and occupational safety awareness should be strengthened by including everyone in the process.

To bring current technologies into OHS applications, a multidisciplinary approach including expertise from engineers, IT specialists, psychologists, ergonomists, social and occupational scientists, medical practitioners, and designers should be adopted [12]. Collaboration should be established among academics, industry, social partners, and governments [17]. In this context, the European Agency for Safety and Health at Work (EU-OSHA) has been conducting comprehensive studies on OHS and digitalization since 2016, aiming to maximize the opportunities offered by digitalization and to create healthier and safer workplaces for everyone in digital workspaces [17, 34].

The COVID-19 pandemic, felt globally, has shifted many priorities, with digitalization being one of the most talked-about subjects. Especially with Industry 4.0, digital technologies have begun to be used in the field of occupational health and safety. With the integration of digitalization into occupational health and safety, it is clear that along with opportunities, challenges will be encountered. In this context, it is believed that transitioning to OHS practices unplanned may not be possible, but with the right and effective approach, digital occupational health and safety processes will have a positive impact. Indeed, protecting against the negative effects of risks and challenges brought about by digital OHS applications and establishing the necessary infrastructure to achieve the desired goal seems likely to take place in the long term.

On the other hand, along with new technologies, both personal hazards such as unsafe behaviors of employees and unsafe conditions will be eliminated. As a result, by minimizing risks, work accidents will decrease significantly. Therefore, for effective occupational health and safety technologies that ensure both the safety of businesses and the health of employees, it is concluded that new approaches and processes suitable for the digital age, considering all factors and designing new technologies with employee-centered approaches due to their negative effects on employees, need to be developed.

CONCLUSION

In conclusion, digitization offers both opportunities and new challenges in the field of occupational health and safety. These challenges range from inadequate regulations to unsafe employee behaviors and the shortcomings in adapting to technology. However, various solutions are available to overcome these challenges. Managing digitization within an ethical framework, providing education and involvement for employees, and implementing regulations that enhance safety are key steps in transitioning occupational health and safety to the digital age. This process requires collaboration across a wide spectrum of expertise, from engineers to psychologists, occupational health specialists to designers. Organizations like the European Agency for Safety and Health at Work (EU-OSHA) also contribute significantly to this area. Therefore, it is evident that a continuous effort is essential to guide the transformation brought by digitization in the safest and healthiest manner possible.

Authors' Contribution

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

Conflict of interest

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

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1520-1528 Case Series

DOI: 10.18621/eurj.1285835 *Pediatric Surgery*

"Should the child be raised as male or female?" The evaluation and management of different causes of ambiguous genital appearance in children

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ABSTRACT

Diagnosing, managing and assigning gender for different causes of ambiguous genitalia in children can be challenging. This article will discuss about the types, diagnosis and treatment of disorders of sex development including congenital adrenal hyperplasia, 46 XY mixed gonadal dysgenesis and 46XY ovotesticular disorder of sex development. This article reports about 3 cases of disorders of sex development. The first case is about a three-year-old girl who was diagnosed with congenital adrenal hyperplasia, genital examination revealed clitoromegaly and a single urogenital sinus; she was managed medically and surgically. The second case is about a one-month-old child with 46XY karyotype, genital examination revealed penoscrotal hypospadias with right palpable and left impalpable gonads. The patient underwent diagnostic laparoscopy in which both female and male internal organs were found; based on these results a diagnosis of 46XY ovotesticular disorder of sex development was made. The third case is about a 3-month-old child with 46XY karyotype, genital examination revealed hypospadias with bilateral impalpable gonads. Diagnostic laparoscopy showed a uterus with a bilateral ovary-looking gonad; histopathology of the bilateral ovary-looking gonads was consistent with testicular tissue. Based on the above, a diagnosis of 46XY mixed gonadal dysgenesis was made. Disorders of sex development are classified into three main categories based on the karyotype, XX, XY and sex chromosome other than XX and XY. Laboratory investigations, karyotype, genetic analysis, imaging, surgery and tissue biopsy, all aid in diagnosing, deciding gender identity and managing different types of disorders of sex development.

Keywords: Ambiguous genitalia, disorders of sex development, ovotesticular disorder, mixed gonadal dysgenesis

Disorders of sex development (DSD) is a broad term that is defined as a congenital discrepancy between the phenotypic and genotypic sex. Disorders of sex development with genital abnormalities sufficient to prompt evaluation occur in approximately 1 in 1000 to 4500 live births [1-3]. The causes of DSD can be classified into 3 categories, DSD in individuals with an XX karyotype, XY karyotype, and sex chromosome complement other than XX or XY. Disorders of sex development in individuals with XX karyotype

Received: ***April 19, 2023;*** *Accepted:* ***July 12, 2023;*** *Published Online:* ***July 31, 2023***

How to cite this article: *Al Khanjari SA, Al Balushi MS, Kanojia RP, Al Sajwani MJ. "Should the child be raised as male or female?" The evaluation and management of different causes of ambiguous genital appearance in children. Eur Res J 2023;9()6:1520-1528. DOI: 10.18621/eurj.1285835*

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e-ISSN: 2149-3189

are caused by high levels of androgens either from the adrenal cortex, gonads, or exogenous source; and therefore, lead to virilization in XX infants. Adrenal overproduction of androgens is caused by either congenital adrenal hyperplasia (CAH) or glucocorticoid resistance. Gonadal overproduction of androgens is caused by either XX testicular/ovotesticular DSD or aromatase deficiency. Other causes of XX chromosome DSD include gestational hyperandrogenism. Disorders of sex development in individuals with XY karyotype are caused by low levels of dihydrotestosterone action either due to gonadal dysgenesis or abnormal androgen synthesis or response (androgen insensitivity). Sex chromosome DSD occurs in sex chromosomes other than XX or XY and examples of this subtype of DSD include mosaicism and chimerism.

This article focuses only on DSD that present with atypical genital appearance discordant with the chromosomal sex (also referred as ambiguous genitalia) and it will cover the causes, diagnosis, and treatment of DSD. The aim of presenting the cases is to overview the different types of DSD in general, to review case scenarios of rare types of DSD including 46 XY ovotesticular DSD and 46 XY mixed gonadal dysgenesis, and to discuss the approach of diagnosing and treating such cases.

We selected the cases who initially presented to the endocrine clinic of our tertiary hospital and then they were referred to us, the pediatric surgery outpatient department, and were managed by the same pediatric surgery consultant. We will discuss all the cases in detail below

CASE PRESENTATION

CASE 1

A three-year-old girl was referred to our pediatric surgery department on 1/2020 from another tertiary hospital that doesn't have pediatric surgery service when the child was 13 days of age as a case of neonatally diagnosed congenital adrenal hyperplasia (21-hydroxylase deficiency) with atypical genital appearance. The mother was healthy, no history of consanguinity, and had another child with congenital adrenal hyperplasia. The patient was born full-term by spontaneous vaginal delivery.

Genital examination revealed a clitoromegaly of 3 cm in length, scrotalized labia, no palpable gonads and a single urogenital sinus (Fig. 1); other systemic examination unremarkable.

Investigations revealed a karyotype of 46XX, laboratory tests showed a cortisol of 179 nmol/L, normal range (NR) 4.4-25 nmol/L, Testosterone of 52 nmol/L (NR 0.1-2.4 nmol/L), ACTH of 601 ng/L at birth and 92 ng/L before treatment (NR 7.2-63.3 ng/L), 17 Hydroxyprogesterone of 600 nmol/L at birth and 265 nmol/L before treatment (NR 0.2-5 nmol/L), Androstenedione of 34.5 nmol/L before treatment (NR 2.6-10.7 nmol/L). Improvement of the above values were noted after the initiation of medical treatment with hydrocortisone and fludrocortisone; Testosterone of 5.22 nmol/L, ACTH of 1.2 ng/L, 17 Hydroxyprogesterone of 0.3 nmol/L, and Androstenedione of 0.35 nmol/L after treatment.

The pelvic ultrasound showed a normally appearing uterus and bilateral ovary with no testis. Genitogram was done and showed two linear tracts filling up a bear shape structure likely representing the vagina and the urinary bladder, the common channel measuring approximately 1.5 cm.

At the age of 3 years, the child underwent clitoral reduction and vaginoplasty with the finding of clitoromegaly of 3.5 cm. After inserting a Foley catheter in both, the vagina and the urethra, a flap was created below the vaginal orifice followed by a vertical mid-

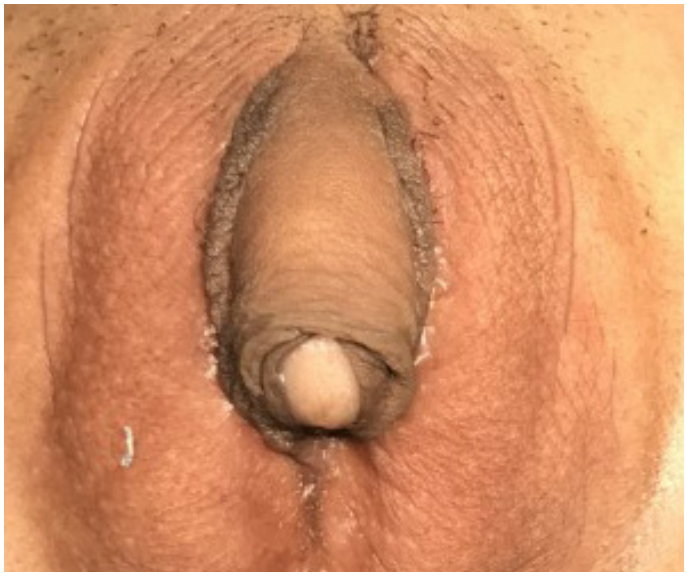

Fig. 1. Clitoromegaly (this picture does not represent the real patient).

line mucocutaneous incision which was made between the vaginal orifice and the flap. Clitoris was fully degloved till the crural separation. The dorsal neurovascular bundle was preserved and parts of the glans and the erectile tissue were excised to reduce their size. The ventral clitoral mucosal tissue was used to create a vestibule and the degloved clitoral skin was used to reconstruct the labia minora. The lateral labio-scrotal skin was used to reconstruct the labia majora. Finally, the flap was sutured along with labia minora and labia majora bilaterally.

CASE 2

A seven-year-old boy was referred to our pediatric surgery department on 1/2016 from a local hospital when the child was one month old due to atypical genital appearance. The boy was born at full-term by spontaneous vaginal delivery. The mother mentioned a history of gestational diabetes which was controlled with diet. There was no prenatal androgens exposure or history of maternal virilization during pregnancy. Also, there was no maternal family history of women who have been unable to conceive or had amenorrhea. The other two siblings of the patient were both normal.

A focused genital examination revealed a peno-scrotal hypospadias with severe penile chordee, right testis was palpable in the scrotum with moderate hydrocele, and left testis was impalpable with hypoplastic bifid scrotum (Fig. 2). Other systemic examination unremarkable.

The laboratory investigations done at one month of age were as follows: chromosomal analysis showed 46XY normal male karyotype, FISH for SRY gene showed the presence of Y chromosome in 100% of the analyzed metaphases, normal 17-OHP and electrolytes, and other hormonal studies were done before and after CGH injection at one year of age and the results were as follows: Testosterone, Androstenedione and Dihydrotestosterone were 0.06 nmol/L (NR 0.1-2.4), 0.53 nmol/L (NR 2-9.3), 0.66 nmol/L (<0.17) before CGH Injection respectively; and 7.23 nmol/L, 0.35 nmol/L, 1.13 nmol/L after CGH Injection respectively. Anti-Mullerian hormone level was 153.5 pmol/L (NR 53-1735). Abdominal ultrasound was done and showed: (The right testis in the right medial inguinal area with hydrocele and the left testis was not clearly seen. Both kidneys appeared normal. A stripe of increased echogenicity seen posterior to the bladder likely represents vaginal tissue. A normal uterus was not seen.). The above information most likely fits the condition of ovotesticular DSD.

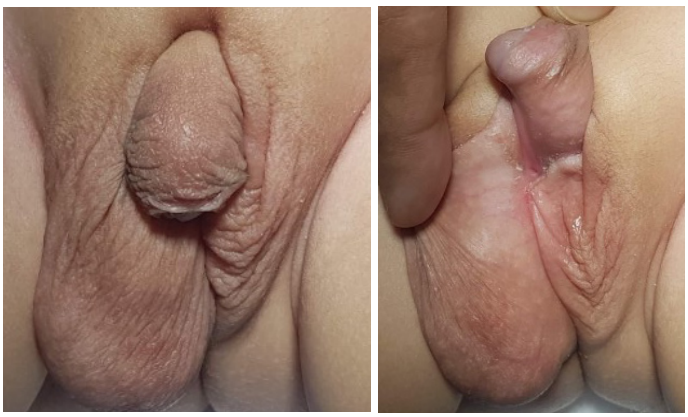

Fig. 2. Genital asymmetry (this picture does not represent the real patient).

At 2 years of age, the patient underwent a laparoscopic left gonadal biopsy, right patent processus vaginalis ligation and right gonadal biopsy; with the following intraoperative findings: on the left side there was a round ligament seen crossing the internal inguinal ring, small atrophic hemi-uterus with fallopian tube and ovary-looking structure was seen, no left testis found. On the right side there were testicular vessels seen crossing the canal passing through the internal inguinal ring, no gonads and no vas deferens were seen. On right inguinal exploration, there was a hernial sac with testis of average size and testicular vessels, but no vas deferens was seen, and small epididymis was seen separated from the testis.

The histopathology report was as follows: the ovarian biopsy was consistent with left ovarian tissue and the testicular biopsy was consistent with right testicular tissue. There was no evidence of gonadoblastoma or other germ cell tumor from both samples.

At six years of age, the patient underwent cystourethroscopy, laparoscopic removal of female internal organs on the left side, and laparoscopic left inguinal herniotomy. The intraoperative findings were atrophic ovarian tissue, atrophic hemi-uterus with soft tissue that looked like atrophic vaginal tissue, and a fallopian tube on the left side. Cystourethroscopic findings were normal urethra with no evidence of urethral fistula. Histopathology of the sample was consistent with atrophic vaginal tissue.

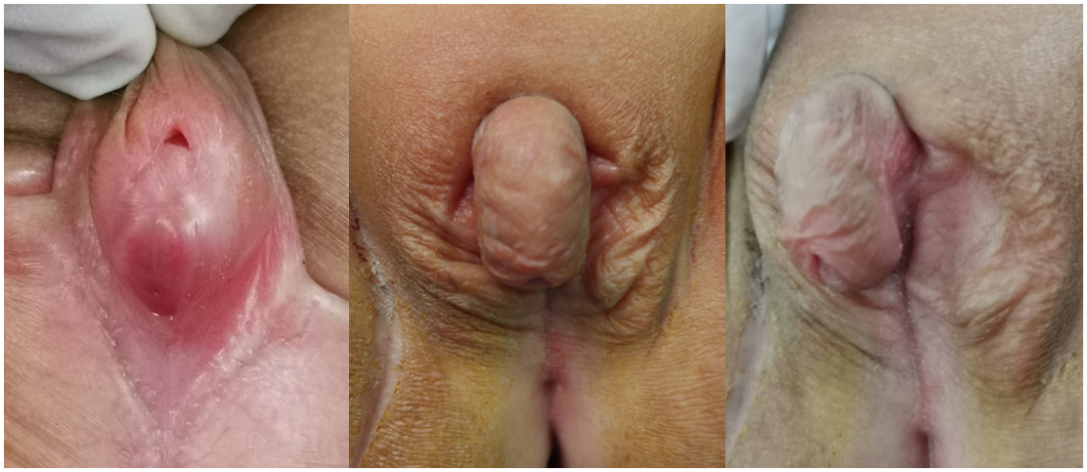

Fig. 3. Coronal hypospadias and hypoplastic scrotum.

CASE 3

A 2-month-old full-term baby was referred to our pediatric surgery outpatient department on 11/2022 from a local hospital due to ambiguous genitalia. The mother was healthy, not on any medications, and no history of maternal virilization. The parents were not consanguineous and no family history of similar condition.

On examination, the child phenotypically looked like a virilized female. Genital examination revealed a phallus of 2.2 cm in length, coronal hypospadias, no vaginal opening, no testicles felt, poorly developed scrotum, and the anus was normal in position (Fig. 3).

Investigations done were as follows: chromosomal analysis of 72 hours of peripheral blood lymphocyte cultures revealed 46XY karyotype with the presence of SRY gene in the Y chromosome. Pelvic ultrasound showed a normal size uterus, normal size bilateral ovaries, no testicles were seen. Laboratory findings were as follows: 17 OH progesterone of 36.5 nmol/L (NR 0.2-5 nmol/L), Androstenedione of 0.47 nmol/L (NR 2-9.3 nmol/L), DHEA-S of 2.82 nmol/L (NR 0.2-4.8 nmol/L), DHT of 0.67 nmol/L (NR < 0.17 nmol/L), Testosterone of 8.35 nmol/L (NR 0.24-0.7 nmol/L). Based on the above findings and information, a diagnosis of partial gonadal dysgenesis was made.

The child underwent diagnostic laparoscopy and cysto-genitoscopy. The cysto-genitoscopy findings were as follows: a 2 cm phallus with mid-penile hypospadias, no verumontanum; at the turn of the urethra, the normal vaginal orifice was seen. The urethra was anterior to the vaginal orifice. Laparoscopic findings were as follows: the uterus seen more well-developed on the right side compared to the left side, both round ligaments were well-formed and entering the in-

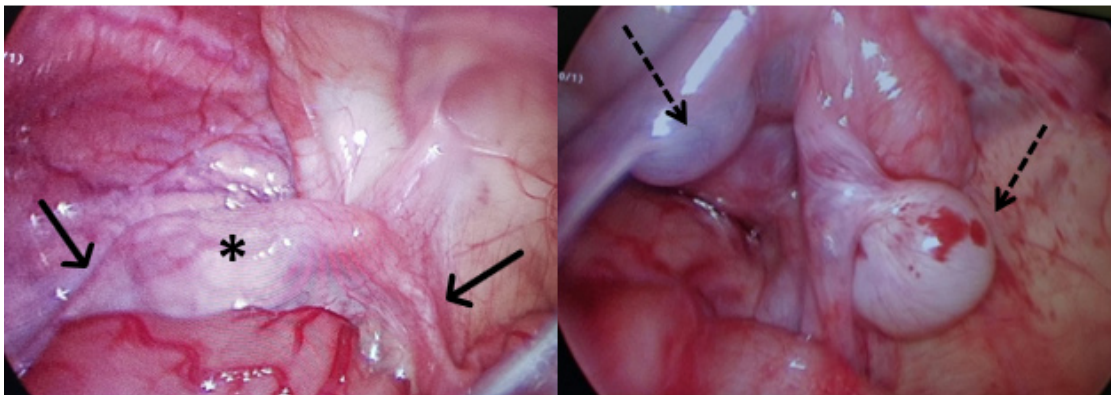

Fig. 4. A) uterus (*) with right and left fallopian tubes (straight line arrow). B) right and left ovaries (dotted arrow)

ternal inguinal ring, both ovaries were normal in size, both fallopian tubes had no fimbriae, two small cysts seen at the junction of the left fallopian tube and the left ovary, no vas deference or testes seen (Fig. 4). Histopathology of the bilateral ovary-looking gonads was consistent with testicular tissue, no ovarian tissue was identified.

DISCUSSION

Disorders of sex development (DSD) should be considered in any infant with frankly atypical genital appearance, in male infants with bilateral nonpalpable gonads, severe hypospadias, or any hypospadias accompanied by unilateral or bilateral cryptorchidism or micropenis; and in female infants with posterior labial fusion, clitoromegaly, urogenital sinus, or gonads palpable in labioscrotal folds or inguinal region.

Initial evaluation of any suspected DSD should include taking a pregnancy and a family history, physical examination, pelvic and abdominal ultrasound, laboratory testing including CAH panel (17-hydroxyprogesterone, 17-hydroxypregnenolone, cortisol, and 11-deoxycortisol), baseline electrolytes, karyotype or fluorescence in situ hybridization (FISH) for SRY, X and Y chromosome probes, and laboratory tests for gonadal function (FSH, LH, testosterone, dihydrotestosterone and AMH).

After the initial evaluation, one would be able to categorize DSD into either XX DSD, XY DSD or mosaicism/chimerism. A subsequent evaluation is then performed to confirm or identify specific causes within a general category of DSD. This includes other steroid precursors to identify rare forms of CAH, ACTH stimulation test (e.g. in XX infants with borderline elevations in 17-OHP), hCG stimulation test, genetic testing and second-line imaging (like retrograde urethrogenitogram). Human chorionic gonadotropin (hCG) stimulation test helps in distinguishing between disorders of abnormal androgen synthesis and abnormal androgen sensitivity; a ratio of testosterone: dihydrotestosterone after hCG stimulation > 10:1 suggests 5-alpha-reductase 2 deficiency [4] and a ratio of testosterone: androstenedione after hCG stimulation < 0.8 suggests 17-beta-HSD3 deficiency [5]. Genetic testing includes AR gene for androgen insensitivity and SRY and NR5A1 genes for testicular/ovotesticular DSD and XY gonadal dysgenesis. Multigene sequencing is becoming increasingly available and cost-effective.

In the first case scenario, history wise there was a positive family history as expected. The clinical examination revealed a clitoromegaly (in XX neonates, clitoral lengths of more than 9 mm are unusual [6-8] and is caused by inappropriate androgen action), a urogenital sinus (one common opening located in the introitus below the clitoris, with internal connection between the vagina and urethra indicating partial but incomplete androgen action) and in some other patients, a posterior labial fusion can also be seen (anogenital ratio > 0.5, which is the distance between the anus and posterior fourchette divided by the distance between the anus and the base of the clitoris/phallus).

The condition was diagnosed neonatally by elevated 17-hydroxyprogesterone. Electrolytes measurement is important for early identification and treatment of salt wasting which is suggested by the findings of hyponatremia, hyperkalemia, hypoglycemia and non-gap metabolic acidosis. Adrenocorticotropic hormone (ACTH) at time of presentation was elevated suggesting primary adrenocortical insufficiency due to CAH. Androstenedione was also elevated. The detection of uterus in XX infants with CAH by pelvic ultrasound is due to the absence of high amounts of AMH produced by Sertoli cells of the testis.

21-hydroxylase deficiency accounts for approximately 95 percent of CAH and it is the most common cause of atypical genital appearance. Other types of CAH include 11-B-hydroxylase deficiency and 3-B-HSD type2 deficiency. Approximately 90 percent of 46XX CAH with Prader II to IV virilization (figure 5) have female gender identity as adults and psychosocial outcomes are generally positive [9]. Surgical treatment for those individuals includes separation of the urinary tract and vagina, labioplasty and clitoroplasty. The separation of the vagina from the common urogenital sinus allows the common urogenital channel to function as the urethra. The separated vagina is then brought out as a separate opening below the urethra (vaginoplasty). This is followed by reconstruction of the labia minora and labia majora next to the opening of the reconstructed vagina. Finally, reduction of the size of the clitoris (for patients with Prader IV or V) using the newer ventral approach technique to pre-

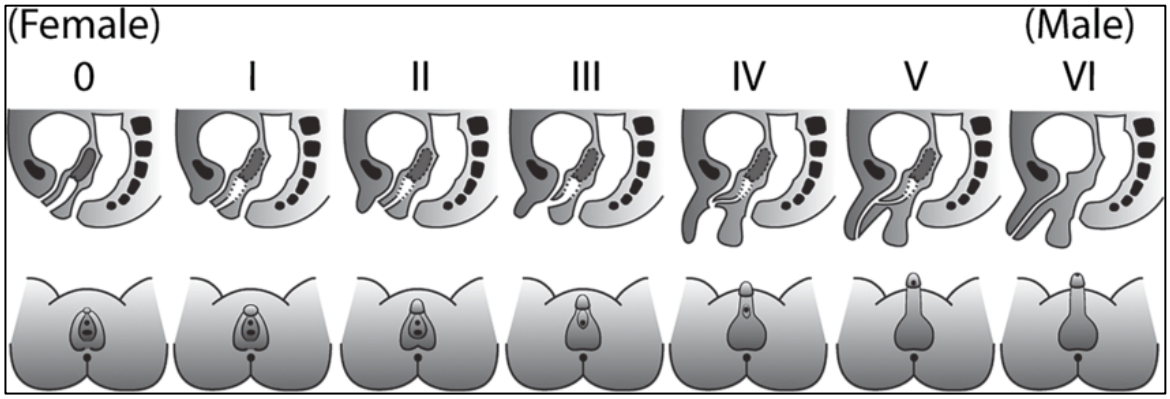

Fig. 5. Grades of Prader scale for XX patients with congenital adrenal hyperplasia [16].

serve sensation and sexual function. Complications of the above-mentioned surgical treatment include vaginal stenosis, which may require additional surgery, typically as a teenager [10], fistulas, urinary incontinence, recurrent urinary tract infection and impaired clitoral sensitivity. In addition, they require lifetime glucocorticoid therapy to minimize the ever-present risk of hyperandrogenemia.

In 46XX CAH with severe virilization and a complete penile urethra, Prader V genitalia (Fig. 5), female sex of rearing is advisable when the diagnosis is made in the neonatal period. A male sex of rearing is reasonable for those whom the diagnosis was made after the neonatal period; with successful reported outcomes [11, 12]. Surgical feminization surgery is challenging due to high insertion of the vagina close to the bladder neck; Robotic and laparoscopic abdominal approaches to the high urogenital sinus have avoided the need for a temporary diverting colostomy and/or the anterior sagittal transrectal approach procedure [13-15]. In those raised as males, they may undergo hypospadias repair in infancy. Treatment with a gonadotropin-releasing hormone (GnRH) analog can be considered before puberty so that a hysterectomy and oophorectomy deferred until later in adolescence when the patient can affirm their gender identity.

In the second case scenario, the patient clinically had a severe form of hypospadias, left impalpable testis and the right testis was palpable in the scrotum. Surgical findings were left sided fallopian tube, atrophic left hemi-uterus and ovary; and the testis was identified in the right-sided inguinal exploration. The ovarian biopsy was consistent with left ovarian tissue and the testicular biopsy was consistent with right testicular tissue. Testosterone and AMH levels were normal. Karyotype showed 46XY chromosome and FISH for SRY probe confirmed Y chromosome in 100% of the analyzed metaphases. The above information most likely fits the condition of ovotesticular DSD (previously known as "true" hermaphroditism). The other differential diagnosis is mixed gonadal dysgenesis (MGD) but it's less likely in this case because Mullerian structures and testicular tissue are usually seen on the same side in MGD; the dysgenetic testicular tissue in MGD secretes inadequate amounts of AMH which may lead to preservation of Mullerian structures on the side of testicular tissue.

Ovotesticular DSD has an incidence of < 10% of all DSD. Out of the total diagnosed ovotesticular disorder cases, 46 XX chromosome is seen in 59.5% of cases and 12.3% have 46 XY chromosome [17]. In this condition, different gene mutation causes both male and female gonadal tissue (testis, ovary, or ovotestis) as well as male and female internal and external structures to coexist. The testes are most often found on the right side. Ovaries are most often found on the left side, and ovotestes -in which both ovarian follicular and testicular tubular tissue are present- can occur on either side. The diagnosis is made based on histology. The phenotype depends on the degree of both testosterone and AMH. Data from small case series suggest that patients reared in either sex can be satisfied with their sex assignment [18, 19] but that gender dysphoria also may occur [20]. Surgery involves the removal of the discordant ovarian tissue if a male sex of rearing is assigned and the removal of testicular tissue if a female sex of rearing is assigned. Gonadectomy may be necessary if ovotestis present to prevent discordant

secondary sex characteristics. The resultant testis cannot produce sperms if it was an XX karyotype due to the lack of y chromosome but the resultant ovotestis may produce oocytes in some cases.

In the third case scenario, the child clinically had a phallic length of 2.2 cm (in a typical XY term infant, penile length is ≥ 2.5 cm [21]), mid penile hypospadias, gonads impalpable bilaterally with underdeveloped scrotum. No Turner's stigmata were visualized. Karyotype was consistent with 46XY chromosome and was positive for SRY gene. Laboratory investigations showed higher than normal levels of basal testosterone and dihydrotestosterone. Cysto-genitoscopy revealed low insertion of the vagina into the urethra. Laparoscopic findings revealed a uterus with a bilateral ovary-looking gonad. No testes were seen. Biopsy from bilateral gonads was sent and histopathology was consistent with testicular tissue from both gonads. Based on the above findings and information, a diagnosis of partial gonadal dysgenesis was made.

XY gonadal dysgenesis is defined by failure of testicular development, resulting in underproduction of testosterone (which is the function of Leydig cell) and underproduction of anti-mullerian hormone (which is the function of Sertoli cell). Anti-mullerian hormone (AMH) causes Mullerian duct regression. Thus, decreased AMH secretion can result in fully or partially developed Mullerian duct structures [22, 23]. Gonadal dysgenesis can be classified as either complete (pure) gonadal dysgenesis (CGD) or partial (mixed) gonadal dysgenesis (PGD) depending on the gonadal morphology [24, 25]. In complete gonadal dysgenesis, there is complete failure of testicular development and thus, patients have a completely female phenotype with typical female external genital appearance due to the lack of any gonadal steroid production with normal Müllerian structures and bilateral streak gonads [26]; If gonadal biopsy is performed, gonadal histology would reveal the presence of bilateral dysgenetic streak gonads. In partial gonadal dysgenesis, there is partial but incomplete gonadal development and can result in a wide range of testicular function and thus, a wide range of phenotypes ranging from isolated infertility, to hypospadias, to an atypical genital appearance, to clitoromegaly. The Mullerian structures may be normal, hypoplastic, or absent.

The most common karyotype seen in PGD is 45X/46XY but 46XY can also be seen. Gonadal histology may reveal either bilateral dysgenetic testes or one streak gonad (usually the left side) and a contralateral dysgenetic or normal-appearing testis (usually the right side). Most individuals with mixed gonadal dysgenesis will have a male gender identity; This is consistent with the moderate level of genital virilization, the presence of a Y chromosome, and the in-utero exposure to higher levels of androgen. The genes commonly involved in XY gonadal dysgenesis include NR5A1 loss-of-function, SRY loss-of-function, WT1 mutation, NR0B1 Duplication and AMH gene mutation.

Regarding the treatment of gonadal dysgenesis, patients with XY CGD are recommended to have bilateral gonadectomy at the time of diagnosis given the high risk of gonadoblastoma reported as 15-35% [27-29]; in patients with XY PGD with nonscrotal gonads that cannot be repositioned surgically into a scrotal position are recommended to have bilateral gonadectomy. Patients with XY PGD with scrotal gonads being reared as males should undergo routine monitoring with self-examination for development of malignancy. In patients with XY PGD who are reared as males with mild under-virilization and gonads that can be repositioned into the scrotum via orchidopexy, one prepubertal gonadal biopsy is recommended at the time orchidopexy is performed and one post-pubertal gonadal biopsy to monitor for malignancy [30]; a normal gonadal biopsy does not completely rule out the presence of a small tumor. Hypospadias surgery is performed at 6 to 15 months of age [31]. Preoperative androgen treatment should be considered if penile size less than average. The Mullerian remnant (hemiuterus) is typically removed at the same time as the streak gonad -if present.

CONCLUSION

Individuals with a congenital discrepancy between the appearance of their external genitalia and gonadal and chromosomal sex are classified as having disorders of sex development (DSD). These disorders are classified into three main categories based on the karyotype; XX DSD (i.e. CAH, ovotesticular DSD), XY DSD (i.e. gonadal dysgenesis, abnormal androgen synthesis or response), and sex chromosome DSD (i.e. mosaicism, chimerism). Laboratory investigations, karyotype, ge-

netic analysis, imaging, diagnostic surgery and histopathology, all aid in diagnosing the specific type of DSD. Decisions about sex of rearing should be based on clinical outcomes for the specific type of DSD, fertility potential and the degree of virilization. The treatment is surgical and/or medical depending on the type of DSD. Several DSD are associated with variable risks for gonadal malignancy and require specific monitoring and management.

Authors' Contribution

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

Ethical approval

The study was approved by The Royal Hospital Ethical Committee, Muscat, Oman.

Informed Consent

Written informed consent was obtained from the families of patients for publication of this case series and any accompanying images or data.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2023;9(6):1529-1532 Case Report

DOI: 10.18621/eurj.1195218 *Thoracic Surgery*

A case developing bilateral bullae due to COVID-19 infection and operated for recurrent pneumothorax

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ABSTRACT

In December 2019, a novel coronavirus (COVID-19) pneumonia emerged in Wuhan, China and has rapidly spread to multiple countries worldwide. Pulmonary parenchymal opacities are often observed during chest radiography. Pneumothorax was reported in 1% of patients in studies. In this paper, we aimed to present a patient with a history of long-term hospitalization in intensive care unit due to COVID-19 pneumonia who was operated for right recurrent pneumothorax secondary to COVID-19 after discharge.

Keywords: COVID-19, pneumothorax, pneumonia

By the end of 2019, a new type of coronavirus (SARSCoV-2) was identified in the city of Wuhan, within the state of Hubei, China and the pandemic caused by this virus affected the entire world. Coronavirus Disease 2019 (COVID-19), is a highly contagious disease with that spreads rapidly [1]. Bilateral ground glass opacities in computed tomography (CT) of thorax is the most frequently seen radiological finding typical for COVID-19. Atypical findings such as bullae and pneumothorax are seen in progression of the disease and it is reported that pneumothorax develops in 1-2% of the patients [2].

In those with COVID-19 infection, pneumothorax was reported in cases without a medical history of underlying lung disease as well. Hence, pneumothorax should be considered in case of sudden development of respiratory distress in patients followed-up for COVID-19 pneumonia [3]. Although the etiopathogenesis of pneumothorax is not fully known in these cases, inflammation with high-level intensity for a certain period of time is thought to cause pneumothorax and/or pneumomediastinum development in addition to severe injury of pulmonary parenchyma [4].

In this paper, we aimed to present a patient with a history of long-term hospitalization in intensive care unit due to COVID-19 pneumonia who was operated for right recurrent pneumothorax secondary to COVID-19 after discharge.

CASE PRESENTATION

Male patient at the age of 42 applied to the hospital with shortness of breath on the 1th of December 2020.

Received: ***November 8, 2022;*** *Accepted:* ***January 19, 2023;*** *Published Online:* ***March 13, 2023***

How to cite this article: *Ersöz Köse E, Boyacıoğlu KA, Alparslan Bekir S, Atinkaya Baytemir C, Yalçınkaya İ. A case developing bilateral bullae due to COVID-19 infection and operated for recurrent pneumothorax. Eur Res J 2023;9(6):1520-1532. DOI: 10.18621/eurj.1195218*

e-ISSN: 2149-3189

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Clinical investigations led to the diagnosis of COVID-19 pneumonia and the patient was internalized to the intensive care unit (ICU) of the hospital due to low oxygen saturation. The patient had a history of chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF) and deep vein thrombosis (DVT). He had been taking inhaler treatment for COPD since 2017. The patient has received treatment in the ICU for about 66 days, 33 of which he spent intubated on a ventilator. The patient was discharged on 12th of February 2021. No pneumothorax was detected in chest x-rays during his ICU stay. The patient applied to the emergency unit with chest pain and shortness of breath on 24th of February 2021 and total pneumothorax on the left lung was detected as a result of clinical investigations. The patient was internalized and left-sided tube thoracostomy was applied. Chest tube was discontinued on the 6th day of follow up and the patient was discharged. He experienced chest pain and shortness of breath once again on 14th of May 2021. Total pneumothorax on the right lung was detected after clinical investigations. He was internalized and right-sided tube thoracostomy was applied. After 12 days of follow up with chest tube, the tube was discontinued and the patient was discharged on 25th of May 2021. The patient applied to our hospital with chest pain on 4th of June 2021 and right pneumothorax was seen on chest x-ray. The patient was internalized to our inpatient clinic and tube thoracostomy was applied for recurrent right pneumothorax (Fig. 1). Computed tomography (CT) of thorax indicated bullae in middle lobe of right lung and lingular segment of left lung (Fig. 2). No bullae was detected in the patient's thoracic CTs prior to COVID-19. The patient was operated on 7th of June 2021. VATS was used to enter the thoracic cavity via right chest tube incision. Bullae on middle lobe was detected upon exploration. An incision of 2 centimeters in length was applied where anterior axillary line meets 3rd intercostal space (ICS). An endostapler was placed under the bullae on middle lobe, but since the stapler would not unlock after cutting, utility incision was switched to mini-thoracotomy in order to prevent parenchymal injury. Linear stapler

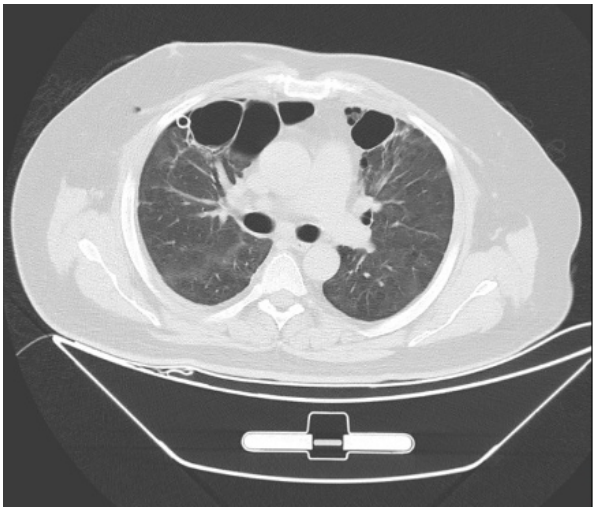

Fig. 2. On thorax CT, bullae are observed in the middle lobe of the right lung and the lingular segment of the left lung.

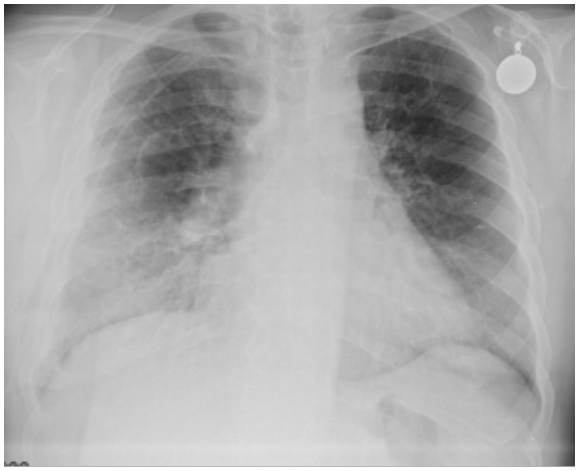

Fig. 1. In the posteroanterior chest radiograph of the patient who underwent tube thoracostomy due to right recurrent pneumothorax, a bullous appearance is observed in the right middle zone.

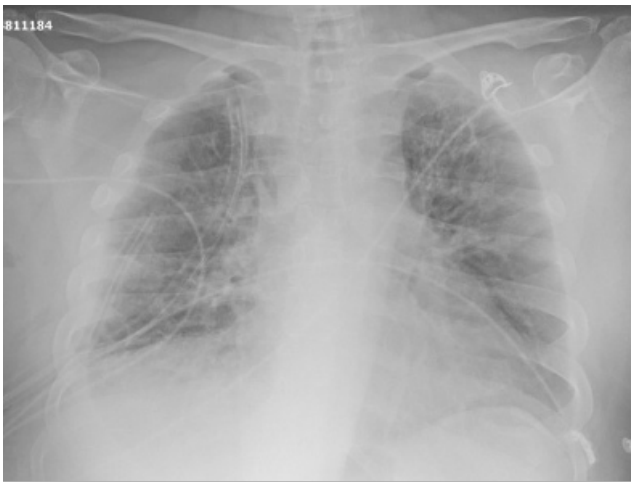

Fig. 3. Chest X-ray of the patient on the first postoperative day.

was placed under the endostapler that was stuck and wedge resection was completed. Partial pleurectomy was applied afterwards. The patient was internalized to intensive care unit for postoperative follow up and no complication such as hematoma or atelectasis was seen on chest X-ray (Fig. 3). The patient was transferred to the inpatient clinic from ICU on postoperative day 1. The basal drain was discontinued on postoperative day 2. Upon observation of no drainage or air leakage, chest tube was discontinued on postoperative day 4 and the patient was discharged. No complication was seen in postoperative follow up of the patient.

DISCUSSION

Pneumothorax is defined as free air inside visseral and parietal pleural linings due to various reasons. It may be spontaneous or secondary to various etiologies such as traumatic, inflammatory, infective, malign, genetic or hormonal causes [5].

Alveolar rupture and diffuse alveolar injury causing air leakage is the predicted pathophysiologic mechanism for pneumothorax development in COVID-19 [6]. Barotrauma due to increase in intrathoracic pressure during intense episodes of cough is also thought to cause pneumothorax development in patients with alveolar structure previously damaged by infection [7]. The development of emphysematous bullae or cavities is thought to probably be caused by endothelial inflammation as a result of pulmonary infarcts [8].

Retrospective studies on COVID-19 patients has shown that pneumothorax may develop in 1% of patients requiring internalization and 2% of patients requiring intensive care [9, 10]. In the large sample study of Martinelli *et al*. [11] development of pneumothorax was reported in 60 patients (0.91%) out of 6574. Bıçak and Salık [12] detected pneumothorax in 11 patients out of 2680 and reported the prevalence for pneumothorax development as 0.41%.

Gonzáles-Pacheco *et al*. [13] have reported development of bilateral pneumothorax 17 days after diagnosis of non-complicated COVID-19 in a previously healthy 45 year-old male. In our patient pneumothorax was detected in left lung 86 days after diagnosis of COVID-19, and it was detected in right lung 135 days later. The patient was operated for recurrent pneumothorax on right lung in addition to bilateral bullae detected on thoracic CT, 169 days after the initial diagnosis of COVID-19.

The existence of bullous lung disease throughout the course of COVID-19 infection is rarely reported. It has been reported that some patients had round cystic changes on thorax CT, which led to the development of bullae after resorption of consolidation. However, bullous lung disease has been described less frequently and only a few cases have been reported in the literature [14].

CONCLUSION

In conclusion, spontaneous pneumothorax is a rare complication of the COVID-19 viral pneumonia. It may appear at any time throughout the course of the disease. In addition, bullous lung disease is scarcely reported. Pneumothorax as a rare but deadly complication of this disease should be considered in the follow up of COVID-19 patients especially in those with sudden respiratory distress. Clinicians should be careful regarding the diagnosis and treatment of this complication.

Authors' Contribution

Study Conception: İY, EEK;CAB; Study Design: EEK, İY; Supervision: EEK, İY, CAB, SAB; Funding: EEK, KAB; Materials: SAB, KAB, İY; Data Collection and/or Processing: İY, KAB; Statistical Analysis and/or Data Interpretation: EEK; Literature Review: EEK; Manuscript Preparation: EEK and Critical Review: CAB, İY, SAB, EEK.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

This paper was presented as a poster at the 11th National Thoracic Surgery Congress, October 24-27, 2021, Antalya, Turkey

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The European Research Journal 2023;9(6):1533-1536 Case Report

DOI: 10.18621/eurj.1165874 *Health Care*

Case report of rare tissue injury and left arm dislocation after incorrect blood pressure measurement during clinical practice

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ABSTRACT

In midwifery education based on theoretical and practical foundations, students encounter midwifery profession education which based on for the first time in the Basic Clinical Skills in Midwifery Course. The aim of the Basic Clinical Skills in Midwifery Course is to help students understand the basic philosophy of midwifery and the role of the midwife in the health system, and to provide the student with knowledge, skills and attitudes about the basic concepts, principles and methods in midwifery care. In the Basic Clinical Skills in Midwifery Course, students are offered learning methods that will enable them to develop behaviors in the cognitive, affective and psychomotor domains. In this context, the course is carried out in skill development laboratories and clinical environments, after the lecturer in the classroom explains the basic concepts, the health system and midwifery, the protection and development of health, the fulfillment of physiological care needs, and the demonstration of the skills related to the psychomotor domain. Clinical practices are an indispensable element of midwifery education. The Midwifery Basic Clinical Skills Course has a very important place in clinical skills training, as students experience the hospital environment for the first time in their education life and experience midwifery practices for the first time on real patients. The aim here is to discuss a case that can be experienced after the correct application of vital sign measurement techniques and erroneous measurement.

Keywords: Blood pressure, clinical practice, erroneous measurement, measurement technique, midwifery student, skills training, vital signs

Vital signs (body temperature, pulse, blood pressure, respiration) reflecting the physiological state of the body provides information about the evaluation of the individual. Accurate evaluation of vital signs and making necessary interventions are essential elements of health care. Blood pressure measurement, which evaluates one of the vital signs, can be an indicator of serious health problems by being affected by many factors. Factors affecting blood pressure are: gender, ethnicity, sympathetic stimulation, daily life, medications, exercise, weight, diet, smoking [1]. For the measurement of blood pressure, firstly, the right tool should be selected for the individual. It can be measured invasively or noninvasively. In non-invasive blood pressure measurement; stethoscope and sphingomanometer are used. In addition to the device se-

Received: ***August 23, 2022;*** *Accepted:* ***September 26, 2022;*** *Published Online:* ***March 9, 2023***

How to cite this article: *Karaman ÖE, Altıparmak YD. Case report of rare tissue injury and left arm dislocation after incorrect blood pressure measurement during clinical practice. Eur Res J 2023;9(6):1533-1536. DOI: 10.18621/eurj.1165874*

e-ISSN: 2149-3189

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lection in measurement, correct region, correct technique and correct interpretation according to the time of hearing the sounds are important. Brachial artery or popliteal artery is used for blood pressure measurement. Usually, if the person does not have a special condition such as mastectomy or loss of arm, measurement is made using the brachial artery [2].

The steps to be considered in blood pressure measurement are briefly as follows:

Exercise, fatigue, eating, smoking, etc. factors should be considered. The measuring arm should be comfortable and clothes should not be tight. During the measurement, information should be given and the person should be asked not to talk, there should be no noise in the environment. The arm should be vertical, level with the apex of the heart, and supported from below with the palm facing up. After feeling the brachial artery, the cuff is wrapped around the arm, 2.5-5 cm above the palpable area and the connecting tube up. It is important that the individual's brachial artery and the artery mark on the cuff should be in line and the cuff should be completely loose [2, 3]. Once the brachial artery has been identified, place the earpiece of the stethoscope over the brachial artery, if the ear receiver. The puvar is taken into the palm of the hand and closed by turning the adjustment knob towards ourselves using the thumb and index finger. While holding the stethoscope over the brachial artery with one hand, with the other hand, inflate the puv regularly and rapidly by 30 mmHg above the individual's previous systolic blood pressure. The hood is turned to the far side and the cuff is emptied slowly, with a 2-3 mmHg second decrease in the air evacuation rate. Too fast or slow emptying of the cuff can cause an error. The first sound heard when the cuff air is emptied is systolic blood pressure, and the closest value to the arrow when the sounds disappear is diastolic blood pressure. After that, the air is completely evacuated [2-4]. If the measurement is to be repeated, it is waited for 30-60 seconds [4]. If a measurement is to be made on the same arm, at least two minutes should be waited between both measurements [5]. Twenty-thirty minutes of the patient after the activity; in the resting state, blood pressure should be measured five minutes later [6, 7]. It is important to measure blood pressure in accordance with the instructions as stated above. Failure to follow these instructions may result in problems. In this article, a case of left arm tissue injury and dislocation after erroneous blood pressure measurement, which is rare during clinical practice, is presented. The aim is to report the case that occurred after blood pressure erroneous measurement during clinical applications and to review the literature.

CASE PRESENTATION

A 21-year-old female patient presented with complaints of pain and numbness in the left arm. In the story of a second-year midwifery student, who was 69

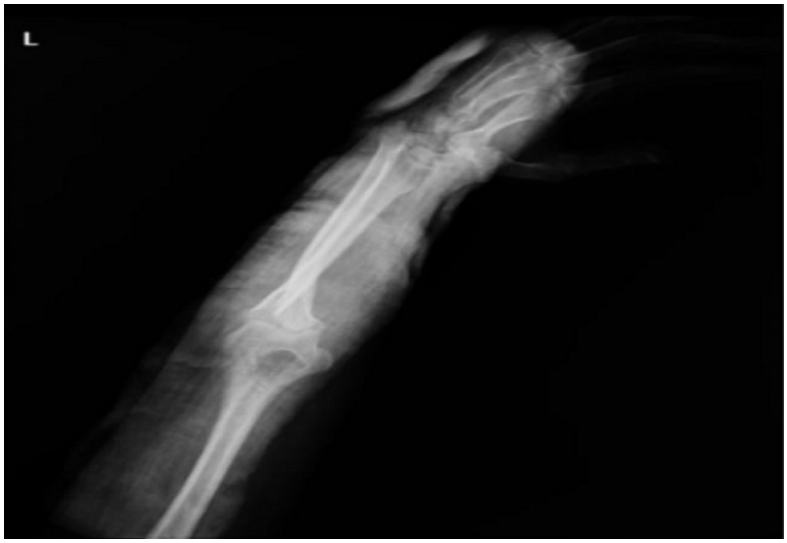

Fig. 1. Left arm CT image.

kg, 1.65 cm tall, and had a body mass index of 25.3 kg/m^2, who did not smoke or use alcohol, it was learned that they had practiced blood pressure measurement with their classmates within the scope of the clinical training program, and that blood pressure was measured on the same arm many times in succession. It could not be learned whether the blood pressure measurement was made at the appropriate pressure and time. As a result of the patient's application to the emergency service; there was no pathology in her physical examination and unidirectional left humerus and bilateral left elbow anteroposterior radiographs. Due to the absence of regression in the patient's complaints and the presence of anterolateral edema, the left arm and elbow were evaluated by computed tomography (CT) imaging by the emergency room doctor. CT reveals pain and soft tissue trauma in the left elbow. The left arm was fixed with a long arm splint and rest for ten days was recommended. Due to the absence of regression in his complaints during the ten day rest period, the patient was admitted to the orthopedics and traumatology outpatient clinic, and left arm dislocation was suspected, and the diagnosis was confirmed by one-sided left arm radiography (Fig. 1). With the appropriate maneuver, the humeral joint space was placed and the necessary interventions performed on the patient. He was discharged after some physical therapy exercises were recommended.

DISCUSSION

Factors such as the fact that patients have different characteristics due to their illness, the severity of their general condition, and that some patients do not volunteer to take part in the learning experience of the students lead to the fact that not all students have equal learning opportunities in the clinical practice environment and have difficulties in the clinical practice process. In addition, the fear of making mistakes in students caused by practicing on real individuals causes students to experience anxiety, and anxiety can prevent students from reflecting their knowledge and skills to real patient care [8-10]. Ensuring that students perform their practices by paying attention to the values and rights of patients during clinical practices is extremely important in terms of ensuring patient safety. For these reasons, the use of skill laboratories is becoming increasingly important in the development of students' clinical skills. It is aimed to better prepare students for clinical practice by observing and practicing clinical skills in skill laboratories, by using traditional tools such as models and teaching methods such as demonstration and role-playing [11-13]. Due to the fact that the practice courses given in the clinical skills laboratory during the pandemic period cannot be conducted face to face, it is seen that the students' practice skills are lacking and their sense of self-confidence cannot be created. For this reason, it was observed that the students made mistakes in the vital sign measurements they made on themselves. In this context, it is seen how important the clinical application laboratory is in the clinical application period. In our case, left arm joint dislocation developed after an incorrect measurement. Elbow joint dislocation is the second most common dislocation of the large joint after the shoulder joint. Posterolateral dislocation often occurs with overloading and forcing the forearm to right or left rotation with excessive pressure. Dislocations are often not accompanied by fractures and are referred to as simple dislocations. Widespread pain, edema and limitation of movement are the main findings in the patient. Diagnosis can be made by radiography of the elbow, CT can be used for further examination. In simple dislocations, intervention with closed reduction followed by two weeks of stability is usually sufficient. There is a need for surgical treatment in complex, recurrent or unreplaceable dislocations that rarely develop [14]. No study similar to our case was found in the literature. Therefore, a comparison could not be made regarding the case.

CONCLUSION

Human life is very important and in this case, the student was damaged during peer education due to the wrong measurement technique. This mistake may also occur in the first clinical practice when confronted with the patient. As a result of the wrong follow-up of vital signs, which is of great importance during patient follow-up, it is revealed that the patient's treatment will be planned incorrectly, as well as the result of harming the patient due to incorrect application. In this context, laboratory practice of basic clinical skills given before the first clinical experience during mid-

wifery education is of great importance. There is a lack of literature evidence due to the fact that this situation is very rare in clinical skills education of blood pressure measurement.

Authors' Contribution

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

Informed Consent

Written informed consent was obtained from the patient for publication of this case and any accompanying images or data.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgments

The authors express their thanks to all the mothers who participated in the study and shared their special moments.

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The European Research Journal 2023;9(6):1537-1540 Case Report

DOI: 10.18621/eurj.1207253 *Chest Diseases*

Cystic fibrosis diagnosed in a nineteen-year-old case

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ABSTRACT

Cystic fibrosis is the most common autosomal recessive hereditary disease in white populations. It is characterized by the formation of abnormal secretions in the exocrine glands located in the sweat and salivary glands, tracheobronchial tree, large intestine, and pancreas. The severity of the clinic depends on the type of "cystic fibrosis transmembrane regulatory protein" gene mutation. Although most cases are diagnosed in infancy or childhood, some patients are also diagnosed during adolescence and adulthood. We report a case a 19-year-old patient who was followed up with a diagnosis of asthma and bronchiectasis since childhood and diagnosed with cystic fibrosis.

Keywords: Adolescence, bronchiectasis, cystic fibrosis

Cystic Fibrosis (CF) is the most common autosomal recessive (OR) inherited disease in the Caucasian race and its frequency is 1 in 2000 to 3000 live births. The main disorder is the formation of abnormal secretions in exocrine glands in sweat and salivary glands, tracheobronchial tree, large intestine, and pancreas [1].

The severity of CF clinic depends on the type of CFTR (Cystic fibrosis transmembrane regulatory protein) gene mutation. Symptoms and signs include recurrent pulmonary infections, pancreatic insufficiency, and high sweat chloride levels [1]. Since the CFTR protein can function, albeit partially, in some patients with CF, their clinics are mild and show atypical symptoms. While most of the cases are diagnosed in infancy or childhood, a few of them are diagnosed in adulthood.

The sweat test is the gold standard method in diagnosis. When the sweat test is found to be normal or borderline in some atypical CF patients, it is recommended to search for genetic mutations [2].

In this case report, a case who was followed up and treated with the diagnosis of recurrent pneumonia and asthma since childhood and diagnosed with CF in adolescence is presented.

CASE PRESENTATION

A 19-year-old female patient was admitted to our outpatient clinic with complaints of cough, sputum, and wheezing that had been going on for about 12 years. She had a history of asthma and chronic sinusitis for seven years. She was still using a combination of inhaled steroids and long-acting beta 2 agonists. She had never smoked and had no history of pet feeding. She wasn't working any job. While breathing room air, oxygen saturation of 98%, pulse rate of 86/min, respiratory rate of 17/min, and body mass index of 22.3 kg/m² were measured by pulse oximetry. In the respiratory examination, inspiratory crackles and squawks were heard in the bilateral upper areas.

Received: **November 19, 2022;** *Accepted:* **April 8, 2023;** *Published Online:* **April 9, 2023**

How to cite this article: *Yılmaz M, Mutlu LC. Cystic fibrosis diagnosed in a nineteen-year-old case. Eur Res J 2023;9(6):1537-1540. DOI: 10.18621/eurj.1207253*

e-ISSN: 2149-3189

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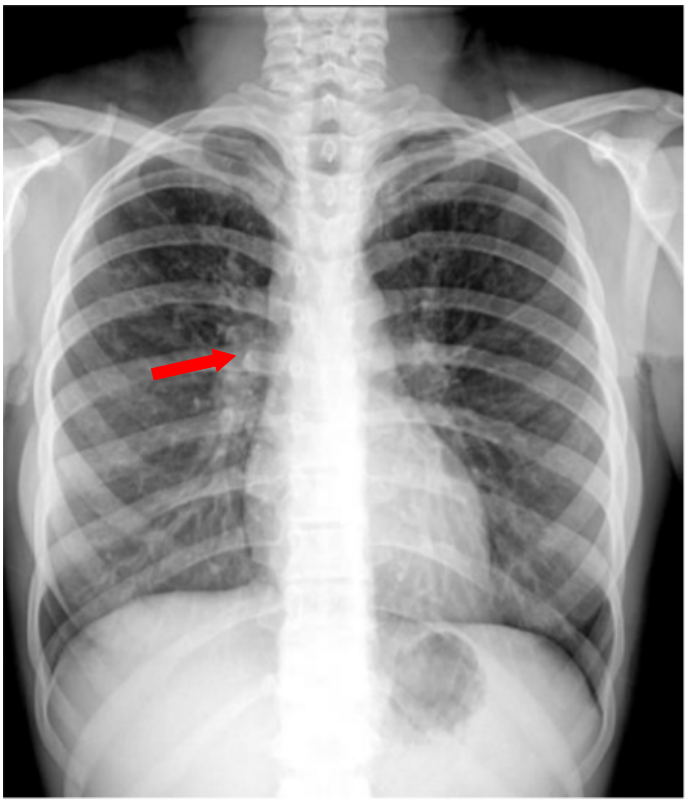

Fig. 1. Enlargement of bilateral upper-middle zones and train rail appearance on PA chest X-ray (red arrow).

Chest X-ray showed increased streaking and train rail appearance in bilateral upper-middle zones (Fig. 1). In the thorax computed tomography taken in an external center six months ago, there was centrally located bronchiectasis in the upper lobes of both lungs, accompanying peribronchial thickening and reticulonodular infiltrates (Fig. 2).

Complete blood count and biochemical parameters were normal, total IgE was 830 IU/mL. There was no growth in nonspecific sputum culture. ARB staining was negative and mycobacterial culture was negative. In the pulmonary function test, FEV1/FVC was 71.75%, FVC: 2.70 L (74.2%), FEV1: 1.94 L (61%), and DLCO 72.6%. Skin prick test was positive for Aspergillus.

Since childhood, the patient has regularly received many inhaler steroid and bronchodilator combinations in various device forms, oral antihistamine and leukotriene receptor antagonists, antitussive drugs, empirical antibiotic treatments, intranasal steroids, oral steroid treatments, but never completely regressed in his complaints. The patient with radiologically upper lobe dominant central bronchiectasis also had a history of not gaining weight and frequent diarrhea when infancy was questioned. In line with this information, genetic testing was requested from the patient with the suspicion of cystic fibrosis. As a result of the genetic test, heterozygous mutations were detected in two genes (p.Phe508del and p.Met1354Lys) for CF. ABPA was not considered in the patient who did not meet the necessary conditions according to ISHAM criteria for allergic bronchopulmonary aspergillus (ABPA). The patient was referred with the diagnosis of cystic fibrosis to be followed up and consulted in an experienced center.

DISCUSSION

Cystic fibrosis is an autosomal recessive inherited disease caused by pathological variants in the CFTR gene located on the long arm of chromosome 7. It is common in the white race. Its frequency is one in 2000 to 3000 live births [3]. Although its frequency is not known clearly in our country, it is considered a rare disease. However, considering consanguineous marriages in our country increase the incidence of this disease with OR transition [4].

The sweat test is the gold standard method in diagnosis. A sweat chloride concentration of > 60 mmol/L is diagnostic for CF. The sweat test may be normal or borderline in some atypical CF patients. It

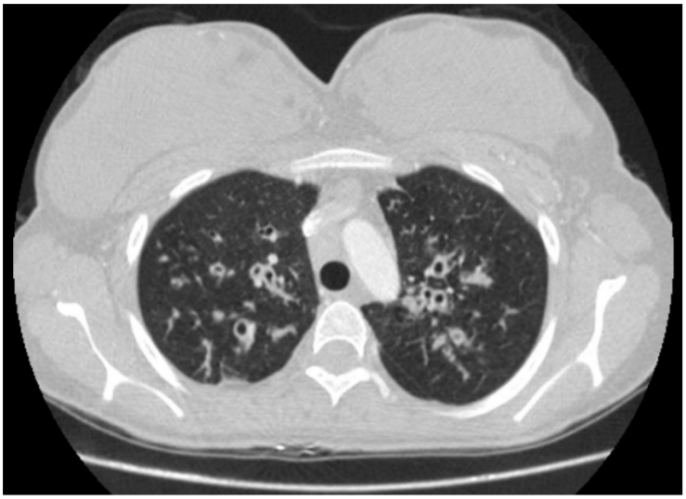

Fig. 2. Centrally located bronchiectasis in the upper lobes of both lungs; accompanying peribronchial thickening and reticulonodular infiltrates on thorax computed tomography.

is recommended to investigate genetic mutations in these patients [2]. The most common type of mutation in the CFTR gene is the deltaF508 mutation, which is 66% common worldwide. However, it has been determined that there are about 2000 mutations of the CFTR gene apart from deltaF508. Different mutations in the CFTR gene cause the disease to occur with different pictures [4].

The early diagnosis affects the life expectancy and quality of patients. Newborn screening was included in the screening program in our country on January 1, 2015. The measurement of immunoreactive trypsinogen (IRT) in a postpartum heel blood sample is used for screening purposes. In case of high values, sweat tests and gene mutation analysis are performed in the follow-up [5].

The disease mainly affects the upper and lower respiratory tract, pancreas, gastrointestinal tract, reproductive organs, and exocrine sweat glands. Chlorine and bicarbonate transport in the epithelial tissue is disrupted, leading to a decrease in moisture in lumen secretions and intensification of secretions [3].

Meconium ileus, prolonged neonatal jaundice, growth retardation, chronic cough, recurrent pneumonia, and recurrent bronchiolitis are seen in infancy. While most of the cases are diagnosed in infancy, patients with the atypical clinical course can be diagnosed in adolescence or adulthood [2]. Our patient was a 19-year-old patient who had asthma, bronchiectasis, and chronic sinusitis since childhood. According to her sister's description, the inability to gain weight in infancy and retardation in development were stated, but this story could not be fully confirmed since the patient was not raised by her parents.

Chronic endobronchial infection and progressive airway obstruction in the early respiratory system lead to impaired airway function. Late complications result in bronchiectasis, respiratory failure, and premature death. Adolescents and adults present with chronic cough and sputum, idiopathic bronchiectasis, chronic sinusitis, nasal polyposis, exercise intolerance, dyspnea, and recurrent pneumonia. Lung complications are the main cause of morbidity and mortality [6]. Our patient had a history of antibiotic use due to recurrent pulmonary infections in the early period, treatment with recurrent wheezing and asthma, and bronchiectasis detected within the last year.

The main goals of treatment are to protect respiratory functions and quality of life and to increase life expectancy. Because lung disease is the leading cause of morbidity and mortality, maintaining respiratory health is the main focus of cystic fibrosis treatment. Airway clearance techniques, mucolytics, treatment of infections with appropriate antibiotics, treatment of the host's inflammatory response with anti-inflammatory drugs to delay airway obstruction, bronchodilator and inhaler steroids in patients with asthma, and steroid therapy in patients with ABPA are the main targets. In addition, caloric support and pancreatic enzyme replacement are required in patients with inadequate exocrine pancreatic functions [6].

In recent years, modulator therapies, which are a new treatment method, aim to improve the production and functions of the CFTR protein. To date, four modulator therapies (ivacaftor, lumacaftor/ivacaftor, tezacaftor/ivacaftor, and tezacaftor/ivacaftor/elexacaftor) have been approved for use in the treatment of patients with specific CFTR mutations [7].

CONCLUSION

Although most of the patients are diagnosed in infancy or childhood, it may have a clinical atypical course in cases where the CFTR protein partially functions. Lung findings may occur at a later stage or CF is not considered due to this atypical course. Our patient was also diagnosed with CF at an adolescent age. CF should be considered in the etiology of all adolescents and adults with bronchiectasis and unexplained signs of chronic lung disease.

Authors' Contribution

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

Informed Consent

Written informed consent was obtained from the patient for publication of this case and any accompanying images or data.

Conflict of interest

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

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The European Research Journal 2023;9(6):1541-1544 Case Report

DOI: 10.18621/eurj.1224955 *Thoracic Surgery*

Tracheobronchopathia osteochondroplastica limited to the trachea: a case report and rewiew of literature

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ABSTRACT

Tracheobronchopathia Osteochondroplastica (TO) is a rare and benign disease with a progressive course. Its clinical manifestation is observed on computed tomography by the presence of diffuse osteocartilage submucosal nodules invading the lumen of the trachea and usually the main bronchi. Bronchoscopic evaluation is necessary for differential diagnosis. Standard treatment has not been determined as few patients have been reported in the literature. A 58-year-old male patient was admitted to our hospital with complaints of cough and dyspnea increasing with exertion. A fiberoptic bronchoscopy demonstrated many protruding lesions along the trachea with no involvement of the bronchial system. The patient was stable at a two-year follow-up.

Keywords: Tracheobronchopathia osteochondroplastica, tracheal stenosis, benign tracheal lesions, bronchoscopy, trachea

Tracheobronchopathia Osteochondroplastica (TO) is an uncommon, progressive, and benign disease of undetected etiology. TO can be distinguished by diffuse osteocartilage submucosal nodules that invade through the lumens of both the trachea and usually the main bronchi [1, 2]. The natural course of the disease is progressive. TO can be distinguished by the presence of diffuse osteocartilage submucosal nodules which can invade both the trachea and main bronchial lumen; occasionally nodules are present only in the lumen of the trachea. In practice, a careful radiological examination is essential for diagnostic purposes, and bronchoscopy helps to eliminate differential diagnoses. There is no consensus on standard treatment as a consequence of few patients being reported in the literature [1-7]. Here, we present a case of TO who was involved with submucosal nodules only in the tracheal lumen and no more progress in the two-year follow-up.

CASE PRESENTATION

A 58-year-old male patient was admitted to our hospital with complaints of cough and exertional dyspnea that had been persisting for the last two years. TO was diagnosed one year ago by tomography, and a follow-up without treatment was recommended. He had no other underlying diseases or history of smoking. Symptoms were stable at the final presentation, and a thorax CT showed no difference from the previous series (Fig. 1A and 1B).

Received: ***December 27, 2022;*** *Accepted:* ***April 6, 2023;*** *Published Online:* ***April 10, 2023***

How to cite this article: *Kargı AB, Atlı E, Kılıç B, Bilgin P. Tracheobronchopathia osteochondroplastica limited to the trachea: a case report and rewiew of literature. Eur Res J 2023;9(6):1541-1544. DOI: 10.18621/eurj.1224955*

e-ISSN: 2149-3189

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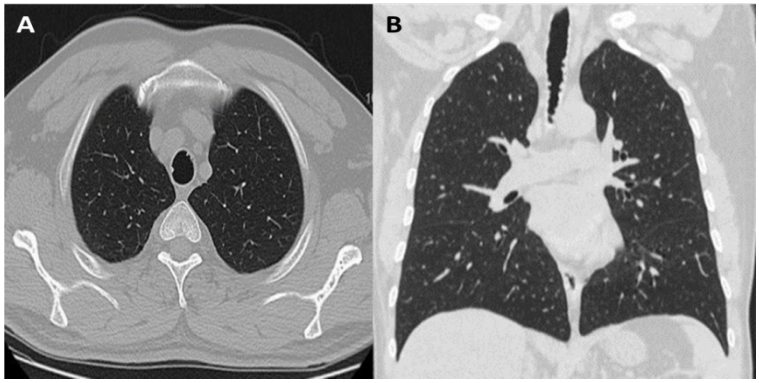

Fig. 1. (A) Axial and (B) coronal CT images showed irregular thickening and nodularity of tracheal cartilage.

A fiberoptic bronchoscopy was performed to evaluate the latest situation under general anesthesia. Several protrusions of lesions were observed throughout the trachea, narrowing down the lumen. The bronchial system was not involved (Fig. 2). Random biopsies were taken from plaques. The patient was discharged on the same day after the procedure, without medication, to come for an annual follow-up. The histopathologic consideration of the biopsies demonstrated osteocartilaginous structures (Fig. 3). There was no evidence of malignancy.

DISCUSSION

TO is an uncommon benign disease that is distinguished by submucosal nodules in the osteocartilaginous structure protruding towards the lumen in the trachea and usually in the main bronchi. The disease was described by Rokintansk in 1855 macroscopically. Wilks investigated microscopically by post-mortem a 38-year-old male who died of pulmonary tuberculosis in 1857 [1]. Von Schroetter proved the diagnosis by using a laryngeal mirror in vivo for the first time in an alive patient in 1896 [1, 2].

Although TO mostly has unknown etiology, some etiopathogenic causes have been identified. However, no genetic transmission has been demonstrated yet. Infections, metabolic diseases, inflammatory disorders, chronic irritation of chemicals, and smoking could be playing a role [2,3,4,7]. Some studies have reported that it may coexist with some malignant diseases [6]. It is more frequently seen in men over 50 years old [1, 2, 7, 8]. Our patient is a 58-year-old male. There were no underlying diseases or any other factors that could alter the etiology.

TO mostly remains asymptomatic [1-3]. Common symptoms are chronic cough, expectoration, dyspnea, recurrent respiratory infection, and wheezing; stridor may be added as the stenosis progresses [1, 2, 6]. He-

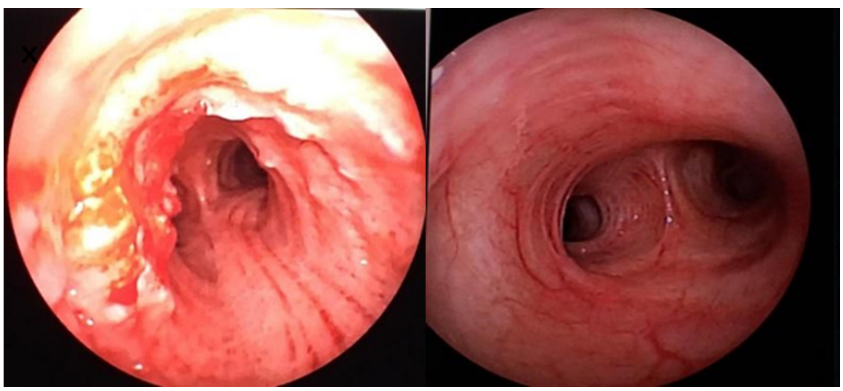

Fig. 2. Bronchoscopic view of the narrowed trachea with protruding lesions on three sides. The bronchial system is not involved by the lesions.

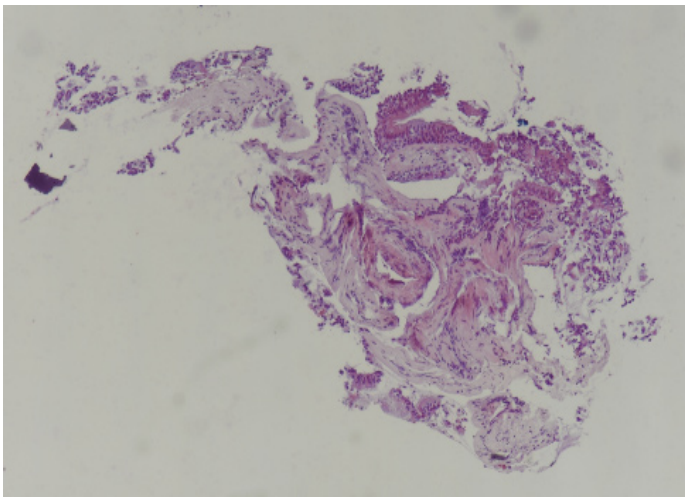

Fig. 3. View by Hematoxylin-eosin staining at 40x magnification. Among the ciliary pseudostratified columnar epithelium of the trachea, osteocartilaginous structures are distinguished.

moptysis is seen in 60% of cases with nodule ulceration or acute infections [9]. Physical examinations of the cases are generally normal [2, 7]. Only a small part of the patients had ventilation anomalies, such as bronchial obstruction findings. But most of the pulmonary function tests are within normal limits [1]. In our patient, there are no specific symptoms and no physical examination findings.

Abnormality and constricting of affected parts of the bronchi and trachea may be observed in a plain chest X-Ray. Calcium deposits can make trachea and bronchial borders as remarkable as lines. CT and bronchoscopy are both considered fundamental in the diagnosis of TO [3, 5, 8]. Radiological findings are fluctuating; however, they are usually overlooked unless observed by an attentive eye. Radiological findings are variable but generally missed unless a focused investigation. Even though CT images are not always diagnostic, CT can be used to detect nodules with irregular calcifications on the lateral and anterior walls of the airway "beaded appearance". Involvement of the posterior wall is not observed [2, 8].

Virtual bronchoscopy, which was started to be used with software added to the CT, provides the opportunity to evaluate the tracheobronchial lumen structures in cases where we cannot image due to stenosis and obstruction with fiberoptic bronchoscopies. Virtual bronchoscopy can also be used to detect possible extensions of pathology outside the lumen. Although tomographic images are sufficient for the diagnosis of TO, bronchoscopy is required. The bronchoscopic appearance is quite distinctive; the 'string of beads' appearance is caused by the submucosal cartilage surface containing abnormal mineralization deposition [2, 3]. In the differential diagnosis, amyloidosis, endobronchial sarcoidosis, calcified tuberculosis lesions, papillomatosis, tracheobronchial calcinosis, and primary or secondary malignant tumors of the trachea should be considered [2, 3]. For this reason, a bronchoscopic biopsy is recommended.

Histopathologically, a calcified protein matrix devoid of cells and submucosal nodules containing cartilage, bone, and blood elements, extending into the bronchial lumen is pathognomonic [1]. The nodules are often followed by squamous metaplasia, which may occur in the mucous columnar epithelium. Microscopic echondrosis may also be observed [1].

TO is a benign condition, so it can be followed without intervention in asymptomatic cases. There is no consensus on treatment because few patients have been reported in the literature. If the lumen narrows over time, interventions such as bougie or stent may be required. Conservative treatments should be applied for the patient's complaints. It has been reported that inhaled corticosteroids can reverse progression in some patients [8]. Reports of successful recovery with dapsone and NSAIDs have been confirmed in some symptomatic cases. The overall prognosis is generally good and the number of patients undergoing further intervention (such as a tracheostomy) is rare. Recommended treatments for severe airway obstruction or recurrent obstructive infection include laser ablation, cryotherapy, surgical resection, stent, and radiotherapy. Neodymium-doped yttrium aluminum garnet (Nd-YAG) laser treatment is considered for patients who develop tracheal stenosis. In case of respiratory tract infection, antibiotics can be added to the treatment [2, 7, 8]. Since our patient has not had any complaints yet, we did not recommend any medication.

CONCLUSION

TO is a rare benign but progressive disease. Although the underlying etiological causes cannot be determined exactly, some factors are thought to play a role. Bron-

choscopy is necessary for a definitive diagnosis. While the trachea and main bronchi are involved in most cases, it can sometimes be seen as isolated tracheal involvement, as in the case we presented. Although definitive treatment has not yet been defined, the approach is generally conservative; with close follow-up and relieving symptoms.

Authors' Contribution

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

Informed Consent

Written informed consent was obtained from the patient for publication of this case and any accompanying pictures or data.

Conflict of interest

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

Financing

The authors disclosed that they did not receive any grant during the conduction or writing of this study.

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