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The role of mid-trimester ultrasound scan: scope and limitations

Christiane Simioni[1], Edward Araujo Júnior[1,2*]

The mid-trimester ultrasound scan is performed mainly for anatomical evaluation of the fetus. In expert hands, most clinically important structural anomalies can be detected. A routine mid-trimester fetal ultrasound examination includes an evaluation of the following: cardiac activity, fetal number (chorionicity and amnionicity in cases of multiple pregnancies), basic fetal anatomy, placental appearance and location, amniotic fluid volume, and gestational age/fetal size[1].

In the second trimester scan, fetal biometrics above the 90th percentile increase the risk of gestational diabetes mellitus (GDM), suggesting that the fetus is affected by abnormal maternal glucose metabolism prior to the diagnosis of GDM[2]. Amniotic fluid index may be preferred in the assessment of polyhydramnios, while the deepest vertical pocket may be preferred in the assessment of oligohydramnios[1].

The use of prenatal ultrasound has been shown to be effective in the prenatal diagnosis of chromosomal abnormalities. The genetic sonogram, which includes a detailed search for sonographic signs of aneuploidy, can be used both to identify fetuses at high risk for aneuploidy and, if normal, to reduce the risk of aneuploidy for a pregnancy in which no sonographic markers are identified[2].

Since the clinical implementation of noninvasive prenatal testing (NIPT) in 2012, there has been a paradigm shift in prenatal screening. Although different approaches have been used to implement NIPT, there is consensus that NIPT should always be offered in combination with a qualified ultrasound scan[3].

A routine mid-trimester ultrasound scan can be performed between approximately 18 and 24 weeks gestation, depending on technical considerations and local legislation. In countries where pregnancy termination is restricted by gestational age, detection rates should be balanced against time[1]. Although many fetal malformations and anomalies can be detected at this mid-trimester scan, some may be missed or become apparent later in pregnancy, even with the best sonographic equipment in the best hands. If the examination cannot be performed completely according to the adopted guidelines, the scan should be repeated to ensure a complete examination, or the patient should be referred to another examiner[1]. Maternal obesity, a growing problem worldwide, has been shown to decrease the accuracy of ultrasound in high-risk pregnancies.

Marginal cord insertion (within 2 cm of the placental margin) occurs in 5–8% of cases, and velamentous insertion (insertion of the umbilical vessels into the amniotic membranes instead of the placenta) occurs in approximately 1% of cases[1]. Although formal assessment of umbilical cord insertion is not part of the routine mid-trimester scan, in our opinion it is recommended to describe it at the earliest opportunity as we may need this information later in the pregnancy. Velamentous cord insertion may be associated with vasa previa and fetal growth restriction (FGR).

A single umbilical artery (SUA) is associated with congenital anomalies and FGR, although it is not an anomaly per se. Therefore, care should be taken not to cause anxiety for the parents if no major anomaly is found at the mid-trimester scan. There is no consensus on the potential impact of SUA on pregnancy outcome.

We also recommend that the cervical length (CL) measurement by transvaginal route be offered to all pregnant women at the time of the mid-trimester scan for screening of preterm birth because of the high association between a short cervix (CL<25 mm) and subsequent preterm birth[4].

There is currently insufficient evidence to support the universal use of uterine or umbilical artery pulsed Doppler evaluation for the screening of low-risk pregnant women. Color Doppler is encouraged and can assist in the examination of the fetal heart and the cord vessels and in the determination of the amount of amniotic fluid[1].

In summary, we support the idea that the mid-trimester scan should be offered to all pregnant women as part of routine antenatal care.

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AUTHORS' CONTRIBUTIONS

EAJ: Conceptualization Methodology, Project administration, Supervision, Validation, Visualization, Writing –review & editing.

CS: Data curation, Investigation, Validation, Visualization, Writing –original draft.

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Use of intra-articular hyaluronic acid in knee osteoarthritis or osteoarthritis

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct research and to assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical condition of each patient.
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Societies: Brazilian Medical Association.

INTRODUCTION

With an estimated worldwide prevalence of 3%, osteoarthritis (OA) is among the most frequent problems in elderly clinical practice. For a long time, it was considered a disease that only involved wear and tear of the articular cartilage, but today, with the advances in the understanding of the disease, the understanding is that the pathophysiological changes involve the joints as a whole (cartilage, bone, synovial membrane, ligaments, adipose tissue, and meniscus), as well as pain processing nerve pathways. Changes may arise due to internal (obesity) and external mechanical loads, joint misalignment (genu varus and genu valgus), metabolic, and genetic factors. Excessive load on the bone can result in spinal cord injuries with microfractures, necrosis, fibrosis, and adipocytes, all suggestive of damage and remodeling in the injured area. Synovitis is commonly observed, and it plays an important role in joint destruction. Factors with pro-inflammatory cytokines (interleukin-6 [IL6]), monocyte chemoattractant protein, vascular endothelial growth factor, protein, and monokine induced by interferon γ are responsible for the progressive destruction due to the stimulation of degradation enzymes, and the growth factors stimulate the production of matrix for remodeling but end up promoting the formation of osteophytosis, thus contributing to subchondral sclerosis. Cytokines are not only the drivers of joint destruction but also potential targets for intervention to modify disease progression. Cartilage, as the only tissue without vascular, nervous, or lymphatic supply, has properties that condition its low intrinsic repair capacity, making repair difficult[1].

The treatment of knee OA begins with clear and consistent information about the history of the disease to patients, clarifying the benefits of exercise, weight loss, and physiotherapy, which are behaviors that have well-established benefits to reduce pain, in addition to anti-inflammatory drugs, administered topically or orally, which are the backbone of pharmacological treatment. Intra-articular (IA) corticosteroid injections provide temporary relief. Hyaluronic acid (HA) injection is also frequently offered, although evidence of its benefit remains controversial[1].

With the discovery of HA in bovine vitreous humor in 1934, it began to play an important role in the repair of wounds and skin damage. Thus, the use of HA in the form of IA injections in patients with OA of the knee, called viscosupplementation, was the first indication for clinical use in orthopedics and traumatology, with the aim of treating joint cartilage injuries by having a lubricating effect, mechanical and biochemical, with the expected result of partial relief of painful symptoms and improvement in function. The effect is not immediate but long-term. Currently, the use of HA is widespread and frequent, but without clear evidence of benefit and with the risk of potential harm[1].

The objective of this study was to evaluate the clinical efficacy and adverse effects of treatment with HA for anterior knee pain caused by grade II and III OA, as it causes discomfort and an inability to perform daily activities. Assessments will be short- and medium-term, measuring different scores.

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METHODOLOGY

In the methodology, we will express the clinical question, the structured question (PICO), eligibility criteria of the studies, consulted information sources, search strategies used, critical evaluation method (risk of bias), quality of evidence, data to be extracted, measures to be used to express results, and the method of analysis.

Clinical question

Is the use of HA in IA application for the treatment of knee OA efficacy and safe?

Structured question

- P (population): Patients with osteoarthritis or osteoarthrosis of the knee
- I (intervention): High or low molecular weight hyaluronic acid
- C (comparison): Placebo or sham or steroid or usual care
- O (outcome): Clinical improvement (overall – pain – stiffness – gait)

Sources of information consulted and search strategies

The searches they were performed in the Medline database (PubMed), with the next terms: (Osteoarthritis OR Osteoarthritides OR Osteoarthrosis OR Osteoarthroses) AND Knee AND (Viscosupplements OR Viscosupplement OR Visco Supplements OR Viscosupplementation OR Viscosupplementations OR Hyaluronic Acid OR Hyaluronate Sodium) AND Random*.

Eligibility criteria

PICO components; randomized clinical trials (RCTs); no period restriction; languages English, Spanish, and Portuguese; full text or abstract with the necessary data; outcomes expressed in absolute number of events or mean/median with variation.

Exclusion criteria

Observational and noncomparative studies, in vitro and/or animal studies, case series or case reports, narrative or systematic reviews, and guidelines.

Risk of bias and quality of evidence

For RCTs, the following risks of bias will be evaluated: focal question, randomization, blinded allocation, double blinding, losses, analysis by intention to treat (ITT), definition of outcomes, sample calculation, early interruption, and prognostic characteristics.

Extracted data

Author, year of publication, study design, characteristics and number of patients, intervention, comparison, and outcomes (clinical improvement and adverse effects). Each study was described individually in a qualitative analysis of the evidence. Evaluation of seven outcomes (adverse and clinical events) with priority for categorical outcomes and/or averages (SD). Subgroup analysis: HA versus CORTICOID and HA versus SALINE SOLUTION (SS). Outcomes – overall WOMAC – pain WOMAC – functional WOMAC – overall KSS – overall VAS. Measured with continuous variables (final mean or mean difference with standard deviation) and dichotomous variables.

Outcome measures

For categorical variables, we will use absolute numbers, percentages, absolute risk, reduction or increase in risk, number needed to treat or number of harm (NNH), and 95% confidence interval (95%CI). For continuous variables, we will use means or the difference of means with a standard deviation.

Expression of results

If it is possible to aggregate the results of one or more included studies regarding one or more common outcomes, a meta-analysis will be performed [RevMan 5.4 software (Cochrane)].

Evidence quality analysis

Comparisons were demonstrated in the risk difference and 95%CI. The inconsistency of effects across interventions was assessed using I^2. The random effects model was used if $I^2>50\%$ and the fixed effects model if $I^2\leq50\%$. To access possible publication biases, Egger's test (funnel plot) was analyzed for asymmetry. The certainty of the evidence was assessed using the GRADEpro guideline development tool and rated as high, moderate, low, or very low.

RESULTS

The results presented will be: study recovery and selection diagram (Figure 1), study characteristics (Tables 1A, B), risk of bias (Tables 2A, B), results (Tables 3A, B), analysis by outcomes (Figures 2–12), quality of evidence (Tables 4 and 5), and synthesis of evidence.

A total of 680 studies were retrieved, of which, meeting the eligibility criteria, 27 studies were selected[2-28], of which 17 were comparisons against saline solution (Table 1A)[2-18] and 10 comparisons against steroids (Table 1B)[19-28]. The main reasons for exclusion were orphan studies and outcomes, technical comparisons, and lack of comparisons.

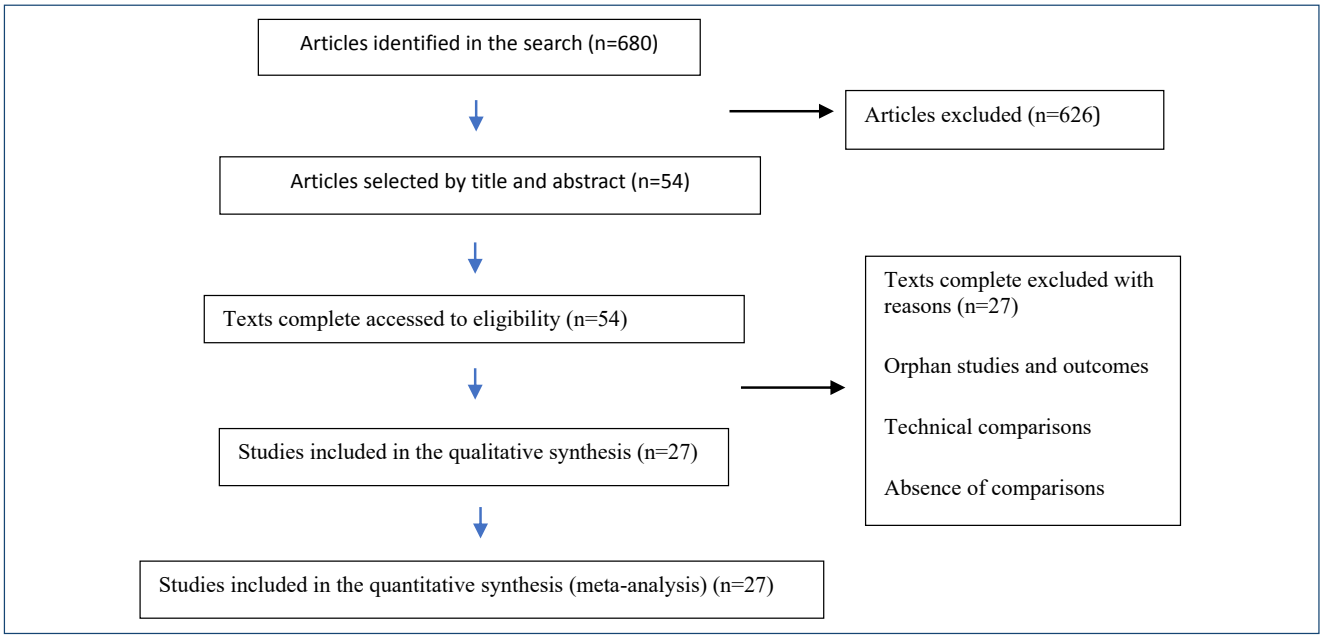

Figure 1. Flowchart of selected works.

Table 1A. Description of studies comparing hyaluronic acid with saline solution (n=17).

Author/year	Patients number		Outcomes measured - Instrument		Adverse effects reported	Molecular weight	Injection number	Follow-up weeks
	Hyaluronic Acid	Saline Solution	Pain	Function				
Altman RD 2004	173	174	WOMAC	WOMAC	Yes	High	1	24
Altman RD 2009	293	295	WOMAC	WOMAC	Yes	High	3	26
Arden N 2013	108	110	WOMAC	WOMAC	Yes	Intermediate	1	6
Baltzer AWA 2008	135	107	WOMAC	VAS	Yes	High	3	26
Brandt KD 2001	114	112	WOMAC		Yes	Intermediate	3	16
Chevalier X 2010	124	129	WOMAC	WOMAC	Yes	High	1	26
Day R 2004	116	124	WOMAC		Yes	High	5	18
Dougados M 1993	55	55	VAS	Lequesne index	Yes	High	4	52
Hangody L 2018	150	69	WOMAC	WOMAC	Yes	Intermediate	1	2
Henderson EB 1994	45	46	VAS	VAS	Yes	High	4	5
Huang TL 2011	98	100	Pain on walking (VAS)	WOMAC	Yes	Low	5	25
Huskisson EC1999	50	50	Pain on walking (VAS)	Lequesne index	Yes	High	5	24
Karlsson J 2002	88	66	VAS	Lequesne index	Yes	High	3	52
Migliore A 2021	347	345	VAS	Lequesne index	Yes	Low/high	1	24
Petterson SC 2019	184	185	WOMAC	WOMAC	Yes	High	1	26
Pham T 2004	131	85	Global pain (VAS)	Lequesne index	No	Intermediate	3	52
Strand V 2012	251	128	WOMAC		Yes	Intermediate	1	1

Table 1B. Description of studies comparing hyaluronic acid with steroids (n=10).

Author/year	Patients number		Outcomes measured - Instrument		Adverse effects reported	Molecular weight	Injection number	Follow-up weeks
	Hyaluronic acid	Saline solution	Pain	Function				
Askari A 2016	71	69	WOMAC	VAS	No	High	1	12
Bisicchia S 2016	75	75	WOMAC		No	High	2	26 and 52
Caborn D 2004	113	102	WOMAC / VAS	WOMAC	No	High	3	26
Maia PAV 2019	16	12	WOMAC	WOMAC	No	High	1	24
Shimizu M 2010	32	29	VAS		No	High	5	24
Skwara A 2009	30	30	VAS	Lequesne index	No	Intermediate	1	12
Tammachote N 2016	50	49	VAS	WOMAC	Yes	High	1	24
Tasciotaoglu F 2003	28	27	VAS	Lequesne index	Yes	High	3	26
Housman L 2014	129	132	WOMAC		Yes	High	1	26
Leighton R 2014	221	221	WOMAC		Yes	Intermediate	1	26

Table 2A. Overall risk of bias in studies comparing HA and saline AI.

Author/year	Randomization	Allocation	Double blind	Evaluator blindness	Losses	Prognostic	Outcomes	Intention to treat	Sample	Interruption
Baltzer AWA 2008										
Chevalier X 2010										
Day R 2004										
Dougados M 1993										
Pham T 2004										
Huskisson EC 1999										
Karlsson J 2002										
Migliore A 2021										
Altman RD 2004										
Altman RD 2009										
Petterson SC 2019										
Brandt KD 2001										
Hangody L 2018										
Huang TL 2011										
Arden NK 2014										
Henderson EB 1994										
Strand V 2012										
Subtitle	Low bias risk				Without information			High bias risk		

Characteristics of the included studies

A total of 5,917 patients with OA or knee osteoarthrosis who underwent IA injection of HA (n=3,101) compared to saline solution (n=2,816) were studied and followed for a period between 8 and 52 weeks. Molecular weight ranged from high to intermediate, and the outcomes measured were pain and functional (WOMAC, Lequesne index, KSS, and VAS) (Table 1A).

A total of 1,677 patients with OA or osteoarthrosis of the knee who underwent IA injection of HA (n=847) compared to steroids (n=830) were studied and followed for a period between

Table 2B. Overall risk of bias in studies comparing HA and steroid AI.

Author/Year	Randomization	Allocation	Double blind	Evaluator blindness	Losses	Prognostic	Outcomes	Intention to treat	Sample	Interruption
Askari A 2016										
Maia PAV 2019										
Caborn D 2004										
Tammachote N 2016										
Skwara A 2009										
Bisicchia S 2016										
Shimizu M 2010										
Tasciotaoglu F 2003										
Housman L 2014										
Leighton R 2014										
Subtitle	Low bias risk				Without information			High bias risk		

12 and 52 weeks. Molecular weight ranged from high to intermediate, and the outcomes measured were pain and functional (WOMAC, Fansne index, KSS, and VAS) (Table 1B).

Risk of bias

The overall risk of bias in studies comparing HA and saline solution AI is high, with most of this risk concentrated in the lack of blinding, losses, and analysis by ITT (Table 2A).

The overall risk of bias in studies comparing HA and steroid AI is high, with most of this risk concentrated in the lack of blinding, losses, and analysis by ITT (Table 2B).

Results of the quantitative analysis by comparison and by outcomes (meta-analysis)

Comparison between HA IA (IA-HA) and saline solution IA (IA-SS) (Figures 2–8)

In this comparison and analysis, it was possible to aggregate the results of 17 studies in relation to seven outcomes: overall WOMAC for pain, pain at rest (VAS), functional index (Lequesne), WOMAC (functional), WOMAC (pain), pain (VAS) walking, and adverse events (Table 3A).

Overall WOMAC for pain at 18 to 26 weeks – IA-HA versus IA-SS (Figure 2)

In pain assessment using the global WOMAC score (Figure 2), comparing IA-HA (n=375) and IA-SS (n=360), three studies were included[2-4]. The analysis identified a benefit of HA with a mean score reduction of -0.16 [95%CI -0.23, -0.10][2-4]. The quality of evidence is very low (Table 4).

Pain at rest (VAS) – IA-HA versus IA-SS (Figure 3)

In the assessment of pain at rest using the VAS score (Figure 3), comparing IA-HA (n=186) and IA-SS (n=140), two studies were included[5,6]. In the analysis, no difference in pain was identified between the -0.27 [-6.34, +5.79] comparisons. The quality of evidence is very low (Table 4).

Lequesne's functional assessment (Figure 4), comparing IA-AH (n=671) and IA-SS (n=601), five studies were included[5-9]. In the analysis, no difference in function was identified between comparisons -0.24 [95%CI -1.24, +0.76]. The quality of evidence is very low (Table 4).

WOMAC – functional subscale (baseline up to 26 weeks) – IA-HA versus IA-SS (Figure 5)

In the functional assessment (WOMAC), comparing IA-HA (n=785) and IA-SS (n=761), four studies were included[2,10-12]. In the analysis, no difference in function (WOMAC) was identified between comparisons -0.18 [95%CI -1.61, +1.26][2,10-12]. The quality of evidence is very low (Table 4).

WOMAC – pain subscale (baseline up to 26 weeks) – IA-HA versus IA-SS (Figure 6)

In the pain assessment (WOMAC), comparing IA-HA (n=830) and IA-SS (n=748), five studies were

Table 3A. Description of results by outcomes (IA-HA versus IA-SS).

Author/ Year	WOMAC function (Baseline REDUCTION) 26 weeks (Median±SD) (N)		WOMAC pain (BASELINEREDUCTION) 26 weeks (Median±SD) (N)		VAS (0-100) PAIN (WALKING) 26-52 weeks (Median±SD) (N)		Lequesne's functional index 26-52 weeks (Median±SD) (N)		Adverse events n/N		VAS (0-100) PAIN REDUCTION (REST) 52 weeks (Median±SD) (N)		WOMAC GLOBAL PAIN 18-26 weeks (Median±SD) (N)	
	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution
Baltzer AWA 2008	3.74 (2.44) (135)	3.94 (2.48) (107)			49.3 (25.9) (135)	48.2 (25.59) (107)			51/135	30/107			3.75 (2.42) (135)	3.93 (2.38) (107)
Chevalier X 2010									70/124	79/129			1.43 (0.06) (124)	1.59 (0.058) (129)
Day R 2004													3.84 (3.27) (116)	4.61 (3.14) (124)
Dougados M 1993					38.9 (30.9) (55)	32.71 (28.8) (55)	4.4(5.1) (55)	2.7 (4.1) (55)	18/55	18/55	17.9 (30.0) (55)	16.9 (23.4) (55)		
Pham T 2004							20.0 (16.5) (131)	18.9 (16.9) (85)			33.5 (28.5) (131)	34.5 (27.4) (85)		
Huskisson EC 1999					39.4 (27.8) (50)	53.7 (29.9) (50)	11.2 (4.4) (50)	12.6 (4.8) (50)	17/50	14/50				
Karlsson J 2002							4.4 (4.1) (88)	4.7 (4.4) (66)	51/88	50/66				
Migliore A 2021					29 (24) (347)	33 (24) (345)	7.4 (4.1) (347)	8.2 (4.3) (345)	187/347	180/345				
Altman RD 2004	5.82 (12.16) (173)	7.42 (13.52) (174)	2.50 (4.00) (173)	2.89 (4.17) (174)					112/173	114/174				
Altman RD 2009	19.6 (31.27) (293)	15.4 (29.33) (295)	19.2 (26.8) (293)	16.3 (26.8) (295)	30.0 (26.1) (293)	36.1 (28.6) (295)			158/293	168/295				
Petterson SC 2019	32.5 (24.8) (184)	33.1 (25.2) (185)			31.9 (22.0) (184)	30.9 (22.9) (185)			121/184	123/185				
Brandt KD 2001			2.1 (0.7) (114)	2.0 (0.7) (112)					76/114	74/112				
Hangody L 2018			39.5 (22.8) (150)	32.9 (23.6) (69)										
Huang TL 2011			29.28 (1.92) (100)	21.52 (1.94) (98)	17.00 (14.32) (100)	21.53 (15.69) (100)			39/100	48/100				
Arden NK 2014									68/108	69/110				
Henderson EB 1994									21/45	10 de 46				
Strand V 2012									172/251	81/128				

Table 3B. Description of results by outcomes (IA-HA versus IA-SS).

Author/ Year	WOMAC PAIN 12 weeks (Median±SD) (N)		WOMAC PAIN 26 weeks (Median±SD) (N)		VAS (0-100) PAIN 12 weeks (Median±SD) (N)		VAS (0-100) PAIN 26 weeks (Median±SD) (N)		WOMAC GLOBAL 26 weeks (Median±SD) (N)		WOMAC GLOBAL 52 weeks (Median±SD) (N)		Adverse events n/N	
	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution	Hyaluronic acid	Saline solution
Askari A 2016	13.22 (4.24) (71)	12.60 (3.69) (69)			6.7 (2.01) (71)	6.56 (2.15) (69)								
Maia PAV 2019	14.3 (3.6) (16)	7.1 (3.9) (12)												
Caborn D 2004			0.7 (0.1) (113)	0.4 (0.1) (102)			28.0 (2.5) (113)	12.4 (2.6) (102)	18.4 (1.7) (113)	10.4 (1.8) (102)			87/113	71/102
Tammachote N 2016			21 (15) (55)	21 (19) (55)			24 (22) (55)	21 (22) (55)						
Skwara A 2009					44.0 (22.3) (30)	45.8 (27.8) (30)								
Bisicchia S 2016							4.0 (2.0) (75)	5.0 (1.0) (75)	27.3 (10.8) (75)	36.0 (7.1) (75)	39.6 (17.9) (75)	42.3 (7.5) (75)		
Shimizu M 2010							21.5 (19.3) (32)	22.6 (18.3) (29)						
Tasciotaoglu F 2003							23.56 (10.11) (30)	26.46 (14.30) (30)					16/30	13/30
Housman L 2014													91/130	81/132
Leighton R 2014													50/221	9/221

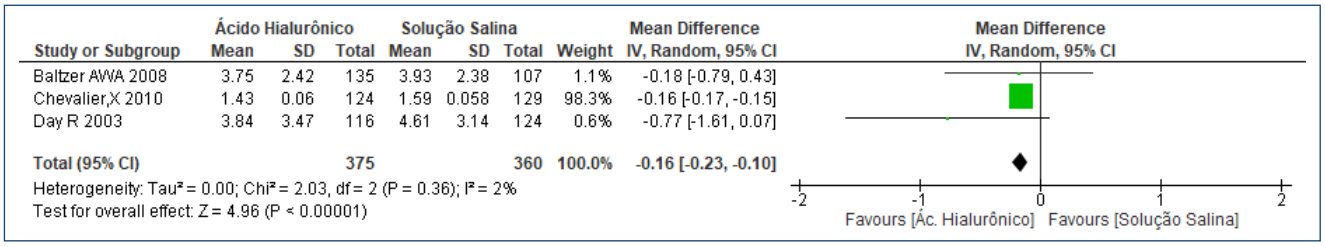

Figure 2. Western Ontario McMaster University Osteoarthritis (WOMAC global) – IA-HA versus IA-SS.

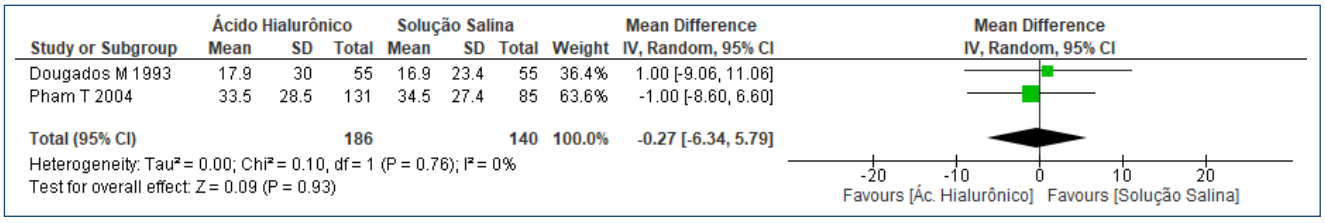

Figure 3. Decreased pain at rest (VAS) – IA-AH versus IA-SS.

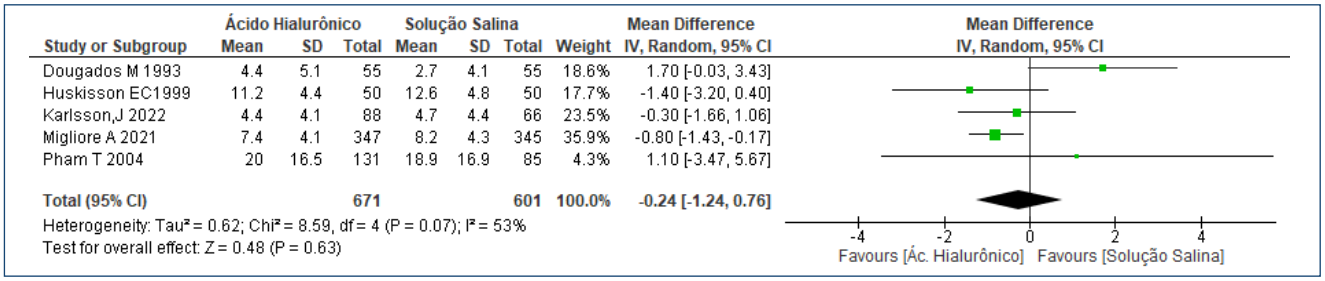

Figure 4. Lequesne's functional index from 26 to 52 weeks – IA-HA versus IA-SS.

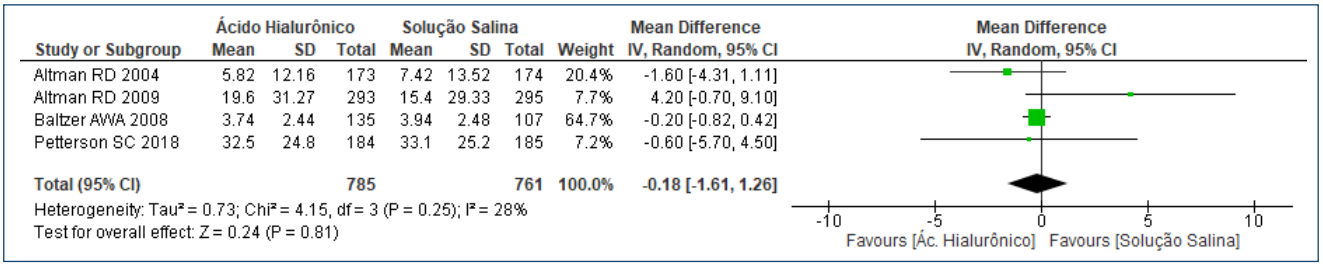

Figure 5. WOMAC (functional subscale) – score decrease – IA-HA versus IA-SS.

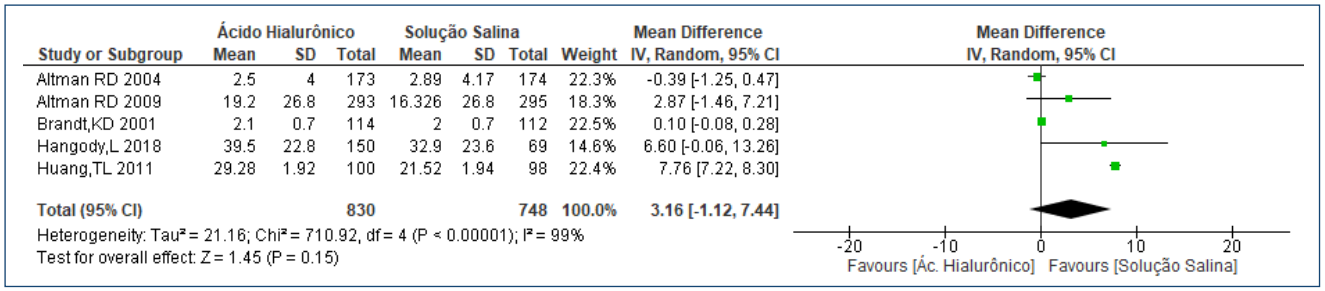

Figure 6. WOMAC (pain subscale) – score decrease – IA-HA versus IA-SS.

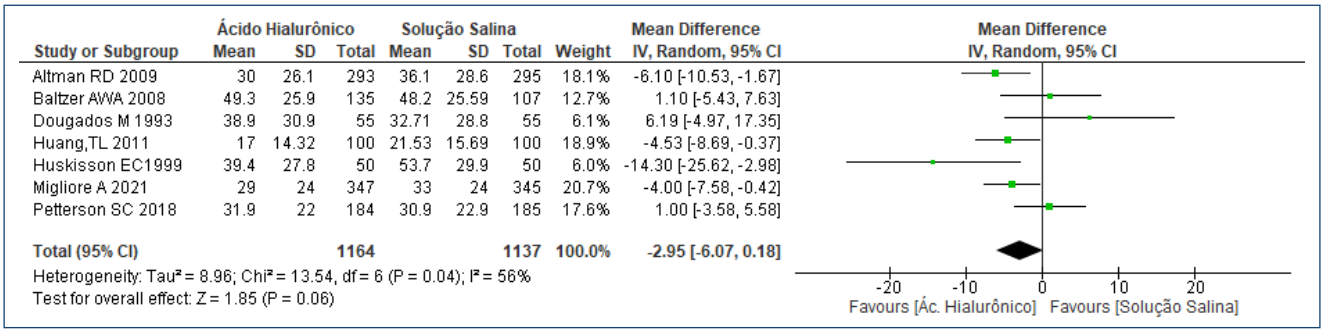

Figure 7. Decreased walking pain (VAS) – IA-HA versus IA-SS.

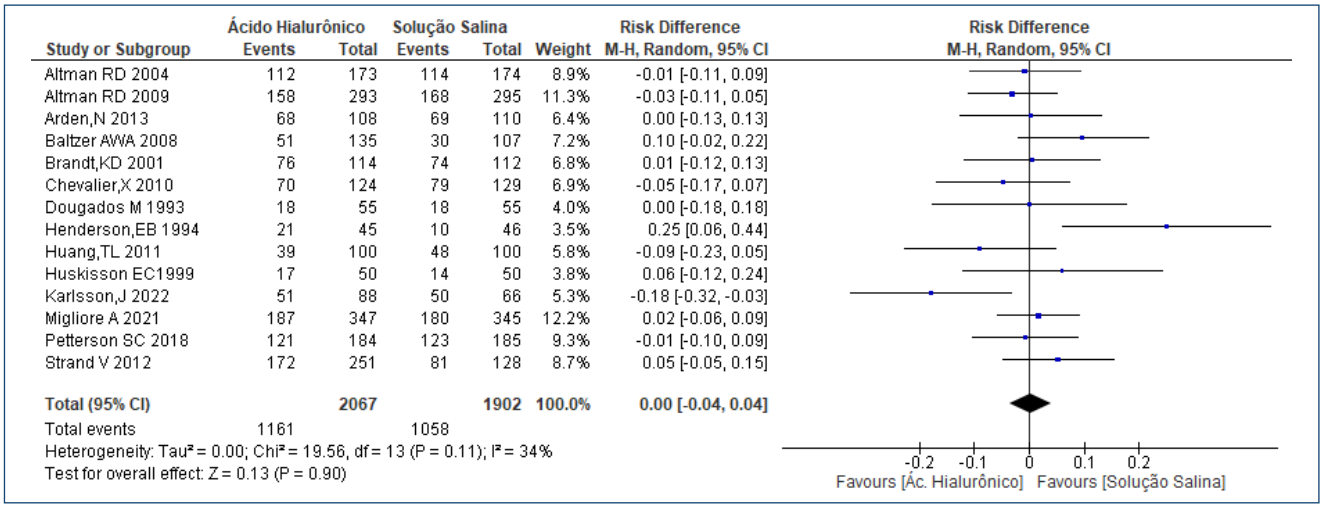

Figure 8. Adverse events – IA-AH versus IA-SS.

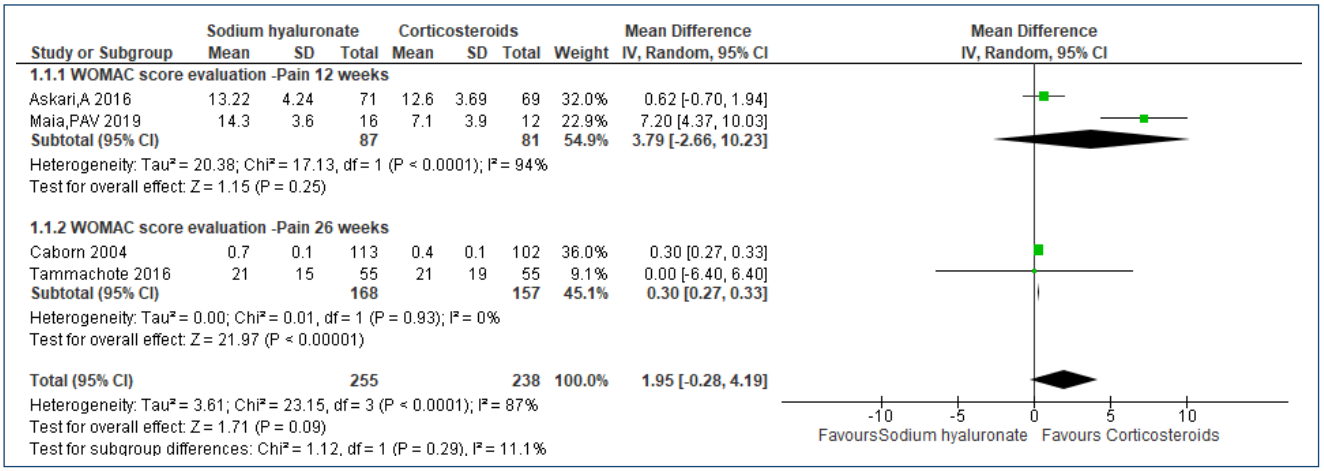

Figure 9. WOMAC pain score (12 and 26 weeks) – IA-HA versus IA-SS.

included[10-11,13-15]. In the analysis, no difference in function (WOMAC) was identified between comparisons +3.16 [95%CI -1.12, +7.44][10-11,13-15]. Very low quality of evidence (Table 4).

Walking pain at 26–52 weeks (VAS) – IA-HA versus IA-SS (Figure 7)

In the assessment of pain on walking using the VAS score (Figure 7), comparing IA-HA (n=1,164) and IA-SS (n=1,137), seven

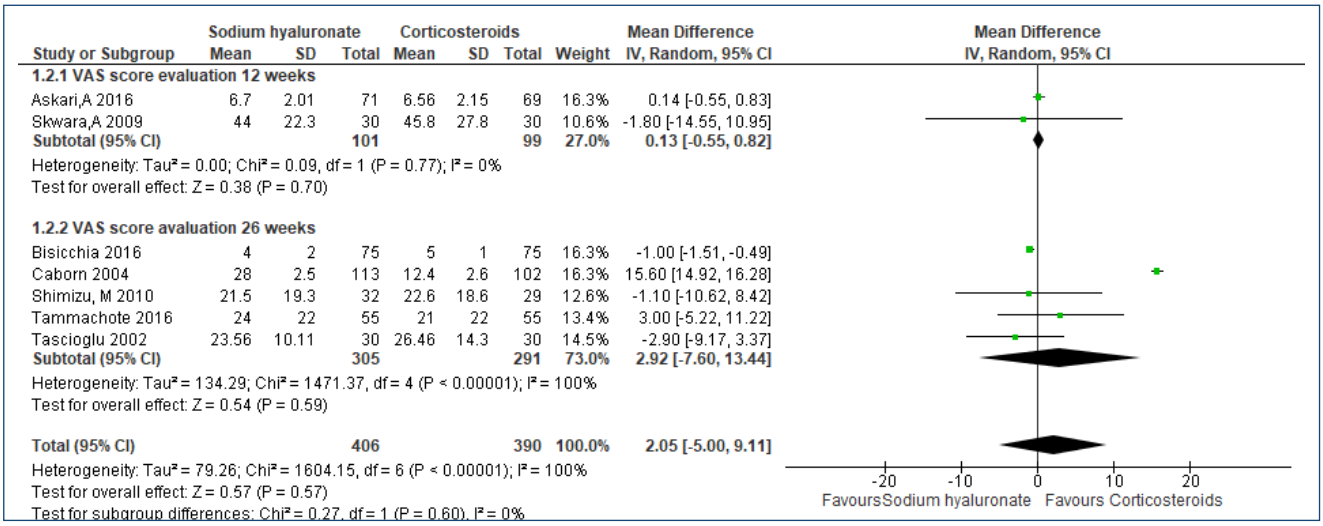

Figure 10. Pain assessment – VAS (12 and 26 weeks) – IA-HA versus IA-SS.

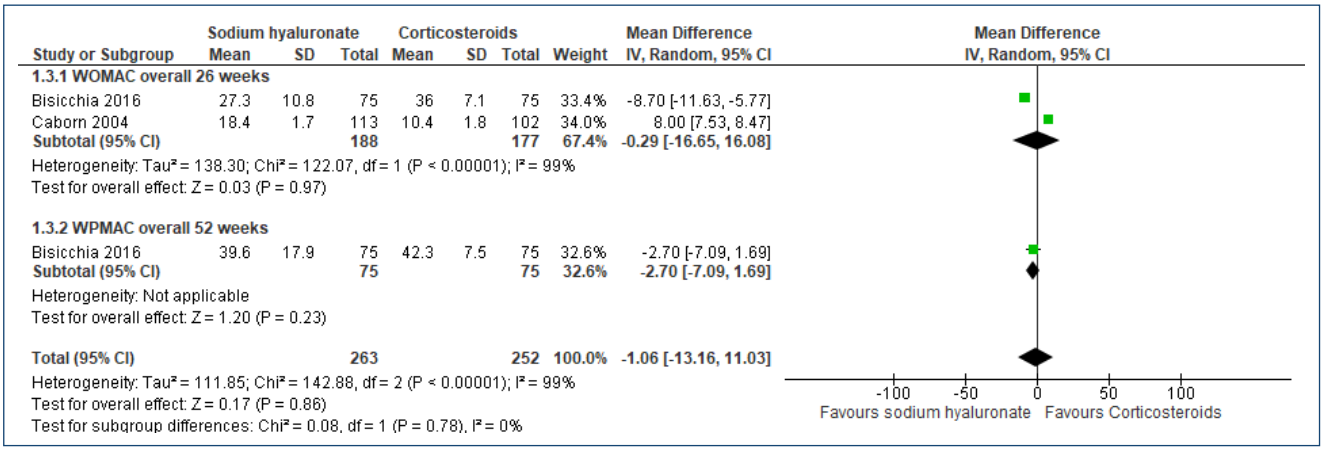

Figure 11. Pain assessment – overall WOMAC (26 and 52 weeks) – IA-HA versus IA-SS.

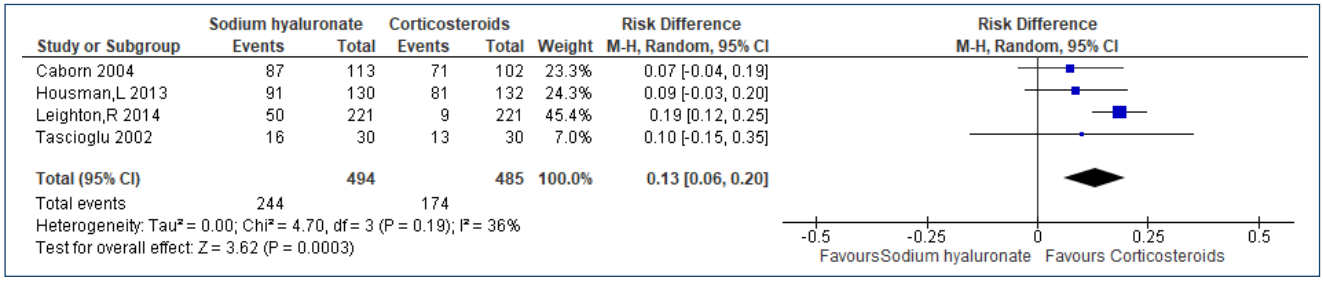

Figure 12. Adverse events – IA-HA versus IA-SS.

studies were included[2,5,7,9,11,12,15]. In the analysis, no difference in pain was identified between the -2.95 [-6.07, +0.18] comparisons. The quality of evidence is very low (Table 4).

Adverse events – IA-HA versus IA-SS (Figure 8)

In the evaluation of adverse events between IA-HA and IA-SS, 14 studies were included with 2,067 patients in the

Table 4. Question: knee infiltration with hyaluronic acid versus saline solution – GRADE.

Certainty assessment							Patients number		Effect		Certainty	Importance
Studies number	Study design	Bias risk	Inconsistency	Indirect evidence	Imprecision	Other considerations	Hyaluronic acid	Saline solution	Relative (95%CI)	Absolute (95%CI)		
WOMAC global – pain – 18–26 weeks												
3	Randomized clinical trials	Serious[a,b]	Serious[c]	Not serious	Serious[d]	None	375	360	–	MD **0.16 lower** (0.23 lower to 0.1 lower)	⊕◯◯◯ Very low	
VAS – pain reduction (rest) – 52 weeks												
2	Randomized clinical trials	Serious[a,b]	Serious[c]	Not serious	Serious[d]	None	186	140	–	MD **0.27 lower** (6.34 lower to 5.79 higher)	⊕◯◯◯ Very low	
Lequesne's Functional Index – 26 and 52 weeks												
5	Randomized clinical trials	Serious[a,b]	Serious[c]	Not serious	Serious[d]	None	671	601	–	MD **0.24 lower** (1.24 lower to 0.76 higher)	⊕◯◯◯ Very low	
WOMAC - Functional – Reduction from Base Line – 26 weeks												
4	Randomized clinical trials	Serious[a,b]	Serious[c]	Not serious	Serious[d]	None	785	761	–	MD **0.18 lower** (1.61 lower to 1.26 higher)	⊕◯◯◯ Very low	
WOMAC - Pain – Reduction from Base Line – 26 weeks												
5	Randomized clinical trials	Serious[a,b]	Serious[c]	Not serious	Serious[d]	None	830	748	–	MD **3.16 higher** (1.12 lower to 7.44 higher)	⊕◯◯◯ Very low	
VAS 0-100 Pain (walking) – 26–52 weeks												
7	Randomized clinical trials	Serious[a,b]	Serious[c]	Not serious	Serious[d]	None	1,164	1,137	–	MD **2.95 lower** (6.07 lower to 0.18 higher)	⊕◯◯◯ Very low	
Adverse events												
14	Randomized clinical trials	Serious[a,b]	Serious[c]	Not serious	Serious[d]	None	1,161/2,067 (56.2%)	1,058/1,902 (55.6%)	L	**0 less by 1,000** (40 less to 40 more)	⊕◯◯◯ Very low	

CI: confidence interval; MD: mean difference. [a]Without intention to treat analysis. [b]Unblided. [c]High heterogeneity. [d]Large confidence interval.

HA group (intervention) and 1,902 in the SS group (control). There was no difference in the risk of adverse events 0.00 [95%CI -0.04, +0.04][2,3,5-7,9-13,15-18]. The quality of evidence is very low (Table 4).

Comparison between HA IA (IA-HA) and Steroid IA (IA-SS) (Figures 9–12)

In this comparison and analysis, it was possible to aggregate the results of 10 studies, in relation to four outcomes:

Table 5. Question: knee infiltration with hyaluronic acid versus steroids – GRADE.

Certainty assessment							Patients number		Effect			
Studies number	Study design	Bias risk	Inconsistency	Indirect evidence	Imprecision	Other considerations	Hyaluronic acid	Steroids	Relative (95%CI)	Absolute (95%CI)	Certainty	Importance
WOMAC score evaluation – Pain												
4	Randomized clinical trials	Not serious	Not serious	Not serious	Not serious	None	255	238	–	MD **1.95 higher** (0.28 lower to 4.19 higher)	⊕⊕⊕⊕ High	
VAS score evaluation – Pain												
7	Randomized clinical trials	Not serious	Serious[a]	Not serious	Serious[b]	None	406	390	–	MD **2.05 higher** (5 lower to 9.11 higher)	⊕⊕◯◯ Low	
WOMAC overall												
2	Randomized clinical trials	Not serious	Serious [a]	Not serious	Not serious	None	150	150	–	MD 1.06 **lower** (13.16 lower to 11.03 higher)	⊕⊕⊕◯ Moderate	
Adverse events												
4	Randomized clinical trials	Not serious	Serious[a]	Not serious	Not serious	None	244/494 (49.4%)	174/485 (35.9%)		**130 less by 1,000** (200 less to 60 less)	⊕⊕⊕◯ Moderate	

CI: confidence interval; MD: mean difference. [a]High heterogeneity. [b]Large confidence interval.

WOMAC (pain) (12 and 26 weeks), pain at rest (VAS) (12 and 26 weeks), WOMAC overall for pain, and adverse events (Table 3B).

WOMAC pain score (12 and 26 weeks) – IA-HA versus IA-SS (Figure 9)

In assessing pain using the WOMAC score and comparing IA-HA and IA-SS, two studies were included in the 12-week evaluation (87 patients in the IA-HA group and 81 in the IA-SS group), and two studies were included in the 26-week evaluation (168 patients in the IA-HA group and 157 in the IA-SS group). The result of the analysis of subgroups by follow-up time does not identify a difference between the comparisons at 12 weeks: 3.79 [95%CI -2.66, +10.23] and results in an increase in the pain score with HA of 0.30 [95%CI +0.27, +0.33] at 26 weeks. In the global analysis (regardless of the follow-up time), no difference was identified between the comparisons: 1.95 [-0.28, +4.19] (Figure 9)[19-22]. High quality of evidence (Table 5).

PAIN assessment (VAS) at 12 and 26 weeks – IA-HA versus IA-SS (Figure 10)

In the assessment of pain using the VAS score comparing IA-HA and IA-SS, two studies were included in the 12-week assessment (101 patients in the IA-HA group and 99 in the IA-SS group), and at 26 weeks, five studies were included (305 patients in the IA-HA group and 291 in the IA-SS group). No differences were identified in the score at the 12-week follow-up [0.13 (95%CI -0.55, +0.82)], the 26-week [2.92 (95%CI -7.60, +13.44)], or in the global analysis regardless of follow-up time [2.05 (95%CI -5.00, +9.11)] (Figure 10)[19-26]. Low quality of evidence (Table 5).

Overall WOMAC for pain at 26 and 52 weeks – IA-HA versus IA-SS (Figure 11)

In pain assessment (global WOMAC score), comparing IA-HA and IA-SS, two studies were included in the 26-week follow-up (188 patients in the IA-HA group and 177 in the IA-SS group), and one study in 52 weeks of follow-up (75 patients in groups IA-HA and IA-SS). There was no difference between the two groups at the follow-up of 26 [-0.29 (95%CI -16.65, +16.08)], or 52 weeks [-2.70 (95%CI -7.09, +1.69)], or at global assessment [- 1.06 (95%CI -13.16, +11.03)] (Figure 11)[21,24]. Moderate quality of evidence (Table 5).

Adverse events – IA-HA versus IA-SS (Figure 12)

In the evaluation of adverse events, in the comparison between IA-HA and IA-SS, four studies were included (494 patients in the IA-HA group and 485 in the IA-SS group). The analysis demonstrates that there is an increase in the risk of adverse events with the 13% HA [95%CI 6–20%][21,26-28]. Moderate quality of evidence (Table 5).

Quality of evidence by comparison and outcome (Tables 4 and 5)

Knee infiltration comparing hyaluronic acid to saline solution (placebo) in osteoarthritis

Outcomes: Overall WOMAC for pain, pain at rest (VAS), functional index (Lequesne), WOMAC (functional), WOMAC (pain), pain (VAS) while walking, and adverse events.

Knee infiltration comparing hyaluronic acid to steroids in osteoarthritis

Outcomes: WOMAC (pain) (12 and 26 weeks), pain at rest (VAS) (12 and 26 weeks), overall WOMAC for pain, and adverse events.

SUMMARY OF EVIDENCE

There were seven analyses (seven outcomes) comparing IA injection with HA and saline solution and four analyses (four outcomes) comparing steroids, with follow-up at different times (8 weeks to 52 weeks). In only two outcomes, there was a difference in effect between the comparisons: (1) In the comparison between HA and saline solution: reduction in the Western Ontario McMaster University Osteoarthritis (global WOMAC) score of 0.16 points favorable to HA on a scale ranging from 0 to 96 points; (2) Increase in adverse events by 13% (NNH: 8) with the use of HA compared to steroids.

RECOMMENDATION

Despite the frequent and disseminate use of IA-HA in the treatment of knee OA, there is no high-quality evidence sustaining this form of treatment.

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A vignette epexegesis of a model for training sonography-guided fine-needle aspirations in thyroidology and thyroidologists: think twice with needle size?

Demet Sengul[1*], Ilker Sengul[2,3]

Dear Editor,

Mater artium necessitas. A Deucalione (Necessity is the mother of invention. Since Deucalione), thyroid gland disorders with their diagnostic options remain their significance in tellurian. Today, thyroidology, *per se*, deals with up-to-date management of nodular thyroid disease demanding the availability of several diagnostic and therapeutic modalities in order to obtain a correct diagnosis and recommend appropriate treatment options.

We read with a great deal and respect the research article entitled "A model for training ultrasound-guided fine-needle punctures". This beneficial research of high quality seems to demand determining in order to evaluate the efficacy of a training program in sonography-guided fine-needle aspiration (FNA) using a cost-effective model[1]. Luz et al.[1] declared in their study published in the 68th volume of *Rev Assoc Med Bras* that no difference had been detected between the two groups of residents, resident physicians from the first year of the course (R1) *vs.* those from the second year of the course (R2), in the time required to perform the procedure before or after the FNA training course. Conversely, the authors reported that the posttraining punctures performed were significantly lower than pretraining ones numerically, regardless of the group of residents, R1 *vs.* R2. In addition, they also stated that the resident physicians' evaluation of the course was positive and that no significant difference between them in their answers to the questionnaire of Q1 to Q6 had been recognized. Nevertheless, the optimal needle size has not been established distinctly and conclusively in thyroidology for an optimal and accurate evaluation of thyroid FNA cytology till now. An indisputable accuracy of needle size has not been declared globally for thyroidologists.

Of note, the debate is still ongoing on this controversial issue utilizing a broad range, from 21 to 27 gauges, of needle sizes[2-4]. Luz et al.[1] emphasized using a 25-gauge needle, without the puncture guide, in their good work. However, the aforementioned size has been known as not much "finer" and also a "larger" one[2-4]. Therefore, would the outcomes of the study in three key points: (i) time to perform the procedure, (ii) the number of punctures on the matrix surface, (iii) answers to the questionnaire of Q1 to Q6, be altered as they had harnessed significantly (i) finer or (ii) larger needle sizes? Of note, does utilizing a unique size affect the evaluations of the relevant parameters of the mentioned study? As such, is it essential to compare at least two, 21 vs. 27 gauges, even three, 21, 25, and 27 gauges, or more in order to design this kind of deducing educational and technical study?[5-10]. Finally, would the outcomes might switch in case of utilizing the capillary sampling technique instead of their used terminology of "puncturing" and in case of comparison of both interventional applications in their study?

To resolve these issues, working with finer and larger sizes, even comparing at least three sizes to each other, which would give shed light on it, might be opted for by thyroidologists. As a matter of fact, this issue merits further investigation. We thank Luz et al.[1] for their valuable study.

ACKNOWLEDGMENTS

We thank all of the study participants.

AUTHORS' CONTRIBUTIONS

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Response to letter to the editor: Comment on "Mutagenic damage among bronchiectasis patients attending in the pulmonology sector of a hospital in southern Brazil"

Daniela Fernandes Ramos[1,2*], Daniel Wenceslau Votto Olmedo[1,2], Katheryne Benini Martins[1,2], Milene Machado Paz[1,2], Caroline Lopes Feijo Fernandes[1,3], Flavio Manoel Rodrigues da Silva Júnior[1,3]

Dear Editor,

We are grateful to have the opportunity to respond to the letter to the editor: "Comment on 'Mutagenic damage among bronchiectasis patients attending in the pulmonology sector of a hospital in southern Brazil'" and equally happy that the authors of the letter are interested in our study. The scientific debate that discusses theoretical and methodological aspects is capable of improving knowledge.

Regarding the content of the letter, de Moraes Malinverni et al.[1] comment on the limitations of using the staining technique with eosin-methylene blue according to Leishman. This point has already been discussed elsewhere[2], and we reinforce here that the study cited by the authors of the letter to the editor[3] did not mention staining using eosin-methylene blue according to Leishman. Furthermore, the study itself does not rule out the possibility of using simple staining techniques such as May-Grünwald. The technique employed by Votto Olmedo et al.[4] is an appropriate mixture for the differential visualization of both the cytoplasm and the cell nucleus and is recommended in the study by Korsakov et al.[5] for use in assessing mutagenicity in oral cells. For a complementary reading in relation to possible cellular artifacts, we recommend da Silva Júnior et al.[2] who already discuss these same aspects brought up here.

Regarding the second point discussed by the authors of the letter, the number of cells analyzed, we apologize to the readers because we did not include in the materials and methods section that 2,000 cells per patient were visualized and the results were expressed as the number of micronuclei in 1,000 cells. The way of describing the methodology was not clear and is certainly a point of confusion for readers. We appreciate the opportunity to clarify this point.

Considering the points highlighted by de Moraes Malinverni et al.[1], regarding the use of "lymphocytes as a result of systemic host response," we would like to highlight that the study was conducted in a hospital environment, including stable patients, where blood collection is not part of the routine consultations, and based on the high correlation of results obtained between oral mucosa and lymphocytes, previously described by Ceppi et al.[6], we chose to use a noninvasive method.

The final point of the letter concerns the investigation of cytotoxicity using metanuclear markers. In fact, it is a very interesting strategy, but it is beyond the scope of our study. In any case, cytotoxicity is always measured through simple techniques using Trypan blue, tetrazolium salts, or resazurine, where viability was always found to be above 90%.

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Comments on "Criteria for selection and classification of studies in medical events"

André Pontes-Silva[1]*

Vieira et al.[1] evaluated the impact of study methodology and evaluation type on the selection of studies during the presentation of scientific events. This article highlighted something worrying for the health sciences in medical events: "The evidence pyramid rule[2]." After the inception of the evidence-based health movement in the 1990s, the evidence pyramid rose from the mud[2]. Inherent in this pyramid is the concept of a hierarchy (less valid evidence is at the bottom of the pyramid and more valid at the top). Thus, a search for an answer to a clinical question should begin at the top of the pyramid (i.e., systematic reviews with meta-analyses of randomized controlled trials)[2].

Systematic reviews with meta-analyses of randomized controlled trials are important to show whether an intervention is effective/efficacy; however, it is important to emphasize that the clinical research question is not always about the effectiveness/efficacy of an intervention. Namely, in some cases, patients and professionals may want to know the risk, prevalence, incidence, or symptoms of a disease but a systematic review with meta-analyses of randomized controlled trials does not reveal these details. Therefore, it is important first to analyze the clinical question in order to decide which is the best study design. Furthermore, there is not just one evidence pyramid[3,4].

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Comments on "Evaluation of functional parameters of the foot and ankle in elderly with sarcopenia"

Cassius Iury Anselmo-e-Silva[1], Leticia Menegalli Santos[1], André Luiz Lopes[2], Erika da Silva Maciel[3], Fernando Rodrigues Peixoto Quaresma[3], Aldair Darlan Santos-de-Araújo[1], André Pontes-Silva[1*]

The paper title does not contain this information[1], but Souza Júnior et al.[1] identified sarcopenic elderly people through the SARC-F score and grip-force strength using a Jamar dynamometer. Traditionally, the Jamar dynamometer is the reference standard tool for grip-force strength evaluations with excellent validity and reliability in the clinic and research[2,3], and many health professionals use it for measuring grip-force strength and recording a single maximal or submaximal grip-force strength value during testing[2,3]. Nevertheless, the Jamar dynamometer is a mechanical measurement tool and only shows instantaneous grip-force strength, which means that it cannot continuously record handgrip force or show changes in the quality of grip-force strength control. The Jamar dynamometer also needs recalibration each year[4], and a study reported that its limited contact area may cause hand pain in subjects, thereby influencing the grip-force measurement when applying higher grip strength[5,6]. Souza Júnior et al.[1] did not report these adjustments.

In the article by Souza Júnior et al., the volunteers' palmar grip was repeated 3× on the right side and 3× on the left side, with an interval of 1 min among repetitions[1]. Afterward, the authors used the highest value obtained on each side. However, due to the variations mentioned above, the best strategy would be to use the median obtained in the evaluations[7,8]. Besides, it is currently necessary to use a novel digital dynamometer with automatic calibration, a larger contact area, automatic grip force data recording, and continuous grip-force strength data collection, which might be more convenient for therapists for measuring the quality of grip-force strength control, such as the MicroFET3 dynamometer[5], an instrument with excellent validity and reliability[9]. Finally, another important variable is the volunteer's positioning. The dynamometer needs to be supported by some object (e.g., a table) to compensate for the weight (force of gravity)[10,11]; otherwise, the volunteers have to make two efforts: one to press the dynamometer and the other to overcome the resistance of gravity (Figure 1)[12].

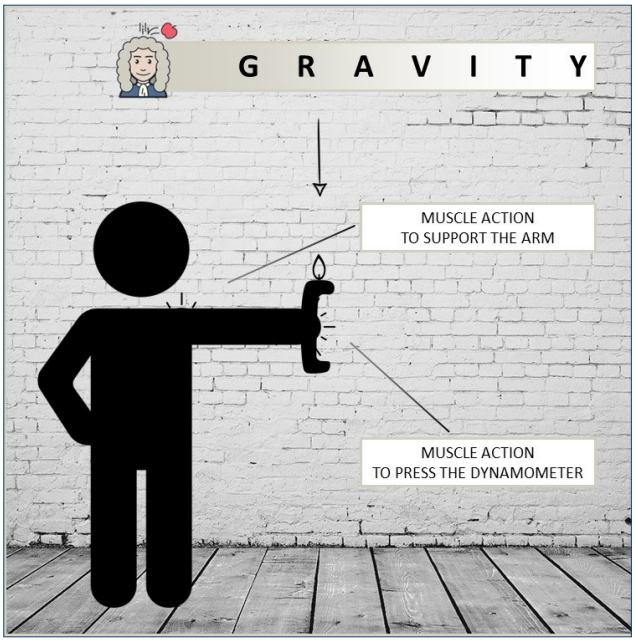

Figure 1. Pressing the dynamometer and overcoming gravity.

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Comment on "How may ChatGPT impact medical teaching?"

Amnuay Kleebayoon[1*], Viroj Wiwanitkit[2,3]

Dear Editor,

We found that the article entitled "How may ChatGPT impact medical teaching?"[1] is interesting. Yoshinari Júnior and Vitorino discussed the usefulness of the emerging ChatGPT technology, specifically the impact of ChatGPT on medical teaching[1]. We both agree that careful consideration is required when using technology responsibly, especially in light of the rapidly advancing field of artificial intelligence (AI). AI should not be used to create, analyze, or approve critical information without human review[2]. The accuracy of the data in ChatGPT is a crucial and debatable subject. But it is important to think about how AI should be used responsibly. Without any user input, the ChatGPT may output information that is immediately useful, which increases the likelihood of additional crimes like plagiarism. Abuse may increase as a result of ineffective intake management techniques. However, it might still be beneficial. For example, it might be used to automatically detect plagiarism and ghostwriting. Everyone agrees that AI needs a stronger foundation. We can all agree that for AI to function properly, a cutting-edge strategy is required. It is now essential to establish the ethically sound and effective use of developing AI.

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Cognitive carbohydrate restriction: a new proposal for the diet mentality in the era of low-carb diets

Jônatas de Oliveira[1]*

Dear Editor,

Low-carb diets have been considered again for weight loss in trials, and their spread goes hand in hand with the increase in unsupervised practices, as seen in intermittent fasting strategies[1]. Even if the results of controlled studies indicate more consistent results, the discussions also reach the lay public, who will make food choices, considering information dissipated on the Internet and by health professionals[2]. Feinman et al. evaluated an online support forum with 86,000 members discussing low-carb, "Active Low-Carber Forums[3]," and recently investigated this practice among university students in Brazil. In 2018, 25% of students practiced low-carb[4].

However, analyses of this behavioral profile and how food choices are made still lack appropriate methodologies to understand the low-carb phenomenon. Considering all the information and the various types of low-carb diets[1], how do these people plan their eating behaviors to ensure this restriction? In other words, eating attitudes (i.e., thoughts, beliefs, and feelings), specifically about the food source of this macronutrient, are elements of choice that determine consumption.

Considering this problem, we adapted the cognitive restriction subscale of the Three Factor Eating Questionnaire, which assesses how willing, in terms of thoughts and behaviors, individuals are to restrict food in order to change shape and body weight[5]. The change was to identify this carbohydrate-directed diet mindset[6]. An example is question #3, "*I do not eat some foods because they make me fat*," which was adapted to "*I do not eat some foods (source of carbohydrates) because they make me fat*." Low-carb dieters showed more remarkable cognitive restraint and more significant cognitive restriction of carbohydrates compared to non-dieters. Nevertheless, the overall score for cognitive restraint on carbohydrates correlated positively with guilt for food cravings in low-carb dieters[7].

These findings indicate that unsupervised and popularly advertised diets will not always be aligned with healthy eating behavior, requiring further studies regarding diet mentality and other ways of thinking about food from an attitudinal point of view, which can be worked on in psychoeducational proposals and treatment programs.

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ORIGINAL ARTICLE

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Association between hyperuricemia and hypertension and the mediatory role of obesity: a large cohort study in China

Changyi Wang[1,2], Pei Qin[3], Yinxing Liu[1,2], Li Wang[1], Shan Xu[1], Hongen Chen[1], Shuhong Dai[1], Ping Zhao[4], Fulan Hu[3], Yanmei Lou[4*]

SUMMARY

OBJECTIVE: This study aimed to investigate the sex-specific association between hyperuricemia and the risk of hypertension and whether obesity mediates this association.

METHODS: This study included 31,395 (47.0% women) adults without hypertension, cardiovascular disease, or cancer at baseline who completed at least one follow-up annual examination between 2009 and 2016. Cox regression models were performed to calculate hazard ratios and 95% confidence intervals. Mediation analysis was conducted to estimate the effect of body mass index on the association between hyperuricemia and hypertension.

RESULTS: During a median 2.9-year follow-up, hyperuricemia was significantly associated with a higher risk of hypertension (HR 1.15, 95%CI 1.07–1.24 for all participants; HR 1.12, 95%CI 1.03–1.22 for men; and HR 1.23, 95%CI 1.02–1.48 for women) after adjustment for potential confounders. Additional adjustment for body mass index attenuated this association (HR 1.09, 95%CI 1.08–1.10 for all participants; HR 1.07; 95%CI 0.98–1.16 for men; HR 1.18; 95%CI 0.96–1.44 for women). Mediation analysis showed that BMI partially mediated the relationship between hyperuricemia and incident hypertension (indirect effect HR 1.09, 95%CI 1.08–1.10; direct effect: HR 1.08, 95%CI 1.02–1.15). The percentage of the mediation effect was 53.2% (95%CI 37.9–84.5).

CONCLUSION: Hyperuricemia is associated with a risk of hypertension in both sexes, and BMI partially mediates hyperuricemia-related incident hypertension.

KEYWORDS: Uric acid. Hypertension. Obesity. Mediation analysis.

INTRODUCTION

Hypertension is a modifiable risk factor for cardiovascular disease[1]. The China Health and Nutrition Survey of 451,755 adults showed that the prevalence of hypertension was 27.9% in China in 2015[2]. In 2010, the global number of people with hypertension was estimated at 1.4 million, and this number is expected to increase to more than 1.6 million by 2025[3].

Serum uric acid (SUA) is the end product of endogenous and dietary purine metabolism[4]. Experimental evidence has suggested that high SUA levels cause damage to renal microcirculation, tubulointerstitial injury, chronic low-grade inflammation, and endothelial dysfunction and promote the development of hypertension[5,6]. Previous observational studies have shown that an elevated SUA concentration may be associated with an increased risk of hypertension[7-13]. However, studies have reported inconsistent results regarding sex-specific differences in the association between SUA concentration and hypertension[8,11,13,14]. Some of these studies showed a significant association in both sexes[8,11,13], and others showed a significant association in women but not in men[14]. Additionally, limited data are available on the interaction of SUA and sex regarding hypertension. Although changes in obesity have been reported to be independently associated with changes in uric acid concentrations, previous metabolic studies and pathophysiology imply a possible interaction between them[15]. A cross-sectional study showed that body mass index (BMI) increased significantly with elevated SUA in 27,009 Chinese middle-aged and older adults[16]. There is also strong evidence from studies showing a positive association between SUA concentrations and obesity in different populations[17-20]. Obesity is an established

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risk factor for hypertension[21]. Therefore, obesity may play a mediatory role in the relationship between hyperuricemia and hypertension. However, this relation remains unclear. There are also no studies demonstrating that BMI as a mediator affects the relationship between SUA and hypertension.

Therefore, we prospectively investigated the sex-specific difference in the association between hyperuricemia and incident hypertension in a large Chinese cohort study. We also examined the mediation effect of BMI on the association between hyperuricemia and the risk of incident hypertension.

METHODS

Study population

This cohort analysis was based on a large ongoing health examination in China called the Xiaotangshan Health Examination Study. In this study, participants without hypertension at first entry were included in the analysis (n=33,529). We excluded 1,045 adults with missing data on SUA levels at baseline. We also excluded 779 adults with a history of myocardial infarction, stroke, coronary heart disease, or heart failure and 310 adults with cancer at baseline. Finally, data from 31,395 adults were analyzed. The Institutional Review Board of Xiaotangshan Hospital approved this study.

Diagnosis of hypertension and baseline serum uric acid measurement

Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the use of anti-hypertension medication according to the National High Blood Pressure Education Program[22]. Hyperuricemia was defined as an SUA concentration ≥7.0 mg/dL for men and ≥6.0 mg/dL for women[23].

Measurement of covariates

Data regarding demographic characteristics, medical history, and the use of medications were collected through a face-to-face standardized questionnaire interview. Anthropometry and clinical and biochemical measures were collected by trained staff. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Overnight fasting blood samples were obtained (at least 8 h of fasting). SUA, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations were measured by an enzymatic colorimetric assay (Type 7600; Hitachi, Tokyo). Fasting plasma glucose (FPG) and alanine aminotransferase (ALT) concentrations were measured using an automated analyzer. Serum creatinine concentrations were measured using the enzymatic method. The estimated glomerular filtration rate (eGFR) was evaluated using an equation developed by adaptation of the Modification of Diet in Renal Disease equation[24]. This equation was as follows: eGFR (mL/min/1.73 m^2)=175×creatinine$^{-1.234}$×age$^{-0.179}$ (if women, ×0.79), where creatinine is the serum creatinine concentration (denoted in mg/dL) and age is denoted in years.

Statistical analyses

The main characteristics of the study population are described by hyperuricemia at baseline or incident hypertension at follow-up (yes or no). Descriptive data are presented as the mean (standard derivation) for normally distributed continuous variables and the median (interquartile range) for skewed continuous variables. Between-group differences in the main characteristics were evaluated using the Student's t-test for normally distributed variables or the Mann-Whitney U test for skewed variables.

The follow-up time was defined as the period between the visit at first entry and the last confirmed follow-up or the date when an event occurred. The associations of hyperuricemia (yes or no) and the risk of hypertension in the overall population, for men and women, were analyzed using Cox proportional hazards models. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. The following models were used with adjustment for increasing degrees of potential confounders.

Mediation analysis using the natural effect model proposed by Lange et al.[25] was performed to address whether the hyperuricemia–hypertension association could be explained by BMI.

Two sensitivity analyses were performed as follows: exclusion of participants aged ³75 years (n=348) and those with hypertension that occurred in the first 2 years of follow-up (n=2,736). All statistical analyses were performed using R 3.5.2 (R Foundation), and a two-sided P<0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The mean age of the participants was 40.00±11.71 years, and 47.0% were women. During a median follow-up of 2.9 years (total person-years: 100,580), 5,023 (16.0%) participants developed hypertension, of whom 3,729 (22.4%) were men and 1,294 (8.8%) were women. The mean SUA level

was significantly higher in men (6.22±1.24 mg/dL) than in women (4.42±0.95 mg/dL) (P<0.001) who had a higher BMI, age, systolic and diastolic blood pressure, and concentrations of creatinine, TC, TG, and LDL-C and had a lower HDL-C concentration and eGFR compared with those without hyperuricemia (all P<0.05) (Table 1).

Table 1. Baseline characteristics of the study population by hyperuricemia and hypertension.

	Hyperuricemia			Hypertension		
	Yes (n=4,943)	No (n=26,452)		Yes (n=5,023)	No (n=26,372)	
Men						
n	4,043	12,587		3,729	12,901	
Age (years)	40.76 (12.48)	41.63 (12.04)	<0.001	45.78 (13.27)	40.16 (11.51)	<0.001
Heart rate (beats/min)	75.44 (9.83)	74.69 (9.53)	<0.001	76.17 (9.57)	74.50 (9.59)	<0.001
BMI (kg/m^2)	26.53 (3.03)	25.07 (3.05)	<0.001	26.69 (3.09)	25.06 (3.02)	<0.001
Weight (kg)	79.24 (10.65)	74.35 (10.16)	<0.001	78.93 (10.83)	74.56 (10.18)	<0.001
Blood pressure (mmHg)						
Systolic	118.07 (10.11)	116.64 (10.53)	<0.001	122.42 (8.87)	115.43 (10.34)	<0.001
Diastolic	75.00 (7.21)	73.37 (7.48)	<0.001	78.52 (6.12)	72.41 (7.23)	<0.001
SUA (mg/dL)	7.88 (0.78)	5.69 (0.82)	<0.001	6.40 (1.32)	6.17 (1.22)	<0.001
FPG (mmol/L)	5.42 (0.92)	5.47 (1.21)	0.002	5.76 (6.12)	5.38 (7.23)	<0.001
TC (mmol/L)	5.04 (0.94)	4.78 (0.89)	<0.001	5.06 (1.41)	4.78 (1.04)	<0.001
TG (mmol/L)	2.22 (1.76)	1.63 (1.26)	<0.001	2.10 (0.93)	1.68 (0.89)	<0.001
HDL-C (mmol/L)	1.21 (0.26)	1.28 (0.28)	<0.001	1.25 (1.73)	1.27 (1.31)	<0.001
LDL-C (mmol/L)	3.13 (0.75)	2.97 (0.73)	<0.001	3.13 (0.27)	2.97 (0.28)	<0.001
Albumin (g/L)	45.69 (3.02)	45.35 (3.06)	<0.001	45.24 (3.01)	45.49 (3.06)	<0.001
ALT (IU/L)	26.6 (19.0–39.1)	21.2 (16.0–30.0)	<0.001	25.0 (18.4–35.8)	22.0 (16.0–31.0)	<0.001
Women						
n	900	13,865		1,294	13,471	
Age (years)	42.83 (14.75)	38.01 (10.6)	<0.001	48.34 (13.27)	37.34 (11.51)	<0.001
Heart rate (beats/min)	76.12 (9.9)	76.33 (9.74)	0.522	76.67 (9.57)	76.29 (9.59)	<0.001
BMI (kg/m^2)	25.48 (3.66)	22.83 (3.11)	<0.001	25.28 (3.09)	22.78 (3.02)	<0.001
Weight (kg)	65.61 (10.01)	59.02 (8.39)	<0.001	64.38 (10.83)	58.95 (10.18)	<0.001
Blood pressure (mmHg)			<0.001			<0.001
Systolic	113.79 (11.64)	108.79 (11.48)	<0.001	120.6 (8.87)	108 (10.34)	<0.001
Diastolic	72.08 (7.93)	68.24 (7.85)	<0.001	76.67 (6.12)	67.69 (7.23)	<0.001
SUA (mg/L)	6.62 (0.62)	4.28 (0.78)	<0.001	4.84 (1.32)	4.38 (1.22)	<0.001
FPG (mmol/L)	5.36 (0.91)	5.08 (0.65)	<0.001	5.43 (1.41)	5.06 (1.04)	<0.001
TC (mmol/L)	5.18 (1.05)	4.63 (0.88)	<0.001	5.12 (0.93)	4.62 (0.89)	<0.001
TG (mmol/L)	1.65 (1.15)	1.05 (0.68)	<0.001	1.42 (1.73)	1.06 (1.31)	<0.001
HDL-C (mmol/L)	1.4 (0.3)	1.54 (0.34)	<0.001	1.47 (0.27)	1.54 (0.28)	<0.001
LDL-C (mmol/L)	3.2 (0.86)	2.73 (0.72)	<0.001	3.11 (0.74)	2.72 (0.73)	<0.001
eGFR (mL/min/1.73 m^2)	95.66 (23.63)	106.97 (24.18)	<0.001	95.49 (17.25)	107.32 (17.92)	<0.001
Albumin (g/L)	44.51 (3.04)	44.37 (3.18)	0.245	43.9 (3.01)	44.43 (3.06)	<0.001
ALT (IU/L)	18.0 (13.0–26.0)	13.7 (11.0–18.0)	<0.001	16.7 (13.0–22.58)	13.5 (11.0–18.0)	<0.001

Data are mean (SD) or median (IQR). BMI: body mass index; SUA: serum uric acid; FPG: fast plasma glucose; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate; ALT: alanine aminotransferase.

Association between hyperuricemia and hypertension

In the age- and sex-adjusted Cox model, hyperuricemia was significantly associated with an increased risk of hypertension (HR 1.43, 95%CI 1.34–1.52; P<0.001). After adjusting for age, sex, heart rate, systolic blood pressure, eGFR, and concentrations of FPG, TC, TG, HDL-C, albumin, and ALT at baseline, this association was weakened but remained significant (HR 1.15, 95%CI 1.07–1.24; P<0.001). Further adjustment for BMI attenuated this association (Table 2).

In the sex-specific analysis, we used the Cox proportional hazards model to assess men and women separately. The age-adjusted Cox regression model showed a significant association in men (HR 1.34, 95%CI 1.25–1.44; P<0.001) and women (HR 1.60, 95%CI 1.36–1.88; P<0.001). After adjusting for mixed factors at baseline, this association was weakened but remained significant for both sexes (HR 1.12, 95%CI 1.03–1.22; P=0.007 for men; HR 1.23, 95%CI 1.02–1.48; P=0.034 for women). (Table 1).

Mediation analysis

In mediation analysis (Figure 1), the total effect of hyperuricemia on hypertension was significant (HR 1.18, 95%CI 1.11–1.26). BMI partially mediated the relationship between hyperuricemia and hypertension, which indicated a significant indirect effect (HR 1.09; 95%CI 1.08–1.10) and a significant

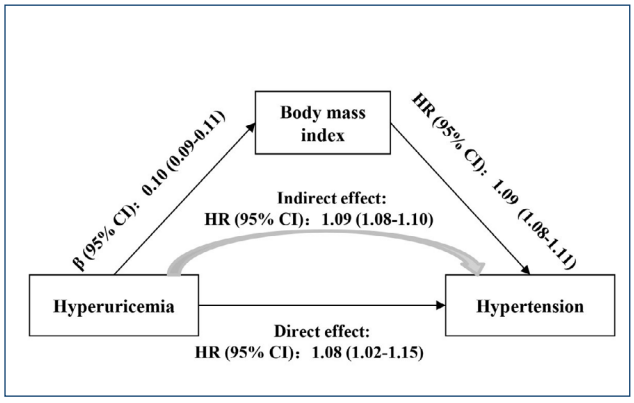

Figure 1. Mediation analysis to determine the relationship between hyperuricemia and hypertension through body mass index (kg/m²).

Table 2. Association between hyperuricemia and risk of incident hypertension.

	Whole cohort		Sensitivity analysis[d]		Sensitivity analysis[e]	
	Hazard ratio (95%CI)	P-value	Hazard ratio (95%CI)	P-value	Hazard ratio (95%CI)	P-value
Overall						
Model 1[a]	1.43 (1.34–1.52)	<0.001	1.44 (1.35–1.54)	<0.001	1.44 (1.31–1.59)	<0.001
Model 2[b]	1.15 (1.07–1.24)	<0.001	1.17 (1.09–1.27)	<0.001	1.19 (1.06–1.33)	0.003
Model 3[c]	1.10 (1.02–1.19)	0.018	1.11 (1.03–1.20)	0.007	1.14 (1.01–1.28)	0.032
Men						
Model 1[a]	1.34 (1.25–1.44)	<0.001	1.36 (1.27–1.46)	<0.001	1.40 (1.26–1.56)	<0.001
Model 2[b]	1.12 (1.03–1.22)	0.007	1.14 (1.05–1.24)	0.002	1.20 (1.06–1.36)	0.004
Model 3[c]	1.07 (0.98–1.16)	0.131	1.08 (0.99–1.18)	0.066	1.15 (1.01–1.31)	0.034
Women						
<50 years						
Model 1[a]	1.37 (1.27–1.47)	<0.001	1.37 (1.27–1.47)	<0.001	1.20 (1.10–1.31)	<0.001
Model 2[b]	1.26 (1.16–1.37)	<0.001	1.26 (1.16–1.37)	<0.001	1.29 (1.16–1.43)	<0.001
Model 3[c]	1.18 (1.07–1.29)	<0.001	1.18 (1.07–1.29)	<0.001	1.25 (1.11–1.40)	<0.001
≥50 years						
Model 1[a]	1.17 (1.09–1.27)	<0.001	1.19 (1.10–1.30)	<0.001	1.42 (1.27–1.59)	<0.001
Model 2[b]	1.12 (1.02–1.24)	0.017	1.13 (1.02–1.25)	0.018	1.23 (1.07–1.41)	0.003
Model 3[c]	1.12 (1.01–1.24)	0.031	1.13 (1.02–1.26)	0.023	1.16 (1.00–1.34)	0.052

CI: confidence interval; HR: hazard ratio. HR (95%CI) was presented in the Cox proportional model. [a]Model 1: adjusted for age at baseline. [b]Model 2: adjusted for age, heart rate, systolic blood pressure, fast plasma glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol, estimated glomerular filtration rate, serum albumin, and alanine aminotransferase at baseline. [c]Model 3: adjusted for BMI plus all the variables in model 2. [d]Sensitivity analysis 1: excluding participants aged >75 years at baseline (n=348). [e]Sensitivity analysis 2: excluding participants without hypertension that occurred in the first 2 years of follow-up (n=2,736).

direct effect (HR 1.08, 95%CI 1.02–1.15). The percentage of the mediation effect was 53.2% (95%CI 37.9–84.5).

Sensitivity analysis

Participants aged >75 years were then excluded due to a substantial potential risk of other comorbidities, but this sensitivity analysis did not obviously affect any of the associations (Table 2). In mediation analysis, the total effect of hyperuricemia on hypertension was significant (HR 1.20; 95%CI 1.13–1.27). BMI partially mediated the relationship between hyperuricemia and hypertension (indirect effect: HR 1.09; 95%CI 1.08–1.10; direct effect: HR 1.10; 95%CI 1.03–1.17). The percentage of the mediation effect was 49.1% (95%CI 36.1–75.6).

We conducted a sensitivity analysis by excluding participants who developed hypertension in the first 2 years of follow-up. This analysis showed a slightly attenuated effect of hyperuricemia on hypertension (Table 2). In mediation analysis, the total effect of hyperuricemia on hypertension was significant (HR 1.27; 95%CI 1.16–1.38). BMI partially mediated the relationship between hyperuricemia and hypertension (indirect effect: HR 1.08; 95%CI 1.07–1.10; direct effect: HR 1.17; 95%CI 1.07–1.29).

In general, the mean of menopause is 50 years[26]. In addition, we performed a subgroup analysis of women's age with a cutoff of 50 years. In a mediation analysis of a population of women <50 years of age, the total effect of hyperuricemia on hypertension was significant (HR 1.30; 95%CI 1.19–1.43). BMI partially mediated the association between hyperuricemia and hypertension (indirect effect: HR 1.18; 95%CI 1.07–1.30; direct effect: HR 1.10; 95%CI 1.07–1.13). The total effect of hyperuricemia on hypertension was significant when women were ³50 years of age (HR 1.16; 95%CI 1.04–1.32). BMI partially mediated the association between hyperuricemia and hypertension (indirect effect: HR 1.13; 95%CI 1.02–1.27; direct effect: HR 1.03; 95%CI 1.00–1.08).

DISCUSSION

This study showed that hyperuricemia was associated with an increased risk of incident hypertension in men and women. BMI partially mediated the association between hyperuricemia and incident hypertension.

SUA levels are different between men and women, but inconsistent findings were found regarding the sex-specific difference in this association. Our study showed a significant association between hyperuricemia and the risk of hypertension in both sexes, which is consistent with previous studies[9,11,13]. We also observed that sex did not modify the association between hyperuricemia and the risk of hypertension. This finding is inconsistent with a cohort study in the USA[14].

This study is the first to examine the mediation effect of BMI on the association between hyperuricemia and the development of hypertension. This finding is critically important to prevent or alleviate hyperuricemia-related hypertension.

The strengths of this study include a large sample size and adequate adjustment for a broad range of potential well-measured confounders. However, the limitations of our study should also be considered. Some confounders were not collected, such as smoking, alcohol drinking, physical activity, and a family history of hypertension, although we controlled for a series of well-measured cardiovascular risk factors. However, an experimental study[27] showed that the ingestion of fructose increased SUA concentrations and caused mitochondrial oxidative stress, thereby stimulating fat accumulation. A population-based study[20] used cross-lagged panel analysis to examine the temporal relationship between hyperuricemia and obesity. Future research is required using multiple time points to investigate the temporal relationship between hyperuricemia and obesity.

In conclusion, hyperuricemia is significantly associated with an increased risk of hypertension for both sexes, and obesity partially mediates the association between hyperuricemia and hypertension. However, the generalizability of our findings is limited because we studied a highly educated Chinese population. Future cohort studies in other ethnic populations are required to further confirm our findings.

DATA AVAILABILITY STATEMENT

Derived data supporting the findings of this study are available from the corresponding author [YLo] on request.

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AUTHORS' CONTRIBUTIONS

CW: Conceptualization, Methodology, Writing – review & editing. **PQ:** Conceptualization, Methodology, Writing – original draft. **YLi:** Formal Analysis, Visualization. **LW:** Formal Analysis, Software. **SX:** Supervision. **HC:** Methodology, Supervision. **SD:** Methodology, Project administration. **PZ:** Data curation. **FH:** Resources, Validation. **YLo:** Funding acquisition, Investigation, Resources.

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ORIGINAL ARTICLE

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Serum galectin-3 levels predict poor prognosis in sepsis and septic shock patients

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SUMMARY

OBJECTIVE: Sepsis and septic shock are clinical conditions with high mortality and an ever-increasing prevalence, and early diagnosis is of great importance in treating these diseases. Increase in serum Galectin-3 protein in septic patients is associated with increased inflammation, which in turn is associated with mortality. This study aimed to investigate the diagnostic importance of serum Galectin-3 levels and its relationship with in-hospital mortality in sepsis and septic shock patients.

METHODS: This prospective cohort study included 44 sepsis and 44 septic shock patients. Sequential Organ Failure Assessment score and Acute Physiology and Chronic Health Evaluation 2 score were calculated. In addition, routine clinical and laboratory parameters along with serum Galectin-3 were evaluated.

RESULTS: Serum Galectin-3 levels were significantly higher in the septic shock group [4.1 (0.1–10.2) vs. 6.0 (0.1–11.3) ng/mL, respectively; p=0.01]. Moreover, patients with a Galectin-3 level <6.94 ng/mL were associated with longer survival [31.4 vs. 23.1 days; hazards ratio, 1.85; 1.03–3.34, p=0.03]. More importantly, the need for mechanical ventilation, the duration of mechanical ventilation, and serum Galectin-3 levels were independent prognostic factors and predicted poor in-hospital survival in both sepsis and septic shock patients.

CONCLUSION: These findings suggest that Galectin-3 levels are higher in septic shock patients and predict mortality. In addition, high serum Galectin-3 levels, together with mechanical ventilation requirement and mechanical ventilation duration, are closely associated with poor in-hospital survival. Therefore, Galectin-3 may be a valuable diagnostic and prognostic biomarker in these patients.

KEYWORDS: Sepsis. Septic shock. Galectin-3.

INTRODUCTION

Sepsis, defined as an excessive immune response to infection, is a clinical condition with high mortality and an ever-increasing prevalence. Septic shock, on the contrary, is defined as tissue hypoperfusion and fluid-resistant hypotension that require vasopressors and is a more severe clinical condition than sepsis[1-3]. Early diagnosis and treatment are vital for sepsis and septic shock patients. Despite several studies reporting promising results with various biomarkers and scoring systems, it is still unclear which biomarker or scoring system is more functional in daily practice[4-6].

Galectins are beta-galactoside-binding lectins expressed in most living organisms and have critical functions in the immune system. In particular, the Galectin-3 protein is widely expressed in many cells and plays a role in cellular vital functions[7]. It is secreted from damaged and inflammatory cells in diseases, including heart diseases, various infectious diseases, and cancer[8-10]. Moreover, recent studies have reported that it is significantly increased in patients with sepsis and septic shock compared to other biomarkers and is associated with mortality[10,11]. However, the precise role of Galectin-3 in sepsis and septic shock patients has not been fully elucidated yet.

Studies investigating serum Galectin-3 levels in patients with sepsis and septic shock and its relationship with mortality are limited in the literature. Thus, in the present study, we aimed to investigate the importance of serum Galectin-3 levels and its relationship with in-hospital mortality in patients with sepsis and septic shock.

METHODS

Study population

This prospective cohort study enrolled 88 patients diagnosed with sepsis or septic shock in the intensive care unit of The Isparta City Hospital. The study consisted of 44 patients with sepsis and septic shock. Diagnoses of sepsis and septic shock were made according to the guidelines entitled, "the Third International Consensus Definition for Sepsis and Septic Shock

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(Sepsis-3): 2016[3]." The clinical criteria for septic shock were the need for vasopressor therapy to maintain a mean arterial pressure of 65 mm Hg or greater and a serum lactate level greater than 2 mmol/L persisting after fluid resuscitation. Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation (APACHE) 2 scores were calculated within the first day[3].

We excluded patients with a history of coronary artery disease, congenital heart disease, dysrhythmia, valvular heart disease, heart failure, peripheral arterial disease, hematological disorders, or a history of malignancy. The study was conducted according to the recommendations of the Declaration of Helsinki on biomedical research involving human subjects. It was approved by the Health Research Ethics Board at Süleyman Demirel University.

Blood sample collection

Blood samples were drawn from an antecubital vein by careful venipuncture without stasis before the diagnoses were made on the first day. Hematological indicators were measured within 30 min of collecting the blood samples in tubes containing dipotassium EDTA. Simultaneously, blood gas was taken from the radial artery for diagnosis and treatment. Biochemical analyses were performed with an Olympus AU-640 (Olympus Diagnostica, Hamburg, Germany). An automatic blood counter (Beckman-Coulter Co., Miami, FL, USA) was used for whole blood counts.

Measurement of galectin-3 levels

Serum Galectin-3 levels were measured with the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human Galectin-3. Samples (or standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human Galectin-3 and Avidin-Horseradish Peroxidase conjugate is added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain human Galectin-3, biotinylated detection antibody, and Avidin-Horseradish Peroxidase conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a stop solution, and the color turns yellow. The optical density is measured spectrophotometrically at a wavelength of 450±2 nm. The optical density value is proportional to the concentration of human Galectin-3. The concentration of human Galectin-3 was measured by comparing the optical density of the samples to the standard curve.

Statistical analysis

Data were analyzed using the SPSS software version 23.0 for Windows (SPSS, Chicago, IL, USA). Continuous variables were expressed as means±standard deviation or medians and 25th–75th percentile values (normally and non-normally distributed, respectively). To compare continuous variables, the Student's t-test or Mann-Whitney U test was used, as appropriate. Categorical variables were compared using the chi-square test. Using Cox's proportional hazards model, univariate and multivariate analyses for survival differences were performed. Survival was calculated from the diagnosis of the patient to either the date of death from any cause or the date of the last follow-up. Receiver operating characteristic curve analysis was used to determine the cutoff value for serum Galectin-3 levels (6.94 ng/mL). The median cumulative survival probability was calculated using the product-limit method of Kaplan-Meier. Differences in survival between groups were determined using the log-rank test. A p-value less than 0.05 was considered statistically significant.

RESULTS

Baseline demographic and clinical features were comparable between the two groups (Table 1). However, in the septic shock group, while the average heart rate was higher, systolic and diastolic blood pressures were lower. In addition, the need for a mechanical ventilator was significantly higher [24 (54%) vs. 38 (86%), respectively; p<0.01], and mechanical ventilator duration was significantly longer [9.4 (0–55) vs. 16.9 (0–62) day, respectively; p<0.01]. Moreover, the APACHE score (20.4±5.5 vs. 23.6±6.5, respectively; p=0.01) and SOFA score (8.8±2.6 vs. 11.2±3.1, respectively; p<0.01) were remarkably higher (Table 1).

Routine biochemical tests were generally comparable among the groups (Table 1). However, in the septic shock group, the serum C-reactive protein levels [13.4 (3.1–28.5) vs. 17.2 (7.0–28) mg/L, respectively; p<0.01] and lactate level measured from blood gas (2.0±1.1 vs. 3.2±1.9 mmol, respectively; p<0.01 mmol/L) were remarkably higher. Most importantly, serum Galectin-3 levels were significantly higher in the septic shock patients compared with the sepsis patients [4.1 (0.1–10.2) vs. 6.0 (0.1–11.3) ng/mL, respectively; p=0.01].

Survival and prognostic factors

At the last follow-up, the number of patients who died in the septic shock group was higher than in the sepsis group [29 (66%) vs. 20 (45%), p=0.04, Table 1]. In Kaplan-Meier analyses, survival was similar in sepsis and septic shock groups [28.9 vs. 28.2 days; hazards ratio (HR) 0.92; 95% confidence

Table 1. Comparison of demographic, clinical, and laboratory characteristics between the patients with sepsis and septic shock.

	Sepsis n=44	Septic shock n=44	p-value
Mean age, years	79±4	78±6	0.80
Male/female, n/n	26/18	22/22	0.52
Systolic BP, mmHg	112±13	63±4.5	**<0.01**
Diastolic BP, mmHg	65±11	42±3.9	**<0.01**
Heart rate, bpm	100±19	112±24	**<0.01**
Mechanical ventilation, n (%)	24 (54)	38 (86)	**<0.01**
Mechanical ventilation time, days	9.4 (0–55)	16.9 (0–62)	**<0.01**
Hospitalized time, days	17.9 (5–55)	21.8 (5–62)	0.16
SOFA score, n	8.8±2.6	11.2±3.1	**<0.01**
APACHE score, n	20.4±.5.5	23.6±6.5	**0.01**
Mortality, n (%)	20 (45)	29 (66)	**0.04**
Glucose, mg/dL	150±49	147±70	0.82
Creatinine, mg/dL	1.51 (0.3–9.1)	1.38 (0.2–3.4)	0.60
Hemoglobin, g/dL	10.3±2.1	10.0±2.2	0.58
WBC, $\times 10^3$/mL	13.3±6.6	13.6±6.8	0.84
Lymphocyte count, 10^3/mL	1.1 (0.2–4.1)	1.13 (0.1–3.9)	0.86
Neutrophil count, 10^3/mL	11.2 (2.1–27.6)	11.1 (1.5–25.8)	0.84
NLR	15.2 (3.2–89.0)	16.3 (0.3–149.2)	0.78
Procalcitonin, ng/mL	6.0 (0.1–68.2)	7.8 (0.1–94)	0.55
Albumin, g/dL	3.4 (1.6–28.0)	2.6 (1.6–3.8)	0.17
C-Reactive protein, mg/L	13.4 (3.1–28.5)	17.2 (7.0–28.0)	**<0.01**
Galectin-3, ng/mL	4.1 (0.1–10.2)	6.0 (0.1–11.3)	**0.01**
pH	7.40±0.10	7.40±0.09	0.56
PaO_2	69±22	69±18	0.87
$PaCO_2$	43±11	45±10	0.40
HCO_3	27±6	28±7	0.41
Lactate	2.0±1.1	3.2±1.9	**<0.01**

APACHE: acute physiology and chronic health evaluation; BP: blood pressure; HCO_3: bicarbonate; NLR: neutrophil/lymphocytes ratio; pH: acidity/alkalinity; PaO_2: partial pressure of oxygen in arterial blood; $PaCO_2$: partial pressure of carbon dioxide in arterial blood; SOFA: Sequential Organ Failure Assessment Score; WBC: white blood cells. Bold values indicate statistical significance at the p<0.05 level.

interval (CI) 0.51–1.62, p=0.76]. The patients with Galectin-3 level <6.94 ng/mL had prominently longer survival (31.4 vs. 23.1 days; HR 1.85; 95%CI 1.03–3.34, p=0.03, Figure 1).

Additionally, prognostic risk factors were evaluated by univariate analysis (Table 2). According to this analysis, the need for mechanical ventilation (MV, p=0.02), MV duration (p=0.002), neutrophil count (p=0.04), neutrophil-lymphocyte ratio (p=0.002), white blood cells (p=0.02), and serum Galectin-3 level (p=0.005) were significantly associated with survival. Subsequently, all significant prognostic factors were evaluated via multivariate analysis using Cox's proportional hazards model. The need for MV (HR 233; 95%CI 25–2198; p<0.001), MV time (HR 0.86; 95%CI 0.82–0.91; p<0.001), and serum Galectin-3 levels (HR 1.09; 95%Cl 1.01–1.19; p=0.03) were independent prognostic factors and predicted poor in-hospital survival in sepsis and septic shock patients. All multivariate survival analyses are presented in Table 2.

DISCUSSION

In septic shock patients, serum Galectin-3 levels were found to be significantly higher, and levels above 6.94 ng/mL were closely associated with poor in-hospital survival. In addition, the multivariate analysis identified serum Galectin-3 levels,

MV requirement, and MV duration as independent prognostic factors associated with in-hospital survival.

Early diagnosis is of great importance in treating sepsis and septic shock diseases. For this purpose, biomarkers such as procalcitonin, C-reactive protein, cytokine levels (such as IL-6, IL-8, and TNF), and scoring systems such as APACHE 2 and SOFA were studied and shown to be associated with mortality[4-6]. However, despite all these studies, which biomarker or scoring system to prefer in daily practice is still controversial[12]. Mammalian galectins can be found in intracellular and extracellular spaces[7,13]. While the extracellular ones are involved in many extracellular processes such as inflammation and cell-cell communication, the intracellular ones participate in various cellular functions such as anti-apoptosis and cell cycle control. In addition, they take part in critical processes such as cell differentiation, host defense, inflammation, and fibrogenesis[7,13]. Studies in recent years have shown that Galectin-3 is not related to age or body mass index, does not show a circadian rhythm, and increases with exercise but returns to normal after a while[7,13]. A recent study showed that Galectin-3 levels were significantly higher in rats with sepsis due to endotoxemia and played a critical role in the development of systemic inflammation, which is the most important component of the pathophysiology of sepsis[14]. In another study, Ferreira et al. investigated the change in serum Galectin-3 concentrations in mice with sepsis and septic shock induced by cecal ligation and puncture. Similar to our study, they found that serum Galectin-3 levels were significantly higher in the septic shock group than those in the sepsis group. They showed that increased serum Galectin-3 levels in septic rats prevented neutrophil migration to the focus of infection, promoted bacterial spread, and worsened the outcome of sepsis, while Galectin-3 deficiency reduced sepsis-induced organ dysfunction. Moreover, they indicated that these data from rat models are compatible with humans and that high serum Galectin-3 levels are associated with the severity of sepsis, suggesting a new potential biomarker that may be valuable for early diagnosis[15]. Similar to these results, in our study, Galectin-3 levels were significantly higher in septic shock patients than those in sepsis patients and were associated with in-hospital mortality. Likewise, another study reported increased levels of extracellularly released Galectin-3 in the lungs of mice with fatal pulmonary infections with the Francisella novicida strain. They reported that Galectin-3 has immune-modulatory properties such as induction of pro-inflammatory cytokines, immune cell chemotaxis, and regulation of cell death[16,17]. Similar to animal studies, human studies have reported that Galectin-3 is secreted from damaged and inflammatory cells and is an important regulator of the inflammatory response and immune system in heart patients, various infectious patients, and cancer patients, which are associated with poor prognosis. Moreover, it has been suggested as a diagnostic or prognostic marker[7-10,18]. Another study investigated the prognostic value of biomarkers, including presepsin, procalcitonin, and sST2, along with Galectin-3, in sepsis patients. In particular, serum Galectin-3 levels were found to predict 30-day mortality better than the SOFA score and procalcitonin. It has even been argued that the combined use of these markers is more beneficial for the

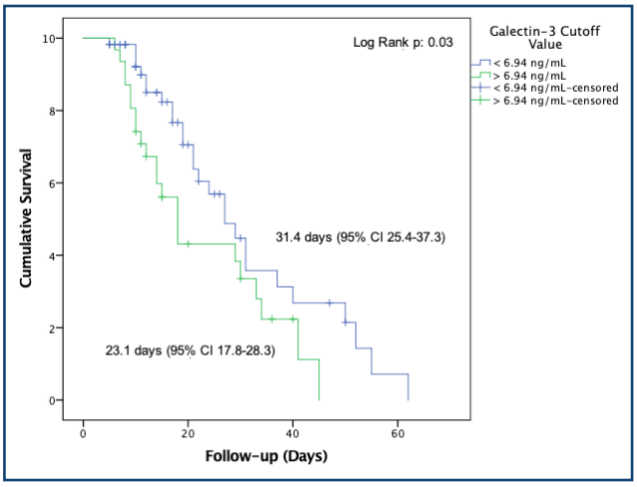

Figure 1. Kaplan-Meier median overall survival curves reflect the difference in survival rates relative to the cutoff galectin-3 values in sepsis and septic shock patients.

Table 2. Results of univariate and multivariate Cox's proportional hazard models regarding overall survival.

Characteristics	Univariate analysis		Multivariate analysis	
	OS HR (95%CI)	p-value	OS HR (95%CI)	p-value
MV	10.4 (1.4–76.1)	0.02	233 (25–2198)	<0.001
MV duration	0.96 (0.94–0.98)	0.002	0.86 (0.82–0.91)	<0.001
Neutrophil count	1.05 (1.00–1.11)	0.04		
NLR	1.02 (1.01–1.03)	0.002		
WBC	1.06 (1.00–1.11)	0.02		
Galectin-3 level	1.09 (1.01–1.18)	0.02	1.09 (1.01–1.19)	0.03

MV: mechanical ventilation; NLR: neutrophil/lymphocyte ratio; OS: overall survival; WBC: white blood cell.

prediction of prognosis[11]. Similarly, in our study, Galectin-3 levels were closely associated with in-hospital mortality in patients with sepsis and septic shock, whereas serum procalcitonin levels, SOFA, and APACHE 2 scores were not. Studies have shown that many sepsis-causing microorganisms contribute to the sepsis process through Galectin-3[19-21].

Several study limitations should be considered. The statistical power of the study may have decreased due to the limited number of patients. Our study does not provide information associated with the long-term results due to the short period of patient follow-up. Only one blood sampling was performed due to the cost. The relationship between serum Galectin-3 and the severity of the disease could be evaluated more clearly by taking multiple blood samples at certain intervals during the course of the disease.

CONCLUSION

Our study showed that serum Galectin-3 levels are higher in septic shock patients than in sepsis patients, and serum levels above 6.94 ng/mL are particularly associated with mortality. Moreover, serum Galectin-3 levels, as well as mechanical ventilator requirement and duration, were closely associated with in-hospital survival. Therefore, we think that Galectin-3 may be a valuable biomarker for early diagnosis and identification of patients with poor prognosis in this disease whose treatment is still not clarified.

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ORIGINAL ARTICLE

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Neuropathy in multiple sclerosis patients treated with teriflunomide

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SUMMARY

OBJECTIVE: Teriflunomide is an oral medication approved for the treatment of patients with multiple sclerosis. The primary effect of teriflunomide is to reduce de novo pyrimidine synthesis by inhibiting mitochondrial dihydroorotate dehydrogenase, thereby causing cell-cycle arrest. We aimed to investigate the occurrence of peripheral neuropathy, a rare side effect of teriflunomide, in patients receiving teriflunomide.

METHODS: Multiple sclerosis patients receiving teriflunomide (n=42) or other disease modifying therapies (n=18) and healthy controls (n=25) were enrolled in this cross-sectional study between January 2020 and 2021. The mean duration of teriflunomide treatment was 26 months (ranging from 6 to 54 months). All participants underwent neurological examination and nerve conduction studies of tibial, peroneal, sural, superficial peroneal, median, and ulnar nerves by using surface recording bar and bipolar stimulating electrodes.

RESULTS: The mean superficial peroneal nerve distal latency and conduction velocity were significantly slower, and the mean superficial peroneal nerve action potential amplitude was lower in patients using teriflunomide (2.50 ms, p<0.001; 47.35 m/s, p=0.030; and 11.05 μV, p<0.001, respectively). The mean peroneal motor nerve distal latency was significantly longer and amplitude was lower in teriflunomide patients (3.68 ms, p<0.001, and 5.25 mV, p=0.009, respectively). During the study period, treatment switching to another disease-modifying therapy was planned in 10 patients, and all neuropathic complaints were reversed after switching.

CONCLUSION: Teriflunomide has the potential to cause peripheral neuropathy. The awareness of peripheral neuropathy, questioning the symptoms, and if suspected, evaluation with electromyography and switching the therapy in patients under teriflunomide treatment are crucial.

KEYWORDS: Multiple sclerosis. Teriflunomide. Electromyography. Nerve conduction. Peripheral neuropathy. Neuropathic pain.

INTRODUCTION

Teriflunomide is the active metabolite of leflunomide that has been used for treating rheumatoid arthritis and psoriatic arthritis for years[1]. Teriflunomide is the second oral disease-modifying therapy (DMT) that was approved for the treatment of adult patients with relapsing-remitting multiple sclerosis (MS) in 2012[2]. It affects the metabolism of pyrimidines by selectively and reversibly inhibiting dihydroorotate dehydrogenase, which is the rate-limiting mitochondrial enzyme for the de novo pyrimidine synthesis[1]. The restriction in pyrimidine synthesis reduces the proliferation of activated T and B cells, thereby controlling the inflammation in the central nervous system[3]. The effect of teriflunomide on inflammation is not only through pyrimidine metabolism. Li et al. showed that teriflunomide considerably decreases the release of some pro-inflammatory cytokines [i.e., interleukin-6 (IL-6), IL-8, and monocyte chemotactic protein-1] from peripheral blood mononuclear cells and monocytes by a different mechanism other than dihydroorotate dehydrogenase-dependent pathway[4].

The most common adverse events (AEs) reported in patients receiving teriflunomide were diarrhea, nausea, increased alanine aminotransferase, and alopecia (hair thinning). Furthermore, peripheral neuropathy (both polyneuropathy and mononeuropathy) was also reported as a rare AE[2]. A previous study has shown that the incidence of peripheral neuropathy is higher (13 of 1002 patients) in patients under teriflunomide treatment compared to the control group (1.4 and 0.4%, respectively)[2]. If peripheral neuropathy is suspected and confirmed as a consequence of teriflunomide treatment, discontinuation of teriflunomide is recommended[2].

People with MS could have co-occurring neuropathy, either as a consequence of autoimmune mechanism other than the autoimmunity causing MS or secondary to some other factors such as vitamin deficiency, malnutrition, immobilization, drug usage, systemic disease, and so on. Although MS is primarily thought to be a central demyelinating disease, some studies showed peripheral demyelinating neuropathies in MS patients[5-7]. Misawa et al. showed demyelinating features in 3 of 60 patients' nerve conduction studies (NCSs)[7]. The medications used to treat MS can also trigger the occurrence of peripheral neuropathy. Axonal or demyelinating neuropathy with interferon treatment has been reported in several studies[8,9]. Diagnosing

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neuropathies may be difficult or sometimes under-diagnosed as sensory and motor symptoms of MS can mimic or resemble neuropathic complaints. Being aware of patients' signs and recognition are critical as some neuropathies are treatable and preventable. Peripheral neuropathy is a rare AE of teriflunomide. In this study, we aimed to investigate the occurrence of peripheral neuropathy in patients receiving teriflunomide with objective NCSs.

METHODS

This cross-sectional study was conducted between January 2020 and 2021 at a tertiary referral hospital. Patients aged between 18 and 65 years with definite MS diagnosis according to the McDonald criteria 2017, followed at our MS outpatient clinic, as well as healthy appearing controls were recruited for the study. Patients having a high degree of disability [Expanded Disability Status Scale (EDSS) score higher than 5.5 or requiring a walking aid to walk about 100 meters] or weakness and history of a disease that can cause neuropathy (i.e., rheumatoid arthritis, hypothyroidism, diabetes mellitus, systemic sclerosis, alcohol abuse, etc.) were excluded. Informed consent was obtained from all the participants, and the study protocol was approved by the local ethics committee, conforming to the ethical guidelines of the 1975 Declaration of Helsinki (Decision Number: 514/192/6). A total of 42 patients receiving teriflunomide (group 1), 18 patients receiving other DMTs (group 2), and 25 controls were recruited for the study. The mean duration of teriflunomide treatment in group 1 was 26 months (ranging from 6 to 54 months). After the neurological examinations of the patient group, clinical information and demographic data, vitamin B12, fasting blood glucose, glycosylated hemoglobin (hemoglobin A1c, HbA1c) levels were noted, and EDSS scores were calculated. Demographic and clinical data of the controls were also noted. Finally, the participants were referred to the electromyography (EMG) laboratory for electrodiagnostic evaluation.

We performed NCS using an EMG device (Nihon Kohden Corporation Neuropack® X1) with surface recording bar and bipolar stimulating electrodes. All participants underwent routine NCS of tibial and peroneal motor nerves, sural and superficial peroneal sensory nerves in lower extremities, and motor and sensory branches of median and ulnar nerves on non-dominant side in upper extremities. Motor distal latency, conduction velocities (CVs), compound muscle action potential (CMAP) amplitudes and F-wave latencies of motor nerves, and sensory CV and sensory nerve action potential (SNAP) amplitudes of sensory nerves were measured. Sensory NCS were performed antidromically. Normative data for electrophysiological studies were compared to 25 healthy controls. For amplitude and velocity, lower limits (5th percentile) were used and, for latency parameters, upper limits (95th percentile) were used.

For statistical analysis, categorical variables were defined using percentages and continuous variables using mean (standard deviation) (SD) or median and interquartile ranges. Chi-square test was used for categorical variables. Student's t-test or analysis of variance (ANOVA) was used if normally distributed for continuous variables, and Mann-Whitney U or Kruskal-Wallis test was used if not normally distributed. The analyses were performed using IBM SPSS, version 20 (Statistical Package for Social Sciences, Chicago, IL).

RESULTS

DMTs other than teriflunomide were interferon beta-1a, glatiramer acetate, dimethyl fumarate, fingolimod, and ocrelizumab, and two of the patients were followed up without receiving any DMT. The age and EDSS values of the patients varied between 24 and 56 years and 0–5.5, respectively. There was no significant difference between these three groups in terms of gender, age, vitamin B12 level, vitamin D level, fasting blood glucose, and HbA1c value. A detailed analysis is given in Table 1. The disease duration [mean (SD)] was 69.7 (48.2) months in group 1 and 84.2 (44.5) months in group 2, and there was no significant difference between groups (p=0.229). The median EDSS value (minimum-maximum) of patients was 2 (0–5).

In group 1, neuropathic complaints described by patients were as follows: seven patients had numbness, one patient had allodynia, and one patient had burning sensation. With neurological examination, hypoesthesia of gloves-socks type and hyporeflexia were detected in nine patients. No patient from the other two groups complained about having sensory symptoms.

Superficial peroneal SNAP amplitude could not be obtained in one patient in group 1 and one control. The patient had hypoesthesia of gloves-socks type. Carpal tunnel syndrome was found in five patients in group 1, one patient in group 2, and five controls. When patient groups and the controls were compared according to sensory nerves (median, ulnar, sural, and superficial peroneal), there was no statistical difference in the analysis of median, ulnar, and sural sensory nerves. The mean values of the superficial peroneal nerve assessment were significantly different in all three groups. After post-hoc analysis, the mean value of superficial peroneal nerve distal latency was significantly longer in group 1 compared to group 2 and controls, and it was also significantly longer in group 2 compared to controls (2.50, 2.09, and 1.69 ms, respectively, p<0.001).

The mean superficial peroneal SNAP amplitude was lower in group 1 than in group 2 and controls (11.05, 15.61, and 19.45 μV, respectively, p<0.001). The mean superficial peroneal SNAP amplitude was lower in group 2 than controls, but there was no significant difference. The mean superficial peroneal nerve CV value was significantly slower in group 1 than controls (47. 35 and 58.70 m/s, respectively, p=0.030). The mean CV of group 2 was slower than controls, but it was not significant. A detailed analysis is given in Table 2.

When patient groups and the controls were compared according to motor nerves (median, ulnar, tibial, and peroneal), only mean peroneal nerve distal latency and amplitude were significantly different between the three groups. With post-hoc analysis, the mean value of peroneal nerve distal latency was significantly longer in group 1 compared to group 2 and controls (p<0.001). The mean amplitude of the peroneal nerve was significantly lower in group 1 than controls (p=0.009) (Table 3).

Table 1. Demographic and clinical characteristics of the patient and control groups.

	Group 1	Group 2	Control group	p-Value
Number of participants	42	18	25	
Gender (female/male)	35/7	15/3	19/6	
Age	40.57 (9.60)	38.83 (0.69)	40 (0.83)	0.799
Medication (number of patients)	Teriflunomide:42	Interferon beta-1a: 2 Glatiramer acetate: 1 Dimethyl fumarate: 6 Fingolimod:6 Ocrelizumab: 1 None: 2		
Vitamin B12 (pg/mL)	273 (204–394)	264.5 (185.75–351.25)	223 (203–317.25)	0.569
Vitamin D (ng/mL)	20.87 (14.63–34)	27.57 (18.28–29.94)	20 (13.88–23.30)	0.218
Fasting blood glucose (mg/dL)	94.5 (84.25–101)	85.5 (83.25–109.5)	95 (90–101.5)	0.346
HbA1c (%)	5.30 (5.0–5.30)	5.10 (4.7–5.50)	5.15 (4.90–5.45)	0.129

Data are shown as mean (standard deviation) or median (interquartile intervals). pg/mL: picograms per milliliter; ng/mL: nanograms per milliliter; mg/dL: milligrams per deciliter; HbA1c: hemoglobin A1C.

Table 2. Comparison of sensory nerve conduction results in the patient and control groups.

Sensory nerve	Parameter	Group 1	Group 2	Control	p-Value	LL/UL of normal (5th or 95th percentile)
Median	Distal latency (ms) Amplitude (μV) Velocity (m/s)	2.46 (0.31) 31.86 (9.75) 53.38 (5.92)	2.27 (0.26) 37.81 (10.96) 53.89 (6.67)	2.42 (0.23) 34.44 (12.84) 53.88 (5.80)	0.067 0.159 0.928	2.90 16.59 42.09
Ulnar	Distal latency (ms) Amplitude (μV) Velocity (m/s)	1.97 (0.30) 28.22 (14.09) 57.41 (10.78)	1.88 (0.21) 36.21 (10.65) 61.35 (6.04)	2.03 (0.22) 32.38 (12.29) 58.26 (5.75)	0.299 0.083 0.275	2.52 24.66 47.06
Sural	Distal latency (ms) Amplitude (μV) Velocity (m/s)	2.44 (0.29) 16.14 (4.99) 58.01 (6.66)	2.48 (0.48) 19.67 (8.05) 58.42 (9.95)	2.47 (0.41) 18.24 (6.81) 57.42 (8.72)	0.890 0.115 0.919	3.53 8.92 43.64
Superficial peroneal	Distal latency (ms) Amplitude (μV) Velocity (m/s)	2.50 (0.41)*,+ 11.05 (6.11)*,+ 47.35 (19.35)*	2.09 (0.29)* 15.61 (6.49) 54.62 (15.36)	1.69 (0.30) 19.45 (5.57) 58.70 (13.95)	**<0.001** **<0.001** **0.030**	2.24 12.49 48.2

Bold p-values are statistically significant. LL, lower limit (for amplitude and velocity); UL, upper limit (for latency parameters). *Data are expressed as mean (SD). *p<0.05 compared with the control group. +p<0.05 compared with group 2.

Table 3. Comparison of motor nerve conduction results in the patient and control groups.

Motor nerve	Parameter	Group 1	Group 2	Control	p-Value	LL/UL of normal (5th or 95th percentile)
Median	Distal latency (ms) Amplitude (mV) Velocity (m/s) Minimum F-latency (ms)	2.87 (0.41) 13.92 (3.53) 59.18 (5.75) 24.07 (2.04)	2.77 (0.33) 14.83 (3.46) 60.73 (7.85) 23.69 (2.20)	2.96 (0.48) 15.19 (4.00) 59.83 (4.95) 24.12 (2.06)	0.334 0.355 0.659 0.790	3.95 8.6 51.3 27.7
Ulnar	Distal latency (ms) Amplitude (mV) Velocity (m/s)	2.14 (0.48) 14.32 (4.22) 62.68 (13.00)	2.14 (0.33) 14.77 (3.71) 67.40 (6.96)	2.19 (0.34) 13.31 (2.55) 63.26 (8.01)	0.881 0.396 0.280	2.87 9.2 48.3
Tibial	Distal latency (ms) Amplitude (mV) Velocity (m/s) Minimum F-latency (ms)	4.14 (0.80) 11.04 (3.86) 48.51 (5.07) 45.10 (3.79)	3.88 (0.93) 12.65 (4.75) 50.97 (6.96) 44.60 (4.48)	3.83 (0.60) 12.55 (4.01) 49.32 (5.37) 42.91 (2.94)	0.226 0.226 0.300 0.077	4.78 5.18 41.7 50.7
Peroneal	Distal latency (ms) Amplitude (mV) Velocity (m/s)	3.68 (0.59)*,+ 5.25 (1.69)* 53.20 (5.00)	2.95 (0.57) 6.02 (2.08) 54.71 (5.58)	2.95 (0.58) 6.72 (1.90) 55.68 (5.12)	**<0.001** **0.009** 0.156	4.33 3.81 51.6

Bold p-values are statistically significant. LL, lower limit (for amplitude and velocity); UL, upper limit (for latency parameters). *Data are expressed as mean (SD). *p<0.05 compared with the control group. +p<0.05 compared with group 2.

Superficial peroneal SNAP amplitude in 15 patients, superficial peroneal sensory CV in 25 patients, and peroneal CMAP amplitude in 11 patients were lower in group 1 (n=42) when compared with controls (normative data). Furthermore, peroneal nerve distal latency in 37 patients and superficial peroneal nerve distal latency in 11 patients were longer in group 1 according to the normative data. When we classified the patients according to the history of myelitis (n=24) or not (n=67), regardless of groups, only mean peroneal nerve distal latency was significantly higher in patients with myelitis than in patients without myelitis (p=0.039).

During the study period, treatment switching was planned in 10 patients (one patient for breakthrough disease activity and nine patients for the development of neuropathic complaints). Teriflunomide was switched to fingolimod (n=1), dimethyl fumarate (n=6), or ocrelizumab (n=1). The treatment could not be switched in two patients who did not come to regular follow-up.

DISCUSSION

In this study, both axonal and demyelinating features were found in sensory and motor NCS of patients receiving teriflunomide. The mean superficial peroneal nerve distal latency was longer and CV was slower, and the mean superficial peroneal nerve SNAP amplitude was lower in patients receiving teriflunomide in sensory NCS. The mean peroneal nerve distal latency was longer, and CMAP amplitude was lower in patients receiving teriflunomide in motor NCS.

The diagnosis of polyneuropathies in people with MS is challenging due to the accompanying neurological complaints related to brain and spinal cord lesions. It is important to distinguish whether neuropathic complaints are due to MS pathology or as a result of a concomitant polyneuropathy. In this instance, electrodiagnostic studies can be beneficial. With the help of NCS, neuronal damage can be demonstrated objectively in patients with suspicious neuropathy. Peripheral neuropathy in MS could be divided into two categories as MS with chronic inflammatory demyelinating polyradiculoneuropathy (MS-CIDP, 21%) and MS with other non-inflammatory polyneuropathies (mainly axonal, 79%)[10]. MS CIDP may be the coexistence of two different diseases; however, there are different opinions on this issue. MS-CIDP can be due to a similar immunopathogenesis, common antigen between central and peripheral nervous system, or a consequence of immunomodulatory treatment. The common antigens between central and peripheral nervous systems could be myelin basic protein, myelin-associated glycoprotein, or neurofascin[11-13]. Both axonal and demyelinating polyneuropathies have been indicated after interferon beta treatment in MS patients[8,9]. In a study conducted with relapse remitting MS patients, Gorgulu et al. found demyelinating type changes in motor NCS[14]. We found both axonal and demyelinating findings in sensory and motor NCS in MS patients treated with teriflunomide. The findings indicating demyelination were as follows: the mean value of superficial peroneal nerve distal latency was significantly longer; the mean superficial peroneal nerve CV was significantly slower; and peroneal nerve distal latency was significantly longer

in group 1. None of the patients with demyelinating NCS findings met the European Academy of Neurology/Peripheral Nerve Society criteria for CIDP. The mean superficial peroneal sensory SNAP amplitude and the mean peroneal CMAP amplitude were significantly lower in group 1, and these results suggest axonal loss. We could not obtain superficial peroneal SNAP amplitude in one patient in group 1 (indicating axonal loss), and with the suspicion of neuropathy clinically, teriflunomide therapy was discontinued and switched to another DMT. In this study, we observed abnormalities in NCS of peroneal motor nerve and superficial peroneal sensory nerve; however, we did not find any significant abnormality with the sural nerve. The sural nerve forms from the terminal branches of the tibial nerve and common peroneal nerve. Sumner showed that larger-diameter myelinated fibers are more durable against neurotoxicity than smaller-diameter fibers. Furthermore, the areas of compression sites increase the risk of neuronal damage as a consequence of impaired blood nerve barrier[15]. Among them, fibular head is the most common area for common peroneal nerve entrapment at the lower limbs[16].

In our study, peroneal nerve distal latency was significantly longer in patients with myelitis, but this could be affected by the presence of central (spinal) lesions. Axons from the motor neurons in the ventral gray matter of the spinal cord constitute the motor roots and fibers in the peripheral nerves. Therefore, any lesion of the primary motor neuron can result in degeneration of motor fibers throughout the peripheral nerve. By this way, a spinal cord lesion can result in abnormalities on motor NCSs. Additionally, F-wave responses could be longer in MS patients[17]. In our study, we evaluated F-wave responses of tibial and median motor nerves, but we could not find any significant difference.

Our study had some limitations, of which small sample size is the most important one. Another limitation is that patients with spinal lesions were not excluded. We did not evaluate the presence of plexopathy or radiculopathy that might affect NCS. The strength of our study is that, to the best of our knowledge, this is the first study in the literature investigating the development of peripheral neuropathy in MS patients receiving teriflunomide with objective NCS findings in daily practice. Further comprehensive studies with larger sample size are needed to investigate the development of neuropathy in patients using teriflunomide.

CONCLUSIONS

Teriflunomide is a widely used agent known to have the potential to cause peripheral neuropathy in the treatment of MS. Our findings support the neuropathy AE of teriflunomide as stated in previous studies. The awareness of possible peripheral neuropathy AEs in patients treated with teriflunomide and, in the follow-up, questioning the symptoms or signs indicative of peripheral neuropathy are important for treatment planning. If neuropathy is suspected, evaluation with EMG and switching the therapy should be considered.

ETHICAL STATEMENT

The author declares that the research has been conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," and the study protocol was approved by the local ethics committee (Decision Number: 514/192/6).

INFORMED CONSENT

Informed consent was obtained from all participants.

AUTHORS' CONTRIBUTIONS

AKK: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing. **AAS:** Data curation, Resources, Software, Validation, Writing – original draft, Writing – review & editing. **AB:** Data curation, Validation, Visualization. **GS:** Data curation, Investigation, Resources, Software.

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ORIGINAL ARTICLE

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Comparison of the fibrosis degree using acoustic radiation force impulse elastography and diffusion-weighted magnetic resonance imaging in chronic hepatitis cases

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the correlation of fibrosis stages in cases of chronic hepatitis by comparing shear wave elastography and diffusion-weighted magnetic resonance imaging.

METHODS: A total of 46 chronic hepatitis patients with an age range of 20–50 years were classified into three groups based on their fibrosis stages. Comparison group 1: the presence of fibrosis (S0 and S1≤); comparison group 2: the presence of significant fibrosis (≤S2 and S3≤); and comparison group 3: the presence of cirrhosis (≤S4 and S6). Shear wave velocities were measured by acoustic radiation force impulse elastography. Diffusion-weighted magnetic resonance imaging was performed on a 3.0 Tesla MRI device.

RESULTS: In comparison group 1 (S0 and S1≤), the area under the curve, sensitivity, and specificity of acoustic radiation force impulse values were 0.784, 87, and 60%, respectively, while these values were 0.718, 80, and 66%, respectively, for apparent diffusion coefficient . In comparison group 2 (≤S2 and S3≤), the area under the curve, sensitivity, and specificity of acoustic radiation force impulse values were 0.917, 80, and 86%, respectively, and the apparent diffusion coefficient values were 0.778, 90, and 66%, respectively. In comparison group 3, the area under the curve, sensitivity, and specificity of acoustic radiation force impulse values were 0.977, 100, and 95%, respectively. There was no statistically significant difference between the apparent diffusion coefficient values of the cases in the three groups (p=0.132).

CONCLUSION: Noninvasive methods are gaining importance day by day for staging hepatic fibrosis. Acoustic radiation force impulse elastography was evaluated as a more reliable examination than diffusion-weighted magnetic resonance imaging in revealing the presence of fibrosis, determining significant fibrosis, and diagnosing cirrhosis.

KEYWORDS: Hepatitis. Elastography. ARFI imaging. Diffusion weighted MRI.

INTRODUCTION

Hepatic fibrosis occurs as a result of chronic liver diseases. In response to liver injury, hepatic lobules collapse, fibrous septa form, and hepatocyte regeneration nodules form. Fibrosis, for which acute pathologies are reversible, progresses to portal hypertension and cirrhosis. Factors that cause liver fibrosis are viral hepatitis (B, C, and D), metabolic causes (hemochromatosis, alpha-1 antitrypsin deficiency, Wilson disease, galactosemia, tyrosinemia, and type IV glycogen storage disease), hepatic venous obstruction, toxins and drugs (alcohol, amiodarone, methotrexate, etc.), primary biliary cirrhosis, autoimmune hepatitis, helminths (schistosomiasis), cryptogenic cirrhosis, and nonalcoholic steatohepatitis, which is a risk factor for the development of hepatocellular carcinoma[1-4]. The incidence of hepatocellular carcinoma increases in patients who develop fibrosis and cirrhosis[5,6]. Additionally, recent studies have reported that

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nonalcoholic fatty liver disease may be a potential risk factor for the development of hepatocellular carcinoma[6,7]. Noninvasive fibrosis grading imaging techniques have recently gained much attention, as advanced diagnostic tools are important in slowing the fibrosis development process and therefore in mitigating the incidence of hepatocellular carcinoma.

Ultrasound (US) is generally the preferred imaging method for radiological evaluation of the liver. US elastography plays an important role in the characterization of diffuse liver diseases and focal liver lesions[8].

Acoustic radiation force impulse (ARFI) is an up-to-date method that has been widely used in recent years. It provides information for tissue characterization according to the response of tissues to applied force. The fact that US elastography can be used to image tissues was first demonstrated in 1987[9,10].

Diffusion-weighted magnetic resonance imaging (DW-MRI) can provide additional information about many pathologies in addition to conventional MRI sequences.

Determining the stage of fibrosis development in chronic hepatitis patients is of great importance in terms of preventing the progression to cirrhosis with appropriate treatment methods and determining the response to the treatment applied. Today, percutaneous liver biopsy remains the gold standard diagnostic test for staging fibrosis. Therefore, this study aimed to investigate the correlation between fibrosis stages in cases of chronic hepatitis by comparing shear wave elastography and DW-MRI.

METHODS

Patient selection

Between September 2014 and May 2015, 46 patients (40 males and 6 females) who were followed up with after receiving a diagnosis of histopathologically proven viral hepatitis (HBV and HCV), steatohepatitis, or autoimmune hepatitis across the various clinics of Health Sciences University, Gulhane School of Medicine, were evaluated prospectively. US examinations, measurement of ARFI values, and DW-MRI examinations were conducted for all patients at least 1 week before biopsy. The patients were classified into three groups as follows: comparison group 1, presence of fibrosis (S0 and S1≤); comparison group 2, presence of significant fibrosis (≤S2 and S3≤); and comparison group 3, presence of cirrhosis (≤S4 and S6).

Approval for this study was obtained from the Institutional Ethical Review Board (dated May 30, 2014, decision no. 8000-259-14/1560), and the study followed the tenets of the Declaration of Helsinki.

Acoustic radiation force impulse elastography analysis

B-mode US and ARFI elastography measurements were evaluated on a Siemens Acuson S3000 device (Siemens Medical Solutions, Mountain View, CA, USA) using a 6C1 HD convex probe with Virtual Touch Quantification (VTQ) software.

The values were obtained in m/s units using a 10×5 mm region of interest (ROI) placed at least 2 cm away from the Glisson capsule. The median values of 10 shear wave velocities measured were used (Figure 1A).

Magnetic resonance imaging protocol

A Sense-XL Torso coil in a 3.0 Tesla (T) superconductive MR (Philips 3T Achieva Release 3.2.3.0) was used. DW images were obtained using the following parameters: eco-planar spin echo, the fat suppression technique, and breath hold: 1241/52 repetition time (TR)/echo time (TE); 90° flip angle; 375×302×255 mm field of view (FOV); 124×100 matrix; and 7 mm thickness. The acquisition time for the images was 3 min and 12 s. Diffusion gradient b values of 0, 100, and 800 were used in DW-MRI. These values were applied to minimize diffusion anisotropy in three orthogonal directions (x, y, and z).

The images obtained were transferred to a separate workstation (DynaCAD Version 2.1.6), and measurements from apparent diffusion coefficient (ADC) maps were made at this station (Figure 1B).

Magnetic resonance imaging analysis

ADC values were measured using circular ROIs of approximately 1 cm^2 from the right liver lobe. ADC values were measured in mm^2/s from the right liver lobe parenchyma. ROIs at least 1 cm away from the Glisson capsule, where there were no main vascular structures, focal lesions, or artifacts, were placed on ADC maps simultaneously.

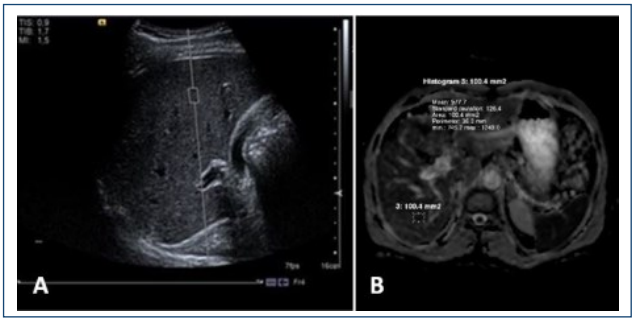

Figure 1. Placement of region of interest in acoustic radiation force impulse elastography (A) and apparent diffusion coefficient value measurement (B).

Evaluation of liver biopsy

The Ishak scoring system[11] was used to stage fibrosis. Accordingly, stage (S)0 was evaluated as no fibrosis, S1–S2 as mild fibrosis, S3–S4 as moderate fibrosis, and S5–S6 as advanced-stage fibrosis[11].

Statistical analysis

SPSS for Windows version 15.00 (SPSS Inc., Chicago, IL, USA) was used. The t-test and the Fisher-corrected chi-squared test were used to determine intergroup differences. For each group, the area under the curve (AUC), according to the ARFI and ADC values, was measured at a 95% confidence interval (CI). p-Values less than 0.05 were considered statistically significant.

RESULTS

The mean age of the 46 patients was 34.52±9.6 years, and the mean body mass index (BMI) was 25.226±3.27. The disease distribution and fibrosis stages are shown in Table 1.

The comparison of patients in group 1 was performed to detect the presence of fibrosis between patients with S0 fibrosis and patients with fibrosis at any stage (S1≤). ARFI elastography was determined to be more effective than DW-MRI in detecting any stage of fibrosis. When the optimal cutoff value for ARFI elastography was determined to be 1.25 m/s in separating the two groups, the sensitivity was 87%, and the specificity was 60%. When the optimal cutoff value for DW-MRI was determined to be 1.110×10^{-3} mm²/s, the sensitivity was 80%, and the specificity was 66%.

Comparison group 2 featured patients with S0–S2 (≤S2) fibrosis and was compared to patients with S3–S6 (S3≤) fibrosis to reveal the presence of significant fibrosis. ARFI elastography was determined to be more effective than DW-MRI in diagnosing significant fibrosis. When the optimal cutoff value for ARFI elastography was determined to be 1.52 m/s in separating the two groups, the sensitivity was 80%, and the specificity was 86%. When the optimal cutoff ADC value for DW-MRI was determined to be 1.063×10^{-3} mm²/s, the sensitivity was 90%, and the specificity was 66%.

Comparison group 3 was created to compare patients with S0–S4 (S4≥) fibrosis and patients with S5 and S6 fibrosis (pre-cirrhosis+cirrhosis) to distinguish cirrhosis cases. ARFI elastography was determined to be more effective than DW-MRI in diagnosing cirrhosis. In separating the two groups, when the optimal cutoff value for ARFI elastography was determined to be 1.8 m/s, the sensitivity was 100%, and the specificity was 95%.

The AUC, optimal cutoff value, sensitivity, specificity, positive predictive value, negative predictive value, and positive-negative likelihood ratio of all three groups are summarized in Table 2.

DISCUSSION

Image parameters, such as magnetic sensitivity, spatial resolution, signal-to-noise ratio, and pathophysiological factors (e.g., cellular density and tissue components), affect ADC[12]. In comparing the 3.0T MRI device to a 1.5T device, a high magnetic field was shown to increase the signal-to-noise ratio and spatial resolution while decreasing imaging time[13]. In our study, we aimed to benefit from these advantages by using the 3.0T MRI device.

In comparison group 1, the AUC value of the ARFI was 0.784, the sensitivity was 87%, and the specificity was 60%. Lupsor et al.[14] reported AUC, cutoff, sensitivity, and specificity values of 0.709, 1.19 m/s, 62.07, and 85.7%, respectively, in 112 chronic hepatitis C cases with F0 and F1≤ fibrosis. The AUC values between Lupsor et al.'s[14] study and the present study were similar. However, Lupsor et al.[14] reported lower sensitivity and higher specificity values.

In comparison group 1, the AUC of the mean ADC, cutoff, sensitivity, and specificity values were 0.718, 1.110×10^{-3} m²/s, 80, and 66%, respectively. Similar to the AUC and sensitivity values in this study, the study by Bonekamp et al.[15] accepted B values of 0–750 s/m² with a 1.5T system; the AUC, cutoff, sensitivity, and specificity values were 0.79, 1.51×10^{-3} m²/s, 75.8, and 78.2%, respectively. The specificity in Bonekamp et al.'s study was found to be higher than in the present study. The different results in our study may be due to differences in

Table 1. Distribution of disease groups according to fibrosis stages.

Pathology	S0	S1	S2	S3	S4	S5	S6	Total
HBV	6	8	8	2	4		1	28 (60.9%)
HCV	1	2						3 (6.5%)
Autoimmune hepatitis			2	2			1	5 (10.9%)
Nonalcoholic steatohepatitis	8		2					10 (21.7%)
Total	15 (32.6%)	9 (19.6%)	12 (26.1%)	4 (8.7%)	4 (8.7%)		2 (4.3%)	46

Table 2. Statistical data of acoustic radiation force impulse and diffusion-weighted magnetic resonance imaging in three comparison groups.

	ARFI elastography	DW-MRI
Comparison group 1 Presence of fibrosis (S0 and S1≤)		
Cutoff value	1.25 m/s	1.110×10^{-3}
Sensitivity (95% confidence interval)	87%	80%
Specificity (95% confidence interval)	60%	66%
Positive likelihood ratio (+LR)	2.17	2.35
Negative likelihood ratio (-LR)	0.22	0.3
Positive predictive value	68.5%	70.1%
Negative predictive value	82.19%	76.7%
AUC (95% confidence interval)	0.784	0.718
Comparison group 2 Presence of significant fibrosis (≤S2 and S3≤)		
Cutoff value	1.52 m/s	1.063×10^{-3}
Sensitivity (95% confidence interval)	80%	90%
Specificity (95% confidence interval)	86%	66%
Positive likelihood ratio (+LR)	5.71	2.65
Negative likelihood ratio (-LR)	0.23	0.15
Positive predictive value	85.1%	72.5%
Negative predictive value	81%	86.8%
AUC (95% confidence interval)	0.917	0.778
Comparison group 3 Presence of precirrhosis+cirrhosis (≤S4 and S5≤)		
Cutoff value	1.8 m/s	Statistically no significant difference (p=0.132)
Sensitivity (95% confidence interval)	100%	
Specificity (95% confidence interval)	95%	
Positive likelihood ratio (+LR)	20	
Negative likelihood ratio (-LR)	0	
Positive predictive value	95%	
Negative predictive value	100%	
AUC (95% confidence interval)	0.977	

the B values, acquisition parameters, susceptibility effects, and magnetic field strengths of the devices.

In comparison group 2, the AUC value of ARFI elastography was 0.917, and that of DW-MRI was 0.778. The sensitivity and specificity values were 80–86% for ARFI elastography and 90–66% for DW-MRI. Friedrich-Rust et al.[16] reported that the AUC, cutoff, sensitivity, and specificity values were 0.87, 1.34 m/s, 79, and 85%, respectively. In the present study, similar sensitivity and specificity values were found.

A US liver elastography consensus statement[17] claimed that most studies using ARFI report that a liver stiffness value of less than 1.5 m/s could help rule out significant fibrosis. For comparison group 2, we assumed that the cutoff value was 1.52 m/s, which is very similar to the value stated in this consensus[17].

Furthermore, in comparison group 2, the AUC of the mean ADC, cutoff, sensitivity, and specificity values were 0.778, 1.063×10^{-3} m²/s, 90, and 66%, respectively. Bonekamp et al.[15] reported that the AUC, cutoff, sensitivity, and specificity values were 0.77, 1.33×10^{-3} m²/s, 84.9, and 71.4%, respectively. Similar AUC, sensitivity, and specificity values were found in this study. Sandrasegaran et al.[18] conducted a study using B values of 50–400 s/m² in a 1.5T system and reported that the AUC, cutoff, sensitivity, and specificity values were 0.686, 1.03×10^{-3} m²/h, 72.6, and 59.3%, respectively. In our study,

it was thought that the high sensitivity and specificity values may be due to the use of the 3.0T MRI.

In comparison group 3, there was no statistically significant difference between the ADC values. There may be several reasons why we found a much better result using ARFI elastography than DW-MRI in determining cirrhosis. One of these reasons is the absence of Ishak S5 patients and the fact that there were only two S6 patients. In the present study, although no statistically significant result was obtained with DW-MRI in this comparison group, in the study by Sandrasegaran et al.[18], when the cutoff value was 0.98×10^{-3}, the AUC, sensitivity, and specificity were 0.656, 51.7, and 71.4%, respectively.

ARFI elastography showed very good performance in distinguishing the cirrhosis group from the other fibrosis groups, with an AUC of 0.977. The sensitivity and specificity values were found to be 100 and 95%, respectively. In patients (4%) with Ishak S6, the mean ARFI values were found to be significantly higher (1.89 and 1.85 m/s). In the study by Friedrich-Rust et al.[16], the AUC of 0.93 was the highest compared to the other comparison groups. The cutoff value was 1.80 m/s, and the sensitivity and specificity values were 92–86%. In this study, the highest AUC was obtained for the differentiation of cirrhosis. Fierbinteanu-Braticevici et al.[19], Karlas et al.[20], and Nierhoff et al.[21] found a sensitivity value of 100% in the differentiation of cirrhosis cases. In these studies, the values for the cirrhosis patient group compared to the whole patient group were reported to be 27[19], 26.5[20], and 6%[21], respectively.

In the US liver elastography consensus statement[17], a cutoff interval of 1.7–2.1 m/s for the ARFI value suggests advanced chronic liver disease, and further testing is required for confirmation. In our study, the cutoff value for this category (1.8 m/s) is compatible with this consensus statement[17].

There were inconsistent results regarding which b value is sufficient in DW-MRI images, especially due to the small number of studies performed with 3.0T MRI. Although a low b value is affected by capillary perfusion, the perfusion effect, especially above 300 s/m², disappears[22]. Therefore, high b values may be more valuable in determining fibrosis[23].

This study has various limitations. First, a larger patient population could not be reached. In particular, the number of patients with advanced-stage fibrosis constituted 4% of the entire study group. The second limitation was that while the liver was evaluated by ADC and ARFI elastography, steatosis, possible effects of iron load, and histological activity index were not taken into account in the comparison. The third limitation was the difficulty in placing the ROI used in ARFI elastography measurements, the difficulty in placing the ROI used during ADC measurements in the same area, and the inability to place the ROI used in ARFI measurements deeper than 8 cm. Another limitation is that the disease groups were not homogeneous because of the inclusion of both viral and non-viral hepatitis patients.

CONCLUSION

Conducting new studies involving larger populations and patient groups using a 3.0 Tesla MRI device with a high signal-to-noise ratio, spatial resolution, and short imaging time will contribute to the diagnosis and treatment follow-up of especially early-stage patients.

ETHICAL APPROVAL

Approval for this study was obtained from the Institutional Ethical Review Board (date: 30.05.2014, decision no: 8000-259-14/1560), and the study followed the tenets of the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

MS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. **MuS:** Conceptualization, Formal Analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **CA:** Resources. **KO:** Resources. **OK:** Resources. **HTS:** Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Diagnostic value of serum signal peptide-CUB-EGF-like domain-containing protein 1 levels in patients with acute appendicitis

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SUMMARY

OBJECTIVE: Acute appendicitis is one of the most common surgical causes of an acute abdomen among patients admitted to the emergency room due to abdominal pain. The clinical diagnosis of acute appendicitis is usually difficult and is made by evaluating the clinical, laboratory, and radiological findings together. The aim of this study was to investigate the diagnostic potential of signal peptide-CUB-EGF-like domain-containing protein 1 as a biomarker for acute appendicitis.

METHODS: A total of 67 adult patients without any comorbidities who presented to the emergency department with abdominal pain and were clinically diagnosed with acute appendicitis were included in the case group. The patients included in the study were classified into the negative appendectomy group and the acute appendicitis group according to their histopathological final diagnosis. In addition, 48 healthy volunteers without comorbidities were included in the control group. Signal peptide-CUB-EGF-like domain-containing protein 1 levels of patients and the control group were measured.

RESULTS: According to postoperative histopathological examinations of the patients, 7 (10.4%) patients were diagnosed with negative appendectomy, and 60 (89.6%) patients were diagnosed with acute appendicitis. Signal peptide-CUB-EGF-like domain-containing protein 1 levels were higher in the patients with acute appendicitis than in negative appendectomy patients (p=0.012). Signal peptide-CUB-EGF-like domain-containing protein 1 levels were also higher in the case group compared to the control group (p=0.001).

CONCLUSION: The admission signal peptide-CUB-EGF-like domain-containing protein 1 level was significantly higher in adults with acute appendicitis. The SCUBE1 level is a novel but promising biomarker that aids in the diagnosis of acute appendicitis.

KEYWORDS: Acute disease. Appendicitis. Biomarkers. Leukocyte count. Membrane proteins. SCUBE1 protein, human.

INTRODUCTION

Acute appendicitis (AA) is one of the most common surgical causes of an acute abdomen among patients admitted to the emergency department (ED) due to abdominal pain[1]. Clinicians diagnose AA by evaluating the results obtained from laboratory and radiological examinations, primarily anamnesis and physical examination findings. In addition, diagnostic evaluation can be supported by adding clinical scoring systems, including physical examination findings and various laboratory markers. Many simple and usable scoring systems (the Alvarado score, the modified Alvarado score, the Appendicitis Inflammatory Response score, the RIPASA score, etc.) have been developed for the prediction of AA risk[2]. But their use alone is controversial[3,4]. The role of ultrasonography (USG) and computer tomography (CT) imaging methods remains important in the diagnosis of AA. Despite all diagnostic methods, negative pathology results ranging from 3 to 25% in patients with AA diagnosis and surgical treatment method can be found[5,6]. New methods are needed to make the correct diagnosis in AA and to reduce the rate of negative surgical treatment methods. For this purpose, studies have been carried out showing the relationship of various biochemical markers with AA[1,7].

SCUBE1 is a glycoprotein found on platelet and endothelial cell surfaces. This is a novel molecule with matrix-bound or soluble forms released from the platelet surface as a result of platelet aggregation, which has been shown to play an adhesive role in platelet-platelet or platelet matrix interaction. Studies were carried out on various levels of cardiovascular diseases, inflammatory events, and ischemic processes[8,9]. Platelet activation has a role in the pathophysiology of thrombosis and

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inflammatory diseases. Various platelet markers have been investigated in association with both thrombosis and inflammation[10]. There are few studies in the literature that investigate SCUBE1 levels in adult patients diagnosed with AA[11]. The aim of this study was to investigate the diagnostic value of serum SCUBE1 levels in adult patients who admit to the ED with abdominal pain and are diagnosed with AA.

METHODS

Study design and patient selection

This is a single-centered, prospective study that examines patients who were admitted to the ED with abdominal pain and have been diagnosed with AA. Approval was received from the local ethics committee (Decision No: 2017/109) before starting the study.

The study included patients aged 18 and over who had been admitted to the ED with abdominal pain, had no accompanying disease, were clinically diagnosed with AA, and underwent surgery. Pregnant women, trauma patients, patients who underwent medical treatment methods in the treatment of AA, and patients who voluntarily did not give the necessary consent to participate in the study were excluded from the study. Participants were informed about the study, and written informed consent was obtained from all participants to participate in the study. Symptoms, physical examination findings, laboratory parameters, imaging findings, Alvarado scores, and clinical and demographic characteristics of the patients included in the study were recorded in the study form. In our study, the postoperative histopathological diagnoses of patients were considered the gold standard.

As a control group, 48 healthy volunteers without comorbidities or an active inflammatory disease were selected. A voluntary consent form was obtained from the individuals in the control group. Plasma samples were taken from patients and the control group to measure SCUBE1 levels at the time of admission. After centrifuging for 10 min at 4,000 rpm, the plasma was separated and stored at -80°C. 24 h before the start of the SCUBE1 examination, the separated plasmas were removed from the -80°C environment and placed at +4 degrees. Dissolved plasmas were brought to room temperature, and SCUBE1 levels were measured.

Determination of signal peptide-CUB-EGF-like domain-containing protein 1 levels

SCUBE1 levels in plasma samples were measured by sandwich immunoassay (Enzyme-Linked Immunosorbent Assay (ELISA)) in accordance with the manufacturer's instructions. Commercially purchased Human SCUBE1 (floor no: E-EL-H5405) ELISA kits were used in the analysis. The results were expressed as ng/mL.

The endpoint of the study

In our study, it was determined to investigate the usability of initial SCUBE1 levels from patients in the diagnosis. For this purpose, the use of SCUBE1 levels in the distinction between a negative appendectomy and an AA diagnosis according to histopathological classification was determined as the primary endpoint. In addition, according to histopathological results, the distinction of SCUBE1 levels between the AA diagnosis and the control group was determined as the secondary endpoint.

Statistical method

Statistical analysis was carried out using the Jamovi v.1.6 statistical program (Jamovi Project Computer Software, Version 1.6, Sydney, Australia). Continuous variables were defined as mean and standard deviation for the data with normal distribution, and for the abnormal distribution with median and interquartile range (IQR). Categorical data were shown as frequency (n) and percentage (%). The Shapiro-Wilk test was used to check whether the data were normally distributed. Normal distribution data were expressed with a mean±SD, and abnormal distribution data were expressed with a median (IQR 25–75). The Student's t-test was used to compare normally distributed data, and Mann-Whitney U test was used to compare data showing abnormal distribution.

RESULTS

According to postoperative histopathological examinations of the patients, 7 (10.4%) patients were diagnosed with negative appendectomy, and 60 (89.6%) patients were diagnosed with AA. In the study, 65% (n=39) of the AA group (n=60) was male; 54.2% (n=26) of the control group (n=48) was male; and 28.5% (n=2) of the negative appendectomy group (n=7) was male. The median age of the AA group was 33 (25.0–43.5) years, while the median age of the control group was 30 (22.5–41.5). The gender and age distribution of patients and control groups is shown in Table 1.

Laboratory data, imaging findings, and Alvarado scores of the patients included in the study were calculated using the values at the time of initial admission and presented in Table 2. When laboratory parameters were examined, leukocyte (p=0.016), neutrophil (p=0.003), and neutrophil-lymphocyte

ratio (NLR) (p=0.004) levels were statistically significantly higher in the group with AA than the negative appendectomy group. Again, Alvarado scores were higher in the AA group and statistically significant (p=0.025). When the imaging findings of the patients included in the study were examined, 18 patients were diagnosed with USG and 49 patients were diagnosed with CT. The appendix diameter measured by CT was larger in the group diagnosed with AA (p=0.049). SCUBE1 levels, which were examined at the time of the first admission of the patients and the control group included in the study, are presented in Table 3. When the SCUBE1 levels of the patients were examined, it was shown that they were higher in the AA group (p=0.012) when compared with the negative appendectomy group. In addition, SCUBE1 levels in patients with AA were higher than those in the control group, and this was found to be statistically significant (p=0.001).

DISCUSSION

AA is one of the most common surgical causes of an acute abdomen. The clinical diagnosis of AA is often difficult and is made by co-evaluation of clinical, laboratory, and radiological findings. To help with diagnosis, a number of scoring systems have been developed that incorporate physical examination findings and various laboratory markers. However, many of

Table 1. Gender and age distribution of the patient and control groups.

	Gender		Age distribution (years)
	Male	Female	Median (IQR 25–75)
Negative appendectomy (n=7)	2 (28.5%)	5 (71.5%)	40 (IQR 28.5–42.0)
Acute appendicitis (n=60)	39 (65.0%)	21 (35.0%)	33 (IQR 25.0–43.5)
Control (n=48)	26 (54.2%)	22 (45.8%)	30 (IQR 22.5–41.5)

IQR: interquartile range.

Table 2. Laboratory, imaging, and alvarado scores of patients according to histopathological classification.

	Negative appendectomy (n=7)	Acute appendicitis (n=60)	p-value
WBC (10^3/μL)	8.6±3.7	13.2±4.7	**0.016**
Neutrophil (10^3/μL)	5.3±3.0	10.5±4.3	**0.003**
Lymphocyte (10^3/μL)	2.4 (IQR 1.5–2.9)	2.0 (IQR 1.2–2.5)	0.296
PLT (10^3/μL)	247 (IQR 226–282)	224 (IQR 203–260)	0.122
MPV (fL)	9.5±0.8	9.8±1.3	0.469
CRP (mg/L)	1.6 (IQR 0.8–6.4)	0.9 (IQR 0.3–4.7)	0.559
Total protein (g/L)	7.7±0.7	7.5±0.5	0.706
Albumin (g/L)	4.9±0.3	4.4±0.3	0.866
NLR	1.44 (IQR 1.17–3.55)	5.83 (IQR 3.23–9.76)	**0.004**
Alvarado score	5 (IQR 4.5–6)	7 (IQR 5–8)	**0.025**
USG (mm) (n=18)	7±0	7.9±0.7	0.143
CT (mm) (n=49)	9.4±2.1	10.2±2.6	**0.049**

WBC: white blood cell; PLT: platelet; MPV: mean platelet volume; CRP: C-reactive protein; NLR: neutrophil lymphocyte rate; IQR: interquartile range; USG: ultrasonography; CT: computed tomography. Bold values indicate statistical significance at the p<0.05 level.

Table 3. Signal peptide-CUB-EGF-like domain-containing protein 1 levels of the patient and control groups.

	Negative appendectomy	Acute appendicitis	p-value
SCUBE1 (ng/mL)	19 (IQR 15.5–19.5)	23.5 (IQR 19–28.3)	0.012
	Control	**Acute appendicitis**	
SCUBE1 (ng/mL)	13.5 (IQR 11–16)	23.5 (IQR 19–28.3)	0.001

IQR: interquartile range.

these scoring systems have not been widely accepted. In addition, diagnostic imaging tools such as USG and CT are also used in the diagnosis of AA[3,7]. CT scanning has now become the gold standard for diagnosing AA. In cases where a CT scan cannot be performed, it has become difficult to reach the correct diagnosis[12]. Despite all diagnostic methods, a 3–25% negative appendectomy is encountered[6]. In our study, similar to the literature, we found a negative appendectomy rate of 10.4%. In order to reduce the negative effects of CT scanning and the negative appendectomy rate, clinicians have tended to investigate the role of clinical scoring systems, USG, and some biomarkers in the diagnosis of AA[1,7,11-15].

SCUBE1 is a cell surface glycoprotein found in platelets and endothelial cells[16]. Studies have been carried out on the possibility that SCUBE1 may have a role in various cardiovascular, metabolic, and ischemic diseases[8,9,17-19]. Güzel and her colleagues found SCUBE1 levels higher in hypertensive patients than in normal, healthy individuals[18]. Türkmen and his colleagues mentioned that SCUBE1 could be used to diagnose the early stage of acute mesenteric ischemia[9]. Erdoğan and his colleagues noted that SCUBE1 levels have the potential to be used to predict mortality in septic patients. They also argued that there would be endothelium damage as a result of severe inflammation, and consequently, SCUBE1 levels would increase[8]. We investigated the utility of SCUBE1 in the diagnosis of AA with the hypothesis that there would be endothelium damage after inflammation and therefore SCUBE1 levels could increase.

The results of our study support our hypothesis that SCUBE1 levels in patients diagnosed with AA were higher compared to both the negative appendectomy group and the control group. According to our study, high levels of SCUBE1 statistically support the AA diagnosis. When we look at the literature, there are a limited number of studies evaluating the availability of SCUBE1 in the diagnosis of AA. Sonmez and his colleagues evaluated SCUBE1 levels in the diagnosis of AA and argued that there was no diagnostic marker. However, they found SCUBE1 significantly higher in the CRP-positive group[11]. In a different study involving pediatric appendicitis patients, the difference between appendicitis and control group SCUBE1 values was found to be statistically significant[20].

Our study has some limitations. The most significant limitation is that it is a single-centered study, and the number of patients included in the study is small. Only patients who underwent an appendectomy were included in the study. Patients who initially suspected appendicitis but were not clinically diagnosed with it were not included in this study.

CONCLUSION

As a result, SCUBE1 levels can be used to help diagnose patients clinically diagnosed with AA. Some biochemical markers have produced promising results to help diagnose AA in adult patients. However, it is obvious that there is a need for a greater number of high-quality evidence-based studies.

AVAILABILITY OF DATA AND MATERIALS

The authors agree to the conditions of the publication including the availability of data and materials in our manuscript.

INFORMED CONSENT

Patients' consents were obtained from the patients before starting the study.

ETHICAL APPROVAL

This study was approved by the Recep Tayyip Erdogan University Clinical Research Ethics Committee (Decision No. 2017/109).

HUMAN RIGHTS

The principles outlined in the Declaration of Helsinki have been followed.

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AUTHORS' CONTRIBUTIONS

GA: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **MA:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Writing – review & editing. **MI:** Data curation, Writing – review & editing. **HAU:** Formal Analysis, Validation, Writing – review & editing. **RB:** Validation, Writing – review & editing. **MKÇ:** Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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The bone mineral density and isokinetic knee strength in amputee soccer players

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SUMMARY

OBJECTIVE: The aim of this study was to examine the isokinetic knee strength, H/Q ratio (%), and bone mineral density values between amputees (n=14; amputee soccer players) and healthy football players (n=14; non-amputee soccer players).

METHODS: A total of 28 amputee soccer players and non-amputee soccer players participated in the study. An isokinetic dynamometer was used to determine the knee flexion/extension forces of the dominant legs of the athletes at 60, 180, and 240°/s. Bone mineral density scans were performed using dual-energy X-ray absorptiometry.

RESULTS: H/Q ratio and 60°/s flexion and 180 and 240°/s flexion/extension strength ($p<0.05$) were found to be high (180°/s, $p=0.03$; 240°/s, $p=0.048$) in the non-amputee soccer player group. Accordingly, the bone mineral density values of the lumbar vertebra, femoral neck, proximal metaphysis of the femur ($p<0.01$), tibia/fibula proximal metaphysis, and tibia/fibula distal metaphysis ($p<0.05$) were found to be high. A correlation was observed between the 60°/s knee extension strength and tibia/fibula diaphyseal bone mineral density ($p=0.025$; $r=0.594$) and tibia/fibula distal metaphysis bone mineral density ($p=0.017$; $r=0.623$) values in the amputee soccer players group. The Z-scores of the amputee soccer players and non-amputee soccer players were in the expected range according to age (>-2).

CONCLUSION: The bone mineral density, H/Q ratio, and all measured angular velocities of isokinetic strength were high in non-amputee soccer players. This finding made us think that lower extremity amputation may also be associated with losing strength. However, it was observed that the relationship between strength and bone mineral density in amputee athletes might vary according to different angular velocities. It is recommended that isokinetic strength measurement can be evaluated together with bone mineral density in athletes.

KEYWORDS: Amputee. Soccer. Bone mineral density. Muscle strength.

INTRODUCTION

Although there are various types of para-sports events, the popularity of amputee football is spreading rapidly around the world and the awareness of the sport is increasing[1]. Amputee football players are required to run forward, move backward, turn around in their own axis, move laterally, show a high level of balance, and jump using single leg and forearm crutches while playing on the field[2].

The low bone mineral density (BMD) experienced by amputees leads to an increased long-term risk of hip fragility fractures[3]. Therefore, physical exercise is extremely beneficial to increase BMD and bone mineral content as a potent protective factor to limit the occurrence of osteopenia, which leads to the development of osteoporosis[4]. Regarding the effects on muscle tissue, many studies investigating sports practice or resistance training during growth have shown a positive relationship between muscle mass and bone health[5-7].

BMD is influenced by a variety of factors such as mechanical forces, hormonal changes, and exercise. The mechanical forces are, in part, influenced by muscle strength[8,9].

When the literature is examined, different findings are found in BMD values in studies conducted with amputees and healthy individuals. However, it has been observed that the findings related to the subject of athletes are insufficient[10,11]. No study has been found in the literature investigating the relationship between isokinetic strength values and BMD (femur/tibia regions) of amputee soccer players (ASPs) and non-amputee soccer players (NASPs). In this study, the isokinetic muscle strength, BMD, and hamstring/quadriceps strength ratio of the dominant legs were evaluated together, and the differences between the groups were analyzed in ASPs and NASPs.

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METHODS

Participants

Male amputee (n:14, age 29.21±5.87 years, height 172.50±8.04 cm, weight 76.71±16.26 kg, years in the sport 8.50±4.53 years, and amputation time 18.07±6.86 years) without any history of knee injury and non-amputee soccer (n:14, age 24.21±3.11 years, height 176.92±5, 92 cm, weight 73.85±8.42 kg, and years in the sport 10.35±0.53 years) voluntarily participated in the study. The lower extremity amputee levels of ASPs were determined as transtibial amputation, knee disarticulation, and transfemoral amputation.

Procedure

All athletes gave their informed consent according to the Declaration of Helsinki, and all experimental procedures were approved by the ethics committee of Ondokuz Mayıs University, Samsun, Turkey (No. 2017/164). All athletes voluntarily participated in the study and signed the voluntary consent form. Isokinetic strength measurements were performed in the sports sciences performance laboratory, and BMD measurements were made in the Nuclear Medicine Laboratory of the University Hospital (13:00 to 14:30). Athletes were asked not to participate in training and to be rested the day before and on the day of the test. Isokinetic knee extension/flexion strength and BMD measurements were performed on the intact leg for ASPs and the dominant leg for NASPs.

Assessment of muscle strength

A computer-controlled isokinetic dynamometer (Humac Norm Testing and Rehabilitation System, CSMI, USA) was used to measure the knee extension and flexion strengths of the athletes. Before the measurement, the athletes were asked to pedal for 5 min on the bicycle ergometer to warm up, and then stretching movements for the lower extremities were performed. The isokinetic strength measurement protocol in Cybex was evaluated as slow (60°/s) and fast (240°/s; 180°/s)[12].

In the fixed protocol of the dynamometer, the knee extension and flexion measurements of the athletes were performed at angular velocities of 60°/s (15 s rest after four repeat trials and five repeats of the main test), 180°/s (four repeat trials, 15 s rest, and then five repeats of the main test), and 240°/s (four repeat trials followed by 15 s rest and 15 repeats of the main test), respectively. The rest intervals of 30 s were given to the athletes during the transitions between angular velocities[13]. The force values obtained in the tests were recorded in Newton meters.

Measurement of bone density

BMD measurements of the athletes were performed using the dual-energy X-ray absorptiometry (DEXA, Hologic QDR-2000, Discovery Series; Hologic, Inc., Waltham, MA, USA) device. For the lower extremity region, the femur and tibia/fibula regions of the dominant leg of the NASPs and the non-amputated leg of the ASPs were visualized, and BMD (g/cm^2) was determined by drawing the relevant areas from each bone as proximal metaphysis, distal metaphysis, and diaphysis.

Data analysis

The SPSS 22.0 package program was used for the statistical analysis of the data obtained. An independent sample t-test was used for the group comparisons, whereas a Pearson correlation test was used to determine the relationships between the parameters. The alpha value was accepted as <0.05. When type I error (α) was 0.05 and type II error (β) was 0.20, at least 13 athletes were calculated by the power analysis with the NCSS-Pass v.2008 software.

RESULTS

BMD values were found to be high in the femur proximal metaphysis (Figure 1A, p=0.0001), tibia/fibula proximal metaphysis (Figure 1D, p=0.021), tibia/fibula distal metaphysis (Figure 1F, p=0.021), lumbar vertebra (Figure 1G, p=0.006), and femoral neck (Figure 1H, p=0.000) in the NASP group. In the NASP group, 60°/s flexion (p=0.018), 180, and 240°/s flexion/extension strength was found to be high (p<0.01). No significant difference was found in the 60°/s extension strength (p=0.56). H/Q ratios of 180°/s (p=0.03) and 240°/s (p=0.048) were found to be significantly higher in the NASP group (Figure 2). In the NASP group, no correlation was observed between isokinetic knee strength parameters and BMD values in the leg regions. In the ASP group, a correlation was observed between the 60°/s knee extension strength and tibia/fibula diaphyseal BMD (p=0.025; r=0.594) and tibia/fibula distal metaphysis BMD (p=0.017; r=0.623) values (Figure 3).

There was no significant differences femur diaphysis (Figure 1B), femur distal metaphysis (Figure 1C), tibia/fibula diaphysis (Figure 1E), radius/ulna (Figure 1I) BMD between ASP and NASP (p>0.05).

DISCUSSION

In this study, the BMD values were examined separately by dividing the lower extremity into separate groups, and for the

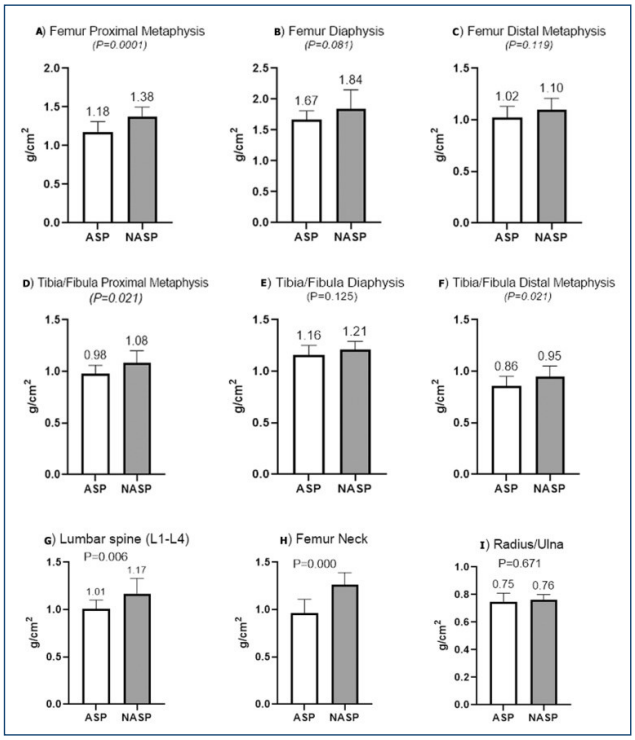

Figure 1. Findings and significance levels of athletes' bone mineral densities. A significant difference was found in the bone mineral density values of femur proximal metaphysis (1.38±0.03), tibia/fibula proximal metaphysis (1.08±0.03), and tibia/fibula distal metaphysis (0.95±0.03) ($p<0.05$). There was no significant difference in the bone mineral density values of femur diaphysis, femur distal metaphysis, and tibia/fibula diaphysis ($p>0.05$). A significant difference was found in the bone mineral density values of the lumbar vertebra and femoral neck ($p<0.05$). There was no significant difference in the radius/ulna bone mineral density values ($p>0.05$). (A) Femur proximal metaphysis. (B) Femur diaphysis. (C) Femur distal metaphysis. (D) Tibia/Fibula proximal metaphysis. (E) Tibia/Fibula diaphysis. (F) Tibia/Fibula distal metaphysis. (G) Lumbar spine (L1-L4). (H) Femur neck. (I) Radius/Ulna.

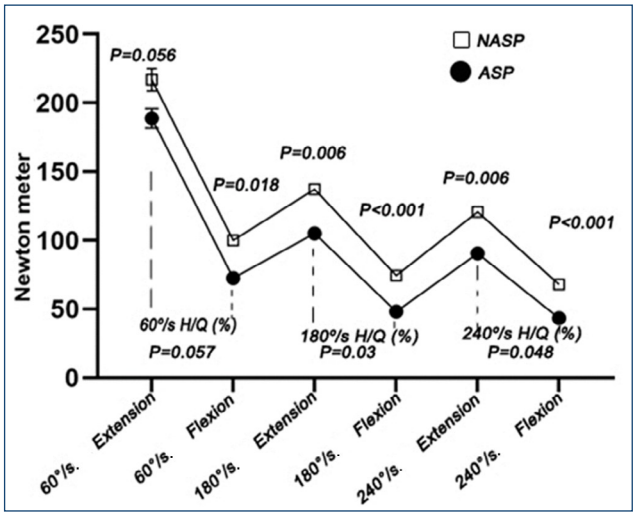

Figure 2. Findings of isokinetic knee strength and H/Q values of the dominant legs of the athletes. The non-amputee soccer players group had a higher flexion strength of 60 (100.07±8.26), 180 (74.57±4.22), and 240°/s (68.07±3.23) ($p<0.05$). Additionally, a significant difference was observed in the 180 (137.21±6.83) and 240°/s (120.64±6.50) extension strength ($p<0.05$). There was no significant difference in 60°/s extension strength ($p>0.05$). A significant difference was found in the 180°/s (54.64±1.89) and 240°/s (57.00±2.37) H/Q ratio ($p<0.05$). No significant difference was found at 60°/s ($p>0.05$).

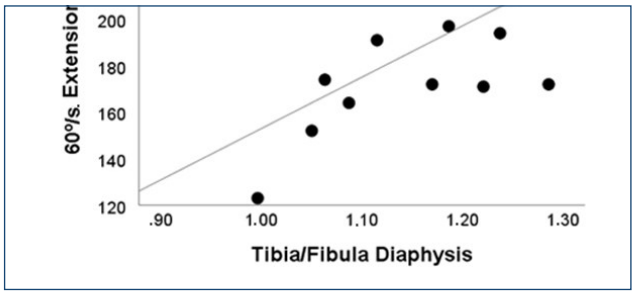

Figure 3. Significant correlation findings between dominant leg bone mineral density values and peak torque in the amputee soccer players group. A correlation was observed between the 60°/s knee extension strength and tibia/fibula diaphysis bone mineral density ($p=0.025$; $r=0.594$) and tibia/fibula distal metaphysis bone mineral density ($p=0.017$; $r=0.623$) values in the amputee soccer players group.

first time, their relationship with the isokinetic knee strength was evaluated.

Among the BMD parameters of the athletes, the lumbar vertebra and femoral neck were found to be significantly higher in the NASP group in our study. No significant difference was detected in the radius/ulna BMD value. BMD increases in athletes who continue high-impact loads in training and competition[14]. The occurrence of situations such as sprinting, jumping, sudden acceleration and deceleration, and change in direction in the soccer game also includes different loads on the muscles and bones and the ground reaction force. Thus, BMD develops most effectively with activities that produce greater loads on the bone, such as soccer[15]. From this point of view, we can predict that players who have been doing soccer training for many years and who are more exposed to loads may also show higher BMD values.

In our study, high BMD values were found in the NASP leg regions of the femur proximal metaphysis, tibia/fibula proximal metaphysis, and tibia/fibula distal metaphysis. No significant difference was found in the BMD values of the athletes' femoral diaphysis, femur distal metaphysis, and tibia/fibula diaphysis.

It is plausible that there is a dynamic and dependent physiological link between muscle strength and BMD. However, it is known that multiple factors also affect BMD apart from the forces originating from muscle contraction.

These factors include nutrition, age, hormones, impact, and genetics[16]. The reason for the high BMD values of NASPs may be that the leg area is exposed to more intense training compared to ASPs.

Some studies have suggested that the skeletal and muscular systems are structurally interdependent, and both adapt to mechanical loading. It is stated that, this way, muscle movement provides a stimulus for bone remodeling by pulling on the bone where the tendon attaches, and the skeleton adapts to the increasing magnitude of loading by accumulating bone[17]. Accordingly, it was thought that the findings of our study also affected the regional training of the athletes and the parts of the muscles at the attachment points.

In our study, 60º/s flexion and 180 and 240º/s flexion/extension strengths were high in the NASP group. H/Q ratios of 180 and 240º/s were significantly high in the NASP group. When the literature was examined, no study was found comparing the isokinetic knee strength values of ASPs and NASPs.

According to the literature, the high muscle strength values of soccer players can be explained by the fact that soccer includes various technical skills such as kicking, jumping, and landing, as well as the fact that strength exercises have an important place in training planning. Based on the findings of our study, it can be said that, due to the less active condition of ASPs, the muscle groups tend to be more atrophic and weaker.

Based on the H/Q ratios obtained in our study, it can be said that the risk of injury in ASPs is higher compared to NASPs as the H/Q ratios of both groups were found to be lower than the norm values indicated in the literature. The norm values of H/Q ratios are accepted as 50–70% for 60°/s[18] and 70–90% for 180°/s[19,20]. The reason for the low H/Q ratios of both ASPs and NASPs can be shown as unilateral exercises in both groups and the neglect of the hamstring muscle group. H/Q ratios outside of the norm can pose a risk for joint and muscle injuries.

In our study, there was no correlation between the isokinetic knee strength parameters and leg region BMD values in the NASP group. In many studies, muscle strength and BMD values of athletes were found to be higher than non-athletic controls. The relationship between muscle strength and BMD was stronger in those with low-to-moderate physical training[21,22]. However, less or no relationship was observed between muscle strength and BMD in highly trained individuals. Such a relationship was not found in women athletes participating in sports that involve heavy body load such as soccer[21]. Such a relationship was not found in ice hockey athletes[17]. Based on these studies, it was concluded that high physical activity weakens this association. However, there are many studies reporting a relationship between muscle strength and BMD[23,24]. These conflicting results may be due to individual differences in athletes and training protocols.

In our study, a correlation was observed between 60º/s knee extension strength value and tibia/fibula diaphysis BMD and tibia/fibula distal metaphyseal BMD values in the ASP group. No correlation was found between the isokinetic knee strength parameters and leg region BMD values.

While few studies were found in the literature on isokinetic knee strength and BMD in amputees, these current studies do not focus on the relationship between isokinetic knee strength and BMD values of the leg regions.

Tugcu conducted a study with individuals with trans-tibial amputation and did not find a relationship between 30 and 120^0/s knee muscle strength and femoral neck, total femur, and tibia BMD values[25]. The lack of strong correlations between many strength measurements and bone densities may be because strength is not only dependent on the size of the muscle attached to the bone but also on neural action to the muscle[14]. This can weaken the relationship between strength and bone.

CONCLUSION

The BMD, H/Q ratios, and all measured angular velocities of isokinetic strength were high in NASPs. This finding made us think that lower extremity amputation may also be associated with losing strength. However, it was observed that the relationship between strength and BMD in amputee athletes might vary according to different angular velocities. It is recommended that isokinetic strength measurement can be evaluated together with BMD in athletes. A limitation of this study is that the biochemical parameters that affect the BMD of the participants were not measured. Additionally, the small sample size and the sample consisting of only males can be seen as limitations of the study. Not being able to determine the effects of training protocols of participants on individual differences is also considered another limiting factor of the study.

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ETHICAL STATEMENT

All athletes gave their informed consent according to the Declaration of Helsinki, and all experimental procedures were approved by the Ethics Committee of Ondokuz Mayis University Samsun, Turkey (No. 2017/164).

AUTHORS' CONTRIBUTIONS

II: Conceptualization, Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **MÇ:** Investigation, Methodology, Validation, Writing – review & editing. **FCT:** Methodology, Resources, Writing – review & editing.

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ORIGINAL ARTICLE

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Effect of pregnancy symptoms on the sexual quality of life

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SUMMARY

OBJECTIVE: This descriptive cross-sectional study aimed to determine the effect of pregnancy symptoms on the sexual quality of life.

METHODS: This study included 150 pregnant women who visited the obstetrics and gynecology outpatient clinic of the hospital between October 1, 2019, and April 1, 2020, and met the inclusion criteria. Data were collected using the Personal and Obstetric Information form, Sexual Quality of Life-Female scale, and Pregnancy Symptom Inventory.

RESULTS: The mean age of the participants was 27.7±5.2 years. As per the collected data, 39.3% of the participants had university- or higher-level education and 21.3% had an income-generating job. A weak negative correlation was found between the scores of Sexual Quality of Life-Female and frequency of pregnancy symptoms and limitation in daily activities (p=0.016 and p=0.020, respectively), whereas a strong positive correlation was found between frequency of pregnancy symptoms and limitation in daily activities. Regression analysis showed that as Sexual Quality of Life-Female scores decreased, frequency of pregnancy symptoms and limitation in daily activities scores increased (p<0.001).

CONCLUSION: Our study showed that, as the frequency of symptoms experienced during pregnancy and their impact on daily life increase, the sexual quality of life decreases. We recommend providing education and counseling services to women and their partners about pregnancy symptoms and its impact on sexual life during pregnancy and implementing effective measures to eliminate the negative effects of these symptoms on the sexual quality of life.

KEYWORDS: Pregnancy. Symptoms. Sexual health.

INTRODUCTION

Sexuality is a very important part of life that is necessary for the continuation of a species. Sexuality in humans is not only associated with genital organs but also includes the whole body and mind, and is shaped according to the perspective of the society[1]. According to the World Health Organization, sexual health is the state of physical, emotional, mental, and social well-being in relation to sexuality[2]. Many people view sexuality as a factor of great importance for their quality of life. For this reason, many people worry that pregnancy and childbirth will have negative and irreversible effects on their sexual life[3].

Although pregnancy is a natural event, it can have significant physiological, metabolic, and psychological effects on the mother's body. Symptoms such as nausea, vomiting, frequent urination, heartburn, fatigue, back pain, constipation, weakness, cramps, breast tenderness, diarrhea, shortness of breath, varicose veins, vaginal discharge, headache, and sleep problems can be observed during pregnancy[4,5]. These symptoms can affect the sexual life of pregnant women and thus their sexual quality of life. Sexuality is interrupted during pregnancy due to the problems experienced during pregnancy, health concerns, and lack of adequate counseling[6]. The aim should be to preserve or improve the sexual quality of life in pregnant women through interventions and education that will reduce the severity of symptoms and counseling services.

The aim of this study was to determine the effect of pregnancy symptoms on the sexual quality of life. The data obtained from the study can be used as a guide for training and consultancy services to be provided for improving the sexual quality of life of pregnant women and helping them go through this delicate period as best as possible.

METHODS

This was a descriptive cross-sectional study that aimed to determine the effects of pregnancy symptoms on the sexual quality

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of life. This study was conducted at the obstetrics and gynecology clinic of the Yozgat City Hospital between October 2019 and April 2020.

The study population included pregnant women who visited the obstetrics and gynecology outpatient clinic of the hospital and met the inclusion criteria. Sample selection was not performed, and all pregnant women were included in the initial sample. The total number of participants was 150. Voluntary consent forms were provided to all participants, and written consent was obtained from all participants. All data were collected during face-to-face interviews.

The inclusion criteria were as follows: consenting to participate in the study; aged ≥18 years; having a single pregnancy; not having a chronic disease; not having any risk factors such as placental anomaly, infection, bleeding, premature rupture of membranes, and threat of premature birth, during pregnancy; not having any psychiatric disorders; not having a communication problem; and no prescribed restriction on sexual life. The exclusion criteria included being illiterate and having treatment-induced pregnancy, such as through *in vitro* fertilization or intrauterine insemination.

After the study was completed, G power analysis determined that the sample size of the study was sufficient [calculation based on the mean scale score, partial eta squared=0.039 power=0.688 for FoPS, partial eta squared=0.038, and power=0.671 for limitation in daily activities (LiDA)].

Data collection tools

Research data were collected using the Personal and Obstetric Information form, the Pregnancy Symptom Inventory (PSI), and the Sexual Quality of Life-Female (SQOL-F) scale.

Personal and obstetric information form

This form was prepared by the researchers according to the relevant literature[7,8]. This form included 20 questions on the sociodemographic (i.e., age, educational status, family type, etc.), obstetric, and sexual life characteristics.

Pregnancy Symptom Inventory

PSI was developed by Foxcroft et al. in 2013. The Turkish validity and reliability of the scale were determined by Can Gürkan and Ekşi Güloğlu in 2018. PSI includes 42 items evaluating the frequency of symptoms experienced during pregnancy and the limitation of daily activities by Frequency of Pregnancy Symptoms (FoPS), the first subdimension of PSI uses a four-point Likert-type scale, and the total score that can be obtained from this subdimension ranges between 0 and 126. Higher scores are interpreted as an increase in the frequency of experienced symptoms. LiDA is the second subdimension of PSI. The total score that can be obtained on LiDA ranges between 42 and 126. Higher scores indicate more LiDA due to symptoms[9,10].

Sexual Quality of Life-Female Scale

The SQOL-F scale is a valid and reliable tool developed in 2005 by Symonds et al. The Turkish validity and reliability of SQOL-F were determined by Tuğut and Gölbaşı in 2010. The SQOL-F scale is a six-point Likert-type scale and includes 18 items based on the participants' sexual life in the last 4 weeks. The scores that can be obtained are in the range of 18–108. A high score indicates a good sexual quality of life[11,12].

Evaluation of data

IBM SPSS Statistics Standard Concurrent User V 25 (IBM Corp., Armonk, New York, USA) was used for statistical analysis of the data.

Shapiro-Wilk test and QQ charts were used to check whether the data were normally distributed. Comparisons of FoPS and LiDA scores according to the patient characteristics were made with an independent two-sample t-test for variables with two categories, and a one-way analysis of variance was used for variables with three or more categories. The effect of FoPS and LiDA scores on SQOL-F scores was evaluated by linear regression analysis. A p-value of <0.05 was accepted as statistically significant in all analyses.

Ethical considerations

Ethics approval was obtained from the Yozgat Bozok University Non-Interventional Clinical Research Ethics Committee (2017-KAEK-189_2019.10.16_04). Written permission was obtained from the hospital's chief physician to conduct the study. Written and verbal consents were obtained from the pregnant women included in the study.

RESULTS

This study was conducted to determine the effect of pregnancy symptoms on the sexual quality of life. Accordingly, the mean age of the pregnant women was 27.7±5.2 years. It was determined that 72.7% of the pregnant women had planned pregnancy, and 48.7% of the participants stated that the problems experienced during pregnancy did not affect their sexual life (Table 1).

The mean FoPS subdimension score of PSI was 45.31±18.01. The LiDA subdimension score of PSI was 68.18±12.92. Finally, the mean SQOL-F score was 75.49±19.71 (Table 2).

Table 1. Descriptive, obstetric, and sexual life characteristics of the pregnant women (n=150).

Characteristics	n (%)
Age groups (Mean: 27.7±5.2) (years)	
≤20	6 (4.0)
21–25	52 (34.7)
26–30	50 (33.0)
31–35	28 (18.7)
≥36	14 (9.3)
Educational status	
Literate/primary school graduate	20 (13.4)
Secondary school graduate	26 (17.3)
High school graduate	45 (30.0)
University graduate and above	59 (39.3)
Having an income-generating job	
Yes	32 (21.3)
No	118 (78.7)
Family type	
Nuclear	119 (79.3)
Extended	31 (20.7)
Parity	
One	57 (38.0)
Two	50 (33.4)
Three	20 (13.3)
Four or more	23 (15.3)
Gestational week	
M (Q_1–Q_3)	34.0 (29.0–37.0)
Min–max	10.0–41.0
Planned pregnancy status	
Planned	109 (72.7)
Unplanned	41 (27.3)
Knowledge about sexual intercourse during pregnancy	
Yes	113 (75.3)
No	37 (24.7)
Do the problems you experience during pregnancy affect your sex life?	
Yes	77 (51.3)
No	73 (48.7)

M: Median; Q_1–Q_3: interquartile range.

As shown in Table 3, a weak negative correlation was found between the SQOL-F and FoPS and LiDA scores (p=0.016 and p=0.020, respectively). The SQOL-F score decreases as the FoPS and LiDA scores increase. A strong positive correlation was found between the FoPS and LiDA scores.

DISCUSSION

Although sexual health is ignored by many people, sexuality cannot be ignored when dealing with the overall health of an individual. Sexuality continues during pregnancy. However, the changes or symptoms experienced during this period can affect the sexual health of pregnant women. Pregnant women may avoid sexual intercourse or experience sexual problems due to various symptoms they experience. The findings obtained in this study conducted to determine the effect of pregnancy symptoms on the sexual quality of life were discussed in line with the relevant literature.

Considering the score range of FoPS (0–126), the mean score obtained in this study (45.31) was below the mid-level. Considering the score range of LiDA (42–126), which represents the level of daily activities of pregnant women due to various symptoms they experience, the mean score obtained in this study (68.18) was also below the mid-level (Table 3). Similarly, Woo also found a mean FoPS score of 36.23. In the study of Ağapınar Şahin, the mean frequencies of pregnancy symptoms (FoPS) and LiDA scores were found to be 53.88 and 41.09, respectively. These results are consistent with our findings[13,14].

The mean SQOL-F (75.49) score of the participants was above the mid-level of the score range (10–100) (Table 3). Similarly, in another study conducted by Kırıkkaleli (2015) with 171 pregnant women, the mean SQOL-F score of pregnant women was found to be 81.59. Furthermore, Bakır et al. reported mean SQOL-F scores which are consistent with our findings. Maasoumi et al. conducted a study on women who were not pregnant or in the menopausal period and found that the mean SQOL-F score was 86.4. Again, in another study conducted by Küt, the mean SQOL-F score

Table 2. Distribution of Sexual Quality of Life-Female scale and Pregnancy Symptom Inventory scores of participants (n=150).

	Cr α	X±ss	Min–Max
Pregnancy Symptoms Inventory-FoPS	0.866	45.31±18.01	0–97
Pregnancy Symptoms Inventory-LiDA	0.876	68.18±12.92	42–108
Sexual Quality of Life-Female Scale	0.891	75.49±19.71	10–100

FoPS: frequency of pregnancy symptoms; LiDA: limitation in daily activities; Cr α: Cronbach's alpha internal consistency coefficient.

Table 3. Relationship between Sexual Quality of Life-Female scale and Pregnancy Symptom Inventory scores.

PSI	SQOL-F	LiDA
Frequency of pregnancy symptoms	rho=−0.196; p=0.016	r=0.873; p<0.001
Limitation in daily activities	rho=−0.189; p=0.020	-

SQOL-F: Sexual Quality of Life-Female; PSI: Pregnancy Symptom Inventory; r: Pearson correlation coefficient; rho: Spearman's correlation coefficient.

was found to be 81.8. Based on our findings and the results of other studies, it can be said that pregnancy reduces the sexual quality of life[8,15,16].

In this study, a weak negative correlation was found between the sexual quality of life and the FoPS and limitation in daily activities. Accordingly, it was found that the sexual quality of life decreased as the FoPS and limitation in daily activities increased. Pregnancy is a period of physiological, psychological, social, and sexual changes for women[17]. Some studies have reported that urinary incontinence, stomach complaints, respiratory problems, leg pain, cramps, nausea, and fatigue complaints cause sexual dysfunction[4,18]. In their study, Cassis et al. reported that the symptoms experienced during pregnancy increase the most in the last trimester and 86.1% of the pregnant women in the last trimester have adverse effects on their sexual functions[19].

Gümüşay found that the positive body image of pregnant women had a positive effect on the sexual function of couples[7]. In another study, it was determined that positive body image during pregnancy was associated with lower sexual dysfunction[20]. As a result, physical or mental changes affect the sexual life during pregnancy.

CONCLUSION AND RECOMMENDATIONS

Our results showed that the symptoms experienced during pregnancy affect the sexual quality of life of pregnant women. Accordingly, we recommend as follows:

Symptoms experienced during pregnancy should be regularly reviewed by healthcare professionals, and appropriate interventions should be performed.

Education and counseling services about sexual life during pregnancy should be provided to all couples.

Sexual quality of life should not be neglected during pregnancy, and effective interventions should be performed to eliminate the negative effects of pregnancy symptoms and other factors on the sexual quality of life.

Further quantitative studies evaluating pregnant women and their spouses together and more comprehensive qualitative studies with detailed interviews should be conducted.

Limitations of the study

The data obtained from the study were based on the self-reports of the participants. The results represent only the pregnant women who participated in the study and cannot be generalized to other health institutions and pregnant women.

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AUTHORS' CONTRIBUTIONS

ENÇM: Conceptualization, Data curation, Methodology, Resources, Writing – original draft. FTG: Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Prevalence of skin lesions in a sample of Brazilian patients with inflammatory bowel disease

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SUMMARY

OBJECTIVE: Inflammatory bowel diseases may have extra intestinal manifestations such as those affecting the skin. This study aimed to study skin manifestations in a cohort of Brazilian patients with inflammatory bowel diseases.

METHODS: Epidemiological and clinical data were obtained through a cross-sectional study of 70 inflammatory bowel diseases patients and a control group comprising 50 healthy individuals. All patients were subjected to dermatological examination and photography of skin lesions.

RESULTS: Out of the 70 inflammatory bowel diseases patients, 50 had ulcerative colitis and 20 had Crohn's disease. Skin lesions occurred in 95.7% of the inflammatory bowel diseases patients and in 88% of individuals in the control group (p=0.001). Alopecia (p<0.0001), xerosis (p=0.03), striae (p=0.02), and acne (p=0.04) were more common in inflammatory bowel diseases patients than in the control group. Alopecia was more frequent in females (p=0.01) than in males. Two male patients, one with ulcerative colitis and the other with Crohn's disease, had pyoderma gangrenosum. Erythema nodosum was not observed in both groups.

CONCLUSION: There was a high prevalence of skin lesions in the Brazilian inflammatory bowel diseases patients. Additionally, alopecia, xerosis, striae, and acne were more common in patients with inflammatory bowel diseases than in those in the control group.

KEYWORDS: Inflammatory bowel disease. Pyoderma gangrenosum. Alopecia. Skin care.

INTRODUCTION

Inflammatory bowel diseases (IBD) are autoimmune diseases affecting the intestinal mucosa and caused by a complex interaction of genetic and environmental factors[1]. In Brazil, IBD is more frequent in females than in males and occurs more frequently during the third to fifth decade of life[2]. IBD is subdivided into Crohn's disease (CD) and ulcerative colitis (UC); location and depth of the affected intestinal wall are some of the differences between them. UC affects the mucosa and submucosa of the gastrointestinal tract and characteristically initiates in the rectum spreading proximally. CD involves the whole thickness of the intestinal wall, from the mucosa to the serosa, and may appear in any part of the gastrointestinal tract[1].

In addition to gastrointestinal tract involvement, IBD may affect several other systems, leading to high morbidity and poor quality of life[1,3]. Skin involvement is among the extraintestinal manifestations of IBD and appears in more than 10% of affected individuals[4].

The skin involvement associated with IBD may be specific, having the same histological characteristics as those of the intestinal disease. The skin lesions share similar pathophysiologic mechanisms with intestinal involvement or may appear as lesions that are secondary either to the treatment or the malabsorption[5]. Specific lesions, including perianal fistula and perianal abscess, are more common in CD. Erythema nodosum (EN), pyoderma gangrenosum, and Sweet's syndrome are found in the group of reactive lesions. Skin manifestations secondary to malnutrition or malabsorption occur due to a shortage of vitamins and microminerals. For example, angular stomatitis is due to deficiencies of vitamin B12 and iron, phrynoderma is due to vitamin A deficiency, scurvy is due to a deficit of vitamin C, and seborrheic dermatitis is due to vitamin E and zinc deficiency. Hair loss and skin rashes or allergic reactions may be secondary to the immune-suppressive treatment. Psoriasis and infections of the skin are associated with anti-TNF-alpha use[2,5,6]. Moreover, there are other skin manifestations that are more common in IBD patients than in the general population. They are usually associated with a special expression of the HLA gene and a chronic inflammatory state. Psoriasis,

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skin vasculitis, autoimmune bullous dermatoses, vitiligo, multiform erythema, phlebitis, lichen planus, urticaria, secondary amyloidosis, and squamous cell carcinoma are some of them[5].

This study aimed to investigate the prevalence of skin manifestations and to identify possible epidemiologic factors associated with their occurrence.

METHODS

Ethical issues

This study was approved by the local Committee of Ethics in Research, under permit number 4.252.1444 and adhered to the principles of the Declaration of Helsinki. All participants signed consent forms.

Sample and study design

This study was a cross-sectional study conducted from October 2020 to March 2022 at a single Gastroenterology Outpatient Clinic that cares for IBD patients from the Brazilian Public Health System.

Patients diagnosed with IBD according to the European Crohn's and Colitis Organization and the European Society of Gastrointestinal and Abdominal Radiology guidelines[7] and older than 18 years of age were included in the study. This is a convenience sample that included all patients that came for a regular consultation during the above period and were invited to participate according to their appointment order and willingness to participate in the study. The control group consisted of their companions who were declared healthy.

Data were obtained from direct questioning or through chart review and included: epidemiological data (age, sex, years of formal education, and phototype according to the Fitzpatrick scale[8]) and clinical data (IBD type, disease duration, comorbidities, and treatment).

Dermatological examinations, consisting of full inspection and palpation of the patient's skin, were performed. These examinations were performed by two researchers trained and supervised by a dermatologist. Each lesion was appropriately annotated and photographed for documentation or further examination in case of diagnostic doubt.

Statistical analysis

The data were saved in Excel and analyzed using the software Graph Pad Prism 5.0. The continuous variables were expressed as mean ± standard deviation or median and interquartile range (IQR). The experimental and control groups were compared using Mann-Whitney or independent samples t-tests. Categorical variables were expressed in percentages and compared between the two groups with the chi-square test or Fisher's exact test. Logistic regression was used to study the independence of the use of treatment drugs and the presence of acne. p-Values less than 5% were considered statistically significant.

RESULTS

Description of studied sample

The studied sample included 70 IBD patients, of whom 45 (64.2%) were females, with a mean age of 44.7±14.7 years. CD were diagnosed in 50 (71.4%) and 20 (28.6%) with UC. About treatment, aminosalicylates were used by 68.2%, azathioprine by 34.2%, biological drugs by 24.2%, glucocorticoids by 10%, methotrexate by 1.4%, and no medications by 7.1% of patients.

As a control group, we invited 50 individuals. Patients and controls were paired, and there are no differences for gender (p=0.84), age (p=0.08), years of formal education (p=0.27), and Fitzpatrick phototype (p=0.57).

Skin findings

Table 1 shows the comparison of the main skin lesions in patients with IBD and a matched control group. The following lesions were found in patients with IBD: dermatofibroma, eczema, dyshidrosis, actinic keratosis, drug lichenoid eruptions, striated lichen, scleroatrophic lichen, hidradenitis, dysplastic nevus, depapillated tongue, keloidal folliculitis, pityriasis versicolor, plantar wart, tinea corporis, chronic pigmented purpura, urticaria, erythema pernio, rosacea, hyperhidrosis, striated melanonychia, varicose ulcer, venous lake, eczematoid, and drug granulomatous reaction. These lesions were excluded from Table 1 due to their low prevalence of 1.4% (only one case of each lesion was found among the 70 patients with IBD).

The comparison of the most prevalent lesions according to the IBD type is shown in Table 2. The only observed difference was in the striae that were more common in CD patients than in UC patients. In addition, the distribution of the most common dermatological lesions according to gender shows that alopecia is more frequent in females.

Table 3 shows the comparison of main skin lesions according to the use of prednisone, azathioprine, and anti-TNF-α; acne was more commonly seen in those using anti-TNF-α and azathioprine. When a logistic regression was done using acne as the dependent variable and azathioprine, prednisone, and anti-TNF-α as independent variables, only anti-TNF-α kept its independence (odds ratio [OR] 9.4; 95% confidence interval [CI] 1.5–58.5).

Skin lesions that have a well-established pathophysiology were grouped into three categories: reactive skin manifestations, cutaneous manifestations secondary to malnutrition or malabsorption, and skin manifestations secondary to treatment.

Table 1. Comparison of main skin lesions in inflammatory bowel disease patients and controls.

	IBD n=70	Controls n=50	p-value
Alopecia	29 (41.4%)	1 (2%)	<0.0001*
Xerosis	19 (27.1%)	5 (10%)	0.03**
Onychomycosis	13 (18.6%)	12 (24%)	0.50
Tinea pedis	12 (17.1%)	11 (22%)	0.80
Striae	8 (11.4%)	0 (0%)	0.02§
Acne	7 (10%)	0 (0%)	0.04§§
Pilar keratosis	7 (10%)	3 (6%)	Ns
Contactdermatites	6 (8.6%)	2 (4%)	Ns
Folliculitis	5 (7.1%)	1 (2%)	Ns
Weak nails syndrome	5 (7.1%)	0 (0%)	0.07
Seborrheic dermatitis	5 (7.1%)	2 (4%)	Ns
Seborrheic keratosis	4 (5.7%)	8 (16%)	Ns
Common wart	4 (5.7%)	4 (8%)	Ns
Plantar keratoderma	3 (4.3%)	3 (6%)	Ns
Hypertrichosis	3 (4.3%)	0 (0%)	Ns
Pioderma gangrenoso	2 (2.9%)	0 (0%)	Ns
Aphtha	2 (2.9%)	0 (0%)	Ns
Amiloidosis	2 (2.9%)	1 (2%)	Ns
Paronychia	2 (2.9%)	1 (2%)	Ns
Sebaceouscyst	2 (2.9%)	2 (4%)	Ns
Telogeneffluvium	2 (2.9%)	0 (0%)	Ns
Onycholysis	2 (2.9%)	0 (0%)	Ns
Fibroma	2 (2.9%)	0 (0%)	Ns
Herpes lesion	2 (2.9%)	1 (2%)	Ns
Hidrocystoma	2 (2.9%)	0 (0%)	Ns

*OR=34.6; 95%CI=4.5–265.7. **OR=3.3; 95%CI=1.1–9.7. §OR=13.7; 95%CI=0.77–243. §§OR=11.2; 95%CI=0.66–214.0.

Two cases of pyoderma gangrenosum were recorded in male patients, one in UC and another in CD (p=0.50). Abnormalities in skin and nails were more frequent in IBD patients (42/70; 60%) than controls (2/50; 4%) with p<0.0001. Skin lesions secondary to treatment, such as xerosis and eczema, were more frequent in IBD patients (27/70; 38.5%) than in controls (9/50; 18%), p=0.02. Cases of skin infections, liquenoid eruptions, and granulomatous reactions were similar in both groups (all p=ns).

DISCUSSION

Our findings indicated a higher prevalence of dermatoses in patients with IBD than in the control population. The main skin findings in patients with IBD were alopecia, xerosis, striae, and acne.

When grouped according to the underlying pathophysiology process, those associated with malnutrition and secondary to the used treatment were prominent. Striae and acne are well-known undesirable effects of glucocorticoid treatment[9], although this association could not be demonstrated in this study. This may have happened because of the low number of individuals using glucocorticoid in this sample and by the cross-sectional design of the study, which did not consider the previous use of this medication. Striae were found to be more common in those with CD than in those with UC. It is possible that this has happened because UC patients may benefit from topical use of this medication, decreasing its systemic use[10].

In the present study, acne was associated with anti-TNF-α therapy and azathioprine use, but only anti-TNF-α was independently associated with this skin lesion. This association has also been found by others[11]. Curiously, this medication has beneficial results in acne manifestations from SAPHO syndrome[12].

Table 2. Dermatological lesions according to inflammatory bowel disease subtype and gender.

	Crohn's disease (n=20)	Ulcerative colitis (n=50)	p-value
Alopecia	10 (50%)	19 (38%)	0.8
Xerosis	6 (30%)	13 (26%)	0.65
Acne	4 (20%)	3 (6%)	0.18
Striae	5 (25%)	3 (6%)	0.03[a]
Onychomycosis	4 (20%)	9 (18%)	0.98
Tinea pedis	5 (25%)	7 (14%)	0.30
	Females (n=45)	**Males (n=25)**	
Alopecia	24 (53.3%)	5 (20%)	0.01[b]
Xerosis	12 (26.6%)	7 (28%)	0.9
Acne	3 (6.6%)	4 (16%)	0.40
Striae	4 (8.8%)	4 (16%)	0.44
Onychomycosis	6 (13.3%)	7 (28%)	0.19
Tinea pedis	5 (11.1%)	7 (28%)	0.38

[a]OR=5.2; 95%CI=1.1–24.4). [b]OR=4.5; 95%CI=1.4–14.3). n: number.

Table 3. Skin lesions in inflammatory bowel disease patients according to the use of prednisone and anti-TNF-α.

	With prednisone (n=7)	Without prednisone (n=63)	p-value
Alopecia	3 (42.8%)	26 (41.2%)	1.00
Xerosis	1 (14.2%)	18 (28.5%)	0.66
Acne	2 (28.5%)	5 (7.9%)	0.14
Striae	1 (14.2%)	7 (11.1%)	1.00
Onychomycosis	0	13 (20.6%)	0.33
Tinea pedis	0	12 (19.0%)	0.34
	With anti-TNF-? (n=14)	**Without anti-TNF-? (n=56)**	
Alopecia	6 (42.8%)	23 (41.0%)	0.90
Xerosis	3 (21.4%)	16 (28.5%)	0.74
Acne	4 (28.5%)	3 (5.3%)	0.02*
Striae	3 (21.4%)	5 (8.9%)	0.19
Onychomycosis	2 (14.2%)	11 (19.6%)	1.00
Tinea pedis	2 (14.2%)	10 (17.8%)	1.00
	With azathioprine (n=24)	**Without azathioprine (n=46)**	
Alopecia	10 (41.6%)	19 (41.3%)	0.97
Xerosis	7 (29.1%)	12 (26.0%)	0.78
Acne	5 (20.8%)	2 (4.3%)	0.04**
Striae	4 (16.6%)	4 (8.6%)	0.43
Onychomycosis	3 (12.5%)	10 (21.7%)	0.51
Tinea pedis	5 (20.8%)	7 (15.2%)	0.73

*OR=7.06; 95%CI=1.3–36.5. **OR=5.7; 95%CI=1.03–32.5. n: number.

No descriptions of the association of acne with azathioprine use have been found in the literature.

Another important dermatological manifestation was alopecia, which was more common in females (82.7% of IBD patients). The occurrence of alopecia in IBD has been reported in a previous study[13], but in this study, it is possible to highlight that its prevalence is higher in females than in males. Immunosuppression and/or use of aminosalicylates, nutritional deficiencies of vitamin B12, iron, and zinc may have also contributed to this finding[13,14]. Unfortunately, micronutrients were not measured in this study, precluding any conclusion on their etiology. Hair is important to a patient's corporal image, and alopecia may have a negative impact on an individual's quality of life, influencing social relationships and self-esteem. These harmful effects are usually more prevalent and important in females[15].

The occurrence of xerosis cutis could have been caused by many factors. Use of drugs such as anti-TNF-alpha may play a role, although it was not possible to prove this association in the present study. Cleynen et al.[16] found xerosis cutis occurred in 10.6% of their IBD patients who used anti-TNF-alpha for a median time of 1.0 year. Nutritional aspects could also contribute to this finding, as xerosis has been a common finding in the skin of patients with anorexia nervosa[17] and in those undergoing bariatric surgery[18]. Skin dryness causes pruritus, which favors abrasions and increases the risk of skin infections[17].

Finally, reactive skin lesions were found in two patients (2.8% of the sample), both of whom had pyoderma gangrenosum. Pyoderma gangrenosum is an autoinflammatory, chronic, and ulcerating condition that may have harmful effects that usually appears in those with severe bowel disease[19]. Pyoderma gangrenosum is regarded as a skin lesion associated with UC and more common in females[4,20], but in this study, one of the patients had CD, and both were males. In patients with CD, pyoderma gangrenosum prevalence has been estimated to be 0.7%[21].

None of the patients in the present study had EN, although it is considered the most common form of reactive lesion in IBD[21].

This study is limited by the small number of patients recruited and its cross-sectional design. However, it is important in that it is the first to report the prevalence of skin lesions in Brazilian patients with IBD.

In conclusion, in this study, there was a high prevalence of skin lesions in the Brazilian IBD patients. Additionally, alopecia,

xerosis, striae, and acne were more common in IBD patients than in the control group.

AUTHORS' CONTRIBUTIONS

TMS: Conceptualization, Data curation, Investigation, Writing – original draft. **MK:** Conceptualization, Data curation, Investigation, Writing – original draft. **KSMP:** Conceptualization, Data curation, Investigation, Writing – original draft. **ORJ:** Conceptualization, Data curation, Investigation, Writing – original draft. **TS:** Conceptualization, Methodology, Formal Analysis Writing – original draft. **RN:** Project administration, Supervision, Formal Analysis , Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Human papillomavirus prevalence and frequency of sexually transmitted diseases in encarcered women by self-sampling approach

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SUMMARY

OBJECTIVE: This study aimed to assess the sociodemographic and clinical profile of women deprived of their liberty and to identify the prevalence of sexually transmitted diseases and human papillomavirus through self-sampling samples.

METHODS: This is an epidemiological, cross-sectional, observational, and descriptive study of the prevalence and correlation of the diagnosis of human papillomavirus infection in 268 encarcered women in Amazonas submitted to self-sampling from June 2019 to September 2020 using the genotyping analysis. Patients with positive and inconclusive results were evaluated by commercialized PCR to detect pathogens causing sexually transmitted diseases. The sample size used was based on a convenience sample.

RESULTS: In 268 women, human papillomavirus DNA was detected in 87 (32.5%) of them. Sexually transmitted diseases were detected in 30 (34.48%) of the 87 women with a positive or inconclusive result for human papillomavirus. Women with more than three pregnancies had a higher risk of human papillomavirus detection (p=0.004).

CONCLUSION: The prevalence of human papillomavirus and other sexually transmitted diseases in encarcered women in Amazonas is 32.5 and 34.48%, respectively. Most women were single (60.4%) and reported having had more than 15 partners (90.8%).

KEYWORDS: Sexually transmitted disease. Screening. Prisoner. Human papillomavirus viruses.

INTRODUCTION

Studies suggest that the association between HPV infection and other sexually transmitted diseases (STDs) represents a public health problem with biological and psychosocial repercussions that would be considered cofactors that favor cervical carcinogenesis[1].

Encarcered women are more exposed to risk factors for STDs such as irregular condom use, multiple partners, sex at risk, use of illicit drugs, and smoking[1,2].

Self-sampling is a screening strategy for encarcered women with difficult access to the health system and may be an alternative to a previous study[3], as it has greater sensitivity for detection of HPV and also the possibility of diagnosing STDs that are considered cofactors for viral persistence, and it may also be a strategy for places where the performance of colpocytology is not possible[4-6].

Encarcered women present peculiarities and complexity that justify studies to improve health care. These women are on the margins of the health system in Brazil, despite being a risk group for HPV infection and other STDs. Thus, this study aimed to assess the sociodemographic and clinical profiles of encarcered women and identify the prevalence of STDs and HPV infection through a self-sampling approach. The data from this study open up a perspective on the use of this screening method, favoring broader screening for populations with difficult access, such as encarcered women.

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METHODS

This is an epidemiological, cross-sectional, observational, descriptive, and analytical study of the prevalence and association of the diagnosis of HPV AND STDs in encarcered women by self-sampling. The women belonged to the female prison system of Amazonas, totaling 281 women from June 2019 to September 2020. Sexually active women who agreed to participate in the research after completing the Informed Consent Form were included. Hysterectomized, virgin, and pregnant women were excluded. The sample size used was based on a convenience sample. The present study was submitted and approved by the Ethics and Research Board of the Federal University of Amazonas (UFAM) with the opinion number 3.421.145, CAAE 15976719.3.0000.5020 on June 27, 2019.

The selected women were instructed to perform self-sampling with the COARI® Kolplast device, and samples were analyzed by Cobas® 4800 HPV CTNG Test (Roche®) following all the manufacturer's specifications. Women who had positive results for HPV 16, HPV 18, and other high-risk and inconclusive types were submitted to the PCR Panel (XGEN MULTI UP Kit MULTIPLEX Plus) by Mobius Life Science for detection of STD-causing pathogens using the same self-sampling sample. The following pathogens were evaluated: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum.*

Orientation for self-sampling samples was carried out by the researchers in the prison system itself. It was evaluated as a characteristic of the sociodemographic and clinical profile of the population studied. The self-samples were stored at room temperature and sent to the IPOG Laboratory in São Paulo, Brazil, for detection analysis and genotyping.

Statistical analysis was performed using the R version 3.5 software (R Core Team, 2019). The difference between the groups was evaluated by the Mann-Whitney or Kruskall-Wallis test. To verify the association between qualitative variables, Fisher's exact test was used. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the 281 women eligible for the study, 12 were excluded for being hysterectomized and 1 for being pregnant. Finally, 268 women were included (Figure 1).

Most women were single (60.4%) and reported having had more than 15 partners (90.8%). None of the patients had received the HPV vaccine (Table 1).

Among the 268 women studied, 87 (32.5%) had detectable HPV DNA, including HPV 16 in 12 women (13.8%), HPV 18 in three patients (3.5%), HPV other in 51 (58, 6%), and 21 women (24.1%) had inconclusive results (Table 2).

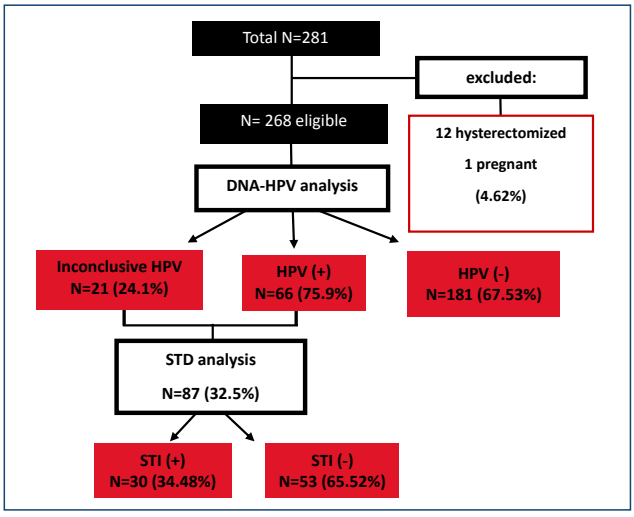

Figure 1. Flowchart of allocation of women in the study.

STDs were detected in 30 (34.48%) of the 81 women with a positive or inconclusive result for HPV (Figure 1). The most frequent pathogens were *Mycoplasma hominis* 23 (26.4%), *Chlamydia trachomatis* 7 (8%), *Ureaplasma urealyticum* 5 (5.7%), and *Neisseria gonorrhoeae* 1 (1%).

Among the 268 women studied, 87 (32.5%) had detectable HPV DNA, with HPV 16 in 12 women (13.8%) (Table 2). The most frequent pathogens were *Mycoplasma hominis* 23 (26.4%) and *Chlamydia trachomatis* 7 (8%).

As shown in Table 1, women with more than three pregnancies had a higher risk of HPV detection compared to those with fewer pregnancies (77.0 versus 23.0%, p=0.004).

DISCUSSION

The prevalence of HPV infection in the population was elevated, the prison system presents greater exposure to transmission, and women have the possibility of acquiring STDs, deserving a differentiated look due to the risks that represent all STDs, especially HPV, which is considered a public health problem[4]. It is worth mentioning that this population was not vaccinated, and this fact may contribute to the high prevalence in this population. By incorporating self-sampling and examination for STDs through PCR in the study, we have the possibility to demonstrate that screening through this methodology can minimize access difficulties, embarrassment, shame, and sociocultural aspects while also improving the sensitivity of the diagnosis. In this way, it becomes a new perspective to reduce the incidence of cervical cancer[5].

Table 1. Associated risk factors in encarcered women in Amazonas (Brazil) with human papillomavirus and detectable sexually transmitted diseases.

Variant	Total n=268 (%)	HPV test result		p-value	Total n=87 (%)	STD test result			p-value
		Positive n=87 (%)	Negative n=181 (%)			Positive n=30 (%)	Negative n=53 (%)	Inconclusive n=4 (%)	
Age (years)									
18–26	74 (27.6)	33 (37.9)	41 (22.7)	0.0926	33 (37.9)	12 (40)	19 (35.8)	2 (50)	0.6758
27–35	97 (36.2)	30 (34.5)	67 (37)		30 (34.5)	10 (33.3)	19 (35.8)	1 (25)	
36–44	64 (23.9)	16 (18.4)	48 (26.5)		16 (18.4)	5 (16.7)	11 (20.8)	–	
45–53	27 (10.1)	6 (6.9)	21 (11.6)		6 (6.9)	3 (10)	2 (3.8)	1 (25)	
Over 53	6 (2.2)	2 (2.3)	4 (2.2)		2 (2.3)	–	2 (3.8)	–	
Number of pregnancies									
Up to 3 pregnancies	173 (64.6)	67 (77)	106 (58.6)	0.0040	67 (77)	21 (70)	42 (79.2)	4 (100)	0.4467
More than 3 pregnancies	95 (35.4)	20 (23)	75 (41.4)		20 (23)	9 (30)	11 (20.8)	–	
Condom use									
No	85 (31.7)	30 (34.5)	55 (30.4)	0.5752	30 (34.5)	9 (30)	19 (35.8)	2 (50)	0.6255
Yes	183 (68.3)	57 (65.5)	126 (69.6)		57 (65.5)	21 (70)	34 (64.2)	2 (50)	
Contraceptive use									
No	144 (53.7)	45 (41.7)	99 (44.7)	0.6954	45 (51.7)	16 (53.3)	26 (49.1)	3 (75)	0.707
Yes	124 (46.3)	42 (48.3)	82 (45.3)		42 (48.3)	14 (46.7)	27 (50.9)	1 (25)	
Number of partners									
Up to 15 partners	233 (86.9)	79 (90.8)	154 (85.1)	0.2463	79 (90.8)	27 (90)	48 (90.6)	4 (100)	1.0000
Over 15 partners	35 (13.1)	8 (9.2)	27 (14.9)		8 (9.2)	3 (10)	5 (9.4)	–	
Age of first sexual intercourse									
Up to 15 years	192 (71.6)	67 (77)	125 (69.1)	0.1949	67 (77)	24 (80)	39 (73.6)	4 (100)	0.6115
Over 15 years	76 (28.4)	20 (23)	56 (30.9)		20 (23)	6 (20)	14 (26.4)	–	
Use of drugs									
No	95 (35.4)	29 (33.3)	66 (36.5)	0.6831	29 (33.3)	11 (36.7)	18 (34)	–	0.4527
Yes	173 (64.6)	58 (66.7)	115 (63.5)		58 (66.7)	19 (63.3)	35 (66)	4 (100)	
Cigarette use									
No	133 (49.6)	47 (54)	86 (47.5)	0.3617	47 (54)	16 (53.3)	30 (56.6)	1 (25)	0.5327
Yes	135 (50.4)	40 (46)	95 (52.5)		40 (46)	14 (46.7)	23 (43.4)	3 (75)	

Table 2. Prevalence of human papillomavirus in self-sampling samples from encarcered women in Amazonas (Brazil).

HPV type	n=87 (%)
HPV 16	12 (13.8)
HPV 18	3 (3.5)
Inconclusive HPV	21 (24.1)
Other HPVs	51 (58.6)

HPV: human papillomavirus.

With regard to the number of pregnancies, in the series, 26.9% had 4–6 pregnancies, similar to data from the study carried out in the prison system in São Paulo, which reported that 68.3% had 1–3 pregnancies[2]. Multiparity is considered a risk factor for cervical cancer; in the series, it showed a slight association with the presence of HPV.

Sexual intercourse at an early age favors the vulnerability of the cervical epithelium to the occurrence of infection by the HPV. In the sample, sexarche in the age group of 15 years was more frequent in 24.6%, corroborating studies carried out in the Prison System in Mato Grosso do Sul[4]; however, the association of HPV infection with early sexarche showed no statistical difference in this population.

In relation to drug use and smoking, the prison population of Amazonas has high rates; however, in the case series, there was no statistical difference regarding the presence of HPV, in disagreement with other studies[6]. Although the association between the number of partners and drug use has shown signs

of dependence for the detection of HPV, this may demonstrate the importance of associated cofactors for infection.

The persistence of HPV infection is the main cause of cervical cancer[1,7], but there are factors that favor its persistence, and sexually transmitted infections are considered cofactors. In the series, the association occurred, with 30% positivity for STDs in women with detectable HPV; similar data from other series showed an association with HPV in 47.4%, with *Chlamydia trachomatis* being the most frequent in the study carried out in Belo Horizonte[7].

In this study, *Mycoplasma hominis* was more prevalent, with the association with HPV 16 being more frequent in 41.7%, especially in the age group of 18–35 years, demonstrating that the microbiome influences the pathophysiology of intraepithelial lesions, especially in this younger age group, and that when associated with Mycoplasma hominis, it can influence HPV infection[7].

The study had limitations as to having performed the HPV DNA test in the entire prison population regardless of age as recommended by the Ministry of Health in women under 30 years of age; however, the strengths of the study allow demonstrating the profile of a population where the health system is not effective and the possibility of the use of self-sampling associated with the HPV DNA Test and STD by PCR to become an effective screening method for these women.

The data from this study open up a perspective on the use of self-sampling, favoring broader screening for populations with difficult access, such as encarcered women.

In conclusion, self-sampling can be an alternative screening method for HPV and other STDs, yet this population is considered a risk group for HPV and other STDs. The prevalence of HPV and other STDs in encarcered women in Amazonas is 32.5 and 34.48%, respectively. Most women were single (60.4%) and reported having had more than 15 partners (90.8%).

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AUTHORS' CONTRIBUTIONS

HFBESAP: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **GPSN:** Data curation, Writing – original draft. **HVP:** Data curation. **KDS:** Data curation. **MMPO:** Data curation. **TCFS:** Data curation, Writing – original draft, Writing – review & editing. **VTA:** Data curation. **VSC:** Data curation. **KLT:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **ALSF:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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The impact of the coronavirus disease 2019 outbreak on decision-making styles and breastfeeding of pregnant women: a cross-sectional study

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SUMMARY

OBJECTIVE: This study was planned to examine the factors affecting the decision-making styles of pregnant women in the coronavirus disease 2019 epidemic, their choice of birth environment, and their decision to start breastfeeding.

METHODS: The study was conducted in a cross-sectional descriptive type. The study was conducted with 631 pregnant women who voluntarily participated between January 2020 and April 2021 and met the sample selection criteria. Women aged 18–45 years who had healthy singleton pregnancies were included. Pregnant women with signs or symptoms of coronavirus disease 2019 or suspected or diagnosed with birth were excluded from the study. The data were collected by the questionnaire method through the links shared with the pregnant women. Data Collection Form, Questionnaire for Birth and Breastfeeding in the coronavirus disease 2019 Period, and Melbourne Decision-Making Styles Scale-II were used as data collection tools.

RESULTS: The mean age of the pregnant women was found to be 28.56±6.36 years. Approximately 50.71% of the participants reported that they preferred normal vaginal delivery. It was reported that 56.1% of the pregnant women had a say in the decision-making process of the delivery method. It has been determined that there is a significant difference between the education status, employment status, pregnancy planning, family type, and the person who has a say in deciding the mode of delivery ($p<0.05$). The results of the analysis of worrying about starting breastfeeding according to the decision-making styles of the pregnant women in the sample group are examined. The difference between the scores of avoidant and procrastinating decision-making style, which is the sub-dimensions of the scale, and worrying about starting breastfeeding is statistically significant ($p<0.029$ and $p<0.029$, respectively).

CONCLUSION: The research findings show that situations such as epidemics affect the decisions of pregnant women, and breastfeeding situations and decision-making styles affect each other. For this reason, education programs and guides including guidance services and support systems should be published and pregnant women should be guided correctly.

KEYWORDS: COVID-19. Pregnancy. Decision making. Breastfeeding.

INTRODUCTION

It is observed that women, who are the most affected and abused side of epidemics, wars, and disasters, are greatly affected by coronavirus disease 2019 (COVID-19)[1,2]. There is limited information available for pregnant women about the COVID-19 outbreak and for now management is as for non-pregnant women[3,4].

Although birth is a physiological event, deciding how to give birth is a great source of stress for women[5,6]. The characteristics of the individual and his social conditions are effective in the decision-making process[7,8]. As COVID-19 infection can cause serious complications such as death and the risk of transmission is high, pregnant women in special periods of their lives are even affected by applying to health institutions for routine pregnancy check-ups, while their delivery preferences are highly affected[9,10]. It is of great importance that the delivery methods are explained to the pregnant by professional health workers and that the pregnant woman takes an active role in determining the birth environment or the health personnel who will help[11]. In addition, having a say in her own body and birth will increase the self-confidence of the pregnant, reduce the anxiety caused by the negative conditions created by the pandemic process, and prevent the pregnancy process from being negatively affected[12,13]. One of the important determinants of the decision-making process is the decision-making style[14]. In one study, three important areas in decision making

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are stated: the variability of the decision of the mode of delivery, the participation of the pregnant women in the decision, and the factors affecting the decision of the mode of delivery. Today, women want to have a say in the mode of delivery and to participate in the decision. It is thought that her own decision-making style is effective in the decision-making process of a woman who has to decide on the mode of delivery, especially for a reason that affects the decision-making process, such as the epidemic. At this stage, the duty of physicians and midwives is to ensure the appropriate participation of the pregnant woman in the birth decision and to encourage her to decide on the right mode of delivery. For this reason, this study was planned considering that there is a need for scientific studies examining the effect of the decision-making styles of expectant mothers on the decision of birth and breastfeeding in important risk factors such as epidemics. This study was planned to examine the factors affecting the decision-making styles of pregnant women in the COVID-19 epidemic, their choice of birth environment, and their decision to start breastfeeding.

METHODS

Study type and population

The study was planned as a descriptive cross-sectional type. The STROBE Statement was used in the planning, implementation, and reporting of the study design. The universe of the study consists of all pregnant women in Istanbul. The study sample consisted of 631 pregnant women who voluntarily participated and met the sample selection criteria. The study was conducted between January 2020 and April 2021. The ethics committee approval was obtained from the Non-Invasive Clinical Research Ethics Committee (Ethics Number: 198, Date: 24.12.2020) before starting data collection. Written informed consent was obtained from the pregnant women to voluntarily participate in the study. The study was conducted in accordance with the Declaration of Helsinki. The survey was anonymous, and pregnant women were able to quit the study at any time.

Inclusion criteria: The inclusion criteria were as follows: (a) volunteered to participate in the study, (b) residing in Istanbul, (c) speaking, reading, and writing Turkish, (d) aged between 18 and 45 years, (e) having a healthy singleton pregnancy, and (f) COVID pregnant women who did not pass 19.

Exclusion criteria: The exclusion criteria were as follows: (a) pregnant women outside of Istanbul, (b) pregnant women who did not want to participate in the study, (c) pregnant women with signs or symptoms of COVID-19 or suspected or diagnosed delivery, (d) pregnant women with any obstetric indications and requiring hospitalization, (e) pregnant women with any existing/previously known psychiatric disorder, and (f) those who were not willing to participate in the research.

Data collection tools

Questionnaire Form: It consists of a total of 35 questions, of which (a) 9 questions about the socio-demographic characteristics of women, (b) 18 questions about obstetric history, and (c) 8 questions about the problems and concerns caused by the coronavirus during pregnancy[11,13].

Melbourne Decision-Making Scale II: The Turkish validity and reliability study of the scale was conducted by Deniz[15,16] Melbourne Decision-Making Scale II. The three-point Likert scale is "1=True, 2=Sometimes, and 3=Not True" and consists of 22 items and measures decision-making styles. There are four sub-dimensions in the scale that evaluate different decision-making styles, and the internal consistency coefficients of the scale sub-dimensions range from 0.65 to 0.80. Within the scope of this study, the internal consistency coefficients of the sub-dimensions of the scale ranged from 0.65 to 0.84. Within the scope of this study, the internal consistency coefficient for the whole scale was determined as 0.88.

Data collection process and bias

After obtaining the necessary ethical approval for the research, the data collection tool prepared on the online platform was shared online by the researcher. Information about the study and the web link to the survey and the pandemic by the researchers were sent to all midwives and nurses working in their hospitals through email and/or text messages, and they were requested to share it with their pregnant women. Electronic data were collected through a secure method of Google surveys, and each survey took an average of 10–15 min to complete. In the first part of the form of the questionnaire link, an explanation was made about the purpose of the study, and their consent was obtained in the digital environment without asking for their identity information. In addition, there was a statement at the beginning of the survey in which the pregnant women confirmed in writing that they were willing to participate in the study. Participation of pregnant women was free, and they were informed that there was no benefit or harm. Participants were able to view the questions after their consent was obtained. Participants were informed in the statement that they had the right to leave the survey while answering the questions. At the beginning of the form, it was possible to move on to other questions according to the questions covering the inclusion and exclusion criteria. Those who did not cover these kits were excluded from the study because they could not see the other

questions. Pregnant women who completed all the questions were considered to have completed the questionnaire. The survey was accessible during the research period, and participants were able to access it on any device they wanted.

Ethical approval

Before starting data collection, the ethics committee approval was obtained from the Non-Invasive Clinical Research Ethics Committee (Ethics Number: 198, Date: 24.12.2020). Written informed consent was obtained from the pregnant women to voluntarily participate in the study. The study was conducted in accordance with the Declaration of Helsinki. The survey was anonymous, and pregnant women were able to quit the study at any time.

Statistical analysis

Statistical Package for Social Science (SPSS) version 21.0 for the Windows software (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. The Kolmogorov-Smirnov test was used to evaluate the distribution of data before statistical analysis. Descriptive statistics were calculated, including frequency, percentage for nominal variables, and mean and standard deviation for continuous variables. The significance level was determined as $p<0.05$.

RESULTS

A total of 631 postpartum mothers (mean age 28.56±6.36 years; mean number of pregnancies 2.17±1.72; mean marriage years 3.72±1.20) were included in the study. It was reported that 41.7% of the participants had a second pregnancy and 76.9% did not have curettage/miscarriage. Approximately 50.71% of the participants reported that they preferred normal vaginal delivery. It was found that the differences between the educational status, employment, planned pregnancy, and income status of the pregnant women participating in the study and Melbourne Decision-Making Scale I–II (MDMS-I–II) were significant ($p<0.005$) (Table 1). It was found that there was no significant difference between birth preferences and those who are effective in birth preferences and MDMS-I–II ($p>0.005$).

The difference between the questions of delaying check-ups, paying attention to hygiene behaviors, and whether there is a difference between pregnancies of pregnant women during the COVID-19 period and the MDMS-I–II is significant ($p<0.05$) (Table 2).

In Table 3, when the results of the analysis of worrying about starting breastfeeding according to the decision-making styles of the pregnant women in the sample group are examined. The difference between the scores of avoidant and procrastinating decision-making style, which is the sub-dimensions of the scale, and worrying about starting breastfeeding is statistically significant ($p<0.05$), and the difference between the scores of avoidant and procrastinating decision-making style, which is the sub-dimensions of the scale, and being worried about the choice of birth environment is statistically significant ($p<0.05$). When the results of the analysis between the birth environment preferences of the pregnant women constituting the sample group are examined, the difference between the sub-dimensions of the scale, avoidant, procrastinating, and panic decision-making style scores and the birth environment preferences is statistically significant ($p<0.05$).

DISCUSSION

The research findings show that situations such as epidemics affect the decisions of pregnant women and breastfeeding situations and decision-making styles. The findings of the study show that there is a significant difference between the anxiety of starting breastfeeding and the birth environment preferences according to the decision-making styles of the pregnant women.

During the pandemic process, it is recommended that pregnant women avoid unnecessary travel, crowds, public transport, and contact with sick people, and more importantly, apply and maintain personal and social hygiene rules. It is recommended to reduce the frequency of follow-up of pregnant women and to continue follow-up by telephone or online, if possible[12,16,17]. According to the findings of this study, it was determined that there was a significant relationship between the total mean score of the Melbourne Decision-Making Scale and the fact that the pregnant women neglected their controls, paid attention to the use of masks, and felt different compared to their previous pregnancies. In a study, it was reported that, during the COVID-19 epidemic, approximately 57% of pregnant women were worried about being infected and 28.3% wanted to reduce the frequency of appointments[18]. It was observed that the research findings and the literature findings were in parallel. It is thought that these results are due to the belief of pregnant women that the risk of transmission will increase in the hospital environment.

Contrary to the onset, the World Health Organization (WHO) guidelines recommend encouraging mothers with suspected or confirmed COVID-19 infection to begin and continue breastfeeding because the benefits of breastfeeding significantly outweigh the potential risks of transmission. The findings of

Table 1. The effect of the sociodemographic variables on the MDMS-I–II scale (n=631).

Parameters	Mean+SD			
Age (years) X±SD (95%CI)	28.56±6.36 [27.03–28.92]			
Time of marriage (years) C±SD (95%CI)	3.72±1.20 [3.01–4.48]			
Number of pregnancies mean±SD (95%CI)	2.17±1.72 [2.29–2.48]			
	n	%	MDMS-I–II Mean+SD	p-value
Education	27	4.2	4.27±1.22	**0.000**
Primary school	128	20.2	5.01±1.30	
Secondary school	231	36.6	5.16±1.21	
High school	246	38.8	5.13±1.56	
University and above	27	4.2	4.27±1.22	
Working status				
Yes	200	31.7	5.09±1.60	**0.000**
No	431	68.3	4.98±1.23	
Income status				
Miscarriage	323	51.2	5.08±1.12	**0.000**
Middle	272	43.1	3.83±2.20	
High	76	5.7	3.83±2.51	
Family type				
Nuclear family	535	84.8	5.09±1.60	**0.000**
Extended family	96	15.2	4.98±1.23	
Planning pregnancy				
Yes	379	60.1	4.48±2.12	**0.008**
No	252	39.9	4.92±1.93	
Post-COVID-19 mode of delivery preference				
Vaginal	320	50.7	4.74±2.16	0.276
Cesarean	311	49.3	4.56±1.95	
Who was influential in the choice of birth				
Doctor	354	56.1	2.65±2.01	0.569
Midwife	71	11.3	5.24±2.03	
Myself	136	21.6	6.82±2.0	
My family and others	70	11.1	5.65±2.04	

MDMS-I–II, Melbourne Decision-Making Scale I–II. Bold indicates statistically significant p-values.

the study show that there is a significant difference in anxiety about starting breastfeeding according to the decision-making styles of pregnant women. The study findings are similar to the literature[17]. In order for the breastfeeding process to be positive for pregnant women, especially in the risky category such as the epidemic, appropriate guidance, counseling, and breastfeeding training are required for the mother.

When deciding whether to give birth in a hospital or at home, women consider factors such as safety and the psychological impact of the place they choose. One of the consequences of the epidemic is that women's views about birth environments have changed. In particular, it is thought that the risk of transmission of the epidemic is perceived as high by women in pandemic hospitals, and the thought that they and their baby

Table 2. The effect of the variables of the participants in the period of COVID-19 on the MDMS-I–II scale (n=631).

Parammeters		n	%	MDMS-I–II Mean±SD	p-value
Disrupting their control	Yes	460	72.90	6.80±.2.76	**0.000**
	No	171	27.09	5.62±2.69	
I didn't go out unless I had to	Yes	589	93.34	5.87±1.85	0.347
	No	42	6.65	5.69±1.78	
I consumed foods that would strengthen my immunity	Yes	515	81.61	5.89±2.79	0.573
	No	116	18.38	5.76±1.76	
I wore a mask and took care of hand hygiene	Yes	575	91.12	5.79±2.73	**0.002**
	No	56	8.87	5.49±2.96	
Have you considered ending your pregnancy due to COVID-19?	Yes	26	4.12	5.77±1.79	0.675
	No	605	95.87	6.79±1.88	
Is there a difference compared to your other pregnancies due to COVID-19?	Yes	459	72.74	5.80±2.76	**0.000**
	No	173	27.41	5.62±2.69	

MDMS-I–II, Melbourne Decision-Making Scale I–II. Bold indicates statistically significant p-values.

Table 3. Comparison of pregnants' breastfeeding and delivery preferences with MDMS-I–II and scale sub-dimensions (n=631).

Variables		Self-esteem* X±SS	Careful Decision Making* X±SS	Avoidant Decision Making X±SS	Procrastinating Decision Making* X±SS	Panic Decision Making* X±SS	MDMS I-II Total avarage X±SS
Worrying about starting breastfeeding	Yes	5.98±2.56	9.86±1.56	5.64±1.57	4.48±2.48	4.69±1.54	6.56±2.11
	No	6.68±2.12	8.87±2.34	4.32±2.42	3.51±1.32	3.50±2.32	6.79±2.24
p		0.121	0.174	**0.029**	**0.003**	0.119	**0.028**
Worrying about the choice of birth environment	Yes	6.41±2.42	10.86±1.34	5.04±1.37	4.20±1.48	5.10±1.47	6.96±2.31
	No	8.58±2.33	9.87±2.38	4.06±1.42	4.11±1.52	3.50±2.11	6.77±2.65
p		**0.032**	0.089	**0.039**	**0.002**	0.443	**0.000**
Birth environment preference	Home	6.79±2.95	8.87±1.36	3.06±1.39	4.22±1.49	3.12±1.49	6.21±2.23
	Hospital	6.68±2.56	9.78±1.31	5.90±2.39	6.03±1.54	5.90±2.41	5.98±2.45
p		**0.032**	0.061	**0.004**	**0.007**	**0.001**	**0.002**
Choice of obstetrician	Midwife	5.98±2.34	8.37±1.66	3.76±2.26	4.22±1.36	3.43±1.21	6.54±2.66
	Doktor	6.17±2.76	10.48±1.43	4.94±2.21	3.43±1.34	4.67±2.65	6.68±2.43
p		0.056	0.170	0.114	0.231	0.570	0.176

MDMS-I–II, Melbourne Decision-Making Scale I–II. Bold indicates statistically significant p-values.

will be harmed is quite high. According to the findings of this study, it was determined that there was a significant relationship between the decision-making styles of pregnant women during the COVID-19 period and their anxiety about their birth environment preferences. In a qualitative study, women planning to give birth at home emphasized the quality of their birth experience and believed in the natural process of childbirth. Women planning to give birth in a hospital believed that access to medical care outweighed their concerns about the physical environment. This study showed that exposure to different situations affects our choices by influencing decision-making styles[19].

Although many associations, organizations, or societies in the world support woman-centered birth, women have stated that they think that a physician should be the person who decides on the mode of delivery, the delivery environment, or who will assist the birth, as, in our country, the primary manager of childbirth and the person who mostly carries out

pregnancy follow-ups is a physician. It was reported that the decision of the physician was mostly effective in the birth preference decisions of the pregnant women in this study. In a study, pregnant women stated that they did not want to take an active role and responsibility in making the delivery decision[17,18].

Limitations and generalizability

In this study, only pregnant women who came to the controls in pandemic hospitals in 10 different provinces across Turkey were included.

The study results can only be generalized to the pregnant women who took part in this study.

CONCLUSION

The findings of the study found that there was a significant difference between the preferences of the birth environment, the choice of personnel to assist the delivery, the anxiety about starting breastfeeding, and the decision-making styles of the pregnant women during the COVID-19 epidemic. The impact of epidemics on pregnant women, especially in the risk group category, is quite high and affects their decision-making styles. Decision making can be adversely affected when the necessary counseling is not received. Therefore, psychological education programs including guidance services and support systems should be conducted and guidelines should be published to help pregnant women cope with stress factors and how they should behave in future epidemics.

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ORIGINAL ARTICLE

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The effect of postnatal breastfeeding education given to women on breastfeeding self-efficacy and breastfeeding success

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SUMMARY

OBJECTIVE: This study was conducted to determine the effect of postnatal breastfeeding education given to women who had normal vaginal and cesarean delivery on breastfeeding self-efficacy and breastfeeding success.

METHODS: This is a pretest-posttest randomized controlled quasi-experimental study. This study included 76 women (38 intervention group and 38 control group) who gave birth in a women's and children's diseases training and research hospital.ClinicalTrials.gov Identifier: NCT 05666817. The data were collected by means of the introductory information form, breastfeeding knowledge level diagnosis form, LATCH scale, and postnatal self-efficacy scale. In the evaluation of the data, independent group t-tests and dependent group t-tests were used.

RESULTS: Research findings indicate that the women's breastfeeding knowledge level, LATCH scale, and postnatal breastfeeding self-efficacy scale scores were statistically higher than the control group in the post-test ($p<0.05$).

CONCLUSION: It was found by the researchers that postnatal breastfeeding education is effective in increasing the level of breastfeeding knowledge, breastfeeding success, and breastfeeding self-efficacy.

KEYWORDS: Breastfeeding. Education. Self efficacy. Women.

INTRODUCTION

The World Health Organization (WHO) recommends that babies be exclusively breastfed for the first six months and breastfed until at least two years of age[1]. In the report prepared within the scope of the WHO and UNICEF Global Breastfeeding Collective, the breastfeeding rates of 194 countries were evaluated; only 44% of babies were exclusively breastfed in the first 6 months, 68% of babies were breastfed until the age of 1 year, and 44% of babies were breastfed until the age of two. It is planned by WHO and UNICEF that the rate of exclusive breastfeeding throughout the world will be above 70% by 2030[2].

Breastfeeding self-efficacy shows the mother's belief that she can breastfeed her child, the effort she can put in, her thoughts on breastfeeding, the mother's belief in herself, and her perceived ability[3]. The concerns of mothers about whether they can breastfeed effectively, whether their milk is sufficient, and how they feel about breastfeeding may affect breastfeeding self-efficacy. In the studies carried out, it has been found that breastfeeding education given to women face-to-face, beginning during pregnancy and continuing after childbirth, increases the perception of breastfeeding self-efficacy[4-6].

Breastfeeding success is an interactive process that allows mother and child to mutually meet each other's needs[7]. In addition to the physical well-being of the mother, it is very important for her to be psychologically ready to breastfeed and to start breastfeeding early after childbirth in order to ensure breastfeeding success[8].

According to the WHO's data, it has been reported that mothers who received breastfeeding education started breastfeeding earlier in the postpartum period, breastfed their babies for a longer period of time, and had higher breastfeeding success than mothers who did not receive education[2]. In the literature, there are studies that have reached positive results on breastfeeding self-efficacy and breastfeeding success by providing breastfeeding education to mothers in the prenatal and postpartum period[9-11]. Although mothers receive prenatal breastfeeding education about potential problems in breastfeeding and the initiation and continuation of breastfeeding, they also need breastfeeding education in the postpartum period because they encounter these problems after giving birth.

Therefore, this study was conducted to determine the effect of postnatal breastfeeding education given to women who had

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normal vaginal and cesarean delivery on breastfeeding self-efficacy and breastfeeding success. The research hypotheses are:

H1a: The breastfeeding success of women in the intervention group is higher than that of the control group.

H1b: The breastfeeding self-efficacy of women in the intervention group is higher than that of the control group.

METHODS

This study was carried out based on a pretest-posttest randomized controlled quasi-experimental design in a women's and children's diseases training and research hospital in Istanbul between October 2021 and January 2022. The study was conducted with the ethics committee's approval (Date: 05.08.2021; Decision No. E.143) and in line with the principles of the Declaration of Helsinki.

For the sample size, a power analysis was performed by considering the monthly number of normal vaginal and cesarean deliveries in the postpartum care unit. In the pretest-posttest quasi-experimental research conducted by Özgüneş in the related literature, the effect size was calculated as 0.86[12]. In order to exceed the 95% value in calculating the power of the study, it was found that 76 women, including 38 interventions and 38 controls, should be reached in the study at a 5% significance level and a 0.86 effect size. In accordance with these criteria, the women participating in the study were randomly determined in such a way that the odd numbers formed the control group and the even numbers formed the intervention group, respectively. The mothers who volunteered to participate in the study were between the ages of 18 and 40, were literate, could read and understand Turkish, had no hearing, speech, or vision impairment, had a normal or cesarean delivery, and had healthy babies. Recommendations from the CONSORT group (Consolidated Standards of Reporting Trials) were followed in this study. This study is registered in The ClinicalTrials.gov Protocol Registration and Results System (PRS) ID: NCT 05666817.

As data collection tools, introductory information form, breastfeeding knowledge level diagnosis form, LATCH scale, and postnatal self-efficacy scale were used. Introductory information form developed by researchers in line with their knowledge of the literature consists of 12 questions in total. Breastfeeding knowledge level diagnosis form: In order to measure the effectiveness of breastfeeding, an expert opinion was obtained before the application for this form was prepared by the researchers in line with the literature, and after the preliminary application, it was rearranged and the final version was created[12]. This form consists of 15 questions about the breastfeeding initiation time, breast milk storage conditions, breastfeeding positions, duration and frequency of breastfeeding, and general information about breastfeeding and breast milk. Each question has three options: yes, no, and I don't know. 1 point is given for each correct answer, and 0 points are given for the incorrect or I don't know answer. The highest total score that can be obtained from the form is 15. Breastfeeding Assessment Tool (LATCH): The scale consists of five criteria. Each of the criteria is evaluated as "0, 1, 2" points. The maximum score that can be obtained is 10, and a high score on the assessment scale indicates that breastfeeding success is high. The Cronbach's alpha reliability coefficient of the original scale was calculated as 0.93. However, in this study, the Cronbach's alpha reliability coefficient of the scale was calculated as 0.74. Postnatal Self-Efficacy Scale: The scale assesses how competent mothers feel about breastfeeding. The 14-item scale includes a 5-point Likert-type scale consisting of "I'm not sure at all: 1, I'm not very sure: 2, Sometimes I'm sure: 3, I'm sure: 4, I'm very sure: 5." The minimum score that can be attained on the scale is 14, and the maximum score is 70. In this study, the postnatal form was applied. The reliability coefficient of the scale was calculated as 0.80 in this study.

Intervention

Data were collected from the mothers participating in the study through face-to-face interviews twice, as a pre-test and a post-test. In the pre-test, the introductory information form, breastfeeding knowledge level diagnosis form, LATCH scale, and postnatal self-efficacy scale were used. In the post-test, the breastfeeding knowledge level diagnosis form, LATCH scale, and postnatal self-efficacy scale were applied.

Within the scope of the research, breastfeeding education was given to mothers in the intervention group in the room where they stayed in the form of a straight narration supported by a puppet and amigurumi breast. The question-and-answer method was used after education. In addition, in order to evaluate whether the mother understood the breastfeeding education after its completion, the mother demonstrated the practices (the way of holding the child, grasping the breast, positioning the child appropriately, and the process of milking and storing breast milk) described by the researcher.

Statistical analysis

The data obtained in the study were analyzed using the SPSS 25.0 (Statistical Package for Social Science) program. Number, percentage, mean, and standard deviation were used as descriptive statistical methods in the evaluation of the data. The differences between sociodemographic and descriptive variables in independent groups were analyzed with chi-square and Fisher's

exact tests. In the examination of the differentiation of the scale scores according to the groups, the independent groups t-test was used, and in the examination of the variation between the pre-test and post-test measurements of the scales within the groups, the dependent groups t-test analyses were used.

RESULTS

The average age of the women participating in the study was 30.82±4.95 years. The intervention and control groups were found to be similar in terms of their descriptive characteristics (Table 1) ($p>0.05$).

In the post-test, the total breastfeeding knowledge level diagnosis form, LATCH scale, and postnatal breastfeeding self-efficacy scale total scores of women in the intervention group were found to be significantly higher than the control group (t^b=-12.310, $p<0.001$; t^b=-7.255, $p<0.001$; t^b=-10.170, $p<0.001$, respectively) (Table 2).

In Table 3, the relationship between the breastfeeding knowledge level diagnosis form, LATCH scale scores, and postnatal breastfeeding self-efficacy scales is given. There is a moderate positive correlation between the LATCH scale pre-test score and LATCH scale post-test score ($r=0.685$, $p<0.001$); a weak positive correlation between the breastfeeding knowledge level diagnosis form pre-test score and postnatal breastfeeding self-efficacy scale post-test score ($r=0.252$, $p<0.05$); a strong positive correlation between the postnatal breastfeeding self-efficacy scale pre-test score and postnatal breastfeeding self-efficacy scale post-test score ($r=0.705$, $p<0.001$); and a weak positive correlation between the postnatal breastfeeding self-efficacy scale post-test score and breastfeeding knowledge level diagnosis form post-test score ($r=0.427$, $p<0.001$) (Table 3).

DISCUSSION

The results of the study revealed that postnatal breastfeeding education given by nurses to women who had normal vaginal and cesarean delivery can increase breastfeeding knowledge level, breastfeeding success, and breastfeeding self-efficacy.

The average age of the women was 30.82±4.95 years in this study. Wang et al. found the average age of women to be 31.3±4.9 years, Gao et al. 31.26±4.22 years, and Magnazi et al. 32.55±4.2 years in their studies. The reason for this difference is thought to be due to the low rate of primiparous women participating in our study[13-15].

In this study, we concluded that 60.5% of the women in the intervention group and 55.3% of the women in the control group had a cesarean delivery. Minharro et al. determined that 52.6% of women and Ergezen et al. determined that 68.6% of women gave birth by cesarean section[10,16]. The research findings show similarities with our research. It is believed that the high rate of cesarean delivery in this study is due to the fact that the study was conducted in a training and research hospital where risky pregnancies were referred. The fact that the rate of cesarean section is above the 10–15% target determined by WHO is considered a sign that prenatal information and efforts to encourage normal birth should be increased[1].

In the study, it was determined that 18.4% of the women in the intervention group received prenatal breastfeeding education, and all of those who received breastfeeding education received it from a nurse. Muda et al. found in their study that 26.3% of women received breastfeeding education, and in the study of Minharro et al. to determine the perception of breastfeeding self-efficacy, 64.3% of women received breastfeeding education during pregnancy[10,17]. The information from the literature shows that women who receive breastfeeding education are able to breastfeed their babies for a longer period of time[17,18]. It is thought that the low rate of prenatal breastfeeding education findings in our study was due to the fact that the mothers participating in the study experienced their pregnancy during the pandemic period, and therefore, this situation also affected their participation in breastfeeding education.

The breastfeeding knowledge level diagnosis form post-test scores of the women in the intervention group were found to be statistically and significantly higher than those of the control group in this study. This significant increase indicates that the breastfeeding education provided is effective in increasing the level of breastfeeding knowledge. In addition, it is believed that the application of the demonstration technique using puppets and amigurumi breasts in the breastfeeding education within the scope of the research contributed to the increase in the post-test scores of the women in the intervention group. The information from the literature also indicates that supporting breastfeeding education with supplementary materials benefits the effectiveness and sustainability of education, and studies in the literature support our findings[13].

In this study, the LATCH scale post-test mean score of the intervention group was found to be significantly higher than the control group. In the study conducted by Gao et al., breastfeeding education was given to women from the 32nd week of pregnancy, and it was seen that the postpartum intervention group underwent the breastfeeding process more successfully than the control group. In the study of Liu et al., primiparous women were given breastfeeding education during pregnancy and after childbirth, and it was stated that the breastfeeding success of women who received education was higher than that of the control group[13,18]. The literature shows that breastfeeding

Table 1. Distribution of introductory characteristics of women with normal vaginal and cesarean delivery (n=76).

Variables	Experimental (n=38)		Control (n=38)		Statistics	
	n	%	n	%	χ^2	p-value
Age (years)						
18–25	5	13.2	5	13.2	2.410	0.492
26–30	10	26.3	16	42.1		
31–35	15	39.5	12	31.6		
≥35	8	21.1	5	13.2		
Educational status						
Primary school graduate	8	21.1	5	13.2	2.065	0.559
Secondary school graduate	10	26.3	10	26.3		
High school graduate	8	21.1	13	34.2		
University graduate/Postgraduate	12	31.6	10	26.3		
Economic status						
High	5	13.2	2	5.3	2.286	0.319
Middle	30	78.9	30	78.9		
Low	3	7.9	6	15.8		
Number of pregnancies						
1	6	15.8	7	18.4	0.869	0.833
2	11	28.9	14	36.8		
3	12	31.6	10	26.3		
≥4	9	23.7	7	18.4		
Mode of delivery						
Vaginal	15	39.5	17	44.7	0.216	0.408
Caesarean section	23	60.5	21	55.3		
Sex of the child						
Girl	20	52.6	16	42.1	0.844	0.245
Boy	18	47.4	22	57.9		
State of wanting pregnancy						
Yes	21	55.3	25	65.8	0.881	0.241
No	17	44.7	13	34.2		
Number of applications to health institution during pregnancy						
0–2	1	2.6	0	0	2.061	0.357
3–5	3	7.9	6	15.8		
≥6	34	89.5	32	84.2		
Breastfeeding education status						
Yes	7	18.4	4	10.5	0.957	0.258
No	31	81.6	34	89.5		
From whom did she/he receive breastfeeding education?*						
Nurse	7	100	4	100		
Desire to receive postpartum breastfeeding education						
Yes	36	94.7	35	92.1	0.214	0.500
No	2	5.3	3	7.9		

χ^2: Chi-square test. *This question was answered by mothers who received breastfeeding education.

Table 2. The differentiation status of breastfeeding knowledge level diagnostic form, LATCH scale, and postnatal breastfeeding self-efficacy scale scores according to groups (n=76).

Breastfeeding knowledge level diagnosis form	Experimental (n=38)	Control (n=38)	Statistics	
	x̄±SS	x̄±SS	t[a]	p-value
Pre-test	10.18±1.59	10.29±1.62	-0.285	0.776
Post-test	13.68±1.14	10.63±1.92	8.414	**0.000**
t[b]	-12.310	-1.04		
p-value	**0.000**	0.303		
LATCH scale				
Pre-test	7.07±1.56	7.07±1.26	0.000	1.000
Post-test	8.31±1.31	7.60±1.28	2.380	**0.020**
t[b]	-7.255	-3.141		
p-value	**0.000**	**0.003**		
Postnatal breastfeeding self-efficacy scale				
Pre-test	54.76±5.26	55.42±6.80	-0.471	0.639
Post-test	60.50±3.90	54.92±6.19	4.694	**0.000**
t[b]	-10.170	0.952		
p-value	**0.000**	0.347		

[a]Independent groups t-test; [b]Dependent groups t-test. Bold values indicate statistical significance at p<0.05 level.

Table 3. The relationship between breastfeeding knowledge diagnostic form, LATCH scale, and postnatal breastfeeding self-efficacy scale (n=76).

Variables	1	2	3	4	5	6
Breastfeeding knowledge level diagnosis form pre-test (1)	1.000					
LATCH scale pre-test (2)	-0.127	1.000				
Postnatal breastfeeding self-efficacy scale pre-test (3)	0.312**	-0.023	1.000			
Breastfeeding knowledge level diagnosis form post-test (4)	0.190	0.017	0.092	1.000		
LATCH scale post-test (5)	-0.213	0.685**	-0.110	0.124	1.000	
Postnatal breastfeeding self-efficacy scale post-test (6)	0.252*	-0.010	0.705**	0.427**	0.121	1.000

Pearson correlation analysis, *<0.05, **<0.001.

success is affected by many factors and that breastfeeding success increases as the mother's education level increases, while the lack of breastfeeding information, negative experiences in previous breastfeeding, and cesarean section as a delivery method reduce breastfeeding success[13,18]. In the study, the fact that there is a positive correlation between the LATCH scale pre-test score and the LATCH scale post-test score proves the effectiveness of the education. H1a hypothesis was accepted according to the study results.

The post-test postnatal breastfeeding self-efficacy scale scores of the intervention group were found to be significantly higher than those of the control group. It is believed that postnatal breastfeeding education given by a nurse, using the face-to-face interview method to women who have had vaginal delivery or cesarean delivery, contributes to an increase in the perception of breastfeeding self-efficacy. Araban et al. concluded in their study that breastfeeding education given to women during pregnancy is beneficial; Shafaei et al. concluded that breastfeeding counseling starts during pregnancy and continues up to 4 months after birth; and Pilus et al. concluded that face-to-face breastfeeding education increases the perception of breastfeeding self-efficacy[4-6]. H1b hypothesis was accepted according to the study results.

CONCLUSIONS

The results of this study suggest that breastfeeding education given by nurses can increase the level of breastfeeding knowledge, breastfeeding success, and breastfeeding self-efficacy. As a result of research findings, it is recommended that regular breastfeeding education be given by nurses in the early postpartum

period, to shape the education plan according to the mother's age, birth type, education level, and previous experience during education, to support education with visual materials such as puppets and amigurumi breasts, and to ensure the standardization of breastfeeding education by having all nurses working in postpartum services participate in breastfeeding counseling programs.

AUTHORS' CONTRIBUTIONS

NBKA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **MK:** Conceptualization, Formal Analysis, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Inflammatory prognostic index predicts new-onset atrial fibrillation and mortality after on-pump coronary artery bypass grafting

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SUMMARY

OBJECTIVE: This study aimed to analyze its predictive role in incipient postoperative atrial fibrillation by constructing an inflammatory prognostic index based on hematological and biochemical parameters in patients undergoing elective isolated coronary artery surgery accompanied by cardiopulmonary bypass.

METHODS: The data of 343 patients who underwent coronary bypass surgery between May 2021 and July 2022 were evaluated. Multivariate logistic regression and recipient study characteristic curve analyses were studied by comparing the patients' hematological indices and basic clinical features between the two groups.

RESULTS: Logistic regression analysis showed that age (p<0.001), hypertension (p=0.01), and inflammatory prognostic index (p<0.001) were independent predictors of new-onset postoperative atrial fibrillation. To predict the development of postoperative atrial fibrillation, a cutoff value of 0.25 (77.8% sensitivity and 69.3% specificity) was determined for inflammatory prognostic index in the receiver-operating characteristic curve analysis (area under curve=0.798, 95% confidence interval 0.752–0.840).

CONCLUSION: Inflammatory prognostic index can be a noninvasive, easily available marker for predicting new-onset atrial fibrillation after coronary artery bypass surgery.

KEYWORDS: Atrial fibrillation. Coronary artery disease. Inflammation.

INTRODUCTION

Coronary artery bypass graft (CABG) operations are the most commonly performed cardiac surgery procedure all over the world, and atrial fibrillation (AF) is the most common arrhythmia after coronary artery surgery. The incidence of postoperative atrial fibrillation (POAF) after CABG varies between 20 and 50%[1]. Many factors are held responsible for the development of POAF after CABG. Use of cardiopulmonary bypass (CPB), cardioplegic agents used, inappropriate use of preoperative antiarrhythmic agents, surgical trauma, hypoxia, and electrolyte disturbances are some of these[1]. Inflammatory reactions that begin after surgical stress are predicted to trigger arrhythmogenic events[2,3]. In many studies, the development of POAF has been shown to be associated with higher costs and increased morbidity and mortality rates due to longer hospital stays[4]. Predicting the risk of developing POAF and identifying high-risk patients are important in terms of taking necessary prophylactic measures. Therefore, there is a need for simple, inexpensive, and reliable biomarkers that can be used in daily clinical practice for the prediction of POAF.

Potential predictive biomarkers for POAF after CABG have been studied and have been shown to predict POAF. White blood cell (WBC)[5], neutrophil/lymphocyte ratio (NLR)[6], platelet/lymphocyte ratio (PLR)[7], C-reactive protein (CRP)[3], and interleukins[3] are frequently used biomarkers. The inflammatory prognostic index (IPI): CRP, NLR, and serum albumin (ALB) levels are evaluated together, and it is a new hematological biomarker that shows the inflammatory and immune status of patients (IPI=CRP×NLR/ALB). This new biomarker has been shown to provide important information about prognosis in oncologic patients, and its high levels are associated with poor outcomes[8-10]. To the best of our knowledge, there is no study in the literature showing the role of IPI in predicting the risk of new-onset POAF in patients after CABG. Therefore, we investigated the possible role of IPI in predicting new-onset POAF after CABG.

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METHODS

Study population and design

For the study, approval was obtained from the local ethics committee of our hospital (No: 2022-10/9, Date: 16.09.2022). We retrospectively evaluated 343 adult patients who underwent elective isolated CABG operations using CPB between May 2021 and July 2022 in our hospital. The patients were classified into two groups: those who did not develop as group 1 POAF (72.3%, n=248) and those who developed as group 2 POAF (27.7%, n=95). The groups were compared in terms of demographic, clinical, and preoperative blood parameters. Preoperatively, the patients' complete blood count parameters (hemoglobin, WBC, thrombocyte, neutrophil, and lymphocyte), biochemical parameters (serum CRP, albumin, urea, creatinine, glomerular filtration rate (GFR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), electrocardiographic, and echocardiographic (ECHO) findings) were recorded. The incidence of POAF in electrocardiography was determined by electrocardiography (ECG) and rhythm monitoring from the day of operation until discharge, considering new-onset POAF in cases of urgent intervention due to an AF rhythm lasting longer than 10 min or an unstable hemodynamic condition. The ECHO findings were determined by measuring left ventricular ejection fraction and left anteroposterior atrial diameter on a parasternal long-axis view. In the intraoperative and postoperative periods, CPB duration, cross-clamp time and number of bypass grafts, infection status, stroke development, intensive care unit (ICU) and hospitalization time, reintubation, reoperation, low cardiac output, and mortality rates were investigated.

Patients who received emergency surgery, reoperative surgery, valve surgery, permanent pacemaker and implantable cardioverter defibrillator, malignancy, sepsis, autoimmune and inflammatory disease, AF history, and preoperative amiodarone treatment were excluded from the study as they were thought to adversely affect the statistical results.

After the operation, ECG, arterial blood pressure, central venous pressure, oxygen saturation, and urine output were continuously monitored in all patients. The cardiac rhythms of the patients were evaluated by taking a standard 12-lead ECG every day. Additionally, radial pulse control was performed four times a day to check for rhythm changes.

Statistical analysis

Data were entered into the Statistical Package for the Social Sciences (IBM® SPSS Statistics for Windows, Version 23.0, Armonk, NY, USA) software package. Whether the distributions were normal or not was determined by Kolmogorov-Smirnov analysis. Student's t-test was used for comparisons between groups. Pearson's chi-square test was used for comparative analysis of qualitative variables; however, Fisher's exact test was used if the sample size was small (≤5). Interquartile range (IQR) results were also given for the values recorded as median. A p-value <0.05 was considered statistically significant. Multivariate analysis was performed using variables that were found to have a statistically significant effect on AF in the univariate analysis ($p<0.05$). In the univariate analysis, only a single variable was included in the multiple logistic regression analysis when there were confounders (e.g., albumin, CRP, NLR, and IPI, or neutrophils, lymphocytes, and NLR) among the variables found to statistically affect the development of AF. Whether IPI predicted AF was analyzed by performing ROC analysis, and the area under the curve (AUC) was calculated.

RESULTS

Group 2 patients were statistically significantly older than group 1 patients, and the median age was 59 (35–78) and 70 (39–86) years for groups 1 and 2, respectively ($p<0.001$). The number of patients with HT and COPD, left atrial size, neutrophil, lymphocyte, hemoglobin, NLR, PLR, CRP, urea, GFR, albumin, ALT, and albumin levels were found to be higher in group 1 compared to group 2. HT, COPD, left atrial size, neutrophils, NLR, PLR, CRP, urea, and IPI were found to be statistically significantly higher in group 2 compared to group 1. The groups were similar in terms of other preoperative variables, and no significant difference was found (Table 1).

In the postoperative follow-ups, the length of stay in the ICU and hospital was found to be longer in the POAF group. It was observed that patients who developed POAF had a statistically higher rate of re-intubation, more low cardiac output syndrome, and a higher rate of surgery-related infection. The mortality rate in patients who developed POAF was found to be higher, close to statistical significance, than that of those who did not develop POAF (Table 1).

A multivariate logistic regression analysis was performed to explore the link between POAF occurrence and independent predictors by controlling relevant variables. Age, HT, and IPI were found to be independent variables determining the development of POAF in multivariate logistic regression analysis (Table 2).

The effectiveness of IPI in predicting the development of AF was examined by performing ROC analysis. IPI was found to have an adequate AUC value (AUC 0.798, 95%CI 0.745–0.852). The threshold value was determined as 0.25 according to the best sensitive and specificity values for IPI (Figure 1).

Table 1. Comparison of demographic, clinical, and laboratory values of group 1 and group 2 patients.

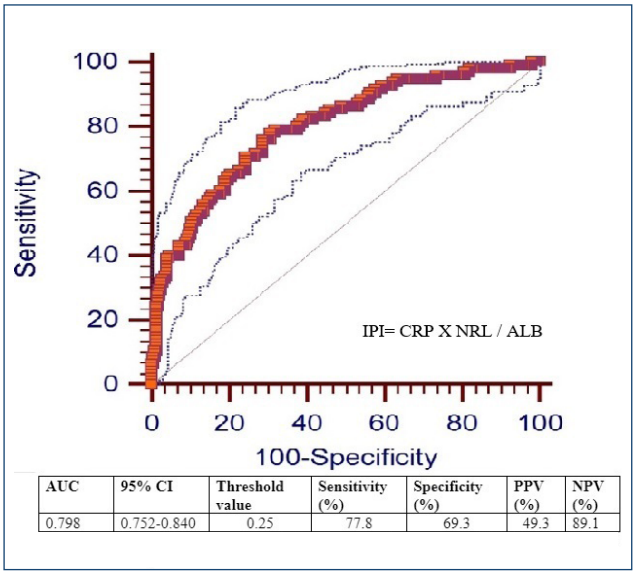
Variable	Group 1 (n=248)	Group 2 (n=95)	p-value
Age (years), median (IQR)	59 (12)	70 (15)	**<0.001**
Gender, n (%)			
Male	191 (77.0)	68 (71.6)	0.295
Woman	57 (23.0)	27 (28.4)	
BMI (kg/m^2), median (IQR)	26.8 (4.7)	27.6 (4.9)	*0.09*
DM, n (%)	98 (39.5)	44 (46.3)	0.253
HT, n (%)	104 (41.9)	61 (64.2)	**<0.001**
COPD, n (%)	18 (7.3)	22 (23.1)	**0.03**
Smoke, n (%)	86 (34.7)	40 (42.1)	0.202
CABG graft count, median (IQR)	4 (1)	3 (1)	0.644
EF (%), median (IQR)	50 (15)	55 (15)	0.975
Left atrium size, median (IQR)	3.9 (0.3)	4.0 (0.2)	**0.009**
CPD time (min), median (IQR)	88 (38)	88 (45)	0.492
Cross time, median (IQR)	57 (25)	60 (21)	0.449
WBC (10^3/μL), median (IQR)	8.8 (3.2)	8.8 (3.0)	0.491
Neutrophil (10^3/μL), median (IQR)	5.6 (2.7)	7.3 (3.7)	**<0.001**
Lymphocyte (10^3/μL), median (IQR)	2.2 (1.1)	2.0 (1.2)	**0.02**
NLR, median (IQR)	2.4 (1.5)	4.3 (3.0)	**<0.001**
Monocyte (10^3/μL), median (IQR)	0.6 (0.3)	0.7 (0.2)	0.292
Eosinophil (10^3/μL), median (IQR)	0.1 (0.2)	0.1 (0.1)	0.931
Hb (g/dL), median (IQR)	13.6 (2.4)	13.0 (2.7)	**0.03**
RDW-SD (fL), median (IQR)	40.1 (4.5)	40.2 (4.4)	0.256
PCT (%), median (IQR)	0.26 (0.10)	0.26 (0.10)	0.922
MPV (fL), median (IQR)	10.2 (1.4)	10.2 (1.4)	0.975
PDW (fL), median (IQR)	11.6 (3.1)	11.6 (2.5)	0.788
Platelet (10^3/μL), median (IQR)	246.5 (97.8)	256.0 (91.0)	0.300
PLR, median (IQR)	113.5 (58.6)	138.3 (89.2)	**0.002**
CRP (mg/L), median (IQR)	2.6 (3.2)	4.7 (6.1)	**<0.001**
Urea (mg/dL), median (IQR)	32.6 (18.3)	35.5 (18.6)	**0.01**
Creatinine (mg/dL), median (IQR)	0.8 (0.2)	0.9 (0.3)	*0.08*
GFR (mL/min/1.73 m^2), median (IQR)	90.0 (26.2)	80.0 (29.6)	**<0.001**
Albumin (g/L), median (IQR)	42.0 (5.4)	35.8 (5.6)	**<0.001**
AST (IU/L), median (IQR)	21.0 (15.8)	20.0 (11.0)	*0.06*
ALT (U/L), median (IQR)	20.0 (13.8)	17.0 (12.0)	**0.01**
Potassium (mmol/L), median (IQR)	4.3 (0.6)	4.3 (0.6)	0.650
IPI, median (IQR)	0.15 (0.21)	0.53 (0.95)	**<0.001**
Reoperation, n (%)	4 (1.6)	4 (4.2)	0.224
ICU stay, day, median	2.5 (1.0)	4.0 (2.0)	**<0.001**
Hospital stay, day, median	7.0 (1.0)	9.0 (3.0)	**<0.001**
Mortality, n (%)	7 (2.8)	7 (7.4)	*0.05*
Re-intubation, n (%)	18 (7.3)	14 (14.7)	**0.03**
Low cardiac output, n (%), n (%)	9 (3.6)	12 (12.6)	**0.002**
Stroke, n (%)	4 (1.6)	3 (3.2)	0.401
Infection, n (%)	11 (4.4)	14 (14.7)	**0.001**

Bold p-values indicate statistical significance. The p-values indicated in italics are values close to statistical significance. Alb: albumin; ALT: alanine transaminase; AST: aspartate aminotransferase; BMI: body mass index; CABG: coronary artery bypass grafting; COPD: chronic obstructive lung disease; CPD: cardiopulmonary bypass; CRP: C-reactive protein; DM: diabetes mellitus; EF: ejection fraction; GFR: glomerular filtration rate; Hb: hemoglobin; HT: hypertension; ICU: intensive care unit; IPI: inflammatory prognostic index; IQR: interquartile range; MPV: mean platelet volume; n: number; NLR: neutrophil lymphocyte ratio; PCT: plateletcrit; PDW: platelet distribution width; Plt: platelet; PLR: platelet lymphocyte ratio; RDW-SD: red blood cell erythrocyte distribution width; WBC: white blood cell.

Table 2. Investigation of independent risk factors affecting the development of atrial fibrillation by multivariate analysis*.

Variable	Odds ratio	95%CI	p-value
Age (for each year)	1.132	1.085–1.184	**<0.001**
HT presence	2.089	1.138–3.834	**0.01**
COPD presence	1.427	0.521–3.515	0.489
Left atrium size (for each unit)	2.636	0.714–9.733	0.146
Hb (for each unit)	1.055	0.885–1.259	0.550
Urea (for each unit)	0.996	0.983–1.010	0.419
GFR (for each unit)	1.001	0.983–1.020	0.877
AST (for each unit)	0.998	0.981–1.016	0.865
IPI (for each unit)	10.880	4.810–24.610	**<0.001**

*In univariate analysis, only a single variable was included in the multivariate analysis when there were confounders (e.g., albumin, CRP, NLR and their IPI, or neutrophils, and lymphocytes and their NLR) that were found to statistically affect the development of AF. Bold p-values indicate statistical significance. AST: aspartate aminotransferase; CI: confidence interval; COPD: chronic obstructive; GFR: glomerular filtration rate; Hb: hemoglobin; HT: hypertension; IPI: inflammatory prognostic index.



AUC	95% CI	Threshold value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0.798	0.752-0.840	0.25	77.8	69.3	49.3	89.1

Figure 1. Receiver operating characteristic curve for inflammatory prognostic index. AUC: area under the curve; CI: confidence interval; IPI: inflammatory prognostic index; NPV: negative predictive value; PPV: positive predictive value.

DISCUSSION

Our study revealed that the patients in the group developing POAF were statistically significantly older than the other group, and the number of patients with HT and COPD was higher. Considering the hematological and biochemical parameters, hemoglobin, lymphocyte, GFR, ALT and albumin values were found to be lower in the POAF group compared to the other group. In addition, the left atrial size, neutrophil, NLR, PLR, CRP, urea, and IPI values were found to be statistically significantly higher in the group with POAF compared to the other group. Age, HT, and IPI values were found to be statistically significant in multivariate analysis. In our study, it was determined that each unit increase in IPI increased the probability of AF 10.8 times, age 1.1 times, and HT 2.08 times. It was concluded that these parameters independently predicted POAF after CABG. The most valuable finding in our study was that, for the first time in the available literature, IPI independently predicted POAF after CABG.

The exact mechanisms of AF after CABG are still unclear. There are studies reporting that systemic inflammation is associated with the occurrence and recurrence of AF in patients undergoing CABG surgery[11-13]. POAF occurs most frequently 2–4 days after surgery, and it is estimated that 96% of cases occur within the first 1 week after surgery[14].

A systematic review and meta-analysis, including 6,098 patients and 22 studies, was conducted to evaluate the relationship between POAF developing after CABG and hematological indices. In this study, it was shown that platelet count, mean platelet volume (MPV), WBC, NLR, and red blood cell distribution width can predict the risk of POAF[15]. In the univariate analysis of our study, in addition to this meta-analysis, we determined that the amounts of neutrophils, lymphocytes, hemoglobin, and PLR would be the predictive hematological index for POAF.

In the prediction of patients at risk of POAF, some clinical scoring methods (CHA2DS2-VASc Score, HATCH Score, etc.) can be used in addition to inflammation and immune-based prognostic scoring methods (systemic immune inflammatory index, etc.)[16-18].

In some studies in the literature, inflammation and immune-based effects of hematological parameters such as CRP, NLR,

and PLR have been shown to predict mortality and morbidity after cardiac surgery[3,11,13]. In addition, a meta-analysis examining 42 studies evaluating its association with POAF following CABG showed that perioperative inflammation is involved in the pathogenesis of POAF[3]. In these studies, it was concluded that the perioperative evaluation of inflammatory markers, especially CRP, would help clinicians in predicting and monitoring POAF. In a study focusing on new-onset POAF after CABG, it was shown that low preoperative albumin levels are a risk factor for the development of POAF[19]. In our study, we showed that IPI composed of CRP, NRL, and albumin predicted POAF more strongly in patients undergoing elective isolated CABG operations.

IPI has clinical importance in predicting prognosis and is a new inflammatory prognostic marker based on CRP, NLR, and serum albumin. IPI was developed for the first time from hematological and biochemical parameters by Dirican et al. to determine the prognosis of patients with non-small cell lung cancer[8]. IPI is a noninvasive, inexpensive, accessible, and easily formulated parameter to determine prognosis. It has been used as an important new marker to determine survival in many studies on oncological patients[8-11]. To the best of our knowledge, there is no published study investigating the relationship between IPI and POAF. We found in our study that IPI predicted POAF and survival in patients who underwent CABG for the first time in the literature. We demonstrated that the cutoff value of IPI was 2.5 in patients who developed POAF after CABG. It estimated incipient POAF with a sensitivity of 77.8% and a specificity of 69.3%.

There are many studies showing that it is associated with POAF developing after CABG, hemodynamic instability, prolonged hospital stay, increased risk of stroke, and increased mortality[20-22]. In our study, it was concluded that, similar to the literature, it increased reintubation rates, development of low cardiac output syndrome, rates of surgery-related infection, and length of stay in the ICU and hospital. Therefore, identifying patients with a high risk of POAF is considered critical for taking the necessary precautions in the preoperative period.

Our study had a few limitations. The most important limitations of our study were that it was single-center and retrospective. Another important limitation is the relatively small number of patients and the limited number of parameters examined. In addition, the lack of correlation analysis with other predictive markers of inflammatory response is an important limiting factor.

Studies on indices derived from hematological parameters in the literature have gained importance recently[3,11,13,15,23,24].

CONCLUSION

Our study revealed that age, HT, and IPI are independent predictive risk factors for POAF developing after elective isolated CABG. The most valuable finding in our study was that, for the first time in the available literature, IPI independently predicted POAF after CABG. To the best of our knowledge, there is no published study investigating the relationship between IPI and POAF. To support our study's findings and provide more precise scientific data, better structured trials with higher patient participation are required.

AUTHORS' CONTRIBUTIONS

SB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **AP:** Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Pregnancy school education program in mother friendly training and research hospital impact on stress and anxiety

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SUMMARY

OBJECTIVE: This study aimed to investigate prenatal education and its relationship with anxiety and stress in pregnant women.

METHODS: This research was carried out between July 1, 2022, and December 1, 2023, at Giresun Gynecology and Pediatrics Training and Research Hospital. Women aged >18 years at >20 weeks of pregnancy were included. Patients were randomized into two groups, and one group received a 4 week training on meditation, breathing exercises, and pregnancy-related health issues. A questionnaire was applied to both groups to analyze sociodemographic characteristics, pregnancy, birth, medical history, the Pregnancy Stress Rating Scale, and the State Trait Anxiety Inventory.

RESULT: The groups were similar in terms of age, educational status, anthropometric characteristics, occupation, economic status, and gestational week. There was no difference between the trained and nontrained groups in terms of the Pregnancy Stress Rating Scale score and the State Trait Anxiety Inventory-state score. The State Trait Anxiety Inventory-trait was significantly lower in the trained group (p=0.033). There were weak positive correlations between Pregnancy Stress Rating Scale score and medication use and between State Trait Anxiety Inventory-state score and age. A negative correlation was found between the State Trait Anxiety Inventory-state score and working status, showing that employed women had lower anxiety scores irrespective of training. Another weak positive correlation was found between the State Trait Anxiety Inventory-trait score and the presence of comorbidities.

CONCLUSION: State Trait Anxiety Inventory-trait anxiety was lower in pregnant women who received training on prenatal meditation, exercise, and pregnancy health; however, State Trait Anxiety Inventory-state and Pregnancy Stress Rating Scale scores were similar in the two groups. Unemployed pregnant women and those with chronic diseases appear to need closer follow-up to reduce anxiety levels.

KEYWORDS: Pregnancy. Maternal health. Prenatal care. Health education. Prenatal education. Anxiety.

INTRODUCTION

Pregnancy brings biological, psychological, and social changes in women, which may lead to stress, anxiety, and depression to varying degrees throughout this period[1]. Care for the emotional state of pregnant women remains a neglected aspect of obstetric medicine. Depression, anxiety, and stress during pregnancy can often go undetected or untreated[2].

Prenatal anxiety symptoms can have negative health consequences not only for the expectant mother, but also for the unborn baby[3]. Low birth weight, a smaller head circumference, spontaneous preterm birth, preterm birth, emotional problems, symptoms of attention deficit hyperactivity disorder, or impaired cognitive development may occur in children of mothers who experienced depression, anxiety, or stress during pregnancy[2,4]. Therefore, it is crucial for the physical and mental health of the mother and the baby to evaluate and reduce the anxiety and stress of pregnant women.

While factors such as young age, low education level, incompatibility in family relationships, low life satisfaction, and lack of social support are among the reported risk factors for stress, anxiety, and depression encountered in the prenatal period, it has been reported that exercise has a protective effect[1]. Providing prenatal training to mothers has been shown to increase the resilience of pregnant women against anxiety and stress, which may lead to a reduced likelihood of depression and anxiety disorders as well as improved physiological characteristics[5].

The aim of this study was to evaluate the levels of anxiety and stress in pregnant women with and without prenatal education.

METHODS

This cross-sectional study was carried out between July 1, 2022 and December 1, 2022, at Giresun Gynecology and Childhood

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Education and Research Hospital. Ethics committee approval for the research (dated June 4, 2022, number: E-50288587-050.01.04-93332) was received from the Scientific Ethics Committee of Giresun University. The authors declared that it has been conducted in accordance with the Declaration of Helsinki.

The research was carried out in Giresun Gynecology and Pediatrics Training and Research Hospital with women aged 18 years and over 40 years who had been pregnant for at least 5 months (>20 weeks of gestation). Pregnant women who applied to the outpatient clinic for routine follow-ups were randomized into two groups with respect to prenatal training. Pregnant women in the training group were enrolled in a "pregnant school" with a training program including meditation, breathing exercises, and theoretical and physical training for childbirth. Ministry of Health pregnant school training is given by a certified nurse for 1 h daily per week for a total of 4 weeks[6]. A structured "controlled frequency breathing technique" helps women in the antenatal period distract themselves from negative emotions through breathing exercises[7]. Pregnant women who had a risky pregnancy, had a history of psychiatric disease or had received medication for a psychiatric condition, had divorced, or did not accept to participate in the study were not included.

After giving detailed information about the purpose and scope of the study to the pregnant women included in the study group, written consent was obtained from those who agreed to participate in the study. As part of the training, breathing and straining techniques were taught to the pregnant women who attended the pregnant school. The lessons were provided in the form of oral lectures, brochures, slide presentations, and practical demonstrations. The knowledge and characteristics of women who received training were assessed after 4 weeks of training using a questionnaire. The results were compared by applying the same questionnaire form to the pregnant women who accepted to participate in the study but were not enrolled in the pregnant school.

The questionnaire included questions about women's sociodemographic characteristics, pregnancy, birth, medical history, the Pregnancy Stress Rating Scale (PSRS-36), and the State Trait Anxiety Inventory (STAI).

Pregnancy Stress Rating Scale

The PSRS, which was used to assess pregnancy-related stress, was developed by Chen and colleagues, and its Turkish validity and reliability study was performed by Akın. The PSRS-36 consists of 5 sub-dimensions and 36 items. Each scale item received a response in a 5-point Likert-type system, scored between 0 and 4. The total score that can be obtained from the scale varies between 0 and 144, and as the score obtained from the scale increases, it is interpreted as an increase in pregnancy-related stress[8,9].

State Trait Anxiety Inventory

The STAI was used to evaluate the anxiety level of pregnant women. The Turkish validity and reliability study of STAI was performed by Öner and Le Compte. The scale consists of 2 sub-dimensions of 20 items each, which assess state anxiety (perceived anxiety in a temporary state) and trait anxiety (characteristic anxiety levels). The feelings and behaviors expressed by the person in the scale items are graded as "never=1, sometimes=2, often=3, always=4." Items 1, 2, 5, 8, 10, 11, 15, 16, 19, and 20 in the STAI-state sub-dimension scored inversely. In the STAI-trait sub-dimension, items 21, 26, 27, 30, 33, 36, and 39 are scored inversely. The score that can be obtained from each sub-dimension of the scale varies between 20 and 80, and as the score increases, the level of anxiety is expected to increase[10,11].

Statistical analysis

All analyses were evaluated with a $p<0.05$ threshold for statistical significance and were performed on IBM SPSS Statistics for Windows, Version 25.0 (IBM, NY, USA). For the normality check, the Kolmogorov-Smirnov test was used. Data are given as mean±standard deviation or median (1st–3rd quartile) for continuous variables according to normality of distribution, whereas absolute and relative frequencies are reported for categorical variables. Normally distributed variables were analyzed with the independent samples t-test. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Spearman correlation coefficients were calculated to evaluate the directional relationships between continuous variables. Categorical variables were analyzed with appropriate chi-square tests (Pearson, Yate's continuity correction) or Fisher's exact tests.

RESULTS

There were 110 pregnant women in the study group, 55 of whom received training and 55 did not. Trained and non-trained women were similar in terms of age (p=0.554), educational status (p=0.180), height (p=612), weight (p=0.893), BMI (p=0.928), occupation (p=0.843), economic status (p=0.101), and gestational week (p=1.000).

Gravidity (p<0.001) and parity (p<0.001) were higher in the control group. There was no difference between the groups in terms of history of abortion (p=0.348) or stillbirth (p=0.271). The frequency of having more than one living child

was higher in the control group (p<0.001). The frequency of medication use was higher in pregnant women who received training (p<0.001). Comorbidities were more common in training recipients (p=0.015). In terms of the PSRS-36 score, there was no difference between trained and nontrained women (p=0.368). While the groups were similar in terms of STAI-state score (p=0.198), the STAI-trait score was significantly lower in training recipients (p=0.033, Table 1).

There was a weak positive correlation between the PSRS-36 score and medication use (r=0.247, p=0.009). The STAI-state score was positively but weakly correlated with age (r=0.240, p=0.011), while it was negatively but weakly correlated with working status (r=-0.328, p<0.001), indicating that employed women had lower anxiety scores. A very weak positive correlation was found between the STAI-trait score and the presence of comorbidity (r=0.191, p=0.046) (Table 2).

Table 1. Summary of individual characteristics with regard to groups.

	Groups		p-value
	Control (n=55)	Trained (n=55)	
Age, years			
18–25	14 (25.4%)	19 (34.5%)	0.554
25–35	32 (58.2%)	27 (49.1%)	
35–40	9 (16.4%)	9 (16.4%)	
Education status			
Primary school	3 (5.5%)	2 (3.6%)	0.180
Secondary school	13 (23.6%)	5 (9.1%)	
High school	16 (29.1%)	22 (40%)	
University	23 (41.8%)	26 (47.3%)	
Body mass index, kg/m^2	28.45±5.18	28.53±3.96	0.928
Occupation			
Unemployed	21 (38.2%)	19 (34.5%)	0.843
Employed	34 (61.8%)	36 (65.5%)	
Economic status			
Above minimum wage	33 (60.0%)	42 (76.4%)	0.101
Below minimum wage	22 (40.0%)	13 (23.6%)	
Gestational week			
20–28	35 (63.6%)	34 (61.8%)	1.000
>28	20 (36.4%)	21 (38.2%)	
Abortus history	14 (25.5%)	9 (16.4%)	0.348
Stillbirth history	6 (10.9%)	2 (3.6%)	0.271
Medication use	7 (12.7%)	26 (47.3%)	**<0.001**
Comorbidities	3 (5.5%)	13 (23.6%)	**0.015**
Diabetes mellitus	0 (0.0%)	2 (3.6%)	0.495
Hypertension	0 (0.0%)	0 (0.0%)	N/A
Hypothyroidism	3 (5.5%)	11 (20.0%)	**0.045**
Other	0 (0.0%)	0 (0.0%)	N/A
Pregnancy Stress Rating Scale-36 score	93.40±28.44	88.93±23.18	0.368
State-Trait Anxiety Inventory			
State score	42 (37–45)	42 (40–47)	0.198
Trait score	49 (44–53)	47 (43–50)	**0.033**

Data are given as mean±standard deviation or median (1st–3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. N/A: non-applicable. Bold values indicate statistical significance at the p<0.05 level.

Table 2. Relationship between assessment scores and other characteristics.

		Pregnancy Stress Rating Scale-36 score	STAI state score	STAI trait score
Age	r	-0.069	**0.240**	0.103
	p	0.474	**0.011**	0.284
Education status	r	0.038	0.005	-0.087
	p	0.690	0.961	0.367
Occupation, employed	r	-0.049	**-0.328**	-0.143
	p	0.613	**<0.001**	0.137
Economic status, below minimum wage	r	0.101	-0.136	0.065
	p	0.295	0.156	0.502
Gestational week	r	-0.096	-0.118	-0.099
	p	0.318	0.219	0.303
Number of pregnancies	r	0.020	-0.025	-0.047
	p	0.833	0.795	0.624
Number of deliveries	r	-0.022	-0.054	0.025
	p	0.821	0.572	0.793
Abortus history	r	0.101	-0.070	-0.169
	p	0.293	0.468	0.077
Stillbirth history	r	0.125	0.078	-0.091
	p	0.195	0.418	0.346
Number of children	r	-0.084	-0.036	0.049
	p	0.384	0.707	0.608
Medication use	r	**0.247**	0.057	0.002
	p	**0.009**	0.556	0.982
Comorbidity	r	0.148	0.073	**0.191**
	p	0.122	0.452	**0.046**

r: Spearman correlation coefficient. Bold values indicate statistical significance at the $p<0.05$ level.

DISCUSSION

Among the effective interventions used to improve the mental health of the mother during pregnancy, interventions such as relaxation techniques are important because they are non-pharmacological[12]. In this study, we found lower scores for STAI-trait among training recipients; however, STAI-state and PSRS scores were similar in the two groups.

Despite similarities in demographic and various other characteristics, the groups in the study showed a heterogeneous distribution in terms of the number of pregnancies and births. The frequency of nulliparous women was significantly higher in those who received training compared to the control group, as demonstrated by the fact that 90.9% of them had never given birth. Pregnancy is considered a period of increased emotional vulnerability, especially when experienced for the first time[13]. According to the results in the literature, it is seen that the stress levels of pregnant women often decrease after training. As such, it is possible that the effect of training in the present study was not observed due to high baseline stress among nulliparous women, illustrating a potential confounder.

In a study by Woods et al. it was reported that having a medical comorbidity was significantly associated with higher psychosocial stress[14]. In another study, it was reported that those with chronic diseases had significantly higher stress[15]. In the presence of a chronic disease or condition that requires medication, pregnant women may perceive themselves as being unhealthy, which could elevate pregnancy-related stress due to concerns for the impact of their disease or medications on the health of their baby. It may be beneficial to closely monitor pregnant women with chronic diseases that will require continuous drug use, particularly with the utilization of intervention

programs that can alleviate concerns regarding chronic conditions and medication use.

Although the correlations revealed by the present study are weak, it may be valuable to mention these findings in the context of available literature. It has been reported that being unemployed and having a comorbidity are risk factors for pregnancy anxiety[16]. In a study by Tang et al. it was reported that the frequency of anxiety was significantly higher in unemployed pregnant women[1], which is consistent with our correlation results. Despite the low effect sizes observed from these analyses, it may be useful to carefully monitor unemployed pregnant women and those with chronic diseases in terms of anxiety disorders.

Antenatal anxiety is a common psychological disorder during pregnancy. In a meta-analysis evaluating the data of 221,974 pregnant women (including studies from 34 countries), the prevalence of any anxiety disorder was estimated to be 15.2%, and the prevalence of generalized anxiety disorder was 4.1% throughout pregnancy[17]. Ideally, for women who develop anxiety or have an anxiety disorder before pregnancy, interventions should begin before pregnancy. Non-pharmacological and pharmacological interventions are available for this purpose. In a study by Ponting et al. it was reported that the level of state anxiety was lower in women who were trained for stress management compared to those who did not complete the intervention[18]. Shen et al. reported that the anxiety level of the pregnant women who were given five needs-based education programs by trained researchers was significantly lower than that of the control group[19]. In another study conducted with pregnant women at risk of preterm birth, it was reported that trait and situational anxiety decreased significantly in women who were given counseling support[20]. A systematic review of psychological interventions described moderate improvements in anxiety-related findings among pregnant women[21]. However, the method and route of training appear to be important factors affecting the uptake of information and the outcomes of training recipients, as shown by unchanged anxiety levels in a systematic review examining pregnant women who received Internet-based training[22]. In the present study, while the trait anxiety score was found to be lower in pregnant women who attended the pregnant school, supporting the literature, the groups were similar in terms of state anxiety. Educational programs to be added to routine prenatal follow-ups in pregnant women may be beneficial in reducing the level of anxiety; however, it is evident from the literature that the methodology of these training programs is crucial for their success.

This research has some limitations. Pregnant women were selected from a single institution, so our results are not representative of the population. Another limitation is that stress and anxiety were assessed via questionnaires without any clinical interviews.

CONCLUSION

This study shows that the presently used training program offered in antenatal care services is associated with lower trait anxiety levels in recipients. Nonetheless, considering the correlation results for STAI-state, this study demonstrates that unemployed pregnant women and those with chronic diseases must be followed more closely to reduce anxiety levels and prevent the development of anxiety disorders. There is a need for population-based, prospective, longitudinal studies to assess pregnancy school practices in different age groups and especially in the disadvantaged sections of society.

AUTHORS' CONTRIBUTIONS

AA: Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **SÖ:** Data curation, Writing – review & editing. **ŞAT:** Formal Analysis, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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What do patients know about healthcare-associated infections? What do they want to know? Ethical evaluation

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SUMMARY

INTRODUCTION: Patients hospitalized for treatment may be exposed to healthcare-associated infections, and these infections can affect patients seriously.
OBJECTIVE: This research was conducted to determine what hospitalized patients know and what they want to know about healthcare-associated infections.
METHODS: This is a full-text original research article. The study was conducted between July and September 2022 with inpatients in all clinics of Kocaeli University Hospital in Turkey. A total of 310 patients participated in this cross-sectional study. The questions were asked by the researcher and the answers were recorded.
RESULTS: Almost all (92.8%) the patients who stated that they knew what healthcare-associated infection was evaluated their knowledge as insufficient. Patients with higher educational levels have more information (p=0.000) about healthcare-associated infections. Young (p=0.000) and highly educated patients (p=0.000) stated that the infection rate of the hospital would affect the choice of hospital.
CONCLUSION: Patients want to learn about healthcare-associated infections, but most do not know about them. Patients should be informed about healthcare-associated infections during hospitalization planning and hospitalization.
KEYWORDS: Patient. Safety. Healthcare associated infection. Ethics.

INTRODUCTION

Hundreds of millions of people around the world are affected by healthcare-associated infections (HAIs), many of which are completely preventable. It has even been stated that there is no country where HAIs are not seen. It has been determined that the incidence of HAIs in the world is 7% in high-income countries and 15% in low- or middle-income countries[1]. In Turkey, 61,745 HAIs were reported within the scope of surveillance in 2017[2]. HAIs depend on the hospital's infection control practices, the patient's immune status, and the prevalence of pathogens. HAIs can be seen in all clinics, mostly in intensive care units. When HAIs are seen, the hospitalization period of the patients is prolonged, and drug use, treatment cost, mortality rate, complications increase, and long-term disabilities are observed. Patients need to protect themselves and their families from germs that can cause infections[3]. It was determined that patients wanted to learn about HAIs, which has negative results; however, most of them were not informed[4]. In fact, it has been claimed that the diagnosis was not disclosed to the patients with the infection, they were not informed about their treatment, and the physicians made unilateral decisions about the treatment[5].

It is important to inform patients about their diagnosis and treatment options. Thus, patients' autonomy and dignity are respected, their awareness of the risks of cross-contamination of infections is increased, and they could get their own infection under control[6]. The first condition for patients' participation in decisions about HAIs is the appropriate disclosure of relevant information. However, studies have shown that a significant portion of patients are not informed about HAIs, and especially information about preventive measures is rarely explained accurately[5,7].

Nurses have important roles in HAIs. For example, nurses can teach patients with respiratory disease not to cough into a tissue, patients with bowel disease to wash their hands thoroughly before and after using the toilet, and a patient with a wound to keep their wound clean and dry. They can provide counseling on what patients should pay attention to when determining the hospital where they will be treated. They may advocate that patients exposed to infection make autonomous decisions to seek treatment[8].

There are very few studies that determine the level of knowledge of patients about HAIs[9,10]. Therefore, this study was conducted to determine what patients know and want to know about HAIs.

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METHODS

Type of research: The research is of cross-sectional type.

Place and time of research: The study was conducted between July and September 2022 with inpatients in all clinics of Kocaeli University Hospital in Turkey.

Population and sample of the research: The population of the study consisted of inpatients treated in all clinics of a 500-bed Kocaeli University Hospital between July and September 2022. The study was completed with 310 patients.

Inclusion criteria:

Volunteering to participate in the research

Being open to communication and cooperation

Being 18 years or older

Exclusion criteria:

Being in pain and not being able to answer the questions asked.

Inability to speak and communicate the same language.

Data collection forms

Introductory characteristics form: This form was prepared by the researchers. It consists of questions about the status of being diagnosed with an HAI at the last hospitalization and the type of infection[4,5].

Questionnaire on infections: A study form consisting of 20 questions was prepared by the researcher. For the preparation of the form, the explanations of the World Health Organization[1] and articles were used[4,5].

Data collection: Data were collected through face-to-face interviews with inpatients in all clinics between July and September 2022.

Analysis of data: The collected data were analyzed through SPSS version 20.0. Data were compared using the chi-squared test or Fisher's exact test, as appropriate.

Ethical aspect of the study: Ethics Committee approval (Approval No: 2022/176) and official permission were obtained from the hospital before starting the study. The study was conducted in accordance with the Helsinki Declaration Principles, and written permission with the "Informed Voluntary Consent Form" was obtained from all patients.

RESULTS

In our study, 57.1% of the patients were females. The mean age was 52.22±17.328 years, with the youngest participant being 18 years old and the oldest being 94 years old. At the time of the study, only 15.5% of the patients who participated in the study developed an infection, and all of them were informed by the physicians that they were exposed to the infection (Table 1).

Table 1. Distribution of patients' information about healthcare-associated infections.

	n	%
What do you know about HAI? (n=97)*		
It is serious	84	86.2
Other**	13	13.8
Do you find their knowledge about HAI sufficient? (n=97)*		
Yes	7	7.2
No	90	92.8
What do you want to know about HAI?***		
Reason of HAI	266	85.8
Signs and symptoms of HAI	265	85.5
Whether they are at risk	262	84.5
How to prevented	262	84.5
Risks and complications	242	78.1
Treatment and cost	224	72.3
Do you think HAI can be prevented?		
Yes	262	84.5
No	48	15.5
Where did you learn about HAI? (n=95)		
From persons with hospital infections	35	36.1
From the healthcare worker	29	32.0
From the Internet	23	23.7
From previous HAI experience	8	8.2
Were patients exposed to HAI while they were hospitalized? (n=310)		
Yes	49	15.5
No	261	84.5
Types of HAI (n=49)		
Surgical site infections	20	40.8
Urinary tract infection	11	22.4
Pneumonia	11	22.4
Bloodstream infections	7	14.3
Do you want to be told if you are exposed to HAI?		
Yes	293	94.5
No	17	5.5
Why do you want to know if you develop HAI?***		
Being aware of what happened to me	276	89.0
Consciously participating in treatment	156	50.3
Protecting other patients from infection	122	39.4
What are their roles and responsibilities in the prevention of HAIs?***		
Washing hands and paying attention to personal hygiene	301	97.1
Not going to other patients' rooms	78	25.2
Notifying the healthcare worker when I realize that I have a problem myself	74	23.9

*Patients answered the relevant question. **HAI develops in every hospitalized patient, treatment of HAI is difficult and expensive, HAI develops in patients hospitalized in intensive care, HAI passes through operating rooms, and HAI passes through the hospital environment. ***Patients reported more than one opinion.

Almost all the patients (92.8%) evaluated their knowledge about HAIs as insufficient. A total of 59.8% of the patients learned about HAIs from people who had been exposed to infection before and from the Internet. Patients wanted to be informed if infection developed.

Most of the male participants (p=0.043) wanted to know the incidence of HAIs in the hospital to which they applied. Those in the 18–37 years age group (p=0.000), those with high school or higher education (p=0.000), and those who stayed in the hospital for 1–6 days (p=0.001) stated that the infection rate of the hospital would affect the choice of hospital. Some patients reported that they would like to be treated in the same hospital even though the infection rate is high (Table 2).

A total of 71.3% of the participants stated that they can warn any healthcare worker if they behave in a way that will expose them to infection. In particular, patients with high school or higher education degree (p=0.000), between 18 and 37 years old (p=0.17), hospitalized in the surgery clinic (p=0.004), and hospitalized for 1–6 days (p=0.014) reported that they could warn more. The majority (35.3%) of the patients who said they would hesitate to warn were 58 years old and over (p=0.034), had less than high school education (p=0.002), hospitalized in the internal medicine clinics (p=0.004), and hospitalized for 1–6 days (p=0.004) (Table 3). A total of 28.1% of the patients who stated that they could not warn stated that they would hesitate to warn, and 10% stated that if they warned, it would negatively affect their treatment.

DISCUSSION

HAIs are explained by the principles of no harm, respect for autonomy, beneficence, non-harming, and justice[5].

Principle of autonomy: Autonomy is a human right to self-determination. In order for the patients who are planning to be hospitalized to decide in which hospital they can be treated, the infection rates of the hospitals should be explained[1]. It has been suggested that, in countries with high HAI-related mortality, patients look at the risk of getting HAIs when choosing the institution to be treated[5]. In our study, patients in the 18–37 years age group (p=0.000), those with high school or higher education degree (p=0.000), and those patients hospitalized for 1–6 days (p=0.001) stated that the infection rates of the hospitals would be effective in choosing the hospital[5]. Some patients reported that they would like to be treated in the same

Table 2. The state of patients wanting to know what healthcare-associated infections are and the healthcare-associated infection rate of the hospital.

	Do you know what HAIs are?			Do you want to know the HAI incidence rate of the hospital?			Does the HAI rate of the hospital affect your choice of hospital?		
	Yes	No	p	Yes	No	p	Yes	No	p
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Education									
Less than high school	46 (23.8)	147 (76.2)	**0.000**	175 (90.7)	18 (9.3)	0.612	98 (50.0)	95 (49.2)	**0.000**
High school and above	51 (43.6)	66 (56.4)		104 (88.9)	13 (11.1)		86 (73.5)	31 (26.5)	
Gender									
Female	55 (31.1)	122 (68.9)	0.924	154 (87.0)	23 (13.0)	**0.043**	111 (62.7)	66 (37.3)	0.119
Male	42 (31.6)	91 (68.4)		125 (94.4)	8 (6.0)		73 (54.9)	60 (45.1)	
Age (years)									
18–37	16 (23.5)	52 (76.5)	0.293	60 (88.2)	8 (11.8)	0.828	52 (76.5)	16 (23.5)	**0.000**
38–57	36 (33.0)	73 (67.0)		98 (89.9)	11 (10.1)		70 (64.2)	39 (35.8)	
58 and above	45 (33.8)	88 (66.2)		121 (91.0)	12 (9.0)		62 (46.6)	71 (53.4)	
Clinic									
Internal medicine clinics	35 (31.5)	76 (68.5)	0.945	101 (91.0)	10 (9.0)	0.844	62 (55.9)	49 (44.1)	0.399
Surgery clinics	62 (31.2)	137 (68.8)		178 (89.4)	21 (10.6)		122 (61.3)	77 (38.7)	
How many days in the hospital									
1–6 days	67 (33.0)	136 (67.0)	0.370	182 (89.7)	21 (10.3)	0.845	134 (66.0)	69 (34.0)	**0.001**
7 days and above	30 (28.0)	77 (72.0)		97 (90.7)	10 (9.3)		50 (46.7)	57 (53.3)	

Statistically significant values are indicated in bold.

Table 3. Situations and reasons of patients wanting to warn the healthcare worker who may expose them to healthcare-associated infection.

	Would you warn the healthcare worker if he or she engages in a behavior that may expose you to HAI?			If you do not warn, what is the reason?					
				I hesitate to warn			If I warn, my treatment will be adversely affected		
	Yes	No	p	Yes	No	p	Yes	No	p
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Education									
Less than high school	124 (64.2)	69 (35.8)	**0.000**	66 (34.2)	127 (65.8)	**0.002**	24 (12.4)	169 (87.6)	0.066
High school and above	97 (82.9)	20 (17.1)		21 (17.9)	96 (82.1)		7 (6.0)	110 (94.0)	
Gender									
Female	128 (72.3)	49 (27.7)	0.645	48 (27.1)	129 (72.9)	0.669	17 (9.6)	160 (90.4)	0789
Male	93 (69.9)	40 (30.1)		39 (29.3)	94 (70.7)		14 (10.5)	119 (89.5)	
Age (years)									
18–37	55 (80.9)	13 (19.1)	**0.017**	13 (19.1)	55 (80.9)	**0.034**	3 (4.4)	65 (95.6)	0.213
38–57	82 (75.2)	27 (24.8)		27 (24.8)	82 (75.2)		12 (11.0)	97 (89.0)	
58 and above	84 (63.2)	49 (36.8)		47 (35.3)	86 (64.7)		16 (12.0)	117 (88.0)	
Clinic									
Internal medicine clinics	68 (61.3)	43 (38.7)	**0.004**	42 (37.8)	69 (62.2)	**0.004**	13 (11.7)	98 (88,3)	0.453
Surgery clinics	153 (76.9)	46 (23.1)		45 (22.6)	154 (77.4)		18 (9.0)	181 (91.0)	
How many days in the hospital									
1–6 days	154 (75.9)	49 (24.1)	**0.014**	47 (23.2)	156 (76.8)	**0.008**	17 (8.4)	186 (91.6)	0.189
7 days and above	67 (62.6)	40 (37.4)		40 (37.4)	(67 (62.6)		14 (13.1)	93 (86.9)	

Statistically significant values are indicated in bold.

hospital, even though the HAI rate was high. As a reason, 48% stated that they trust their physician, and 52% stated that they believe that the university hospital is always better than other hospitals. In a study, it was shown that patients were influenced by positive recommendations about the hospital (31.8%), and they preferred the hospital they applied to just because they knew the physician (31.1%)[11]. This result is obtained because patients might think that university hospitals will meet their needs although the infection rate is high, and some patients need services that require advanced expertise and want to be treated by a physician who is good in their field[12].

Benefit principle: For the benefit of patients, patients should be explained how to protect themselves from HAIs. However, it has been explained that accurate information is rarely given to patients about HAIs[1]. In this study, 31.3% of the patients had information about HAIs. Almost all of those who stated that they had knowledge (92.8%) evaluated their knowledge as insufficient. In the study conducted in Sydney, 8 out of 15 patients were shown to have sufficient knowledge about HAIs[7]. In our study, the educational level of the patients who had knowledge about HAIs was high (p=0.000). In the study of Merle et al., patients with a high level of education defined HAIs correctly, similar to the findings of our study[13]. This may be due to the increase in the research and comprehension skills of the patients as their educational level increases. In our study, the fact that most of the patients with a high level of education knew that HAIs may enable them to take precautions to prevent infections. Patients must obtain information about infections from reliable sources[8]. In this study, 68.0% of the patients learned about infections from the Internet and people with experience of infection. In a study conducted with surgical patients, it was shown that patients acquired most of the information about infections outside the hospital[4]. In another study, it was shown that only 14% of the patients were informed by the healthcare worker[14]. The fact that the patients could not learn information about HAIs from the healthcare professionals suggested that they could not reach the correct and up-to-date information. The reason why patients are not informed about HAIs may be because healthcare professionals are worried that patients will be worried[6]. Almost all patients wanted to know about all aspects of HAIs. In the study of Merle et al., 76.9% of the patients wanted to get information

about hospital infections[13]. Patients' lack of knowledge about HAI may increase the risk of infection[5].

To prevent and control the risk of HAI, patients should have some roles and responsibilities. In this study, 98.1% of the patients stated that they also have roles and responsibilities in the prevention of infections, and 97.1% associated these roles and responsibilities with handwashing and personal hygiene. In the study by Seale et al., the participants stated that they have a role in preventing infections in the hospital and this role is to protect their personal hygiene[7]. In our study, it may be due to patients' assumption that they should pay more attention to hand hygiene and the pandemic's emphasis on the importance of hand hygiene information on social media.

Do no harm: One of the basic ethical principles in the provision of healthcare services is to do no harm[3]. HAI is a completely preventable infection. However, patients may be exposed to HAIs and may be harmed. WHO stated that the incidence of HAIs in low-income countries is 10%[15]. In our study, it was found that 15.5% of the patients had HAIs. This result shows that the rate of HAIs is high in the hospital where the research was conducted. When necessary precautions are taken to prevent infections, patients' lives can be saved and treatment costs can be reduced[3].

Explaining the truth: Telling the truth is a mandatory requirement unless it causes significant harm to patients. In this study, it was explained to all the infected patients that they were exposed to the infection. Patients with high school or higher education degree (p=0.033) and hospitalized in the surgery clinic (p=0.036) wanted to be informed in case of infection in order to consciously participate in the treatment. Informing all patients exposed to HAIs suggested that physicians were telling the truth to their patients and that patients' right to abstain from non-consensual treatments and their autonomy were respected[1].

Patients should have the knowledge and courage to ask healthcare professionals about hand hygiene[1]. In a study, 48.8% of patients stated that they were willing to remind physicians and 49.9% of them to nurses to wash their hands[16]. In our study, this rate was high, and 71.3% of the patients stated that they could warn any healthcare worker if they acted in a way that would expose them to infection. The higher rate of patients who stated that they could warn the healthcare worker in our study may be because the patients witnessed deaths during the pandemic and cared about their health. A total of 35.3% of the patients who said they would hesitate to warn the healthcare professional were 58 years old and over (p=0.034), had less than high school education (p=0.002), and were treated in the internal medicine clinics (p=0.004). This difference may be because educated and young patients are conscious. A total of 28.1% of the patients who stated that they could not warn stated that they would not dare to warn, and 10% stated that, if they were warned, it would negatively affect their treatment. These thoughts of the patients may be because some healthcare professionals discourage patients from asking questions, dislike being told what to do, and have unequal power in the patient-physician relationship. In particular, it may be beneficial to address the fears and concerns of elderly and low-educated patients and to inform them about patient rights.

Nurses have important roles in HAIs. Nurses can encourage patients to take responsibility for their own health and safety and explain their rights to patients[1].

CONCLUSION

None of the patients are informed about HAIs when they are hospitalized. It may be recommended that the educational level of society should be increased in order to increase the awareness and participation of patients on healthcare services-associated infections.

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ORIGINAL ARTICLE

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Does resveratrol reduce cisplatin-induced ovarian damage?

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SUMMARY

OBJECTIVE: The objective of this study was to investigate the protectiveness of resveratrol on cisplatin-induced damage to the ovary using experimental models.

METHODS: A total of 30 female Wistar-Albino rats constituted the research material. The rats were categorized into three groups: Group 1 was administered one milliliter of 0.9% NaCl solution, Group 2 was administered 7.5 mg/kg cisplatin, and Group 3 was administered 7.5 mg/kg cisplatin and 10 mg/kg resveratrol. Ovaries were extirpated in all groups and subjected to biochemical and histopathological tests. Cisplatin-induced damage to ovarian tissue was graded and scored as the total histopathological findings score. The ovarian function was assessed using immunohistochemical staining for c-kit expression. Rats' malondialdehyde, catalase, and superoxide dismutase levels were determined.

RESULTS: The histopathological finding score was significantly higher in Group 2 than in other groups ($p<0.05$). The superoxide dismutase and catalase levels were significantly higher in Group 3 than in Group 2 ($p<0.001$ for both cases). The malondialdehyde level was significantly higher in Group 2 than in Group 3 ($p<0.001$).

CONCLUSION: The study findings demonstrated that resveratrol reduced ovarian injury and enhanced biochemical parameters following cisplatin-induced ovary damage in experimental models.

KEYWORDS: Cisplatin. Resveratrol. Rat. Ovary.

INTRODUCTION

Cisplatin is a platinum-based anticancer chemotherapy medication widely used to treat malignancies. However, it may adversely affect normal tissues and organs along with cancerous cells. The kidney, ovary, and liver are the main organs where cisplatin toxicity has been observed[1]. The accumulation of the platinum component of cisplatin may lead to a DNA complex, resulting in cell injury[2]. Elevated levels of reactive oxygen species and free radicals give rise to disruption of the cellular structure[3].

Resveratrol, an antioxidant found in fruits and red wine, has been shown to protect against free radical damage and is beneficial in the treatment of many diseases[4]. Den Hartogh et al.[5] reported that resveratrol enhances antioxidant activity by reducing inflammation and oxidative stress. Thus, resveratrol has become known as a cytoprotective nutrient. In addition, resveratrol, a free-radical scavenger, increases endothelial cell activity and exhibits potent antioxidant activity by blocking the DNA damage induced by free radicals, mainly through regulating major antioxidant enzymes[6].

Although advances in cancer treatments have improved survival, they cause premature ovarian failure, which presents as infertility in premenopausal women[7,8]. Both the increase in the frequency of female cancer and the fact that it is seen at a younger age have brought to the fore the negative effects of the chemotherapeutic drugs used on the reproductive system[7]. The invasiveness, high cost, and difficulty of implementing cryopreservation methods used for the preservation of ovarian tissue and the continuation of fertility limit their use. This situation has led to research into alternative treatment methods. A few experimental studies showed the beneficial effects of resveratrol on the number of primordial and primary follicles[9,10]. Extremely active nano-formulations of resveratrol regenerated the non-functional, chemoablated ovaries and testes in

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mice[11]. Resveratrol ameliorated oxidative stress, inflammation, and apoptosis secondary to cisplatin administration in female rats' ovarian and uterine tissues by preventing granulosa cell loss and controlling inflammation[7,8,12,13].

In view of the foregoing, this study was carried out to investigate the efficacy of resveratrol in reducing the ovarian damage caused by cisplatin using immunohistochemistry (IHC) and biochemical methods based on the hypothesis that resveratrol could protect the ovaries from the toxicity of cisplatin.

METHODS

Cisplatin (Ebewe-Liba, Istanbul, Turkey) and resveratrol (Sigma-Aldrich, Oakville, ON, Canada) were obtained from a pharmacy. Cisplatin was administered intraperitoneally at a dose of 7.5 mg/kg as previously described by Ibrahim et al.[8]. Intraperitoneal injections of resveratrol (10 mg/kg) were given based on the treatment protocol published previously[10].

Animals and experimental procedure

The study protocol was approved by the Kirikkale University Animal Experiments Local Ethics Committee (18.06.2020–2020/03-16) and supported by the Ahi Evran University Scientific Research Projects Unit (TIP.A4.21.001). A total of 30 female Wistar-Albino rats (150–220 g) aged 8–12 weeks were included in the study. All animals were kept for 1 week at approximately 24°C and fed an ad libitum laboratory diet. The rats were categorized into three groups, with 10 rats in each group. Group 1 (control group): A single intraperioneal dose of 1 ml/kg of 0.9% NaCl was given. Group 2 (cisplatin group): A single intraperioneal dose of 7.5 mg/kg cisplatin was given. Group 3 (cisplatin+resveratrol group): A single dose of 10 mg/kg resveratrol, followed by a single dose of 7.5 mg/kg cisplatin 1 h later, was administered intraperioneally.

Surgical procedures were initiated on the seventh day of the study. Ketamine/xylazine hydrochloride was used to achieve anesthesia in the rats. The ovaries on the right side were surgically removed and fixed in 10.0% formaldehyde. At the end of the procedure, the sacrification procedure was performed by cervical dislocation. Ovarian tissue samples were embedded in paraffin blocks and cut at 4 µm thickness. Sections were stained using hematoxylin and eosin (H&E) or with c-kit, also known as cluster of differentiation 117 (CD117) dye. Histopathological findings (HPF) were evaluated by a pathologist blinded to the experimental groups using a light microscope (Olympus CX41 microscope, Tokyo, Japan). At least 10 ovary areas were analyzed and assessed for IHC differences.

Intracardiac blood samples were centrifuged for biochemical analysis and stored in Eppendorf tubes at -80°C.

Immunohistochemistry

A polyclonal rabbit anti-human CD117 antibody (1:400; Dako, Glostrup, Denmark) was used to grade the immunohistochemical staining for c-kit expression. The results of c-kit staining (cytoplasmic/membranous staining in the ovarium) were scored as follows: negative (0), weak (1), moderate (2), and intense (3)[14].

A 5-point (0: None, 1: Minimal, 2: Mild, 3: Moderate, 4: Severe) scoring system was used in histopathological scoring. In this way, each area of the ovary was scored via a semi-quantitative analysis. The pigmentation, inflammation, fibrosis, congestion, and hemorrhage scores were used to determine the degree of damage, that is, the total HPF score.

Biochemistry

Blood samples were analyzed for malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels through absorbance using a spectrophotometer (Shimadzu UV 1800, Japan). A thiobarbituric acid test was used to calculate the MDA levels[15]. SOD enzyme activity was calculated as described by Marklund et al.[16], whereas CAT enzyme activity was calculated as described by Aebi[17].

Statistical analysis

Descriptive statistics obtained from the collected data were expressed as mean±standard deviation values in the case of continuous variables determined to conform to the normal distribution, as median and minimum-maximum values in the case of continuous variables determined not to conform to the normal distribution, and as numbers (n) and percentage (%) values in the case of categorical variables. The Fisher-Freeman-Halton test was used to compare the differences between categorical variables in RxC tables. The Kruskal-Wallis test was used to compare more than two independent groups where numerical variables did not conform to the normal distribution. In analyses featuring nonparametric tests, the differences between the groups were evaluated by the Dwass-Steel-Critchlow-Fligner test. The Jamovi project 2.2.5.0 and JASP 0.16.1 software packages were used in the statistical analyses. The probability (p) of ≤0.05 was deemed to indicate statistical significance.

RESULTS

Compared to the control subjects (Figure 1A), pigmentation, inflammation, fibrosis, congestion, and hemorrhage were

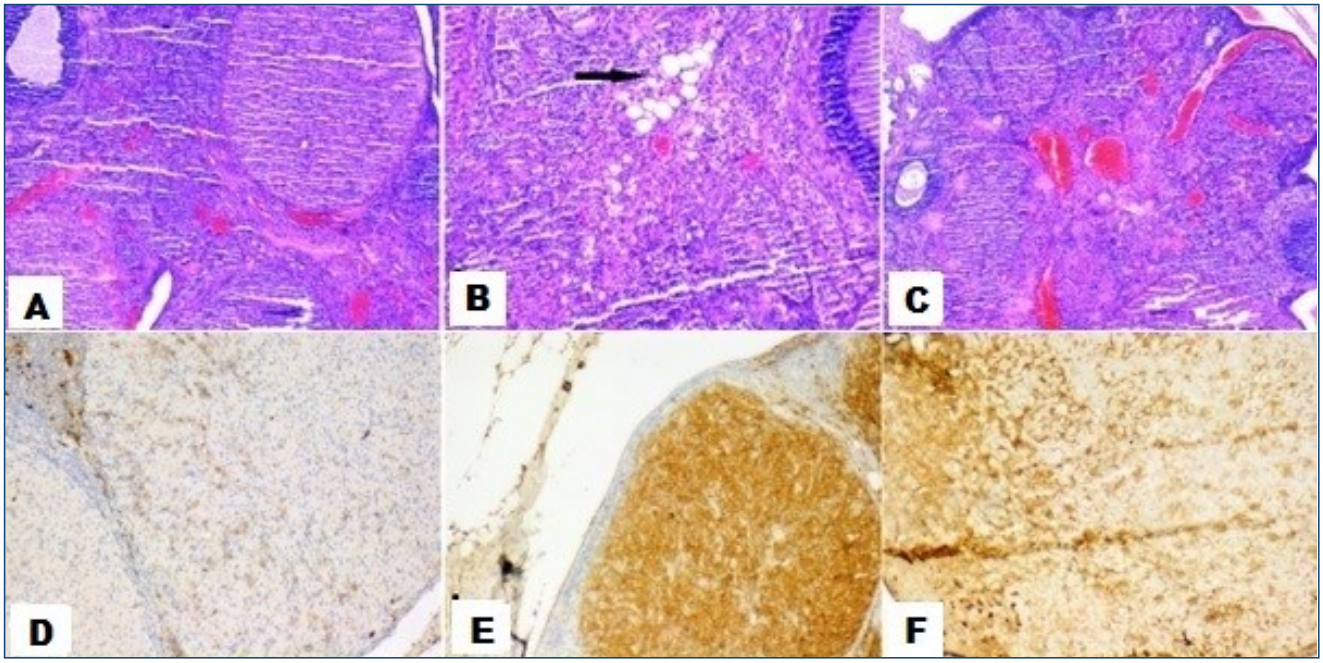

Figure 1. Light microscopic appearance of ovary **(A–C)** and immunohistochemical staining of rats by using c-kit **(D–F)**. **(A)** Minimally fibrosis and congestion in the ovarian stroma of the rat from the control group (H&E, ×50). **(B)** Lipoid cell storage (arrow), fibrosis, and lymphocyte infiltration in the ovarian stroma of the rat from the cisplatin group (H&E, ×100). **(C)** Mild fibrosis, congestion, and lymphocyte infiltration in the ovarian stroma of the rat from the cisplatin+resveratrol group (H&E, ×50). **(D)** Negative staining with c-kit (cd117) in the ovary of the rat from the control group (×100). **(E)** Focal 10% staining with c-kit (cd117) in the ovary of the rat from the cisplatin group (×100). **(F)** Focal 10% but less than cisplatin group staining with c-kit (cd117) in the ovary of the rat from the cisplatin + resveratrol group (×100).

detected in the ovarian tissues of the rats in Group 2 (Figure 1B), which were morphologically normal, and minimal fibrosis, congestion, and lymphocyte infiltration were observed in the rats in Group 3 (Figure 1C).

The total median HPF scores were 2, 12, and 6 in Groups 1, 2, and 3, respectively. There was a significant difference between the groups in HPH scores ($p<0.001$). The HPF score of Group 2 was significantly higher than those of Groups 3 ($p=0.003$) and 1 ($p<0.001$). The total HPF score of Group 3 was significantly higher than that of Group 1 ($p=0.004$). The comparison of the histopathological parameters revealed significant differences between the groups ($p<0.05$). There was no significant difference between Groups 2 and 3 in the distribution of pigmentation and fibrosis scores. As for congestion and hemorrhage, there were significantly more animals with low grades of congestion and hemorrhage in Group 3 than those in Group 2. The degree of inflammation was significantly higher in Group 2 than that in Group 3 (Table 1).

There were no animals with negative c-kit expression in Groups 2 and 3. However, there was a significant difference in the distribution of the c-kit expression grades between the three groups ($p<0.001$) and there was no significant difference in the percentage of different grades of c-kit expression in the ovarian tissues between Groups 2 and 3 (Table 2).

There were significant differences in serum MDA, SOD, and CAD levels between the groups ($p<0.001$). Group 1 had significantly lower levels of MDA and higher levels of SOD and CAD than Groups 2 and 3 ($p<0.001$ for all cases). The SOD and CAD levels were significantly higher in Group 3 than those in Group 2 ($p<0.001$ for both cases). In addition, Group 2 had significantly higher MDA levels than Group 3 ($p<0.001$) (Table 2).

Immunohistochemical staining of rats using c-kit dye revealed more ovarian damage in Group 2 (Figures 1D–F).

DISCUSSION

The study findings demonstrated that the degree of inflammation, congestion, and hemorrhage after cisplatin therapy was attenuated using resveratrol. Additionally, resveratrol increased the activities of SOD and CAT enzymes and reduced MDA activity. These findings have shown that resveratrol has protective potential against ovarian damage due to cisplatin-induced oxidative stress.

Table 1. Histopathological scoring of the findings evaluated in the ovarian tissue.

	Group 1 (Control) (n=10)	Group 2 (Cisplatin) (n=10)	Group 3 (Cisplatin+Resveratrol) (n=10)	p-value
Pigmentation‡				
None	7 (70.0)[a]	0 (0.0)[b]	0 (0.0)[b]	**<0.001***
Minimal	3 (30.0)[a]	3 (30.0)[a]	5 (50.0)[a]	
Mild	0 (0.0)[a]	6 (60.0)[b]	4 (40.0)[b]	
Moderate	0 (0.0)[a]	1 (10.0)[a]	1 (10.0)[a]	
Inflammation‡				
None	7 (70.0)[a]	0 (0.0)[b]	2 (20.0)[b]	**<0.001***
Minimal	3 (30.0)[a,b]	2 (20.0)[b]	7 (70.0)[a]	
Mild	0 (0.0)[a]	4 (40.0)[b]	1 (10.0)[a,b]	
Moderate	0 (0.0)[a]	4 (40.0)[b]	0 (0.0)[a]	
Fibrosis‡				
None	10 (100.0)[a]	0 (0.0)[b]	0 (0.0)[b]	**<0.001***
Minimal	0 (0.0)[a]	3 (30.0)[a,b]	7 (70.0)[b]	
Mild	0 (0.0)[a]	5 (50.0)[b]	3 (30.0)[a,b]	
Moderate	0 (0.0)[a]	1 (10.0)[a]	0 (0.0)[a]	
Severe	0 (0.0)[a]	1 (10.0)[a]	0 (0.0)[a]	
Congestion‡				
Minimal	7 (70.0)[a]	0 (0.0)[b]	6 (60.0)[a]	**0.002***
Mild	3 (30.0)[a]	3 (30.0)[a]	4 (40.0)[a]	
Moderate	0 (0.0)[a]	4 (40.0)[b]	0 (0.0)[a]	
Severe	0 (0.0)[a]	3 (30.0)[a]	0 (0.0)[a]	
Hemorrhage‡				
None	3 (30.0)[a]	0 (0.0)[a]	0 (0.0)[a]	**<0.001***
Minimal	6 (60.0)[a]	1 (10.0)[b]	7 (70.0)[a]	
Mild	1 (10.0)[a]	2 (20.0)[a]	3 (30.0)[a]	
Moderate	0 (0.0)[a]	6 (60.0)[b]	0 (0.0)[a]	
Severe	0 (0.0)[a]	1 (10.0)[a]	0 (0.0)[a]	
HPF total§	2.0 [1.0–6.0]	12.0 [6.0–17.0]	6.0 [4.0–9.0]	**<0.001***

‡n (%), §median [min–max]. HPF: histopathological findings. [a,b]Different letters showing significant differences between the groups. *Fisher Freeman Halton test. Bold indicates statistically significant values.

Table 2. Distribution of the c-kit expression levels and results of the biochemical parameters in the groups.

	Group 1 (Control) (n=10)	Group 2 (Cisplatin) (n=10)	Group 3 (Cisplatin+Resveratrol) (n=10)	p-value*
C-kit expression‡				
Negative	7 (70.0)[a]	0 (0.0)[b]	0 (0.0)[b]	**<0.001**
Weak	3 (30.0)[a]	1 (10.0)[a]	3 (30.0)[a]	
Intermediate	0 (0.0)[a]	4 (40.0)[b]	5 (50.0)[b]	
Strong	0 (0.0)[a]	5 (50.0)[b]	2 (20.0)[a,b]	
Biochemical parameters p-value**				
MDA§	3.2 [3.0–3.5]	8.2 [7.4–8.7]	5.0 [4.7–5.3]	**<0.001**
SOD§	30.1 [26.7–33.4]	12.3 [10.8–13.0]	19.4 [17.0–20.8]	**<0.001**
CAD§	65.9 [59.2–71.0]	17.1 [15.0–19.1]	37.1 [33.2–41.8]	**<0.001**

‡n (%). [a,b]Different letters showing significant differences between the groups. *Fisher Freeman Halton test. §median [min–max]. **Kruskal-Wallis H test, Dwass-Steel-Critchlow-Fligner test for pair-wise comparisons. Bold indicates statistically significant values.

Cisplatin has been used to treat cancers of many tissues and organs, such as the testes, ovaries, and lungs. However, its clinical use has been restricted due to its toxic effects. Dixit et al. reported gonadotoxicity and ovarian damage in approximately 40% of their patient cohort[18]. Many studies have shown that cisplatin results in ovarian tissue damage or injury[19,20]. Our results are also similar.

Resveratrol, a natural plant product, has been extensively studied. In one of these studies, Hascalik et al. investigated resveratrol in ovary torsion using a rat model and demonstrated that resveratrol reduced lipid peroxidation[21]. Antioxidants have often been used in experimental studies to antagonize the adverse effects caused by cisplatin. An example is resveratrol, which has been proven beneficial in ameliorating cisplatin-induced oxidative stress, inflammation, and apoptosis in ovarian and uterine tissues of female rats through its antioxidant, anti-inflammatory, and anti-apoptotic characteristics[8-10,22,23].

The resveratrol dosage used in studies varied from 5 to 50 mg/kg, depending on the target organ, including the liver, ovaries, or testes[9,10,12,24,25]. In comparison, the rats included in this study were administered 10 mg/kg/day of resveratrol. Consequently, it was concluded that resveratrol ameliorated, albeit not significantly, the severity of inflammation, congestion, and hemorrhage caused by cisplatin in the ovarian tissues. Nevertheless, the extent of the amelioration achieved by resveratrol provided sufficient evidence for its potential use against the toxic effects of cisplatin.

In a similar study conducted by Chinwe et al. resveratrol significantly increased SOD and CAT levels in the ovarian tissue in a dose-dependent manner and provided protection against the toxic effects of cisplatin by significantly reducing the MDA levels[9]. Administration of 10 mg/kg/day of resveratrol to the rats included in this study resulted in similar changes. The findings of this study have shown that it reduces oxidative stress by increasing SOD and CAT activity and decreasing MDA levels.

The histopathologic structure was more protected, as there was less inflammation, hemorrhage, and congestion in Group 3 compared to Group 2. The histopathological analysis revealed the highest scores in the group that received only cisplatin (Group 2), which decreased with the addition of resveratrol to the treatment regimen (Group 3). All these findings provided substantial evidence that resveratrol reduced and prevented cisplatin-induced ovarian toxicity in rats.

Compared to several growth factors, cytokines, and gonadal hormones, which are used to estimate normal ovarian function, c-kit expression is directly related to ovarian function and reserve. They thought that C-kit could be one of the new potential biomarkers of ovarian senescence[26]. In contrast, no significant difference was found between the groups investigated in this study in c-kit expression. More studies are needed to shed light on this subject.

The limitations of this study are as follows: first, it is a preclinical animal experiment. Second, there are no multi-dose groups of cisplatin and resveratrol. Third, due to the short experimental period, the parameters for evaluating the reproductive functions could not be studied (AMH, FSH, etc.). The strong aspect of this study is that the biochemical parameters were studied in blood instead of ovarian tissue in similar studies.

CONCLUSION

The study findings demonstrated that resveratrol reduced the histopathological damage and reversed the inflammatory response related to cisplatin. In our opinion, resveratrol has minimized the negative effects on ovarian tissue by reducing the oxidative stress caused by cisplatin. In order for the results of our study to be applied to medical practice, studies evaluating the effect of resveratrol and cisplatin at multiple doses in tumor tissue are also needed.

AUTHORS' CONTRIBUTIONS

BC: Conceptualization, Data curation, Formal Analysis, Project administration, Writing – original draft. **EGT:** Conceptualization, Data curation, Formal Analysis, Investigation, Writing – original draft. **OK:** Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **GD:** Formal Analysis, Investigation, Methodology, Writing – review & editing. **MMA:** Data curation, Writing – review & editing. **YS:** Conceptualization, Data curation, Project administration, Formal Analysis, Writing – review & editing. **YKD:** Data curation, Formal Analysis, Investigation, Methodology, Validation. **MK:** Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Writing – original draft.

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ORIGINAL ARTICLE

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Improved quality of life (EHP-30) in patients with endometriosis after surgical treatment

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SUMMARY

OBJECTIVE: This study aimed to evaluate the quality of life of patients with endometriosis before and after surgical treatment.

METHODS: An observational, longitudinal, and prospective study was conducted with 102 women with pelvic pain and endometriosis that was unimproved by clinical treatment and indicated for surgical treatment. The patients' quality of life was assessed using the 30-item Endometriosis Health Profile (EHP-30) questionnaire before and 3 and 6 months after surgery. The statistical tests were analyzed using the Statistical Package for Social Sciences version 17.0, and the Friedman test was used.

RESULTS: There was a reduction in EHP-30 scores 3 and 6 months after surgery compared to before surgery, as well as 6 months after surgery compared to 3 months after surgery, in the central questionnaire (PART 1) and in Sections A, B, C, E, and F ($p<0.0001$). For Section D, there was a reduction in scores 6 months after surgery compared to before surgery ($p<0.0001$).

CONCLUSION: Surgical treatment of endometriosis improves quality of life in several areas assessed by the EHP-30 questionnaire.

KEYWORDS: Endometriosis. Surgical procedure. Quality of life.

INTRODUCTION

Endometriosis is a common benign gynecological disorder defined by the presence of fibrotic lesions outside the uterine cavity that are morphologically similar to the endometrium, most commonly in the organs of the female pelvis[1,2]. The clinical presentation of this pathology is mainly characterized by pelvic pain and infertility; its etiology is undefined, and its overall incidence is approximately 10% in women of reproductive age[3,4].

The delay in the diagnosis of endometriosis leads to chronic pelvic pain, centralization of pain, anxiety, and depression, with consequent suffering and loss of quality of life as the disease progresses[5,6]. To assess the quality of life of patients with endometriosis, three instruments have been developed to date: the *Endometriosis Health Profile Questionnaire* (EHP-30) developed by Jones et al.; the instrument developed by Colwell et al. 1998; and the instrument developed by Bodner et al. in 1997[7,8]. Of these, only the EHP-30 includes items that were generated directly from interviews with patients. The relevance of this method for the construction of its items arises from literature findings that indicate that patients' evaluations of their health and well-being differ from those performed by health professionals[7,8].

This study aimed to evaluate in the most diverse ways the quality of life of women with endometriosis that was unsuccessfully clinically managed and who underwent surgical treatment.

METHODS

This is a longitudinal and prospective analytical study of the evolution of the quality of life of women with endometriosis who underwent surgical treatment between September 2020 and May 2022.

The inclusion criteria were as follows: patients from the chronic pelvic pain and endometriosis outpatient clinic of the São Domingos Hospital, São Luis, Maranhão, Brazil, who voluntarily sought treatment; had a clinical picture and imaging test results compatible with endometriosis of various forms; were clinically treated for more than 3 months with no improvement in pain; had indications for surgical treatment via laparoscopy with intraoperative confirmation and pathological anatomy

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consistent with endometriosis; and agreed to participate in the study and signed an informed consent form.

The following patients were excluded from the study: those with suspected endometriosis alone or with infertility without pain; those with suspected endometriosis with or without pelvic pain that was not confirmed during laparoscopy and/or histological analysis; those who did not return for outpatient follow-up; those with previous or ongoing neoplastic pathologies; those with incomplete surgeries, major uncontrolled psychiatric disorders, or surgical or spontaneous menopause; those who underwent robotic surgeries; and those who refused to participate in the study.

The validated Portuguese-language version of the EHP-30 questionnaire was administered on the day of admission for surgery and 3 and 6 months after the surgical procedure, at the outpatient level[8].

The EHP-30 consists of a central questionnaire comprising 30 items that evaluate 5 dimensions (pain, control and powerlessness, emotional well-being, social support, and self-image) and a modular questionnaire comprising 23 items distributed across 6 scales (sexual relations, work, medical profession, infertility, relationship with children, and treatment)[8]. Each scale yields a score from 0 to 100, and lower scores indicate better quality of life[7]. The prospective examination of the patients' quality of life entailed three applications of the questionnaire: during the preoperative period and 3 and 6 months after surgery.

The surgical procedures entailed the removal of all endometriotic lesions, according to Koninckx et al.[9], and the Working Group of the ESGE, ESHRE, and WES et al.[10]. The endometriomas were treated according to the Working Group of the ESGE, ESHRE, and WES et al.[11].

The data were organized using Microsoft Excel 2010® software for the preparation of databases, tables, and graphs. The statistical tests were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0. Absolute and relative frequency measurements were used to quantify the numerical and categorical variables. The Kolmogorov-Smirnov test was used to assess the normality of the EHP-30 questionnaire data. Since the distribution of the data was nonnormal, nonparametric data are expressed as the median (25th–75th percentile). For the comparisons among the different time points (before and 3 and 6 months after surgery), the Friedman test was used, followed by the Dunn posttest. Spearman's correlation was used to assess the correlation between the degree of endometriosis and the EHP-30 scores.

This study was evaluated and approved by the Research Ethics Committee of São Domingos Hospital through the Brazil Platform, with the Research Ethics Appraisal Certificate (CAAE) number 11808919.2.0000.5085, process approval number 3.334.498.

RESULTS

The final sample consisted of 102 patients with a mean age of 35.96±6.309 years of whom 34 (33.3%) were single and 68 (66.6%) were married. Regarding color, 18 (17.6%) patients were white, 70 (68.6%) were brown, and 14 (13.8%) were black. Regarding the classification of endometriosis according to the rASRM, 9 (8.8%) patients had minimal endometriosis, 18 (17.6%) had mild endometriosis, 35 (34.3%) had moderate endometriosis, and 40 (39.3%) had severe endometriosis (Table 1).

The types of surgery that the patients underwent were as follows: 21 (20.5%) underwent endometriosis, 30 (29.5%) underwent endometriosis+myomectomy, 23 (22.6%) underwent endometriosis+hysterectomy, 23 (22.5%) underwent endometriosis+rectosigmoidectomy+hysterectomy, and 5 (4.9%) underwent endometriosis+myomectomy+rectosigmoidectomy (Table 1).

Table 1. Characteristics of patients before and 3 and 6 months after laparoscopic surgical treatment of all forms of endometriosis.

Variables	No. (%)	Standard deviation
All cases	102 (100%)	–
Age	–	35.96±6.309
Marital status		
Single	34 (33.3%)	–
Married	68 (66.6%)	–
Race		
White	18 (17.6%)	
Brown	70 (68.6%)	
Black	18 (17.6%)	
rASRM classification of endometriosis		
Stage I (minimum)	9 (8.8%)	
Stage II (mild)	18 (17.6%)	
Stage III (moderate)	35 (34.3%)	
Stage IV (severe)	40 (39.3%)	
Surgeries performed		
Edt alone	21 (20.5%)	
Edt+mio	30 (29.5%)	
Edt+hta	23 (22.6%)	
Edt+hta+rectosig	23 (22.6%)	
Edt+mio+rectosig	5 (4.9%)	

rASRM: revised endometriosis classification of the American Society of Reproductive Medicine; Edt: endometriosis; myo: laparoscopic myomectomy; hta: total hysterectomy and bilateral laparoscopic salpingectomy; rectosig: laparascopic rectal, segmental, or shaving rectosigmoidectomy.

The EHP-30 data are shown in Table 2. There were reductions in the scores 3 and 6 months after surgery compared to presurgery, and at 6 months after surgery compared to 3 months after surgery, on the questionnaire (Part 1) and on Sections A, B, C, E, and F ($p<0.0001$). For Section D, there was a reduction 6 months after surgery compared to before surgery ($p<0.0001$).

There was no moderate or strong correlation between the degree of endometriosis and the EHP-30 quality of life scores before surgery and 3 and 6 months after surgical treatment (Spearman's correlation).

DISCUSSION

Due to the lengthy diagnostic process and consequent loss of quality of life for women with endometriosis, it is necessary to evaluate the quality of life of patients with symptomatic endometriosis along several parameters to improve their prognosis and offer both medical and multidisciplinary care[3,12,13]. As the results of this study show, in addition to providing a good prognosis for clinical symptomatology, surgical management led to a significant improvement in the quality of life 3 and 6 months after surgical treatment of endometriosis for the women who participated in this study.

Most of the participants were between 30 and 40 years old, reflecting a delay in diagnosis and treatment similar to what has been reported worldwide[6]. The participants were predominantly brown, compatible with the mixed ethnicities of the region where the study was conducted (the extreme north of Brazil), and the most common marital status was married.

Regarding the classification of endometriosis according to the rASRM, approximately 73.6% of the cases were moderate and severe, showing that the more advanced forms of this pathology are difficult to control with clinical treatment; this finding is reinforced by the fact that most of the patients required more comprehensive surgeries, such as myomectomies, hysterectomies, or rectosigmoidectomies.

Next, we will analyze other endometriosis studies that assessed quality of life with the EHP-30 questionnaire. Our study did not aim to compare clinical and surgical treatments, as the failure of clinical treatment was an inclusion criterion; however, it seems that both surgery and clinical treatment are valuable options to improve the harmful impact of dysmenorrhea associated with endometriosis[14].

Among the prospective studies that evaluated the treatment of intestinal endometriosis with surgeries performed by a multidisciplinary team experienced with the management of endometriosis, the results indicated significant improvement in quality of life 1 year after the surgical procedure, with no difference between the types of intestinal approach[15]. In a study that included only patients with deep endometriosis with or without intestinal resection, both groups showed a significant improvement in quality of life after surgery[16,17].

Furthermore, in a prospective study that evaluated quality of life in 22 patients with deep endometriosis who underwent surgical treatment, the EHP-30 results showed significant improvement for the items pain, control and powerlessness, emotional well-being, social relationships, sexual relations, and relationships with medical providers, but no significant changes in self-image, work, or relationships with the children[18,19]. Although both that study and our study were prospective and had equivalent study durations, our study had a larger sample and included patients with all forms of endometriosis, not just deep endometriosis, and we observed significant improvement in all areas except relations with medical providers; in this area, improvement was noted only between the preoperative scores and the scores 6 months after surgery.

Table 2. Evaluation of quality of life (EHP-30) in patients before and 3 and 6 months after laparoscopic surgical treatment of all forms of endometriosis.

EHP-30	Before surgery	3 months after surgery	6 months after surgery	p-value
Part 1	46.67 (29.16–46.67)[a,b]	16.25 (8.33–30)[a,c]	7.5 (3.198–15.83)[b,c]	<0.0001
Section A	35 (10–60)[a,b]	10 (0–25)[a,c]	0 (0–10)[b,c]	<0.0001
Section B	50 (0–62.50)[a,b]	0 (0–25)[a,c]	0 (0–12.50)[b,c]	<0.0001
Section C	50 (20–75)[a,b]	15 (0–50)[a,c]	7.5 (0–15)[b,c]	<0.0001
Section D	0 (0–0)[b]	0 (0–0)	0 (0–0)[b]	<0.0001
Section E	41.66 (0–66.66)[a,b]	16.66 (0–25)[a,c]	0 (0–8.33)[b,c]	<0.0001
Section F	50 (25–75)[a,b]	25 (6.25–56.25)[a,c]	6.25 (0–25)[b,c]	<0.0001

EHP-30: 30-item Endometriosis Health Profile; Friedman test, followed by the Dunn posttest. [a]Significant difference according to Dunn's posttest before surgery and 3 months after surgery; [b]significant difference according to Dunn's posttest before surgery and 6 months after surgery; [c]significant difference according to Dunn's posttest between 3 months after surgery and 6 months after surgery.

Studies have shown that removal of the unaffected uterus does not seem to improve the quality of life of patients with endometriosis[18,20]. A study that evaluated the quality of life of 61 patients who underwent surgical treatment that included the removal of the foci of endometriosis, hysterectomy, and oophorectomy compared with that of a group without hysterectomy and ovarian preservation found significant improvement on all 5 scales of the EHP-30 at 4 weeks postsurgery, and this improvement persisted for up to 6.8 years[20]. Although this was a longer observation period than that of our study, the results are in agreement with our findings.

In a multicenter, randomized clinical trial, we investigated the differences between the conventional robotic and laparoscopic pathways in the surgical treatment of endometriosis. Patients with all forms of endometriosis were evaluated. One of the comparison variables was quality of life, measured with the EHP-30 questionnaire; we found no difference between these two pathways, and both groups showed improved quality of life[5]. In the present study, our sample included patients with all forms of endometriosis, and the results were similar, but we did not include patients who underwent robotic surgery.

A literature review showed that endometriosis impairs quality of life, especially in the domains of pain and psychological and social functioning, and that therapies alleviate symptoms and improve the quality of life of these patients, but further research is needed to evaluate the impact of endometriosis on patients' lives[21]. In this study, we confirmed that surgical treatment improves the quality of life of patients with surgical indications for the treatment of endometriosis that has not been relieved by clinical treatment. The cases were not separated by the type of surgical intervention performed. All patients who underwent the removal of peritoneal, ovarian, or deep endometriosis with or without hysterectomy or rectal, segmental, or shaving rectosigmoidectomy were included, providing an overview of all patients who require surgical therapy.

Based on the results obtained and analyzed, surgery offers a good prognosis for improving the quality of life of women with endometriosis, and it is justified to relieve the suffering, limitations of daily activities, and anxiety and depression that affect patients with endometriosis[6].

In conclusion, when indicated for women with endometriosis, surgery generally improves their quality of life in several areas analyzed by the EHP-30 questionnaire.

AUTHORS' CONTRIBUTIONS

JNN: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology. **VGM:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **LCSL:** Conceptualization. **CMBO:** Conceptualization, Data curation, Project administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **MVLRC:** Conceptualization. **LMRSG:** Data curation, Project administration, Resources, Software, Supervision, Validation. **GIMF:** Data curation, Project administration, Resources, Software, Supervision, Validation. **LCS:** Data curation, Project administration, Resources, Software, Supervision, Validation. **PCL:** Data curation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ECRM:** Formal Analysis, Funding acquisition, Investigation, Methodology, Supervision.

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ORIGINAL ARTICLE

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Relationship between IL-17, TNF-α, IL-10, IFN-γ, and IL-18 polymorphisms with the outcome of hepatitis B virus infection in the Turkish population

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SUMMARY

OBJECTIVE: Hepatitis B virus is a global threat that can lead to liver cirrhosis and hepatocellular carcinoma. For the treatment of chronic hepatitis B virus, polymorphisms might be an option for gene treatments. This study aimed to investigate the effects of IL-17, TNF-α, IL-10, IFN-γ, and IL-18 gene polymorphisms on hepatitis B virus infection in the Turkish population.

METHODS: The genotypes and allele distribution of 75 patients exposed to hepatitis B virus and 50 healthy control individuals were analyzed. The real-time polymerase chain reaction method was used for identification.

RESULTS: A correlation was observed between susceptibility to hepatitis B virus infection and IL-17 Exon 3/3'UTR (rs1974226) C, IL-17 Exon 3 (rs763780) A, IL-18 (-607) (rs1946518) A alleles, and IL-17 Exon 3 (rs763780) AA genotype (p=0.006, p=0.009, p=0.025, and p=0.008, respectively). Furthermore, IL-18 (-137) (rs187238) TT genotype and TNF-α-308 (rs1800629) G and A alleles, were associated with protection against hepatitis B virus infection (p=0.0351 and p=0.032, respectively).

CONCLUSION: This study demonstrated that TNF-α (-308), IL-17 (Exon 3/3' UTR), IL-17 (Exon 3), and IL-18 (-607) polymorphisms are associated with hepatitis B virus infection. Therefore, these may serve as potential therapeutic targets for chronic viral hepatitis in the Turkish population.

KEYWORDS: IL-17. TNF-alpha. IL-10. IFN-gamma. IL-18. Hepatitis B.

INTRODUCTION

Hepatitis B virus (HBV) continues to be a global health burden despite the availability of highly protective vaccines and effective antiviral drugs. The World Health Organization reported that, in 2019, 820,000 people died of cirrhosis and liver cancer, and 296 million were chronic hepatitis B patients[1].

The dynamics between virological, environmental, and host genetic factors determine the outcomes of HBV infection[2]. In some individuals, the host response is viral clearance against HBV infection, whereas the reason for developing chronic HBV infection in other individuals is unknown. This difference has been associated with single-nucleotide polymorphisms (SNPs) in the regulatory region of cytokine genes. Genetic variations such as SNPs, which cause cytokine structure and expression changes, can increase the risk of infection and affect disease outcomes and treatment responses[3].

Among cytokines, IL-17 synthesized by the next-generation T helper 17 (Th17) cells plays a critical role in the pathogenesis of chronic HBV[4]. In cases of chronic HBV, a significant increase in the blood levels of Th17 cells and a rise in the number of Th17 cells in liver tissue in proportion to the hepatitis activity index have been observed[5]. However, few studies have investigated the relationship between HBV and IL-17 SNPs. Unlike IL-17, polymorphisms in the TNF-α are the best-characterized genetic variations associated with HBV[6]. Park et al. found that the antiviral effect of TNF-α was associated with the activity of p22-FLIP (cellular FLICE inhibitory protein), which restricts the HBV life cycle by inhibiting HBV DNA replication[7]. Furthermore, TNF-α SNPs could be important genetic biomarkers for liver pathogenesis[8].

In chronic HBV, a new subset of regulatory B (Breg) cells that can regulate CD8+T cell immunity and produce IL-10

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was identified. Increased IL-10 levels with Breg cell number in chronic HBV were associated with viral load and liver inflammation dynamics[9]. In support of this information, IL10 SNPs have been reported to be associated with the chronicity of hepatitis B and liver damage caused by HBV[10]. Like TNF-α, IFN-γ suppresses HBV replication in infected cells and reduces viral load by activating the antiviral effect of CD8+T lymphocytes[11]. The IFN-γ +874T/A polymorphism has been associated with HBV risk, particularly in the East Asian population[12]. IL-18, known as a potent inducer of IFN-γ, was found to be associated with the Th1 polarization of HBV-specific T cells and promote viral clearance[13]. In many studies, IL-18 polymorphisms have also been associated with susceptibility to HBV and reported to have a therapeutic value[13].

Viral hepatitis clinical outcomes vary in different populations, and genetic background is likely one of the reasons behind the differences. Genetic variability could be utilized to predict disease outcomes and treatment responses. Turkey is in the middle endemic region of the world in terms of HBV infection[14]. However, just several studies on this subject have been conducted in the region. Therefore, this study aimed to evaluate the effect of SNPs in IL-17, TNF-α, IL-10, IFN-γ, and IL-18 gene regions on the clinical course of HBV infection in the Turkish population.

METHODS

The study group included patients who were followed up for HBV infection for at least 18 months or longer in Suleyman Demirel University Faculty of Medicine, Infectious Diseases polyclinic. According to the criteria, 75 patients exposed to HBV and 50 healthy individuals were included in the study[15]. Patients group were classified into subgroups as "naturally immune," "inactive carriers," and "chronic active hepatitis." Individuals co-infected with hepatitis C and D and human immune deficiency virus and patients with different chronic liver diseases were not included in the study. The study was carried out with the approval of the Suleyman Demirel University Faculty of Medicine Ethics Committee (2/14). Informed consent was obtained from all participants, and the research protocol was performed in accordance with the ethical rules of the Declaration of Helsinki.

DNA Isolation

Peripheral venous blood samples taken from the individuals in the study group were transferred to tubes containing ethylene diamine tetra acetic acid (EDTA). Genomic DNA was extracted from a 200 µL peripheral venous blood sample according to the manufacturer's recommendations (High Pure PCR Template Preparation Kit, Roche). Isolated DNA samples were stored at -20°C for use in real-time polymerase chain reaction (RT-PCR) analysis.

Identifying polymorphisms

RT-PCR was used to identify SNPs in the IL-17, TNF-α, IL-10, IFN-γ, and IL-18 gene regions. The Light Cycler 2.0 RT-PCR system (Roche, Germany) was used for SNP analysis in accordance with protocols specified by the manufacturer. For DNA amplification, target site-specific primers and hybridization probe sequences (TIB Molbiol, Berlin, Germany) were designed.

RESULTS

Allelic and genotypic distributions of SNPs in the HBV-infected case group and the healthy control group included in the study were analyzed. Allelic distributions of the patient and control groups were determined by RT-PCR melting curve data according to polymorphisms (Figure 1).

The frequency of the TNF-α (-308) G and A alleles was higher in the control group than in other groups, especially significantly considerably higher when compared with the group that was naturally immune to HBV (p=0.032) (Table 1).

When the control group and all patients group were compared, we found that IL-17 (Exon 3/3' UTR) C allele was more common in the all-patients group infected with HBV and the A allele in the control group, and the difference was statistically significant (p=0.006) (Table 1). Also, in subgroup analysis (according to their clinical status), a comparison of the patient groups with control group showed that the C allele was more common in natural immune and asymptomatic carriers, while the A allele in control group had a statistically significant difference (p=0.033 and p=0.018, respectively) (Table 1).

The IL-17 (Exon 3) A allele was found to be expressed at higher levels in all patients infected with HBV group compared to the control, and the G allele was found to be expressed at a higher level in control group (p=0.0099) (Table 1). In genotype association analysis between control and all-patient groups infected with HBV, IL-17 (Exon 3) AA (homozygous wild type) genotype was observed at a higher frequency and statistically significant in the all-patient group (p=0.008). In subgroup analysis, IL-17 (Exon 3) AA genotype was more common in the naturally immune to HBV and asymptomatic carriers' groups compared to the control group (p=0.045 and p=0.045, respectively). Conversely, IL-17 (Exon 3) AG (heterozygous) genotype was observed at a lower frequency in the groups, naturally immune to HBV, asymptomatic carriers, and

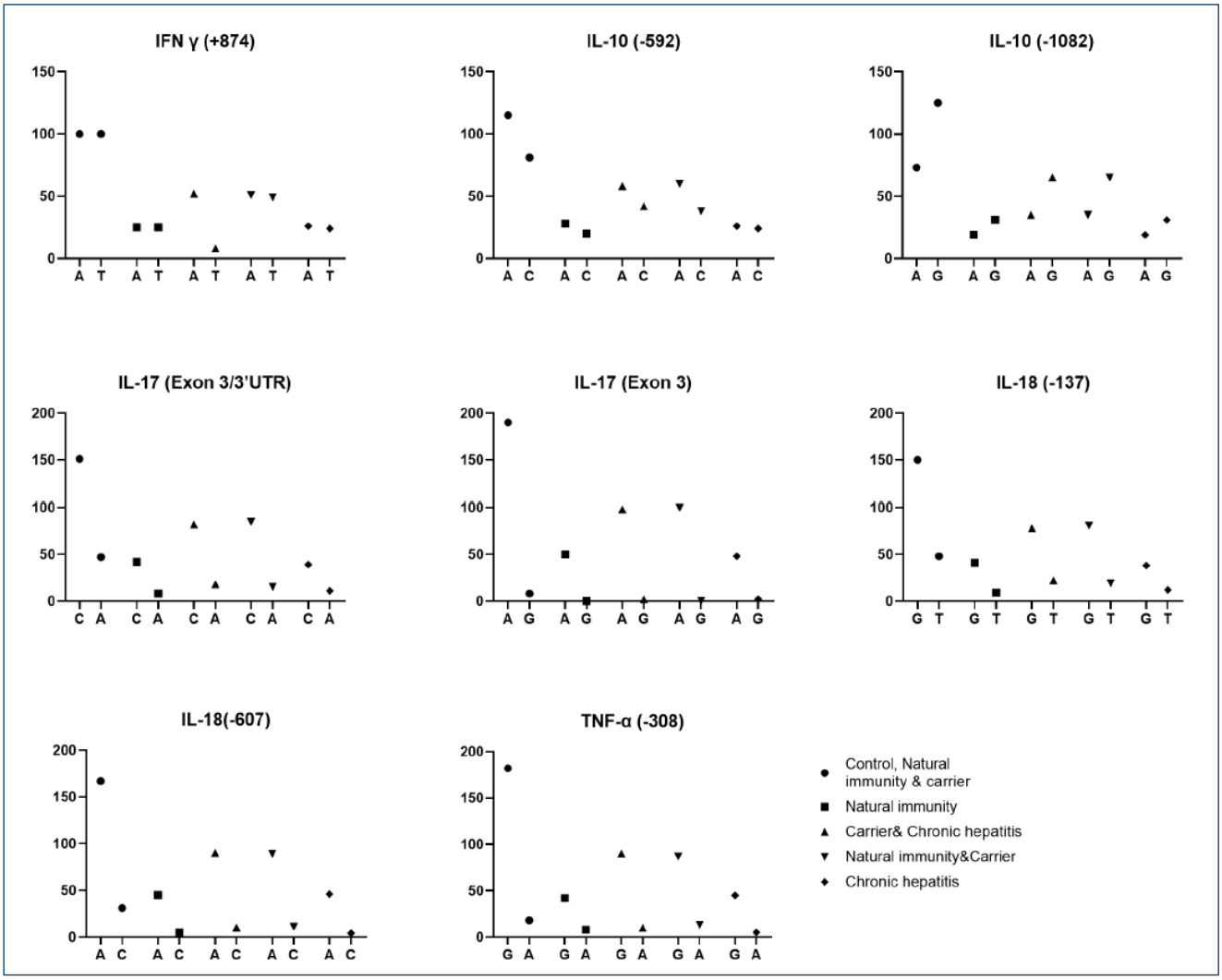

Figure 1. Allele distribution graph in the created groups.

all patients infected with HBV groups than the healthy control group (p=0.045, p=0.045, and p=0.008, respectively) (Table 2).

We determined that IL-18 (-607) C allele significantly increased in the group of patients infected with HBV, while the A allele increased in the control group (p=0.025) (Table 2). Moreover, there was no noticeable difference in allele level for IL-18 (-137), whereas, in the genotype association analysis, the CC genotype was significantly higher in the control group compared to the group with all HBV-infected patients (Table 2).

DISCUSSION

The development of molecular genetics has stimulated studies evaluating the impact of human genome variability on immune response and disease mechanisms. Numerous studies have shown that cytokine gene polymorphisms have an impact on the prognosis of HBV infection in the general population[3,16]. Our study revealed that TNF-α (-308) G and A alleles, which were found to be higher in the healthy control group than in subjects exposed to HBV, were associated with protection against HBV. However, no significant relationship was found between the genotypic level of TNF-α (-308) G/A polymorphism and HBV infection. Unlike our results, the TNF-α (-308) G allele was found to be higher in the chronic hepatitis group in two studies conducted in Turkey[17,18]. In a meta-analysis by Zhang et al. in which studies conducted on the Chinese population were compiled, the TNF-α (-308) A allele was associated with a decrease in the chronicity of HBV, similar to our study[19]. In addition to these results, in a Brazilian study, the TNF-α (-308) A allele and AA genotype were associated with

Table 1. Allele distribution, frequencies, and statistical analysis between the control and patient groups.

Gene	Allele	Control (2n=100) (F) OR (95%CI)	Natural immunity (2n=50) (F) p-value OR (95%CI)[a]	Carrier (2n=50) (F) p-value OR (95%CI)[b]	Chronic hepatitis (2n=50) (F) p-value OR (95%CI)[c]	All patients (2n=150) (F) p-value OR (95%CI)[d]
TNF-α (-308) rs1800629	G	95 (0.95)	42 (0.84)	45 (0.9)	45 (0.9)	132 (0.88)
	A	5 (0.5)	8 (0.16)	5 (0.1)	5 (0.1)	18 (0.12)
	NA	0	0	0	0	0
			p=0.032 3.61 (1.11–11.71)	p=0.302 2.11 (0.58–7.66)	p=0.302 2.11 (0.58–7.66)	p=0.074 2.59 (0.92–7.22)
IFN-γ (+874) rs2430561	A	49 (0.49)	25 (0.5)	26 (0.52)	26 (0.52)	77 (0.51)
	T	51 (0.51)	25 (0.5)	24 (0.48)	24 (0.48)	73 (0.49)
	NA	0	0	0	0	0
			p=10.96 (0.49–1.89)	p=0.862 0.88 (0.45–1.75)	p=0.862 0.88 (0.45–1.75)	p=0.796 0.91 (0.55–1.51)
IL-10 (-592) rs1800872	A	55 (0.56)	28 (0.58)	32 (0.64)	26 (0.52)	86 (0.58)
	C	43 (0.44)	20 (0.42)	18 (0.36)	24 (0.48)	62 (0.42)
	NA	2	2	0	0	0
			p=0.859 0.91 (0.45–1.84)	p=0.382 0.72 (0.36–1.45)	p=0.727 1.18 (0.60–2.34)	p=0.793 0.92 (0.55–1.54)
IL-10 (-1082) rs1800896	A	38 (0.39)	19 (0.38)	16 (0.32)	19 (0.38)	54 (0.36)
	G	60 (0.61)	31 (0.62)	34 (0.68)	31 (0.62)	96 (0.64)
	NA	2	0	0	0	0
			p=1 1.03 (0.51–2.08)	p=0.472 1.35 (0.65–2.76)	p=1 1.03 (0.51–2.08)	P=0.687 1.12 (0.67–1.90)
IL-17 (Exon 3/3'UTR) rs1974226	C	66 (0.67)	42 (0.84)	43 (0.86)	39 (0.78)	124 (0.83)
	A	32 (0.33)	8 (0.16)	7 (0.14)	11 (0.22)	26 (0.17)
	NA	2	0	0	0	0
			p=0.033 0.39 (0.16–0.93)	p=0.018 0.34 (0.14–0.83)	p=0.188 0.58 (0.26–1.28)	p=0.006 0.43 (0.23–0.79)
IL-17 (Exon 3) rs763780	A	90 (0.92)	50 (1)	50 (1)	48 (0.96)	148 (0.99)
	G	8 (0.8)	0	0	2 (0.04)	2 (0.01)
	NA	2	0	0	0	0
			p=0.051	p=0.051	p=0.495 0.47 (0.10–2.29)	p=0.0099 0.15 (0.03–0.73)
IL-18 (-137) rs187238	G	69 (0.70)	41 (0.82)	40 (0.8)	38 (0.76)	119 (0.79)
	T	29 (0.30)	9 (0.18)	10 (0.2)	12 (0.24)	31 (0.21)
	NA	2	0	0	0	0
			p=0.164 0.52 (0.22–1.21)	p=0.241 0.59 (0.26–1.35)	p=0.561 0.75 (0.34–1.64)	p=0.129 0.62 (0.34–1.11)
IL-18 (-607) rs1946518	A	78 (0.8)	45 (0.9)	44 (0.88)	46 (0.92)	135 (0.9)
	C	20 (0.2)	5 (0.1)	6 (0.12)	4 (0.08)	15 (0.1)
	NA	2	0	0	0	0
			p=0.163 0.43 (0.15–1.23)	p=0.257 0.53 (0.20–1.42)	p=0.061 0.34 (0.11–1.05)	p=0.025 0.43 (0.21–0.89)

[a]Control vs. natural immunity. [b]Control vs. carrier. [c]Control vs. chronic hepatitis. arrier. [d]Control vs. all patients. NA: missing allele; F: frequency; OR: odds ratio; CI: confidence intervals.

Table 2. Genotype distribution, frequencies, and statistical analysis in the control and patient groups.

Gene	Genotype	Control (n=50) (F)	Natural immunity (n=25) (F) p-value OR (95%CI)[a]	Carrier (n=25) (F) p-value OR (95%CI)[b]	Chronic hepatitis (n=25) (F) p-value OR (95%CI)[c]	All patients (n=75) (F) p-value OR (95%CI)[d]
TNF-α (-308) rs1800629	GG	45 (0.90)	18 (0.72) p=0.091 3.5 (0.98–12.8)	21 (0.84) p=0.728 1.71 (0.41–7.04)	20 (0.8) p=0.286 2.25 (0.58–8.65)	59 (0.79) p=0.142 2.44 (0.83–7.16)
	GA	5 (0.10)	6 (0.24) p=0.164 0.35 (0.10–1.29)	3 (0.12) p=1 0.81 (0.18–3.72)	5 (0.2) p=0.286 0.44 (0.11–1.70)	14 (0.19) p=0.213 0.48 (0.16–1.44)
	AA	0	1 (0.04) p=0.33	1 (0.04) p=0.333	0 p=1	2 (0.02) p=0.516
	NA	0	0	0	0	0
IFN-γ (+874) rs2430561	AA	10 (0.20)	5 (0.2) p=1.00 1 (0.3–3.3)	6 (0.24) p=0.768 0.79 (0.25–2.5)	7 (0.28) p=0.559 0.64 (0.21–1.96)	18 (0.24) p=0.665 0.79(0.33–1.89)
	AT	29 (0.58)	15 (0.6) p=0.534 0.9 (0.34–2.44)	14 (0.56) p=1 1.08 (0.41–2.86)	12 (0.48) p=0.466 1.50 (0.57–3.93)	41 (0.55) p=0.854 1.14 (0.56–2.36)
	TT	11 (0.22)	5 (0.2) p=0.547 1.13 (0.34–0.84)	5 (0.2) p=0.547 1.13 (0.34–0.84)	6 (0.24) p=1 0.89 (0.28–2.78)	16 (0.21) p=1.00 1.04 (0.44–2.48)
	NA	0	0	0	0	0
IL-10 (-592) rs1800872	AA	6 (0.12)	4 (0.17) p=4.720 0.70 (0.18–2.75)	7 (0.28) p=0.113 0.36 (0.11–1.217)	1 (0.04) p=0.411 3.35 (0.38–29.48)	12 (0.16) p=0.610 0.72 (0.25–2.07)
	AC	43 (0.88)	20 (0.83) p=0.720 1.43 (0.36–5.65)	18 (0.72) p=0.113 2.79 (0.82–9.45)	24 (0.96) p=0.411 0.30 (0.03–2.63)	62 (0.84) p=0.610 1.38 (0.48–3.98)
	CC	0	0 p=1.00	0 p=1	0 p=1	p=1
	NA	1	1	0	0	1
IL-10 (-1082) rs1800896	AA	9 (0.18)	5 (0.2) p=1 0.9 (0.25–3.04)	2 (0.08) p=0.314 2.58 (0.51–13.02)	3 (0.12) p=0.749 1.35 (0.33–5.46)	10 (0.13) p=0.611 1.46 (0.54–3.91)
	AG	20 (0.41)	9 (0.36) p=0.802 1.23 (0.45–3.32)	12 (0.48) p=0.624 0.75 (0.28–1.97)	13 (0.52) p=0.460 0.64 (0.24–1.68)	34 (0.46) p=0.712 0.83 (0.40–1.72)
	GG	20 (0.41)	11 (0.44) p=0.808 0.88 (0.33–2.32)	11 (0.44) p=0.808 0.88 (0.33–2.32)	9 (0.36) p=0.802 1.23 (0.45–3.32)	31 (0.41) p=1 0.98 (0.47–2.03)
	NA	1	0	0	0	0
IL-17 (Exon 3/3'UTR) rs1974226	CC	26 (0.53)	18 (0.72) p=0.14 0.44 (0.16–1.24)	19 (0.76) p=0.078 0.36 (0.12–1.05)	15 (0.6) p=0.627 0.75 (0.28–2.00)	52 (0.69) p=0.087 0.50 (0.24–1.03)
	CA	14 (0.29)	6 (0.24) p=0.785 1.27 (0.42–3.83)	5 (0.2) p=0.576 1.6 (0.50–5.10)	9 (0.36) p=0.598 0.71 (0.25–1.98)	20 (0.27) p=0.839 1.10 (0.49–2.46)
	AA	9 (0.18)	1 (0.04) p=0.149 5.4 (0.64–45.3)	1 (0.04) p=0.149 5.4 (0.64–45.3)	1 (0.04) p=0.149 5.4 (0.64–45.3)	3 (0.04) p=0.031 4.40 (1.36–17.1)
	NA	1	0	0	0	
IL-17 (Exon 3) rs763780	AA	41 (0.84)	25 (1) p=0.045	25 (1) p=0.045	23 (0.92) p=0.478 0.45 (0.09–2.28)	73 (0.97) p=0.008 0.14 (0.03–0.69)
	AG	8 (0.16)	0 (0) p=0.045	0 (0) p=0.045	2 (0.08) p=0.478 0.45 (0.09–2.28)	2 (0.03) p=0.008 7.12 (1.44–35.13)
	GG	0	0 p=1	0 p=1	0 p=1	p=1
	NA	1	0	0	0	0
IL-18 (-137) rs187238	GG	25 (0.51)	16 (0.64) p=0.330 0.59 (0.22–1.58)	15 (0.6) p=0.622 0.69 (0.26–1.84)	14 (0.56) p=0.807 0.82 (0.31–2.15)	45 (0.60) p=0.358 0.69 (0.34–1.43)
	GT	19 (0.39)	9 (0.36) p=1 1.13 (0.41–3.06)	10 (0.4) p=1 0.95 (0.35–2.54)	10 (0.4) p=1 0.95 (0.35–2.54)	29 (0.39) p=1.00 1.00 (0.48–2.10)
	TT	5 (0.10)	0 (0) p=0.160	0 (0) p=0.160	1 (0.04) p=0.428 2.73 (0.30–24.7)	1 (0.01) p=0.0351 8.40 (0.95–74.3)
	NA	1	0	0	0	
IL-18(-607) rs1946518	AA	38 (0.78)	22 (0.88) p=0.357 0.47 (0.12–1.87)	22 (0.88) p=0.357 0.47 (0.12–1.87)	22 (0.88) p=0.357 0.47 (0.12–1.87)	66 (0.88) p=0.139 0.47 (0.18–1.24)
	AC	2 (0.4)	1 (0.04) P=1 1.02 (0.09–11.84)	0 (0) p=0.546	2 (0.08) p=0.560 0.48 (0.06–3.70)	3 (0.04) p=1.00 1.02 (0.16–6.34)
	CC	9 (0.18)	2 (0.08) p=0.314 2.58 (0.51–13.02)	3 (0.12) p=0.532 1.65 (0.40–6.73)	1 (0.04) p=0.149 5.4 (0.644–45.3)	6 (0.08) p=0.097 2.58 (0.86–7.80)
	NA	1	0	0	0	0

[a]Control vs. natural immunity. [b]Control vs. carrier. [c]Control vs. chronic hepatitis. [d]Control vs. all patients. NA: missing allele; F: frequency; OR: odds ratio; CI: confidence intervals.

chronic HBV infection as well as susceptibility to severe fibrosis and increased necro-inflammatory activity[20]. It is thought that gene variants affecting TNF-α expression may play a role in different clinical outcomes, such as elimination, resolution, or fibrosis in HBV infection.

In chronic hepatitis B patients, despite the increase in serum IL-17 levels, lower IL-17R expression levels and a weaker response to IL-17 were detected in vitro compared to the healthy control group[4,5]. This phenomenon may be due to the reduction in IL-17R expression depending on the negative effect of high levels of IL-17 on cells expressing IL-17R. In light of this information, we argue that IL-17 level and genetic polymorphisms affecting this level may be associated with the clinical consequences of HBV infection. In our research, a correlation was observed between susceptibility to HBV and IL-17 (Exon 3) AA genotype, IL-17 (Exon 3/3'UTR) C, and IL-17 (Exon 3) A alleles. Additionally, a correlation was observed between protection against HBV and IL-17 (Exon 3/3' UTR) A and IL-17 (Exon 3) G alleles. However, in our literature review to compare our results, we determined that minimal studies evaluate the relationship between IL-17 genetic polymorphisms and HBV. In contrast to our results, in two studies conducted in Iran and China, the IL-17 (Exon 3) SNP was found not to affect the clinical course of HBV[21,22]. Our results will form the basis for studies evaluating the effect of IL-17 polymorphisms on the clinical course of HBV.

IL-18 directly activates CD8+ T cells, which are central to viral clearance, and genetic variations can affect pro-IL-18 production, conferring susceptibility to viral hepatitis[23]. In our study, a correlation was observed between susceptibility to HBV with IL-18 (-607) A allele, while the IL-18 (-607) C allele and IL-18 (-137) CC genotype were associated with protection against HBV infection. There is only one study conducted in the Turkish population, and unlike our study, no association was found between HBV with 607/-137 genotypes and alleles[24]. In studies on IL-18, the roles of polymorphisms in the -607 and -137 regions are controversial. Some studies have associated genetic variations with lower transcriptional activity, lower IL-18 production from hepatic macrophages, and less inhibition of HBV replication, while other studies have associated protection from HBV[25]. However, during the antiviral response, IL-18, which is considered the determining factor in controlling the Th1/Th2 balance, may have effects that may change the individual disease risk[23].

The research is precious because there are minimal studies on this subject in the Turkish population. In addition, our study is the first to investigate polymorphisms in the IL-17 gene in Turkish people. However, the study had some potential limitations. First, all the data for this study were obtained from a single-center study and may not represent the entire target population. The second is the failure to evaluate cytokine levels. Overall, this study should be considered exploratory and should be validated in subsequent studies.

CONCLUSION

This study demonstrated a correlation between susceptibility to HBV and IL-17 Exon 3/3'UTR C, IL-17 Exon 3 A, IL-18 (-607) A alleles, and IL-17 Exon 3 AA genotype, whereas IL-18 (-137) TT genotype, TNF-α-308 G, and A alleles were associated with protection against HBV. Genome-wide association studies are a practical approach to understanding how polymorphisms affect diseases. Polymorphisms might be potential targets for gene therapies in treating chronic HBV infection. Our findings will form the basis for studies on the immunopathogenesis and pharmacogenetics of HBV infection.

AUTHORS' CONTRIBUTIONS

ENT: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **FZA:** Conceptualization, Methodology, Validation, Visualization. **VC:** Formal Analysis, Investigation, Methodology, Validation. **GB:** Formal Analysis, Investigation, Methodology, Validation. **MYT:** Software, Validation, Visualization.

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ORIGINAL ARTICLE

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Expression of sirtuin 2 and 7 in placenta accreta spectrum

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SUMMARY

OBJECTIVE: This study aimed to investigate the expression levels of sirtuin 2 and sirtuin 7 in the placenta accreta spectrum to reveal their role in its pathogenesis.

METHODS: A total of 30 placenta accreta spectrum, 20 placenta previa, and 30 controls were experienced. The sirtuin 2 and sirtuin 7 expression levels in the placentas of these groups were determined by Western blot. sirtuin 2 and sirtuin 7 serum levels in the maternal and fetal cord blood were examined by enzyme-linked immunosorbent assay.

RESULTS: It was found that sirtuin 7 in placenta accreta spectrum was significantly lower in the placenta compared to the control and placenta previa groups ($p<0.05$). However, a significant difference was not observed between the sirtuin 2 and sirtuin 7 levels in the maternal and fetal cord serum samples of those three groups ($p>0.05$).

CONCLUSION: Sirtuin 7 may play an important role in the formation of placenta accreta spectrum. The effect of decreased expression of sirtuin 7 might be tissue-dependent in the placenta accreta spectrum and needs to be investigated further.

KEYWORDS: Epithelial-mesenchymal transition. Placenta accreta. Placenta previa. Sirtuin 2. Sirtuins.

INTRODUCTION

Placenta accreta spectrum (PAS) is the aberrant invasion of the placenta by trophoblasts into the myometrium[1]. PAS is histopathologically divided into three types according to the degree of attachment to the myometrium, namely, placenta accreta, increta, and percreta[2]. In placentation, extravillous trophoblasts (EVTs) containing interstitial and endovascular cells invade the superficial myometrium and cause remodeling of the basilar and spiral arteries[3,4]. The most common risk factors that increase the probability of PAS formation are total or partial absence of decidua and intrauterine surgical scars[5-7], presence of the placenta previa (PP), and advanced maternal age[8]. PAS has a serious effect on maternal health and increases maternal mortality and morbidity up to 0.7 and 46.9%, respectively[9,10].

The process known as epithelial-mesenchymal transition (EMT) transforms motionless epithelial cells into migratory mesenchymal cells[11]. Therefore, it is very critical for the adherence of the placenta to the myometrium during the first trimester. Although, in the second and third trimesters, EMT should not be continued[12], it was reported that if EMT is presented in the second and third trimesters, it may contribute to the formation of PAS[13].

It was observed that sirtuin 2 (SIRT2) is weakly expressed in placental disorders[14], and along with the SIRT2, sirtuin 7 (SIRT7) is known as having roles in the EMT[15]. Besides, SIRT2 has been shown to increase the abnormal proliferation and migration of cancer cells by promoting the expression of EMT-related genes[16]. Transforming growth factor-β (TGF-β) is also an important regulator of EMT, and SIRT7 is known to modulate EMT/TGF-β signaling[17]. Therefore, our study aimed to investigate the expression levels of SIRT2 and SIRT7 in the placental tissues, maternal and fetal cord of the control, PP, and PAS groups.

METHODS

Study subjects

This study was approved by and conducted with the decision of the Inonu University Clinical Research Ethics Committee (2020/51-13.05.2020), and informed consent was obtained

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from all participants. Clinical samples including placenta, fetal cord serum, and maternal serum of 80 women were used, and they were supplied by the Department of Obstetrics and Gynecology of Dicle University Faculty of Medicine. Samples were divided into three groups. The first group named the PP group (n=20) consists of patients with no previous history of cesarean section or uterine surgery but diagnosed with PP without invasion. The second group named the PAS group (n=30) has the patients who had at least one previous cesarean section along with the PP and invasion. The third group, the control group (n=30), includes healthy women with similar demographic features (Table 1). Exclusion criteria were as follows: (a) patients with PP marginalis or inferior placenta, (b) patients who underwent surgery before the 24th week of pregnancies, (c) patients who gave birth under 500 g, (d) patients under the age of 18 years, (e) patients having multiple pregnancies, (f) patients having pregnancy complications with thyroid dysfunction, hypertension, epilepsy, gestational diabetes mellitus, type 1 and type 2 diabetes mellitus, patients using any medications that may affect the cardiovascular system, and pregnant women with kidney disease were not included in the study. To make a preoperative diagnosis, abdominal, transvaginal, and Doppler ultrasonography were used. PAS or PP was defined and diagnosed according to the current American College of Obstetricians and Gynecologists[18] and Society for Maternal-Fetal Medicine guidelines as well as FIGO consensus guideline[19]. PAS was also diagnosed with the pathology results. Age of patients, gravidity, parity, pregnancy week, newborn's gender, newborn's weight, and other patient information were recorded (Table 1). Placental tissues were collected immediately after the cesarean section and stored at -80°C until Western blot analysis.

Western blot analysis

Placenta samples were removed from the -80°C and crushed in liquid nitrogen. Then, cold RIPA buffer containing protease-phosphatase inhibitor cocktail and nuclease (Thermo Scientific) was added to the sample. The total cellular protein concentration of lysates was determined by BCA protein assay kit (TaKaRa). Total cellular proteins (20 μg) were separated using the 10% SDS-PAGE (BIO-RAD), and the separated proteins were transferred to the PVDF transfer membrane. The membranes were incubated with anti-SIRT2 antibody (STJ25534) and anti-SIRT7 antibody (STJ25536) for 2 h at room temperature, and β-actin (Mouse IgG2b-643802-Biolegend) was used as a loading control. Appropriate HRP-conjugated secondary antibodies (Biolegend) were used to visualize the specific bands by ECL (Advansta) and the images were taken by using G: Box (Syngene).

Enzyme-linked immunosorbent assay

Maternal peripheral venous blood was obtained before the administration of anesthesia. Fetal cord blood was taken from the umbilical artery after the umbilical cord was clamped and stored at -80°C before use. Serum levels of SIRT2 and SIRT7 were examined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (SunRed bio, Shanghai, PR China). The optical density (OD) of each well was identified with a microplate reader at 450 nm.

Statistical analysis

Line intensities obtained from the Western blot analysis were evaluated by the Image J. The OD values resulted from ELISA were analyzed via the Myassays program. Statistical analysis was performed by the SPSS® 11.5 (SPSS Inc.; Chicago, IL, USA) program. After the analyses, the numerical data were given as mean±standard deviations. Normally distributed data among

Table 1. Demographic and clinical characteristics of patient groups.

Demographic and clinical characteristics	Control	PP	PAS	p-value
	Mean±std	Mean±std	Mean±std	
Age	33.3±4.8	33.4±6.0	33.9±4.9	0.883
Gravidity	4.7±1.9	3.2±2.3	5±1.8	0.009*
Parity	3.2±1.4	1.45±1.5	3.3±1.7	0.0002**
Previous cesarean section	2.7±1.1	0	2.3±1.0	0
Birth weight (g)	3,076±420	2,954±574	2,788±430	0.062
Birth week	37.2±1.2	36.5±2.4	36.3±1.2	0.106
Hemoglobin	11.7±1.4	11.3±1.6	11.5±1.2	0.689
Hematocrit	36.1±3.6	36.5±4.7	34.9±3.5	0.505
Thrombocyte	224±58	197±40	227±51	0.103

*p<0.05, **p<0.01.

the multiple groups were analyzed with the one-way ANOVA test. A value of p<0.05 was considered statistically significant.

RESULTS

In this study, we investigated the expression levels of SIRT2 and SIRT7 in control, PAS, and PP placentas. In addition, we determined the maternal and fetal cord serum levels of these three groups. The control, PP, and PAS groups have similar demographic and clinical characteristics (i.e., age, birth weight, week of birth, hemoglobin, hematocrit, and thrombocyte in all groups) (Table 1).

Our results showed that SIRT7 expression levels in placenta were lower in patients with PAS compared to the control and PP groups (*p<0.024) (Figure 1). In addition, SIRT2 expression levels were found to be decreased in PAS patients compared to the control and PP groups. However, this decrement was not statistically significant (p>0.05) (Figure 1).

SIRT2 and SIRT7 protein levels were identified by ELISA in all maternal and fetal cord sera. According to the results, there was no statistically significant difference between SIRT2 levels in both maternal serum samples of the control (n=30, mean: 1.7 ng/mL, ±std: 1.0), PP (n=20, mean: 1.5 ng/mL, ±std: 0.8), and PAS groups (n=30, mean: 2.3 ng/mL, ±std: 1.7) (p=0.0549) (Figure 2A) and fetal cord sera of the control (n=30, mean: 4.5 ng/mL, ±std: 3.13), PP (n=20, mean: 3.85 ng/mL, ±std: 2.91), and PAS groups (n=30, mean: 4.14 ng/mL, ±std: 3.09) (p=0.6932) (Figure 2B). When SIRT7 levels were compared in the maternal sera, there were no differences between control (n=30, mean: 2.2 ng/mL, ±std: 1.2), PP (n=20, mean: 2.3 ng/mL, ±std: 1.2), and PAS groups (n=30, mean: 2.5 ng/mL, ±std: 1.6) (p=0.6574) (Figure 2C). The SIRT7 levels in fetal cord serum were also measured, and a similar trend was found in three groups as in maternal sera of control (n=30, mean: 2.2 ng/mL, ±std: 1.0), PP (n=20, mean: 2.0 ng/

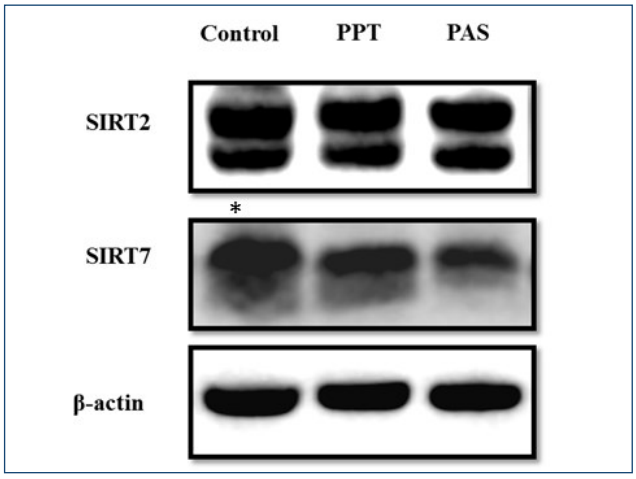

Figure 1. Expression level of sirtuin 2 and sirtuin 7 in placentas of control, placenta previa, and placenta accreta spectrum groups. sirtuin 2 expression levels were found to be not decreased statistically significant in placenta accreta spectrum patients compared to control and placenta previa groups. β-actin was used as a loading control. Sirtuin 7 expression levels were significantly lower in placental tissue of placenta accreta spectrum patients compared to the control and placenta previa groups (*p<0.024).

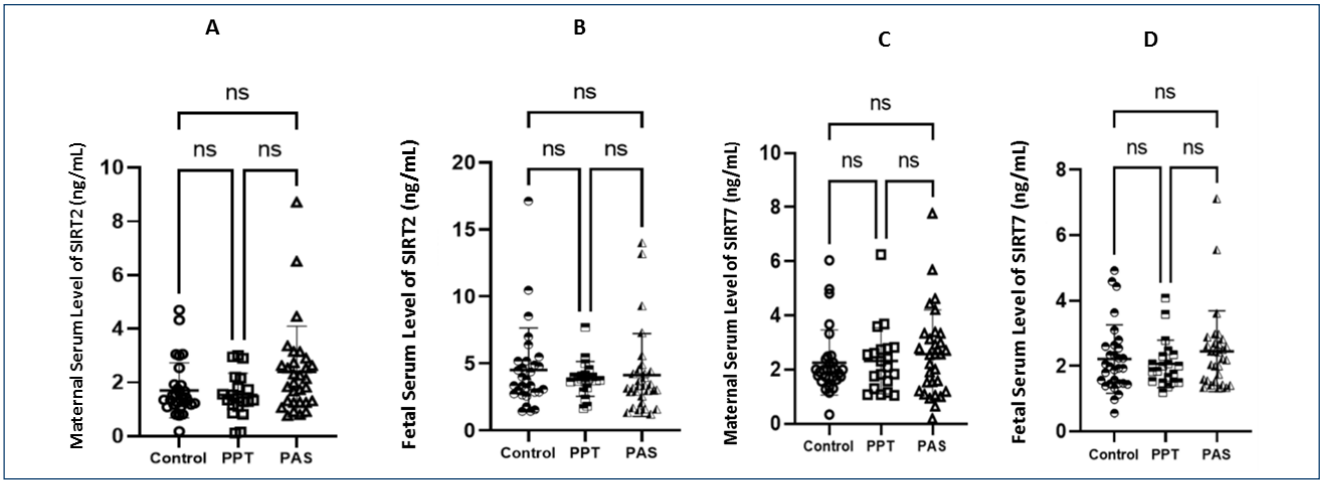

Figure 2. Maternal and fetal serum levels of sirtuin 2 and sirtuin 7 in control, placenta previa, and placenta accreta spectrum groups. (A) Comparison of sirtuin 2 levels in maternal sera from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. (B) Comparison of sirtuin 2 levels in fetal sera from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. (C) Comparison of sirtuin 7 levels in maternal sera from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. (D) Comparison of sirtuin 7 levels in fetal serum from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. One-way ANOVA was used for comparisons among the three groups.

mL, ±std: 0.7), and PAS (n=30, mean: 2.4 ng/mL, ±std: 1.2) (p=0.5880) (Figure 2D). Finally, no significant difference was found in the serum levels of the three groups.

DISCUSSION

Placental adhesion anomally is a condition in which the placenta adheres to the uterine wall in various degrees. Although the development of PAS is a complex and multi-factor process, the molecular mechanism behind the PAS is still unknown.

As the lack of the decidua or basal layer, improper maternal revascular patterning, and excessive EVT invasion are among the postulated theories for the emergence of PAS[20], many studies have investigated the EMT markers in PAS. Although EMT is necessary for proper placental invasion and attachment to the myometrium in the first trimester, it should not continue throughout pregnancy[12]. It has been hypothesized that excessively vigorous EMT that persists during pregnancy contributes to the development of PAS[13]. N-cadherin, ZEB1, and Snail are also markers of EMT[21]. The loss of the crucial E-cadherin is the most visible symptom of EMT. The expression of E-cadherin was decreased in the chorionic villi of the invasive part of the placenta, whereas the expression of Snail and TGF-β increased in the decidual cells of the invasive region[22]. These data imply that EMT may have an important role in PAS. Sirtuins have been shown to affect epithelial plasticity by reprogramming transcription at the EMT, leading to invasion and metastasis. That is why we proposed to investigate SIRT2 and SIRT7 in PAS as they govern in EMT.

In this study, we examined SIRT2 and SIRT7 expression in the placenta, maternal serum, and fetal cord serum of PAS. Our result showed that there is a reduced expression of SIRT7 in the placental tissue of PAS patients compared to the PP and healthy groups. Likewise, it has been showed that SIRT7 is significantly downregulated in breast cancer lung metastases in humans and mice, deacetylates beta-transducin repeat containing E3 ubiquitin protein ligase (β-TrCP1), mediates SMAD family member 4 (SMAD4) degradation and SIRT7 deficiency, activates TGF-β signaling, and increases EMT[23]. It was also demonstrated that resveratrol antagonizes TGF-β signaling by activating SIRT7 deacetylase activity, inhibiting breast cancer lung metastases and increasing survival. Besides, SIRT1 can regulate SMAD4 with SIRT7 in breast cancer metastasis[23]. Interactions between the TGF-β protein family have been shown to contribute greatly to the regulation of EVT invasion[17]. As SIRT7 expression was found to be reduced in the placenta of the PAS group, but not in maternal and fetal sera in our study, and the serum level of TGF-β expression was found to be significantly higher in the placenta accreta group by another research[24], the effect of decreased expression SIRT7 and its contribution to the EMT may be the tissue depended on PAS, which needs to be further investigated.

We have also evaluated SIRT2 expression in placenta, maternal, and fetal cord of PAS patients and compared with the PP and control groups. SIRT2 is known to have dual roles in many different tumors via regulation of EMT as SIRT7. SIRT2 positively regulated EMT and upregulated the protein levels of the mesenchymal markers such as N-cadherin and vimentin and the levels of MMP2 and MMP9 in osteosarcomas[25]. However, an increase in MMP9 was observed in SIRT2-null mouse embryonic fibroblasts as was a decrease in E-cadherin, which promotes cellular migration and invasion. Even though it has also revealed an increase in the expression of MMP9 in PAS regulated by SIRT2[22], we could not detect any differences in SIRT2 levels in placental tissue, maternal, and fetal cord sera of PAS patients. Those data imply that MMP9 increase may be regulated by another way rather than SIRT2 in PAS.

CONCLUSION

Our results revealed that the expression of SIRT7 was reduced in PAS. Although this is the first study showing the relationship between the SIRT2 and SIRT7 with PAS, further studies are still needed for understanding the exact role of SIRT2 and SIRT7 in PAS.

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AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

AUTHORS' CONTRIBUTIONS

IIT: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **MSI:** Data curation, Resources, Writing – original draft. **FMF:** Data curation, Resources. **SG:** Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **DCD:** Resources, Writing – review & editing.

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ORIGINAL ARTICLE

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Decreased growth differentiation factor 9, bone morphogenetic protein 15, and forkhead box O3a expressions in the ovary via ulipristal acetate

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SUMMARY

OBJECTIVE: Folliculogenesis is a complex process involving various ovarian paracrine factors. During folliculogenesis, vitamin D3 and progesterone are significant for the proper development of follicles. This study aimed to investigate the effects of vitamin D3 and selective progesterone receptor modulator ulipristal acetate on ovarian paracrine factors.

METHODS: In the study, 18 female Wistar-albino rats were randomly divided into three groups: control group (saline administration, n=6), vitamin D3 group (300 ng/day vitamin D3 oral administration, n=6), and UPA group (3 mg/kg/day ulipristal acetate oral administration, n=6). Ovarian tissue was analyzed by histochemistry and immunohistochemistry. For quantification of immunohistochemistry, the mean intensities of growth differentiation factor 9, bone morphogenetic protein 15, and forkhead box O3a expressions were measured by Image J and MATLAB. Blood samples were collected for the analysis of serum anti-Müllerian hormone levels by ELISA.

RESULTS: Atretic follicles and hemorrhagic cystic structures were observed in the UPA group. After immunohistochemistry via folliculogenesis assessment markers, growth differentiation factor 9, bone morphogenetic protein 15, and cytoplasmic forkhead box O3a expressions decreased in the UPA group ($p<0.05$). Anti-Müllerian hormone level did not differ significantly between the experimental groups ($p>0.05$).

CONCLUSION: Ulipristal acetate negatively affects folliculogenesis via ovarian paracrine factors. The recommended dietary vitamin D3 supplementation in healthy cases did not cause a significant change.

KEYWORDS: Ovarian follicles. Immunohistochemistry. Progesterone. Vitamin D.

INTRODUCTION

Folliculogenesis occurs in the ovaries which is a crucial organ of the female reproductive system. This mechanism is a complex process involving various growth factors and signaling molecules. Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are the factors involved in the development of the preantral follicle from the primary follicle[1]. These two factors not only improve the developmental competence of the oocyte but also act directly on the granulosa cells. GDF9 and BMP15 have critical effects on granulosa cell proliferation, differentiation, apoptosis, and cumulus expansion[2]. Forkhead box O3a (FOXO3a) is involved in various processes such as cell proliferation, apoptosis, differentiation, and metabolism. Activation of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) signaling pathway inhibits these transcription factors. Akt hyperphosphorylation results in the nuclear export of FOXO3a and primordial follicle activation[3]. Active FOXO3a causes infertility with insufficient oocyte and follicular development leading to anovulation. It has also been shown that inhibition of Akt leads to FOXO3a-dependent apoptosis[4]. Another factor, anti-Müllerian hormone (AMH), is an inhibitory regulator of primary and preantral follicle development[1].

Progesterone is one of the hormones involved in the control of ovulation. It is synthesized from the corpus luteum and follicle. The progesterone receptor (PR) is a nuclear receptor and is expressed in the granulosa cells of the Graffian follicle. Another progesterone-binding protein, PGR membrane component 1 (PGRMC1), is synthesized in the granulosa cells of developing follicles. PGRMC1 controls the antiapoptotic and antimitotic effects of progesterone on granulosa cells[5]. Progesterone regulates follicle

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growth through PI3K/Akt and mitogen-activated protein kinase (MAPK) signaling pathways[6]. According to previous research, progesterone at periovulation concentration was shown to stimulate primary follicle development[5]. UPA is a 19-norprogesterone derivative and a selective PR modulator. Selective PR modulators act as antagonists when the progesterone level increases, preventing the LH peak and ovulation. UPA inhibits ovulation possibly by suppressing the expression of PR-dependent genes critical for the process. Antiproliferative and apoptotic effects are the researched effects of UPA[7]. However, there are limited studies on the effect of folliculogenesis. According to a study conducted in 2000, the single dose administration of UPA in the mid-follicular phase suppressed lead follicle growth. They presented that high dose causes luteinized unruptured follicles[8]. In the literature, UPA effects on folliculogenesis markers are not elucidated. Effects via these factors are significant to understand the underlying mechanism.

Vitamin D3 (VitD3) is converted from 7-dehydrocholesterol in the skin under UV light. VitD3 regulates cellular functions via the VitD3 receptor (VitD3R). VitD3R binds to the vitamin response element region in DNA. These binding sites are involved in the regulation of many genes[9]. VitD3R is expressed in many organs, including the ovary. Previous studies have reported that VitD3 plays a significant role in the functions of the ovaries, including follicular development. VitD3 supplementation increases preantral follicle survival, antral follicle growth, and survival[10]. In VitD3-deficient rats, follicular development is stalled[11]. From the analyzed data, it is determined that VitD3 has a fundamental role in ovarian functions. However, VitD3 and folliculogenesis-associated mechanism is still not elucidated. It requires comprehensive research regarding the mechanism of action on ovarian paracrine signals[12]. In the literature, it was reported that 600,000 IU VitD3 single-dose administration led to an increment of BMP15 and GDF9 levels in follicular fluid[13]. The relationship between VitD3 and AMH according to a recent meta-analysis is controversial[14].

Ovarian paracrine factors originating from different compartments in the ovary play roles in follicular developmental stages. Considering progesterone and VitD3 significance for follicular development, we investigated the effects of recommended dietary VitD3 supplementation and emergency contraceptive UPA on ovarian factors. To contribute to the literature, the variations of GDF9, BMP15, FOXO3a, and AMH by UPA and VitD3 are analyzed. The observed changes and inferences of each group are given from a statistical perspective.

METHODS

Animals

We used 18 adult female Wistar albino rats (180–240 g, 6–8 weeks). The rats were housed in cages under a 12 h light/12 h dark cycle at a temperature of 22±2°C with free access to food and water. The experimental procedures were approved by the Ethics Committee of Gazi University (G.Ü.ET-20.055), and the study was conducted at Gazi University Experimental Research Center.

Experimental design

The rats were randomly divided into three groups:

Control group (n = 6): 0.05 mL/rat/day saline (peroral) per day,

VitD3 group (n = 6): 3 weeks oral VitD3 (300 ng/day) administration[15],

UPA group (n = 6): 5 weeks oral UPA (3 mg/kg/day) administration[16].

Saline, VitD3, and UPA gavage administrations for each group continued once a day and at a certain time (10.00–11.00 a.m.) throughout the week continuously. UPA and VitD3 doses were prepared to be nontoxic in accordance with the literature. As VitD3 is given to healthy rats, the application time is shorter to avoid toxic effects in rats[15,16]. VitD3 300 ng/day for a 200 g body-weighted rat is equivalent to 15.81 µg/day for a 60 kg human[17]. The recommended dietary allowance of VitD3 for adults through to 70 years is 600 IU daily (15 µg)[18]. UPA 3 mg/kg/day for a 200 g body-weighted rat is equivalent to 31.62 mg/day UPA for a 60 kg human[17]. UPA's daily dose in adults as an emergency contraceptive is 30 mg[19]. After the experiments, rats were anesthetized. Rats were euthanized by intracardiac blood collection.

Histological examination

Ovarian tissue samples were fixed in neutral buffered 10% formalin for 48 h. Samples were dehydrated through an ascending alcohol series, cleared with xylene, and embedded in paraffin. Paraffin-embedded ovarian samples were then sliced into 5 µm sections, rehydrated, and stained with hematoxylin and eosin. Ovarian sections were examined under a bright-field microscope. Images were acquired using Leica DCM 4000 (Germany).

Immunohistochemistry

For immunohistochemical analyses, the sections were deparaffinized, rehydrated, and incubated with pH 6.0 citrate buffer. Endogenous peroxidase activity was denatured with hydrogen peroxide. After Ultra V block (Thermo Scientific), the sections

were incubated with primary antibody at a dilution of 1:200 in BMP15 (E-AB-62302, Elab, USA), 1:100 in GDF9 (bs4720R, Bioss, USA), and 1:100 in FOXO3A (bs-1548R, Bioss, USA) for overnight. The sections were then washed and incubated with the biotinylated secondary antibodies (Thermo Scientific). Immunoreactive signals were detected using streptavidin-HRP (Thermo Scientific) and diaminobenzidine (Thermo Scientific). Sections were also counterstained with hematoxylin. Images were acquired using Leica DCM 4000 (Germany). For quantification of IHC staining, the mean immunoreactivity intensity for an ovarian section was measured. After color deconvolution via ImageJ software (ImageJ), images are inverted. The mean density was measured with the MATLAB software (MATLAB). Six ovaries from each group and five areas from each ovary were evaluated[20].

ELISA

Rats were euthanized by intracardiac blood collection after experiments. Serum AMH levels were measured by ELISA in these blood samples. The blood samples were centrifuged at 4,500 rpm for 15 min. The obtained serum samples were stored at -20°C before analysis. Serum AMH levels were determined using the Rat AMH ELISA kit (E0456Ra, BTLAB, China) following the manufacturer's kit procedures.

Statistical analysis

Statistical analysis was performed using an SPSS program (IBM SPSS Statistics 20). The Kruskal-Wallis method with the Dunn-Bonferroni post-hoc test was used for statistical analysis of immunoreactivity density among nonparametric groups. ANOVA test was used for statistical analysis of serum AMH levels. A p-value less than 0.05 was considered statistically significant.

RESULTS

Histomorphological findings

In the histological examination of the control group, cortex and medulla structures were observed. Normal follicles were detected through all developmental stages in the cortex (Figures 1A, B). Follicles at various developmental stages in the VitD3 group were normal in the cortex (Figures 1C, D). In the UPA group, hemorrhagic cyst structures were observed (Figure 1E). Follicles in their normal developmental stage have become atretic (Figure 1F). The number of these atretic follicles relatively increased in the UPA group compared with the other groups.

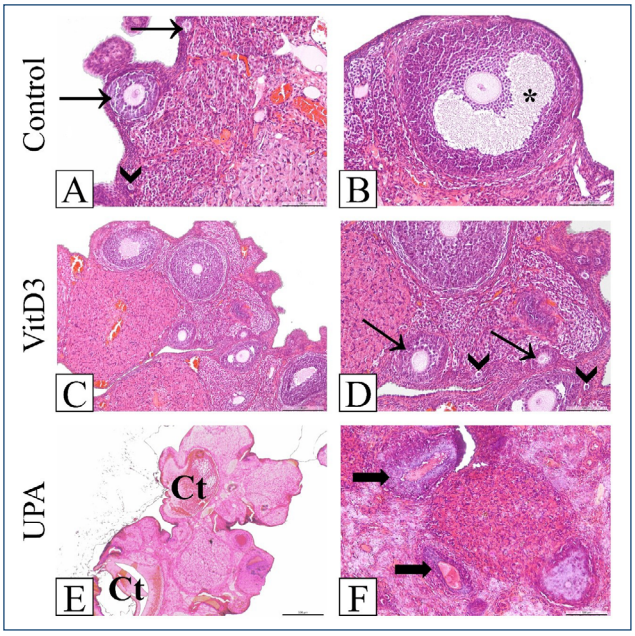

Figure 1. Hematoxylin & eosin stained sections belonging to the groups. Control group: **(A)** Primordial follicle (black arrowhead) and preantral follicles (black thin arrow). Scale bar: 100 µm. **(B)** Antral follicle (*). Scale bar: 100 µm. Vitamin D3 group: **(C)** Normal follicles at various developmental stages in the cortex. Scale bar: 200 µm. **(D)** Primordial follicle (black arrowhead) and preantral follicle (black thin arrow). Scale bar: 100 µm. UPA group: **(E)** Hemorrhagic cyst (Ct) structures. Scale bar: 500 µm. (F) Atretic follicles (black thick arrow). Scale bar: 100 µm.

Immunohistochemical results

Immunohistochemical studies were performed to evaluate the expression of BMP15, GDF9, and FOXO3a in the ovary. BMP15 expression was predominantly localized in the oocyte cytoplasm and granulosa cells of follicles. BMP15 expression was also detected in the corpus luteum. It was noticed that BMP15 expression decreased in the corpus luteums of the UPA group (Figure 2A). The mean pixel density of BMP15 throughout the ovary decreased in the UPA group compared with the other groups ($p<0.05$) (Figure 2D). The GDF9 expression was mainly detected in the oocyte cytoplasm. The GDF9 immunoreactivity was also detected in the corpus luteum structures. The GDF9 immunoreactivity decreased in the corpus luteums and preantral and antral follicles of the UPA group (Figure 2B). The mean pixel density of GDF9 decreased throughout the ovary in the UPA group compared with the other groups ($p<0.05$) (Figure 2D). FOXO3a expression was predominantly detected in the oocyte cytoplasm of the preantral follicles. It was observed that its expression decreased in antral follicles. UPA group shows weak FOXO3a immunoreaction in the antral and preantral follicles (Figure 2C). The mean pixel density of FOXO3a decreased throughout the ovary in the UPA group compared with the VitD3 group ($p<0.05$) (Figure 2D).

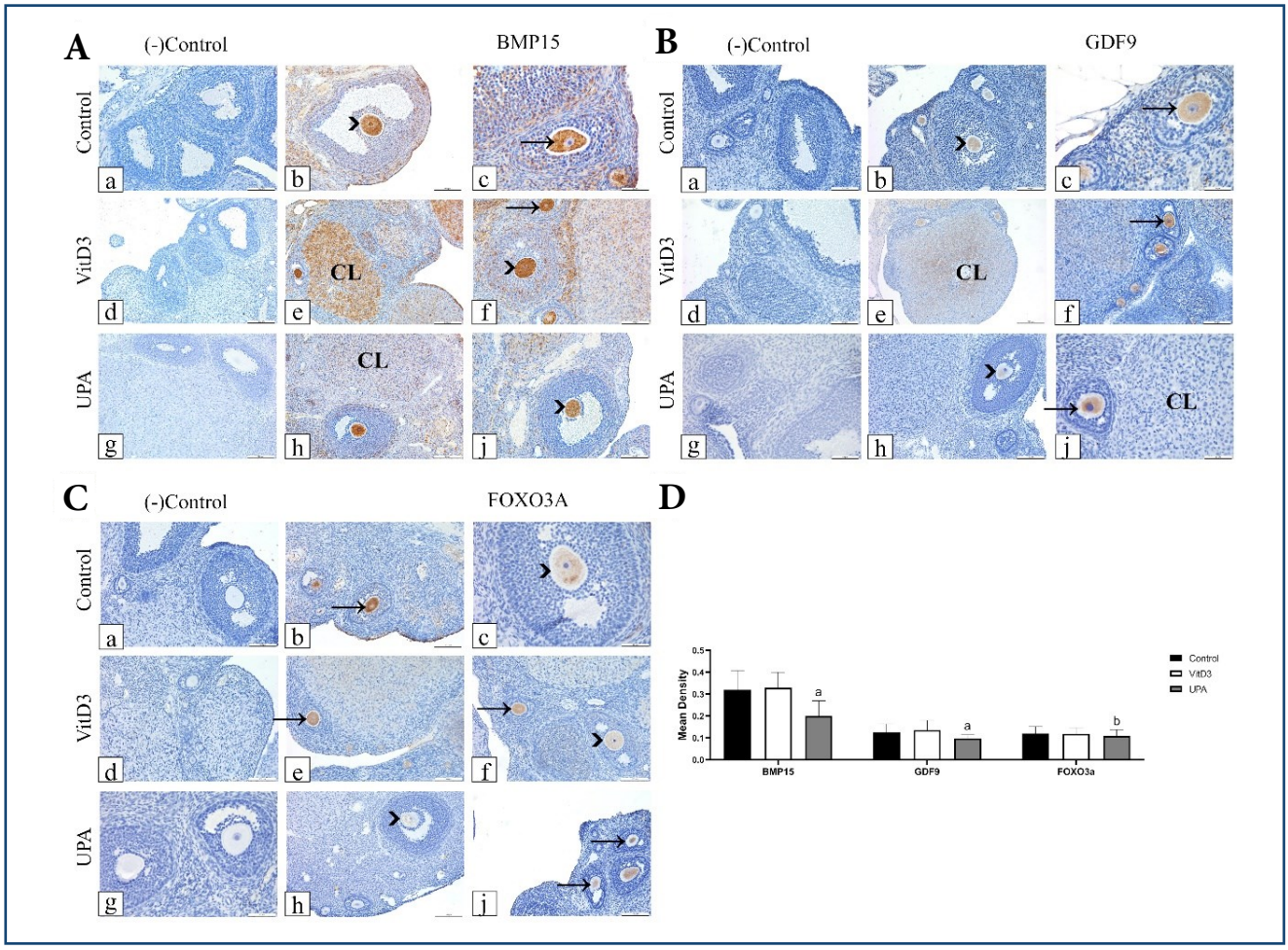

Figure 2. Bone morphogenetic protein 15, growth differentiation factor 9, and forkhead box O3a immunohistochemical staining of the groups and mean pixel density assessment. The first panels of immunohistochemical stains present negative controls **(a,d,g)**. Immunoreactivity is represented in the ooplasm of preantral follicle with a black arrow and antral follicle with a black arrowhead. **(A)** Bone morphogenetic protein 15 immunohistochemical staining; **(b)** bone morphogenetic protein 15 expression in the oocyte cytoplasm (black arrowhead). **(c)** Predominant bone morphogenetic protein 15 expression is localized in the oocyte cytoplasm (black arrow) and granulosa cells. **(e)** Vitamin D3 group shows strong immunoreaction in the corpus luteum. **(f)** Bone morphogenetic protein 15 expression in the oocyte cytoplasm (black arrowhead, black arrow). (h) UPA group shows weak immunoreaction in the corpus luteum. **(j)** Bone morphogenetic protein 15 expression in the oocyte cytoplasm (black arrowhead). Scale bars: Panel **(c)**—50 μm, panels **(d,e)**—200 μm, and others—100 μm. (B) Growth differentiation factor 9 immunohistochemical stain. **(b)** Growth differentiation factor 9 expression in the oocyte cytoplasm (black arrowhead). **(c)** Predominant growth differentiation factor 9 expression is localized in the oocyte cytoplasm (black arrow). **(e)** Growth differentiation factor 9 immunoreaction in the corpus luteum of the vitamin D3 group. **(f)** Growth differentiation factor 9 expression in the oocyte cytoplasm (black arrow). (h) UPA group shows weak immunoreaction in the antral follicle (black arrowhead). **(j)** UPA group shows weak immunoreaction in the preantral follicle (black arrow). Expression of growth differentiation factor 9 in corpus luteums decreased in this group. Scale bars: Control **(c,g,j)**—50 μm, **(e)**—200 μm, and others—100 μm. **(C)** Forkhead box O3a immunohistochemical stain. **(b)** Control group shows strong immunoreaction in the preantral follicle (black arrow). **(c)** Control group shows weaker immunoreaction in the antral follicle (black arrowhead) compared with preantral follicles. **(e,f)** Forkhead box O3a expression in the oocyte cytoplasm (black arrowhead, black arrow). (h) UPA group shows weak immunoreaction in the antral follicle (black arrowhead). **(j)** UPA group shows weak immunoreaction in the preantral follicles (black arrow). Scale bars: Panels **(c,f,g)**—50 μm and others—100 μm. **(D)** Immunoreactivity assessment via mean pixel density of the groups throughout the ovary; Kruskal-Wallis test and post-hoc Dunn-Bonferroni. Bone morphogenetic protein 15, growth differentiation factor 9, and forkhead box O3a mean pixel density decreased in the UPA group. **(a)** significant difference compared with the control and vitamin D3 groups. **(b)** Significant difference compared with the vitamin D3 group. Statistical significance: $p<0.05$.

Serum anti-Müllerian hormone levels

After the evaluation of serum AMH levels with ELISA, there was no statistically significant difference between the groups ($p>0.05$) (Table 1).

DISCUSSION

Studies on follicular development will provide comprehensive data on female reproductive life and facilitate the development of new therapeutic approaches against reproductive

Table 1. Comparison of serum anti-Müllerian hormone levels (ng/mL).

AMH (ng/mL)					
Groups	**Mean**		**Std. deviation**		**Std. error**
Control	6.245		4.14		1.85129
VitD3	4.963		2.072		0.92645
UPA	5.574		2.762		1.23535
Source of variation	**Sum of squares**	**Degree of freedom**	**Mean square**	**F**	**p-value**
Between groups	4.111	2	2.055	0.212	0.812
Within groups	116.233	12	9.686		
Total	120.344	14			

One-way ANOVA test. Statistical significance: $p<0.05$.

aging[1]. In the scope of this study, the effects of UPA and VitD3 on ovarian paracrine factors were examined immunohistochemically and biochemically. Progesterone affects granulosa cells via the PGRMC1 receptor, and its concentration correlates with follicle development. Komatsu et al. showed that when the variation of the progesterone concentration level was imitated, periovulation concentration stimulated primary follicle development[5]. According to a study conducted in 2000, Stratton et al. found that the single dose administration of antiprogestin UPA in the mid-follicular phase suppressed follicular growth[8]. In our study, we observed atretic follicles in the UPA group. By considering related studies, it was deduced that the specified situation presumably is related to the inhibition of required progesterone concentration by UPA for proper follicle development. In 2009, Tamura et al. demonstrated that mifepristone, a PR antagonist, caused luteal cysts and large corpus luteum structures at doses of 20 mg/kg or more in rats[21]. Large hemorrhagic cyst structures were also detected in our UPA group.

BMP15 is involved in normal follicular development as well as being mitogenic for somatic cells. Another crucial factor for preantral follicle development is GDF9[22]. In 2018, Xu et al. showed that VitD3 causes increased expression of BMP and GDF9 in preantral follicles[10]. There is no information in the literature about UPA effects on folliculogenesis markers. In our study, GDF9 and BMP15 expressions did not show a significant difference with recommended dietary VitD3 dose (approximately 15 µg/day for an adult human) in healthy cases[18]. UPA administration with an emergency contraceptive dose (approximately 30 mg) suppressed these expressions[19].

The phosphorylated FOXO3a by PI3K-Akt is an inactive form and localized in the cytoplasm. Its active non-phosphorylated form is localized in the nucleus and induces apoptosis[23]. In this study, decreased cytoplasmic FOXO3a expression in the UPA group represents inactive form and it is parallel with atretic follicle findings. We conclude that UPA causes atresia, possibly by inducing FOXO3a-dependent apoptosis. In the literature, it was concluded that activated FOXO3a reduces the expression of BMP15[24]. In our study, decreased expressions of BMP15 and inactive FOXO3a in the UPA group were consistent with the association of these factors.

The relationship between vitamin D and AMH in the literature is controversial[14]. In our study, recommended dietary VitD3 supplementation did not change serum AMH levels. In 2017, Dennis et al. indicated that the amount of AMH produced per follicle is only a minor determinant of circulating AMH level[25]. In this study, the absence of a significant change in serum AMH values between the groups was attributed to this deduction.

CONCLUSION

The effects of UPA and VitD3 on ovarian paracrine factors were studied in this work by considering studies addressing the corresponding topic. During histological analyses, UPA decreased inactive form of FOXO3a expression and caused follicular atresia. The recommended dietary VitD3 supplementation in healthy cases did not cause significant changes in GDF9 and BMP15 expressions. However, UPA suppressed these expressions. It was concluded that UPA negatively affects folliculogenesis via ovarian paracrine factors.

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AUTHORS' CONTRIBUTIONS

DGF: Data curation, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. **GTK:** Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **GNA:** Data curation, Methodology, Project administration. **NBAA:** Data curation, Project administration.

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ORIGINAL ARTICLE

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A needful, unique, and in-place evaluation of the injuries in earthquake victims with computed tomography, in catastrophic disasters! The 2023 Turkey-Syria earthquakes: part I

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SUMMARY

OBJECTIVE: This study was carried out to evaluate the injuries in pediatric earthquake victims due to the 2023 Turkey-Syria earthquakes with computed tomography and determine the anatomotopographic distribution of injuries.

METHODS: The material of this retrospective study consisted of the computed tomography findings of 257 pediatric cases injured in the 2023 Turkey-Syria earthquakes, and those were divided into subgroups based on their age group, i.e., 0–4, 5–9, 10–14, and 15–18 years, and the type of injury, i.e., head, maxillofacial, thoracic, abdominal, pelvic, and spinal injuries.

RESULTS: Earthquake-related injuries had been detected in 102 (39.6%) patients. Of the 29 patients with multiple injuries, 17, 10, and 2 had injuries in two, three, and four topographic regions, respectively. The most common injury was a head injury, which was detected in 48 (18.7%) cases, followed by thoracic injury, spinal injury, pelvic fracture, abdominal injury, and maxillofacial fracture, which were detected in 40 (15.6%), 22 (8.5%), 19 (7.4%), 10 (3.9%), and 6 (2.3%) patients, respectively. The cranial bone fractures and intracranial injuries were significantly more frequent in the 0–4 years age group compared with other age groups (p=0.028 and p=0.024, respectively). The rib fractures with spinal and pleural injuries were significantly more common in the 15–18 years age group compared with others (p=0.016, p=0.004, and p=0.002, respectively).

CONCLUSION: The head injury was the most common earthquake-related injury in pediatric cases. Herein, it was more common in younger children compared with other age groups, whereas rib, spine, and pleural injuries were more common in older children.

KEYWORDS: Earthquake. Pediatrics. Tomography. Radiology. Surgery.

INTRODUCTION

With regret, on February 6, 2023, at 4:17 a.m., an earthquake with a magnitude of at least 7.7 occurred, deeply affecting the South and East of Turkey and the North and West of Syria. Straight after, 9 hours later the first earthquake, a second earthquake with a magnitude of 7.6 occurred in the same region, triggered by the first earthquake. According to the data released by the Turkish Ministry of Interior Affairs, the death toll in earthquakes rose to 50,096 and the number of injured to 107,204 on March 20, 2023. Children are the most vulnerable population during such a major disaster[1]. According to the data on previous earthquakes, approximately one-third of earthquake victims are children, and the earthquake-related mortality rate is higher in pediatric cases than in adults[2,3].

Multiple traumas such as bone fractures, soft tissue, and organ injuries caused by the collapse of buildings or falling objects constitute the most common reasons for hospital admissions after a major earthquake[4]. Pediatric cases are more likely to suffer serious and multiple injuries in earthquakes as they have less subcutaneous fat and larger head and solid abdominal organs compared with the body[5]. In pediatric trauma cases, X-ray, ultrasonography, and computed tomography (CT) are used in the first place to detect damage quickly. The next-generation CT scan with multiplane reconstruction is an extremely

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valuable diagnostic tool for detecting head, thoracic, spinal, abdominal, and pelvic injuries[6,7].

Patients injured in the earthquakes affecting 11 provinces in Turkey were immediately transferred to neighboring cities for treatment management, where they were evaluated according to trauma guidelines, utilizing CT scans in most. The Teleradiology System of the Ministry of Health is a system that allows accessing the radiological examination records 7 days 24 h on the web, reporting these examinations, performing teleconsultation between radiologists, and evaluating the medical images and reports in terms of quality (https://teleradyoloji.saglik.gov.tr). Under the coordination of the Ministry of Health, the CT examinations performed in hospitals that serve trauma patients after earthquakes began to be reported immediately by radiologists across Turkey through the Teleradiology System. In this way, it was aimed to alleviate the burden of physicians in the regions affected by such a devastating disaster.

In this study, we purposed to evaluate the CT scans of pediatric patients via Teleradiology System in order to assess the characteristics of earthquake-related injuries in these cases based on the relevant age groups.

METHODS

Ethical aspects

This study was conducted according to the declaration of Helsinki and approved by the Clinical Research and Ethics Committee linked to the Ministry of Health-Giresun University Education and Research Hospital, under the approval number 210227920/10.KAEK-44/2023.

Study design

The study population consisted of pediatric cases aged 0–18 years who had undergone CT scans due to injuries related to the 2023 Turkey-Syria earthquakes. Patients' demographic characteristics, including age and gender data, had been recorded. Ten patients with insufficient CT image quality due to motion artifacts and five who underwent surgery before CT examination were excluded from the study.

Data collection

The CT scans' axial and multiplanar reformatted sagittal and coronal images that had been preserved in the Ministry of Health Teleradiology System (https://teleradyoloji.saglik.gov.tr) were independently evaluated by two experienced radiologists, and inconsistencies between their evaluations were resolved by consensus. Accordingly, the patients were divided into six subgroups based on the type of injury, i.e., the head, maxillofacial, thoracic, abdominal, pelvic, and spinal injury groups. In the head and maxillofacial injuries, craniomaxillofacial soft tissue, bones, and brain had been reviewed, and anatomical distributions or types of injuries were recorded. Furthermore, in the thoracic injuries, the thoracic cage, pulmonary parenchyma, and pleura had been evaluated, whereas in the abdominal and pelvic ones, the abdomen was classified as retroperitoneal and intraperitoneal spaces and the pelvis had been interpreted for pelvic fractures. Finally, in the spinal injuries, the presence of vertebral corpus, transverse process, spinous process, and articular process fractures in each vertebral region, as well as the degree of narrowing of the spinal canal, had been interpreted.

Statistical analysis

All the patients' data were entered into a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet. Afterward, the statistical analyses of these data were performed comparatively according to the subgroups created based on the age groups, i.e., 0–4, 5–9, 10–14, and 15–18 years, using SPSS 13.0 (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL, USA). The Pearson χ^2 test was used to identify the differences between age groups in the respective anatomical regions. As such, the data were presented as n (%), and the probability (p) statistics of ≤0.05 were deemed to indicate statistical significance.

RESULTS

A total of 257 pediatric patients with earthquake-related injuries who had undergone CT examinations between February 6 and 11, 2023, had been included in this study. The mean age of the study group was 9.7±5.1 (0–17) years, and earthquake-related injuries were detected in 102 (39.6%) patients. Of the 29 patients with multiple injuries, 17, 10, and 2 had injuries in two, three, and four anatomical regions, respectively. The most common injury was a head injury, which was detected in 48 (18.7%) patients, followed by thoracic injury, spinal injury, pelvic fracture, abdominal injury, and maxillofacial fracture, which were detected in 40 (15.6%), 22 (8.5%), 19 (7.4%), 10 (3.9%), and 6 (2.3%) patients, respectively. In the anatomotopographic distribution of injuries between the age groups, head injuries were more common in the 0–4 years age group, while spine injuries were in the 15–18 years age group (p=0.028 and p=0.004, respectively) (Table 1 and Figure 1).

Head and maxillofacial injuries

Cranial bone fractures and intracranial injuries were significantly more common in the 0–4 years age group compared with other

Table 1. Distribution of earthquake-related injuries according to age groups.

	0–4 years (n=57)	5–9 years (n=57)	10–14 years (n=83)	15–18 years (n=60)	Total (n=257)	p
Male	30 (52.6)	28 (49.1)	48 (57.8)	28 (46.7)	134 (52.1)	0.570
Female	27 (47.4)	29 (50.9)	35 (42.2)	32 (53.3)	123 (47.9)	
Head injury	17 (29.8)	13 (15.6)	13 (15.6)	5 (8.3)	48 (18.7)	**0.028**
Scalp hematoma	10 (17.5)	10 (17.5)	11 (13.2)	3 (5)	34 (13.2)	0.145
Bone fractures	8 (14)	5 (8.7)	3 (3.6)	1 (1.7)	17 (6.6)	**0.028**
Frontal	3 (5.3)	2 (3.5)	2 (2.4)	0 (0)	7 (2.7)	0.357
Parietal	3 (5.3)	2 (3.5)	2 (2.4)	0 (0)	7 (2.7)	0.357
Temporal	2 (3.5)	1 (1.8)	1 (1.2)	0 (0)	4 (1.6)	0.485
Occipital	1 (1.8)	1 (1.8)	1 (1.2)	0 (0)	3 (1.2)	0.789
Intracranial injury	9 (15.8)	4 (7)	3 (3.6)	2 (3.3)	18 (7)	**0.024**
SAH	1 (1.8)	1 (1.8)	0 (0)	0 (0)	2 (0.8)	0.470
EDH	1 (1.8)	1 (1.8)	1 (1.2)	0 (0)	3 (1.2)	0.789
SDH	5 (8.8)	2 (3.5)	2 (2.4)	1 (1.7)	10 (3.9)	0.177
Cerebral edema	2 (3.5)	1 (1.8)	0 (0)	1 (1.7)	4 (1.6)	0.432
Contusion	4 (7)	0 (0)	0 (0)	1 (1.6)	5 (1.9)	**0.015**
Maxillofacial injury	0 (0)	2 (3.5)	3 (3.6)	1 (1.7)	6 (2.3)	0.490
Spine injury	0 (0)	3 (5.2)	8 (9.6)	11 (15)	22 (8.5)	**0.004**
Cervical	0 (0)	0 (0)	1 (1.2)	1 (1.7)	2 (0.8)	0.636
Thoracal	0 (0)	0 (0)	1 (1.2)	3 (5)	4 (1.6)	0.089
Lumbar	0 (0)	1 (1.8)	2 (2.4)	7 (11.7)	10 (3.9)	**0.004**
Sacral	0 (0)	2 (3.5)	4 (4.8)	4 (6.7)	10 (3.9)	0.289
Thorax injury	7 (12.2)	8 (14)	9 (10.8)	16 (26.7)	40 (15.6)	0.055
Bone fractures	1 (1.8)	2 (3.5)	3 (3.6)	6 (10)	12 (4.7)	0.151
Rib	0 (0)	1 (1.8)	3 (3.6)	6 (10)	9 (3.5)	**0.016**
Clavicula	1 (1.8)	1 (1.8)	0 (0)	0 (0)	2 (0.8)	0.470
Scapula	0 (0)	0 (0)	0 (0)	1 (1.7)	1 (0.4)	0.348
Pulmonary parenchyma	7 (12.3)	5 (8.8)	8 (9.6)	7 (11.7)	27 (10.5)	0.913
Contusion	7 (12.3)	5 (8.8)	8 (9.6)	7 (11.7)	27 (10.5)	0.913
Laceration	0 (0)	1 (1.8)	0 (0)	2 (3.3)	3 (1.2)	0.234
Pleura	2 (3.5)	4 (7)	5 (6)	13 (21.7)	24 (9.3)	**0.002**
Pneumothorax	1 (1.8)	1 (1.8)	4 (4.8)	7 (11.7)	13 (5.1)	**0.045**
Hemothorax	0 (0)	2 (3.5)	1 (1.2)	3 (5)	6 (2.3)	0.257
Hemo-pneumothorax	1 (1.8)	1 (1.8)	0 (0)	3 (5)	5 (1.9)	0.203
Abdomen injury	2 (3.5)	3 (5.2)	4 (4.8)	1 (1.7)	10 (3.9)	0.730
Liver	1 (1.8)	0 (0)	1 (1.2)	1 (1.7)	3 (1.2)	0.807
Renal	0 (0)	2 (3.5)	0 (0)	0 (0)	2 (0.8)	0.070
Hemoperitoneum	1 (1.8)	3 (5.3)	1 (1.2)	1 (1.7)	6 (2.3)	0.422
Pneumoperitoneum	0 (0)	0 (0)	1 (1.2)	0 (0)	1 (0.4)	0.551
Pelvic injury	1 (1.8)	4 (7)	7 (8.4)	7 (11.7)	19 (7.4)	0.222

SAH: subarachnoid hemorrhage; EDH: epidural hemorrhage; SDH: subdural hemorrhage.

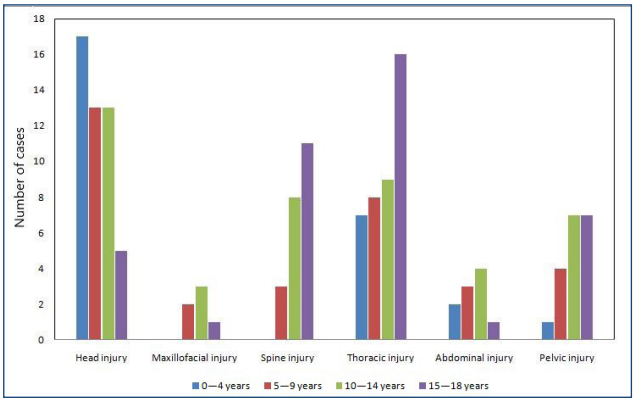

Figure 1. The cluster bar graphs exhibit the different anatomotopographic injuries recognized in the different pediatric earthquake victims' age groups.

age groups (p=0.028 and p=0.024, respectively). No statistically significant difference between age groups in maxillofacial injury (p=0.490) has been recognized. The intracranial injuries were present in one (16.6%) of the six cases with maxillofacial fractures, ten (71.4%) of the 14 with cranial bone fractures, and five (13.8%) of the 36 with scalp hematoma without fractures.

Thoracic injuries

The rib fractures were significantly more common in the 15–18 years age group compared with other age groups (p=0.016). No significant difference between age groups in terms of pulmonary parenchymal injuries (p=0.913) has been observed. Among pleural injuries, pneumothorax was significantly more common in the 15–18 years age group compared with other age groups (p=0.045).

Abdominal and pelvic injuries

Of the two cases with retroperitoneal injury, one had a renal subcapsular hematoma, and the other had a renal laceration. The liver laceration was present in three cases with intraperitoneal solid organ injuries, whereas no significant difference between age groups in terms of abdominal and pelvic injuries (p=0.730 and p=0.222, respectively) has been exhibited.

Spinal injuries

Among spinal injuries, the most and the least affected were the vertebras, the lumbar and sacral (45.4%), and the cervical (9%), respectively. Of the four (18.1%) cases, two had burst, one had a translation, and one had an unstable vertebral fracture in the form of distraction, all of which fractures were observed at the lumbar levels, accompanied by a narrow spinal canal. Other vertebral fractures were stable fractures such as transverse and spinous process fractures, corpus anterior column fractures, and non-displaced sacral fractures.

DISCUSSION

The most common earthquake-related injury in the study group was head injury (18.7%), followed by chest injury (15.6%), spinal injury (8.5%), pelvic fracture (7.4%), abdominal injury (3.9%), and maxillofacial fracture (2.3%). Head injuries were more common in the 0–4 years age group, whereas rib fractures and spine and pleural injuries were more common in the 15–18 years age group. Of note, the types and localization of injuries in pediatric cases differ from those of adults due to physiological and anatomical differences[5]. As such, CT imaging provides systematic and rapid diagnosis in pediatric earthquake victims, shortening the time to initiate lifesaving treatment[8].

Earthquake-related head injuries are often caused by building collapses and falling objects. Bai and Liu[9] reported that children are more vulnerable to head injuries. In studies conducted with pediatric patients on earthquake-related head injuries, Farfel et al.[10] reported that 3.2% of the 155 cases had head injuries, whereas Zhao et al.[11] reported that 12% of the 192 cases had head injuries. However, to the best of our knowledge, no study in the literature describes in detail the types of earthquake-related head injuries in pediatric cases. The most common type of earthquake-related injury observed in pediatric cases included in this study was head injury (18.7%). As the age of the patients decreased, the incidence of craniofacial fracture and intracranial injury increased. This finding might be attributed to the bigger head size compared with the body in early childhood. Moreover, most (71.4%) cases with cranial bone fractures had accompanying intracranial injuries, the most common being subdural hemorrhage. As such, this finding is in line with the relevant literature data, which indicates that the most common intracranial hemorrhage among blunt head traumas is subdural hemorrhage[12].

The incidence of earthquake-related thoracic injury in pediatric patients reported in the English-language literature varies between 2 and 9.4%[5]. In this study, the thoracic injury was the most common injury after head trauma, 15.6%. As the thorax ossifies with children's age, the incidence of rib fractures increases[6]. Similarly, in this study, rib fracture incidence had been significantly augmented with age. Pulmonary contusion was the most common CT finding among earthquake-related thoracic injuries in this cohort. On the contrary, the incidence of pulmonary laceration was low, which might be attributed to persistent heavy compression being more likely to result in pulmonary contusion than a pulmonary laceration. Some authors reported the incidence of pneumothorax in earthquake-related thoracic injuries as 3.2 and 51.9%, respectively[13,14]. In this study, the rate of pneumothorax among thoracic injuries was 32.5% and this rate was significantly higher in advanced pediatric age groups.

Given this sudden surge of patients in the emergency services after the earthquake, difficulties have arisen in delivering diagnostic and treatment services. An earthquake-related abdominal injury has been associated with high mortality[15-18]. In the retroperitoneal space, kidney or perirenal injury might occur more frequently than injuries of other retroperitoneal organs in the event of an earthquake. Liver injuries in the peritoneal cavity were reportedly more frequent than injuries in other intraperitoneal organs[19]. In our study, the abdomen was the least injured anatomical region due to earthquakes after maxillofacial injuries (3.9%). In line with the incidence of earthquake-related abdominal injuries in adult cases reported in the literature, the most common intraperitoneal and retroperitoneal organ injuries in this study were observed in the liver and kidney, respectively.

Spinal fractures were detected in 8.5% of pediatric cases, mainly at the lumbosacral level. Spinal injuries were the most frequent (50%) in the 15–18 years age group. Conversely, no spinal injury in the 0–4 years age groups had been recognized. As reported in the literature, most victims fall to the ground when an earthquake occurs and are trapped in the prone fetal position[20]. The high incidence of lumbosacral fractures can be explained by the higher probability of objects falling with high force and hitting the lower back. In addition, spinal cord injuries may emerge in 4.9–9.0% of patients in the early post-fracture period[21]. Unstable fractures, in particular, are the types of fractures that should be paid attention to in terms of spinal cord injury. As a matter of fact, unstable fractures constituted 18.1% of the vertebral fractures in this study.

Limitations

First, given that this study was an imaging study based on teleradiology data, the trauma scores of the cases assessed at admission were not available. Second, considering that earthquake-related extremity injuries are evaluated mainly by direct radiography, extremity injuries had been not assessed in this CT study. Finally, the patient's morbidity and mortality data had not been available.

CONCLUSION

The types of earthquake-related injuries in pediatric patients differed between age groups due to anatomical differences and different mechanisms of injury. Head injuries were more frequent in younger children, while rib fractures with spinal and pleural injuries were more common in older children. The characteristics of pediatric earthquake-related injuries detected by CT imaging in this study may help to accurately diagnose and treat earthquake-related trauma cases in the future. With its ability to provide detailed and accurate images of the anatomic internal structures, CT helps physicians to identify injuries of pediatric earthquake victims that may not be visible on their physical examinations or X-rays.

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AUTHORS' CONTRIBUTIONS

IMC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **IS:** Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **TB:** Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **GT:** Investigation, Validation, Visualization. **UE:** Visualization, Writing – original draft. **ROO:** Investigation, Validation, Visualization. **IA:** Formal analysis, Writing – original draft. **AEB:** Resources, Software, Validation, Visualization. **MT:** Validation, Visualization. **DS:** Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – review & editing. **SA:** Formal analysis, Investigation.

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Anatomical features of sella turcica with comprehensive literature review

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SUMMARY

OBJECTIVE: This study aimed to explore the relationship between skeletal patterns and the frequency of sella turcica bridging in a sample of young Turkish adults in order to provide a better understanding of the relationship between craniofacial morphology and sella turcica abnormalities.

METHODS: A total of 90 individuals aged between 18 and 25 years were examined in this study. The individuals were classified according to their skeletal pattern, specifically Class I, Class II, and Class III. Each group consisted of 15 males and 15 females. The length, depth, and anteroposterior diameter of sella turcica were calculated. The shape and bridging of sella turcica were estimated using lateral cephalometric images. All data were correlated and statistically analyzed according to skeletal patterns, genders, and age.

RESULTS: The mean length, depth, and anteroposterior diameter of sella turcica were 7.02±2.13, 7.56±1.38, and 10.54±1.3 mm in Classes I–III, respectively. There was no significant difference between the dimensions of sella turcica according to gender and age ($p>0.05$). The length of sella turcica was larger in Class III, and the depth of sella turcica was larger in Class II individuals ($p<0.05$). A total of 44.4% of the individuals had normal sella turcica, while the remaining 56.6% had other types of sella turcica. It was determined that 31.1% of the individuals have no calcification, 62.2% had partial calcification, and 6.7% had total calcification.

CONCLUSION: The normal dimensions, shape, and bridging of the sella turcica can be used by the orthodontist for diagnosis, treatment planning, and evaluation of various pathological conditions associated with the sella turcica.

KEYWORDS: Abnormalities, craniofacial. Morphology. Cranium. Sella turcica.

INTRODUCTION

The sella turcica is crucial in the radiological assessment of the craniofacial and neurocranial regions. It is situated on the upper surface of the sphenoid bone's body[1-3]. The three parts of this structure, namely, the dorsum sella, tuberculum sella, and fossa hypophysialis, were given the name "sella turcica" because they approximate the shape of a Turkish saddle. The pit in which the pituitary gland sits is called the hypophysial or pituitary fossa, which is found in the middle cranial fossa. The anterior wall of the pituitary fossa is formed by the tuberculum sella, while the posterior wall is formed by the dorsum sella. The processes on both sides of the tuberculum sella are called the middle clinoid process, the superior-lateral corners of the dorsum sella are called the posterior clinoid process, and the posterior processes of the lesser wings (of sphenoid bones) are the anterior clinoid process[1]. As a result, the pituitary gland and the sella turcica are found in a close relationship. Moreover, the development of the pituitary gland is faster than the sella turcica. For this reason, the development of the pituitary gland is completed before the sella turcica is entirely finished. Any pathology that occurs in the pituitary gland during this development will also affect the morphology of the sella turcica[4,5]. Therefore, knowing the morphology of the sella turcica is essential not only for the evaluation of craniofacial morphology but also for the diagnosis of pituitary gland anomalies and various syndromes[6].

The sella turcica has clinical relevance in various medical fields, including endocrinology and neurology. In recent years, the evaluation of sella turcica has gained significant attention due to its diagnostic and management implications in a variety of conditions. One of the areas where sella turcica morphology can help is in the diagnosis and management of genetic syndromes such as acromegaly and empty sella syndrome. A recent systematic study has discussed the sella turcica's morphology in patients with genetic syndromes[7]. The study reviewed 15 articles and found that patients with genetic syndromes such as Down syndrome, Marfan syndrome, and Turner syndrome had

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variations in sella turcica morphology compared with individuals without genetic syndromes. These variations included increased or decreased size, altered shape, and increased bridging. The authors suggest that knowledge of these variations could aid in the diagnosis and treatment of genetic syndromes, as well as aid in understanding the underlying genetic mechanisms that influence craniofacial development. Another reported study[8] has analyzed the empty sella syndrome, which is a condition characterized by a partially or completely empty sella turcica. Sella turcica's volumetric assessment can also aid in the diagnosis and management of pituitary tumors, which may cause hormonal imbalances[9]. Furthermore, the evaluation of sella turcica can help in transnasal endoscopic approaches, which are becoming increasingly popular in pituitary surgery[10]. In addition, the morphology of the sella turcica can be evaluated as a part of craniofacial growth monitoring, particularly in children with unilateral cleft lip and palate (UCLP), to assess sella turcica bridging and tooth agenesis. The relationship between sella turcica bridging and tooth agenesis in children with UCLP has been reported in the literatüre[11]. One hundred and sixteen UCLP patients' dental and radiographic records were analyzed, and a significant correlation was found between sella turcica bridging and tooth agenesis. The findings suggested that sella turcica bridging can be used as a predictor of tooth agenesis in children with UCLP, which can aid in early identification and treatment planning[11]. The evaluation of sella turcica can be performed by X-ray or computed tomography (CT). Both of these are commonly used diagnostic imaging techniques that use ionizing radiation to create images of the body's internal structures. While CT has advantages such as detecting abnormalities and producing detailed 3D images, it also has drawbacks such as higher radiation exposure, longer procedure time, and higher cost compared with X-ray. X-ray, on the contrary, is quick, easy, and cost-effective and is routinely used in today's orthodontic diagnosis and treatment planning.

In this study, we aimed to assess the morphology of sella turcica using lateral cephalometric X-ray images. The study focused on evaluating sella turcica morphology in orthodontic patients but acknowledges the broader clinical implications of this assessment in other medical fields. Hence, we hope to contribute to the growing body of literature on the clinical relevance of sella turcica evaluation and its potential applications in various medical fields.

METHODS

This retrospective study was conducted after obtaining ethical approval from the Inonu Health Sciences Clinical Research Ethics Committee (date: 05.01.2021; number: 1339). The lateral cephalometric X-ray images recorded as skeletal Class I, Class II, and Class III at Inonu University Faculty of Dentistry, Department of Orthodontics between 01.01.2017 and 01.12.2020 were included. Young adults aged between 18 and 25 years with no history of orthognathic surgery and no head and neck trauma were included in the study. First, the anteroposterior angle between the maxilla and mandible was detected which is an angle known as ANB angle. This angle refers to a cephalometric measurement used in orthodontic diagnosis to determine the relationship between the maxilla and the mandible. It represents the angle formed by the intersection of two lines: the AN line, which connects the A point (the most anterior point of the maxilla) and the Nasion (the point at the junction of the frontal and nasal bones), and the NB line, which connects the Nasion and the B point (the most anterior point of the mandible). This measurement is commonly used to assess skeletal discrepancies and plan orthodontic treatment. In this way, according to the ANB angle, the classification was determined as skeletal Class I, Class II, and Class III. For each group, the images of 90 Turkish individuals registered in the archive were selected randomly, taking into account equal gender distribution (45 male and 45 female). Afterward, the length, the depth, and the anteroposterior diameter of sella turcica were calculated. For the length, the distance between the apex of the tuberculum sella and dorsum sella was considered. The length of the perpendicular line drawn from the length of sella turcica to the deepest point of the floor of the sella turcica was taken into account for the calculation of the depth of sella turcica. Finally, the last morphometric parameter (anteroposterior diameter) was measured between the tuberculum sella and the furthest point on the posterior-inner wall of the pituitary fossa (Figure 1). After morphometric measurements, morphological evaluations of sella turcica were made as follows:

1. Determination of the shape of sella turcica: According to the classification of Axelsson et al.[9], it was classified as normal, double contour of the floor, oblique anterior wall, pyramidal shape of dorsum sella, bridging of sella turcica, and irregular shape of sella turcica.

2. Determination of bridging of sella turcica: According to the classification of Leonardi et al.,[10] the bridging of sella turcica was classified into three types based on the morphology and extent of the bony bridge. In Type I, the bridging was characterized by the absence of calcification of the bony bridge. In this type, the bony bridge was composed of fibrous tissue without any evidence of mineralization, also known as incomplete or uncalcified bridging. Type II was characterized by the partial calcification of the bony bridge where the

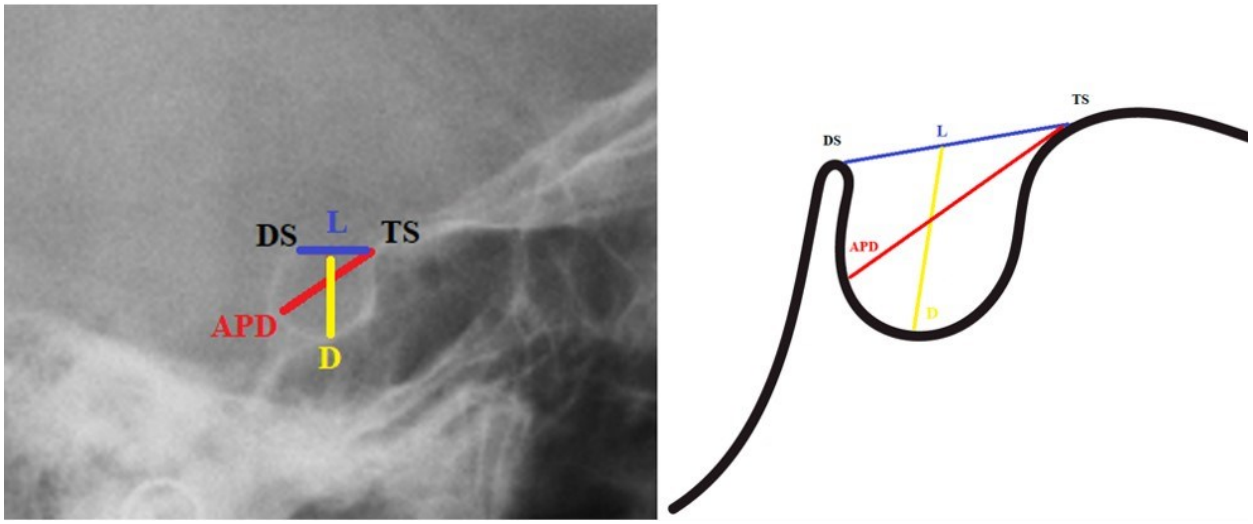

Figure 1. Demonstration of morphometric measurements of sella turcica. The length of sella turcica (blue line): the distance between the apex of the tuberculum sella and dorsum sella; the depth of sella turcica (yellow line): the length of the perpendicular line drawn from the length of sella turcica to the deepest point of the floor of the sella turcica; the anteroposterior diameter of sella turcica (red line): the distance between the tuberculum sella and the furthest point on the posterior-inner wall of the pituitary fossa. TS: tuberculum sella; DS, dorsum sella; L, length of sella turcica; APD, anteroposterior diameter of sella turcica; D, depth of sella turcica.

bony bridge contained both fibrous tissue and calcified tissue. This type is also known as partially calcified bridging. Finally, in Type III, the bridging was characterized by the complete calcification of the bony bridge. The bony bridge was fully composed of calcified tissue, which is known as completely calcified bridging.

The morphological evaluations and morphometric measurements were performed by the same specialist dentist and researcher A.E., who used the Planmeca Romexis 3.5.1.R program. The X-ray images were taken with a Planmeca branded X-ray device with serial number RPP11161, with the person's head fixed to the cephalostat, the Frankfort horizontal plane parallel to the ground, the central beam perpendicular to the patient's mid-oxal plane, and the teeth in the centric occlusion position.

Statistical analysis

The "independent-sample t-test" and "Mann-Whitney U test" were used to compare two independent groups, while the "one-way ANOVA test" and the "Kruskal-Wallis H test" were used to compare more than two independent groups. Examination of the relationships between the scales was determined by "Spearman's rank differences correlation coefficient." Values less than $p<0.05$ were considered statistically significant. The SPSS v26 (IBM Inc., Chicago, IL, USA) statistical package program was used for statistical analysis of the data.

RESULTS

Our study included 45 males and 45 females of skeletal Class I (30), Class II (30), and Class III (30) aged 20.46±2.27 years. The mean length, depth, and anteroposterior diameter of the sella turcica were 7.02±2.13, 7.56±1.38, and 10.54±1.31 mm, respectively. There was no significant difference between the morphometric measurements and the genders or age ($p>0.05$). However, the mean length, depth, and diameter of Class I were 6.63±2.16, 7.52±1.35, and 10.25±1.56 mm. The mean values of these morphometric results of Class II and Class III were found as 6.41±1.56, 7.91±1.24, 10.73±1.05 and 8.02±2.29, 7.25±1.52, 10.63±1.27 mm, respectively. The statistical results have shown a significant difference between the skeletal classification (Class I–Class II–Class III) and the length or depth measurements ($p<0.05$), while the results of the anteroposterior diameter between the skeletal classification have not shown a significant correlation ($p>0.05$) (Table 1).

The results of morphological evaluation of sella turcica according to the shape were observed as normal, oblique anterior wall, bridging of sella turcica, double contour of the floor, pyramidal, and irregular dorsum sella in 44.4% (18 in males; 22 in females), 6.7% (5 in males; 1 in females), 13.3% (5 in males; 7 in females), 15.6% (6 in males; 8 in females), 16.7% (8 in males; 7 in females), and 3.3% (3 in males; 0 in females), respectively (Figure 2).

Table 1. Comparison and measurement of sizes of sella turcica in different skeletal patterns.

Unit (mm)	Class	n	Median (min–max)	Mean±SD	p-value
Length	Class I	30	6.80 (1.80–10.70)	6.63±2.16	0.017*
	Class II	30	6.45 (2.90–10.50)	6.41±1.56	
	Class III	30	7.45 (3.40–11.90)	8.02±2.29	
Depth	Class I	30	7.80 (3.20–9.20)	7.52±1.35	0.042*
	Class II	30	8.25 (4.80–9.40)	7.91±1.24	
	Class III	30	7.35 (3.20–10.60)	7.25±1.52	
Diamater	Class I	30	10.35 (6.70–14.00)	10.25±1.56	0.322
	Class II	30	10.90 (8.70–12.70)	10.73±1.05	
	Class III	30	10.55 (7.40–13.10)	10.63±1.27	

SD: standard deviation. *The results were evaluated at 95% reliability, p<0.05 significance level.

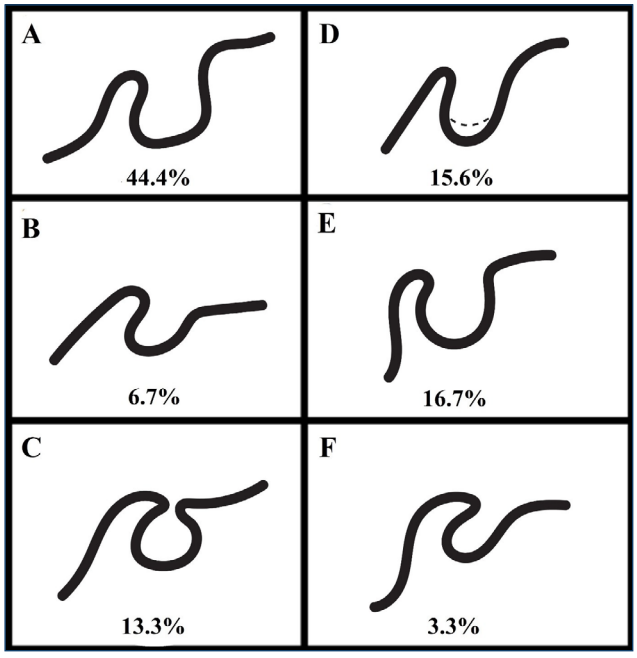

Figure 2. The frequency of types of sella turcica. (A) Normal type. (B) Oblique anterior wall. (C) Sella turcica bridging. (D) Double contour of the floor. (E) Pyramidal shape of the dorsum sella. (F) Irregularity on the posterior part of the sella turcica.

Finally, the frequency of bridging of sella turcica according to the Type I (no calcification), Type II (partial calcification), and Type III (total calcification) was recorded. In 31.1% (n=28) of 90 participants, the sella turcica had no calcification, while partial (n=56) and total (n=56) calcifications were recorded equally for the remaining 112 participants. The results showed that, among individuals with Class I skeletal patterns, 35.7% (n=10) had Type I bridging of the sella turcica, 32.1% (n=18) had Type II bridging, and 33.3% (n=2) had Type III bridging. For those with Class II skeletal patterns, 14.3% (n=4) had Type I bridging, 42.9% (n=24) had Type II bridging, and 33.33% (n=2) had Type III bridging. Finally, among those with Class III skeletal patterns, 50% (n=14) had Type I bridging, 25% (n=14) had Type II bridging, and 33.3% (n=2) had Type III bridging.

DISCUSSION

The sella turcica serves as a stable reference point for measuring the relative positions of other craniofacial structures. This information can then be used to diagnose various dental and skeletal abnormalities and plan orthodontic treatment. The S point, also known as the sella point, is a cephalometric landmark located at the deepest point on the sella turcica. It is used in orthodontics to evaluate the position of the maxilla relative to the cranial base. The distance between the "S point" and the anterior cranial base can be measured, and this measurement can provide valuable information in the diagnosis and treatment of various dentofacial anomalies[1,12-14].

The results of mean depth and anteroposterior diameter of our study were found similar to the findings reported by Muhammed et al.[15], while the length mean was reported as higher. The mean of the length, depth, and anteroposterior diameter of our study were observed similar to the findings reported by Shestra et al.[16], while the results reported by Ghaida et al.[17] and Yasir et al.[18] have shown a few millimeter differences. It is thought that this difference is due to ethnic diversity, environmental factors, sample size, and so on. It was determined that the length, depth, and anteroposterior diameter of sella turcica did not show a statistically significant difference between the genders. Similarly, Shrestha et al.[16] and Akolfide[19] reported that there was no significant correlation according to gender. However, in studies with a wider age range, it has been reported that the length of the sella turcica differs between the genders[6,20,21]. According to the correlation between age and

morphometric results of sella turcica, the findings had no significant correlation in our study, while the results of Acheson and Archer[22] showed significant differences between the genders. In a study by Axelsson et al.[21], it was concluded that the depth and diameter of the sella turcica raised with puberty, but the change in the length of the sella turcica was insignificantly small. In our study, it was concluded that there was no significant relationship between the size of sella turcica and age. It can be thought that this result is because the individuals included in our study were after puberty.

The relationship between skeletal patterns and dimensions of sella turcica is still unclear in the current literature. Alkofide[18] stated that the diameter of the sella turcica was larger in Class III individuals than in Class II individuals, while Shresta et al.[16] stated that both the diameter and length of the sella turcica were greater in Class III individuals than in Class II individuals. Similarly, Sathyanarayana et al.[6] reported that the diameter of sella turcica had a larger diameter in Class III individuals than in Class II individuals. On the contrary, there is also a study that skeletal patterns and dimension of sella turcica have no significant correlation[23,24].

While 44.4% of the individuals included in our study had normal type sella turcica, 56.4% had other types of sella turcica. Axelson et al.[21] reported that 68% of the individuals included in their study had normal type sella turcica. Similar frequency (61–67%) has been reported by Sathyanarayana et al.,[6] Alkofide[19], and Shah et al.,[24] while Magat and Sener[23] reported 39% frequency in the Turkish population. In our study, the normal sella turcica type was the most frequent type found in 44.4%, while the least frequent sella turcica type was the irregular sella turcica (3.3%).

Finally, according to the literature, Type I, Type II, and Type III classifications were found as 56.4, 33.7, and 23.5% by Leonardi et al.[25] and 65, 23.3, and 11.67% by Shrestha et al.[16], respectively. However, in our study, Type II was found as the highest with 62.2%.

Even so, some authors suggested that the frequency of sella turcica bridging increased in the rate of severe craniofacial anomaly or by receiving surgical treatment Becktor et al[26].

In addition, the pituitary gland is important because of its proximity to the hypothalamus and its relationship with the optic chiasm, sphenoid sinus, and cavernous sinus[1]. Recent developments in endoscopy have allowed trans-nasal endoscopic approaches to be performed in and around the sellar region, especially in the posterior clinoid processes. As advanced radiological examinations are required for the three-dimensional evaluation of anatomical structures in the preoperative period, determining the presence of any sella turcica variation on lateral cephalometric radiographs may be useful as preliminary information and this may be a guide in the preoperative period[27].

CONCLUSION

There was no statistically significant difference between the morphometric measurements of sella turcica according to age and gender. The length values of the individuals in Class III were statistically higher than the individuals in Class II, and the depth values of the individuals in Class II were statistically higher than the individuals in Class III. The most common type of sella turcica was normal sella turcica, and the most common type of bridging was partial calcification. It is important to reveal the morphology and variations of sella turcica in terms of raising awareness among orthodontists. Similarly, we suppose that it can contribute to the evaluation of surgical procedures for radiologists and surgical clinicians.

ETHICAL STATEMENT

This study was approved by the Inonu Medical Faculty Local Ethics Committee. (No. 2021/1339).

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AUTHORS' CONTRIBUTIONS

VÖ: Conceptualization, Validation, Visualization, Writing – original draft, Writing – review & editing. **AE**: Data curation, Formal Analysis, Investigation, Methodology. **GNC**: Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **AMT**; Data curation, Formal Analysis, Resources, Software.

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ORIGINAL ARTICLE

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Temporomandibular joint involvement in elderly onset and young onset rheumatoid arthritis patients

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SUMMARY

OBJECTIVE: There are studies showing clinical and laboratory differences between elderly-onset rheumatoid arthritis and young-onset rheumatoid arthritis. Temporomandibular joint involvement in rheumatoid arthritis is not rare. In this study, we aimed to examine the temporomandibular joint involvement and magnetic resonance imaging findings in elderly-onset rheumatoid arthritis and young-onset rheumatoid arthritis patients.

METHODS: A total of 87 rheumatoid arthritis patients were investigated retrospectively. The onset ≥60 years was considered elderly-onset rheumatoid arthritis. Erosion, flattening, and resorption of the condyle, narrowing of the joint space, joint effusion, synovial hypertrophy, and synovitis were interpreted as temporomandibular joint involvement with magnetic resonance imaging. Patients' age, gender, rheumatoid factor, and anti-cyclic citrullinated peptide positivity, extra-articular findings, medical treatment, and disease activity score were noted.

RESULTS: A total of 15 (17.2%) patients had elderly-onset rheumatoid arthritis. Temporomandibular joint involvement was detected in 67 (77%) patients; 9 (60%) of them were in the elderly-onset rheumatoid arthritis group (n=15) and 58 (80.6%) of them were in the young-onset rheumatoid arthritis group (n=72). Patients with temporomandibular joint involvement were significantly higher than those without temporomandibular joint involvement in both the elderly-onset rheumatoid arthritis and young-onset rheumatoid arthritis groups (p<0.001). No significant difference was found between elderly-onset rheumatoid arthritis and young-onset rheumatoid arthritis for the temporomandibular joint involvement (p=0.100). In the young-onset rheumatoid arthritis group, rheumatoid factor positivity and anti-cyclic citrullinated peptide positivity were more frequent in the patients with temporomandibular joint involvement (p=0.011, p=0.024). A comparison of the elderly-onset rheumatoid arthritis and young-onset rheumatoid arthritis patients showed no significant difference in the magnetic resonance imaging findings except for the resorption of the condyle.

CONCLUSION: According to our findings, elderly-onset rheumatoid arthritis is not much different from young-onset rheumatoid arthritis in terms of temporomandibular joint involvement, magnetic resonance imaging findings, and clinical and laboratory features.

KEYWORDS: Elderly. Rheumatoid arthritis. Temporomandibular joint.

INTRODUCTION

Although there are no clinical findings, rheumatoid arthritis (RA) is a disease in which subclinical inflammation continues[1]. The prevalence of RA is known to be 0.5–1%[2]. Although RA can be diagnosed in all age groups and in all ethnic populations, it has an increasing prevalence with increasing age, and this frequency rises to 2% in the geriatric population[3].

The terminology is unclear; however, patients with RA whose clinical symptoms begin after the age of 60 or 65 years are considered elderly-onset RA (EORA)[3,4]. As life expectancy increases in developed countries, the number of people ≥60 years old is increasing rapidly; this indicates that the number of EORA patients will increase in the future[5]. There are studies showing clinical and laboratory differences between EORA and young-onset RA (YORA)[6].

The temporomandibular (TMJ) joint is a synovial joint formed between the mandible and the temporal bones. Problems related to TMJ have been reported as the second most common complaint after low back pain[7]. Since RA is an inflammatory arthritis involving synovial joints, it is not surprising that it involves TMJ. The incidence of TMJ involvement in RA patients ranges from 5 to 86%[8]. In this study, we aimed to examine the TMJ involvement and magnetic resonance imaging (MRI) findings in EORA and YORA patients.

METHODS

A total of 99 patients who were followed up in the rheumatology clinic and diagnosed with RA according to the 2010 American College of Rheumatology-Rheumatoid Arthritis

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(ACR-RA) classification criteria[9] and who were examined due to the TMJ complaints in the dental clinic between January 2020 and July 2022 were retrospectively analyzed. The records of patients with consent were reviewed. Those under the age of 18 years, without consent, and those who did not have an MRI were excluded from the study. The onset age of ≥60 was accepted as EORA and those <60 were considered as YORA[4].

Patients' age (years) and gender were recorded. Rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) values were obtained. RF and anti-CCP tests were considered positive or negative based on laboratory reference values[9]. Those who had extra-articular findings accompanying RA were noted. Steroid and disease-modifying anti-rheumatic drugs (DMARDs: methotrexate, sulfasalazine, hydroxychloroquine, leflunomide, biologics) usage were investigated. Disease activity scores calculated with erythrocyte sedimentation rate (DAS 28) values were noted.

Erosion, flattening, and resorption of the condyle, narrowing of the joint space, joint effusion, synovial hypertrophy, and synovitis were interpreted as TMJ involvement with MRI[10].

It was decided whether the data were normally distributed or not by evaluating the skewness and kurtosis values and normality plots[11]. Mean and standard deviation values were given for normally distributed data, and median and minimum-maximum values were given for non-normally distributed data. Mann-Whitney U test was used to compare non-normally distributed data, while the t-test was used for normally distributed data. Chi-square and Fisher's exact tests were used to determine the difference between the two groups. Kruskal-Wallis test was used to determine the difference of more than two between the groups. Significance level was accepted as $p<0.05$. SPSS version 22 was used for statistical analysis (SPSS Inc., IBM Co., and Chicago, IL, USA).

The study protocol was approved by the Ondokuz Mayıs University Faculty of Medicine ethics committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

A total of 4 patients without consent and 12 patients who did not have MRIs were excluded. Overall, 87 patients were included in the study, 9 of whom were males (10.3%). The mean age of the patients was 48.56±13.98 years. The disease duration was 80.59±56.79 months. Notably, 15 (17.2%) patients had EORA; TMJ involvement was detected in 67 (77%) patients, 9 (60%) of them were in the EORA group (n=15) and 58 (80.6%) of them were in the YORA group (n=72) (Table 1).

Table 1. Comparison of elderly-onset and young-onset rheumatoid arthritis patients.

	EORA, n=15	YORA, n=72	p-value
Age (years)	67.4±3.31	44.64±12	<0.001*
Gender, male (%)	3 (20)	6 (8.33)	0.182
Disease duration (months)	40.2±23.91	89±58.12	0.002*
DAS 28	3.72±1.43	3.59±1.33	0.590
TMJ involvement (%)	9 (60)	58 (80.6)	0.100
RF positive (%)	3 (20)	32 (44.4)	0.079
Anti-CCP positive (%)	6 (40)	40 (55.6)	0.272
Rheumatoid nodule positive (%)	1 (6.7)	4 (5.6)	1.000
Methotrexate+glucocorticoid (%)	1 (6.7)	4 (5.6)	0.943
Methotrexate+sulfasalazine (%)	9 (60)	41 (56.9)	
Leflunomide (%)	1 (6.7)	3 (4.2)	
Biologics (%)	4 (26.7)	24 (33.3)	
Erosion (%)	9 (60)	52 (72.2)	0.365
Resorption of the condyle (%)	7 (46.7)	10 (13.9)	0.008*
Flattening of the condyle (%)	6 (40)	28 (38.9)	0.936
Narrowing of the joint space (%)	4 (26.7)	24 (33.3)	0.765
Joint effusion (%)	1 (6.7)	18 (25)	0.174
Osteophytes (%)	7 (46.7)	44 (61.1)	0.301
Disk perforation (%)	0	8 (11.1)	0.341

Significance level *p<0.05; n: number of subjects; EORA: elderly-onset rheumatoid arthritis; YORA: young-onset rheumatoid arthritis; DAS 28: disease activity score 28; TMJ: temporomandibular joint; RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide.

Patients with TMJ involvement were significantly higher than those without TMJ involvement in both the EORA and YORA groups (p<0.001 for each).

The age of EORA patients was significantly higher (p<0.001), and disease duration was significantly lower (p=0.002) than that of YORA patients (Table 1). Although the number of patients who had TMJ involvement was numerically higher in the YORA group, no statistically significant difference was found between EORA and YORA for TMJ involvement (p=0.100, Table 1).

In the EORA group with TMJ involvement, disease duration was significantly shorter (p=0.004). In the YORA group, patients with TMJ involvement were significantly older than patients without TMJ involvement (p=0.001). Also, in the YORA group, RF positivity and anti-CCP positivity were more frequent in patients with TMJ involvement (p=0.011 and p=0.024). Comparisons of the patients with and without TMJ involvement are shown in Table 2.

Comparison of the EORA and YORA patients showed no significant difference in the MRI findings except for the resorption of the condyle (Table 1). Comparisons of the patients with and without TMJ involvement according to MRI findings are shown in Table 3.

DISCUSSION

In our study, all the patients had TMJ complaints. According to the MRI findings, we detected TMJ involvement in 67 (77%) patients. Patients with TMJ involvement were significantly higher than those without TMJ involvement in both the EORA and YORA groups. TMJ problems can affect up to 5–12% of the entire population[12]. There are no data about the comparison of EORA and YORA for TMJ complaints. EORA is associated with higher disease activity and accelerated joint destruction compared with YORA[6]. Although TMJ involvement is numerically found to be higher in YORA patients than in

Table 2. Comparison of the patients with and without temporomandibular joint involvement according to demographic, clinical, and laboratory findings.

		EORA		YORA		p-value
		TMJ involvement positive n=9	TMJ involvement negative n=6	TMJ involvement positive n=58	TMJ involvement negative n=14	
Age (years)		67±3.87[ab]	68±2.44[cd]	46.93±11.18[bde]	35.14±10.83[ace]	<0.001*
		p=0.586		p=0.001*		
Gender	Male	3	0	6	0	0.062
	Female	6	6	52	14	
		p=0.229		p=0.589		
Disease duration (months)		27±8.88[a]	60±26.29	91.86±58.03[a]	36 (12–156)	0.002*
		p=0.004*		p=0.330		
DAS 28		2.58 (1.98–5.49)	4.05 (2.58–5.49)	3.57±1.31	3.7 (1.68–5.71)	0.591
		p=0.328		p=0.754		
RF	Positive	3	0	30[a]	2[a]	0.011*
		p=0.229		p=0.011*		
Anti-CCP	Positive	4	2	36	4	0.094
		p=1.000		p=0.024*		
Rheumatoid nodule	Positive	1	0	4	0	0.611
		p=1.000		p=0.580		
Methotrexate+glucocorticoid		0	1	4	0	0.394
Methotrexate+sulfasalazine		6	3	35	6	
Leflunomide		0	1	1	2	
Biologics		3	1	18	6	
		p=0.315		p=0.097		

Significance level *p<0.05; [a,b,c,d,e]: same letters in the same rows show where the significant differences found by comparing four groups with Kruskal-Wallis test; n: number of subjects; EORA: elderly-onset rheumatoid arthritis; YORA: young-onset rheumatoid arthritis; DAS 28: disease activity score 28; TMJ: temporomandibular joint; RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide.

Table 3. Comparison of the patients with and without temporomandibular joint involvement according to magnetic resonance imaging findings.

	EORA		YORA		p-value
	TMJ involvement positive n=9	TMJ involvement negative n=6	TMJ involvement positive n=58	TMJ involvement negative n=14	
Erosion positive	9^{ab}	0^{ac}	52^{cd}	0^{bd}	<0.001*
	p<0.001*		p<0.001*		
Resorption of the condyle positive	7^{abc}	0^{a}	10^{b}	0^{c}	<0.001*
	p=0.007*		p=0.193		
Flattening of the condyle positive	6^{a}	0	28^{b}	0^{ab}	0.001*
	p=0.028*		p=0.001*		
Narrowing of the joint space positive	4	0	24^{a}	0^{a}	0.007*
	p=0.103		p=0.003*		
Joint effusion positive	1	0	18^{a}	0^{a}	0.030*
	p=1.000		p=0.015*		
Osteophytes positive	7^{ab}	0^{ac}	44^{cd}	0^{bd}	<0.001*
	p=0.007*		p<0.001*		
Disk perforation positive	0	0	8	0	0.226
	Not computed		p=0.341		

Significance level *p<0.05; ^{a,b,c,d}: same letters in the same rows show where the significant differences found by comparing four groups with Kruskal-Wallis test; n: number of subjects; EORA: elderly-onset rheumatoid arthritis; YORA: young-onset rheumatoid arthritis.

EORA patients, this difference was not statistically significant. There was no difference between the groups in terms of disease activity in our study. It seems possible that there may be a difference in terms of TMJ involvement. TMJ involvement may be higher in patients with long disease duration. In addition, the tendency to progress more aggressively may be associated with the higher incidence of TMJ involvement.

There was no difference between those with and without TMJ involvement in the EORA group in terms of mean age, gender, DAS 28, RF positivity, anti-CCP positivity, extra-articular findings, or use of DMARDs. Only the disease duration of the patients with TMJ involvement was shorter, which we expected to be the opposite. These findings suggest that clinical and laboratory features do not differ so much in EORA patients with and without TMJ involvement.

Erosion, resorption, flattening of the condyle, and osteophytes were the most common MRI findings in the EORA patients with TMJ involvement. Erosion, flattening of the condyle, narrowing of the joint space, joint effusion, and osteophytes were the most common MRI findings in the YORA patients with TMJ involvement. There was no difference between EORA and YORA patients with TMJ involvement in terms of MRI findings.

In our study, the proportion of EORA was found to be higher (17.2%) than in the literature. In the literature, the frequency of RA in the geriatric population is reported as 2–2.4%[3,4]. Our finding may be due to the fact that our study population was not reflecting the general population. TMJ disorders and complaints are reported to be more frequent in elderly population[13], and only the patients with TMJ complaints who were referred to a dental clinic were included in this study. However, we should keep in mind that patients now have easier access to experienced rheumatologists and dentists dealing with TMJ involvement and/or the elderly population that has increased over the years, which indicates that the number of EORA patients increases.

In our study, the disease duration of the YORA patients was significantly higher than that of the EORA group. Also, the mean age of the YORA patients with TMJ involvement was higher than the patients without TMJ involvement. This may indicate that disease duration and advancing age can be counted among the determining factors in terms of TMJ involvement in young patients.

Female dominancy of RA and its lower incidence in men indicate that there is a hormonal effect on this disease[14]. The incidence in men rose steeply with age, whereas the incidence in women fell in the very elderly. In our study, male/female ratio of EORA group was higher than that of the YORA group, but this difference was not significant. When we looked at the literature, we identified studies

showing the gender distribution of patients with EORA and YORA similar[15-18].

Since being positive for anti-CCP and RF is an important prognostic factor, the variable findings of studies complicate to explain the different prognosis and clinical course seen in patients of different ages. In our study, RF positivity was numerically low in the EORA group, but this difference was not statistically significant. Also, in the YORA group, RF positivity and anti-CCP positivity were more frequent in patients with TMJ involvement. Similar to our finding, Calvo-Alen et al.[16] stated that the frequency of RF was similar in EORA and YORA patients. We know that RF increases with age. However, there are studies that found less RF positivity in EORA compared to YORA[19]. In our study, anti-CCP positivity was similar in both groups. Besides our study, in the studies of Cho et al.[18] and Krams et al.[17], anti-CCP was evaluated. Contrary to our study, the anti-CCP was found to be lower in the elderly in these two studies.

In our study, we could not find a significant difference in the use of DMARDs. Similar to our study, Cho et al.[18] found no difference between EORA and YORA in terms of DMARD use, but they found steroid doses higher in YORA patients. Takeda et al.[20] claimed that low-dose use of methotrexate is more common in elderly patients. Contrary to our results, Calvo-Alen et al.[16] detected the use of DMARDs and the use of combined DMARDs less in EORA patients than in YORA patients. Here, the difference in the treatment regimens is due to the difference in the management of side effects and complications. The limitation of our study is that the doses and the preference for biologic therapy were neglected.

In our study, when we compared the mean DAS 28 score of EORA and YORA patients, we could not find a difference. There are different results in the literature in terms of disease activity in EORA and YORA. Some studies reported that the disease activity in EORA and YORA patients was found to be similar, which is consistent with our study[16,18]. Contrary to our study, Krams et al.[17] found the median value of the Simplified Disease Activity Index to be higher in EORA patients than in YORA patients.

To the best of our knowledge, this is the first study to examine whether there was a difference between YORA and EORA according to TMJ involvement and MRI findings. One of the limitations of our study is that it was designed retrospectively. It will be possible to obtain more reliable results through prospective multicenter studies with long follow-up periods. Another limitation is the small number of patients included in the study, particularly in the EORA group.

CONCLUSION

According to our findings, EORA is not much different from YORA in terms of TMJ involvement, MRI findings, and clinical and laboratory features, but we would like to emphasize the importance of evaluating TMJ involvement in each RA patient.

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AUTHORS' CONTRIBUTIONS

MI: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **II:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICE

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Determination of the relationship between self-care agency and sleepiness in chronic hemodialysis patients

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SUMMARY

OBJECTIVE: This study was conducted to determine the relationship between self-care agency and sleepiness in chronic hemodialysis patients.

METHODS: The study was conducted with 75 patients with chronic renal failure in the hemodialysis unit of a training and research hospital in our country. In the descriptive study, the data were collected through a face-to-face questionnaire. The IBM SPSS Statistics 22.0 program was used to evaluate the data.

RESULTS: It was determined that there was no significant relationship between self-care agency and sleepiness total scores in chronic hemodialysis patients ($p>0.05$) and a significant relationship between sleepiness and drug use compliance and mental status in female patients and between diet compliance and sleepiness in patients younger than 52 years of age ($p<0.05$).

CONCLUSION: As a result, it was observed that there was no relationship between self-care agency and sleepiness in chronic hemodialysis patients. We think that working with a larger sample group can lead to clearer results.

KEYWORDS: Hemodialysis. Sleep. Self-care.

INTRODUCTION

Chronic kidney disease (CKD) is observed as a common health problem all over the world, and its frequency is reported to be increasing[1]. The primary replacement therapy for CKD is hemodialysis. The aim of the hemodialysis treatment was to correct the electrolyte and fluid imbalance of the patients and to increase the self-care agency of the patients and the quality of life of the individuals[2]. Although the life span of individuals is prolonged with hemodialysis[3], the fact that they constantly come to the institution on certain days of the week for treatment and have to stay connected to the device and personnel negatively affects the quality of life and social life of the patients[4]. Depending on this process, the self-care agency of these patients is adversely affected.

Self-care agency is an individual's ability to initiate or implement health activities to maintain his or her life, health, and well-being[5]. Self-care agency levels are of great importance in the control of disease symptoms of individuals undergoing hemodialysis. Studies have shown that individuals' self-care agencies are affected by their bio-psycho-social status[6-8]. As with all risky diseases, sleep disorders are frequently encountered in patients with CKD and undergoing hemodialysis[9,10]. As a result, the mental health of individuals is adversely affected. In addition, it has been determined that gender, age, and anxiety are related to sleep in hemodialysis patients[11]. When we review the literature, it is observed that sleep problems are frequently experienced in hemodialysis patients. However, a limited number of studies have been reached on how much these patients' self-care agencies are affected due to the sleep problems they have experienced. This study was conducted to determine the relationship between self-care agency and sleepiness in chronic hemodialysis patients and to contribute to future experimental studies.

METHODS

Study design: The study was conducted as descriptive and cross-sectional.

The sample of the study: The sample of the study consisted of 75 patients with chronic renal failure who were accepted to work at the Dialysis Unit of a Training and Research Hospital in Turkey, aged 18 years and over. Those who used drugs that affect sleep were excluded from the study.

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Data collection method: Data were collected by the face-to-face survey method between January 18, 2023 and February 18, 2023. While collecting the questionnaires of foreign nationals, support was obtained from the translator of the hospital.
Data collection: A form describing the personal characteristics of the patients, the Self-Care Scale for Chronic Hemodialysis Patients, and the Epworth Sleepiness Scale were used while collecting the data.

Personal data identification form of patients

The data were created by scanning the literature and consist of six items related to age, gender, educational status, marital status, medications used, and number of months they underwent hemodialysis.

Self-care agency scale for chronic hemodialysis patients

The self-care agency scale was developed by Oren in 2010, and its validity and reliability were made. In Oren's study, it is stated that Cronbach's alpha values are between 0.56 and 0.68 based on sub-dimensions and 0.75 in the whole scale. The self-care agency scale consists of 22 items. It is a three-point Likert scale scored between 0 and 2. The statements in the scale consist of five sub-dimensions related to the use of drugs, diet, self-monitoring of the patient, hygienic care, and mental state. Low scores obtained from the scale are considered poor self-care agency, and high scores are considered good[12,13]. In this study, the Cronbach's alpha coefficient of the Self-Care Scale was determined as 0.702.

Epworth sleepiness scale

It is a simple and self-reported scale. It questions the general sleepiness level of the individual. It aims to evaluate the chances of falling asleep or napping in eight different daily life situations. It is a simple, easy-to-understand, eight-item scale with proven validity and reliability in assessing the general sleepiness level in adults. A total score of "10" or more is considered increased daytime sleepiness, and scores above "15" are considered pathological sleepiness. Cronbach's alpha coefficient was found to be 0.870 for 150 individuals with sleep apnea and 0.860 for 60 healthy individuals. In this study, the Cronbach's alpha coefficient was determined as 0.738[14].

Analysis of data: The IBM SPSS Statistics 22.0 program was used for statistical analysis in the study. While evaluating the study data, in addition to descriptive statistical methods (i.e., mean, standard deviation, frequency, and percent). Pearson and Spearman correlation analyses were used to evaluate the correlation between variables. The results were evaluated at the 95% confidence interval and the significance level of $p<0.05$.

Ethical aspect of the study: Before starting the study, permission was obtained from Istanbul Gelişim University Ethics Committee with decision number 2023-02-44 dated 18.01.2023. Participants who voluntarily accepted to participate in the study were informed about the research and their rights as necessary, and their "informed consent" was obtained before the research. All the rights of the participants were respected, and the principles of voluntariness and confidentiality were paid attention to.

RESULTS

The descriptive features of chronic hemodialysis patients are shown in Table 1. It was determined that 54.7% of the participants were women, 84% were married, 38.7% were primary school graduates, their mean age was 52.41±15.90 years, and they underwent hemodialysis for an average of 28.71±22.24 months (Table 1).

The self-care agency sub-dimensions, self-care, and sleepiness scale total scores of chronic hemodialysis patients are shown in Table 2. It was determined that the patients' self-care agency total score was moderate (29.07±5.35) and the daytime sleepiness total score was at the borderline (9.64±4.79).

Table 1. Descriptive characteristics of chronic hemodialysis patients (n=75).

	n	%
Gender		
Female	41	54.7
Male	34	45.3
Age (average) (years)	52.41±15.90	
Marital status		
Married	63	84
Single	12	16
Educational status		
Illiterate	17	22.7
Literate	5	6.7
Primary school	29	38.7
Middle school	9	12
High school	12	16
Bachelor degree	3	4
Number of months they underwent dialysis	28.71±22.24	

The correlation between the sub-dimensions of the self-care agency scale, self-care agency, and sleepiness scale total scores among chronic hemodialysis patients was examined (Table 3). In addition, the correlation between these scales was evaluated with the relationship between gender, age, and the months of starting dialysis. In the selection of patient groups, ≥52 and <52 years were taken as ≥28 and <28 months in the months of dialysis. The reason for taking it this way is that the mean age of the patients was 52.41±15.90 years, and the months of starting dialysis were 28.71±22.24 (Table 3).

In the correlation of the scales in terms of gender of the patients, while no correlation was found in male patients, a negative linear relationship was found between sleepiness and drug use compliance (p=0.001; r=-0.525) and a positive linear relationship between mental states and sleepiness (p=0.047; r=0.343) in female patients. In the correlation in terms of age, while no correlation was found in ≥52 patients, a negative linear relationship was found between the patients' adherence to diet and sleepiness in <52 patients (p=0.020; r=-0.380). It was found that there was no significant correlation between the two groups in terms of the months of starting dialysis (≥28 and <28 months) (p>0.05) and no significant correlation between the total self-care agency scale and the sleepiness scale (p>0.05) (Table 3).

Table 2. Self-care agency sub-dimensions, self-care, and sleepiness total score averages of chronic hemodialysis patients (n=75).

	Min-max points	Scale mean scores
Self-care agency sub-dimensions		
Drug use	0–12	7.69±2.19
Diet	0–10	6.45±1.67
Self-monitoring	0–8	5.64±1.66
Hygienic care	0–8	6.37±1.46
Mental state	0–6	2.91±1.65
Self-care agency total	0–44	29.07±5.35
Sleepiness total	0–24	9.64±4.79

DISCUSSION

It was determined that the studies conducted between sleepiness and self-care agency were limited in number. There was no significant correlation between the total self-care agency scale and the sleepiness scale (p>0.05) (Table 3). When we look at the studies in the literature that look at the relationship between self-care agency and sleepiness, Zhu et al.[15] found a positive correlation (R^2=0.51, p<0.001) between sleepiness and self-care in 64 patients with type 2 diabetes[15]. In the systematic review of Spadela et al.[16], no relationship was found[16] between the two scales in the study of cardiac patients in three studies[17], while, in the other two studies[18,19], relationship was determined. The result of this study supports the research result of Kessing et al[17]. Reigel et al.[19]

Table 3. Correlation between the self-care agency scale sub-dimensions, self-care agency, and sleepiness scale total scores of chronic hemodialysis patients in terms of gender, age, and months of starting dialysis.

				Self-care agency scale sub-dimensions					Self-care agency scale total score
				Drug use	Diet	Self-monitoring	Hygienic care	Mental state	
Women		Sleepiness scale total score	r	-0.525	-0.110	-0.025	-0.139	0.343	-0.211
			p	0.001	0.534	0.890	0.433	0.047	0.232
Men		Sleepiness scale total score	r	0.130	-0.282	-0.240	0.213	-0.279	-0.211
			p	0.419	0.074	0.130	0.182	0.077	0.232
Age	≥52 years	Sleepiness scale total score	r	-0.104	-0.045	-0.066	-0.002	-0.109	-0.110
			p	0.534	0.790	0.695	0.989	0.514	0.509
	<52 years	Sleepiness scale total score	r	-0.149	-0.380	-0.251	0.142	0.051	-0.212
			p	0.379	0.020	0.134	0.401	0.764	0.208
Months of starting dialysis	≥28	Sleepiness scale total score	r	-0.271	-0.212	-0.038	-0.039	0.028	-0.198
			p	0.141	0.252	0.839	0.833	0.882	0.286
	<28	Sleepiness scale total score	r	-0.014	-0.194	-0.197	0.157	-0.059	-0.111
			p	0.927	0.206	0.200	0.309	0.706	0.472
In all patients		Sleepiness scale total score	r	-0.125	-0.214	-0.148	0.065	-0.035	-0.157

conducted their studies with 29 patients with chronic heart failure, Kamrani et al.[18] conducted their studies with 180 elderly patients with heart failure, and Zhu et al.[15] conducted their studies with 64 patients with type 2 diabetes. This study was conducted with hemodialysis patients with chronic renal failure. As the chronic diseases of the patients in the sample group in the literature studies and the chronic diseases of the patients in this study are different, it is thought to affect the result.

No study has been found in the literature examining the correlation between self-care agency sub-dimensions and sleepiness. In this study, in the correlation of the scales in terms of gender of the patients, while no correlation was found in male patients, a negative linear relationship was found between sleepiness and drug use compliance (p=0.001; r=-0.525) and a positive linear relationship between mental states and sleepiness (p=0.047; r=0.343) in female patients. In the correlation in terms of age, while no correlation was found in ≥52 patients, a negative linear relationship was found between the patients' adherence to diet and sleepiness in <52 patients (p=0.020; r=-0.380).

There was no significant correlation between the two groups (p>0.05) in terms of the months of starting dialysis (≥28 and <28 months). It is known that gender differences and different age groups in individuals are affected by sociocultural levels, lifestyles, hormonal differences, and participation in business life. In addition, the fact that in the female sample group (54.7%), primary school graduates (38.7%) and married (84%) are higher is considered to affect the results.

CONCLUSION

It was determined that there was no significant relationship between self-care agency and sleepiness total scores in chronic hemodialysis patients and a significant relationship between sleepiness and drug use compliance and mental status in female patients and between diet compliance and sleepiness in patients younger than 52 years of age. These results are important for future multicenter studies with larger samples and clinical applications. Self-care agency of individuals is the agency to perform physical activities, eating behaviors, drug use, and many other activities. With the increase in daytime sleepiness, there will be a possibility that the individual will not be able to perform these activities. We think that conducting future studies with larger sample groups to examine this potential relationship will lead to clearer results.

ETHICS COMMITTEE APPROVAL

Before starting the study, permission was obtained from Istanbul Gelişim University Ethics Committee with decision number 2023-02-44 dated 18.01.2023. Participants who voluntarily accepted to participate in the study were informed about the research and their rights as necessary, and their "informed consent" was obtained before the research. All the rights of the participants were respected, and the principles of voluntariness and confidentiality were paid attention to.

AUTHORS' CONTRIBUTIONS

NK: Conceptualization, Data curation, Methodology, Supervision, Resources, Validation, Writing – original draft, Writing – review & editing. **MR:** Writing – original draft. **EC:** Conceptualization. **IC:** Data curation. **MK:** Supervision, Resources, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Prognostic value of age, creatinine, and left ventricular ejection fraction risk score in patients evaluated with fractional flow reserve: a cross-sectional study

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SUMMARY

OBJECTIVE: In this study, we investigated the relationship between age, creatinine, and left ventricular ejection fraction risk score and the severity of coronary lesions detected by applying fractional flow reserve in the patient group presenting with chronic coronary syndrome. Also, we presented long-term follow-up results in patients whose age, creatinine, and left ventricular ejection fraction score was evaluated by the fractional flow reserve procedure.

METHODS: This study was planned retrospectively and in two centers. For this purpose, 114 patients who met the study criteria and who underwent elective fractional flow reserve between January 2014 and January 2019 were included in the study. Age, creatinine, and left ventricular ejection fraction was calculated as age/left ventricular ejection fraction +1 (if estimated glomerular filtration rate<30 mL/min).

RESULTS: They were divided into two groups according to the cutoff value of the age, creatinine, and left ventricular ejection fraction score. A total of 76 patients had an age, creatinine, and left ventricular ejection fraction score of ≤1.17 (Group I) and 38 patients had an age, creatinine, and left ventricular ejection fraction score of >1.17 (Group II). The number of patients with severe lesions in fractional flow reserve was significantly higher in Group II compared with Group I (60.5 vs. 32.9%, p=0.005). According to the Kaplan-Meier analysis, a significant increase was observed in major adverse cardiac events and mortality during the follow-up period in the group with a high-risk score (Log Rank: 15.01, p<0.001 and Log Rank: 8.51, p=0.004, respectively).

CONCLUSION: In light of the data we obtained from our study, we found a correlation between the severity of the lesion detected in fractional flow reserve and the age, creatinine, and left ventricular ejection fraction scores. In addition, we found that patients with high age, creatinine, and left ventricular ejection fraction scores had higher mortality and major adverse cardiac events rates during follow-up.

KEYWORDS: Stable angina pectoris. Myocardial fractional flow reserve. Major adverse cardiac events. Creatinine. Ventricular ejection fractions.

INTRODUCTION

Coronary angiography (CAG) is the gold standard method in the diagnosis and treatment of coronary artery lesions. However, sometimes, quantitative measurements are needed to evaluate the severity of the lesion detected in the coronary arteries. It is important to measure fractional flow reserve (FFR) in the coronary arteries, especially when the stenosis level is evaluated as 40–70% (i.e., moderate). FFR is a reliable method, especially for the functional assessment of lesion severity[1]. The development and progression of coronary atherosclerosis can be influenced by many clinical factors[2]. In addition, it is important to predict the short- and long-term prognosis of coronary artery patients. For this purpose, various risk-scoring systems have been developed. One of these scores is the ACEF score, which consists of three independent factors such as age, creatinine, and left ventricular ejection fraction (LVEF). The ACEF score was first used by Ranucci et al. in patients undergoing elective coronary artery bypass surgery (CABG)[3]. It has been reported that this scoring may be a similar or better predictive value for mortality compared with more complex risk scores[3]. Also, it is thought that this risk score may be an alternative predictive value to EuroSCORE[3]. Wykrzykowska et al., in the LEADERS study, reported that ACEF score was a predictor of mortality and myocardial infarction (MI) risk in the group of patients who underwent percutaneous coronary intervention (PCI)[4]. Similarly, it has been reported to be a good prognostic marker in high-risk patients who underwent PCI for lesions such as bifurcation lesions and chronic coronary total occlusion[5,6]. Studies on the ACEF risk score in the literature mostly focused on the patient group presenting with acute coronary syndrome (ACS). Studies on the long-term predictive value of the ACEF risk score in the patient group presenting with chronic coronary syndrome (CCS) are insufficient.

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In this study, we investigated the relationship between the ACEF risk score and the severity of coronary lesions detected by applying FFR in the patient group presenting with CCS. Also, we presented long-term follow-up results in patients whose ACEF score was evaluated by the FFR procedure.

METHODS

Study population

This study was planned retrospectively and in two centers. For this purpose, a total of 121 consecutive patients who underwent elective FFR between January 2014 and January 2019 were analyzed. Seven patients who did not meet the inclusion criteria were excluded from the study. A total of 114 patients were included in the study. Inclusion criteria for the study were as follows: it was determined as the patients who were evaluated as CCS and underwent the FFR procedure under elective conditions. Exclusion criteria for the study were as follows: ACS, severe arrhythmia, hemodynamic instability, high risk of bleeding (i.e., active internal bleeding, hemorrhagic stroke, intracranial neoplasm, arteriovenous malformation or aneurysm, and ischemic stroke in last 3 months), patients with CABG in last 3 months, moderate/severe heart valve pathology, acute decompensated and/or severe heart failure, liver failure, active infection, malignancy, hematologic diseases, patients receiving steroid therapy, familial history of hyperlipidemia, rheumatologic disease, life expectancy <1 year, and ages between <18 and >90 years. The study was designed in accordance with the principles of the Declaration of Helsinki. Approval was obtained from the local ethics committee before starting the study.

Definitions and age, creatinine, and left ventricular ejection fraction score

A detailed medical history was taken from all patients at the time of admission. Hypertension was defined as systolic blood pressure≥140 mmHg or diastolic blood pressure (DBP)≥90 mmHg or using antihypertensive medication. Diabetes mellitus (DM) was defined as a fasting glucose level of 126 mg/dL or the use of antidiabetic agents or HbA1c>7%. Dyslipidemia was defined as a total cholesterol level>200 mg/dL or a low-density lipoprotein level>130 mg/dL. Smoking was defined as current smoking. Peripheral vascular disease was defined as >50% stenosis in peripheral arteries. LVEF was evaluated from the apical four- and two-chambered views using the biplane Simpson method[7]. The ACEF score was calculated as follows: ACEF=age/left ventricular ejection fraction+1 [if estimated glomerular filtration rate (GFR) <30 mL/min][8]. The equation obtained from the Modification of Diet in Renal Disease (MDRD) study was used, and estimated GFR was calculated considering the initial serum creatinine value[9].

Coronary angiography and fractional flow reserve

Selective CAG was performed on the patients with a right-left femoral or radial approach, using 6F or 7F catheters with the Judkins technique. CAG images were evaluated by two experienced cardiologists, who were unaware of the laboratory values and clinical features of the patients. The degree of stenosis in the coronary arteries was decided based on the projection showing the greatest stenosis. Evaluation by applying the FFR is left to the discretion and discretion of the cardiologists. After an intra-arterial bolus of 5,000 units of heparin, the coronary arteries were visualized using a guide catheter without side holes. A 0.014 inch pressure monitoring guidewire (PrimeWire, Volcano, San Diego, CA, USA) was placed distal to the stenosis after calibration. Before FFR measurements, 200 μg bolus nitroglycerin was administered intracoronally. Initially, distal intracoronary pressures of the patients were recorded. Hyperemia was triggered by administering gradually increasing doses of intracoronary adenosine until the last value where the FFR value decreased. FFR value was determined as the ratio between the mean distal intracoronary pressure and the mean aortic pressure, at which time the highest level of hyperemia was observed. FFR value<0.80 was defined as functionally significant. Patients with a critical FFR were treated as recommended in the European Society of Cardiology guidelines[10].

Major adverse cardiac event

All-cause death and MI were considered major adverse cardiac events (MACE). All tracking data, hospital epicrisis, national data recording system, patients' families, or family doctors (face-to-face or telephone interview) were reached by interviewing. Follow-up period was defined as the time from the time of admission to our clinic for CAG until death from any cause. The study was terminated at the end of the 96-month follow-up period.

Statistical analysis

SPSS (IBM, USA, version 25) was used for statistical analysis. The distribution of continuous variables was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables were expressed as mean±standard deviation (mean±SD) or median (interquartile range) in case of skewed distribution. Continuous variables between two independent groups were analyzed using the Student's t-test or Mann-Whitney U test as

appropriate. Categorical variables were presented as percentages (%), and their statistical analysis was performed using the chi-square test or Fisher's exact test. Cox proportional hazards model analysis was used to determine the potential risk factors for MACE, and the results were presented as hazards ratio and 95% confidential interval (CI). Discrimination performance of ACEF score for FFR severity was accessed by receiver operating characteristic (ROC) curve analysis, and their areas under the curve (AUC) were compared using a nonparametric approach. Kaplan-Meier curve with Log Rank test was applied to detect the difference in event-free survival rates between the two groups. A univariable and multivariable analysis for predictors of ACEF score was applied and also plotted in a graph. Variables with a p-value of <0.05 were considered significant.

RESULTS

Baseline characteristics

They were divided into two groups according to the cutoff value of the ACEF score. A total of 76 patients had an ACEF score of ≤1.17 (Group I) and 38 patients had an ACEF score of >1.17 (Group II). The mean age in Group II was significantly higher than in Group I (62.89±7.12 vs. 54.74±8.62 years, p<0.001). Compared with Group I, in Group 2, DM (55.3 vs. 34.2%, p=0.031), cerebrovascular disease (13.2 vs. 2.6%, p=0.040), chronic kidney disease (10.5 vs. 1.3%, p=0.042) was significantly higher. LVEF was significantly higher in Group I (57.64±4.97 vs. 48.39±8.86%, p<0.001) (Table 1). Other clinical and demographic characteristics are summarized in Table 1.

The mean creatinine was higher in Group II compared with Group I, but no significant difference was found (0.87 vs. 0.85, p=0.467) (Table 2). The results of other hemogram and biochemical parameters are summarized in Table 2.

Fractional flow reserve and follow-up data

The mean FFR was higher in Group I compared with Group II (82.47±6.06 vs. 78.47±7.47%, p=0.003). In addition, the number of patients with severe lesions in FFR was significantly higher in Group II compared with Group I (60.5 vs. 32.9%, p=0.005). Mortality (15.8 vs. 2.6%, p=0.016) and MACE (26.3 vs. 3.9%, p=0.001) rates were significantly higher in Group II compared with Group I (Table 1).

Table 1. Demographic, baseline characteristic, and clinical endpoints results.

Parameters	All patients (n=114)	Group I (n=76)	Group II (n=38)	p-Value
Age (years)	57.46±8.99	54.74±8.62	62.89±7.12	**<0.001**
Gender, male, n (%)	76 (66.7)	52 (68.4)	24 (63.2)	0.574
Body mass index (kg/m^2)	26.72±3.08	27.06±3.17	26.03±2.80	0.093
Hypertension, n (%)	65 (57)	39 (51.3)	26 (68.4)	0.082
Diabetes mellitus, n (%)	47 (41.2)	26 (34.2)	21 (55.3)	**0.031**
Hyperlipidemia, n (%)	61 (53.5)	39 (51.3)	22 (57.9)	0.507
Smoking, n (%)	45 (39.5)	33 (43.4)	12 (31.6)	0.223
Cerebrovascular disease, n (%)	7 (6.1)	2 (2.6)	5 (13.2)	**0.040**
COPD, n (%)	18 (15.8)	11 (14.5)	7 (18.4)	0.586
CKD, n (%)	5 (4.4)	1 (1.3)	4 (10.5)	**0.042**
PVD, n (%)	6 (5.3)	3 (3.9)	3 (7.9)	0.317
LVEF (%)	54.56±7.83	57.64±4.97	48.39±8.86	**<0.001**
Mortality	8 (7.0)	2 (2.6)	6 (15.8)	**0.016**
Myocardial infarction in follow-up	3 (2.6)	1 (1.3)	2 (5.3)	0.257
Revascularization	2 (1.8)	0	2 (5.3)	0.109
MACE	13 (11.4)	3 (3.9)	10 (26.3)	**0.001**
FFR value (%)	80.74±6.98	82.47±6.06	78.47±7.47	**0.003**
Critical lesion (FFR value ≤0.80)	48 (42.1)	25 (32.9)	23 (60.5)	**0.005**

COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; PVD: peripheral vascular disease; LVEF: left ventricular ejection fraction; MACE: major adverse cardiac event; FFR: fractional flow reserve; Group I: ACEF score≤1.17 (low risk); Group II: ACEF score>1.17 (high risk). Bold indicates statistically significant values.

Table 2. Hemogram and biochemical parameter results.

Parameters	All patients (n=114)	Group I (n=76)	Group II (n=38)	p-Value
Urea, mg/dL	34.22±11.64	33.26±11.09	36.14±12.60	0.214
Creatinine, mg/dL	0.86 (0.30)	0.85 (0.20)	0.87 (0.28)	0.467
Uric acid, mg/dL	5.40±0.78	5.35±0.76	5.51±0.81	0.306
Total cholesterol, mg/dL	188.09±41.34	183.15±38.08	197.97±46.14	0.071
Triglyceride, mg/dL	157.75±49.08	153.51±45.90	166.21±54.54	0.194
HDL, mg/dL	39.88±9.72	40.18±9.53	39.28±10.19	0.645
LDL, mg/dL	116.66±38.50	112.27±35.32	125.44±43.36	0.085
Hemoglobin, g/dL	13.53±1.48	13.68±1.52	12.98±1.27	**0.017**
Platelet, $\times10^3/\mu L$	260.95±56.18	264.51±58.70	253.84±50.75	0.341
Leukocyte, $\times10^3/\mu L$	8.08±1.67	7.93±1.72	8.37±1.54	0.194
MPV, fL	8.41±0.91	8.38±0.90	8.49±0.94	0.533
Neutrophil, $\times10^3/\mu L$	5.14±1.54	5.10±1.52	5.20±1.59	0.749
Monocyte, $\times10^3/\mu L$	0.87±0.23	0.88±0.25	0.85±0.20	0.635
Lymphocyte, $\times10^3/\mu L$	2.35±0.77	2.40±0.79	2.24±0.73	0.286
Fasting glucose, mg/dL	100.31±15.66	98.61±10.58	103.71±22.45	0.102
TSH, μIU/mL	1.87 (1.71)	1.88 (2.05)	1.85 (1.38)	0.568
T4, ng/dL	1.28 (0.51)	1.30 (0.60)	1.26 (0.29)	0.469
AST, U/L	21.0 (12.5)	21 (13)	22.5 (12.2)	0.269
ALT, U/L	20.0 (10.5)	21 (14)	19 (7.25)	0.415

HDL: High-density lipoprotein; LDL: low-density lipoprotein; MPV: mean platelet volume; TSH: thyroid-stimulating hormone; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Group I: ACEF score≤1.17 (low risk); Group II: ACEF score>1.17 (high risk). Bold indicates statistically significant values.

According to the Kaplan-Meier analysis performed to examine the relationship between ACEF score and MACE and mortality during the follow-up period, a significant increase was observed in MACE and mortality during the follow-up period in the group with a high-risk score (Log Rank: 15.01, p<0.001 and Log Rank: 8.51, p=0.004, respectively).

In the cox regression analysis; we found that ACEF (OR:15.58; 95%CI: 4.79–50.64, p<0.001) and FFR (OR:6.64; 95%CI: 1.37–32.21, p=0.019) parameters were independent predictors of mortality (Figure 1).

In the multivariable regression analysis performed among all causes affecting the ACEF score, we found that MACE (OR: 5.89; 95%CI: 1.23–28.09, p=0.026) and DM (OR:2.49; 95%CI: 1.02–6.07, p=0.044) parameters are independent predictors (Figure 1).

ROC analysis was used to evaluate the power of the ACEF score to predict MACE rates. ACEF predicted MACE rates with 62.5% sensitivity and 66.7% specificity (AUC: 0.708; 95%CI: 0.615–0.802, p<0.001).

DISCUSSION

In our study, we found a correlation between patients with a high ACEF risk score and the severity of the lesion detected in FFR. We found that patients with high ACEF scores had significantly higher mortality and MACE rates in the long-term follow-up. In addition, we showed that the severity of the lesion detected in FFR may be an independent predictor of mortality in the long-term follow-up. Studies on the ACEF score in the literature have generally focused on ACS patients. However, the majority of patients who underwent CAG are CCS patients. Studies examining the effect of the ACEF score on CCS patients are limited. Therefore, in our study, we examined patients who applied with CCS and were evaluated with FFR. In this respect, we wanted to examine its effect on quantitative data, and to the best of our knowledge, this is the first study in the literature. In a study, it was reported that the ACEF risk score was a better predictor than other risk scores in patients with non-ST-elevation MI in whom all treatment strategies were applied[11]. In another study, it was reported that the ACEF value at

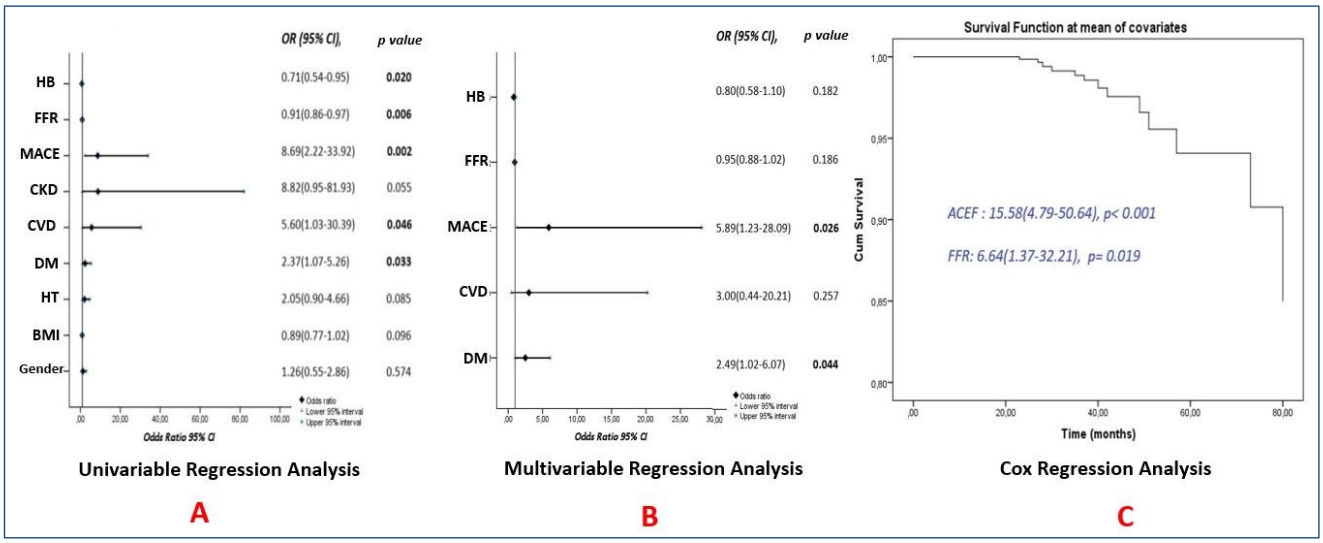

Figure 1. Forest plot of univariable **(A)** and multivariable **(B)** analyses showing correlation of parameters with age, creatinine, and left ventricular ejection fraction risk score. Cox regression analysis **(C)** examining the effect of age, creatinine, and left ventricular ejection fraction and fractional flow reserve parameters on mortality.

admission could predict the 1 month and 1 year cardiac mortality rate after emergency PCI in STEMI patients aged ≥75 years[12]. In contrast, Chichareon et al. stated that the ACEF score may be a better predictive marker in ACS patients compared with CCS patients[13]. The ACEF score, which consists of three vital parameters: age, creatinine, and LVEF, is simple and easy to calculate[3]. Advanced age and renal insufficiency can lead to a deterioration in diastolic parameters and a long-term decrease in LVEF. In addition, the probability of development of calcified stable atheroma plaques is high. Symptoms may not always develop due to silent ischemia and collateral development. Therefore, we want to emphasize that ACEF score can be a prognostic marker not only in ACS patients but also in CCS patients that may cause critical stenosis. Pyxaras et al. showed that the ACEF score can predict MACE rates in the 1 year follow-up of severe calcific coronary lesions undergoing PCI[14]. In a study conducted on CCS patients, it was reported that the ACEF score is a predictor of mortality and MACE rates in the long-term follow-up[15]. However, in our study, we also evaluated the severity of lesions in CCS patients with FFR and conducted our research on quantitative values. This is a strong aspect of our work.

The relationship between inflammatory parameters and cardiovascular diseases has been frequently investigated. However, there are serious limitations regarding such parameters. More powerful and generalizable clinical scoring systems have been investigated in order to determine the prognosis. For this purpose, the ACEF score is one of the simplest prognostic models in the field of cardiology. Therefore, it can be easily calculated in the vast majority of patients, especially in CCS patients undergoing elective percutaneous coronary procedures. Identifying a patient group at high risk of mortality and incorporating ACEF score calculation into routine clinical practice may help improve postprocedural clinical management. In patients considered to be at high risk according to the ACEF score, applications such as cardioverter-defibrillator implantation may be beneficial for more frequent monitoring of kidney and cardiac functions, strict adherence to guidelines in terms of medical treatments, and prevention of sudden death[15].

Limitations

Our study has some limitations. The sample size was relatively small and this was a retrospective study. Prospective studies with larger patient groups are needed. The ACEF score at the time of admission to the hospital was taken into account. The effect of changes in the ACEF score during the follow-up period could not be excluded. In this study, we only looked at the prognostic value of the ACEF score, we did not use other scoring systems. In addition, some data were obtained from the hospital system and national data recording systems. Errors may have occurred in this respect.

CONCLUSION

In light of the data we obtained from our study, we found a correlation between the severity of the lesion detected in

FFR and ACEF scores. In addition, we found that patients with high ACEF scores had higher mortality and MACE rates during follow-up. It may be beneficial to increase the frequency of follow-up in high-risk patients, especially in terms of changeable parameters.

AUTHORS' CONTRIBUTIONS

AA: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **TG**: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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A needful, unique, and in-place evaluation of the injuries in earthquake victims with computed tomography, in catastrophic disasters! The 2023 Turkey-Syria earthquakes: part II

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SUMMARY

OBJECTIVE: This study aimed to determine the computed tomography findings associated with very recent catastrophic 2023 Turkey-Syria earthquake-related injuries and their anatomotopographic distribution in the adult population.

METHODS: The incorporated computed tomography scans of 768 adult cases who had been admitted to the hospital and had undergone computed tomography imaging after these tragic disasters had been examined on the Teleradiology Reporting System of the Turkish Ministry of Health. To this end, the injuries were classified into six categories: head, thoracic, spinal, pelvic, extremity, and abdominal injury, with three age groups (18–34, 35–64, and ³65 years) and four different imaging intervals (<24, 24–48, 49–72, and >72 h).

RESULTS: This study incorporated 316 (41.1%) cases on the first day, 57 (7.5%) on the second day, 219 (28.5%) on the third day, and 176 (22.9%) on the fourth day after the earthquake or later. Of the 768 cases, 109 (14.2%) had a head injury, 100 (13.0%) had a thoracic injury, 99 (12.9%) had a spinal injury, 51 (6.6%) had a pelvic injury, 41 (5.4%) had an extremity injury, and 11 (1.4%) had an abdominal injury.

CONCLUSION: In these regrettable earthquake disasters, we determined a high ratio of head injuries, which was closely followed by thoracic and spinal injuries, in our preliminary outcomes for the pediatric population, Part I. The frequency of abdominal injuries was low among individuals who experienced the earthquake. Last but not least, we have noticed a higher likelihood of spinal injury in individuals older than 65 years in the studied population.

KEYWORDS: Earthquake. Adult. Tomography. Radiology. Surgery.

INTRODUCTION

Ab imo pectore, a regrettable and catastrophic earthquake with a magnitude of at least 7.8 on February 6, 2023, at 4:17 a.m., deeply affecting the south and east of Turkey and the northern and western parts of Syria. Straight after, 9 h after the first earthquake, a second tragic earthquake with a magnitude of 7.6 occurred in the same geographic regions. Official reports state that the earthquake left more than 50,000 people dead and tens of thousands more wounded[1]. The earthquake, *per se*, is estimated to have caused $84.1 billion US dollars' worth of damage, making it one of the costliest natural disasters ever recorded. In the aftermath of such an unpredictable, huge, and wide-ranging disaster, with regret, thousands of houses collapsed, dozens of hospitals became unusable, tens of thousands of people died, and hundreds of thousands were injured.

After a massive earthquake, multiple traumas such as bone fractures, soft tissue injuries, and organ injuries due to the collapse of buildings or damage by falling objects are the most common reasons for hospital admission[2-4]. In trauma cases, X-rays and computed tomography (CT) are used in the first place to detect damage quickly[5,6]. After the disaster affected 11 cities in Turkey, patients were transferred to neighboring

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cities immediately for treatment management. Patients evaluated according to trauma guidelines were frequently examined via CT scan.

The Teleradiology Reporting System (TRS) of the Ministry of Health is a system that allows accessing images of radiological examinations on the web 7×24, reporting these images, conducting teleconsultations between radiologists, and evaluating medical images and reports in terms of quality (https://teleradyoloji.saglik.gov.tr). After the earthquakes, with the coordination of the Ministry of Health, CT examinations carried out in hospitals serving trauma patients in earthquakes began to be reported immediately by radiologists all over Turkey via the TRS. Thus, it was aimed to alleviate the burden on physicians in the regions affected by such a painful disaster.

Herein, we purposed to investigate CT imaging features seen in adult individuals from areas damaged by this massive earthquake based on images carried out by the TRS.

METHODS

Study design

This present study included patients aged 18 years or older who had a history of trauma associated with the 2023 Turkey-Syria Earthquake and had undergone CT scan imaging between February 6 and February 11, 2023, according to the TRS of the Ministry of Health. Of note, two cases who underwent surgical treatment before CT examination and six who underwent CT imaging for non-earthquake-related causes were excluded from the present study design.

Image analysis

The CT images of the cases consisting of axial sections, multiplanar reconstruction, coronal, and sagittal sections had been evaluated by two authors using the TRS of the Ministry of Health. As such, the injuries were classified into six categories: (i) head injury, (ii) thoracic injury, (iii) abdominal injury, (iv) spinal injury, (v) pelvic injury, and (vi) extremity injury. Moreover, patient demographic data concerning age, gender, anatomotopographic distribution, and types of injuries had been recorded. Herein, the differences in interpretation have been resolved with the consensus on the relevant issues. To this end, the cranial subcutaneous soft tissue, bones, and brain parenchyma had been examined for head injuries, while the bones, pulmonary parenchyma, and pleura for thoracic; the solid organs, retroperitoneal and intraperitoneal spaces for abdominal; the pelvic bones fractures for pelvic; and the vertebral bodies, the transverse and spinous processes, had been examined for spinal injuries.

Statistical analysis

The data from each patient were input into a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) chart, and data analysis was conducted on a personal computer using statistics software (SPSS for Windows, version 23.0; SPSS, Chicago). The relevant injuries in six categories were examined with regard to three age groups (18–34, 35–64, and 65 years and older) and four different imaging intervals (<24, 24–48, 49–72, and >72 h) (Figure 1). The Pearson χ^2 test was used in order to determine the differences associated with the age groups and imaging intervals in the involved body parts. The data were presented as n (%), and p-value lower than 0.05 were considered statistically significant.

RESULTS

This study included 768 patients aged 18 or older who had a tragic earthquake-related history of trauma and had undergone CT scans between February 6 and February 11, 2023, according to the TRS of the Ministry of Health. The anatomical distribution of the injuries was evaluated regarding the four different imaging intervals and three age groups (Table 1), and the injuries were detected in 300 of these 768 cases. Of the 300 patients with injuries, 222 (74%) had injuries in a single anatomical region, 61 (20.3%) had injuries in two anatomical regions, and 17 (5.6%) had injuries in three or more anatomotopographic regions.

This study included 316 (41.1%) patients examined on the first day, 57 (7.5%) patients examined on the second day, 219 (28.5%) patients examined on the third day, and 176 (22.9%) patients examined on the fourth day after the earthquake or later. A total of 425 female and 343 male patients were included in the study with an age range of 18–95 years and a mean age of 46.1 years. The present study included 262 (34.2%) cases

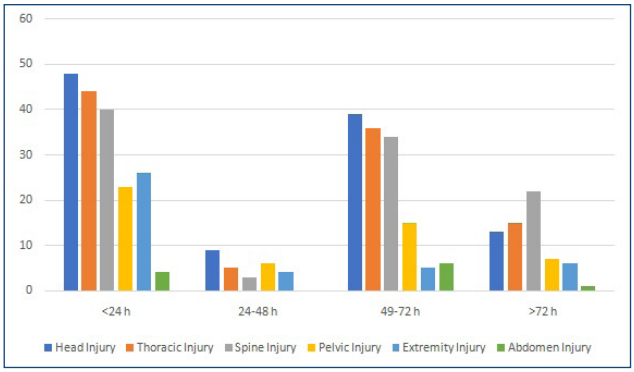

Figure 1. The cluster bar graph shows the distribution of earthquake-related injuries by imaging intervals.

aged between 18 and 34 years, 355 (46.2%) aged between 35 and 64 years, and 151 (19.6%) aged 65 years or older. There was no significant difference between male and female patients in terms of the anatomic location of the injury.

Of the 768 patients included in the present study, 109 (14.2%) had a head injury, while 100 (13.0%) had a thoracic injury, 99 (12.9%) had a spinal injury, 51 (6.6%) had a pelvic injury, 41 (5.4%) had an extremity injury, and 11 (1.4%) had an abdominal injury. The most common types of injury across the six anatomical localizations were, in descending order of frequency, scalp hematoma in 75 patients (7.60%), calvarial bone fracture in 52 (7.60%), and rib fracture in 47 (7.60%). In patients with head injuries, the most common finding was Scalp Hematoma, found in 75 patients, and the most common fracture was a parietal bone fracture. We determined the calvarial bone fractures in 52 cases, the intraparenchymal hematoma in 9, the subdural hematoma in 9, the subarachnoid hemorrhage in 8, the cephalic contusion in 8, the pneumocephalus in 4, the cephalic edema in 3, and the epidural hematoma in 1 patient. Eleven cases had parietal, 10 had frontal, 8 had temporal, 7 had nasal, 5 had zygomatic, 4 had occipital, 3 had maxillary, 2 had ethmoid, and 2 had mandibular fractures. Of these, 62.1% were linear, whereas 22.9% were depressed, and 15% were mixed fractures (Table 2).

The most common finding detected in patients with thoracic injuries was a rib fracture, which affected 47 cases. In addition, 36 cases had lung contusions, 36 hemothorax, 19 pneumothorax, 13 scapular fractures, 5 clavicular fractures, 3 pneumomediastinums, 1 sternal fracture, and 1 lung laceration.

In those with spinal injuries, fractures in levels L1, L3, and L2 were the top three most frequent fractures. The most frequently affected level was the lumbar level, while the least commonly affected level was the cervical level. Of these cases, 16 had bursts, one had translations and one had distraction-type unstable vertebral fractures. These fractures were found in the thoracolumbar levels. Of note, 92 of them are compression fractures in the anterior column of the vertebral corpus and the other vertebral fractures are stable fractures located in the transverse and spinous processes. The levels of spinal fractures are indicated in Table 2.

Of the 40 patients with pelvic injuries, 24 had pubic, 24 had sacral, 11 had acetabular, 6 had iliac, and 2 cases each had coccyx and ischial fractures. Among patients with extremity injuries, the upper extremities were injured in 37, while the lower extremities were injured in 10, involving femur fractures in 21, tibia fractures in 8, and talus fractures in 5. The levels of the other fractures are presented in Table 2. Eight patients with abdominal injuries had hemoperitoneum, two had liver lacerations, and one had a renal laceration.

DISCUSSION

Natural disasters are ecological phenomena that dramatically disrupt the normal order of life in a population. In comparison to other natural disasters such as floods, landslides, and avalanches, earthquakes can impact a greater area within a short time, resulting in the loss of both life and property[7]. Minimizing their effects requires urgent and organized aid, and the burden of loss associated with a major earthquake is also major[8]. The 1999 Marmara earthquake in Turkey resulted in more than 17,000 casualties. Meanwhile, for this earthquake, a death toll exceeding

Table 1. Distribution of earthquake-related injuries by age, gender, and imaging intervals.

	<24 h (n=316)	24–48 h (n=57)	49–72 h (n=219)	>72 h (n=176)	Total (n=768)
Male	169 (53.5)	28 (49.1)	82 (37.4)	64 (36.4)	343 (44.7)
Female	147 (46.5)	29 (50.9)	137 (62.6)	112 (63.6)	425 (55.3)
Head injury	48 (15.1)	9 (15.8)	39 (17.8)	13 (7.4)	109 (14.2)
Thoracic injury	44 (13.9)	5 (8.8)	36 (16.4)	15 (8.5)	100 (13)
Spinal injury	40 (12.7)	3 (5.3)	34 (15.5)	22 (12.5)	99 (12.9)
Pelvic injury	23 (7.3)	6 (10.5)	15 (6.8)	7 (4)	51 (6.6)
Extremity injury	26 (8.2)	4 (7)	5 (2.3)	6 (3.4)	41 (5.4)
Abdominal injury	4 (1.3)	0 (0)	6 (2.7)	1 (0.6)	11 (1.4)
18–34 years	106 (33.5)	29 (50.9)	79 (36.1)	48 (27.3)	262 (34.1)
35–64 years	145 (45.9)	21 (36.8)	104 (47.5)	85 (48.3)	355 (46.2)
>65 years	65 (20.6)	7 (12.3)	36 (16.4)	43 (24.4)	151 (19.7)

Table 2. Distribution of earthquake-related injury types.

Injury	n	%
Head injury types		
SCALP hematoma	75	68.8
Parietal fracture	11	10
Frontal fracture	10	9.2
Intraparenchymal hemorrhage	9	8.2
Subdural hematoma	9	8.2
Temporal fracture	8	7.3
Subarachnoid hemorrhage	8	7.3
Cephalic contusion	8	7.3
Nasal fracture	7	6.4
Zygoma fracture	5	4.5
Pneumocephalus	4	3.6
Occipital fracture	4	3.6
Maxilla fracture	3	2.7
Cephal oedema	3	2.7
Ethmoid fracture	2	1.8
Mandibular fracture	2	1.8
Epidural hematoma	1	0.9
Thoracic injury types		
Rib fracture	47	47
Pulmonary contusion	36	36
Hemothorax	36	31
Pneumothorax	19	19
Scapula	13	13
Clavicular fracture	5	5
Pneumomediastinum	3	3
Sternum fracture	1	1
Laceration	1	1
Spinal injury types		
L1	37	37.4
L3	34	34.3
L2	30	30.3
T12	19	19.2
T11	14	14.1
L4	13	13.1
L5	8	8.1
T5	7	7.1
T10	6	6.1
T4	5	5.1
T6	5	5.1
T3	5	5.1
C6	5	5.1
T9	4	4
C7	4	4
T1	3	3
T8	3	3
T2	2	2
C1	2	2
T7	2	2
Pelvic injury types		
Pubic fracture	40	78.4
Sacrum fracture	24	47.1
Acetabulum fracture	11	21.6
Iliac fracture	6	11.8
Coccyx fracture	2	3.9
Ischium fracture	2	3.9
Extremity injury types		
Femur fracture	21	51.2
Tibia fracture	8	19.5
Talus fracture	5	12.2
Humerus fracture	4	9.7
Radius fracture	3	7.3
Ulna fracture	2	4.8
Patella fracture	2	4.8
Fibula fracture	1	2.4
Carpal fracture	1	2.4
Abdominal injury types		
Hemoperitoneum	8	72.7
Liver	2	18.2
Renal	1	9.1

45,000 was reported within the borders of Turkey at the time of the present study. For earthquake victims that can be rescued with injuries, a systematic and rapid assessment of the extent of the injury is important. Of note, CT is one of the most frequently employed imaging methods in the evaluation of earthquake-related injuries, in accordance with the current evidence in light of the studies in the literature[9-12]. Therefore, we selected CT as the imaging modality in order to be utilized for the determination of earthquake-related injury profiles in the present study.

The most common injuries occurring in earthquake-related trauma patients were reported as extremity injuries[13-15]. However, due to the distribution of anatomical regions in our study, which is not compatible with the English-language literature, we encountered head trauma cases, similar to the very recent study our group conducted with pediatric cases[12]. Of note, the lower rate of extremity injuries in adults compared to the literature is attributable to the exclusion of patients who underwent direct radiography but not CT for the diagnosis of isolated extremity injuries, which affected the representation of extremity injuries in the present study.

In the present study, the majority of the cases underwent imaging within 72 h, which is parallel to the study on the 1999 Marmara earthquake in this regard[13]. In this study, head injury, spinal injury, and thoracic injury were the most common injuries encountered within the first 72 h, in descending order of frequency. However, after 72 h, spinal injury, thoracic injury, and head injury were encountered the most frequently, which leads to the conclusion that physicians would have a greater responsibility for patient intervention after a natural disaster.

Determining the treatment priority for patients with injuries in multiple anatomical regions is of vital importance for the patient[11]. In the present study, approximately 25.9% of earthquake-related trauma cases had injuries affecting two or more regions of the body. Multiple injuries are higher than the 5% reported after the 2008 Sichuan earthquake and the 15% reported after the 1995 South Hyogo and 2005 Kashmir earthquakes[16,17].

We also specified the topographic sites of injury according to age groups. The most affected anatomical regions in cases aged 18–34, patients aged 35–64, and patients aged 65 or older were the head, the thorax, and the spine, respectively. The ratio of spinal injury was slightly higher above the age of 65, which may be explained by the reduced bone density in those older than 65. As such, the TRS of the Ministry of Health collects CT scans in a pool, and these scans can be interpreted by radiologists approved by the ministry in a very short period of time. Considering that the high number of patient scans would further increase the workload of the radiologists living in the earthquake zone in trauma cases and emergency conditions[18-21], who are also victims, and that they might at times experience delays in reporting the scans, the use of the TRS of the Ministry of Health in disasters such as earthquakes offers great benefit in terms of the workload and access to CT reports. On the other hand, potential interruptions to infrastructure, including the Internet, during disasters such as earthquakes may pose a disadvantage for the TRS.

Limitations

Our study has the following three limitations: (i) as a retrospective study, we did not have trauma score data or patient outcomes such as mortality, (ii) only patients with CT images were included in the present study, and (iii) the exclusion of earthquake victims who did not undergo CT scans but were diagnosed using imaging methods such as direct radiography, sonography, or MRI.

CONCLUSION

In these regrettable earthquake disasters of extreme severity, with a magnitude of at least 7.8 and 9 h after the first earthquake, a second earthquake with a magnitude of 7.6 affecting the southern and eastern parts of Turkey and the northern and western parts of Syria, we determined a high ratio of head injuries, which was closely followed by thoracic and spinal injuries in the adults, which is similar to the preliminary results of our former study in the pediatric population, Part I. Herein, a low ratio of abdominal injuries among those who experienced the earthquake has been recognized. In addition, those older than 65 years were noticed to have a greater likelihood of experiencing a spinal injury. Herewith, we might hope our results will be useful in the development of protocols, even guidelines, and disaster preparedness for future high-magnitude earthquakes. This issue merits further investigation.

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AUTHORS' CONTRIBUTIONS

GT: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization. **DS:** Investigation, Methodology, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **TB:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **IS:** Investigation, Methodology, Resources, Software, Supervision, Visualization, Writing – review & editing. **IMC:** Investigation, Resources, Validation, Visualization. **ROO:** Investigation, Validation, Visualization. **DET:** Investigation, Validation, Visualization. **UE:** Validation, Visualization. **IA:** Formal Analysis, Validation. **ECAV:** Investigation, Software, Supervision, Visualization, Writing – review & editing. **SA:** Formal Analysis, Validation.

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REVIEW ARTICLE

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Comparison of safety and efficacy of dapagliflozin and empagliflozin in type 2 diabetes mellitus patients in India

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INTRODUCTION

Approximately 422 million people are suffering from diabetes worldwide. Most of the population is living in third world or developing countries, and about 2 million deaths are directly or indirectly caused by diabetes each year. Among the adult population of India, there are 73 million cases of diabetes[1]. "From 11.9 and 14.2% of adults in urban India have diabetes; in rural India, the prevalence is between 3.0 and 7.8%, with a much higher incidence among people over 50" (ICMR-INDIAB Study)[2].

The mechanism of action of novel antidiabetic drugs known as sodium glucose co-transporter (SGLT-2) inhibitors differs significantly from those of traditional antidiabetic medications[3]. Anti-glucose reuptake inhibitors like SGLT-2 achieve their hypoglycemic effect by increasing glucose excretion in the urine. A medication that inhibits SGLT-2 would be perfect in her case because her kidneys reabsorb around 90% of the glucose they filter throughout the PCT process[4]. The FDA has so far approved canagliflozin, dapagliflozin, and empagliflozin as drugs in this group[5]. Numerous studies have shown that SGLT-2 inhibitors have favorable effects on body weight (BW), blood pressure, dyslipidemia, and fatty liver disease in addition to lowering the risk of hypoglycemia. Positive results from several clinical studies on the subject of cardiovascular (CV) and renal safety have been reported[6,7]. According to the current guidelines, SGLT-2 inhibitors should be used as second-line antidiabetic drugs when first-line antidiabetic medications fail to adequately control blood sugar levels. However, they may be used well alone as a therapy. Patients with type 2 diabetes were investigated to determine the safety and tolerability profile of SGLT-2 inhibitors (dapagliflozin and empagliflozin)[8].

METHODS

To collect the information for this review, searches were performed in Scopus, Web of Science, Embase, PubMed, and MEDLINE for "Comparison of safety and effectiveness of Dapagliflozin with Empagliflozin in patients with type 2 DM in India." Articles published worldwide between 2010 and 2022 were included.

HBA1C REDUCTION

The majority of Indian patients (56.3%) who started using dapagliflozin at the beginning had HbA1c levels between 8 and 10% with a mean±SD value of 9.11±1.44. Patients who started on empagliflozin had an HbA1c% of 7.92±0.7018 (mean±SD)[9].

Statistically substantial reductions in HbA1c were observed at 3 months (1.00%) and 6 months (1.49%) in Indian patients who had started dapagliflozin therapy. Therefore, across all of his HbA1c stratified groups (i.e., 8, 8–10, and >10%) from baseline to 3 and 6 months, the HbA1c value considerably decreased ($p<0.001$) in patients taking dapagliflozin. The mean (SD) HbA1c level was 9.11% (1.44%) at baseline, 8.11% (1.22%) at 3 months, and 7.62% (1.04%) at 6 months. Patients using 10 mg of empagliflozin had a 0.81% decrease in HbA1c levels, while those using 25 mg had a 1.11% reduction by week 76. In patients with baseline HbA1c values considerably greater than 7%, both 10 and 25 mg of empagliflozin were able to lower HbA1c levels to below 7% after 76 weeks of therapy (20.8 and 28.0%, respectively)[10].

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WEIGHT REDUCTION

Indian patients using dapagliflozin lost an average of 1.14 (2.21) kg after 3 months and 1.86 (3.04) kg after 6 months (SD). Individuals with a BMI greater than 30 lost their maximum weight [mean (SD): 1.60 (2.50) kg] at 3 months and [2.56 (3.50) kg] at 6 months[9].

Therapy with either empagliflozin 10 or 25 mg for 76 weeks resulted in a decrease in BW in Indian patients (1.41 and 1.50 kg, respectively)[10].

BLOOD PRESSURE AND HEART RATE

After 3 and 6 months of therapy, patients using dapagliflozin had decreases in systolic blood pressure (SBP) of 3.24 (11.44 mmHg) and 3.77 (12.22 mmHg), respectively, from baseline[8]. The SBP of patients using empagliflozin was observed to decrease by 3.3 and 3.8 mmHg, respectively, when the drug is given in doses of 10 and 25 mg[9]. Although dapagliflozin reduced diastolic blood pressure (DBP) by 1.13 (7.67) and 1.46 (8.30) mmHg after 3 and 6 months, respectively, empagliflozin reduced DBP by 1.0 mmHg after 10 mg and 1.6 mmHg after 25 mg. However, these reductions in SBP and DBP are non-significant[9,10].

Heart rate reductions are non-significant with both the drugs in any doses and for any duration of treatment.

ADVERSE EFFECTS

Only 2.9% Indian patients on dapagliflozin treatment had one adverse event, while 2.2% Indian patients on empagliflozin had one adverse event. One patient on dapagliflozin and no patient on empagliflozin had greater than one serious adverse event requiring hospitalization. Adverse events such as vulvovaginitis were reported in 0.4% of patients using dapagliflozin and 0.6% of patients using empagliflozin. Fungal infections are also common in empagliflozin 0.3% than dapagliflozin (0.2%). The incidence of urinary tract infections is equal in both groups (0.2%).

Mild adverse events such as headache, constipation, and temperature are infrequent but present in both groups.

Hypoglycemia is an important side effect that should be mentioned, which is 0.2% associated with dapagliflozin and none with patients using empagliflozin[9,10].

DISCUSSION

Not only there are very small data regarding safety and efficacy of dapagliflozin and empagliflozin at the national level, but also the trials and papers addressing this issue are also inconclusive. Thus, the only comparison left is with western world trials. Studies from southern Europe showed that there is a role of geographical diversity in dapagliflozin effect on decreasing HbA1c levels, as well as CV and renal outcomes. As we all know, India is also a country of geographical diversity, so this may be true in Indian perspective also. There are differences between CV and renal effects in northern as well as southern parts of India because of cultural, dietary, and religious differences, so there is no uniformity in the effects of these two drugs.

The mean HbA1c level of dapagliflozin-treated patients was 7.62% after 6 months of treatment, which is close to the ADA-recommended target HbA1c level of <7.0%. Some studies showed a much higher reduction in HbA1c levels from basal HbA1c levels after 6 months (1.49%), which may be due to higher basal HbA1c levels. Results from various studies that are conducted at different parts of the world have shown a positive association between basal HbA1c levels[11-13] (Table 1). However, a study on Chinese patients treated with dapagliflozin reported similar decrease in HbA1c levels[14]. (The percentage of patients who responded well to medication was 1.04 and 1.11%, respectively, with $p<0.0001$ for both dapagliflozin doses when compared with the placebo group.) Indian patients using empagliflozin have a mean reduction of 0.8–1.1% in HbA1c levels, which is equal to that reported in studies by Ferrannini et al.[15] and Rosenstock et al.[16]. The less reduction in HbA1c levels may be due to lower HbA1c baseline levels. In a meta-analysis, it was shown that a small but non-significant drop in the HbA1c levels was observed in both Asian and non-Asian patients treated with the same dose of SGLT-2 inhibitors. However, when analyzed per patient's baseline HbA1c value, the reduction in HbA1c levels was very clear. For individuals with higher baseline HbA1c levels, the decreases in HbA1c levels at 3 and 6 months were greater. Early intervention with SGLT-2 inhibitors may assist individuals with long-standing type 2 diabetes achieve their HbA1c goals more rapidly as their basal HbA1c levels are often higher than usual[10,15-17] (Table 2).

At 6 months, Indian patients using dapagliflozin had lost an average weight of 1.86 kg. Most of the weight loss occurred in patients with BMIs greater than 30, who lost an average of 1.60 kg after 3 months and 2.56 kg after 6 months[9]. At 76 weeks of therapy, patients using empagliflozin had a significant reduction in BW of 1.50 kg in adjusted mean weight[10]. Neeland et al. carried out the same study throughout the course of two distinct cohorts, at 12 and 24 weeks. As seen here, after 12 weeks of using empagliflozin, the average weight loss was 1.7 kg, and after 24 weeks, the average weight loss was 1.9

Table 1. Comparison of dapagliflozin as monotherapy and combination therapy in different trials.

Clinical trial	Population	No. of patients	Comparison drug	Primary end point	Results	Weight change in kg	Adverse effects
Viswanathan et al.[9] Dapagliflozin *vs.* placebo (Indian Population)	Treatment naïve patients insufficiently managed on diet and exercise	1,941	Dapagliflozin 10 mg *vs.* placebo	Median HbA1c rise or fall between 3 and 6 months	At 3 and 6 weeks: HbA1c reductions in dapagliflozin and placebo w ere -1.00, -1.04, and +0.02%, respectively.	At 3 and 6 weeks: Reductions in weight with dapagliflozin and placebo were -1.14, -1.86, and -0.72 kg, respectively	Urogenital infections (mainly vulvovaginitis and fungal infection) were more frequent with dapagliflozin than placebo
Bailey et al.[11] Dapagliflozin with metformin *vs.* placebo with metformin (North and South American population)	Diabetic patients inadequately controlled with metformin alone	564	Dapagliflozin 10, 5, 2.5 mg, and placebo with metformin (≥1,500 mg/day)	Percentage reduction from baseline HbA1c at 102 weeks	At 102 weeks: Dapagliflozin 10, 5, 2.5 mg, and placebo reduced hemoglobin A1c by 0.78, 0.58, 0.48, and 0.02%, respectively	Weight loss was 2.86 pounds with dapagliflozin plus metformin and 0.89 pounds with placebo	Genital infections were more frequent with dapagliflozin than placebo
Ferrannini et al.[12] Dapagliflozin*vs.* placebo (Multi-national population)	Treatment naïve patients insufficiently managed on diet and exercise alone with HbA1c between 7 and 10%	485	Dapagliflozin 2.5, 5, and 10 mg daily *vs.* placebo	Percentage reduction from baseline HbA1c at 24 weeks	At 24 weeks: Hemoglobin A1c (HbA1c) decreases for dapagliflozin 2.5, 5, and 10 mg were 0.58, -0.77, -0.89, and -0.23%, respectively	In comparison to the placebo, dapagliflozin 10 mg caused a 3.16-pound weight loss	No major episode of hypoglycemia and signs and symptoms suggestive of urogenital infection were more common in the dapagliflozin group
Nauck et al.[13] Dapagliflozin with metformin *vs.* glipizide with metformin (Multi-national population)	Diabetic patients inadequately controlled with metformin alone	814	Dapagliflozin (≤10 mg/day) with metformin (≥1,500 mg/day) *vs.* glipizide (≤20 mg/day) with metformin (≥1,500 mg/day)	Percentage reduction from baseline HbA1c at 52 weeks	At 52 weeks, HbA1c equally reduced -0.52% from baseline	Dapagliflozin with metformin *vs.* glipizide with metformin -3.22 and +1.44 kg, respectively	Dapagliflozin with metformin *vs.* glipizide with metformin (hypoglycemia 3.4 *vs.* 39.7%) and (urogenital infection 12.3 *vs.* 2.7%)

kg[18]. Similarly, in a clinical study by Bolinder et al., patients using dapagliflozin had a significant reduction in BW of 4.54 kg over a period of 152 weeks[19].

Similar to the outcomes of the trial by Papadopoulou et al.[20], Indian patients using dapagliflozin had a decrease in SBP of 3.24 mmHg at 3 months and 3.77 mmHg at 6 months. Indian individuals using 10 or 25 mg of empagliflozin had smaller reductions in SBP (3.3 and 3.8 mmHg, respectively) compared to the results of the research by Kario et al.[21]. This may be due to the geographical difference in study cohorts. However, the DBP reduction in Indian patients using dapagliflozin and empagliflozin was insignificant.

Various complications can occur in diabetic patients, but the most common is genito-urinary infections that occur mainly due to glycosuria and more common in females. Usually, the patients had mild episodes and resolved with conservative management. Studies in Indian patients had shown that genito-urinary infections were common with dapagliflozin when compared to empagliflozin (2.9 vs. 2.2%)[9,10]. Similar findings were reported by Ridderstråle et al.[17].

Safety analyses of dapagliflozin from many double-blind, placebo-controlled trials found that patients using dapagliflozin had an increase in urine output of around 10%. This effect was observed at recommended dosages of both dapagliflozin

Table 2. Comparison of empagliflozin as monotherapy and combination therapy in different trials.

Clinical trial	Population	No. of patients	Comparison drug	Primary end-point	Results	Weight change	Adverse effects
Gupta et al.[10] empagliflozin 10, 25 mg daily, ***vs.*** placebo ***vs.*** sitagliptin (Indian population)	Type 2 diabetes patients (T2DM) who opt to treat their condition organically (with diet and exercise alone)	108	Empagliflozin 10, 25 mg daily ***vs.*** placebo ***vs.*** sitagliptin	Exploratory effectiveness goals were set using changes from baseline in HbA1c, fasting plasma glucose, body mass, systolic and diastolic blood pressure, and blood sugar levels	At 76 weeks: A significant reduction in hemoglobin A1c was seen with daily empagliflozin 10 and 25 mg, with respective values of -0.81 and -1.11% from baseline, compared to +0.58% in the placebo group and -0.31% in the sitagliptin collective	Compared to placebo and sitagliptin, weight reduction with empagliflozin 10 mg/day was larger (0.39 vs. 0.43 kg) (1.01 vs. 1.16 kg)	When compared to placebo, sitagliptin has a similar effect, although UTIs and vaginal infections occur more frequently
Ridderstråle et al.[17] Empagliflozin ***vs.*** glimepiride (Multi-national population)	Type 2 DM patients insufficiently managed on metformin, diet and exercise with HbA1c of HbA1c ≥7 and ≤10%	1,549	Glimepiride 1–4 mg daily vs. empagliflozin 25 mg daily	Percentage reduction from baseline HbA1c at 104 weeks	Empagliflozin25 mg and glimepiride 1–4 mg/day both reduced HbA1c by 0.11% from baseline after 104 weeks	Empagliflozin superior to glimepiride in reducing weight	Hypoglycemic events 2 and 24% in empagliflozin and glimepiride, respectively
Ferrannini et al.[15] Empagliflozin ***vs.*** metformin (Multi-national population)	Patients with a body mass index (BMI) of 40 kg/m^2 and inadequate glycemic management (HbA1c >7.0 to <10.0).	224	Empagliflozin 5, 10, and metformin	Percentage reduction from baseline HbA1c at 78 weeks	At 78 weeks: HbA1c reduction in empagliflozin 5 mg, 10 mg, and metformin were -0.34, -0.47, and -0.56%, respectively	Empagliflozin 5, 10 mg, and metformin -2.2, -2.6, and -1.3 kg, respectively	Genital infections were more frequent with empagliflozin than metformin
Rosenstock et al.[16] Empagliflozin with insulin ***vs.*** placebo ***vs.*** insulin (Multi-national population)	Obese patients (BMI >30 and <45 kg/m^2) with T2DM and insufficient glycemic control (HbA1c >7.5 to <10% at screening) despite diet and exercise counseling and treatment with MDI insulin (total daily dose>60 IU) alone or in combination with metformin (immediate or extended release, equal or more than 1,500 mg/day	563	Empagliflozin 10, 25 mg, and placebo with basal insulin in each group	Percentage reduction from baseline HbA1c at 52 weeks	At 52 weeks: HbA1c changes in empagliflozin 10 ,25 mg, and placebo were -1.18, -1.27, and -0.81%	Empagliflozin 10 mg, 25 mg, and placebo were -1.95, -2.04, and +0.44 kg, respectively	Empagliflozin vs. placebo hypoglycemia 15.4 ***vs.*** 15.6% Genital infection 58 ***vs.*** 51.1%

and empagliflozin. This is also the case with empagliflozin, suggesting that euglycemic ketoacidosis is a potential adverse effect while using SGLT-2 inhibitors. The FDA and EMA have issued warnings about SGLT-2 inhibitors that these agents can cause diabetic ketoacidosis (DKA) (the body produces large amounts of ketone bodies, namely, acetone, acetoacetate, and beta-hydroxybutyrate), which is a serious complication that may require hospitalization. Patients with a history of DKA, those with type 2 diabetes and low C-peptide levels, those with LADA, chronic pancreatitis, severe dehydration, severe alcoholism, and acute medical and surgical illnesses, and those with decreased food intake are also at risk. The safety of these drugs and their dosage in patients with high risk of DKA[22] is continuously investigated by the FDA.

CONCLUSION

Diabetes is one of the several difficult-to-manage chronic diseases in the world. The development of new medications is underway with the expectation that they will have fewer negative effects and allow for more regulatory precision. Complications from diabetes may be avoided with good glycemic control. As a result, we need additional medications to maintain normal blood sugar levels. In the fight against type 2 diabetes, novel adjuvants that inhibit SGLT-2 are being used. These SGLT-2 inhibitors are investigational kidney-specific diabetic treatments. By increasing the glucose excretion in the urine, dapagliflozin and empagliflozin enhance the glycemic control. Additionally, the diuretic actions of these medications lower blood pressure and BW. Improved lipid values have also been observed.

AUTHORS' CONTRIBUTIONS

AV: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **RR:** Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft.

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Where it reads:
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It should read:
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