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Anxiety and Fear Caused by the COVID-19 Pandemic During the Intervention of Emergency Surgical Cases in Surgical Physicians in Türkiye

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Abstract

Aim: We aimed to determine the anxiety and fear of surgical physicians regarding COVID-19, their fears in emergency surgical cases and their views on the precautions taken.

Material and Methods: Anxiety and fear scales adapted to Turkish and a questionnaire with 20 questions prepared by ourselves were used. Online questionnaires were applied to 275 physicians using the Google forms application. Mann-Whitney U, Chi-square, Kruskal Wallis, linear and binary logistic regression tests were applied.

Results: A significant difference was found in total anxiety and fear score between gender, additional personal protective equipment (PPE) use or not, COVID-19 effect in medical decisions or not and having total anxiety and total fear or not. Surgeons who thought they were infected with COVID-19 infection from the hospital had higher fear score means than those who did not. Surgeons with two children had higher anxiety scores In binary logistic regression, having female gender increased fear of COVID-19, using additional PPE increased COVID-19 anxiety and fear of COVID-19, having COVID-19 fear-anxiety increased fear of COVID-19. İn lineer regression the fear of COVID-19 explained the level of COVID-19 anxiety as much as 39% of the variance.

Conclusion: Anxiety and fears may be more common in surgical branches. While hospitals are performing their duties, physicians and healthcare professionals should absolutely obey the rules and not show the slightest negligence.

Keywords: Anxiety, fear, surgical branches, gender, COVID-19

INTRODUCTION

Pandemic declared on March 11.2020 by the World Health Organization (WHO). Physicians working in the most risky units in Turkey have also been affected by the COVID-19 pandemic, which has devastating effects all over the world. They experience intense anxiety and fear due to the pandemic (1). Surgical physicians come first among the groups of physicians at risk (2). Although the Ministry of Health has postponed emergency surgical cases other than elective cases, there are many cases that fall under the definition of emergency surgery cases. All surgical physicians continue to perform emergency operations related to their specialty. In emergency surgical cases, it is necessary to be fast because the life of the patient is in question and some very important security procedures can be ignored (3).

Medical treatment should be preferred if possible. If urgent treatment is needed (haemodynamic instability, life-threatening complications etc…), the surgeon should check whether the operating room and the technical team are adequately equipped and suitable. In this case, the golden rule is to work with the least number of personnel who have taken security measures (4). Cancer cases if not operated the stage will jump, benign diseases, who are urgent need for surgery and patients, who are life threatening if not operated, should be evaluated within the framework of a multidisciplinary approach by anesthesia, related surgery and internal branches (4). Patients who do not show COVID-19 symptoms, have no radiological findings, and have a negative PCR test can be taken into the operating room with standard precautions. COVID-19 testing and risk assessment should be performed at regular intervals by the entire team (5). The names of everyone in the surgical

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team participating in the surgery should be recorded to facilitate contact follow-up (6-7).

Unfortunately, from the beginning of the pandemic, many surgeons have been infected with COVID-19 infection. Not only are they at risk, they also put their immediate environment and families at risk in terms of infection. They fear that the infection will be transmitted to them and to their relatives and loved ones (8).

In this study, we aimed to determine the anxiety and fear of surgical physicians in intervening emergency surgical cases related to COVID-19 infection, whether they make medical treatment decisions because of COVİD-19 and their opinions about the precautions to be taken.

MATERIAL AND METHOD

Design

This study was a descriptive study. The sample account was made through the open epi info website. We calculated the sample account at 90% confidence interval, p=0.05, q=0.05. When we searched the Ministry of Health Statistics, we found 55000 surgical specialists and emergency medicine specialists in Turkey. We found that when the 55000 number was taken as the population, we need to survey at least 270 physicians. Online questionnaires were applied to 275 physicians using the Google forms application. Consent was obtained from people online while applying the survey. Permission was obtained from the Non-Invasive Ethics Committee of Cumhuriyet University Faculty of Medicine. Decision No: 2021-2/25 Date: 10.02.2021

Statistic

The sociodemographic data form created by us, the COVID-19 Anxiety Scale (CAS) and the COVID-19 Fear Scale were applied to the participants by the researchers. The necessary permissions for the scales were obtained from their authors. The cronbach alpha value of the CAS was 0.889 and the cronbach alpha value of the COVID-19 Fear Scale was 0.893.

Sociodemographic data form; It consists of a total of 20 questions questioning age, gender, marital status, number of children, living place, presence of chronic desease, occupation year, specialty, hospital and physicians' status during the pandemic process.

CAS is a reliable scale with robust factorial (single-factor; invariance between sociodemographics) and structural (correlate with anxiety, depression, suicidal ideation, and substance /alcohol coping) validity (>0.90). Each item is rated on a 5-point scale from 0 (not at all) to 4 (almost daily) based on experience over the past two weeks. CAS total score ≥9 indicates dysfunctional anxiety associated with coronavirus. High scores on a particular item or a high total scale score (≥9) may indicate an individual's problematic symptoms that may require further evaluation and /or treatment (9). It was adapted to Turkish by Evren et al (10).

The COVID-19 Fear Scale was developed by Ahorsu et al. (11) to measure the fear levels of individuals during the COVID-19 pandemic. The adaptation of the scale to Turkish was carried out by Haktanır et al. (12) The Turkish version of the scale was used in our study. The answer to each question in the COVID-19 Fear Scale, which is a Likert-type scale consisting of 7 questions, consists of five scores ranging from 1 (strongly disagree) to 5 (strongly agree). The minimum score on the scale is 7, the maximum score is 35, and high scores indicate that individuals have more fear associated with coronavirus. In our study participants were divided into two groups (0-15 as low, ≥16 as high) based on the median (was calculated as 15) fear value.

Spss 21 program (IBM SPSS Corp.; Armonk, NY, USA) was used for analysis. Whether the data was normally distributed or not was determined by the Kolmogorov-Smirnov test. The differences between the two groups in terms of anxiety and fear levels were analyzed using the Mann-Whitney U test. The nonparametric conditions between the means of more than two groups were determined using the Kruskal Wallis test. Binary and simple lineer logistic regression tests were applied. While conducting the logistic regression models, COVID-19 anxiety and fear scores were accepted as two groups as low (reference category) and high. To determine the relationship between the COVID-19 anxiety, fear levels and sociodemographic characteristics of surgical physicians; age, gender, marital status, number of children, occupation year, living place and having chronic desease were included in the model. To determine the relationship between the COVID-19 anxiety, fear levels of surgical physicians and categories related to pandemic process; overcoming COVID-19 disease, COVID-19 hospital contagion, COVID-19 fear-anxiety, creating COVID-19 algorithm for hospital surgeries, measures taken are sufficient, pre-operative analysis test, PPE provided in the hospital, use of additional PPE and medical treatment decision making were included in the model. $p<0.05$ was considered statistically significant.

RESULTS

To this survey 59 general surgery (21.5%), 45 orthopedics and traumatology (16.4%), 32 gynecology and obstetrics (11.6%), 23 eye diseases (8.4%), 21 ear-nose-throat diseases (7.6%), 19 urology (6.9%) physicians, 3 jaw surgeons (1.1%) participated.

Of the participating physicians, 85 were in a training and research hospital (30.9%), 83 were in a state hospital (30.2%), 51 were in a private hospital (18.5%), 24 were in a city hospital (8.7%), 23 of them were working in university hospitals (8.4%), 3 were in numune hospitals (1.1%), 3 were in branch hospitals (1.1%), 3 were in office (1.1%).

Of the physicians participating in the study, 234 (85.1%) thought that patients did not experience victimization due to COVID-19, and 41 (14.9%) of them thought that they suffered from COVID-19.

Of the physicians participating in the study, 209 (76%) stated that the rules that should normally be followed in operating rooms began to be followed more strictly due to

COVID-19, it had a good effect, and 69 (24%) stated the opposite.

Table 1 shows the distribution of surgical physicians' sociodemographic characteristics according to total COVID-19 anxiety and total COVID-19 fear score means. Total anxiety (p=0.003) and fear (p=0.005) score means of female surgeons were higher than male surgeons. The anxiety score means of surgeons with two children were higher than surgeons with one (p=0.028) or three and more children (p=0.009).

Table 2 shows the distribution of surgical physicians' characteristics related to COVID-19 pandemic according to total COVID-19 anxiety and total COVID-19 fear score means. Surgeons who thought they were infected with COVID-19 infection from the hospital had higher fear score means than those who did not think of hospital contagion (p=0.033). Surgeons who stated that they experienced anxiety and fear due to COVID-19 while intervening in emergency surgical cases, who stated that they obtained and used additional PPE other than PPE provided by the hospital, and who stated that they applied postponement or medical treatment due to the anxiety and fear of COVID-19 in cases where they could not postpone surgery or apply medical treatment before the pandemic had higher COVID-19 anxiety and fear score means (p=0.001).

Table 1. Distribution of surgical physicians' sociodemographic characteristics according to total COVID-19 anxiety and total COVID-19 fear score means

Characteristic	n	%	Total Anxiety (X ± SD)	Total Fear (X ± SD)
Total	**275**	**100.0**	**2.5±3.6**	**16.9±6.4**
Age				
20-30	22	8.0	2.4±3.7	17.4±6.6
31-50	226	82.2	2.4±3.5	16.8±6.3
51 and Over	27	9.8	3.1±4.5	17.9±6.7
			KW=1.317 p=0.518	KW=0.529 p=0.768
Gender				
Male	232	84.4	2.2±3.5	16.5±6.1
Female	43	15.6	3.7±4.1	19.8±7.2
			U=6330.5 p=0.003	U=6326.5 p=0.005
Marriage				
Married	239	86.9	2.4±3.5	16.8±6.1
Widow / Divorced	36	13.1	3.1±4.2	18.3±7.7
			U=4629.0 p=0.441	U=4645.0 p=0.440
Number of children				
0	44	16.0	2.9±4.5	17.4±7.1
1	89	32.4	1.9±2.9	16.3±6.0
2	111	40.4	2.9±3.6	17.7±6.3
3 and over	31	11.3	1.9±3.7	15.9±6.4
			KW=8.947 p=0.030*	KW=3.511 p=0.319
***Significant difference (U; p)= 3 and over-2 (40.255; 0.009), 1-2 (-23.691; 0.028)**				
Living place	189	68.7	2.8±3.9	17.4±6.4
Metropolis	63	22.9	1.7±3.0	16.1±6.4
City	23	8.4	2.2±2.3	15.5±5.9
District			KW=5.795 p=0.055	KW=3.451 p=0.178
Chronic desease				
Yes	57	20.7	2.9±3.6	18.2±6.1
No	218	79.3	2.4±3.6	16.6±6.4
			U=5361.5 p=0.095	U=5248.0 p=0.071
Occupation year				
0-5	22	8.0	3.0±4.9	17.5±5.9
6-10	76	27.6	1.9±2.8	15.7±5.4
11-15	82	29.8	2.2±3.1	16.7±5.6
16 and over	95	34.5	2.9±4.2	18.1±7.5
			KW=1.502 p=0.682	KW=4.703 p=0.195

X mean, SD standard deviation, U Mann-Whitney U Test, KW Kruskal-Wallis Test

Table 2. Distribution of surgical physicians' characteristics related to COVID-19 pandemic according to total COVID-19 anxiety and total COVID-19 fear score means

Characteristic	n	%	Total Anxiety (X ± SD)	Total Fear (X ± SD)
Overcoming COVID-19 disease				
Yes	121	44.0	2.2±3.2	16.9±5.7
No	154	56.0	2.8±3.9	16.9±6.8
			U=9896.5 p=0.353	U=9091.0 p=0.730
COVID-19 hospital contagion				
Yes	65	23.6	2.7±3.3	18.3±5.2
No	26	9.5	2.4±4.7	15.0±5.1
Keep up with COVID-19	184	66.9	2.4±3.6	16.8±6.8
			KW=2.619 p=0.270	KW=8.280 p=0.016*
*Significant difference (U; p)= No- Yes (46.827; 0.033)				
COVID-19 fear-anxiety				
Yes	207	75.3	2.9±3.7	18.2±6.3
No	68	24.7	1.2±3.1	13.3±4.9
			U=4555.5 p=0.001	U=3787.0 p=0.001
Creating COVID-19 algorithm for hospital surgeries				
Yes	224	81.5	2.5±3.7	16.9±6.5
No	51	18.5	2.3±3.5	17.3±5.9
			U=5435.5 p=0.571	U=5996.5 p=0.578
Measures taken are sufficient				
Yes	146	53.1	1.9±3.1	16.3±6.2
No	129	46.9	3.0±4.1	17.2±6.5
			U=10602.0 p=0.059	U=10663.5 p=0.058
Pre-operative analysis test				
Yes	169	61.5	2.2±3.3	17.0±6.3
No	106	38.5	2.9±4.0	16.9±6.5
			U=9519.5 p=0.358	U=8866.0 p=0.887
Is PPE provided in the hospital?				
Yes	219	79.6	2.2±3.4	16.9±6.4
No	56	20.4	3.4±4.4	17.5±6.3
			U=6927.0 p=0.116	U=6558.0 p=0.422
Use of additional PPE				
Yes	138	50.2	3.4±4.1	19.1±6.5
No	137	49.8	1.6±2.8	14.8±5.4
			U=6600.5 p=0.001	U=5720.5 p=0.001
Medical treatment decision making				
Yes	129	46.9	2.9±3.8	17.9±6.7
No	146	53.1	2.0±3.4	16.1±5.9
			U=7692.5 p=0.006	U=7901.5 p=0.021

X mean, SD standard deviation, U Mann-Whitney U Test, KW Kruskal-Wallis Test, PPE personal protective equipment

Table 3 shows the total COVID-19 anxiety and fear scores distribution of surgical physicians. Most of the surgeons involved in our study had a low COVID-19 anxiety level (90.9%), but most had a high COVID-19 fear level (53.8%).

Table 4 shows the odds ratio between the COVID-19 anxiety, fear levels and sociodemographic characteristics of surgical physicians. No significant relationship was found between COVID-19 anxiety levels and sociodemographic characteristics. But having a female gender increased the COVID-19 fear 2.27 times (p=0.040).

Table 5 shows the odds ratio between the COVID-19 anxiety, fear levels of surgical physicians and categories related to pandemic process. Using additional PPE increased the COVID-19 anxiety 5.04 times (p=0.006) and increased the COVID-19 fear 2.97 times (p=0.001). Having COVID-19 fear-anxiety increased the COVID-19 fear 2.34 times (p=0.008).

Table 6 shows simple linear regression analysis results of the predictive effect of COVID-19 fear on COVID-19 anxiety level. It was observed that COVID-19 fear significantly affected COVID-19 anxiety (β=0.628; t=13.320; p=0.001). Fear of COVID-19 explains the level of COVID-19 anxiety up to 39% of the variance (R2=0.394, F=177.423, p=0.001). In other words, it was found that as the COVID-19 fear levels of surgeons increased, COVID-19 anxiety levels increased.

Table 3. Total COVID-19 anxiety and fear scores distribution of surgical physicians

	Total Anxiety Scores*			Total Fear Scores**		
	Scores	**n**	**%**	**Scores**	**n**	**%**
Low	8 and less	250	90.9	15 and less	127	46.2
High	≥ 9	25	9.1	≥ 16	148	53.8
	Total	275	100.0	Total	275	100.0

* According to the scale, if the total anxiety score is 9 and above, it means that anxiety is significant
** The higher the total score according to the scale, the higher the fear. Participants were divided into two groups based on the median (was calculated as 15) fear value

Table 4. The odds ratio between COVID-19 anxiety, fear levels and sociodemographic characteristics of surgical physicians (n= 275)

	Total Anxiety[1] OR(95%CI)	Total Fear[1] OR(95%CI)
Gender[2]		
Female	1.92(0.64-5.82)	2.27(1.04-4.98)*
Age[3]		
20-30	1.26(0.12-13.17)	1.81(0.41-7.99)
31-50	1.15(0.26-5.18)	1.22(0.46-3.22)
Marital status[4]		
Married	1.17(0.25-5.48)	1.31(0.47-3.63)
Number of children[5]		
1	0.85(0.17-4.34)	1.18(0.43-3.23)
2	0.82(0.15-4.54)	1.17(0.40-3.43)
≥ 3	1.05(0.13-8.28)	0.93(0.26-3.26)
Occupation year [6]		
6-10	0.31(0.06-1.63)	0.70(0.25-1.94)
11-15	0.34(0.06-2.04)	1.14(0.38-3.38)
≥16	0.83(0.15-4.64)	1.44(0.47-4.42)
Chronic desease [5]		
Yes	0.56(0.17-1.88)	1.15(0.59-2.21)

n Number of participants, OR Odds ratio, CI Confidence interval, *p<0.05. Reference categories; [1]=Low, [2]=Female, [3]=≥51, [4]=Single + Widow, [5]=None, [6]=≤5

Table 5. The odds ratio between the COVID-19 anxiety, fear levels of surgical physicians and categories related to pandemic process (n= 275)

	Total Anxiety[1] OR (95% CI)	Total Fear[1] OR (95% CI)
Overcoming COVID-19 disease[2]		
Yes	0.35 (0.09-1.36)	0.80 (0.39-1.65)
COVID-19 hospital contagion[3]		
Yes	2.59 (0.59-11.32)	2.02 (0.87-4.70)
No	3.09 (0.53-18.02)	0.72 (0.25-2.10)
COVID-19 fear-anxiety [2]		
Yes	1.54 (0.39-6.03)	2.34 (1.24-4.42)*
Creating COVID-19 algorithm for hospital surgeries[2]		
Yes	3.10 (0.81-11.89)	1.18 (0.58-2.43)
Measures taken are sufficient[2]		
Yes	0.38 (0.14-1.01)	0.72 (0.40-1.31)
Pre-operative analysis test[2]		
Yes	0.76 (0.31-1.90)	1.18 (0.68-2.05)
Is PPE provided in the hospital?[2]		
Yes	0.84 (0.29-2.46)	0.94 (0.44-2.02)
Use of additional PPE[2]		
Yes	5.04 (1.58-16.06)*	2.97 (1.72-5.11)**
Medical treatment decision making[2]		
Yes	0.98 (0.40-2.44)	1.09 (0.64-1.85)

n Number of participants, OR Odds ratio, CI Confidence interval, PPE personal protective equipment, *p<0.05, **p<0.001
Reference categories; [1]=Low, [2]=No, [3]=Keep up with COVID-19

Table 6. Simple linear regression analysis results of the predictive effect of COVID-19 fear on COVID-19 anxiety level

Model	B	Ss	β	t	p
Total fear	0.358	0.027	0.628	13.320	0.001

R^2=0.394; F=177.423; p=0.001

DISCUSSION

The unexpected results were that the fear scores were 15 and less in 127 phisician (46.2%) and the anxiety scores were 8 and less in 250 phisician (90.9%). A study conducted with the 451 participants in Turkey, during COVID-19 pandemic, it was concluded that about 40% of individuals generally had high level of anxiety (13). This was a finding inconsistent with the finding in this study. In this study, anxiety levels were found to be extremely low. It can be expected that COVID-19 will not increase their anxiety levels, especially since it was a study performed by surgical physicians and they are routinely busy with an alarming job. There were no significiant difference between total anxiety and total fear scores by age, by marital status, by place of life, by profession year, by specialty branch, total anxiety and fear scores according to the hospital studied, the total anxiety and fear scores due to overcoming COVID-19, between the total anxiety and fear scores according to the algorithm creation in hospitals, according to the situation of taking adequate precautions. There were no significant difference between total anxiety and total fear scores according to preoperative analysis and examination ability, and between procurement of PPE. In a study conducted in healthcare professionals, no significant difference was found between overcoming COVID-19 and insomnia and anxiety. This was a parallel finding with this study (14). In a study conducted with emergency healthcare workers, no significant difference was found between marital status, title, having a chronic disease, overcoming COVID-19 or not, and anxiety score. Similar results were found in this study. There are also studies with significant differences in terms of marital status. When looking at the effect of the marital status of the participants on the scales in a study conducted with 371 participants; the single group average was found to be significantly different and greater than the married group average (15). There are studies in the literature where there was no difference between title (16), having a chronic disease (17) and anxiety score. No

significant difference was found between PPE supply and anxiety and fear scores. However, in some studies, it was stated that when there was a problem in PPE supply, this increased anxiety and fear (18). In this study there was no significant difference because Turkey provided protective equipment. Already, 79.6% of the participants stated that PPE was given in the hospital. In a study conducted with emergency health care workers, a significant difference was found between the professional year and the level of anxiety. Anxiety level was found to be higher in those with at least 11 years of experience. In this study, a significant effect of the professional year was not observed. It can be thought that the experience decreases the level of anxiety, since the study was conducted with surgeons predominantly in surgery (19). In a study conducted on anesthesiologists in Bursa, the fear of COVID-19 was found to be significantly different according to gender, occupational experience, title, hospitals, and having a chronic disease. İn this study total anxiety and total fear scores of Covid-19 was statistically different between gender, additional PPE use, COVID-19 effect in medical treatment decision and total anxiety and fear scores (20). In this study total anxiety score was statistically different between number of children. (KW=8.947 p=0.030, Significant difference (U;p)= 3 and over-2 (40.255;0.009), 1-2 (-23.691;0.028), total fear score was statistically different between COVID-19 hospital contagion (KW=8.280 p=0.016*, Significant difference (U;p)= No- Yes (46.827; 0.033). Significantly high anxiety and fear scores of those who have more children and think that they are infected by hospital are expected findings. In a study with anesthesiologists, fear of COVID-19 was statistically significantly higher in women, specialist physicians, and participants with chronic diseases (p=0.003, p=0.024 and p=0.014, respectively). The study conducted on anesthesiologists consisted of 65.2% female participants (20). When evaluated in terms of gender variable in a study conducted on 371 participants; COVID-19 fear mean scores of women were found to be significantly different and higher than men. A significant difference was found between the levels of anxiety in favor of female employees in a study conducted with emergency health care workers (15). There are studies in the literature that support this study's finding in favor of men in the study (21). This study found a difference in favor of men. A significance in linear regression was found between total anxiety and total fear scores.

A modeling has been established that the fear of COVID-19 affects COVID-19 anxiety by 39%. The relationship between the COVID-19 anxiety, fear levels and sociodemographic characteristics of surgical physicians. In a binary logistic regression model, having a female gender increased the COVID-19 fear 2.27 times (p=0.040), using additional PPE increased the COVID-19 anxiety 5.04 times (p=0.006) and increased the COVID-19 fear 2.97 times (p=0.001), having COVID-19 fear-anxiety increased the COVID-19 fear 2.34 times (p=0.008). The effects within the model are consistent with the Mann Whitney U and Kruskal Wallis test results.

Of the physicians participating in the study, 234 (85.1%) thought that patients did not experience victimization due to COVID-19, and 41 (14.9%) of them thought that they suffered from COVID-19. In a study conducted on neurosurgery patients, it was found that patients experienced moderate fear due to pandemic and 16.1% of the patients postponed their follow-up dates at least once due to this fear. In this study, physicians stated that 14.9% of patients experienced victimization due to COVID-19. Findings that support each other can be called. Due to COVID-19, the majority of the healthcare organization deals with pandemic patients, so routine follow-ups and surgeries are disrupted (22).

The rate of those who stated in the study that COVID-19 was effective in making medical treatment decisions is 46.9%. They also stated that 14.9% of the patients were victims. The total anxiety and total fear scores of those who said COVID-19 effective while making a medical treatment decision were also found to be significantly higher. Due to COVID-19, some of the patients may lose the chance of surgical treatment or their disease may progress. If patients who can be treated with surgery at an early stage due to COVID-19 are directed to medical treatment, poor results in terms of mortality and morbidity occur. According to COVID-19, when making a decision on surgical treatment and medical treatment, by creating guidelines, making decisions will reduce patients. In addition, it is necessary not to put the surgeons and the operating room team at risk while evaluating the patients sensitively.

53.1% of those participating in the study stated that adequate precautions were taken in their hospitals, 81.5% of them that algorithms were created for operating rooms, and 76% of the rules in operating rooms were followed more strictly after COVID-19. In a study conducted in Jordan, only 28.2% of the doctors were satisfied with the infection control policy in their institutions and only 19.8% felt safe in the workplace (23). This difference may be due to the fact that this study was carried out close to the first year of COVID-19 and the time required for taking adequate measures has passed.

The rate of those who purchased PPE other than PPE provided by their hospitals and used additional PPE themselves is 50.2%. It also made a significant difference in terms of fear and anxiety scores. In a survey study conducted on anesthesiologists, approximately one fourth of anesthesiologists stated that they did not have difficulty in procuring any PPE and only 18% stated that they did not buy any materials themselves. In the study, it was determined that PPEs such as respirator masks, glasses and visors were purchased by themselves (24). In this study, the rate of additional PPE procurement was found to be much lower. However, anxiety and fear were found to be significantly higher in those who provided additional PPE. The reason for this difference may be that this study was conducted in March 2021 and the other study was

conducted in August 2020.

CONCLUSION

In this study, anxiety and fear scores were found to be lower than expected. The low level of anxiety and fear is a great advantage when performing surgeries, especially in surgical branches. In order to sustain this situation and reduce anxiety and fears, hospitals must obey the algorithms created for COVID-19, provide complete PPEs, and make all necessary arrangements in operating rooms. While hospitals are performing their duties, physicians and healthcare professionals should absolutely obey the rules and not show the slightest negligence.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Permission was obtained from the Non-Invasive Ethics Committee of Cumhuriyet University Faculty of Medicine. Decision No: 2021-2/25 Date: 10.02.2021*

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MEDICAL RECORDS-International Medical Journal

Research Article

Clinical and Histopathologic Efficiency of Sucralfate and Ursodeoxicolic Acid in Pediatric Duodenogastric Reflux Disease

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Abstract

Aim: The treatment approach, long-term consequences and surveillance protocols of duodenogastric reflux disease (DGRD) are not well established in the pediatric population. The aim of this study was to evaluate the histopathological and clinical responses to treatment with Ursodeoxicolic Acid (UDCA) and Sucralfate in children diagnosed with DGRD.

Material and Methods: This is a retrospective pre-post design study performed with children admitted to our clinic with reflux symptoms and diagnosed with duodenogastric reflux disease according to endoscopic and histopathologic evaluation. Patients were treated with Sucralfate 60mg/kg/day orally and UDCA at 10 mg/kg/day orally, for 6 months. We compared symptoms/findings, presence of Helicobacter pylori and histopathologic grade of disease before and after treatment.

Results: The presence of all symptoms statistically significantly decreased after treatment. The presence of Helicobacter pylori decreased from 43.8% to 21.9%. There was also statistically significantly histopathologic improvement after six-month treatment of Sucrafate and UDCA.

Conclusion: Six-month treatment of Sucralfate and UDCA provided valuable improvements in clinical and histopathologic features in pediatric patients with DGRD.

Keywords: Duodenogastric reflux disease, sucralfate, ursodeoxycholic acid

INTRODUCTION

While there is a consensus on the definition of GERD, there is limited guidance on the definition of duodenogastric reflux (DGR) and/or duodenogastric reflux disease (DGRD) in children. DGR is described as the reflux of duodenal contents into the stomach and it becomes a pathological entity when it is extreme and lasts for a long time (1). DGR is associated with gastric surgery in adults (2). However, the incidence and etiology in children is not clear. In a study with 1120 children, 92 (8.21%) had bile reflux on endoscopy (3). Primary DGR has not been documented in children and concluded that this was due to difficulties in diagnosis (4).

DGRD is important as the injuries can predispose to gastric ulcers and there is a possibility of malignant transformation (5,6). Gross anatomic and histopathologic changes in DGR are diverse. Gastric mucosa inflammation, ulceration, intestinal metaplasia can be seen (3). In a 2012 study, which investigated the histological features of DGR in children showed lymphatic follicles, intestinal metaplasia, foveolar hyperplasia, interstitial edema, vascular congestion and fibroproliferation (5).

The treatment of DGR is mainly symptomatic in children. There are treatments in children with sucralfate and cisapride (4). Sucralfate adheres to mucosal surface and promote healing and also protect the surface from peptic injury. However, sucralfate is not recommended for treatment of chronic GERD (7). In the past, some studies suggested ursodeoxycholic acid (UDCA) in the treatment of DGR (8,9).

MATERIAL AND METHOD

Study design and setting

The study was carried out in conformity with the Declaration of Helsinki after obtaining the approval of

CITATION
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This is a retrospective before-after trial, in which we studied the efficiency of the treatment of UDCA and Sucralfate on clinical and histopathological findings of the DGRD in a pediatric population. Medical records of patients who admitted with reflux sign and symptoms between January 2014 and June 2017 who underwent upper GI endoscopy, were scanned. Data was obtained from the electronic medical records of the hospital. The hospital is a tertiary level university hospital with a bed capacity of 983, and has a pediatric gastroenterology clinic which offers diagnostic and treatment services, including pediatric endoscopy and biopsy, with experienced medical staff.

Sample size and patients

We did not estimate a priori minimum sample size, we intended to include all eligible patients according to inclusion and exclusion criteria. Inclusion criteria of the study were: (1) admission to our clinic with reflux signs and symptoms, and (2) undergoing esophagogastroduodenoscopy and being diagnosed with DGRD. We defined diagnosis of DGRD as the presence of bile in stomach in the esophagogastroduodenoscopy. Exclusion criteria of the study were: (1) having gastroenteritis or upper respiratory tract infection at the time of endoscopy, (2) receiving long-term NSAID treatment, (3) having received prior *Helicobacter (H) pylori* eradication therapy, and (4) missing patient data.

Endoscopic evaluation

Upper GI endoscopy was performed in all patients by an 12 year experienced pediatric gastroenterologist using EG-530WR endoscopic equipment (Fujifilm Co., Japan). Patients were fasted for 6 to 8 hours before endoscopy. The patients were sedated initially with 0.1mg/kg of midazolam and 1mg/kg of ketamine intravenously, during procedure, an additional dose of sedation was given as necessary. During endoscopy, esophagus, Z line, cardia, fundus, corpus, antrum, pylorus, bulbus and duodenum were examined, respectively. All patients were assessed for findings of endoscopic gastritis, such as erythema, hyperemia, atrophy, and mucosal nodularity following the criteria of the Houston-updated Sydney system. A minimum two histopathological sampling (one each from the antrum and the duodenum) were performed, and sent for the histopathological examination in Hollande solution.

Histopathologic evaluation

The histopathologic examinations were performed by a 25-year experienced pathologist using a light microscope (Olympus Corp., Tokyo, Japan) with 100X and 200X magnifications. Five-micrometer-thick sections were prepared from all obtained biopsy specimens, and the tissue sections were stained with hematoxylin and eosin and Diff-Quick stain for histopathological examination. Histopathologic evaluation included detection of *H. pylori* and histopathologic findings of inflammation. The biopsies were graded using the Houston-updated Sydney system: normal (Grade 0), mild inflammation (Grade 1), moderate inflammation (Grade 2), and severe inflammation (Grade 3).

Treatment and follow-up

All the patients were treated with sucralfate 60mg/kg/day (suspension or tablet) orally and UDCA 10mg/kg/day orally, for 6 months. Patients were followed-up with a planned visit once a month for the continuity of treatment, and for the presence of the reported signs and symptoms. At the end of the six-month treatment period, all patients underwent upper GI endoscopy, and control samples were taken for histopathologic evaluation.

Variables and outcomes

Patients' demographics, baseline characteristics, magnetic resonance cholangiopancreatography (MRCP) reports, endoscopic findings, and follow-up changes of these features, were recorded. We calculated body mass index (BMI) by dividing a patient's weight in kilograms by the square of height in meters, and then, we defined the weight status using age and sex specific percentiles for BMI which were defined for Turkish children (10). We categorized patients who were less than the 5th percentile as underweight, who were between 5th percentile and less than 85th percentile as normal weight, who were between 85th percentile and less than the 95th percentile as overweight, and who were 95th or greater percentile as obese. We reviewed the MRCP reports to determine the presence of any anatomic abnormality of biliary tract and pancreatic duct.

There were two primary outcomes in the study; changes in clinical features and histopathological changes. We defined the changes in clinical features as the changes in the prevalence of signs and symptoms (epigastric burning pain, dyspepsia, nausea, vomiting with bile, loss of appetite, and weight loss or inadequate weight gain), and the histopathological changes as the presence of *H. pylori* and histopathologic grade of disease. We categorized the histopathologic grade as normal, mild, moderate and severe based on the Houston-updated Sydney system (11).

Statistical analysis

Statistical analyses were run using SPSS version 20 (IBM Corp. in Armonk, NY). Descriptive data are displayed as frequency and percentage for categorical variables, and median with interquartile range for numerical variables. Related-Samples McNemar Test was used for comparing dichotomous variables, and Related-Samples Marginal Homogeneity Test was applied for comparing ordinal variables among pre and post treatment evaluations. A value of $p<0.05$ was considered statistically significant.

RESULTS

This retrospective study was performed with the patients admitted to our clinic with reflux signs and symptoms, underwent esophagogastroduodenoscopy and diagnosed with DGRD. 4000 patients who underwent endoscopy were scanned through archive scanning in digital media, 3958 of them admitted with reflux signs and symptoms and 186 patients diagnosed with DGRD according to the endoscopic and histopathologic evaluation. After excluding 154 patients, 32 patients were included in the study (Figure 1).

In the present study, a total of 32 pediatric patients with DGRD, all are in adolescence period, were included, treated and followed during 6 months. We discuss the effectiveness of a combined treatment with sucralfate 60mg/kg/day (suspension or tablet) orally and UDCA at 10mg/kg/day orally, for 6 months. We demonstrated the statistically significant improvement in every clinical signs and symptoms, including epigastric pain and nausea, dyspepsia, vomiting, weight loss and loss of appetite. Also, upper GIS endoscopy were repeated after the 6-months treatment period in all patients and we showed statistically significant decrease in the presence of *H. pylori* and statistically significant improvement in histologic features of gastric mucosa.

The median age of the children was 15.0 years(min:13.0-max:17.0), and girls were 81.3% of the study population. Ten patients were underweight, 14 had normal weight, 3 were overweight and 5 were obese. Six of the patients had kindredship between mother and father, and one patient had a family history for DGRD. Median age at the time of diagnosis was 13.5 years(min:10.3–max:15.0), while the median time from symptom onset to receiving the treatment was 15.0 months(min:12.0–max:33.0). We detected anatomic abnormality in three patients on MRCP (Table 1). One of these patients had prominence in the intrahepatic biliary tract, one underwent cholecystectomy, and one had biliary sludge and dilatation in the intrahepatic biliary tract (data not shown).

The signs and symptoms of the patients before and after treatment, are shown in Table 2. The two most common complaints were epigastric pain and nausea. Dyspepsia, vomiting, weight loss and loss of appetite were other complaints. The presence of all signs/symptoms statistically significantly decreased after treatment (Table 2).

The presence of *H. pylori* statistically significantly decreased from 43.8% to 21.9% (p=0.016). Before the treatment, three patients had normal histologic appearance, 21 patients had mild and 8 patients had moderate histopathological changes. However, the number of the histologically normal patients increased to 13, and the percentage of the mild and moderate histopathologic grades decreased. These changes were statistically significant (p=0.002) (Table 3).

Table 1. Demographics and baseline characteristics

Characteristics (n=32)	
Age (years), median (IQR)	15.0 (13.0-17.0)
Sex (female), n (%)	26 (81.3)
BMI, n (%)	
Underweight	10 (31.3)
Normal weight	14 (43.8)
Overweight	3 (9.4)
Obese	5 (15.6)
Kindredship between mother and father, n (%)	6 (18.8)
Family history, n (%)	1 (3.1)
Age of diagnosis (year), median (IQR)	13.5 (10.3-15.0)
Time from symptom onset to treatment (month), median (IQR)	15.0 (12.0-33.0)
Anatomic abnormality on MRCP, n (%)	3 (9.4)

Note: IQR: Interquartile range, BMI: Body mass index

Table 2. Comparison of signs and symptoms among pre and post treatment period

Signs and Symptoms (n=32)	Pre-treatment, n (%)	Post-treatment, n (%)	p*
Epigastric burning pain	28 (87.5)	6 (18.8)	<0.001
Dyspepsia	21 (65.6)	5 (15.6)	<0.001
Nausea	27 (84.4)	9 (28.1)	<0.001
Vomiting	20 (62.5)	7 (21.9)	<0.001
Loss of appetite	14 (43.8)	7 (21.9)	0.016
Weight loss or inadequate weight gain	15 (46.9)	1 (3.1)	<0.001

* Related-Samples McNemar Test was used

Table 3. Comparison of histopathologic features among pre and post treatment period

Features (n=32)	Pre-treatment, n (%)	Post-treatment, n (%)	p
Presence of Helicobacter pylori	14 (43.8)	7 (21.9)	0.016*
Histopathologic grade			
Normal	3 (9.4)	13 (40.6)	0.002**
Mild	21 (65.6)	17 (53.1)	
Moderate	8 (25.0)	2 (6.3)	

* Related-Samples McNemar Test was used.
** Related-Samples Marginal Homogeneity Test was used

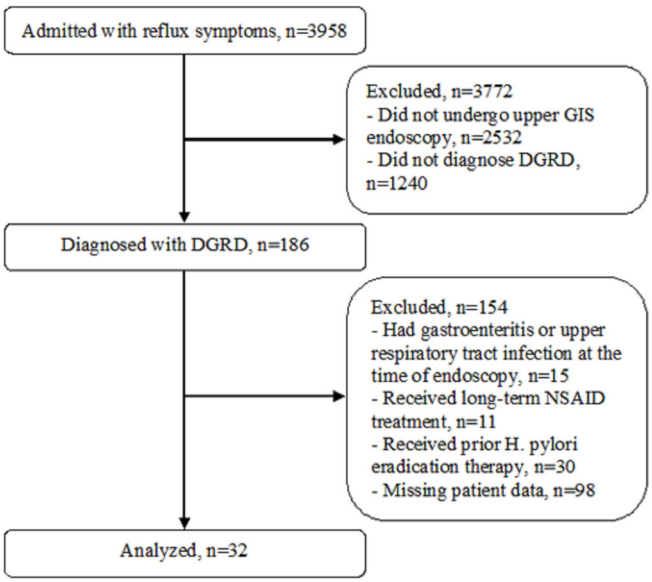

Figure 1. Flow diagram of the study

DISCUSSION

Frequency, clinical implications, treatment approach, long-term outcomes, and monitoring procedures of DGRD are incompletely understood in the pediatric population. In a study, UDCA relieved the symptoms, however, did not make change on histopathological changes, when compared to placebo (8). In our study we showed histopathological and clinical changes in patients. Based on this limited literature, the objective of this study was to investigate the histopathological and clinical responses to combined treatment of UDCA and sucralfate in children diagnosed with DGRD.

The primary medical therapeutic approach for the reflux disease is that proton pump inhibitors reduce acid reflux and relieve reflux symptoms such as epigastric pain (12). However, these drugs are not as good at alleviating all symptoms of patients with reflux disease (13). It was emphasized in a review, the belief that proton pump inhibitors could completely prevent metaplasia was inconsistent, since the proof was all secondary and not supported in randomized controlled trials (14). Therefore, novel therapeutic agents are required for treating reflux disease and prevent its long-term consequences such as metaplasia. Sucralfate is one of the commonly added agents to treat reflux disease because of its selective properties to form a protective antacid layer and its effectiveness against bile acids (4). Our study supports these studies and shows that sucralfate is effective.

Souza has investigated some possible new therapeutic approaches for reflux esophagitis and Barrett's esophagus (15). It was shown that patients with Barrett's esophagus have significant esophageal exposure to bile acids, and some bile acids are more harmful than others (2,16). In support of these studies, in our study, it was shown that the content of bilirubin in patients diagnosed with DGRD harms the stomach and esophagus. In a rat study, refluxed acid and toxic bile salts trigger the production of inflammatory cytokines and that induce cytokine mediated DNA damage in Barrett's cells, and likely contributing to carcinogenesis. Souza, demonstrated that altering bile acids composition with oral treatment of UDCA, which is a hydrophilic bile acid and is not genotoxic, reduces the esophageal DNA damage and cytokine activation resulting from toxic bile acids. (15). In our study, it was shown that UDCA is effective in the treatment of DGRD and plays an important role in both symptom relief and histopathological improvement. On the other hand, the author emphasized that preventing cytokine mediated inflammation -and not just controlling gastric acid- should be another therapeutic approach to relieve reflux induced symptoms and moreover. It was shown in other study that the pretreatment with UDCA decreases oxidative stress, DNA damage and cytokine activation in patients with Barrett's esophagus (17). In a meta-analysis, it was shown that UDCA had therapeutic feature for preventing the inflammatory bowel disease-associated colon cancer in patients with primary sclerosing cholangitis (18). In a way that both supports these studies and makes a new addition, In light of abovementioned fact, we added UDCA therapy to sucralfate as a new therapeutic agent to treat pediatric patients with DGRD.

DGRD is a common physiological process that is generally described as the transition of duodenal material from the duodenum to the stomach (19). Duodenal content leads to proliferation in inflammatory cells in the gastric mucosa, hyperplasia of gastric mucous cells and changes in glandular morphology. Therefore, DGRD has been involved in the pathogenesis of upper gastrointestinal

disorders such as esophagitis, gastritis, gastric ulcers, gastric adenocarcinoma and also intestinal metaplasia of the gastric mucosa (19,20). In adults, diagnostic approach, treatment and follow-up procedures are arranged and widely performed. We think that early diagnosis and effective treatment should be performed for all DGRD patients including pediatric patients. We believe that more comprehensive studies reporting pediatric patients with DGRD should be conducted so as to completely comprehend the natural course of this illness and its leading consequences.

The upper GI endoscopy is the primary method to evaluate the amount and depth of tissue damage of DGRD and it is performed routinely in our center. This process should be performed gently, especially for risk groups such as pediatric patients, as it can cause disturbance in gastric and duodenal motility and may cause undesirable side effects (19). All pediatric patients were sedated before the procedure, the experienced pediatric gastroenterologist performed the procedure, and no side effects were observed after procedure and during follow up period.

The frequency of *H. pylori* in pediatric populations is variable. (21-23). The age-related rise in the seroprevalence of *H. pylori* was shown by Wu et al. They demonstrated that the seroprevalence of *H. pylori* infection sharply elevated in young adolescence: 18.6% at age 15 years, 28.1% at age 16 years, 32.4% at age 17 years and 41.0% at age 18 years, respectively. The marked increase in social activities during school age was thought to be the main cause of *H. pylori* infection (14). In another study, it was demonstrated that an age-related increase *H. pylori* occurrence both in symptom free and in dyspeptic children, and a significantly higher rate in dyspeptic children aged 12-15 years, 38 % of children was found positive for *H. pylori* (14). In our study, similar to these studies the prevalence of *H. pylori* was found to be 43.8% and 21.9% in pre-treatment and post-treatment period, respectively. We think that the prevalence of *H. pylori* in our study group is compatible with literature and our combined treatment can be effective on *H. pylori* prevalence.

There were a number of limitations of the study. First of all, this study is a single-center study with a quite low sample number and the results concerning our study population cannot be generalized to other pediatric populations suffering reflux symptoms related the other health problems than DGRD. The clinical and histopathological improvements as the outcomes of the study are valid for short follow-up period, and may not reflect the long-term effects of the treatment. Also, another limitation is having no control group because of the nature of the pre-post design of the study. These limitations should be taken into account when interpreting the outcomes of the study.

If we look at the strengths of the study

1. In this study, imaging with MRCP to investigate gender, family history, parental consanguinity, and etiological anatomical pathologies in terms of risk factors that may cause DGRH.
2. Evaluation of DGRH together with the control group to investigate the effect of *H. pylori* infection.
3. Separate evaluation of symptoms before and after treatment.
4. Clinical staging of patients according to the presence of symptoms and evaluation of improvement in staging.
5. Performing histopathological evaluation together with the clinic.

CONCLUSION

UDCA and Sucralfate treatment with a six-month period, provides valuable histopathological and clinical improvements in patients with DGRD. Further clinical trials with a relatively large sample size and with a longer follow-up period using long-term outcomes, are needed to validate the results of our study.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *The study was carried out in conformity with the Declaration of Helsinki after obtaining the approval of Akdeniz University Clinical Research Ethics Committee (Approval date: 01.11.2017, No:622).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Evaluation of Thoracic Region Complications Associated with Cardiopulmonary Resuscitation Applied to Cases of Fall From Height

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Abstract

Aim: The most common causes of blunt trauma are traffic accidents and falls from height. Thoracic region complications may be seen in cardiopulmonary resuscitation (CPR). The aim of this study was to examine thoracic complications developing in cases applied with CPR following a fall from height with no direct chest trauma.

Materials and Methods: The thoracic complications of CPR were examined in cases with CPR applied after a fall from height following the exclusion of cases where death occurred and an autopsy was performed.

Results: Evaluation was made of 109 cases who met the study criteria, comprising 82 males and 27 females. Fractures of the sternum and costa were seen at a higher rate than reported in studies in literature. No statistically significant difference was determined between the genders in respect of fracture rates. As age increased, there was found to be a statistically significant increase in fractures.

Conclusion: This is the first study to have examined CPR complications following a fall from height. Just as there are direct effects of trauma on the body in general, there are also known to be some indirect effects. The results of this study showed an in increase in thoracic region complications developing after CPR applied to cases who developed cardiac arrest following a fall from height.

Keywords: Resuscitation, autopsy, complication, thorax, costa fracture

INTRODUCTION

Trauma is separated into two groups of blunt trauma and penetrating trauma according to the effect mechanism. Events such as falls from height and traffic accidents are classified in the blunt trauma group, and the penetrating trauma group includes firearms injuries and sharp blade injuries, etc (1). Traffic accidents and falls are the most common causes of blunt trauma (2,3). Falls have been seen to generally be the leading cause of presentations at the Emergency Department as a result of trauma and injury. Falls from height are a significant cause of morbidity and mortality in all age groups. It has been seen that the chest region is protected in trauma associated with falls from height, in contrast to other high kinetic energy traumas (4,5).

Unwanted complications may occur in cardiopulmonary resuscitation (CPR), such as costa and sternum fractures in particular. Although rare, injuries can also occur in internal organs such as the lungs, liver, heart, or stomach. The frequency of complications can show variability according to the person performing CPR, the surface on which the patient is, the localisation where CPR is performed (in-hospital or out of hospital), and the educational level and the skill of the person performing CPR (6-10). Prevention through the early identification of various complications that can develop associated with CPR is of clinical importance, and the determination in medicolegal autopsies of complications developing as a result of CPR has contcostauted to clinical studies (7,10).

Although there are general studies related to CPR complications, to the best of our knowledge there is no study in literature of patients with cardiac arrest following

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a fall from height. It is thought that in addition to the direct trauma to the chest region after a fall from height, there may also be indirect effects. In bones exposed to high kinetic energy in a fall from height, it is thought that there could be an increase in bone fragility after changes which can occur in the internal structure of the bones.The aim of this study was to examine thoracic complications developing in cases applied with CPR following a fall from height with no direct chest trauma.

MATERIAL AND METHOD

A retrospective examination was made of 4327 autopsy records in an autopsy centre in the east of Turkey between 2012 and 2018. From these, 215 autopsies and records were re-examined of deaths resulting from a fall from a distance of at least their own height. The medical records and autopsy reports were evaluated together.

The medical documents were examined in respect of the examinations made on presentation at the hospital, radiological imaging, follow up and whether or not CPR was applied. In the autopsy reports, the external and internal examination findings were examined.

Patients were excluded from the study if any ecchymous grazed dermabrasions, or crepitation were determined in the examination of the thoracic region or if any costa or sternum fracture was determined with imaging methods. Patients with no trauma determined in the thoracic region in the examination and who were applied with CPR during follow up were included in the study. From examinations of all the medical documents and autopsy reports, it was decided whether the chest trauma had occurred before or after resuscitation. Trauma findings determined in the chest region after a fall from height were accepted as direct trauma, and indirect trauma was defined as trauma originating from secondary rotational or tensile forces which could occur distant from the region that was struck. The decision as to whether the chest trauma occurred before or after resuscitation was made by the physician who conducted the first autopsy together with the researchers by re-examining all the medical documents, autopsy reports and photographs taken during the autopsy. All the autopsies in this study were performed by experienced Forensic Medicine specialists. The majority of the autopsies were performed within 4-8 hours of death, and in general within the first 24 hours.

Taking bone development into consideration, the cases were classified in two age groups of younger and older than 24 years (11). Subcutaneous ecchymosis, costa fractures, sternum fracture, pneumothorax, hemothorax, and lung injuries following CPR were accepted as complications.

Ethical permission was obtained with the decision of İnönü University Scientific Research and Publication Ethics Committee Health Sciences Non-Interventional Clinical Research Ethics Committee dated 02/07/2019 and numbered 2019/268.

Statistical Analysis

Data obtained in the study were analyzed statistically using IBM SPSS vn. 25.0 software. The Pearson Chi-square test, Yates' corrected Chi-square test (continuity correction), and Fisher's Exact Chi-square test were used in the statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Of the 4327 cases examined, 215 were cases of fall from height, of which 106 were excluded from the study because of fractures in the thoracic region associated with the fall. Evaluation was made of 109 cases that met the study criteria. These cases comprised 82 (75.2%) males and 27 (24.8%) females with a mean age of 45.60 years (range, 1-96 years). When classified in age groups, 24 (22%) cases were aged <24 years and 85 (78%) were aged >24 years.

In the cases that developed complications, injury to the left lung was determined in 22 (20.2%) and to the right lung in 18 (16.5%). Pneumothorax developed on the left in 10 (9.2%) cases and on the right in 4 (3.7%). Hemothorax developed in the left thoracic cavity in 21 (19.3%) and in the right in 24 (22%). There was seen to be ecchymosis in the resuscitation area in 33 (30.3%) cases, and sternum fracture in 39 (35.8%). The rate of sternum fractures was found to be statistically signficant in cases aged >24 years ($p<0.05$). No statistically significant difference was determined between the genders in respect of ecchymosis in soft tissue, costa fracture, or sternum fracture ($p>0.05$).

In the left hemithorax, the left 3rd and 4th costa were seen to be fractured most, at the rate of 40.4% (n:44), followed by the 5th, 2nd, 6th, 7th and 8th costas, respectively. The 11th and 12th costas were fractured least in the left hemithorax at the rate of 1.8% (n:2). As a result of the statistical analyses, fractures of left costa 1-8 were found to be statistically signficant in cases aged >24 years ($p<0.05$). No statistically significant difference was determined between the genders in respect of left hemithorax costa fractures ($p>0.05$) (Table 1).

In the right hemithorax, the 4th costa was seen to be fractured most, at the rate of 34.9% (n:38), followed by the 5th, 3rd, 2nd, and 6th costas, respectively. The 10th, 11th and 12th costas were fractured least in the right hemithorax at the rate of 0.9% (n:1). As a result of the statistical analyses, fractures of right costa 2-5 were found to be statistically signficant in cases aged >24 years ($p<0.05$). No statistically significant difference was determined between the genders in respect of right hemithorax costa fractures ($p>0.05$) (Table 2).

When the localisation of costa fractures was examined, there were seen to be costa fractures in a single region and costa fractures in more than one region. The highest rate was in 43 cases with fractures from the midclavicular line, followed by fractures in the parasternal, anterior axillary, and paravertebral regions. The lowest rate of fractures was seen to be from the posterior axillary and midscapular regions (Table 3).

Table 1. Number and location of costa fractures in the left hemithorax Left hemithorax costa number

Left hemithorax costa number	Percentage and (number) of cases	According to age groups	Gender
		p	p
1	12.80% (n:14)	0.037	0.745
2	34.9% (n:38)	0.000	0.613
3	40.40% (n:44)	0.000	0.469
4	40.40% (n:44)	0.000	0.469
5	39.40% (n:43)	0.000	0.401
6	32.10% (n:35)	0.000	0.693
7	24.80% (n:27)	0.004	0.676
8	17.40% (n:19)	0.012	0.077
9	7.30% (n:8)	0.196	0.406
10	2.80% (n:3)	1.000	0.573
11	1.80% (n:2)	1.000	1.000
12	1.80% (n:2)	1.000	1.000

* Most cases had more than one costa fracture. Yates's corrected Chi-square test and Fisher's Exact Chi-square test were applied.

Table 2. Number and location of costa fractures in the right hemithorax

Right hemithorax costa number	Percentage and (number) of cases	According to age groups	Gender
		p	p
1	11.5% (n:21)	0.065	0.487
2	24.80% (n:27)	0.017	0.148
3	28.40% (n:31)	0.006	0.370
4	34.90% (n:38)	0.004	0.968
5	32.10% (n:35)	0.010	0.384
6	22% (n:24)	0.120	0.766
7	11.90% (n:13)	0.290	0.732
8	7.30% (n:8)	0.682	1.000
9	5.50% (n:6)	1.000	0.160
10	0.90% (n:1)	1.000	1.000
11	0.90% (n:1)	1.000	1.000
12	0.90% (n:1)	1.000	1.000

* Most cases had more than one costa fracture. Yates's corrected Chi-square test and Fisher's Exact Chi-square test were applied

Table 3. Localisation of costa fractures

Localisation of costa fractures	No of cases - percentage
Midclavicular	43-39.45%
Parevertebral	8–7.33%
Anterior axillary	9–8.25%
Midscapular	2–1.83%
Posterior sternal	3-2.75%
Mid axillary	4-3.66%
Parasternal	10-9.17%
Posterior axillary	2–1.83%

* in some cases there was more than one costa fracture localisation at the same time

DISCUSSION

Cardiopulmonary resuscitation (CPR) is a life-saving procedure in cases with traumatic or non-traumatic cardiac arrest. However, just as in all medical interventions, there are some complications in CPR. It is important that complications which could develop are reduced to a minimum as far as possible and that treatment can be started by diagnosis after the development. To the best of our knowledge, this is the first study to have examined complications developing in cases applied with CPR after a fall from height with no direct trauma to the chest region.

Fractures have been reported to be more often in the left hemithorax and in the upper costas (except the 1st costa). More fractures have been reported in the 3rd, 4th , and 5th costas in particular (9,10,16). In the current study, fractures

were seen more often in the upper costas (except the 1st) and located in the left hemithorax. That the most frequent fractures were of the left and right 4th costas was consistent with findings in literature. It was an expected result that the fractures costas were those in the area where CPR was applied.

There are various studies in literature related to the localisation of costa fractures developing associated with CPR. In studies conducted in Turkey, fracture locaalisation has been reported to be mostly in the mid-clavicular line (7, 8,17), whereas studies from other countries have stated that the fracture localisation occurs in the axillary line and sterno-chondral junction (10,15). The fracture localisations in the current study were seen to be mostly in the mid-clavicular line, consistent with the previous Turkish studies. The difference in fracture localisation can be attcostauted to differences in ethnicity or the application of CPR.

It has been shown in literature that females have a smaller and thinner sternum than males and the sternum is more frequently fractured (14). In literature, elderly individuals have been reported to be at higher risk of costa fractures (15). It has also been reported that while the incidence of costa fractures increases with age, this does not apply to sternal fractures (9). The results of the current study showed that sternal and costal fractures statistically significantly increased as age increased. However, in contrast to the literature, no significant difference was determined between the genders in respect of sternal and costal fractures. The effects such as rotational or tensile forces to which individuals are exposed in a fall from height were seen to change the effect on fractures with gender but not with increasing age.

In the current study, the complications that developed most were seen to be ecchymosis forming in the soft tissue and fractures in the sternum and costas. The rates of complications seen (sternum and costa fractures) were higher than those in most studies in literature (7,9,12,13). Bone fractures are seen with the direct effect of general body trauma. In addition, because the skeletal system is exposed to high kinetic energy in these traumas, this is thought to decrease resistance and increase fragility.

CONCLUSION

Previous studies in literature of CPR complications have been conducted on non-traumatic cases or without differentiation of traumatic and non-traumatic cases. In this context, the current study is of value as the first study to have examined CPR complications after a fall from height.

Just as there are direct effects of general body trauma, there are also known to be some indirect (rotational or tensile forces) effects. The results of this study demonstrated an increase in thoracic region complications after CPR applied to cases that developed cardiac arrest following a fall from height. Therefore, more care must be taken when applying CPR to patients who develop cardiac arrest following a fall from height. This increase in bone fragility needs to be investigated with new prospective studies.

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Research Article

Development of Artificial Intelligence Based Clinical Decision Support System on Medical Images for the Classification of COVID-19

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Abstract

Aim: The first imaging method to play an vital role in the diagnosis of COVID-19 illness is the chest X-ray. Because of the abundance of large-scale annotated picture datasets, convolutional neural networks (CNNs) have shown considerable performance in image recognition/classification. The current study aims to construct a successful deep learning model that can distinguish COVID-19 from healthy controls using chest X-ray images.

Material and Methods: The dataset in the study consists of subjects with 912 negative and 912 positive PCR results. A prediction model was built using VGG-16 with transfer learning for classifying COVID-19 chest X-ray images. The data set was split at random into 80% training and 20% testing groups.

Results: The accuracy, F1 score, sensitivity, specificity, positive and negative values from the model that can successfully distinguish COVID-19 from healthy controls are 97.3%, 97.3%, 97.8%, 96.7%, 96.7%, and 97.8% regarding the testing dataset, respectively.

Conclusion: The suggested technique might greatly improve on current radiology-based methodologies and serve as a beneficial tool for clinicians/radiologists in diagnosing and following up on COVID-19 patients.

Keywords: COVID-19, image processing, convolutional neural networks, classification

INTRODUCTION

The new Coronavirus pneumonia, which arose in Wuhan, China, in December 2019 and was subsequently called COVID-19, is extremely infectious and pathogenically distinct from SARS-CoV, avian flu, influenza, MERS-CoV and other widespread respiratory viruses (1,2). Later, within a few months, this illness spread quickly from one nation to another, and COVID-19 has been notified a global pandemic by the World Health Organization (WHO) (3,4). With the outbreak of COVID-19, many undesirable situations such as economic crisis, loss of life, etc., have emerged. Furthermore, no clinically licensed antiviral medication or vaccination for COVID-19 has been established (5).

COVID-19 clinical symptoms include muscular soreness, shortness of breath, fever, cough, sore throat, headache, lethargy, and others. If a person with similar symptoms is encountered, it is tested with real-time reverse transcriptase-polymerase chain reaction (RT-PCR), which is employed to determine viral nucleic acids and is considered the gold test to diagnose such viruses (6). However, this procedure often takes many hours, if not days, to diagnose the illness. In addition, the sample must be tested more than once at regular intervals for the results to be reliable. So in the case of a severe infection, this process can be harmful. Chest X-rays may thus be a less expensive, quicker, and more validated to routine COVID-19 testing (7-9). On the other hand, early diagnosis of COVID-19 is required to limit the

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spread of the disease and hinder transmission by isolating infected individuals and tracking and quarantining close contacts. In addition, accurate monitoring of the course of infection in infected patients is an essential element in managing the disease. Thus, chest X-ray medical imaging plays a vital role in confirming a positive diagnosis for COVID-19 pneumonia and monitoring the course of the disease (10,11).

With the rapidly developing computer technology, image processing technology is commonly utilized in the field of medicine to support medical diagnoses. Artificial intelligence (AI) is a subfield of computer science that can evaluate huge amounts of medical/clinical data. Thanks to its ability to identify meaningful associations from a dataset, AI can be used to predict diagnosis, treatment, and outcome in many clinical scenarios (7,12-14). Deep learning (DL) networks are mathematically built networks that are taught to categorize illnesses into one of many subgroups using particular inputs. Rapid DL models must be trained and evaluated in the present COVID-19 epidemic to give quick support and reliable findings. So far, the RT-PCR test's inadequacy, high cost, and delay to get findings have been major constraints. Deep learning combined with chest X-ray pictures may assist address such issues and overcome the shortcomings of RT-PCR (15,16).

The current study aims to create a successful DL model that can distinguish COVID-19 from healthy controls using chest X-ray images. Thanks to the created model, clinicians and radiologists can use it as an auxiliary tool in diagnosing and following COVID-19 infection to screen patients in emergency medical support services.

MATERIAL AND METHOD

Data collection and features

The current study employed an open-source dataset containing augmented X-ray images for COVID-19 (https://data.mendeley.com/datasets/2fxz4px6d8). The relevant dataset contains 1824 X-ray scan images, of which 912 are COVID-19 positive and 912 COVID-19 negative patients based on the RT-PCR test results. Figure 1 presents the sample X-ray images of COVID-19 negative and positive individuals in the relevant data set (17).

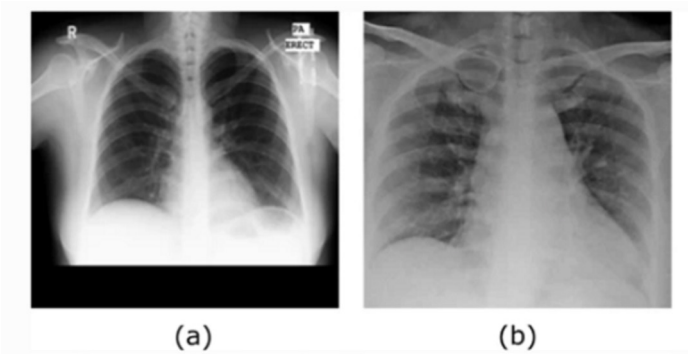

Figure 1. a) COVID-19 Negative X-ray image, **b)** COVID-19 Positive X-ray image

Convolutional Neural Networks (CNNs)

CNNs have gained popularity in machine learning in recent years because to their great predictive capacity in classification tasks requiring extremely high dimensional data and hundreds of distinct classes. CNN is a simple expansion of the multilayer perceptron (MLP) (18). The interest in CNN started with the AlexNet (Krizhevsky et al. 2012) deep learning architecture winning the 2012 ILSVRC ImageNet Large-scale image recognition competition held in 2012. CNN recognizes significant traits without human intervention and is computationally efficient. It has three fundamental layers: the convolution layer, the subsampling layer, and the fully linked layer. Depending on the implementation, the convolution and subsampling layers are iterated and eventually linked to the fully related layer. Utulizing a collection of filter masks, the convolution layer transmits 2D convolution on the pictures in the relevant dataset. All negative values are converted to zero via a non-linear function known as the linear unit (ReLU). The downsampling layer conducts curved image downsampling, lowering spatial resolution. Maximum pooling is the most often utilized subsampling method. Subsampling's purpose is to make the network more resilient and immutable. With ReLU and downsampling, many convolution rounds are performed. Flattening and connecting the last convolutional layer to the fully related layer. This information is subsequently passed on to the categorization layer (7,19).

Transfer Learning and VGG-16

CNNs are recognized to provide excellent results when given a big amount of data with thousands of examples. Because this is not realistic in most circumstances, transfer learning is utilized, in which a previously trained network is taught employing data from the novel dataset. Transfer learning has the significant advantage of reducing the time required to construct and train an algorithm by reusing the weights of previously generated model(s) (20).

VGG-16 is one of the deep CNN networks that aired in 2015 (21). VGG stands for "Visual Geometry Group" in VGG-16. The University of Oxford's Visual Geometry Group came up with the concept for the bespoke 16-layer mesh and trained it using the ImageNet dataset. A conventional VGG-16 model is made up of numerous 33 core size filters that improve the depth of the mesh and assist the model in learning more complicated characteristics. Three fully linked layers follow the convolutional layers in VGG (22).

Modeling and performance assessment

All coding was performed on Intel (R) Xeon (R) Gold 5122 server with 1.5 TB RAM, 16 core @ 3.60 GHz CPU, and Tesla P40 24GB GPU on a virtual server with Python software. Keras and Tensorflow 2.0 libraries in Python programming language were used for program coding. The input size of X-ray images was initially resized to 224x224 pixels for compatibility with deep learning models. 80% of the image dataset, the training set, and 20% of the image dataset

were randomly split as the testing set to validate the deep learning model. Transfer learning was performed by a pre-trained VGG-16 model in the study. Softmax was used as the activation function. In the model, the lot size was set to 16, the learning rate to 0.0001, and the period value to 20. Accuracy, F1 score, sensitivity, specificity, positive and negative predictive values (PPV and NPV) were estimated to evaluate performance of the algorithm(s) (23).

RESULTS

The dataset in the study consists of the subjects with 912 negative and 912 positive PCR results. The relevant dataset includes 1824 X-ray scan pictures, 912 of which are from patients with COVID-19, and 912 of which are from patients without COVID-19. Table 1 presents the confusion matrix in the testing dataset for the VGG-16 model.

Table 1. Performance measures obtained in the testing dataset

Test / True	True Positive	True Negative	Total
Test Positive	178	6	184
Test Negative	4	177	181
Total	182	183	365

Table 2 presents the accuracy, F1 score, sensitivity, specificity, NPV and PPV obtained from the VGG-16 model and their confidence intervals regarding the testing dataset.

The accuracy, F1 score, sensitivity, specificity, PPV and NPV from the model that can successfully distinguish COVID-19 from healthy controls are 97.3%, 97.3%, 97.8%, 96.7%, 96.7%, and 97.8% regarding the testing dataset, respectively.

Table 2. Performance metrics obtained in the testing dataset

Metric	Value	95% Confidence Interval Lower Limit	95% Confidence Interval Upper Limit
Accuracy	0.973	0.956	0.989
F1 Score	0.973	0.956	0.989
Sensitivity	0.978	0.945	0.994
Specificity	0.967	0.93	0.988
PPV	0.967	0.93	0.988
NPV	0.978	0.944	0.994

DISCUSSION

Medical imaging methods such as computed tomography (CT) and X-rays are critical in the worldwide battle against COVID-19, while artificial intelligence technologies enhance the power of imaging instruments and aid specialized clinicians. By accurately identifying infections on X-ray and CT images, work efficiency can be increased and subsequent evaluations facilitated. Artificial intelligence is a system that is quickly being utilized in various sectors to increase performance, accuracy, time efficiency, and cost efficiency all at the same time. In medicine, this technology is used for better patient care through early diagnosis and treatment, improved workflow, reduced medical errors, reduced medical costs, and reduced morbidity and mortality (24,25). It is widely recognized that AI technologies can potentially play a vital role in facilitating and expediting the classification of COVID-19 patients.

COVID-19 infects millions of people all over the world and still causes the death of hundreds of thousands of individuals. A major problem in controlling the spread of this disease is the inefficiency and lack of medical testing and other diagnostic methods. Recently, there has been a substantial surge in attempts to build AI and DL-based systems to diagnose COVID-19 based on several medical imaging (X-ray, CT, MR, and so on). A machine learning-based medical assistance platform may help radiation physicians make clinical choices as well as screen, diagnose, and treat patients. In a study, Xception and Xception + SVM models were used on 1102 chest X-ray images, and the diagnostic accuracy values were 96.75% and 99.33%, respectively (26). Another research contrasted deep learning-based feature extraction, which is often used to construct an automated model of COVID-19 categorization. MobileNet, DenseNet, Xception, ResNet, InceptionV3, InceptionResNetV2, VGGNet, and NASNet were opted from a pool of deep CNNs to achieve the most accurate feature. The models' performance was verified using a publicly accessible COVID-19 data of chest X-ray and CT images. While the deep learning model created with the bagging tree classifier DenseNet121 feature extractor has the highest accuracy with 99.0% classification accuracy, the deep learning model created with the ResNet50 feature extractor with LightGBM classifier has the second-highest accuracy with 98.0% classification accuracy (5). Finally, in a study by Rasheed et al., the developed models that automatically diagnose COVID-19 infection with high oerformance metrics from X-ray images with Logistic Regression (LR) and CNNs, which are frequently utulized ML methods. The models were developed using 500 X-ray images, and a dimensionality reduction method based on principal component analysis (PCA) was also used to accelerate the learning process and boost prediction accuracy by selecting highly differentiating properties. According to the experimental data, the LR and CNN models for positive case identification had an overall accuracy of 95.2 percent to 97.6 percent without PCA and 97.6% to 100% with PCA, respectively (27). The present study intends to assess the prediction performance and classification of COVID-19 disease using transfer learning and VGG-16 with X-ray images. The model predicted COVID-19 infection with 97.3% accuracy. In addition, in the model test set, F1-score, sensitivity, specificity, PPV and NPV were obtained as 97.3%, 97.8%, 96.7%, 96.7%, and 97.8%, respectively.

CONCLUSION

Given the high classification rate achieved for the diagnosis of COVID-19, the resulting model could be advantageous in

assisting in the screening of patients in emergency medical support services and assisting clinicians for emergencies. Overall, It is concluded that the suggested technique may greatly enhance the current radiology- established methodology and may be a useful tool for medical practitioners/radiologists to assist them in diagnosing and following up on COVID-19 patients.

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MEDICAL RECORDS-International Medical Journal

Research Article

Vitamin D, VDR, and VDBP Levels Correlate with Anti-inflammatory Cytokine Profile in FMS Patients

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Abstract

Aim: The major target of this research is to examine whether there is any connection between the levels of vitamin D and anti-inflammatory mediators in patients with fibromyalgia syndrome (FMS).

Materials and Methods: The study contains 30 FMS diagnosed and 25 healthy female individuals and the determination of FMS was made according to the standards of 2010 American College of Rheumatology (ACR). Vitamin D, vitamin D receptor (VDR), vitamin D binding protein (VDBP) levels, and anti-inflammatory cytokine (IL-4, IL-10, TGF-β) levels in the serum of patients with FMS and healthy individuals were measured using enzyme-linked immunosorbent assay (ELISA).

Results: The concentrations of vitamin D, VDR, and VDBP were determined to be higher in healthy controls than in patients with FMS ($p<0.001$). Correlating with this, IL-4, IL-10, and TGF-β levels were measured remarkably higher in the healthy group than in the FMS patients ($p<0.001$).

Conclusion: Low vitamin D levels may cause a decrease in anti-inflammatory cytokine levels and their immunosuppressive effect in FMS.

Keywords: Anti-inflammatory cytokines, vitamin D, fibromyalgia syndrome

INTRODUCTION

The definition of “chronic widespread pain” is widely used for some pain-related diseases whose pathogenesis has not been fully resolved. One of the most well-known subgroups of these diseases is fibromyalgia syndrome (FMS). Fibromyalgia is defined as a pain processing disorder resulting from abnormal conditions in the pain signaling pathways opening to the central nervous system (1). The most prominent symptoms of FMS include chronic widespread pain, extreme fatigue, sleep disturbance, increased pain when touching the inflamed area, tingling in the skin, and prolonged muscle spasm (2). All these symptoms are actually related to inflammation, and studies suggest that inflammation has a noticable task in the pathogenesis of FMS. The most effective mediators involved in the formation of inflammatory responses are cytokines. These immune mediators can be divided two groups as pro-inflammatory and anti-inflammatory (3). Anti-inflammatory cytokines are regulatory molecules that work in coordination with pro-inflammatory cytokines and have immunosuppressive effects on various markers. In this study, the efficacy of anti-inflammatory mediators TGF-β, IL-4, IL-10, in FMS was discussed. IL-10 is an anti-inflammatory cytokine that controls the expression of several pro-inflammatory mediators (IL-1, IL-6, TNF-α etc.). In recent studies, IL-10 and IL-4 levels were found to be low in the blood of patients with chronic widespread pain, and it has been thought that these cytokines may have a prominent function in patients with chronic pain (4). TGF-β can be found in different regions such as peripheral ganglia, choroid plexus, and meninges, which are the basic elements of the nervous system (5). Its main function is to suppress cytokine release by inhibiting macrophage and T cell activity (6). It also participates in the regulation of nitric oxide (NO) production in macrophages. NO has an important action in the functioning of neuropathic pain pathways (7). In recent studies, it is thought that TGF-β with anti-inflammatory activity and other agents that stimulate this cytokine can be shown as therapeutic targets in cases related to neuropathic pain. On the other hand, there are many new perspectives on molecular markers that have critical assignments in the development and treatment of chronic pain. One of them is the immunoregulatory activity of vitamin D. The anti-inflammatory potential of vitamin

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D suggests a remarkable relationship with the immune system (8). Vitamin D acts through intracellular receptors and works with its associated connectors. Especially vitamin D receptor (VDR), and vitamin D binding protein (VDBP) are major contributors to vitamin D activation. VDR is expressed in various tissues in the body and involved in events such as regulation of gene transcription and calcium transport (9). Many immune cell types can express VDRs on their surfaces. In this way, the VDR has a significant function in the orchestration of immune reactions (10). VDBP is the main transport molecule for all vitamin D metabolites, and is involved in immune regulation by binding to the surface of leukocytes and activating the complement system (11). Recent studies on FMS have been suggested that vitamin D concentrations are generally lower in patients than in healthy individuals, and this may be associated with neuropathic pain (12,13). On the other hand, studies on chronic pain reveal that one of the most important factors underlying FMS and similar diseases is immunological parameters (14). The fundamental goal of this study is to evaluate whether there is any connection between vitamin D and anti-inflammatory cytokine profile in FMS patients and to research the therapeutic use of this condition in a case of chronic widespread pain.

MATERIAL AND METHOD

Study Groups

This study was accepted by the Uludağ University Faculty of Medicine Ethics Committee and an informed permission form was gotten from whole individuals. A total of 55 female people, including 25 healthy controls and 30 individuals with FMS, took part in this research. The patients were known to have FMS in the last 1 year and were determined according to American College of Rheumatology (ACR) diagnostic standards. All FMS patients were included in the study without using any FDA-approved fibromyalgia medication for at least 2 weeks. In addition, patients with autoimmune disease, inflammatory disease, infectious disease, cancer and those receiving anti-inflammatory drug therapy were not included in the research. In the healthy group, people who did not have any chronic diseases and did not use any medication were included.

Serum samples of patients

Blood samples were taken into sterile tubes of 20 ml and was centrifuged at 3000 x g for 10 minutes to get serum of patients. Collected samples were transferred to eppendorf tubes and stored at -80°C until the date of use.

Quantification of vitamin D and cytokine levels

Vitamin D, VDR, VDBP, and cytokine (IL-4, IL-10, TGF-β) levels in samples were obtained by ELISA method. The ELISA kit procedure used in the study was applied (BT-LAB, Shangai, China). Each sample was measured twice and the results were calculated according to the standard curve. Validated detection limits were 0.23ng/ml for vitamin D, 2,51pmol/L for VDR, 5.41µg/ml for VDBP, 2,53 ng/L for IL-4, 2,59 pg/ml for IL-10 and 5,11 ng/L for TGF-β, respectively.

Statistical Analysis

The Shapiro Wilk test was used to examine how the variables were distributed. Continuous variables were presented as median and mean±standard deviation values and Mann Whitney U test was performed for comparison of FMS patients and healthy controls. Correlations between variables were evaluated by Spearman correlation test. ROC analysis method was applied to measure the range of values that can be predicted in diagnosing the disease. Statistical analysis was evaluated using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) and p value <0.05 was considered to indicate a statistically significant difference.

RESULTS

There was no notable difference in terms of mean age values in 25 healthy individuals (48.60±10.59 years old) and 30 FMS patients (50.93±9.92 years old) who participated in the study. Details showing vitamin D, VDR, VDBP, and cytokine concentrations are displayed in Table 1. Vitamin D concentration was obtained to be higher in the healthy group (18.06±8.41) than in the FMS patients (15.67±1.83) ($p<0.001$). It was observed that there was an important difference between the patient (120.83±22.06) and healthy (142.02±67.61) groups in terms of VDR values ($p=0.002$) and VDBP values were also different from each other in both groups; patients (310.77±33.68) and healthy individuals (368.03±188.92) ($p<0.001$). VDR and VDBP concentrations of the patients with FMS were lower than healthy group. All the levels of IL-4 (130.47±56.70), IL-10 (182.84±57.02) and TGF-β (547.28±252.27) were also reported to be higher in the healthy group compared to FMS patients (104.03±14.63, 156.83±20.97, 440.71±54.39, respectively) and there was a remarkable difference among the groups in terms of cytokine concentrations ($p=0.001$, $p<0.001$, $p<0.001$, respectively). In addition, a noticable correlation was detected between the values of vitamin D, VDR, VDBP, and IL-4, IL-10, TGF-β ($p<0.05$). As vitamin D, VDR and VDBP concentrations decrease, a decline is observed in IL-4, IL-10, TGF-β values (Table 2). According to the ROC analysis results, vitamin D, VDR, VDBP, and cytokine values were determind to be significant ($p<0.001$). In table 3, cut-off values for vitamin D, VDR, VDBP, and anti-inflammatory cytokines that can be predicted in patient diagnosis are demonstrated. It has been shown that people with or below these values can be diagnosed with FMS (Table 3).

DISCUSSION

In the recent study, we reported that the levels of vitamin D, VDR, and VDBP were correlate with anti-inflammatory cytokine profile (IL-4, IL-10, TGF-β) and all markers were higher in the healthy group than in the FMS patients ($p<0.05$).

Table 1. Values of the FMS patients and healthy controls

	Healthy Controls (n=25)	FMS Patients (n=30)	p-valuea
Vitamin-D	18.06 (8.41)	15.67 (1.83)	<0.001
VDR	142.02 (67.61)	120.83 (22.06)	0.002
VDBP	368.03 (188.92)	310.77 (33.68)	<0.001
IL-4	130.47 (56.70)	104.03 (14.63)	0.001
IL-10	182.84 (57.02)	156.83 (20.97)	<0.001
TGF-β	547.28 (252.27)	440.71 (54.39)	<0.001

FMS: Fibromyalgia Syndrome, VDR: Vitamin D receptor, VDBP. Vitamin D Binding Protein, TGF-β:transforming growth factor beta, IL: Interleukin
Data was presented as median(IQR). a:Mann-Whitney U Test p<0.05

Table 2. Comparison of vitamin D, VDR, VDBP and cytokine levels between the healthy controls and FMS patients

	Healthy Controls (n=25)						FMS Patients (n=30)						Total (n=55)					
	Vitamin-D		VDR		VDBP		Vitamin-D		VDR		VDBP		Vitamin-D		VDR		VDBP	
	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p
IL-4	0.90	**<0.001**	0.92	**<0.001**	0.82	**<0.001**	0.70	**<0.001**	0.82	**<0.001**	0.89	**<0.001**	0.86	**<0.001**	0.91	**<0.001**	0.87	**<0.001**
IL-10	0.87	**<0.001**	0.73	**<0.001**	0.71	**<0.001**	0.55	**<0.001**	0.71	**<0.001**	0.66	**<0.001**	0.82	**<0.001**	0.79	**<0.001**	0.77	**<0.001**
TGF-β		**<0.001**	0.94	**<0.001**	0.83	**<0.001**	0.89	**<0.001**	0.81	**<0.001**	0.85	**<0.001**	0.91	**<0.001**	0.90	**<0.001**	0.89	**<0.001**

FMS: Fibromyalgia Syndrome, VDR: Vitamin D receptor, VDBP. Vitamin D Binding Protein, TGF-β: transforming growth factor beta, IL: Interleukin
rs: Spearman correlation coefficient p<0.05

Table 3. ROC Analysis results

	AUC	p-value	Cut-off Value	Sensitivity	Specificity	PPV	NPV
Vitamin-D	0.799	**<0.001**	≤17.13	86,21	80	83,3	83,3
VDR	0.741	**<0.001**	≤120.83	51.72	96	93,7	63,2
VDBP	0.784	**<0.001**	≤319.07	68.97	88	87	71
TGF-β	0.821	**<0.001**	≤478.84	82.76	88	88.9	81.5
IL-10	0.786	**<0.001**	≤169.75	79.31	80	82.1	76.9
IL-4	0.763	**<0.001**	≤113.13	79.31	80	82.1	76.9

AUC: Area under the ROC curve, PPV:Positive predictive value, NPV:Negative predictive value

Current approaches to FMS have determined that the underlying causes of this disease are more than one. It is emphasized that one of these reasons may be immunological factors and attention should be paid to this issue. Although the mechanism of FMS has not been fully resolved, there are limited studies on the connection of the disease with the immune system. In current study, our main purpose was to obtain the concentrations of vitamin D and anti-inflammatory mediators in FMS patients and to research the link between them. In recent years, it has been discovered that there is a much wider area where vitamin D benefits beyond its known functions such as bone development, regulation of Ca level, and contribution of muscle functions. Additionally, there are several studies showing that vitamin D has a remarkable activity in the pathogenesis of FMS and pain related disorders (15,16). New approaches suggest that vitamin D has an active role in supporting neurological pathways, reducing inflammation and preventing the risk of many chronic diseases (17,18). Vitamin D shows its effectiveness by binding to VDR and modulates the immune system through vitamin D responsive elements (19). Besides, in current studies, it has been determined that the level of VDBP in the serum decreases in liver diseases, kidney-related disorders, and various trauma situations (20). In a study on vitamin D function pulmonary tuberculosis patients, it was shown that vitamin D increased IL-10 production by down-regulating Th1 and Th17 cytokines (21). In addition, it inhibits immune functions by suppressing the transcription factor nuclear factor kappa B (NF-κB) (22). Another possible mechanism is that vitamin D down-regulates toll-like receptor (TLR) expression by reducing pro-inflammatory cytokine release

(23). In line with these studies, it is thought that vitamin D has a suppressive effect on the activity of immune system cells through various inhibitory mechanisms (24). Clinical research on the link between chronic pain and vitamin D is limited. However, there are strong evidences to suggest that vitamin D has a potential role in pain-related conditions. In studies conducted, vitamin D levels were found to be related with symptoms such as headache, muscle-joint, chest and back pain, and were found to be low in patients with FMS (25). Besides, it has been determined that long-term vitamin D deficiency is associated with a weakened immune system and chronic inflammation. Inflammatory responses have a prominent effect in the formation of various pain pathways in the peripheral and central nervous systems. The major molecules involved in the occurance of these responses are cytokines. In addition to being effective in the regulation of immune responses, their physiological and pathological roles in inflammation are becoming increasingly important (26). The anti-inflammatory cytokines discussed in this study are generally those that have an inhibitory effect and act in the direction of suppressing the inflammatory response. Recent studies have shown that low blood concentrations of IL-10 and another anti-inflammatory cytokine, IL-4, are detected in patients with chronic widespread pain, which is a remarkable finding (27). In addition, studies have shown that IL-10 suppresses spinal-mediated pain facilitation in acutely administered animal models. It has also been reported that neuropathic pain can be prevented when spinal IL-10 is blocked (28). TGF-β inhibits macrophage and T cell cell activation thus suppresses cytokine release. It also antagonizes nitric oxide (NO) release in macrophages. NO has a prominent task in neuropathic pain pathways. This anti-feature of TGF-β, which affects cytokine release, suggests that it can be used in neuropathic pain therapy (29). In a study performed on microglia and astrocytes, vitamin D was found to upregulate TGF-β and IL-4 (30). Mahon et al. reported that vitamin D inhibited CD4+ Th2 cell proliferation in animal models by promoting IL-4 production and reducing pro-inflammatory cytokine expression (31). Additionally, it is known that very low doses of circulating vitamin D cause a decrease in Treg cells. However, the function of vitamin D in immune responses is quite complex and depends on the involvement of various factors such as innate immunity, brain pathways, gut microbiome. It has been observed that enriched Treg cells in patients with SLE increase the Th2 type immune response following long-term vitamin D supplementation (32). In this study, vitamin D, VDR and VDBP concentrations were detected to be lower in FMS patients than in healthy individuals, in correlation with the anti-inflammatory mediators IL-4, IL-10 and TGF-β. There are some limitations in this study. It was a single center research prevents the interpretation of the results throughout the country. In addition, the fact that it was studied only in women does not give an idea about the course of the disease in men. Some of the anti-inflammatory cytokines have been studied so further extended studies with other groups of anti-inflammatory cytokines may be done.

CONCLUSION

In conclusion, we would like to emphasize that this is the first study to correlate vitamin D, VDR, and VDBP levels with anti-inflammatory cytokine profile in patients with fibromyalgia. The results we obtained from the study suggested that there was a remarkable link between vitamin D and anti-inflammatory cytokine profile. This finding may help to understand the role of vitamin D and cytokines in pathological process of fibromyalgia and can be a guide for similar disease states.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This study was accepted by the Uludağ University Faculty of Medicine Ethics Committee and an informed permission form was gotten from whole individuals.*

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Research Article

Oxidative Stress and Inflammation Markers in Undescended Testes Patients

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Abstract

Aim: Undescended testis is a congenital genitourinary system pathology characterized by the absence of testis in the scrotum. In this disease, the heat stress caused by the testes not being at the optimal temperature can trigger oxidative stress and inflammation. Our study investigated the status of oxidative stress and inflammation markers between patients with undescended testes and healthy infants.

Materials and Methods: Fifty pediatric patients with undescended testes and a control group who applied to Pediatric Surgery Clinic were included in the study. From the blood samples, oxidative stress and inflammation status were examined. Interleukin 1 beta, interleukin 6, and tumor necrosis factor alpha levels of inflammation parameters were measured by the ELISA method using commercial kits. Total oxidant status, total antioxidant status, total thiol, and native thiol levels were measured photometrically with commercial kits. Oxidative stress index and disulfide levels were calculated with a mathematical formula. Oxidative stress and inflammation marker levels of the patient and healthy groups were compared statistically.

Results: Total antioxidant status, total thiol, and native thiol levels were statistically significantly lower in the patient group than the healthy group ($p<0.05$). Total antioxidant status, oxidative stress index, disulfide levels, and interleukin 1β, interleukin 6 levels were also statistically significantly higher in the patient group ($p<0.05$). There was no difference in tumor necrosis factor-α levels between the groups.

Conclusion: In our study, it was observed that oxidative stress and inflammation were higher in patients with undescended testes. Since this situation may lead to systemic diseases in the future, more extensive studies are needed.

Keywords: Undescended testes, Oxidative Stress, Inflammation, Cryptorchidism

INTRODUCTION

Undescended testes (UT) are one of the most common (1-2%) congenital pathologies of the genitourinary system, characterized by the lack of at least one testicle in the scrotum (1). In congenital or acquired UT cases, the risk of developing infertility (2) and testicular cancer (3) is relatively high. It has been reported that the risk of developing infertility is six times higher in those with bilateral UT (4). A diagnosis of UT is found in 5-10% of males with testicular cancer (5).

Studies in experimental animals have shown that UT causes spermatogenic damage by increasing oxidative stress (OS) and inflammation, which in turn causes infertility (6). In cases of UT, the testis is prevented from reaching the optimal temperature. In this situation, an increase in temperature is observed. Elevated temperature can trigger cell death. It has been suggested that excessive death of the gonocytes and spermatogonia is the cause of infertility seen in UT (7). Elevated scrotal temperature can lead to male infertility by impairing spermatogenesis and steroidogenesis. According to research, OS plays a significant role in the pathophysiology of male infertility (8). The increase in scrotal temperature in UT leads to oxidative damage (9). It has also been reported that OS may cause testicular cancer by changing the environment

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of the testis and sperm parameters (10,11).

Total oxidant status (TOS) is commonly used to measure the body's total oxidation state (12). Similarly, total antioxidant status (TAS) is used to assess the body's overall antioxidant level (13). It is challenging to measure directly because of the low half-life and serum concentrations of ROS. Oxidative protein damage causes an increase in protein carbonyl levels and a reduction in protein thiol levels. This decrease is associated with reduced antioxidant levels. Therefore, thiol-disulfide hemostasis is used as a novel method for evaluating OS. Cytokines (e.g., interleukin 1 beta, Tumor Necrosis Factor-alpha) are biomolecules that mediate inflammatory responses, provide intercellular communication, and play an essential role in reproductive physiology. It is released in inflammatory cells in response to infection and has local and systemic effects (14).

UT can affect male infertility and the risk of developing cancer by increasing OS and inflammation. TAS, TOS, disulfide (DS) homeostasis, interleukin 1 beta (IL1ß), and Tumor Necrosis Factor-alpha (TNF-α) levels, which are OS and inflammatory indicators, have not been studied in patients with UT. This study evaluated the OS and inflammation parameters biochemically in children with UT.

MATERIAL AND METHOD

Study Population

The study was initiated after approval of the Ethics Committee (No: 21/564). Patients with a diagnosis of UT who underwent elective undescended testis operation in the pediatric surgery operating room were included after obtaining written informed consent from their parents. Cryptorchid patients with high scrotal and inguinal canal localization were included. It was calculated that there should be at least 50 volunteers in each group, to achieve 80% power at the α=0.05 significance level by power analysis of the groups. The case group consisted of patients aged 12-24 months, whereas the control group included on healthy infants with the same demographic characteristics. The study did not include patients with neurological, neuromuscular, convulsive, and bleeding coagulation disorders, opioid and local anesthetic drug allergy, and additional pathology.

Approximately 3mL of blood was taken into BD Vacutainer® blood collection tubes in the study. After the routinely requested blood was studied, the remaining inert blood was studied. The blood samples were centrifuged at 3000x g for 10 minutes.

Biochemical Analyses

Oxidative Stress Analyses

Serum samples were analyzed spectrophotometrically in the multi-plate reader for OS parameters. TAS and TOS in serum samples were measured using commercially available kits. According to the standard curve, the serum TAS levels were presented as mmol ascorbic acid Eq/mL, and the tissue TOS levels were computed as μmol H_2O_2 Eq/mL protein. Oxidative stress index (OSI) was calculated as TOS/TAS (15).

Thiol-Disulfide Homeostasis

As new OS biomarkers, serum and disulfide are tested. NaBH4 converted dynamic disulfide links (−S−S−) in the blood sample to NT groups (−SH). Commercial kits were used to assess serum total thiol (TT) and NT levels (Rel Assay, Gaziantep, Turkey). A spectrophotometer was used to measure biomarkers. The molar extinction coefficient of 14.100 mol/L-1 cm-1 of 5-thio-2-nitrobenzoic acid (TNB) calculated total and free thiol levels. The disulfide level (DS) was calculated as μmol/L using the formula (total thiol-free thiol)/2.

Inflammation Biomarkers

IL1β, IL6, and TNFα cytokines, which are inflammation biomarkers, were investigated in serum samples. Cytokines levels were measured spectrophotometrically with ELISA kits in a multi-plate reader.

Statistical Analysis

The IBM SPSS 25.0 version application was used to perform statistical analyses. The data distribution was determined using the Kolmogorov-Smirnov test. Mean ± standard deviation values were used to express continuous variables. When the variable was normally distributed, the independent sample t-test was performed to compare variables between the two groups. The confidence intervals (95%) were used to show the differences between groups. Statistical significance was defined as $p<0.05$.

RESULTS

The mean age of all participants was 17.48 ± 3.69 months. In the classification of undescended testicles, 40 patients had bilateral and 10 unilateral undescended testes. OS and thiol-disulfide hemostasis parameters were compared between infants with UT and the control group (Table 1). TOS and OSI values in the undescended testes group were higher, but TAS was lower. It was found that TT and NT values were lower in patients with UT, but DS levels were higher.

Table 1. Oxidative stress biomarkers between UT and controls

Oxidative Stress Biomarkers	UT (mean±SD)	Control (mean±SD)	p[a]
TAS (mmol Ascorbic Acid Eq/L)	1.02±0.07	1.23±0.06	0.001*
TOS (μmol H2O2 Eq/L)	11.1±2.2	8.4±0.8	0.006*
OSI (AU)	10.9±2.7	6.8±1.1	0.001*
TT (μmol/L)	475±126	545±95	0.008*
NT (μmol/L)	300±100	455±75	0.001*
DS (μmol/L)	87.5±2.2	45.0±0.8	0.001*

TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, TT: Total Thiol, NT: Native Thiol, DS: Disulphide, a: Independent sample t-test, *: p<0.05, SD: Standard deviation

The difference between the levels of inflammation parameters IL1ß, IL6, and TNFα between the two groups was compared (Figure 1). IL1ß (p=0.001) (Figure 1A) and IL-6 (p=0.031) (Figure 1B) levels were found to be significantly higher in patients with UT compared to controls. Although the TNFα level (Figure 1C) was higher in the UT group, this difference was not statistically significant (p>0.05).

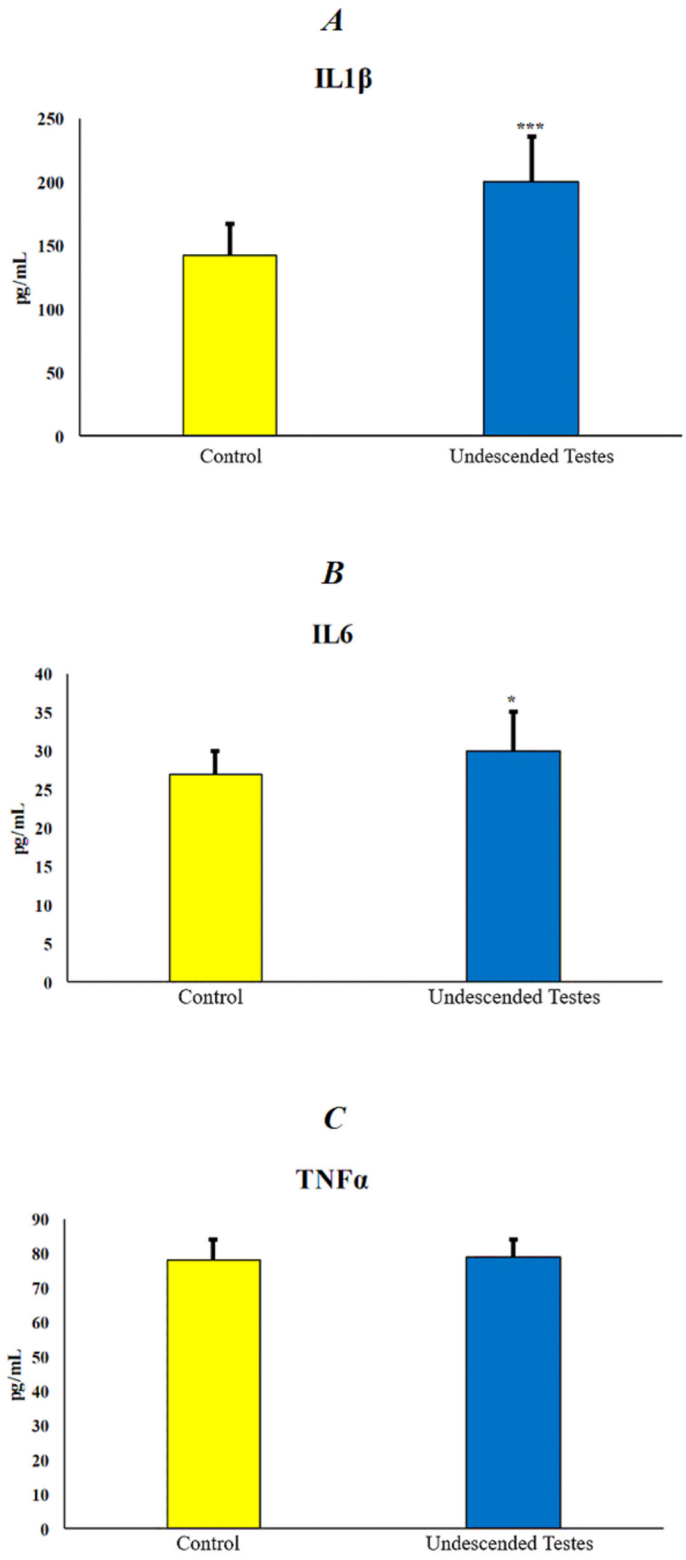

Figure 1. IL1ß, IL6, and TNFα levels between undescended testes and the control group. *: p<0.5, **: p<0.01, ***: p<0.001; Independent Sample t-test

DISCUSSION

Undescended testes are one of the congenital genitourinary system diseases seen in 1-2% of boys worldwide (1). Increased OS and inflammation may occur in UT because the testis is not at optimal temperature (9). In our study, it was observed that OS and inflammation were higher in patients with UT.

The testis should be at a lower temperature than body temperature. In cases of UT, the testis does not reach the scrotum. In UT, heat stress occurs because the testis cannot reach the optimal temperature. Heat stress can contribute to elevated intracellular reactive oxygen species (ROS) levels, creating a signal that triggers apoptosis (16). The imbalance between ROS generation and elimination by current antioxidant mechanisms causes OS to develop. Temperature increase in the testis in UT cases is associated with OS (17). Li et al., in their study (18), examined ROS production and gene expression in adult mice with a UT model. Increased germ cell death was identified in the research, as well as alterations in the expression of genes linked to redox reactions, stress response, energy, and lipid metabolism.

TAS, TT, and NT levels were low in infants with UT in our research, whereas TOS, OSI, and DS levels were high. In parallel with our findings, Imamoğlu et al., In their case-control study, found that IL-6 and malondialdehyde (MDA) levels were higher in blood samples from patients with UT compared to the control (8). These findings show that OS and inflammatory responses are increased in patients with UT. Similarly, MDA levels were greater in individuals with UT in a study of 59 cases and 30 controls (19). Avci et al. planned on 30 patients with UT and 40 healthy controls, they found high levels of lipid peroxidation, oxidative DNA damage, ischemia-modified albumin, and nicotinamide adenine dinucleotide phosphate oxidase 4, which are parameters associated with OS (20).

OS, which occurs when ROS concentrations exceed physiological needs, may cause infertility. OS induced by increased scrotal temperature in UT cases may lead to deterioration of spermatogenesis and steroidogenesis at later ages (7). In addition, OS can negatively affect the structural and functional integrity of sperm. It damages the proteins and lipids in the plasma membrane of the sperm cell, and as a result, the DNA integrity is impaired. Thus, OS can affect the cell membrane fluidity and permeability, leading to disruptive effects on sperm function (21). These damages to spermatogenesis, steroidogenesis, and sperm cells can cause male infertility. Indeed, it is known that OS is associated with many conditions related to male infertility. OS can affect male infertility by causing changes in testicular blood flow, endocrine pathways, and germ cell apoptosis (17). It involves the plasma membrane integrity and causes premature capacitation. These conditions may inhibit the fertilization of spermatozoa (22).

ROS also initiates inflammation by inducing the activation of transcription factors and pro-inflammatory genes (23).

In our study, IL-1β and IL-6 cytokine levels, which are inflammation parameters, were found to be high. IL-6 is a cytokine that regulates temperature during inflammation (22). Koçak et al. In their study, it was observed that IL-6 levels in the seminal plasma of infertile men were higher than those of fertile ones (24). It is reported that IL-1β induces apoptosis in semen and decreases sperm motility (25). Inflammation can reduce semen quality by causing impaired gland functions, inhibiting sperm transport, and affecting spermatogenesis (25). Therefore, inflammation in UT may also affect infertility at later ages.

CONCLUSION

OS and inflammation are more prevalent in people diagnosed with UT, according to the findings of this study. This situation poses a risk in terms of infertility in older ages. It may also pave the way to forming other systemic disorders in the long term. For this reason, children diagnosed and treated with UT should be followed at later ages in terms of possible risks.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *The study was initiated after approval of the Ethics Committee (No: 21/564).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Orbitofrontal Cortex Volumes in Patients Diagnosed with Somatic Symptom Disorder

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Abstract

Aim: Somatic symptom disorder (SSD) is a psychiatric disorder with unknown etiopathogenesis that is still under investigation. The results of neuroimaging studies on SSD have shown that some brain regions may be associated with it. In this connection, this study aims to explore the orbitofrontal cortex (OFC) morphometric changes in patients with SSD to better comprehend the etiopathogenesis.
Material and Methods: The study enrolled 20 patients and 20 healthy controls. All study participants were administered a sociodemographic and clinical questionnaire, the Hamilton Depression Rating Scale (HAM-D) and the Hamilton Anxiety Rating Scale (HAM-A). The volumes of total brain, OFC, total white matter, and total gray matter were measured by a magnetic resonance imaging (MRI)-based method in studied patients.
Results: Orbitofrontal cortex volume was significantly smaller in the patient group than in healthy controls ($p<0.05$). No significant difference between the two groups could be observed in total brain, white matter and gray matter volumes ($p>0.05$).
Conclusions: The OFC was markedly smaller in SSD patients than in healthy controls, suggesting that the OFC may be associated with SSD pathophysiology. Future studies examining the functional features of the OFC using imaging and cognitive function tests will likely shed more light on this issue.

Keywords: Somatic symptom disorder, volume, orbitofrontal cortex, MRI

INTRODUCTION

Somatic symptom disorder (SSD) is a mental disorder characterized by disproportionate thoughts about the severity of one or more somatic complaints that significantly impair functionality (1). It was classified into the somatoform disorder group and included in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (2). It was then renamed "somatic symptom disorder" (1) in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) (1). Prevalence rates for SSD in general population varies from 5% to 7% (3).

Several efforts have been directed towards elucidating the etiology of SSD, but it remains one of the mental disorders about whose biological basis we have very little information. Somatic symptoms may result from a tendency to interpret certain bodily sensations as signs of disease because of an increased perception of these sensations (4). The limited number of neuroimaging studies in SSD patients is based on relatively old data. According to these data, some brain regions become prominent in the physiopathogenesis of SSD. Some studies have shown that SSD patients have non-dominant hemispheric dysfunction in the cerebral hemispheres and dominance of the right hemisphere (5). In other studies, SSD symptoms were thought to be related to the caudate nucleus, hippocampus, and putamen, and volume changes were found in the right and left amygdala (6,7). Bilateral amygdala volume has been reported to be decreased in SSD patients (7). In a functional magnetic resonance imaging (fMRI)-based study examining patients with somatoform pain disorder, it was observed that patients had atypical activation of the precentral gyrus at rest compared with healthy controls (8). In another fMRI study, lower activity was observed in the bilateral parahippocampal gyrus and cerebellum and the left

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amygdala, postcentral gyrus, superior temporal gyrus, and posterior insula in SSD patients compared to control subjects (9). A 2016 study found that the medial prefrontal cortex and anterior cingulated cortex may provoke emotional dysregulation in SSD patients (10).

The orbitofrontal cortex (OFC) has enhanced connections to the basal ganglia and amygdala. The OFC additionally has a key role in cognitive function assessment. Various literature studies have confirmed that the OFC is impaired not only in patients with SSD but also in many psychiatric disorders, including hypochodriasis, body dysmorphic disorder, panic disorder, anorexia nervosa, and obsessive-compulsive disorder (11-15).

It has been suggested that SSD patients respond similarly to relevant and irrelevant stimuli and that their selective attention deteriorates (5). Accordingly, it was hypothesized by the authors of this study that the OFC, the region associated with cognitive function, may be important in SSD patients.

SSD is a disease that is sometimes difficult to diagnose, and therefore it is not uncommon for SSD patients to spend a long time going door to door to find an answer to their condition. The use of imaging, along with the necessary diagnostic tools, can both help diagnose patients early and reduce unnecessary healthcare costs (16). The results of neuroimaging studies in SSD show certain differentiations that may be related to etiology. Therefore, these implications are very important for diagnosing and treating SSD patients. The present study aim is to assess the total brain, OFC, total gray matter, and total white matter volumes in SSD population to determine neuroanatomical causes of SSD etiology. Detection of these regions may offer insight into disease etiology, allowing further studies to determine MRI as a reliable and efficient screening tool for these patients.

MATERIAL AND METHOD

The patient group enrolled in the study consisted of 20 patients admitted to the Psychiatric Clinic of Fırat University, Faculty of Medicine Hospital, diagnosed with SSD based on DSM-IV-TR diagnostic criteria, treated as inpatients or outpatients, and who fulfilled the study inclusion criteria. In addition, 20 healthy subjects who fulfilled the study inclusion criteria and conformed to the patient group in age and gender were classified as a control group. Exclusion criteria for the patient group were the presence of a comorbid personality disorder, the presence of a neurological disorder or a history of a neurological disorder or treatment for a neurological disorder, a history of head trauma, a contraindication to MRI examinations, the presence of significant somatic pathology that could affect the distribution of the patient's ongoing psychiatric disturbances, and a history of alcohol or substance abuse disorder in the past six months. Exclusion criteria for healthy controls were the presence of psychiatric disorders, neurological diseases, and psychoactive drug use in the participants or their first-degree relatives. All participants were administered a sociodemographic and clinical questionnaire designed by the study's authors in agreement with the aims of this study and in agreement with clinical experience and knowledge from the literature. The sociodemographic and clinical questionnaire is a semi-structured form that collects sociodemographic characteristics such as age, marital status, educational level, occupation, gender, place of residence, economic status, and family structure, and clinical data such as duration of disease, number of hospitalizations, and psychosocial stressors at disease onset. Participants' depressive symptoms were measured with the Hamilton Depression Rating Scale (HAM-D) (17), whereas anxiety scores were measured with the Hamilton Anxiety Rating Scale (HAM-A) (18). Signed informed consent was given by all participants.

A power analysis performed with G*Power 3 with a power of 80% for the minimum sample size within the 95% confidence interval (CI) indicated that the study group had to consist of 32 participants, of whom 16 were patients and 16 were controls (19).

Fırat University Ethics Committee (2010/07) granted ethical approval for the study. The current study was undertaken between April 2012 and September 2012 in line with the tenets of Helsinki Declaration.

MRI Procedure and Volumetric Measurement

Procedure: For the imaging studies, a 1.5 Tesla scanner GE SIGNA (GE Medical System) was used to acquire three-dimensional (3D) T1-weighted MRI images with the following parameters: 1.5 mm sagittal cross sections, echo time (TE): 15.6 ms; repetition time: 14.4 ms; excitation number: 1; field of view (FOV): 240 mm; rotation angle: 20 degrees; bandwidth: 20.8; cross-sectional thickness: 2.4 mm; and resolution: 0.9375 x 0.9375 x 2.4 mm. Images achieved with these parameters were processed in the workstation software.

Volumetric Measurements: Volumetric measurements of the total brain, gray matter, white matter, and orbitofrontal regions of each patient and control subject were obtained by MRI. The area of the orbitofrontal cortex was examined in coronal, sagittal, and axial planes using the workstation GE. First, coronal slices were visually inspected and marked, and then they were measured.

The line running from the anterior commissure to the posterior commissure also forms the anterior region of the corpus callosum genu with its superior boundary. Alternatively, a horizontal line can be used to define more posterior sections showing most of the lateral OFC. Fixed geometric boundaries were created by using all sections on the line of the anterior commissure posterior commissure forming its upper boundary. Thus, the upper dimension of the lateral orbital sulcus was also included in the OFC. The posterior OFC boundary was localized with coronal images in sections starting from the first visible sulcus

olfactorius. The inferior boundary was mostly determined considering the inferior portion of the cortex (20, 21). The drawing was completed in the coronal plane to include the temporafrontal junction. Two different evaluators who were blinded to participant gender and diagnosis made the drawing and volumetric measurements.

Some examples of the volumetric measurements are shown in Figures 1 and 2.

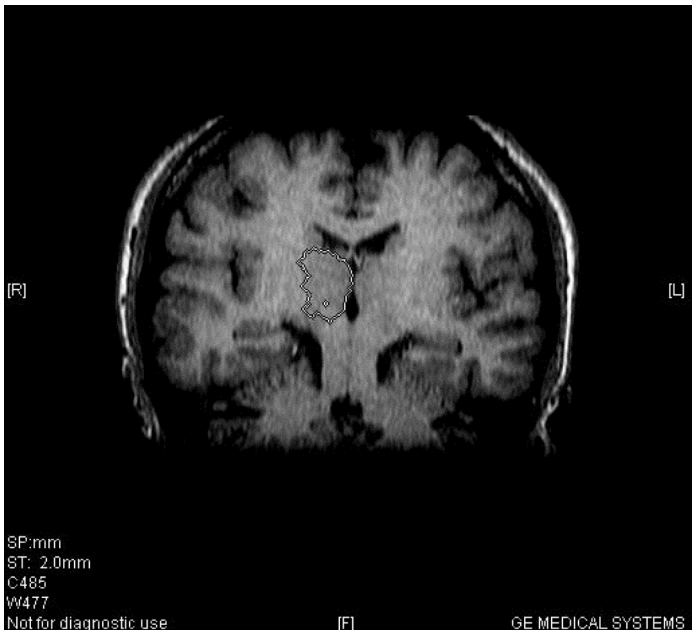

Figure 1. OFC MRG image

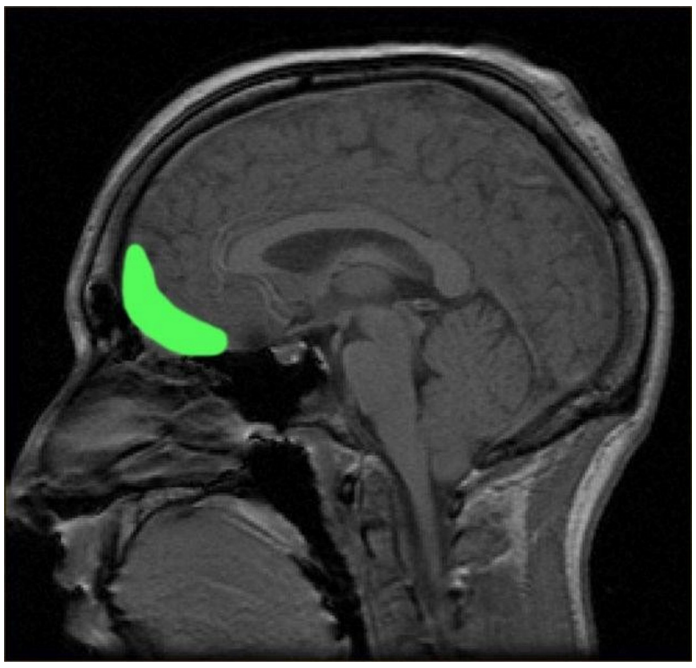

Figure 2. OFC MRG image, sagittal cross-section

Statistical Analysis

Study group data were presented as mean ± standard deviation (mean ± SD). Analysis of covariance (ANCOVA), Student's t-test, and chi-square tests were performed for statistical analysis. The association of volumetric values observed in the groups with age and disease duration was tested using Spearman correlation analysis. The SPSS 13.0 software package was applied for statistical analyses. Statistical significance was regarded as $p < 0.05$.

RESULTS

Twenty female patients were enrolled in the patient group. The study participants were between 30 and 62 years, presenting a mean age of 43.6±8.032. The control group also consisted of 20 healthy female subjects. The mean age of the control subjects was 40.0±3.90, ranging between 24 and 40 years. The patient and control groups had no significant differences regarding age ($p>0.05$). Most patients had either an elementary or middle/high school degree, were married, and had a middle or low socioeconomic status. The distribution of sociodemographical data among the study groups is shown in Table 1.

Table 1. Sociodemographic Characteristics of Patients and Controls

	Control (n=20)	Patient (n=20)
Age	43.6±8.32	40.0±3.90
Gender (M/F)	0/20	0/20
Disease duration		
0-5 y	-	9
6-10 y	-	2
11-15 y	-	5
≥16 y		4
Educational status		
Illiterate	-	2
Literate	-	2
Primary school	3	12
Secondary School	6	4
University	11	-
Marital Status		
Married	8	17
Single	12	3
Socioeconomic status		
Good	15	-
Moderate	5	16
Poor	-	4
Residency		
County	15	16
District	3	2
Village/Town	-	2

M: male, F: Female

The mean HAM-D scores in the patient and control groups were 15.35±3.32 and 7.85±1.92, respectively. In addition, the mean HAM-A scores in the patient and control groups were 11.15±2.97 and 6.15±2.20, respectively. HAM-D and

HAM-A scores did not vary significantly between groups (p>0.05 for both cases). The mean disease duration was 4.14±2.97 years. Statistical tests could not reveal any significant differences between the patient and control groups in total brain, gray matter, and white matter volumes (p > 0.05).

Volumetric measurements yielded that the mean OFC volume in the patient group was 13.21±0.90 ml on the right and 12.76±2.55 ml on the left, whereas in the control group it was 15.48±2.55 ml on the right and 16.16±2.06 ml on the left. Accordingly, statistically significant differences existed between the studied groups in OFC volume, both right and left (p<0.05 for both cases). No laterality was observed in both groups in terms of OFC volume. The distribution of the OFC measurement results between the patient and control groups is presented in Table 2. Some samples taken from sections during the volumetric measurements are illustrated in Figure 1 and Figure 2.

Table 2. OFC Volume in Patient and Control Groups

	Control (n=20)	Patient (n=20)	p
OFC Volume (ml)			
Right	15.48±2.55	13.21±0.90	p<0.05
Left	16.16±2.06	12.76±2.55	p<0.05

OFC: orbitofrontal cortex, ml: milliliter, p: probability statistic

DISCUSSION

Due to the limited number of studies on this issue, we would like to start the discussion by presenting our main study results. These results include that the mean OFC volume in SSD patients was 13.21±0.90 ml on the right and 12.76±2.55 ml on the left and was significantly smaller than in healthy controls.

A meta-analysis suggested that somatoform disorders are characterized by selective alterations in large-scale brain networks involved in cognitive control, emotion regulation and processing, stress, and somatic-visceral perception (22). Valet et al. found a decrease in orbitofrontal cortex volume in patients diagnosed with SSD, similar to our study (23). Perez et al. (24) stated that the orbitofrontal cortex is associated with SDD due to its emotional processing and recognition task. Considering fMRI methods in patients with SSD, there have been few studies on this topic. In this regard, neuroimaging studies with SSD have mentioned slow metabolism in the frontal lobes and non-dominant cerebral hemispheres (25). In a study conducted with SSD patients, regional blood flows in the brain were examined and hyperactivity was determined in the right parietal lobe. This brain region is thought to contain important cortical networks for the control of attention to external stimuli, and in this context, increased attention to external stimuli has been indicated in SSD patients (26). In a study conducted in Northern Finland, significantly higher levels of somatic anxiety were found in patients with right hemispheric tumors than in patients with left hemispheric tumors. Dizziness and palpitations were the most frequent specific signs in patients with right hemispheric tumors (27). Of studies that used structural neuroimaging methods, the 2004 study by Hakala et al. (6), which used MRI to examine 10 female patients with SSD and undifferentiated somatoform disorders and 16 healthy female controls, measured the volume of the caudate nucleus, hippocampus, and putamen, finding an increase in the volume of the putamen, a decrease in the volume of the hippocampus, and a significant increase in the volume of the caudate nucleus. Similarly, the Atmaca et al. study (7), conducted with 20 SSD patients and 20 control subjects, determined that the volume of the right and left amygdala was significantly smaller in patients than in healthy controls. In conjunction with previously reported related data, the present study results suggest that morphological changes in these regions, which are associated with stress and emotion regulation, may lead to alterations in emotional perception in these patients.

Regarding the findings related to the orbitofrontal cortex, this study found a significant reduction in OFC volume in SSD patients compared with control subjects. In comparison, studies from the related literature reported changes in the OFC not only in SSD patients but also in patients with somatoform disorders. In a 2009 study of 16 patients and 16 control subjects, patients with hypochondriasis were concluded to have smaller left and right OFC volumes and larger left thalamic volume than control subjects (11). These results have been reported to suggest that the OFC and thalamus may play an essential role in hypochondriasis pathophysiology. In an MRI study, it was pointed out that the volume of the OFC was smaller, whereas the volume of the white matter was larger in patients with body dysmorphic disorder than in controls. In addition, an association of disease duration with OFC volume was shown in the aforementioned study (12). In another study, single photon emission computed tomography (SPECT) detected elevated blood flow in the cingulum in a patient with body dysmorphic disorder (28).

The SSD patients included in this study had mild depressive symptoms. Somatic symptom disorder can often co-exist with depressive disorder. Moreover, SSD patients may often have symptoms that overlap with depressive disorder symptoms. In parallel, a reduction in OFC volume and a 7.2 % reduction in frontal lobe was observed in patients with depressive disorders compared with healthy control subjects, which was also the case in SSD patients (29). The results of another study, which found a volume reduction of approximately 8.6 %, are consistent with the aforementioned study (30). In a meta-analysis by Koolschijn et al. (31), which included studies with depression patients, it was found that the volume decrease of the prefrontal, anterior cingulate, and OFC regions was higher than the volume decrease of the hippocampal region. This finding suggests that frontal lobe structures and cingulate cortex play a role in or may be affected by the pathophysiology of

depression at least as much as the hippocampus. In light of the above stated, it can be speculated that OFC functions play as important a role in the etiology of SSD as they do in the etiopathogenesis of depression. However, further studies, such as functional neuroimaging studies in SSD patients, are needed to reach more definitive conclusions.

The current study has some potential limitations. First, the low sample size limits the power of our results. In addition, differences in measurement methods between our study and other studies may have affected our results. Another factor that complicates the interpretation and generalizability of our results is that few volumetric studies have been performed on SD patients.

CONCLUSION

In conclusion, the findings of this study indicated the abnormalities pertaining to OFC, which might also be related to the pathophysiology of SSD. Employing imaging modalities such as MRI may reduce the potential clinical diagnostic burden in cases where SSD can be bypassed. However, further studies with larger sample groups are needed to corroborate the findings of this study.

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MEDICAL RECORDS-International Medical Journal

Research Article

Comparison of the Results of Intragastric Gastric Balloon Application Versus Sleeve Gastrectomy on the Quality of Life in Patients Who Were Diagnosed as Asthma

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Abstract

Aim: We have aimed to compare the postoperative outcomes of asthmatic patients who have underwent laparoscopic sleeve gastrectomy (LSG) versus intragastric balloon(IGB) application.

Materials and Methods: Total of asthmatic 84 patients who have underwent LSG versus IGB due to morbid obesity between March 2019 and February 2021 in the tertiary surgery department have retrospectively analyzed. Demographic findings, global symptom scores, and length of the stay in hospital of patients were evaluated. The patients in present resarch have similar in terms of BMI, age, and gender. Statistically significant results were accepted as $p<0.05$.

Results: The mean age of the patients participating in the study were 42.32±6.51 (range 28–54), mean BMI were 42.3±6.7 kg/m² (range 41–52). There were no major complications and mortality were observed in patients. Age and gender were defined as independent risk factors by multivariant logistic regression analysis, Age, p:0.054, (OR (95%CI): 2.017), BMI, p:0.067. (OR (95%CI): 1.379), Gender was determined as p:0.110 (OR (95%CI): 0.928.

Conclusion: We have concluded that although the morbidity of sleeve gastrectomy is higher than the gastric balloon application process, LSG provides more improvement in quality of life than IGB process.

Keywords: Sleeve gastrectomy, gastric balloon, score

INTRODUCTION

Morbid obesity is common significant health problem in the world, but it is a complex and multifactorial disease that continues increasing rapidly (1). The fact that asthma and morbid obesity have a parallel increase all over the World, many researchs have work for potential relationships for both diseases. Although some studies demonstrate that both diseases may have similar origins, the majority of epidemiological and clinical studies in this area have focused on obesity as a causal factor in the development of asthma (2). The relationship between these conditions remains unclear, whether asthma influences the onset of obesity(3). While LSG (laparoscopic sleeve gastrectomy), which has become almost conventional process in bariatric surgery, plays a primary role, gastric balloon application (IGB) is currently applied in patients who have not benefited from first-line treatment (4).

The studies conducted in our hospital, it is seen that the prevalence of obesity in the adult population exceeds the critically high rate of 45%, and although the prevalence of obesity is higher in women, the rapid increase in men in recent years has been noted (1). In the United States, the prevalence of asthma in morbid obese populatıon is 14.6% and 7.9%, consecutıvely (4). Studies have shown that obesity has a dose-dependent effect on asthma risk, and also greater the body mass index (BMI) correlate with increased risk of asthma (5).

In addition to this information, the LSG procedure is more effective than IGB, but it is an irreversible procedure that can cause more morbidity. In our study, we aimed to compare the efficacy of asthma patients who underwent LSG and IGB operation with the Global general symptom (DGB) and reflux quality of life (RQS) scores (6,7).

CITATION

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MATERIAL AND METHOD

Local Ethics Committee has been approved this retrospective research (2021 / 7–decision number 151). Totally 84 patients who have applied to our clinic with morbid obesity and performed LSG and IGB procedures according to their BMI results between March 2019 and February 2021 were evaluated retrospectively. All patients also had their informed consent form read before the procedure and their consent was obtained. Necessary consultations (endocrine, psychiatry, chest, dietitian) were made for our patients who applied before the procedure, and necessary information was given to the patients for the operation. The procedures of the patients were performed by a team experienced in bariatric procedures.

Study protocol

The patients participated in the study in accordance with the Declaration of Helsinki, and approval was obtained from the local ethics committee within this framework. First of all, the Global General Symptom (GGB) questionnaire was applied to the patients, it consisted of a scale with 7 points graded upper gastrointestinal symptoms. The patients were evaluated in terms of epigastric pain, heartburn, acid regu- lation, bloating, nausea, early satiety, postprandial bloating parameters. The Quality of Life in Reflux (RQS) questionnaire was filled, which was a 5-part questionnaire consisting of 25 questions, in which emotional stress, social and physical functions, eating and drinking problems, and sleep problems were questioned. The questionnaires were repeated 12 months after the procedure.

Exclusion criteria

Psychiatric diseases and frequent exacerbations (at least 3 attacks per month) were found in the history of the patients. Demographic findings, duration of the procedure, complication rates, and hospital treatment durations of the patients who underwent the procedure were evaluated. The mean follow-up times of the patients were 1, 3, 6 and 12 months after the procedure.

Bariatric Procedures

Pre-procedural information was given to the patients, both procedures were explained, and their consent was obtained. Patients were advised to fast 12 hours before the procedure. The patients were intubated in the bariatric surgery procedure and placed in the obesity surgery position. LSG procedure was applied to the patients at a distance of 4 cm from the pylorus. In the IGB procedure, the stomach was filled with 550cc of methylene blue and a gastric balloon under sedation. After the procedure, the patients were kept under observation for 45 minutes and were sent with recommendations.

RESULTS

A total of 84 followed-up asthma patients without frequent exacerbations were included in the study. All patients agreed to fill out the questionnaire. 54 of the patients were female (64.7%) and 30 were male (35.3%), mean BMI was 42.32±6.51 (age range 28–54). The mean duration of the procedure was 55.5±8.2 minutes in LSG (range 43–84 minutes), and 18±5.6 minutes (13-48) in IGB. Bleeding was observed in the early postoperative period in 2 patients who underwent LSG in the postoperative period, and the existing bleeding of the patients was controlled with supportive treatment. No additional complication was observed in the patients who underwent IGB. The symptoms of the patients were evaluated at 1,3,6 and 12 months. Independent risk factors of GERD were evaluated in multivariate regression analysis. Considering the effects of GERD in the postoperative period, it was observed that there was a significant improvement and regression in LSG patients compared to the RQS questionnaire (Table 1, 2).

Table 1. Demographic and characteristic features of the patients

	LSG(n:43)	IGB(n:41)	P value
Number of patients	43	41	
Age	41.4(28-52)	43.6(32-61)	0.723
Gender,(Y/F)	32/11	22/19	0.849
BMI, kg/m²	40.7(40-55)	44.2(42-51)	0.903
Hypertension	16	15	0.629
Drugs used			
Inhaler	43	41	0,692
Steroid	11	14	0.754
PPI	21	27	0.472
NSAID	32	34	0.571
Addictions			0.718
Tobacco	11	8	

LSG:lap.sleeve gastrectomy, IGB:Intragastric balloon

Table 2. Scores and postoperative findings of the groups 12 months later the process

	LSG	IGB	P value
Loss of excess weight (LEW%)	35%	24%	<0.05
RQS score posttop	4.5	2.5	<0.05
GGS score postop	4.4	1.7	0.041

RQS: reflux quality of life score, GGS: global general symptom

Statistics

Mann–Whitney U test was used to compare categorical data. All data in the study were analyzed with the SPSS version 25.0 data package. Data are given as mean ± standard deviation (Sd), percent. The patients in our study group were accepted as randomized because they were similar in terms of BMI, age, gender and number. Statistically significant results were accepted as p<0.05.

Table 3. Regression analysis of independent predictive values of reflux symptoms

Predictive factor	P value	Odds ratio (95%CI)
Age	0.054	2.017 [0.872, 3.671]
BMI	0.067	1.379 [0.911, 3.472]
Gender	0.110	0.928 [0.851, 1.325]

DISCUSSION

Asthma is common worlwide disease and also affects nearly half million people all oveer the world (8). The prevalence of asthma in the world is 1.5, with 16.7% in the adult population and approximately 10% in the pediatric population.

In the literature, there are some studies showing that obesity may cause asthma by triggering hyperresponsiveness of airway, similar genetic familiarity for both diseases in the immune system of human, as well as studies involving medical and behavioral mechanisms such as asthma onset time and drugs used in the treatment. Most of the studies which have evaluated the relationship between asthma and physical activity have mentioned the role of asthma as an independent variable affecting physical activity in young people (9).

In the reviewed researchs, it was reported that there is a positive relationship between asthma and overweight. Relative and absolute criteria have varied in the methodology of studies evaluating the relationship between asthma and weight gain. Significant associations varied according to weight status, definition of pulmonary dysfunction, or gender. In a case-control study reporting a higher probability of being overweight among asthmatics compared to controls (10). In a cross-sectional study which investigates the relationship between asthma and BMI, it was reported that there was a stronger relationship between asthma and BMI (11,12).

Among the methods applied for morbidity, the most effective method stands out today as surgery. Although bariatric surgery is a beacon of hope for these patients, it has been shown by studies that there is a positive improvement in patients and a decrease in the number of attacks. Today, especially LSG is a frequently applied method. There is a decrease in the frequency of reflux in patients after LSG. In most of the morbidly obese patients before bariatric surgery, drugs such as proton pump inhibitors are used frequently and their quality of life indexes. IGB application has been performed for morbidly obese people who are afraid for the complications of surgery, it also leads to effective weight loss (13). The European Community Respiratory Health Survey (ECRHS) found a relationship between asthma and obesity and showed that this relationship carries a higher risk in women than in men (14,15).

In multicentric studies, it has been determined that weight loss achieved non-surgical methods has a positive effect on asthma, and reduces the asthma exacerbations and (16-18). In a large retrospective study, it was shown that in LSG applied procedures, 39.3% patients have stopped to use drugs and 42% of patientsstopped to use bronchodilators (19,20). Bariatric surgery has also been shown to improve lung function and reduce exacerbations in these patients (21,22).

In our study, reflux rates and survey results showed a regression compared to the preoperative period after 12 months postoperatively, and a significant difference was found in LSG process compared to the IGB procedure. In the BAROS quality of life index study which is conducted by Alley et al., reported that obesity primarily impairs quality of life (23-25). In present study, more effective and successful results were obtained in LSB in postoperative follow-ups related to reflux. In the regression analysis results of the patients, preoperative BMI was evaluated as an independent risk factor of age and gender.

In the meta-analysis report of Driscoll et al., reported that the quality of life index improved after bariatric surgery. In the LSG method, which is one of the procedures applied in our study, a significant difference was observed compared to the IGB group in terms of quality index (24). We are in the same opinion that the RQS and GGS scores provide sufficient and effective information in terms of evaluating the obese patient profile, similar with the Driscoll study. Of course, there are some limitations in our study. First of all, they were a retrospective study, the patient group was smaller, and it was not a long-term study.

As a result, we concluded that there was a statistically significant improvement in RQS indices compared to IGB in the postoperative 12-month follow-up of the patients who underwent LSG (Table 3).

CONCLUSION

According to the results of our study, LSG procedure is irreversible compared to IGB in the morbidly obese patient population with asthma, and it is more effective procedure for improving reflux and reducing asthma exacerbations. Although it is not suitable to be applied in every patient due to high morbidity. Prospective and comprehensive studies are needed in the future.

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MEDICAL RECORDS-International Medical Journal

Research Article

Long-Term Results of Tragal Cartilage Type 1 Tympanoplasty

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Abstract

Aim: Tympanoplasty is one of the most frequently performed operations by ear, nose, and throat specialists. Surgical treatment of tympanoplasty aims to obtain a healthy ear membrane and minimise hearing loss. The purpose of this study was to evaluate the success of the graft and functional hearing outcomes of patients applied with tragal cartilage type 1 tympanoplasty operation in our clinic.

Materials and Methods: This study included 43 patients who underwent tragal cartilage type 1 tympanoplasty operation between January 2016 and November 2020. A retrospective evaluation was made of the preoperative pure tone audiometry (PTA) values and the operative findings. In the follow-up examination, the graft status and PTA results were recorded. The graft success and improvements in hearing were evaluated in all the patients.

Results: A total of 43 patients were evaluated, comprising 24 males and 19 females with a mean age of 31.2 years (range, 13-58 years). The operation was performed on the right ear of 22 patients and the left ear of 21. The tympanoplasty operation was performed with an endaural approach in 6 cases and a postauricular approach in 37. The mean follow-up period was 20.8 months (range, 12-39 months). The rate of graft success of all the patients was 88.4%. The mean air conduction threshold, air-bone gap, and bone conduction threshold values at 500, 1000, 2000, and 4000 Hz were determined to have statistically significantly decreased postoperatively in comparison with the preoperative values ($p<0.05$).

Conclusion: In tragal cartilage type 1 tympanoplasty operations, high rates of graft success were obtained and a dramatic gain in hearing postoperatively.

Keywords: Cartilage type 1 tympanoplasty, hearing gain, graft success

INTRODUCTION

Chronic otitis media (COM) is an ear disease frequently seen in both children and adults. The clinical findings of the disease are tympanic membrane perforation and intermittent recurrent ear discharge. In the suppurative period of the disease, medical treatment is applied, but the ultimate treatment of COM is surgery. Tympanoplasty is the most frequently performed operation in the treatment of COM. The primary purpose of treatment with tympanoplasty is to obtain a healthy ear membrane and middle ear mucosa, and to correct hearing loss (1).

The factors important in the success of tympanoplasty are operation preparation, the localisation and dimensions of the perforation, the surgical technique used, the functional status of the Eustachian tube, the presence of nasal pathologies, the skill of the surgeon, and the graft materials used. Tympanoplasty was first applied by Wullstein in 1952, followed by Zoellner in 1955, and since then various graft materials have been used to repair perforations in the tympanic membrane (2,3). These graft materials include skin, periostium, perichondrium, dura mater, cartilage, vessels, fat tissue, and temporal muscle fascia (4). In recent years, cartilage has been used more as graft material (5). Cartilage has the properties of resistance to graft retraction, resorption, and negative pressure, has good connections with the surrounding tissue, and appropriate elasticity for sound transmisson (6,7).

The purpose of this study was to compare the preoperative and postoperative pure tone audiometry results of patients applied with tragal cartilage type 1 tympanoplasty operation, to determine the postoperative hearing gain and evaluate the postoperative tragal cartilage graft success.

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MATERIAL AND METHOD

The study included 43 patients on whom tragal cartilage type 1 tympanoplasty operation was performed for a diagnosis of non-suppurative chronic otitis media in the Ear, Nose, and Throat Clinic of Karaman Training and Research Hospital in the period January 2016 - November 2020. The patients forming the study group were those with no cholesteatoma, intact ossicle chain, healthy middle ear mucosa and no ear infection for a minimum of 3 months preoperatively. The study exclusion criteria were defined as damage or fixation in the ossicle chain, a history of mastoidectomy or revision tympanoplasty, the presence of sensorineural or mixed type hearing loss, or incomplete preoperative audiometry results or non-attendance of postoperative follow-up examinations.

The study protocol was approved by the Ethics Committee of Karamanoğlu Mehmetbey University Medical Faculty (decision no:02, dated:07.12.2020). All procedures were applied in compliance with the principles of the Helsinki Declaration. All the study participants provided written informed consent.

The preoperative pure tone audiometry (PTA) values and the operative findings were retrieved from the hospital archives and recorded. To be able to evaluate the long-term graft success and hearing outcomes, the graft status was evaluated with endoscopic ear examination and hearing outcomes with the PTA test at the postoperative 12-month follow-up examination. The air conduction thresholds, the mean air conduction thresholds, the mean bone conduction thresholds, and the mean air-bone gap thresholds in the PTA test at 500, 1000, 2000, and 4000 Hz were recorded preoperatively and postoperatively. Then the preoperative and postoperative results were compared. The air and bone conduction threshold mean values were calculated as the arithmetic mean of the values at 500, 1000, 2000, and 4000 Hz.

Surgical Technique

All the graft materials were prepared from tragal cartilage. The general reconstruction was made using a perichondrium cartilage island graft, starting with the harvesting of the cartilage from the tragal area (8). In order not to disrupt the cosmetic appearance, an incision was made leaving a 2mm cartilage crown in the dome of the tragal cartilage. Protecting the perichondrium on both surfaces of the cartilage, it was dissected from the surrounding tissues, excised and removed. By elevating the perichondrium on the convex surface of the tragal cartilage, the perichondrium formed the cartilage island graft. Cartilage excisions were made from the edges of the tragal cartilage in line with the size of the perforation in such as way as to preserve the perichondrium. Then at the point where the tragal cartilage meets the manubrium mallei, an incision was made to open a space. De-epithelialisation of the perforation edges of the tympanic membrane was performed using a pick. A tympanomeatal flap was formed and elevated, then the middle ear space below the anulus was reached. The integrity of the ossicle chain, mobility, and middle ear mucosa were checked by dissecting the tympanic membrane from the manubrium mallei. The perichondriun cartilage island graft prepared from the tragal cartilage was placed using the underlay technique. The cartilage graft was supported by placing Gelfoam and Spongostan in the middle ear space. The tympanomeatal flap was then laid out in place and it was checked whether or not the perforation was fully covered by the graft. After ensuring that the perforation was fully covered, the tympanomeatal flap was supported with the placement of Gelfoam and Spongostan.

Statistical Analysis

Statistical analyses were performed using IBM SPSS vn. 22.0 software. Conformity of continuous variable to normal distribution was assessed with Q-Q and normal curve with histogram plots. Normally distributed continuous variables were analyzed with the independent samples t-test. The level of statistical significance was set as $p<0.05$.

RESULTS

A total of 43 patients were evaluated, comprising 24 (55.8%) males and 19 (44.2%) females with a mean age of 31.2 years (range, 13-58 years). The operation was performed on the right ear of 22 (51.1%) patients and the left ear of 21 (48.9%). The tympanoplasty operation was performed with an endaural approach in 6 (14%) cases and a postauricular approach in 37 (86%). The mean follow-up period was 20.8 months (range, 12-39 months).

The tympanic membrane perforation was determined to be fully covered in 38 (88.4%) cases, and in the 5 (11.6%) cases where the tympanic membrane perforation was not closed, the perforation was observed to be minimal (Table 1). Ossicle chain reconstruction was not performed in any patient.

Table 1. Patient demographic data

Age (years)		**31.2 (13-58)**
Gender	Female	19 (44.2%)
	Male	24 (55.8%)
Operated ear	Right	22 (51.1%)
	Left	21 (48.9%)
Graft success	Intact	38 (88.4%)
	Perforated	5 (11.6%)

In the preoperative PTA test of the 43 ears applied with tympanoplasty, the air conduction mean threshold values were recorded as 29.53±6.97 dB at 500 Hz, 27.79±6.2 dB at 1000 Hz, 28.13±6.72 dB at 2000 Hz, and 33.25±7.55 dB at 4000 Hz. The postoperative air conduction mean threshold values were seen to be 20.81±5.66 dB at 500 Hz, 20.23±5.34 dB at 1000 Hz, 18.83±6.34 dB at 2000 Hz, and 27.79±6.66 dB at 4000 Hz. The improvements in postoperative hearing levels at all frequencies were determined to be statistically significant ($p<0.05$). Preoperatively, the bone conduction threshold was mean 12.76±5.34 dB, the air conduction threshold was mean 30.06±5.2 dB and the air-

bone gap threshold value was mean 17.65±4.71 dB. The postoperative values were determined to be mean bone conduction threshold of 11.3±4.87 dB, mean air conduction threshold of 22.18±4.24 dB and air-bone gap threshold value of 10.88±4.07 dB. With the exception of the bone conduction value, all the other findings were determined to be statistically significant (p<0.05) (Table 2).

Table 2. Preoperative and postoperative pure tone audiometry results

	Preoperative (dB)	Postoperative (dB)	P value
500 Hz (air conduction threshold)	29.53±6.97	20.81±5.66	<0.001
1000 Hz (air conduction threshold)	27.79±6.2	20.23±5.34	<0.001
2000 Hz (air conduction threshold)	28.13±6.72	18.83±6.34	<0.001
4000 Hz (air conduction threshold)	33.25±7.55	27.79±6.66	<0.001
Mean air conduction threshold	30.06±5.2	22.18±4.24	<0.001
Mean bone conduction threshold	12.76±5.34	11.3±4.87	0.188
Mean air-bone gap threshold	17.65±4.71	10.88±4.07	<0.001

dB: Decibel, Hz: Hertz

DISCUSSION

The aim of surgical treatment for chronic otitis media is to obtain a healthy ear and functional hearing (9). To be able to achieve this, the graft must be intact after the operation, and the incoming sound waves must be able to be sufficiently conducted to the middle ear. Various surgical techniques and graft materials are used in tympanoplasty operations to obtain this success.

As the structure of cartilage grafts used until recently is hard and thick, there was the belief that the hearing outcomes of patients applied with cartilage tympanoplasty would be less successful compared to other tympanoplasty grafts. However, recent studies have shown that in addition to the successful membrane coverage of cartilage tympanoplasty, audiologically it has provided similar hearing results as structurally thinner and more flexible grafts such as the temporal fascia and perichondrum (10,11). Of all autografts, cartilage graft is the most resistant to absorption and is well connected to the surrounding tissue (12). It is appropriate for sound vibration in Eustachian tube dysfunction because of the hard structure and resistance to negative pressure and flexibility (13).

In an extensive study by Dornhoffer, published in 2003, the functional results of cartilage tympanoplasty were shown to be very good (14). Yurttas et al. reported a 93% membrane success rate in cartilage tympanoplasty (15), and in another study, Abdelhameed et al. also reported graft success rate of 92% (16). In the current study, the tragal cartilage graft success rate was found to be 88.4%. In 5 patients in the current series, an opening was determined in the anterior of the graft, but the postoperative membrane perforation was seen to be extremely small in these patients compared to the size of the preoperative perforation. The hearing functions were observed to be better than the preoperative results. Thus, the success rate of cartilage tympanoplasty graft in this study was consistent with previous findings in literature.

Karaman et al. reported that cartilage was a highly appropriate graft material in tympanoplasty in respect of hearing and graft success. Graft success was reported to be 97%, and of the functional results, there were improvements in the air-bone gap with values reported of 20.2 dB at 500 Hz, 23.58 dB at 1000 Hz, 22.23 dB at 2000 Hz, and 24.79 dB at 4000 Hz (17). Yilmaz et al. evaluated cartilage tympanoplasty in both adult and pediatric patients. Graft success rate was found to be 92.6%, and the preoperative and postoperative mean air conduction threshold values were found to be 34.8±8.5 dB and 23.4±9.1 dB, respectively in adults, and 30.6±7.7 dB and 17.8±7.8 dB in children (7). The cartilage graft success and hearing improvements in the current study were evaluated in the follow-up period of mean 20.8 months (range, 12-39 months). The success of the tragal cartilage graft was found to be 88.4%. In the postoperative PTA test, the mean air conduction threshold values were found to be 20.81±5.66 dB at 500 Hz, 20.23±5.34 dB at 1000 Hz, 18.83±6.34 dB at 2000 Hz, and 27.79±6.66 dB at 4000 Hz. The postoperative air conduction threshold was determined to be 22.18±4.24 dB and the air-bone gap was 10.88±4.07 dB. The mean postoperative air conduction threshold values at 500, 1000, 2000, and 4000 Hz, and the mean air-bone gap values were observed to be statistically significantly lower than the preoperative values (p<0.05). Approximately 7 dB closure was determined in the mean air-bone gap.

CONCLUSION

The findings of this study demonstrated that the long-term results of the tragal cartilage tympanoplasty technique were extremely good in respect of graft success and hearing improvements. It can be considered that the recent positive results of perforation closure rates and hearing gains of cartilage tympanoplasty will ensure that this

technique becomes more widespread.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *The study protocol was approved by the Ethics Committee of Karamanoğlu Mehmetbey University Medical Faculty (decision no:02, dated:07.12.2020).*

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Research Article

Evaluation of Factors Associated with the Clinical Course and Prognosis of Patients with Guillain-Barre Syndrome

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Abstract

Aim: This study aims to investigate the clinical, laboratory, electrophysiological, and demographic characteristics of patients with Guillain-Barre Syndrome (GBS) who were admitted to our clinic and underwent treatment and the factors contributing to the prognosis at discharge.

Materials and Methods: The study included 138 patients admitted to our clinic for treatment between January 2013 and December 2017, whose patient records were reviewed retrospectively. The Hughes scores, demographic characteristics, and clinical and laboratory data of the patients at admission and discharge were recorded.

Results: The study sample comprised 61 female (44.2%) and 77 male (55.8%) patients with a mean age of 58.1 years. In evaluations of the Hughes scores at admission and discharge, 117 patients were considered to have a good prognosis and 21 patients to have a poor prognosis at discharge. In the poor prognosis group, advanced age (p=0.028), being in the acute motor axonal neuropathy (AMAN) subtype (p=0.001), development of sepsis (p=0.007), need for mechanical ventilation (p<0.001), high Hughes scores on admission (p<0.001), extended hospitalization (p=0.030), increased WBC count (p=0.033), presence of hyponatremia (p<0.001), abnormal liver function test (p=0.08) were higher than the good prognosis group.

Conclusion: Early identification of GBS patients who may have a poor prognosis and rapid application of appropriate treatment methods are essential in creating positive effects on the clinical course and prognosis in this patient group.

Keywords: Guillain-Barré syndrome, prognostic factors, clinical course

INTRODUCTION

Guillain-Barré Syndrome (GBS) is an inflammatory disease of the peripheral nervous system and is the most common cause of acute flaccid paralysis. The reported global incidence is 1-2 per 100.000 (1). GBS is more common in males than females, and the incidence increases with age (1). Up to 60% of patients have an infectious event history. The most important triggers are diarrhea caused by Campylobacter (C.) jejuni and upper respiratory tract infections (2). The pathophysiology of the disease involves severe immune mechanisms, including cellular and humoral immunity, complement deposition, proinflammatory cytokines, and other inflammatory mediators (3).

During the disease, neurological symptoms that are typically sensory start with or before weakness. Most patients experience paresthesias, such as burning and prickling in the hands and feet. Characteristically, the symptoms are highly symmetrical and often progressive. Deep tendon reflexes (DTRs) may be preserved in the early period but are absent in up to 90% of cases, and weakness usually starts in the lower extremity and spreads upward. Manifestations of autonomic nervous system involvement may accompany the disease. Cranial neuropathy is observed in some cases, while respiratory distress and the need for respiratory support occurs in 20–30% of patients (4). Patients with GBS reach maximum disability in two weeks, while the disease enters a plateau phase after the initial progressive phase that can last from days to weeks or even months (5).

The diagnosis of GBS is based on patient history, neurological examination findings, electrophysiological findings, and cerebrospinal fluid (CSF) studies (6). Electrophysiological studies are of great importance in differentiating between the disease subtypes, such as acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN), and acute inflammatory demyelinating polyneuropathy (AIDP) (6).

GBS is a monophasic disease with an expected relapse

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in only 2–5% of cases. Poor prognostic factors that negatively affect the clinical course include subtypes with axonal involvement, accompanying diabetes mellitus (DM), hypertension (HT), rapid progression in the early period, early respiratory depression, and the need for mechanical ventilation (MV), hyponatremia, hypoalbuminemia, elevated leukocyte (WBC) counts, abnormal liver function tests (LFTs) and acute kidney injury (AKI) (7,8).

The early identification of predictive factors that may result in poor prognosis would enable more effective and aggressive treatment in the early stages of the disease when nerve dysfunction is potentially reversible.

The present study assesses the clinical, laboratory, and electrodiagnostic findings of patients followed up for GBS and evaluates the prognostic factors based on the Hughes scores determined at discharge.

MATERIAL AND METHOD

Included in the study were 138 patients (61 female, 77 male) admitted to our clinic for treatment between January 2013 and December 2017. Patient records were reviewed retrospectively, and the age at admission, sex, presenting complaints, pre-disease status, CSF findings, examination findings, electrophysiological study results, and treatment protocols of all patients were recorded.

GBS was diagnosed according to the Electroneuromyographic (ENMG) examination defined by Asbury and Cornblath (9). The GBS subtypes and variants were differentiated and classified as AIDP, AMAN, AMSAN, and Miller-Fisher Syndrome (MFS). Inclusion criteria were new-onset symmetrical sensory loss and/or weakness and reduced or absent deep tendon reflexes. Patients with clearly defined sensory level loss, accompanying DM, rheumatic disease, toxic substance use or exposure, lesions identified on magnetic resonance imaging that may cause the clinical picture, and other known muscle diseases or neurological diseases were excluded from the study.

The patients' GBS disability scores (Hughes scores) were calculated at admission and discharge and defined as Grade 0: Normal; Grade 1: Minor symptoms, capable of running; Grade 2: Able to walk 10 m without support; Grade 3: Able to walk 10 m with support; Grade 4: Confined to bed or chair-bound; Grade 5: Requiring assisted ventilation for any part of the day; Grade 6: Death. Grade 2 and below were classified as good prognosis, and Grade 3 and above as poor prognosis (10).

Factors that may be effective in terms of prognosis were retrospectively reviewed. These were; age, gender, previous infection or vaccination, subtypes according to EMG findings, treatment modality, presence of complications, need for a mechanical ventilator, CSF protein level, presence of cranial nerve involvement, presence of facial paralysis, Hughes score at admission, serum glucose level at hospitalization, duration of hospitalization, WBC count, serum albumin level and presence of hypoalbuminemia (hypoalbuminemia defined as serum albumin level lower than 3.4g/dL), presence of hyponatremia (defined as serum sodium level lower than 135 mmol/L), and presence of abnormal liver or renal function test [defined as abnormal aspartate aminotransferase-alanine aminotransferase (AST-ALT) and abnormal blood urea nitrogen-creatinine (BUN-Cr) levels].

The study was approved by the Clinical Research Local Ethics Committee of Erciyes University (No: 2018/41). Due to the study's retrospective design, informed consent was not obtained.

Statistical Analysis

All statistical analyses were made in IBM SPSS Statistics (Version 26.0. Armonk, NY: IBM Corp.). Data were presented as the number of patients, percentage, mean, median, and standard deviation. The normality of the data was analyzed with a Shapiro-Wilk test. Parametric tests were used for normally distributed data, and non-parametric tests were used for non-normally distributed data. The significance of the difference between categorical variables was assessed with Chi-square and Fisher's exact tests. A p-value of <0.05 was considered statistically significant.

RESULTS

The study sample of 138 patients admitted with Guillain-Barré Syndrome included 61 (44.2%) females and 77 (55.8%) males, with a mean age of 58.1±19.7 years. The clinical characteristics and demographic data of the patients are presented in Table 1.

There was no statistically significant difference in the seasonal distribution of the patients. 61 (44.2%) patients had a history of disease prior to the event. There was a history of upper respiratory tract infections (URTIs) in 25 (18.1%) patients, gastroenteritis in 24 (17.3%) patients, urinary tract infections (UTIs) in six (4.3%) patients, surgery in three (2.1%) patients, vaccination in two (1.4%) patients, and Herpes zoster in one (0.7%) patient.

All patients underwent an ENMG examination during their hospital stay, revealing AIDP in 83 (60.1%) patients, AMAN in 23 (16.7%) patients, AMSAN in 28 (20.3%) patients, and MFS in four (2.9%) patients.

All patients underwent a lumbar puncture (LP), and the cerebrospinal fluid (CSF) studies revealed a mean CSF protein value of 108 mg/dL (20-692) in the patients. Intravenous immunoglobulin (IVIG) alone was administered to 59 (42.8%) patients, plasma exchange (PD) alone to 42 (30.4%) patients, IVIG followed by PD to 22 patients (15.9%), PD followed by IVIG to seven (4.4%) patients. In contrast, eight patients received no treatment as the symptomatology included only sensory symptoms.

Table 1. Main demographic and clinical characteristics of the patients

Variables	N (%)
Age (years, mean ± SD)	58.1 ± 19.7
Sex	
Female	61 (44.2)
Male	77 (55.8)
Seasonal distribution Spring	
Spring	36 (26.1)
Summer	39 (28.3)
Fall	26 (18.8)
Winter	37 (26.8)
Previous disease or event	
Surgery	3 (2.1)
Vaccination	2 (1.4)
URTI	25 (18.1)
Gastroenteritis	24 (17.3)
UTI	6 (4.3)
Herpes zoster	1 (0.07)
ENMG findings	
AIDP	83 (60.1)
AMAN	23 (16.7)
AMSAN	28 (20.3)
MFS	4 (2.9)
Treatment	
IVIG	59 (42.8)
PE	42 (26.6)
PE+IVIG	7 (5.1)
IVIG+PE	22 (15.9)
No treatment	8 (5.8)
Complications	
AKI	1 (0.7)
Ileus	1 (0.7)
Pneumonia	1 (0.7)
Infection	8 (6)
Sepsis	2 (0.7)
Pancreatitis	1 (0.7)
Need for MV	15 (10.9)
CSF protein, median (min-max)	108 (20-692)
Cranial nerve involvement	17 (12.3)
Facial paralysis	12 (8.8)
Mortality	10 (7.2)

N: Number, SD: Standard deviation, URTI: Upper respiratory tract infection, UTI: Urinary tract infection, ENMG: Electroneuromyography, AIDP: Acute inflammatory demyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, AMSAN: Acute motor and sensory axonal neuropathy, MFS: Miller-Fisher syndrome, IVIG: Intravenous immunoglobulin, PE: Plasma exchange, AKI: Acute kidney injury, MV: Mechanical ventilation, CSF: Cerebrospinal fluid

Table 2. Factors affecting the prognosis of patients according to the Hughes scores at discharge

Variables	Good prognosis (Hughes score ≤2 N=117	Poor prognosis (Hughes score >2)N=21	p value
Age (years, mean ± SD)	57.7 ± 20.2	65.8 ± 14.1	**0.028**
Sex			0.732
Female	51 (43.6)	10 (47.6)	
Male	66 (56.4)	11 (52.4)	
Seasonal distribution			0.323
Spring	29 (24.8)	7 (33.3)	
Summer	34 (29.1)	5 (23.8)	
Fall	20 (17.1)	6 (28.6)	
Winter	34 (29.1)	3 (14.3)	
Previous event or disease			0.231
No previous disease or event	62(53.9)	12 (60.0)	
Surgery	2 (1.7)	1 (5.0)	
Vaccination	1 (0.9)	1 (5.0)	
URTI	20 (17.4)	5 (25.0)	
Gastroenteritis	23 (20.0)	1 (5.0)	
UTI	6 (5.1)	0	
Herpes zoster	1 (0.9)	0	
ENMG findings			
AIDP	78 (66.7)	5 (23.8)	**0.001**
AMAN	14 (12.5)	9 (42.9)	**0.001**
AMSAN	22 (18.8)	6 (28.6)	0.80
MFS	3 (2.6)	1 (4.8)	0.95
Treatment			
IVIG	56 (47.9)	1 (14.3)	**0.08**
PE	34 (29.1)	8 (38.1)	0.96
PE+IVIG	4 (3.4)	3 (14.3)	0.35
IVIG+PE	17(14.5)	5(23.8)	0.88
No treatment	6 (5.1)	2 (9.5)	0.96
Complications			
No	110 (94)	14 (66.7)	**0.03**
Sepsis	0 (0)	2 (9.6)	**0.07**
Pneumonia	0 (0)	1 (4.8)	**0.45**
Infection	6 (5.1)	2 (9.5)	1.00
AKI	0 (0)	1 (4.8)	0.45
Pancreatitis	1(0.9)	0 (0)	1.00
Ileus	0 (0)	1 (4.8)	0.45
Need for MV	3 (2.6)	12 (57.1)	**<0.001**
CSF protein	104.8 (132.8)	140.9 (171.9)	0.430
Cranial nerve involvement	14 (12.1)	3 (15.0)	0.714
Facial paralysis	11 (9.4)	1 (4.8)	0.487
Hughes score at admission	2.1 (0.9)	3.6 (0.5)	**<0.001**
Serum glucose, median (min-max)	80 (48-301)	105.5 (47-329)	0.057
Length of hospital stay (days)	15.8 (9.9)	22 (19.5)	**0.030**
WBC count	9.9 (7.8)	14.7 (15.1)	**0.033**
Albumin	4.1 (3.0)	4.8 (5.3)	0.462
Hyponatremia	4 (3.4)	9 (42.9)	**<0.001**
Abnormal liver function tests	7 (6)	5 (23.8)	**0.08**
Abnormal kidney function tests	0 (0)	1 (4.8)	0.152

SD: Standard deviation, URTI: Upper respiratory tract infection, UTI, Urinary tract infection, ENMG: Electroneuromyography, AIDP: Acute inflammatory demiyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, AMSAN: Acute motor and sensory axonal neuropathy, MFS: Miller-Fisher syndrome, IVIG: Intravenous immunoglobulin, PE: Plasma exchange, AKI: Acute kidney injury, MV: Mechanical ventilation, CSF: Cerebrospinal fluid, WBC: White blood cell count (p-value, in bold those statistically significant)

A need for mechanical ventilation (MV) developed in approximately 10.9% of the patients at follow-up, and among these, ten died (6 male, 4 female) while five were discharged. The patients who developed ileus and AKI as complications died. At admission, complications included infections, pneumonia, sepsis, ileus, pancreatitis, and acute kidney injury.

The Hughes scores were assessed at admission and discharge, and the factors affecting the scores at discharge were analyzed. Accordingly, 117 patients were considered to have a good prognosis and 21 patients to have a poor prognosis. In terms of prognosis, the groups were divided into two as good and bad prognosis. Accordingly, there was no difference between the groups in terms of gender, seasonal distribution, previous events or disease history, CSF protein, cranial nerve involvement, presence of facial paralysis, serum glucose level, serum albumin value, and kidney function tests ($p>0.05$).

Presence of hyponatremia ($p<0.001$), presence of abnormal liver function test ($p=0.08$), need for a mechanical ventilator ($p<0.001$), presence of sepsis among complications ($p=0.07$), presence of AMAN subtype in EMG ($p=0.001$), high Hughes score at admission ($p<0.001$), advanced WBC count ($p=0.033$), extended hospital stay ($p=0.030$) and advanced age ($p=0.028$) were detected more frequently in the poor prognosis group.

The parameters that differ in the groups with good and bad prognosis according to the Hughes score results are shown in Table 2.

DISCUSSION

The present study identified in the poor prognosis group at discharge, advanced age, being in the AMAN group according to EMG findings, development of sepsis, need for mechanical ventilation, high Hughes scores at admission, extended hospital stay, increased WBC count, presence of hyponatremia, and abnormal liver function test were higher than the good prognosis group. Concerning the sex distribution of the study patients, the male sex was diagnosed with GBS 1.3 times more frequently, which is consistent with the literature, although sex did not affect the Hughes score at discharge (1,11,12). Previous studies have emphasized the negative effects of the female sex on long-term prognosis, especially in terms of functional independence (13,14). In the evaluation of prognosis at discharge in the present study, the effects on functional prognosis could differ in the long-term prospective follow-up of the patients.

Most patients in the study presented during the spring and summer, although there are conflicting data in the literature on this subject. While some studies found the autumn and winter months to be riskier, others reported spring and summer to be riskier (15,16). Studies have attributed this to the incidence of C. jejuni or influenza virus, which varies by season and even months. It has been suggested that the seasonal distribution changes because C. jejuni is mainly seen in summer and autumn, and influenza in winter, both in our country and worldwide (17-19). Similar studies in our country have reported higher admissions in the summer season (20,21). The present study found no significant relationship between the admission season and prognosis at discharge, and similarly, Çetiner et al. reported that the season did not affect the 3-month prognosis (20).

In this study, 44.2% of patients had a triggering event, such as vaccination or infection, prior to the disease, with the majority of these events being URTIs and gastroenteritis. Our study did not find a significant relationship between any previous event in terms of prognosis. In previous studies, URTIs and gastroenteritis have also been reported as preceding events in the etiology. However, none of the preceding events identified in our study had a positive or negative effect on prognosis at discharge (1,22). Cetiner et al., on the other hand, associated gastroenteritis with a poor prognosis, while another study reported adverse effects on a 6-month prognosis in those with a history of diarrhea (20,23).

In our country, the most common GBS subtype is AIDP, and this was the case also in the patient population in the present study (20,24). While some publications report no difference in prognosis between the subtypes or a greater need for mechanical ventilation in the early period in the demyelinating subtype, others report the axonal subtype to be associated with a more severe course and a poorer prognosis (20,21,25,26). In our study, we found a statistically significantly higher rate of AIDP variant in the good prognosis group and AMAN variant in the poor prognosis group.

Most of our patients were administered IVIG treatment alone or in combination with PD, while around 5.8% were followed up without treatment as their complaints were limited to isolated sensory symptoms. All of these untreated patients with mild sensory complaints had a good prognosis. Examining the effects of IVIG, PD, and combined PD-IVIG treatments on prognosis revealed that those who received IVIG treatment had a better prognosis than those who received plasmapheresis and combined plasmapheresis-IVIG treatments. In our clinic, the first-choice treatment for all patients admitted with GBS is IVIG if there are no contraindications, and plasma exchange is administered to those who cannot receive IVIG. Plasmapheresis is also used in patients with a severe disease course who do not benefit from IVIG alone. This is attributed to the better disease course observed in patients receiving IVIG compared to PD alone or IVIG combined with PD. Previous studies have stated that none of these two treatment options is superior to the other and that steroids are ineffective (27).

Various complications developed in 13 of the 138 patients in the present study, the most common of which were infections of various types (such as urinary tract infections, catheter site infections, etc.). Among the patients who developed complications, two with ileus and AKI died, while sepsis, on the other hand, had a negative effect on

prognosis at discharge. Two people who developed sepsis were also in the poor prognosis group. The absence of any complication in the clinical course was found to be quite high in the good prognosis group. The need for MV was identified as a negative prognostic factor in the present study, consistent with the literature (28). A prolonged hospital stay may also predispose to the development of complications. In addition, patients who needed MV and required intensive care unit admission stayed in the hospital for longer durations. So a prolonged hospital stay was identified as a poor prognostic factor in our study.

There was no significant effect of CSF protein levels, serum glucose levels, facial paralysis, or other cranial nerve involvement on prognosis. At the same time, an elevated WBC count was higher in the poor prognosis group, supporting similar studies in the literature (7,29). The absence of a relationship between hospitalization serum glucose values and prognosis groups in our study was interpreted as the fact that we did not include diabetic patients. A high Hughes score at admission also suggested a poor prognosis at discharge, and the clinical course was also poor in patients with severe disease onset and primarily motor symptoms (20,21).

There was one patient with an abnormal kidney function test, and acute kidney injury developed in that patient. This patient, who developed acute renal failure, later died. No abnormality was detected in other patients. No significant difference between the group was found when serum albumin values were compared. However, hyponatremia and increased liver function tests were significantly higher in the poor prognosis group. Hyponatremia increased liver and kidney function tests, and low albumin values have been associated with poor prognosis (7).

One of the limitations of our study is the evaluation of patients only according to their prognosis at discharge due to the retrospective study design. At the same time, the long-term follow-up would have provided more valuable information, especially regarding long-term motor prognosis. Furthermore, our study only assessed prognosis according to the Hughes scale and did not use other functional scales. Another limitation is the low number of patients. More extensive and prospective studies are needed of this issue are required.

CONCLUSION

In conclusion, the GBS patient series in the present study mainly consisted of those with AIDP, with the demyelinating subtype being predominant, which is in line with the literature. The parameters associated with poor prognosis at discharge were identified as the need for MV, prolonged hospital stay duration, elevated WBC count, hyponatremia, elevated liver function tests, presence of sepsis, and advanced age.

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Research Article

The Effect of Surgical Menopause on Vasomotor Symptoms and Anxiety in Women: A Prospective Study

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Abstract

Aim: To investigate the effect of of adding oophorectomy on patients who underwent abdominal hysterectomy in the perimenopausal period on menopause, sexual function and mental status.

Materials and Methods: The study was designed prospectively. Women who underwent total abdominal hysterectomy and bilateral salpingectomy (TAH+BS) and total abdominal hysterectomy and bilateral salpingo-ophorectomy (TAH+BSO) in the perimenopausal period for benign indications were included in the study. Three months after surgery, menopausal symptoms (such as vasomotor symptoms, vaginal dryness and/or dyspareunia, memory and sleep problems) were investigated. Beck Anxiety Inventory (BAI) scores were investigated one day before the operation and three months after the operation.

Results: 51 patients with TAH+BS and 55 patients with TAH+BSO included in study. Vasomotor symptoms and postoperative BAI scores were significantly higher in the oophorectomy group ($p<0.001$ and $p=0.009$, respectively). Vaginal dryness and/or dyspareunia, which adversely affect sexual function, were significantly higher in the oophorectomy group ($p=0.005$). Memory and sleep problems were higher in the oophorectomy group ($p=0.009$ and $p<0.001$, respectively). Postoperative BAI scores were found to be correlated with postmenopausal symptoms (vasomotor symptoms, vaginal dryness and/or dyspareunia, memory problems, sleeping disorders) in the TAH+BSO group.

Conclusion: Vasomotor symptoms, vaginal dryness and/or dyspareunia, memory and sleeping problems, and anxiety levels were significantly higher in patients who underwent bilateral salpingo-ophorectomy with hysterectomy compared to patients who underwent only hysterectomy and bilateral salpingectomy. It seems useful to inform the patients who are planned for the operation regarding these effects before the decision of oophorectomy.

Keywords: Hysterectomy, oopherectomy, menopause, sexual dysfunction

INTRODUCTION

Hysterectomy is performed frequently in women and is the second most common major surgery after cesarean section in women (1). Hysterectomy surgeries are performed to treat benign indications such as abnormal uterine bleeding, uterine fibroids, adenomyosis, as well as gynecological malignancies such as cervical, endometrial, and ovarian cancers (2,3). It can be performed abdominally, laparoscopically, or vaginally, depending on the surgeon's experience and the patient's previous surgical history or the size of the uterus (4). Abdominal hysterectomy is the excision of the uterus via laparotomy. The surgery can be performed as a total hysterectomy (including the cervix) or a subtotal (supracervical) hysterectomy. The ovaries may or may not be excised during a hysterectomy. A study conducted in 2009 showed that 68% of the patients underwent unilateral or bilateral oophorectomy during abdominal hysterectomy, 60% of patients underwent unilateral or bilateral oophorectomy in laparoscopic hysterectomy, and 26% of patients underwent unilateral or bilateral oophorectomy was performed in during vaginal hysterectomy (5). A 2014 study showed that 44 percent of patients younger than 51 years of age had an

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oophorectomy during a hysterectomy for benign indications (6). The choice of surgical technique and method depends on clinical findings, surgeon's expertise and patient preference. Excision of normal ovaries in premenopausal women during hysterectomy is still controversial. There are no accepted criteria for the preservation or removal of the ovaries or the removal of a single ovary. The main purpose of oophorectomy is to protect against ovarian cancer. However, surgical menopausal side effects limit this view.

Surgical menopause after total abdominal hysterectomy and bilateral salpingo-ophorectomy (TAH+BSO) causes a sudden increase in follicle stimulating hormone (FSH) levels, a sudden drop in estrogen and progesterone levels (7). In contrast, the transition to natural menopause is characterized by varying levels of estradiol and FSH levels in conjunction with ovulatory and anovulatory cycles, and the variability in hormone levels can persist for up to 10 years (8). In surgical menopause, sudden hormonal changes are associated with more severe menopausal symptoms. With the transition to menopause, symptoms such as sleep disorders, decreased sexual activity and memory problems can be observed in women. Vasomotor symptoms are the hallmarks of the perimenopausal-menopausal period. These symptoms are observed in approximately 80% of women and seriously affect the patient's quality of life (9,10). Although vasomotor symptoms may be observed more severely in surgical menopause, sexual dysfunction and related depressive symptoms are likely to be observed more frequently (11).

The aim of this study was to investigate the effect of of adding oophorectomy on patients who underwent abdominal hysterectomy in the perimenopausal period on menopause, sexual function and mental status.

MATERIAL AND METHOD

Study type

This study was a prospective study conducted between October 1, 2020 and July 1, 2021 at the Department of Obstetrics and Gynecology, University of Health Sciences Tepecik Training and Research Hospital. Power analysis was performed with G-power for the number of samples. Accordingly, a minimum limit of 38 people was found for each group.

Study group

This study included women who underwent total abdominal hysterectomy and bilateral salpingectomy (TAH+BS) and total abdominal hysterectomy and bilateral salpingo-ophorectomy (TAH+BSO) in the perimenopausal period for benign indication. In our clinic, oophorectomy option is offered to patients aged 45 and over who are found suitable, and additional consent is obtained from patients who accept before surgery. Patients with previous psychiatric disease, sexual dysfunction, memory or sleep problems, and patients who underwent hysterectomy for malignant indications, and receiving hormonal therapy for menopausal reasons were excluded.

Variables and data collection

Demographic and medical data of the patients such as age, gravida, parity, smoking and body mass index (BMI) were recorded. Three months after surgery, menopausal symptoms (such as vasomotor symptoms, vaginal dryness and/or dyspareunia, memory and sleep problems) were investigated. Beck Anxiety Inventory (BAI) scores of the patients were investigated one day before the operation and three months after the operation. The data were filled in by face-to-face interviews with the women with the help of the forms prepared by the researchers according to the literature and the form consisting of questions questioning the post-operative complaints of the women.

Procedures

Beck et al. developed a Beck Anxiety Inventory (BAI) and used it to assess the patient's mental changes (12). It was validated in Turkey by Hisli (13). The scale consists of 21 questions. In this test, patients answer the questions by selecting one of four options: "none, mild, moderate, severe". None is 0 points, mild is 1 point, moderate is 2 points, and severe is 3 points. Points are calculated at the end of the test. Total score; A score of 8-15 is categorized as mild anxiety symptoms, a score of 16-25 as moderate anxiety symptoms, and a score of 26-63 as severe anxiety symptoms.

	Not At All	Mildly but it didn't bother me much.	Moderately - it wasn't pleasant at times	Severely – it bothered me a lot
Numbness or tingling	0	1	2	3
Feeling hot	0	1	2	3
Wobbliness in legs	0	1	2	3
Unable to relax	0	1	2	3
Fear of worst happening	0	1	2	3
Dizzy or lightheaded	0	1	2	3
Heart pounding/racing	0	1	2	3
Unsteady	0	1	2	3
Terrified or afraid	0	1	2	3
Nervous	0	1	2	3
Feeling of choking	0	1	2	3
Hands trembling	0	1	2	3
Shaky / unsteady	0	1	2	3
Fear of losing control	0	1	2	3
Difficulty in breathing	0	1	2	3
Fear of dying	0	1	2	3
Scared	0	1	2	3
Indigestion	0	1	2	3
Faint / lightheaded	0	1	2	3
Face flushed	0	1	2	3
Hot/cold sweats	0	1	2	3
Column Sum				

Figure 1. Beck Anxiety Inventory (BAI) (12)

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) v26.0 program was used for data analysis. The Kolmogorov-Smirnov test was used to determine the distributions of normality. Independent T test was applied in the analysis of normally distributed variables and data were given as mean±SD. The Mann Whitney U test was applied in the analysis of non-normally distributed variables and the data were given as median (min-max). Categorical variables were estimated with the Chi-square test. Spearman's correlation test was applied to evaluate the correlation between groups. $p<0.05$ assumed to be significant.

Ethics

Before the study was started, written permissions were obtained from the patients. This study was approved by the University of Health Sciences Tepecik Training and Research Hospital Ethics Committee (approval number: 2021/10-20).

RESULTS

A total of 106 patients who met the criteria were included in the study. TAH+BS was applied to 51 of the women participating in our study, and TAH+BSO was applied to 55 of them. All patients did not have any previous psychiatric disease, sexual dysfunction, memory or sleep problems.

The average age of the patients in the TAH+BS group was 43±5 years, and the average age of the patients in the TAH+BSO group was 50±7 years, and the difference between them was significant (p<0.001). The gravidity of the TAH+BS group was 3 (0-11), and the TAH+BSO group was 4 (1-14), and the difference between them was not significant. While there was no nulliparous patient in the TAH+BSO group, there were 4 nulliparous patients in the TAH+BS group. While all of the TAH+BSO group consisted of multiparous patients, 92.2% of the TAH+BS group consisted of multiparous patients, and there was a significant difference between the parity of the groups (p=0.034). The mean BMI was 26±3.7 in the TAH+BS group, 26.5±3.1 in the TAH+BSO group, and there was no significant difference. While 23.5% of the TAH+BS group smokers, 25.5% of the TAH+BSO group smokers, and the difference was not significant (Table 1).

Table 1. Demographic and medical characteristics of TAH+BSO and TAH+BS groups

	TAH+BSO (n=55)	TAH+BS (n=51)	p value
Age (mean±SD)	50±7	43±5	<0.001
Gravidity median (min-max)	4 (1-14)	3 (0-11)	0.081
Parity (n,%)			0.034
Nulliparous	0	4 (7.8%)	
Multiparous	55 (100%)	47 (92.2%)	
BMI (kg/m²) (mean±SD)	26.5±3.1	26±3.7	0.790
Smoking (n,%)	14 (25.5%)	12 (23.5%)	0.818

Abbreviations: BMI: body mass index

Vasomotor symptoms were questioned. These symptoms were observed in 11.8% (6 patients) of the TAH+BS group and 70.9% (39 patients) of the TAH+BSO group, and were significantly higher in the TAH+BSO group (p<0.001). Vaginal dryness and/or dyspareunia were present in 21.6% (11 patients) of the TAH+BS group and 47.3% (26 patients) of the TAH+BSO group, and the symptoms were higher in the TAH+BSO group (p=0.005). Memory problems such as attention disorder and forgetfulness were questioned and it was determined that 7.8% of the TAH+BS group (4 patients), and 34.5% (19 patients) of the TAH+BSO group had significant differences between them (p=0.009). Sleeping disorders such as insomnia, fatigue, and sleepiness were questioned. It was detected in 7.8% (4 patients) of the TAH+BS group and 34.5% (19 patients) of the TAH+BSO group (p<0.001). While the mean of preoperative BAI was 5±3 in the TAH+BS group, it was 4±3 in the TAH+BSO group, and the mean postoperative BAI was 8±6 in the TAH+BS group and 10±6 in the TAH+BSO group; the mean postoperative BAI was higher in the TAH+BSO group than the TAH+BS group (p=0.009) (Table 2).

Table 2. Menopausal symptoms and BAI scores of TAH+BSO and TAH+BS groups

	TAH+BSO (n=55)	TAH+BS (n=51)	p value
Vasomotor symptoms [a] (n,%)	39 (70.9%)	6 (11.8%)	<0.001
Vaginal dryness and/or dyspareunia (n,%)	26 (47.3%)	11 (21.6%)	0.005
Memory problem [b] (n,%)	15 (27.3%)	4 (7.8%)	0.009
Sleeping disorder [c] (n,%)	19 (34.5%)	4 (7.8%)	<0.001
Preop BAI (mean±SD)	4±3	5±3	0.117
Postop BAI (mean±SD)	10±6	8±6	0.009

Abbreviations: BAI: Beck Anxiety Inventory
[a]Include hot flashes and night sweats
[b]Include attention disorder and forgetfulness
[c]Include insomnia, daytime fatigue and sleepiness

Postoperative BAI scores were found to be correlated with postmenopausal symptoms (vasomotor symptoms, vaginal dryness and/or dyspareunia, memory problems, sleeping disorders) in the TAH+BSO group (Table 3).

Table 3. Correlation of postoperative TAH+BSO group BAI score with menopausal symptoms

	r value	p value
Vasomotor symptoms [a] (n,%)	0.486	<0.01
Vaginal dryness and/or dyspareunia (n,%)	0.644	<0.01
Memory problem [b] (n,%)	0.555	<0.01
Sleeping disorder [c] (n,%)	0.474	<0.01

[a]Include hot flashes and night sweats
[b]Include attention disorder and forgetfulness
[c]Include insomnia, daytime fatigue and sleepiness

DISCUSSION

Bilateral salpingo-opherectomy, which is electively added to hysterectomies for benign diseases, can often be performed to reduce the risk of malignancy or other adnexal pathologies. In a study conducted in the USA, it was shown that oophorectomy is added to operations for these reasons in approximately half of hysterectomies (14,15). In North America, 250.000 women enter surgical menopause each year due to bilateral oophorectomy (16). The rationale for this approach was that ovariectomy significantly reduces the risk of ovarian cancer and the

requirement for future ovarian surgery and that ovarian preservation offers little benefit as patients in this age group are near or past menopause (17,18). However, the menopausal side effects of this approach have led to discussion of this issue, and contrary to these findings, some studies suggest that prophylactic oophorectomy is an unnecessary practice that deprives women of the benefits of ovarian function (19,20).

Bachmann, showed that vasomotor symptoms in surgically menopausal women are more severe and persist for longer than in physiologically menopausal women (21). Marra et al. in their study that all women (100%) with surgical menopause experience a wide variety of vasomotor symptoms (22). According to their studies, 90% of women experience more severe hot flashes than those who are going through natural menopause. In our study, proportion of patients with vasomotor symptoms was significantly higher in the TAH+BSO group than in the TAH+BS group, showing that we obtained results consistent with the literature. It is thought that the rapid hormonal change that occurs in surgical menopause causes this condition (7,8).

Compared to natural menopause, which is a slow process, the withdrawal of estrogen, progesterone and androgens is abrupt in surgical menopause. Studies have shown that TAH+BSO operation performed in the premenopausal period has negative effects on psychosexual health (23,24). In the study by Lonnee-Hoffmann et al., it was shown that after oophorectomy performed in the perimenopausal period, more dyspareunia, more sexual reluctance, vaginal dryness and less sexual satisfaction were observed (25). Similarly, in our study, a relationship was found between vaginal dryness and/or dyspareunia and surgical menopause, and sexual comfort was negatively affected in patients who underwent oophorectomy.

In our study, memory problems (attention disorder and forgetfulness) were significantly higher in the TAH+BSO group. Similar to our study, some studies have shown that surgical menopause may be associated with memory problems (9,26). In their meta-analysis of 18.867 samples, Georgakis et al. showed that surgical menopause with bilateral oophorectomy at the age of ≤45 years was associated with an increased risk of dementia and cognitive decline (27). The natural menopause transition takes place more smoothly. Observational studies have shown that a natural transition to menopause is not associated with significant changes in cognitive abilities (16). However, both natural and surgical postmenopausal estrogen therapy does not improve cognitive status. Therefore, the mechanisms about menopause and cognitive status remain unclear.

Sleep problems are quite common in menopausal women. Poor sleep quality and insomnia are the main sleep problems in menopause (28). The levels of vasomotor symptoms and hormonal changes in postmenopausal women may affect insomnia symptoms (29). Studies have shown that surgical menopause can cause serious sleep disorders in women (30). In a recent study, Cho et. al. showed that sleep disorders were 2.13 times more common in women with surgical menopause than in women with natural menopause (29). Similarly, in our study, significant sleeping disorders (insomnia, daytime fatigue and sleepiness) were detected in patients who underwent oophorectomy together with hysterectomy (34.5% vs. 7.8%). Since sleep quality is more affected in women who have undergone surgical menopause, it seems necessary to evaluate them in terms of sleep disorders in their postoperative follow-up.

Another finding of this study was the change in the classification made according to the score obtained in the anxiety scale in both groups. While the preoperative BAI scores were similar in the TAH+BSO and TAH+BS groups, the postoperative BAI score was significantly higher in the TAH+BSO group. Menopause draws attention as a critical transition period in a woman's life due to biological, social and psychological changes. Since the process occurs suddenly in surgical menopause, menopausal symptoms are more severe. This can trigger anxiety (31). In our study, a significant correlation was also observed between BAI score and menopausal symptoms (such as vasomotor symptoms, vaginal dryness and/or dyspareunia, memory and sleep problems). Many studies have shown a positive relationship between vasomotor symptoms and depressive complaints during the transition to menopause (32,33). However, some other studies suggest that anxiety symptoms are not clearly related to any specific stage of menopause (34). In another study, it was showed that women under the age of 46 who underwent oophorectomy had higher depression scores after surgery (24).

Recent studies have shown that clinical practice is beginning to change as our understanding of the possible long-term health risks of elective oophorectomy and the potential benefits of elective oophorectomy in premenopausal patients (35). For benign disease, the opinion that the decision to leave or remove the tubes and ovaries should be made by considering the long-term health effects and not removing the ovaries in cases under the age of 51 has been presented over the years (36).

There were some limitations of this study. Firstly, the study was conducted only on women who had an abdominal hysterectomy. Previous studies investigated whether sexual function results after hysterectomy were affected by the procedure used (25). However, data comparing the results of sexual function outcomes between vaginal, laparoscopic, and transabdominal hysterectomy are limited. Our study was conducted with a single center and a limited number of patients. The generalizability of the study is limited. Multicenter studies with larger patient populations are needed.

CONCLUSION

In conclusion, vasomotor symptoms, vaginal dryness and/or dyspareunia, memory and sleeping problems, and anxiety levels were significantly higher in patients who underwent bilateral salpingo-ophorectomy with hysterectomy compared to patients who underwent only

hysterectomy and bilateral salpingectomy. More studies are needed to balance the health risks and benefits of prophylactic bilateral oophorectomy performed to reduce the risk of ovarian benign pathologies and cancer.

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Research Article

Adult Onset Langerhans Cell Histiocytosis: A Single Center Experience

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Abstract

Aim: Langerhans cell histiocytosis (LCH) is a proliferative disease caused by the abnormal proliferation of histiocytes in the bone marrow dendritic cell structure. LCH is very rare in adults and its incidence is 1-2 cases per million. Therefore, there is still no clear management plan for adults. It was aimed to evaluate the very rare adult LCH patients.

Materials and Methods: Ten LCH patients who were followed up at Erciyes University Hematology Department between January 2010 and January 2020 were included.

Results: Eight (80%) of them were male and 2 (20%) were female. The median age of the patients was 34.5±8.4 (23-52) years. Although the most common involvement at the time of diagnosis was bone in 7 patients (70%) and lung in 3 patients (30%); pituitary, tympanic membrane, liver, and brain involvement were also observed. Three (30%) patients had single system involvement and 7 (70%) had multisystem. Four (40%) patients had relapsed and all had multisystemic involvement. The BRAF V600E mutation could be performed in 3 patients. It was negative in two patients and positive in 1 patient. All patients still have remission.

Conclusion: Unlike children, LCH has a better course in adults. The most important approach is to determine single or multisystem involvement. In our patients with single system involvement, we obtained response with corticosteroid and surgery alone. We have observed that vinblastin plus methylprednisolone treatment is a good option for multisystemic involvement. In relapsed patients, we obtained a significant response with clofarabine.

Keywords: Adult, BRAF V600E mutation, clofarabin, histiocytosis, langerhans cell, radiotherapy, treatment, vinblastin plus methylprednisolone

INTRODUCTION

Langerhans Cell Histiocytosis (LCH) is an idiopathic group of diseases that cause damage due to local or diffuse accumulation of various tissues such as bone, lymph nodes, skin, lung, liver, and spleen (1). LCH is diagnosed most frequently in children under 3 years old but it can be seen at any age. LCH is very rare in adults and its incidence is 1-2 cases per million (2).

The diagnosis is made by biopsy taken from the involved tissue. After diagnosis, the most important factor is to evaluate whether the disease is single-system or multisystemic. In single-system LCH, curettage of bone lesions, topical treatment for skin, and prednisone are sufficient for treatment, while multisystemic LCH requires chemotherapeutic agents (3,4).

There is still no clear approach in the treatment of multisystemic LCH in adults. Vinblastin-methylprednisolone (VB+MP), cladribine, cytarabine, clofarabine, and vincristine are the most preferred treatments (5-7). In addition, vemurafenib is used by identifying BRAF V600E mutations in LCH patients (8).

LCH is an extremely rare disease in adults, whose diagnosis,

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treatment and clinical course are still unclear. Therefore, in order to increase the awareness of LCH and to evaluate this disease better, we retrospectively evaluated the data of 10 patients who were followed up in our clinic for the last 10 years.

MATERIAL AND METHOD

Ten LCH patients who were followed up at Erciyes University Hematology Department between January 2010 and January 2020 were included in this study. All patients received the diagnosis of LCH by histopathological examination of the samples from the involved tissue. All information about patients was recorded retrospectively from patient files and the hospital automation system.

Age, gender, laboratory features, follow-up time, symptoms at diagnosis, biopsy region, BRAF mutation, bone marrow biopsy, treatments, and response status were evaluated. Also, relapse status, relapse location and post-relapse treatments were evaluated. Patients with and without relapse were compared. All patients were screened with Positron Emission Tomography/Computed Tomography (PET/CT). Pituitary Magnetic Resonance Imaging (MRI) was performed in patients who were considered or diagnosed with diabetes insipidus (DI).

Kolmogorov Smirnov test was used for the normal distribution of the data. Mann Whitney U test was used to compare nonparametric data; Student t-test was used to compare parametric data. Chi-square or Fisher's Exact test was used to compare categorical data. The data were analyzed by using Statistical Package for the Social Sciences (SPSS) for Windows computer program (release 25.0; SPSS Inc., Chicago, IL, USA). $p<0.05$ was considered significant in all tests.

RESULTS

Ten patients with LCH were included in our study. Eight (80%) of them were male and 2 (20%) were female. The median age of the patients was 34.5±8.4 (23-52) years. The first symptoms at the time of diagnosis were headache in 3 patients (30%), bone pain in 2 patients (20%), otalgia in 1 patient (10%), stomachache in 1 patient (10%), polyuria in 1 patient (10%), lymphadenopathy in 1 patient (10%) and swelling of the vulva in 1 patient (10%) (Table 1).

Table 1. Demographic Data of Patients with Langerhans Cell Histiocytosis

Case no	Age at the diagnosis	Gender	Comorbidities	Smoke p/y	Symptom at the time of diagnosis	Biopsy location	PET/CT İnvolvements areas	Pituitary MRI	Bone marrow biopsy	BRAF V600E mutation
1	26	Male	-	4	Bone pain	Symphysis pubis	Bone	N/A	N/A	N/A
2	37	Female	DI	15	Swelling of the vulva	Vulva	Vulva, Lung, pituitary gland	Involvement+	N/A	N/A
3	23	Male	-	-	Headache	Temporal lobe	Brain, Bone, Lung	N/A	N/A	N/A
4	52	Female	DI	-	Polyuria	Jaw	Bone	Involvement+	N/A	N/A
5	33	Male	-	-	Lymphadenomegaly	Left 5. Rib	Lymph node, Bone	Normal	N/A	N/A
6	27	Male	-	10	Bone pain	Left spina scapula	Bone, Lung	N/A	N/A	N/A
7	39	Male	DM, Bipolar disorder	15	Stomach ache	Liver	Liver	N/A	Normocellular	N/A
8	36	Male		15	Headache	Occipital bone	Bone, Lymph node	N/A	Normocellular	negative
9	39	Male	HT	-	Otalgia	Right tympanic membrane	Tympanic membrane	Normal	N/A	positive
10	30	Male	-	-	Headache	Temporal bone	Bone	N/A	N/A	negative

DI: Diabetes insipidus, DM: Diabetes mellitus, HT: hypertension, p/y:pockets/year, MRI: Magnetic resonance imaging, N/A: not applicable, PET/CT: Positron Emission Tomography/Computed Tomography

Organ involvement at the time of diagnosis, bone in 7 patients (70%), lung in 3 patients (30%), pituitary and DI in 1 patient (10%), lymph node in 1 patient (10%), liver in 1 patient (10%), brain in 1 patient (10%) and 1 patient (10%) tympanic membrane involvements were developed. Bone involvements were observed in skull 3 (42.8%), rib 1 (14.3%), scapula 1 (14.3%), jaw 1 (14.3%) and symphysis pubis 1 (14.3%). The BRAF V600E mutation could be performed in 3 patients. It was negative in two patients and positive in 1 patient.

Three (30%) patients had single system involvement and 7 (70%) had multisystem. Patients with osteolytic lesions were given monthly zoledronic acid. One of the patients with single system involvement only had bone involvement and was treated with surgical curettage. The other patient with single system involvement only had tympanic membrane involvement and only received methylprednisolone therapy. In our third patient with single system involvement, only temporal bone involvement was present, but VB +MP treatment was given because of the high risk for the central nervous system (CNS) disease. All of these 3 patients are still in remission.

All patients with multisystemic involvement received systemic chemotherapy. Radiotherapy was given to 2 patients. As an initial treatment, 1 patient (14.3%) received cytarabine and the other 6 patients (85.7%) received VB+MP (Table-2). The patient who received cytarabine as an initial treatment relapsed after 3 years. VB+MP were given after relapse but the patient had again relapse and clofarabine was started and and still treatment is ongoing. Complete response was obtained in 3 of 6 patients who started VB+MP as an initial treatment. The other 3 patients had relapse. Clofarabine treatment was given to 2 of 3 recurrent patients. Cytarabine was started in the other patient, but relapsed again and clofarabine was given. All of the recurrent patients are now in remission.

Four (40%) patients had relapsed but 6 (60%) did not have. The symptoms and location of involvement in all recurrent patients were pain and bone. Patients with and without relapse were compared. The median age of the patients with relapse was 37.5±11.9 years and 1 (25%) was female. The median age of the patients without relapse was 32±5.3 years, and 1 (16.6%) was female (p values 0.34 and 1.00 respectively). Treatments and laboratory values are provided in Table-3. The median hemoglobin level was 13.7±2.1 g/dL in patients with relapse, 15.6±1.6 g/dL in patients without relapse (p=0.13). The median alkaline phosphatase level was 113.7±37.9 g/dL in patients with relapse, 71.6±23.4 g/dL in patients without relapse (p=0.60). While 3(100%) patients with single system involvement didn't have relapse, 3 (42,8%) patients with systemic involvement didn't have relapse (p=0.20). Four (66.6%) patients without relapse received VB+MP treatment and 4 (100%) patients with relapse received VB+MP treatment (p=0.47).

The median overall survival (OS) was 34.4±11.2 months (95% CI, 12.6 to 56.3). All of our patients are still alive.

Table 2. Treatments. outcomes. and clinical course of patients

Case no	Treatment type	Treatment Time	RT	Relapse and time	Symptom at the relapse	Relapsed areas	Treatment after relapse	Final situation
1	Curettage		-	-	-			Remission
2	VB+MP	25 Weeks	-	-	-		-	Remission
3	VB+MP	39 Weeks	-	+: 9 Months	Headache	Bone. Lung	Clofarabine (6 cures)	Remission
4	VB+MP	25 Weeks	+: Cranium	+:12 Months	Bone pain	Bone	Cytarabine (6 cures) Clofarabine (3 cures) Cytarabine+Clofarabine (3 cures)	Remission
5	VB+MP	25 Weeks	-	-	-		-	Remission
6	VB+MP	25 Weeks	-	-	-		-	Remission
7	VB+MP	25 Weeks	+: Shoulder	+: 9 months	Headache		Clofarabine (6 cures)	Remission
8	Cytarabine	6 Months	-	+:3 years	Headache	Bone	Vinblastine (9 cures) Clofarabine (6 cures)	Remission
9	MP	5 Weeks	-	-	-	-	-	Remission
10	VB + MP	25 Weeks	-	-	-	-	-	Remission

VB: Vinblastine, MP: Methylprednisolone

Table 3. Data of LCH patients with and without relapse

	Patients without Relapse	Patients with Relapse	p
Gender	K: 1. E: 5	K: 1. E: 3	1.0[c]
Treatment			
VB+MP	4.66.6%	4.100%	0.47[c]
Cytarabine	0	2.50%	0.13[cc]
Methylprednisolone	5.83.3%	4.100%	1.00[c]
Excision	2.33.3%	0	0.47[c]
Radiotherapy	1.16.6%	1.25%	1.00[c]
Clofarabine	1.16.6%	3.75%	0.20
Disease Involvement			
Single system	3.50%	0	0.52[c]
Multisystemic	3.50%	4.100%	
Age	32±5.3	37.5±11.9	0.34[t]
WBC (103/µL)	8.9±2.8	6.6±1.1	0.16[t]
Hb (g/dL)	15.6±1.6	13.7±2.1	0.13[t]
PLT (103/µL)	312.3±110.6	212.5±71.3	0.30[t]
Creatinin (mg/dL)	0.72±0.12	0.76±0.07	0.57[t]
Albumin (g/dL)	4.76±2.5	4.64±0.33	0.68[t]
LDH (u/L)	208±41.5	175.5±15.2	0.18[t]
ALP (u/L)	71.0±234	113.7±37.9	0.60[t]
Uric Acid (mg/dL)	5.17±0.6	6±2.2	0.76[m]

cChi-square, M: Mann-Whitney u Test, T: Independent Sample t Test
ALP: alkaline phosphatase, Hb: hemoglobulin, PLT: platelet, LDH: lactate dehydrogenase, MP: methylprednisolone, VB: vinblastine, WBC: white blood cell

DISCUSSION

LCH is a disease with unknown etiology caused by the abnormal proliferation of histiocytes in dendritic cells derived from bone marrow with a variable number of leukocytes, neutrophils, eosinophils, lymphocytes, and giant multinucleated cells causing tissue damage. Langerhans type cells express CDla, langerin and S100 protein, and have Birbeck granules. This disease was previously called histiocytosis-X, diffuse reticuloendotheliosis, Hashimoto-Pritzker syndrome, Letterer-Siwe disease, Hand-Schüller-Christian disease, and eosinophilic granuloma but is now called LCH.

LCH is diagnosed more frequently between the ages of 1 to 3 and is clinically severe in this age group but very rare in adults, and the clinical course is milder. There is an approximately 2-3 times male predominance (9) but some demonstrated female predominance. In our study, the female/male ratio was 1/4.

LCH involvement can occur almost anywhere at the time of diagnosis. In a prospective study involving 1741 patients, 77% bone, 39% skin, 19% lymph nodes, 16% liver, 10% lung and 6% CNS involvement were observed (10). In our study, 70% bone, 30% lung, 10% lymph nodes, 10% liver and 10% CNS involvement were present at the time of diagnosis. Although many studies show that skulls are the most common bone involvement, one study has been shown to be jaw. DI was detected in 1 patient at the time of diagnosis and in 1 patient during follow-up. In other studies, DI was the most common endocrinological disorder in LCH, with a frequency of 4% before diagnosis, and 18% during or after diagnosis (11,12).

According to the Histiocyte Society, the most important approach after diagnosing LCH is to determine whether the disease is a single system or multisystem disease. Single system disease treatment includes prednisone, curettage of bone lesions and topical therapy. Topical nitrogen mustard and topical corticosteroids are effective in patients with single skin involvement (13). It was also demonstrated that oral thalidomide and oral methotrexate treatments were effective in the same group of patients (14). Orbital, mastoid, ethmoid, sphenoid or temporal bone involvement should be treated as a multisystemic disease, since the involvement is a risk factor for CNS even if it is only a single system disease.

Multisystemic disease should be treated with systemic chemotherapy. VB +MP, cytarabine, clofarabine, cladribine,

vindesine, vincristine, cyclophosphamide and etoposide are used in multisystemic disease (5,7,15,16). In our patients with multisystemic LCH, VB+MP treatment was given to 6 (85.7%) of them and cytarabine was given to 1 (14.3%). Complete response was observed in all our patients, but 4 patients had relapsed. Our relapsed patients had lower hemoglobulin levels and higher alkaline phosphatase levels. However, since the number of our patients was low, a statistically significant difference was probably not observed. All of our relapsed patients had multisystemic involvement. Also in other studies, relapse frequently developed especially in multisystemic disease.

Single-agent and combination therapies are recommended in the treatment of relapse. Combination treatments include adding oral methotrexate and mercaptopurine to VB+MP (17), and adding vincristine and prednisone to cytarabine. VB + MP, cytarabine, clofarabine, and cladribine are used as the single agent treatment (4,7). In our study, VB+MP was given to 1 (%25), cytarabine was given to 1 (%25) and clofarabine was given to 4 (100%) patients with relapsed. The response was received from all relapsed patients after clofarabine. In other studies, a 90% survival rate was obtained in patients with relapsed /refractory LCH after clofarabine (7,18).

Radiotherapy (RT) is a highly effective treatment for LCH bone lesions. LCH has been reported to be more than 70% effective in adults in the treatment of bone involvement and pain (19). Two patients with bone relapse were treated with RT and their pain decreased and also received remission. Bisphosphonate therapy is effective in reducing pain in bone lesions and improving functional conditions (20). Bone involvement was 70% at the time of diagnosis and 100% at the relapsed of our patients and zoledronic acid was given to all of them.

The BRAF V600E mutation is increasingly reported in LCH and has often been associated with poor prognosis (21). Therefore, BRAF inhibitors such as vemurafenib, dabrafenib and trametinib are taking more place in the treatment of LCH (22,23). In addition, some studies have reported that MEK inhibitors are effective (24). BRAF V600E mutation was performed in our 3 patients. Only one of them was positive. This patient had a single bone lesion and was treated with only curettage and is still in remission.

CONCLUSION

The most important factor in LCH is to identify single or multiple system involvement. In our patients with single system involvement, we obtained response with corticosteroid and surgery alone. We have observed that VB+MP treatment is a good option for multisystemic involvement. In relapsed patients, we obtained a significant response with clofarabine. Our results are very promising however, since our study has a single center experience and the number of patients is low, they have limitations, and further large-scale multicenter studies are needed.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *The study was carried out with the permission of Clinical Research Ethics Committee of Erciyes University. (date: 29.01.2020, Decision No: 2020/63)*

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Research Article

Perceived Stress and Hopelessness in COVID-19 Contacts

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Abstract

Aim: We aimed to determine the perceived stress and hopelessness levels in COVID-19 patient contacts.

Materials and Methods: The study included all COVID-19 contacts who presented to a family health center in Niğde, Turkey between August and October 2020. The data were collected from contacts who were reached daily for a period of 14 days using the Beck Hopelessness Scale (BHS) and the Perceived Stress Scale (PSS). The data were analyzed using the SPSS package program, and $p<0.05$ was considered significant.

Results: While 55.8% of the participants were female, 71% were married, and 46.9% had a chronic disease. The mean age of the participants was 53.44 years. Their mean BHS and PSS scores were 4.40±3.33 and 25.07±5.98, respectively. A statistically significant relationship was found between the participants' places of residence and occupations and their mean BHS loss of motivation subscale scores ($p<0.05$). Among the participants, homemakers, those living in districts, towns, or villages, and those with chronic diseases had significantly higher PSS total scale and.stress-distress subscale mean scores than the others. A statistically significant positive correlation was found between the ages of the participants and their PSS total scale and stress-distress subscale scores ($p<0.05$).

Conclusion: Although the hopelessness levels of the participants were found low, their stress levels were determined to be high, and most of them thought the pandemic was exaggerated. Due to the psychological consequences of the COVID-19 pandemic such as shock, denial, anxiety, worry, and stress in people, it is important to strengthen crisis and stress management efforts and increase awareness, coping and social support resources by prioritizing high-risk groups such as healthcare workers, women, the elderly, those with chronic diseases, and COVID-19 contacts.

Keywords: COVID-19, contacts, hopelessness, stress

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19), which is caused by the "Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)" and spreads rapidly all around the world, still has widespread effects on the world's population, causing not only physiological but also psychological problems (1). Psychological problems caused by the COVID-19 pandemic have rapidly increased its public health burden. Pandemics can trigger depressive and bipolar disorders in people. Experience from previous pandemics has shown that depressive symptoms, varying degrees of anxiety disorders, and post-traumatic stress disorders can develop not only in people with anxiety disorders and panic attacks but also in people who have not had such complaints before (2). Therefore, health employees who are in contact with COVID-19 patients should be evaluated in this regard. Contact-tracing efforts made at the early stages of epidemic diseases in various regions have shown that most secondary infections occur in cases of contact inside the home, and the rate of secondary attacks is found to reach up to 10% (3,4). In a study that was conducted in the United States, the rate of secondary attacks among the 445 close contacts of 10 confirmed cases was found as 0.45%, while this rate was found 10.5% for contacts at home (3). A study carried out in the context of the COVID-19 pandemic showed that frontline healthcare workers, who are among people in contact with confirmed and suspected cases, experienced stress more intensely, while another study examining the psychological problems experienced by nurses revealed that nurses experienced stress and anxiety under intense

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pressure in this period (5,6). Bohlken et al. reported that healthcare workers and those with close contact with infected individuals experienced higher levels of stress (7).

As the COVID-19 pandemic causes both physical health issues and mental health problems such as anxiety, panic, and stress, it should be evaluated not only as a medical health crisis but also as a mental health emergency. Epidemic diseases affect not only people's physical and psychological health but also the well-being of the entire population. In the early days of the COVID-19 pandemic, mostly its physical consequences received attention, and therefore, its mental health consequences were not emphasized. However, even after the pandemic ends, its psychological effects will likely last for months or even years (8). It not only causes serious threats to the physical health and lives of many people around the world but also triggers the emergence of a wide variety of psychological problems, and the increase in perceived stress and hopelessness levels also facilitate the emergence of problems such as panic disorders, anxiety disorders, grief, loss, and depression (9).

In this study, within the scope of examining the effects of the COVID-19 pandemic on the psychological states of individuals, it was aimed to determine the perceived stress and hopelessness levels in the contacts of individuals infected in the pandemic period to understand and plan the necessary interventions.

MATERIAL AND METHOD

Design and Sample

The sample of the study consisted of individuals who had been in contact with COVID-19 patients who were registered to the Kemerhisar Family Health Center No. 1 in Niğde, Turkey and monitored daily for a period of 14 days in line with the relevant guideline. After the COVID-19 contacts were asked about their symptoms and recommended social distancing, their answers to BHS and PSS were recorded on the data collection form, and their scale scores were evaluated by the researcher. Each interview lasted about ten to fifteen minutes. No sample selection was performed within the scope of the study, whereby all COVID-19 contacts who were over 18 years old and registered to the family health center between August and October 2020 were included in the study.

Data Collection Tools

The data were obtained using a 22-item questionnaire, including questions about the participants' sociodemographic characteristics, as well as the Beck Hopelessness Scale and the Perceived Stress Scale. Verbal consent was received via daily routine control phone calls from the participants after they were provided with the necessary information about the study.

Beck Hopelessness Scale (BHS): The scale was designed by Beck et al. and adapted into Turkish by Durak et al. The item-total correlation coefficients of the scale were reported to range from 0.39 to 0.76, and its reliability coefficient was 0.93. It consists of 20 true-false statements with 11 true and nine false answers. This is a self-reported scale, where one point is given for each compatible answer, and zero points are given for each incompatible answer. The arithmetic total scale score is considered the "hopelessness score", ranging from zero to 20. The scale consists of three subscales, namely feelings about the future, loss of motivation, and future expectations. The propositions include emotional, motivational, and cognitive factors (10).

Perceived Stress Scale (PSS): The scale was designed by Cohen Kamarck and Mermelstein, and its validity and reliability in Turkish were tested by Eskin et al. The item-total correlation coefficients of the scale were reported to range from 0.41 to 0.59. The scale consists of 14 items and is designed to measure how stressful individuals perceive certain situations in their lives to be. It has two subscales, namely perceived insufficient self-efficacy and perceived stress-distress. This is a five-point Likert-type scale on which the scoring options are in the range from zero points "never" to four points "very often". Seven items have positive statements and are inversely scored. Total scale score ranges from zero to 56. High scores indicate higher levels of stress. The internal consistency coefficient of the scale was reported as 0.84 (11).

Statistical analysis

The data were analyzed using the SPSS program. Descriptive statistics analyzed using frequency, percentage, and mean values. The Mann-Whitney U test and the Kruskal-Wallis test used to determine the relationships between descriptive statistics and scale scores for independent groups. To determine the distribution characteristics of the data, the Kolmogorov-Smirnov test was used, and $p<0.05$ was considered statistically significant.

Ethical considerations

For conducting the study, ethical approval (dated 23/07/2020 and numbered 27988) was obtained from the Ethics Committee of the Faculty of Medicine at Harran University, and institutional permission (dated 14/06/2020) was obtained from the family health center where the study would be carried out.

RESULTS

Table 1 shows the sociodemographic characteristics of the participants. While 55.8% of the participants were female, 71% were married, 23.9% were smokers, 41.6% were homemakers, 70.8% had detached houses, 88.5% lived outside the city center, 64.6% had primary or secondary education, and 46.9% had chronic diseases. The mean age of the participants was 53.44 years.

Table 2 shows the descriptive characteristics and BHS total and subscale scores of the participants. The mean total BHS

score of the participants was 4.40±3.33. The relationships between the genders, ages, marital statuses, educational levels, and chronic disease statuses of the participants and their mean BHS scores were not statistically significant (p>0.05). On the other hand, their mean loss of motivation subscale scores were significantly related to their places of residence and occupations (p<0.05). The participants who were single, those with bachelor's or higher degrees, those who were civil servants and tradespeople, those living in the city center, and those with no chronic diseases had higher hopelessness levels, while these differences were not statistically significant.

Table 3 shows the sociodemographic characteristics and PSS total and subscale scores of the participants. The mean total PSS score of the participants was 25.07±5.98. No significant relationship was determined between the mean PSS total and subscale scores of the participants and their marital statuses or educational levels (p>0.05). The female participants, those who were single, and those who were illiterate had higher mean total PSS scores than others, while these differences were not statistically significant (p>0.05). The participants who were homemakers, those living in districts, towns, or villages, and those with chronic disease had significantly higher PSS total and stress-distress subscale mean scores than others (p<0.05). The sociodemographic characteristics of the participants did not have a significant effect on their perceived insufficient self-efficacy subscale scores (p>0.05). A statistically significant positive relationship was determined between the ages of the participants and their mean PSS total and stress-distress subscale scores (p<0.05).

Table 4 shows the distributions of the views of the participants about the COVID-19 pandemic. All participants reported that hand washing is important in preventing infections, 99.1% stated that they washed their hands whenever possible, social distancing is important for protection from the disease, the disease is transmitted even by shaking hands, and they cared about personal hygiene during isolation. Additionally, 61.9% of the participants thought that the pandemic was exaggerated, 97.3% stated that the disease is transmitted more in common living areas, and 96.5% reported that it was not difficult for them to follow the rules during isolation.

Table 1. Sociodemographic Characteristics of the Participants

Sociodemographic Characteristics		n	%	Sociodemographic Characteristics		n	%
Gender	Male	50	44.2	Occupation	Homemaker	47	41.6
					Retired	29	25.7
					Civil Servant, Tradesperson	11	9.7
	Female	63	55.8		Laborer	12	10.6
					Other (Self-employed, student, etc.)	14	12.4
Marital Status	Married	71	62.8	Housing Type	Apartment	33	29.2
					Detached house	80	70.8
	Single	19	16.8	Place of Residence	City center	13	11.5
					District, Town, Village	100	88.5
	Widowed / Divorced	23	20.4	Education status	Illiterate	11	9.7
					Literate with not formal degree	11	9.7
Smoking	Yes	27	23.9		Primary or secondary education	73	64.6
	No	86	76.1		University or above	18	15.9
Age	$\bar{X}$±SD	Min	Max	Has a Chronic Disease	Yes	53	46.9
	53.44±21.01	19.00	88.00		No	60	53.1

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Table 2. Comparisons of the BHS Total and Subscale Scores of the Participants

			Beck Hopelessness Scale											
		n	Feelings about the future			Loss of motivation			Future expectations			BHS total scale score		
			$\bar{X}$±SD	Min-max	Median	$\bar{X}$±SD	Min-max	Median	$\bar{X}$±SD	Min-max	Median	$\bar{X}$±SD	Min-max	Median
Gender	Male	50	0.46±0.88	0.00-4.00	0.00	1.60±1.41	0.00-6.00	1.00	1.86±1.06	0.00-5.00	2.00	4.00±2.78	0.00-11.00	4.00
	Female	63	0.57±1.14	0.00-5.00	0.00	1.42±1.62	0.00-6.00	1.00	1.88±1.40	0.00-5.00	2.00	4.01±3.73	0.00-16.00	3.00
	Total	113	0.52±1.03	0.00-5.00	0.00	1.50±1.53	0.00-6.00	1.00	1.87±1.26	0.00-5.00	2.00	4.40±3.33	0.00-16.00	3.00
	p, Z		p=0.875, Z= -0.157			p=0.248, - Z=-1.154			p=0.785, Z=-0.272			p=0.411, Z=-0.823		
Marital Status	Single	71	0.47±0.96	0.00-5.00	0.00	1.59±1.52	0.00-6.00	1.00	1.95±1.26	0.00-5.00	2.00	4.12±3.26	0.00-16.00	3.00
	Married	19	0.42±0.83	0.00-3.00	0.00	1.36±1.21	0.00-3.00	1.00	1.78±1.08	0.00-4.00	2.00	3.68±2.60	0.00-9.00	3.00
	Divorced, Widowed	23	0.73±1.35	0.00-4.00	0.00	1.34±1.79	0.00-6.00	1.00	1.69±1.39	0.00-5.00	1.00	3.91±4.12	0.00-14.00	3.00
	p, KW		p=0.879, KW=0.258			p=0.473, KW=1.496			p=0.577, KW=1.100			p=0.625, KW=0.939		
Education status	Illiterate	11	0.36±0.92	0.00-3.00	0.00	1.36±1.74	0.00-6.00	1.00	1.63±1.20	0.00-4.00	1.00	3.54±3.83	0.00-14.00	3.00
	Literate	11	0.63±1.20	0.00-4.00	0.00	1.45±1.91	0.00-6.00	1.00	1.72±1.27	0.00-4.00	2.00	3.81±3.51	2.00-14.00	2.00
	Primary or secondary education	73	0.53±0.98	0.00-4.00	0.00	1.45±1.36	0.00-6.00	1.00	1.91±1.26	0.00-5.00	2.00	4.04±3.12	0.00-13.00	3.00
	University or above	18	0.50±1.24	0.00-5.00	0.00	1.83±1.85	0.00-6.00	2.00	1.94±1.34	0.00-5.00	2.00	4.27±3.96	0.00-16.00	4.00
	p, KW		p=0.679, KW=0.773			p=0.739, KW=0.604			p=0.714, KW=0.673			p=0.657, KW=0.839		
Occupation	Homemaker	47	0.53±0.99	0.00-4.00	0.00	1.06±1.30	0.00-6.00	1.00	1.72±1.36	0.00-5.00	1.00	3.46±3.38	0.00-14.00	3.00
	Retired	29	0.48±0.98	0.00-4.00	0.00	1.58±1.63	0.00-6.00	1.00	1.75±1.18	0.00-4.00	1.00	3.86±3.11	0.00-14.00	3.00
	Civil Servant, Tradesperson	11	0.90±1.81	0.00-5.00	0.00	2.72±1.84	0.00-6.00	3.00	2.63±1.20	1.00-5.00	3.00	6.36±4.34	1.00-16.00	6.00
	Laborer	12	0.25±0.22	0.00-2.00	0.00	2.16±1.58	0.00-6.00	2.00	2.25±1.05	1.00-5.00	2.00	4.75±2.73	1.00-11.00	4.00
	Other (self-employed, student, etc.)	14	0.50±0.75	0.00-2.00	0.00	1.28±1.06	0.00-3.00	1.00	1.71±1.13	0.00-4.00	2.00	3.64±2.67	0.00-8.00	3.50
	p, KW		p=0.887, KW=1.143			p=0.009, KW=13.630			p=0.111, KW=7.515			p=0.054, KW=9.313		
Place of Residence	City center	13	0.84±1.46	0.00-5.00	0.00	2.38±1.89	0.00-6.00	2.00	2.38±1.50	0.00-5.00	2.00	5.61±4.35	1.00-16.00	6.00
	District, Town, Village	100	0.48±0.96	0.00-4.00	0.00	1.39±1.44	0.00-6.00	1.00	1.81±1.22	0.00-5.00	2.00	3.80±3.14	0.00-14.00	3.00
	p, Z		p=0.329, Z=-0.977			p=0.049, Z=-1.968			p=0.165, Z=-1.388			p=0.106, Z=-1.617		
Has a Chronic Disease	Yes	53	0.56±1.08	0.00-4.00	0.00	1.37±1.54	0.00-6.00	1.00	1.79±1.21	0.00-5.00	2.00	3.83±3.38	0.00-14.00	3.00
	No	60	0.48±0.99	0.00-5.00	0.00	1.61±1.51	0.00-6.00	1.00	1.95±1.30	0.00-5.00	2.00	4.16±3.30	0.00-16.00	3.50
	p, Z		p=0.518, Z=-0.646			p=0.285, Z=-1.069			p=0.559, Z=-0.584			p=0.454, Z=-0.749		
Age		113	53.44±21.01	19.00-88.00	56.00	53.44±21.01	19.00-88.00	56.00	53.44±21.01	19.00-88.00	56.00	53.44±21.01	19.00-88.00	56.00
	p, r		p=0.669, r=0.037			p=0.414, r=0.78			p=0.901, r=0.012			p=0.730, r=0.033		

Table 3. Comparisons of the PSS Total and Subscale Scores of the Participants

			Perceived Stress Scale								
		n	Perceived insufficient self-efficacy			Perceived stress/distress			PSS total scale score		
			$\bar{X}$±SD	Min-max	Median	$\bar{X}$±SD	Min-max	Median	$\bar{X}$±SD	Min-max	Median
Gender	Male	50	13.48±3.40	4.00-19.00	14.00	10.76±5.27	1.00-24.00	10.00	24.24±5.21	12.00-34.00	25.00
	Female	63	14.31±3.66	6.00-21.00	15.00	11.42±5.43	1.00-26.00	12.00	25.74±6.48	12.00-41.00	27.00
	Total	113	13.94±3.55	4.00-21.00	15.00	11.13±5.35	1.00-26.00	11.00	25.07±5.98	12.00-41.00	26.00
	p, Z		p=0.216, Z=-1.237			p=0.436, Z=-0.780			p=0.175, Z=-1.355		
Marital Status	Single	71	14.05±3.47	7.00-21.00	14.00	11.25±5.24	1.00-24.00	11.00	25.30±5.84	12.00-41.00	26.00
	Married	19	14.15±3.05	8.00-18.00	15.00	8.57±4.75	1.00-16.00	9.00	22.73±5.63	12.00-32.00	22.00
	Divorced, Widowed	23	13.43±4.25	4.00-20.00	14.00	12.86±5.57	5.00-26.00	13.00	26.30±6.38	14.00-35.00	27.00
	p, KW		p=0.895, KW=0.222			p=0.075, KW=5.193			p=0.120, KW=4.237		
Education status	Illiterate	11	14.63±3.88	9.00-20.00	16.00	13.63±5.74	6.00-26.00	13.00	28.57±5.53	19.00-35.00	28.00
	Literate	11	12.45±3.23	8.00-18.00	12.00	13.54±4.36	7.00-21.00	13.00	26.00±4.75	15.00-32.00	27.00
	Primary or secondary education	73	14.20±3.57	4.00-21.00	15.00	10.78±5.43	1.00-24.00	11.00	24.98±6.19	12.00-41.00	26.00
	University or above	18	13.38±3.44	7.00-18.00	14.50	9.55±4.68	1.00-20.00	9.00	22.94±5.46	14.00-33.00	24.00
	p, KW		p=0.367, KW=3.168			p=0.113, KW=5.964			p=0.126, KW=5.724		
Occupation	Homemaker	47	14.65±3.65	6.00-21.00	16.00	11.70±4.56	2.00-21.00	12.00	26.36±5.99	13.00-41.00	28.00
	Retired	29	13.20±3.64	4.00-19.00	14.00	13.20±5.67	2.00-26.00	13.00	26.41±5.36	14.00-35.00	27.00
	Civil Servant, Tradesperson	11	13.63±3.80	7.00-18.00	15.00	9.90±6.25	1.00-20.00	9.00	23.54±6.71	16.00-36.00	24.00
	Laborer	12	14.41±2.90	9.00-20.00	14.50	7.91±5.96	1.00-19.00	7.00	22.33±5.89	12.00-30.00	22.00
	Other (Self-employed, student, etc.)	14	12.92±3.22	8.00-18.00	14.00	8.64±3.91	1.00-15.00	9.50	21.57±4.76	12.00-29.00	21.50
	p, KW		p=0.352, KW=4.419			p=0.019, KW=11.819			p=0.014, KW=12.561		
Place of Residence	City center	13	12.76±3.70	7.00-17.00	15.00	8.07±4.64	1.00-17.00	9.00	20.84±5.11	14.00-29.00	21.00
	District, Town, Village	100	14.10±3.52	4.00-21.00	14.50	11.53±5.33	1.00-26.00	11.50	25.63±5.88	12.00-41.00	26.00
	p, Z		p=0.345, Z=-0.945			p=0.027, Z=-2.206			p=0.006, Z=-2.762		
Has a Chronic Disease	Yes	53	14.30±3.94	4.00-21.00	15.00	12.98±5.39	2.00-26.00	13.00	27.28±5.85	14.00-41.00	29.00
	No	60	13.63±3.17	7.00-20.00	14.50	9.50±4.79	1.00-20.00	10.00	23.13±5.43	12.00-36.00	24.00
	p, Z		p=0.298, Z=-1.040			p=0.002, Z=-3.101			p=0.000, Z=-3.881		
Age		113	53.44±21.01	19.00-88.00	56.00	53.44±21.01	19.00-88.00	56.00	53.44±21.01	19.00-88.00	56.00
	p, r		p=0.770, r=0.028			p=0.01, r=0.320			p=0.000, r=0.730		

Table 4. Thoughts of the Participants on the Pandemic

Thoughts on the COVID-19 pandemic	Yes		No	
	n	%	n	%
Hand washing is important in preventing the disease	113	100	0	0
I wash my hands whenever possible	112	99.1	1	0.9
I think the pandemic is exaggerated	70	61.9	43	38.1
Social distancing is important for protection from the disease	112	99.1	1	0.9
The disease is transmitted even by shaking hands	112	99.1	1	0.9
The disease is transmitted more in common living areas	110	97.3	3	2.7
It is not difficult for me to follow the rules during isolation	109	96.5	4	3.5
I care about my personal hygiene during isolation	112	99.1	1	0.9

DISCUSSION

In addition to the evaluation of the societal impact of the pandemic on mental health among infected individuals, it is also important to evaluate people at risk of COVID-19 infection. The psychological evaluation of COVID-19 contacts is neglected as the treatment of COVID-19 patients is prioritized. We aimed to determine the perceived stress and hopelessness levels in individuals who had had contact with COVID-19 patients. The mean BHS and PSS scores of the participants of our study were 4.40±3.33 and 25.07±5.98, respectively. Studies have reported that both family members and contacts of COVID-19 patients have mental issues as they are isolated or quarantined, and these individuals feel shame, guilt, or stigma. Studies have also reported that the frequency of post-traumatic stress disorder and depression increases in the family and close contacts of COVID-19 patients (12,13,14). Although the stress levels of the COVID-19 contacts in our study were in parallel with those reported in the literature, the hopelessness levels of our participants were not very high. This may be because of the possibility that the participants of this study considered that their personal protective measures would protect them from the disease. Additionally, 61.9% of the participants thought that the pandemic was exaggerated, and 96.5% stated that it was not difficult for them to abide by the rules, which may have reduced their hopelessness levels. Although the hopelessness and stress levels of the female participants were higher than those of the male participants, the differences between them were not statistically significant. Furthermore, almost all participants were aware of the importance of washing hands and social distancing during the pandemic period, the risks of commonly shared areas, and the value of personal hygiene. Göksu and Kumcagiz conducted a study on perceived stress and anxiety in individuals during the COVID-19 pandemic period and found higher anxiety and stress levels in female participants (15). One study on psychological reactions and related factors in the first phase of the COVID-19 pandemic revealed that women had higher anxiety levels than men did (12). Another study of healthcare workers found higher stress and anxiety levels in female workers during the pandemic (5). This may be because the working life requirements and social and domestic roles imposed on women lead them to have higher stress levels than men.

Although the participants of our study who were single were more stressed and hopeless than those who were married, the difference between them was not statistically significant. Göksu and Kumcağiz found higher stress levels in single individuals (15). This may be because family support reduces hopelessness and stress levels among married people. The results of our study revealed that occupation did not affect hopelessness, but the retired participants had a significantly higher mean total PSS score than others. Likewise, there was a significant positive relationship between the ages of the participants and their perceived stress levels. Our result was supported by one study about the effects of COVID-19 on mental health (16). Tian et al. determined that people aged 50 and over in China had phobic anxiety, more obsessive-compulsive symptoms, psychotic symptoms, and interpersonal sensitivity during the pandemic (17). Another study emphasized psychological problems in the elderly during the pandemic as they had a higher level of fear of becoming infected and dying (18). Stress increases by age as older people stay at home more than other age groups do during the pandemic, and the disease causes more deaths in the elderly. Studies in the literature have stated that isolation at home increases depression, health anxiety, financial anxiety, and feelings of loneliness (19,20). The results of the present study showed higher perceived stress levels in the participants who lived outside the city center (district, village, town) and those had chronic diseases. Although the COVID-19 pandemic has caused unemployment and loss of welfare in all segments of society, it had a greater impact on some risk groups (21,22). Wilner et al. reported that the pandemic affected people with chronic diseases more, causing them to have higher stress levels (23). Cao et al. also emphasized that staying in an urban area instead of a rural area has a protective effect during the

pandemic (24). People living outside the city center and those with chronic diseases have higher perceived stress levels due to their inability to access health services, the need for private or rental cars for hospital transportation, and the emergence of additional nutritional needs to keep immunity strong.

CONCLUSION

The COVID-19 pandemic has caused unemployment and loss of welfare in all social segments, but it has had more severe impact on some risk groups such as COVID-19 contacts. In our study, the mean BHS and PSS scores of the COVID-19 contacts were 4.40±3.33 and 25.07±5.98, respectively. Moreover, 61.9% of the participants thought that the pandemic was exaggerated, and 96.5% stated that it was not difficult for them to obey the rules in place. Furthermore, almost all participants were aware of the importance of washing hands and social distancing during the pandemic period, the risks of commonly shared areas, and the value of personal hygiene.

Due to the psychological consequences of the COVID-19 pandemic like shock, denial, anxiety, worry, and stress in people, it is important to strengthen crisis and stress management and increase awareness, coping, and social support resources by prioritizing high-risk groups like the aged, women, health employees, those with chronic diseases, and COVID-19 contacts.

Social workers should increase the awareness of these risk groups regarding pandemic-related problems, identify their needs, evaluate the effects of these problems on individuals, and help them develop rational coping strategies.

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Research Article

Effects of Auditory Stimulation and Exercise on Gender Hormones in GMOs-Fed Rats

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Abstract

Aim: In this study, it was aimed to investigate the effects of auditory stimuli and exercise on structural measurements, functional characteristics and sex hormones of rats fed with genetically modified organisms (GMO).

Materials and Methods: A total of 64 8-week-old Spraque Dawley rats, 32 male and 32 female, were used in the study. GMO feeding, auditory stimulus and exercise were applied to both male and female rats. Control groups were also formed at the same time as the experimental groups. GMO application; It was fed by mixing 20ml water+20ml corn syrup per day. Auditory application; Segah and Hüseyni maqams were played at 55 decibels loudness for 60 minutes a day. Exercise app; It was applied as challenging swimming in an 80 cm long and 40 cm wide pool.

Results: It was determined that the body weights of all rats treated with GMO showed a significant increase ($p<0.05$). Significant increases in blood lactate levels were observed in exercise-treated rats ($p<0.05$). It was determined that musical auditory stimuli were effective on sex hormones and significant decreases occurred in estrogen levels of female rats fed GMOs ($p<0.05$).

Conclusion: It was observed that the preference of feeding with GMO significantly affected the body composition of the subjects. It was determined that swimming and sound stimuli were effective on sex hormones. It was determined that there was an increase in testosterone levels in male rats fed with exercise and GMO. A significant decrease was determined in the estrogen levels of female rats, especially in those fed with GMOs. For this reason, it was seen that the changes in the sex hormones caused by the high fructose-containing GMO diet can cause very important health problems. It was determined that more research on the subject should be done in order to explain the system response mechanisms of the organism, the relationship between exercise and auditory stimuli and GMO nutrition.

Keywords: GMO, auditory stimulus, exercise, lactate, testosterone, estrogen

INTRODUCTION

The organism, which is derived from the manipulation of an organism by gene sequencing or by trying to give it a new feature that is not in its own, is called a genetically modified organism (GMO) *(Cast, I 2006)*. Genetically modified organisms (GMOs) are organisms that are subject to a genetic change by using various techniques, by changing the gene sequence of the organism or by providing a feature that is not inherent to the gene transfer (Pamuk S 2010, Sungkyoung Lee 2020). The food industry is the most commonly used area of GMO. GMO is preferred in nutrients for many reasons, such as increasing efficiency, prolonging shelf life. The most common GMO product is corn. GMO corn; the main oil is used in the production of flour, starch, glucose syrup and fructose syrup. These products are made of corn, biscuits, pretzels, pudding, waffles, chocolate, candy, soda, chips, ketchup, coated nuts, food, mayonnaise, meat juice tablet, instant soup, coke, it is included in many products, such as juice. It also enters the body through animals consuming GMO corn with animal food *(Costa-Font J 2007, Ozer I 2003, Pusztai A. 2003)*.

The impact of GMO on the human body is a controversial issue. However, studies have been very broad in the literature that GMO has a negative impact on health. Among the things that are said to have caused a change in

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metabolism with unexpected gene interactions, resulting in weight gain, as well as negativity at various blood parameters and hormone levels (Hug, 2008; *Domonkos E, 2017)* can also cause consequences such as cancer, antibiotic resistance and allergies (Hug, 2008).

It is clear that certain work must be carried out to minimize the risks posed by GMO on health. For this reason, there are different ways to recycle the harmful effects caused by GMO. It is known that exercise plays a very effective role in human health. It is often referred to as a method of treatment of metabolic problems or diseases due to hormone imbalance (Niemiro et al., 2020; Woodward et al., 2020). Given the effects of the exercise on hormonal and metabolic levels, it is also possible that GMO will have an effect on these changes. Another remarkable parameter today, besides medication, is the reduction of symptoms of disease with auditory stimuli. They showed that music and nature sounds in front of you reduce pulse rate (Smurf Sky, 2015) in a study on intensive care patients.

In this study, it is aimed to examine body weight and change of gender hormones in rats fed with high fructose corn syrup and to investigate the effect that is produced by exercise and auditory warnings and recycling.

MATERIAL AND METHOD

In the study, 32 female and 32 male Spraque Dawley rats in eight weeks randomly, control, genetically modified organism, auditory stimulus, exercise, the genetically modified organism+auditory stimulus, genetically modified organism+exercise, genetically modified organism+auditory stimulus+exercise groups are divided into eight groups (Table 1). GMO groups were given a mix of 40 ml 50% corn syrup a day, while auditory stimulants played Segah and Huseyni at 60 minutes 55 decibel sound a day, while exercise groups were treated a challenging swimming swim in a 80cm long 40cm pool. Study protocol and experimental methods *Erciyes* University Animal experiments were approved by the Local Ethics Board (Erciyes University 09.03.2016). During the course of the experiment, the rats were kept up to 24±2°C, 12 hours at 12 hours a night, and hours a day in a vinegar rhythm, in plastic cages with wire covers, to receive air above them. at the end of the 28 days, the subjects were silamine (80mg/kg) and xyleasine (5mg/kg) were carried out under anesthesia. Lactate, testosterone and estrogen hormone values were measured on blood samples from abdominal aorta after the laceration.

Table 1. Experimental prosedure and groups. CONT; control group, GDO; Genetically modified organism group, EXC; Exercise group, AUDI; Auditory group, GDO+ AUDI; Genetically modified organism+ Auditory group, GDO+EXC; Genetically modified organism+Exercise group, EXC+AUDI; Exercise and Auditory group, GDO+EXC+AUDI; Genetically modified organism+Exercise+Auditory group

Groups	Number of the subject	Experimental prosedure
CONT	8	The group fed with standard feed with no treatment
GMO	8	GMO-containing feed group
AUDI	8	The group that is listened to music and fed with standard feed
EXC	8	The group subjected to the swim test and fed with standard feed
GMO+EXC	8	The group subjected to the swim test and fed with GMO-containing feed
GMO+AUDI	8	The group that is listened to music and fed with standard feed
EXC+ AUDI	8	The group that is listened to music and the subjected to the swim test and fed with standard feed
GMO+EXC+AUDI	8	The group that is listened to music and the subjected to the swim test and fed with GMO-containing feed

Preparation of GMO food, auditory and exercise applications

A food containing 55% fructose, 42% glucose and 3% glucose polymers was prepared in the preparation of the nutrient that was applied to GMO groups, mixed with high-fructose corn syrup and tap water, containing 50% corn syrup. The reduction was reported by putting this nutrient in the leeches of the animals in the group to be applied to GMO.

Audiovisual alerted groups were played by mobile phone from 10:00 to 11:00 every day, at 55-decibel volume, at the Segah office and the Hüseyni office.

A mandatory swimming test is used for exercise application. The rats were included in the program in groups and exercised in plastic containers with a cylindrical water temperature of 25°C, 80 60cm in diameter of 40cm in 40 minutes for four weeks.

Measurement of hormone levels

The lactate meter was used to measure the lactate level. Blood samples from subjects were carried out using disposable strips, by the instrument instructions. For

lactate measurements, the reference range is 0.5–25 mmol/L (49).

ELISA kits (Sunrise/ Shanghai Chine) were used to measure testosterone and estrogen levels. Blood samples from the abdominal aorta were centrifugated at 3000g for 10 minutes. The serum samples obtained were tested by the procedure specified in the kits and measurements were performed with plate reader assistance.

Statistical Analysis

The data obtained at the end of the operation was evaluated using the Statistical Package for the Social Sciences 22.0 (SPSS 22.0) program in the computer environment. The Shapiro-Wilk test was evaluated and the Independent-Samples t-test was used for parametric data in the cross-group comparisons, whether the parameters have a normal distribution. In cases where variance shows the homogeneous distribution, post hoc Tukey testing was conducted and in cases where homogeneous distribution was not shown, multiple comparisons were made with Tamhane T2 testing. Kruskall Wallis test was used to compare groups in non-parametric data, and was statistically recognized at $p<0.05$.

RESULTS

Weight change

When examining the values obtained from the test start and end of the test, the average weight measurements of the subjects of GDO groups were significantly increased at the end of the experiment ($p<0.05$) (Figure 1).

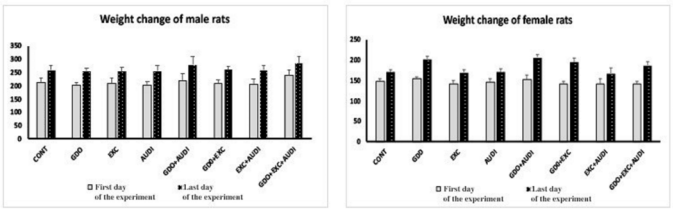

Figure 1. The average weight values of the subjects at the beginning and end of the experiment are shown. CONT; control group, GDO; Genetically modified organism group, EXC; Exercise group, AUDI; Auditory group, GDO+AUDI; Genetically modified organism+Auditory group, GDO+EXC; Genetically modified organism+Exercise group, EXC+AUDI; Exercise and Auditory group, GDO+EXC+AUDI; Genetically modified organism+Exercise+Auditory group

Hormone analysis results

The hormone levels for all subjects are shown in the figure below (Figure 2). Given the data of male rats, a noticeable difference was observed in the exercise groups at serum lactate levels ($p<0.05$). In addition, in comparison with GMO and GMO+AUDI groups, the lactate level in the GMO + AUDI group is lower than the GMO group ($p<0.05$). It has also been observed that the lactate level in the EXC+AUDI and GMO+ EXC group has increased compared to the GMO group ($p<0.05$).

When the testosterone levels of male rats were examined, only the reduction in the AUDI group compared to the CONT group was observed ($p<0.05$). There is no statistically significant difference between other groups ($p<0.05$). Analysis of estrogen levels shows a reduction in GMO +AUDI group compared to the CONT group; there is an increase in the EXC+ AUDI group compared to the GMO group ($p<0.05$).

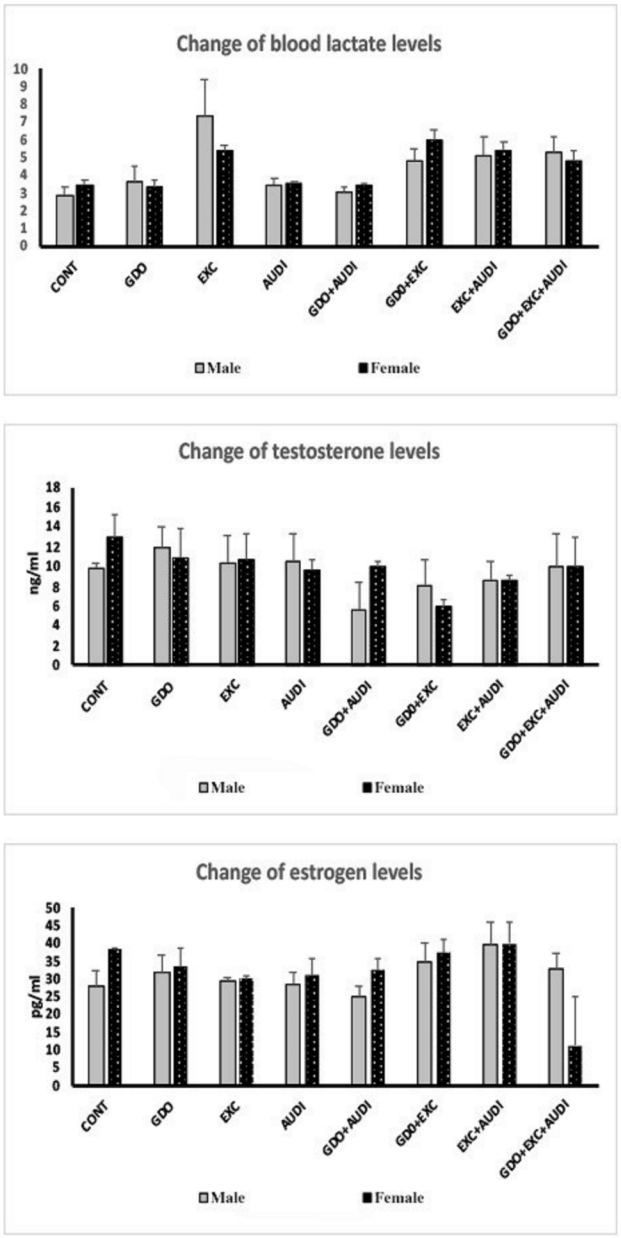

Figure 2. The results (± SEM) of serum hormon level in all groups. A. Serum laktat level results (±SEM) of all groups are showed.There was a significant diffirences between EXCs and CONT group, GDO and GDO+AUDI groups on the male rats. There was a significant diffirences between EXCs and CONT group on the female rats. B. Serum testosteron level results (±SEM) of all groups are showed.There was a significant diffirences between AUDI and CONT group on the male rats, and CONT and EXC+AUDI groups on female rats. C. Serum estrogen level results (±SEM) of all groups are showed.There was a significant differences between GDO+AUDI and CONT groups, GDO and EXC+AUDI groups on the male rats. There was not differences among all groups on the female rats

In a hormone-level analysis of female rats, an increase in serum lactate levels in all EXC groups has been observed compared to the CONT group ($p<0.05$). In testosterone levels, a reduction in the EXC+AUDI group was observed compared to the CONT group ($p<0.05$). While estrogen levels do not have a statistically significant difference between groups, there are easy-to-follow changes ($p\geq0.05$).

DISCUSSION

GMOs is a species of organism that has entered our lives and bodies with food source consumed in our daily lives. GMOs, which are located in many packaged foods, are also available in the food of animals such as chickens. This increases the rate of body ingestion.

The literature on the effects of GMO on health has much questionable information (D'Agnolo, 2005). However, it is known that when an organism is genetically modified, it will have toxic effects if it is not suitable for the composition or operation of the human body (Paparini and Romano-Spica, 2004). Toxic nutrients are the basis for anti-nutrients, autointocchosis or food poisoning. Studies have drawn attention to the fact that GMO has adverse effects on health such as allergic reactions, hormonal changes. In this study, it is intended to demonstrate how much these changes can be affected by changes in the hormone level of rats and exercise and auditory stimulation, using high fructose corn syrup from genetically modified corn.

It is stated in many studies, that the weight of the subjects fed with corn syrup causes an increase or decrease (with behaved, 2014; Mor, 2014). In a study, a total of 20 (12-14 weeks) Swiss albino mice with 10 females and 10 males reported a 2.20g increase in the mice and 3.40g in the females of the mice, the control group fed with corn without GMOs. A weight increase of 1.5g in the males of mice fed with GMO corn and a weight reduction of 4.00g in the females. In mice fed with corn without GMOs, it was determined that the weight increase was higher compared to mice fed with GMOs corn. It has been determined that these increases are statistically significant in the female and male of the control group according to the teeth of the GMO group, and the increase in the men of the control group is not statistically significant compared to the males of the group fed by GMO corn. In terms of gender, it has been determined that there is more weight increase and decrease in the females of both groups, which are fed by GMOs and GMOs corn (Karakuslu S, 2014). In the study, after the fatty diet was given to the groups, there was a significant increase in the weight of the mice in the experiment group according to the control group (Purple B 2014). In this study, GMOs were observed to have a significant weight increase in the teeth of all the groups given. This shows that GMO consumption increases weight and supports many of the studies in this literature. In the weight increase of male rats, the increase was observed, although not as seen in the females in the GMOs. There is no significant difference between the starting and end of the experiment in the GMO+AUDI group only. It was purple and his friends.

The blood is sensitive to changes in lactate concentration, exercise intensity and duration (Beneke R, 2011). Therefore, it is a common variable to estimate the intensity of exercise (Beneke, R 2001). Studies have been conducted on laboratory animals and the blood lactate concentration has been used to determine the exercise intensity (Voltarelli FA, 2002). In a study, it has been reported that the blood lactate level has increased significantly according to the control group in mice undergoing swimming exercise. This study has seen an increase in lactate in all exercise groups in both female and male mice. This result supports the studies in the literature. GMO consumption did not cause a lactate serum level change. However, while the group fed with GMO and auditory was not differentiated with the CONT group, a lactate level decrease was observed compared to the GMO group. Since blood lactate concentration is known to increase by folding with the exercise intensity, it is believed that an increase in lactate levels may be possible, given the soothing properties of music used for auditory stimulation (Voltarelli FA, 2002; Smurf Sky, 2015).

GMO is also thought to have an impact on the reproductive system. In some studies, GMO may have an effect on sterile due to endometriosis, gender hormonal disorder, endocrine-metabolic anomaly (Aris and Paris, 2010; I'm not. Séralini and Moslemi, 2001; Séralini et al., 2012). There are also studies that show that the increase in GMO consumption has negative GMO is also thought to have an impact on the reproductive system. In some studies, GMO may have an effect on sterile due to endometriosis, gender hormonal disorder, and endocrine-metabolic anomaly (Aris and Paris, 2010; I'm not. Séralini and Moslemi, 2001; Séralini et al., 2012). There are also studies that show that the increase in GMO consumption has negative effects in the developmental process (Tyshko et al., 2011; Zhou et al., 2012). This study has examined the change of testosterone and estrogen hormones that play a significant role in the reproductive system with the GMO effect. In testosterone levels, no difference was observed between CONT and GMO groups of both types. Although female rats do not make a statistically significant difference, there are decreased levels of testosterone in the GMO group. There was no study in the literature regarding the effects of GMO on testosterone levels. Although the results of this study show that GMO does not affect testosterone, there is a requirement for further studies to be carried out, given the effects on GMO (Séralini and Moslemi, 2001). Other androgens evaluated in this study are the estrogen hormones. The level of estrogen hormones did not make a significant difference in the group fed by GMOs in both male and female rats. However, the results may not be statistically significant, but the GMO has caused reductions in estrogen levels in female rats; male rats are seen to have caused an increase. These results suggest that the effects of GMO can also make a difference between the sexes. In a study where the effects of the consumption of GMOs plants in the literature are examined, significant increases

in estrogen levels were observed in subjects fed by GMOs plants (Séralini et al, 2012). Given all this, the effect of GMO on estrogen levels is also necessary for a detailed review. Both warnings show different effects. Exercise and auditory members were used in this study to minimize the effects of GMO. Analysis of hormonal levels shows that auditory stimulation causes decreased testosterone levels in the analysis of data from male rats; in female rats, there was no statistical difference, but a decrease was observed. There are many studies that study the effects of music on hormonal secretions (take Doi et, 2018; Kreutz et al., 2012, Borniger et al., 2013). Music is thought to be effective in the neuroendocrine secretions of the brain. A study shows that sophisticated music in men has a negative effect on the testosterone level in saliva; in another study, it has been reported that the level of testosterone is high in women who are dealing with music (Borniger et al., 2013; Doi et al., 2018). The difference in the results of the study suggests that the testosterone and the musical relationship should be investigated in detail. In addition, the exercise is in studies that show that the testosterone level is reduced. It reduces testosterone levels of cortisol increased by exercise (Brownlee et al., 2005). While this study shows a decrease in testosterone levels in exercise groups, this decrease did not make a statistically significant difference. In addition, a significant difference in testosterone levels has been observed in the group that has both auditory warning and exercise stimulus for female rats. These results support that the exercise is associated with the testosterone level.

Studies show that music affects levels of steroids such as estrogen, receptor genes and related proteins (Fukui and Toyoshima, 2008). In this study, estrogen levels were measured in both male and female rats. While estrogen levels have decreased in the auditory quasi-given group in male rats, but this decrease does not make any statistical meaning, the increase in GMOs is the exact opposite of GMO+ AUDI group and results in a statistically significant decrease, this increase does not mean statistically significant. However, this increase is very meaningful when considering cognitive effects on estrogen level and musical capability in females (Fukui and Toyoshima, 2008). The increase in the exercise warning in the females has increased the mi estrogen levels, but this increase has not statistically expressed meaning. Estrogen is a hormone that plays an important role in muscle contractions and repair. Although the mechanisms in which estrogen applies its effect on skeletal muscle damage, inflammation, and repair indicators are not fully illuminated, it is believed that the effects of estrogen are potentially preventive by acting as antioxidants (Enns and Tiidus, 2010). it's being watched. This is to be said that auditory stimulation causes a decrease in the estrogen level in men and the female rats.

CONCLUSION

lthough there is a change in the hormonal level, given all the results in our work, many of these changes do not mean statistically. This can be explained as the difference between individuals in groups and the high deviation values. In this study, there have been differences between the sexes in hormonal changes. This indicates that GMOs and auditory and exercise stimulants have different effects between sexes. In addition, in the light of all analyzes and literature, detailed and comprehensive work has been planned to describe the effects of both GMO nutrition and hormonal changes and their mechanisms.

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MEDICAL RECORDS-International Medical Journal

Research Article

Anxiety Status in Parents of Infants Referred During National Newborn Hearing Screening

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Abstract

Aim: This study aims to investigate the anxiety status in parents of infants who received pass and refer results during newborn hearing screening (NHS).

Materials and Methods: The study was conducted on parents (mother and father) of a total of sixty infants who came to the NHS. All infants underwent automated (A)-ABR. Forty parents of 20 infants who were born healthy and received NHS-pass results were included in the study as group I. Forty parents of 20 infants who were born healthy and received NHS-refer results were included in the study as group II. Forty parents of 20 infants hospitalized in the neonatal intensive care unit (NICU) for at least five days and received NHS-refer results were included in the study as group III. Beck Anxiety Inventory was administered to all parents in the groups.

Results: When the anxiety levels were evaluated according to the groups, the anxiety scores of the parents in group III were higher than those in group I and group II ($p<0.05$). However, no difference was found between the parents' anxiety levels in group I and group II. When the anxiety scores were compared according to the genders, there was no difference in the anxiety scores of the fathers between the groups ($p>0.05$). However, mothers in Group III had higher anxiety scores than mothers in Group I ($p<0.05$).

Conclusion: Mothers of infants hospitalized in the NICU who received the NHS-refer result had higher anxiety levels than mothers who were born healthy and received the NHS-pass result. In order to keep the anxiety level of mothers of babies hospitalized in NICU under control, training can be organized for these mothers.

Keywords: Hearing, screening, ABR, audiology, newborn

INTRODUCTION

Hearing loss in healthy newborns is between 1-6 per thousand (1,2). In newborns who need intensive care, this rate rises to 2-4% (3-6). According to the World Health Organization, there are 7.5 million children with hearing loss (<5 years) worldwide (7), and hearing loss is one of the leading causes of the global non-fatal burden of disease (8). Most children with hearing loss live in low-income countries and can not access the necessary therapy (9). Childhood hearing loss affects language acquisition and children's social, motor and cognitive development and quality of life (10). For this reason, rapid diagnosis and intervention are essential in babies with hearing loss.

Newborn hearing screening (NHS) aims to identify infants with congenital or early-onset hearing loss. The result of NHS, usually implemented using Automated-Auditory Brainstem Responses (A-ABR) (and/or otoacoustic emission), can be a 'pass' or a 'refer'. Babies who fail the NHS (at least one ear) receive a refer result, and a refer result is a potential alarm for hearing loss. It was reported that parents who received the NHS- refer felt sad, angry, depressed, shocked, and had higher anxiety levels (11,12). On the other hand, some studies (13,14) stated that the anxiety status of the parents of babies with refer and pass results was similar. These emotional states can be affected by social differences, educational level, financial opportunities or cultural differences. To the best of our knowledge, no study in Turkey investigates the anxiety status of parents of infants with pass and refer results during newborn hearing screening.

This study aims to investigate the anxiety status in parents

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of infants who received the pass and refer results during newborn hearing screening.

MATERIAL AND METHOD

This study was planned prospectively. Verbal and written consent was obtained from all parents participating in the study. In addition, permission was obtained from the ethics committee of the University for the study (ethics committee project no: 2022/782).

The study was carried out on the parents (mother and father) of 60 babies, 40 of whom were born healthy and 20 of whom were hospitalized in the neonatal intensive care unit (NICU). Parents who applied to the NHS were grouped according to the test results of their babies. Forty parents of 20 infants who were born healthy and received NHS-pass results were included in the study as group I. Forty parents of 20 infants who were born healthy and received NHS-refer results were included in the study as group II. Forty parents of 20 infants who had been hospitalized in the NICU for at least five days and received NHS-refer results were included in the study as group III. The NHS protocol and groups applied in Turkey are shown in (Figure 1). The study did not include parents with psychiatric, neurological, orthopedic diseases or divorced parents. One week after the Automated (A)-ABR test was applied to the infants and the test result was reported to the parents, the Beck Anxiety Inventory was administered to the parents.

Automated-Auditory Brainstem Responces (A- ABR)

A-ABR test was applied to infants with Otometrics (Madsen Accuscreen) device. Electrode locations were cleaned with cleansing gel. Electrodes were placed on the forehead, cheek and neck of neck. If the impedance is <8 kOhm, the test is started. The click stimulus was automatically presented at an intensity of 35 dB nHL and applied to both ears. The noise-weighted pattern matching method was used as the test method.

Beck Anxiety Inventory

The Turkish validity and reliability study of the Beck Anxiety Inventory was conducted by Ulusoy et al. (14) and consists of 21 questions. Each question can be answered none (0 scores), mild (1 score), moderate (2 scores), and severe (3 scores). The total score is calculated as over 63.

Statistical analysis

SPSS 21 program was used for statistical analysis. The normal distribution of the data was checked with the Shapiro Wilk test. The One Way ANOVA test was used to compare the three groups if the data were normally distributed, and the Tukey test was used to compare the subgroups from the post hoc tests to compare the paired groups. If the data were not normally distributed, the Kruskal Wallis test was used. $p<0.05$ was considered statistically significant.

RESULTS

The mean age of parents in group I was 31.95±4.99 (22-43), the mean age of parents in group II was 30.20±5.73 (23-48), and the mean age of parents in group III was 31.98±3.71 (25-39). There was no significant difference between the groups in terms of the age variable ($p>0.05$). Age distributions by groups are shown in Table 1.

Table 1. Age distribution by groups

	Group I Mean±ss (min-max) n:40	Group II Mean±ss (min-max) n:40	Group III Mean±ss (min-max) n:40	p* value
Age	31.95±4.99 (22-43)	30.20±5.73 (23-48)	31.98±3.71 (25-39)	0.063
Mother	31.25±5.79 (22-43)	28.50±4.78 (23-38)	31.20±3.45 (25-36)	0.114
Father	32.65±4.06 (24-38)	31.90±6.14 (25-48)	32.75±3.89 (26-39)	0.439

* Kruskal Wallis test

Considering the results of NHS-refer babies, 14 (70%) babies in Group II failed unilaterally, and 6 (30%) failed bilaterally. Fifteen (25%) babies in Group III failed unilaterally, and 5 (75%) failed bilaterally. The mean duration of hospitalization in the NICU of the babies in Group III was 14.66±7.39 (6-36) days. There was no relationship between the length of stay of the babies in the NICU and the anxiety level of the parents ($p=0.664$). When the anxiety levels were evaluated according to the groups, the anxiety scores of the parents in group III were higher than those in group I and group II ($p<0.05$, Table 2). However, no difference was found between the parents' anxiety levels in group I and group II. When the anxiety scores were compared according to the genders, there was no difference in the anxiety scores of the fathers between the groups ($p>0.05$). However, mothers in Group III had higher anxiety scores than mothers in Group I ($p<0.05$, Table 2). 38 (31.6%) of the parents were primary or secondary school graduates, 38 (31.6%) were high school graduates, and 44 (36.6%) were university graduates. The anxiety score of the parents who were secondary or primary school graduates was 5.00±5.04, the anxiety score of the parents who graduated from high school was 3.66±3.21, and the anxiety score of the parents who graduated from university was 3.82±4.43. There was no difference in anxiety scores according to the educational status of the parents ($p=0.874$).

Table 2. Anxiety scores by groups

	Group I (min-max) n:40	Group II (min-max) n:40	Group III (min-max) n:40	p* value	P (Pairwise Comparison)
Total Anxiety Score	2.50 (0-8)	2.00 (0-14)	5.50 (0-26)	0.003	0.367^{X} 0.003^{Y} 0.048^{Z}
Mother	2 (0-8)	4.70±3.97	7.00±4.53	0.002	0.060^{X} $<0.001^{Y}$ 0.094^{Z}
Father	2.90±2.38	1.50 (0-12)	5.00 (0-26)	0.203	

* Kruskal Wallis Test, X: Group I- Group II, Y: Group I- Group III, Z: Group II-Group III

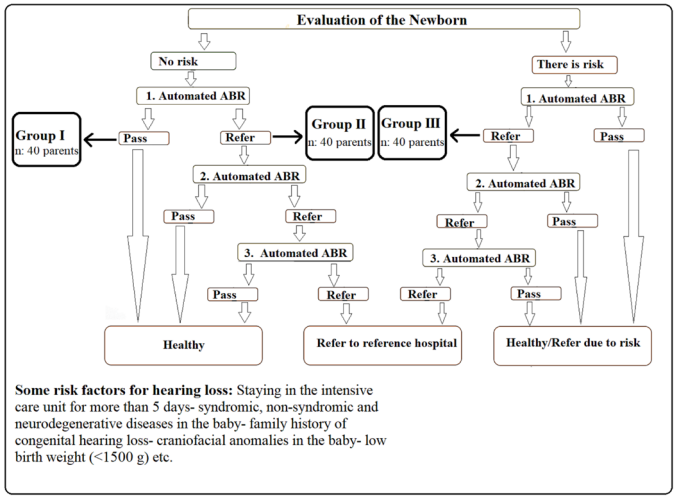

Figure 1. The newborn hearing screening protocol applied in Turkey is shown in the flow chart (11)

DISCUSSION

NHS, whose primary purpose is to diagnose hearing loss early, can be applied differently according to countries. In some countries, it can be combined OAE and A-ABR, while in others, it can be only OAE or only A-ABR. These applications may have advantages or disadvantages compared to each other. Combined OAE and Automated-ABR are excellent methods to predict some auditory disorders such as auditory neuropathy. However, combined administration is time-consuming, and tests are complex for babies who are already sensitive to sleep. It is also costly to administer the two tests. It is inexpensive to apply only OAE, but with this method, diagnosing disorders such as auditory neuropathy becomes challenging. In addition, the false negative rate increases with this application. The protocol applied in Turkey consists of 3 steps, and only Automated-ABR is applied to newborns (Figure 1). According to the protocol, babies who fail the first screening should be tested two more times within a month. Babies born healthy and failing the NHS applied three times (at least one ear) are referred to reference hospitals. Babies with risk factors for hearing loss, such as the NICU, are referred to reference hospitals even if they pass through the NHS. We included only the parents of healthy infants who received the pass and refer result from the screening test performed in the first step and the parents of infants hospitalized in the NICU who received the NHS-refer result. In this way, we aimed to investigate the effect of only hearing screening on anxiety in parents. In our study, it was determined that the parents in group III were more anxious than the parents in group I and group II. When analyzed by gender, it was found that the anxiety level of mothers in group III was higher than in group I. However, there was no difference between the groups in terms of fathers' anxiety levels.

Many factors that cause babies to be hospitalized in the NICU can also cause hearing loss. These include various infections, anomalies and ototoxic agents. The risk of hearing loss increases more than 10 times in infants hospitalized in the NICU (15). Beaula Vincy et al. (12) investigated the anxiety status of parents of healthy-born babies with NHS-pass and NHS-refer results and parents of babies hospitalized in the NICU who had NHS-pass and NHS-refer results in their study. In the study, it was determined that parents of healthy born and NHS-refer babies had higher anxiety levels than parents of healthy born and NHS-pass babies; Parents of NHS-pass babies hospitalized in the NICU had higher anxiety levels than parents of healthy-born and NHS-pass babies; the parents of NHS-referred infants hospitalized in the NICU had higher anxiety levels than the parents of healthy born and NHS-pass infants, and the mothers had higher anxiety levels than the fathers. As a result, the authors stated that NHS-refer result increases anxiety levels in parents of both healthy babies and NICU babies, and providing parents with preliminary information about the NHS can reduce anxiety. Mohd Khairi et al. (16) reported that 18% of mothers of infants who failed the first step of NHS had moderate or severe anxiety. Authors were stated that this group of mothers should be identified and supported psychologically.

The NHS-refer result does not mean that babies have definitive hearing loss. It has been reported that parents' anxiety level due to NHS-refer is generally related to parents' failure to understand the test result and their assumption that their baby has definite hearing loss (17). NHS-refer is only an alert for hearing loss. This information can be told to parents before the test. However, care should be taken when informing parents. Underestimating the NHS too much can reduce parents' trust in the NHS and alienate parents from NHS follow-ups. For this reason, when giving

information to parents about the NHS, parents' interest in the NHS should be kept as high as possible, but it should be explained that the result is only a risk situation for hearing loss.

Unlike studies stating that NHS-refer results in infants cause anxiety in parents, Suppiej et al. (14) evaluated 288 parents and noted that NHS-refer did not cause anxiety. The authors stated that there was no difference in anxiety levels between parents of infants with NHS-pass and refer results hospitalized in the NICU. In addition, there was no difference in anxiety between parents of high-risk NHS-refer infants and parents of low-risk NHS-refer infants. Our study did not find any difference in anxiety levels between the parents of healthy born and NHS-pass babies and the parents of healthy born and refer babies. However, in our study, the anxiety level of parents of NHS-referred infants hospitalized in the NICU was higher than that of parents of healthy-born NHS-pass and NHS-refer infants. In terms of gender, the anxiety level of mothers of NHS-referred infants whose infants were hospitalized in the NICU was higher than that of mothers of healthy-born and NHS-pass infants. However, there was no difference between the anxiety level of mothers of NHS-referred infants hospitalized in the NICU and mothers of healthy-born and NHS-referred infants. When the fathers were examined, it was observed that there was no difference in the anxiety levels of the fathers between the groups. Parents of infants hospitalized in the NICU are often concerned about the infant's overall development due to other health conditions (12). Thus, it can be thought that the direct NHS result from our study did not increase the anxiety level of the parents, and the NICU created more anxiety.

This difference between studies may be due to social and cultural differences. Some societies can be more cold-blooded and they can meet the referral result more calmly. In some societies, superstitious and local beliefs or religious dogmas may be at the forefront (12). In addition, our study showed no relationship between parents' education levels and anxiety levels.

Limitation of this study: According to the NHS protocol implemented in Turkey, babies with risk factors are referred to reference audiology clinics for further audiological examinations, even if they pass through the NHS. Referral of infants to other hospitals may affect parents' anxiety state and may impair the methodology of our study. For this reason, infants hospitalized in the NICU and NHS-pass were not included in our study because they were referred to reference audiology clinics.

CONCLUSION

In this study, the effect of NHS administered to infants on parents' anxiety was investigated, and it was found that healthy-born and NHS-refer results did not increase anxiety in parents. However, mothers of babies hospitalized in the NICU who received the NHS-refer result had a higher anxiety level than the mothers of the babies who were born healthy and received the NHS-pass result. In order to keep the anxiety level of mothers of babies hospitalized in the NICU under control, trainings can be organized for these mothers.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Verbal and written consent was obtained from all parents participating in the study. In addition, permission was obtained from the ethics committee of the University for the study (ethics committee project no: 2022/782).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Linear Combination of Leukocyte Count and D-Dimer Levels in the Diagnosis of Patients with Non-traumatic Acute Abdomen

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Abstract

Aim: Rapid intervention is required in patients with non-traumatic acute abdominal pain. It is very important to distinguish between surgical and non-surgical pathologies during this intervention. This study aimed to increase the diagnostic accuracy by combining the leukocyte count and D-dimer levels used in this evaluation with linear combination methods.

Materials and Methods: Logistic regression, scoring, min-max, minimax, Su & Liu, Pepe & Thompson, Pepe, Cai & Langton, and Todor & Saplacan methods were used as linear combination methods. The data set was divided into 70% training set and 30% test set. Parameter optimization was performed on the training data by 5 fold cross-validation method using 10 repeats. The area under the ROC curve, sensitivity, selectivity, accuracy, positive and negative predictive value, and positive and negative likelihood ratio statistics were used in the performance evaluation.

Results: The area under the ROC curve statistic for D-dimer level and log-transformed leukocyte count variable were obtained as 0.71 and 0.70, respectively. The accuracy rate was 0.69 for the D-dimer level and 0.73 for log-transformed leukocyte count. For the linear combination methods, the area under the ROC curve was between 0.77 and 0.81, and the accuracy statistics were between 0.72 and 0.79. The best performance was obtained with the min-max method.

Conclusion: In patients with non-traumatic acute abdominal pain, leukocyte count and D-dimer levels can be evaluated together by using linear combination methods in differentiating surgical and non-surgical pathologies. The obtained results showed that the diagnostic performance of the combined results with the min-max procedure was higher than the leukocyte count and D-dimer levels.

Keywords: Abdominal pain, D-dimer level, linear combination, nontraumatic acute abdomen

INTRODUCTION

Patients with acute abdominal pain constitute a significant portion of patients admitted to the emergency department. Therefore, it is very important to determine whether there is an urgent need for surgery in these patients and to determine the follow-up period for those who do not need urgent surgery. A delay in diagnosis will increase the mortality of the patients (1-3).

Patient history and complete physical examination are compulsory for immediate therapy. In addition, methods such as laboratory tests, ultrasonography, computed tomography, and magnetic resonance imaging (MRI) contribute to this evaluation. However, the reason that the ultrasonography method does not have high sensitivity for all conditions and is recommended for use in right upper quadrant pains; allergic reactions, renal insufficiencies, and technical problems for computed tomography, and the high cost and lack of immediate availability of the MRI method increase the need for new diagnostic tests (3,4).

Akyıldız et al. (3) performed measurements of leukocyte counts and D-dimer levels in patients with this symptom. As a result of the study, the authors stated that D-dimer levels performed better in the differential diagnosis of patients with acute abdominal pain than the leukocyte count. In another study, using the same study data, Zararsiz et al. (4) combined these two tests using various machine learning methods. The built machine-learning models outperformed the performances of individual markers. Although high accuracy results are obtained with machine learning models, interpretation of the estimated results is very difficult since most models are based on the black-box concept. Especially in medical applications, there is a need to interpret the models to confirm the results, apart from obtaining highly accurate results (4,5).

CITATION
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Another approach for combining diagnostic tests is the linear combination methods. These methods combine the results of two or more diagnostic tests with the help of linear models and aggregate them into a single variable. The performance of the combined diagnostic test is calculated on this newly generated variable. One of the most important advantages of linear combination methods is that they can increase diagnostic performance compared to individual diagnostic tests and produce simple and interpretable results (6-8).

In this study, we applied and assessed the performance of eight linear combination methods to increase the diagnostic accuracy of nontraumatic acute abdomen.

MATERIAL AND METHOD

Dataset

In our study, we used the data of previously published studies (3,4). This data contains 225 patients who applied to Erciyes University Hospital with acute abdominal pain. The patients were divided into two groups based on their medical history, prompt diagnostic modalities, physical examination, and laboratory test results. The first group included 110 patients who needed immediate laparotomy, while the second group included 115 patients without the need for immediate laparotomy. The data includes leukocyte count and D-dimer levels markers. D-dimer concentrations were measured with the quantitative immunofiltration assay method (MDA® D-dimer, bioMérieux Inc., Durham, NC, USA). Due to its highly skewed distribution and discrete count nature, a logarithmic transformation was applied (base 10) to leukocyte counts.

The primary output of the study is the patient's need for immediate laparotomy (0: not needed, 1: needed). The used biomarker measurements were D-dimer levels and the leukocte counts on log scale. We did not find any information about randomization or blinding in the study where the data was taken (3,4). As far as we can see, no power analysis has been carried out. For this reason, we decided to make an evaluation with the power analysis performed at the end of the study.

Considering an inequality test for two ROC curves, we calculated post-power statistics using the area under ROC curves of the D-dimer level, leukocyte counts and min-max linear combination model. In our hypothesis, which is the subject of post power analysis, we evaluated whether the AUC value obtained as a result of combining with the min-max model was found to be significantly higher than the AUC of D-dimer level and log (leukocyte count). For this purpose, we used Hanley and McNeil approach to compute the effect sizes. For type-I error rate of 5%, the post power statistics for the two hypothesis were computed as 0.953 and 0.971. Power Analysis and Sample Size Software, version 11.0. (PASS, NCSS Statistical Software, Kaysville, UT, USA: https://www.ncss.com/software/pass/).

The study was approved by the local ethics committee in accordance with the Declaration of Helsinki (2022/703). Since the study was designed as retrospective, no informed consent was obtained from participants.

Linear Combination Methods

We used eight linear combination methods to combine leukocyte count and D-dimer levels. In this section, we describe the background of these methods. Let x_1 and x_2 the quantitative values of the first and second markers, respectively. Then, a combination score (s) can be obtained using linear combination methods as follows:

Logistic regression: A binary logistic regression model is fitted using the maximum-likelihood method. The combination score will be the posterior probabilities obtained from the following logistic regression model:

$$s = \frac{e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2}}{1 + e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2}}$$

Scoring based on logistic regression: The same binary logistic regression model is used. However, this time, for a more straightforward interpretation, slope values are rounded to a given digit number, and the combination score is computed as follows:

$$s = \beta_1 x_1 + \beta_2 x_2$$

Pepe & Thompson's method (9): This method is a ranking score-based approach that does not include any distribution assumptions in determining the linear combination of diagnostic tests. The combination score is obtained as follows:

Control group: $C_i = (C_{1i}, C_{2i}), i = 1,2,\ldots,n,$

Disease group: $D_j \leq (D_{1j}, D_{2j}), j = 1,2,\ldots,m,$

$$maximize\ W(\lambda) = \frac{1}{mn}\sum_{j=1}^{m}\sum_{i=1}^{n} I[D_{1j} + \lambda D_{2j} \geq C_{1i} + \lambda C_{2i}]$$

$$s = x_1 + \lambda x_2$$

Pepe, Cai & Langton's method (10): Pepe, Cai, and Langton combination score is obtained by using AUC as the parameter of a logistic regression model:

Control group: $C_i = (C_{1i}, C_{2i}), i = 1,2,\ldots,n,$

Disease group: $D_j <= (D_{1j}, D_{2j}), j = 1,2,\ldots,m,$

$$maximize\ W(\lambda) = \frac{1}{mn}\sum_{i=1}^{n}\sum_{j=1}^{m} I(D_{1j} + \lambda D_{2j} > C_{1i} + \lambda C_{2i}) + \frac{1}{2} I(D_{1j} + \lambda D_{2j} = C_{1i} +$$

$$s = x_1 + \lambda x_2$$

Su & Liu's method (11): Su and Liu's combination score is obtained by using Fisher's discriminant function under the assumption of a multivariate normal distribution model and proportional covariance matrices. Let μ the mean vector, Σ the covariance matrix for the corresponding group, and σ^2 unknown scaling factor. Then, the combination score is calculated as follows:

Control group: $C \sim N(\mu_c, \Sigma)$

$$\text{Disease group: } D \sim N(\mu_D, \sigma^2 \Sigma)$$

$$\text{Fisher' s discriminant coefficient: } (\alpha,\beta)\alpha(\mu_D-\mu_C)^T \Sigma^{-1}$$

$$s = \alpha x_1 + \beta x_2$$

Minimax method (12): Minimax method is an extension of Su & Liu's method. In this case, the combination score is obtained with the minimax procedure:

$$\text{Control group: } C_i = (C_{1i}, C_{2i}),\ i = 1,2,\ldots,n,$$

$$\text{Disease group: } D_j = (D_{1j}, D_{2j}),\ j = 1,2,\ldots,m,$$

$$(b_1, b_2) = [t\Sigma_D + (1-t)\Sigma_C]^{-1} (\mu_D - \mu_C)$$

$$s = b_1 x_1 + b_2 x_2$$

In this formula, t is a constant which takes values between 0 and 1. This value can be optimized by maximizing the AUC from the combination score by trial and error method.

Min-Max method (13): This method linearly combines the minimum and maximum values of the markers by finding a parameter λ that maximizes the corresponding Mann-Whitney statistic.

$$\text{Control group: } C_i = (C_{1i}, C_{2i}),\ i = 1,2,\ldots,n,$$

$$\text{Disease group: } D_j = (D_{1j}, D_{2j}),\ j = 1,2,\ldots,m,$$

$$\text{maximize } W(\lambda) = \frac{1}{mn}\sum_{i=1}^{n}\sum_{j=1}^{m} I(D_{j,max} + \lambda D_{j,min} > C_{i,max} + \lambda C_{i,min})$$

$$s = x_{max} + \lambda x_{min}$$

Todor & Saplacan's method (14): Todor and Saplacan's method uses trigonometric functions to calculate the combination score. The combination score is obtained by the θ value that optimizes the corresponding AUC.

$$s = \sin(\theta) x_1 + \cos(\theta) x_2$$

Statistical Analysis

Histogram, q-q plots, and Shapiro-Wilk's test were performed to test data normality. In addition, the Levene test was used to assess variance homogeneity. To compare the distribution of leukocyte counts and D-dimer levels in patients with or without need of immediate laparotomy, a two-sided Mann-Whitney U test was applied. Analyses were conducted using TURCOSA (Turcosa Analytics Ltd. Co., Turkey, www.turcosa.com.tr) statistical software. A p-value less than 5% was considered statistically significant.

Model Building and Performance Assessment

The data was split into training (70%) and test (30%) sets. All model-building steps were conducted in training data, while the performances of each model were assessed in test data. The training data included 158 (immediate laparotomy needed 77, immediate laparotomy not needed 81) patients. The test data included the data of the remaining 67 (immediate laparotomy needed 33, immediate laparotomy not needed 34) patients. In training data, five-fold cross-validation was performed with 10 repeats to optimize the model parameters. Logistic regression, scoring, min-max, minimax, Su & Liu, Pepe & Thompson, Pepe, Cai & Langton, and Todor & Saplacan methods were used as linear combination methods. The combination scores for each method were calculated as described in the previous section. Performances of the built models were assessed using the area under the ROC curves. Moreover, the Youden index was used to identify the optimal cut-points, sensitivity, selectivity, accuracy, positive and negative predictive value, and positive and negative likelihood ratio statistics were also calculated for performance assessment. All analyses were conducted using R programming language 4.2.0 (www.r-project.org) with self-generated R scripts.

RESULTS

The distribution of leukocyte counts, as well as D-dimer levels, were found to be statistically higher in patients who need immediate laparotomy (p<0.05) (Table 1, Figure 1). These markers were combined using linear combination models described in the Material and Methods section. The model parameters were optimized, and the detected optimal parameters are given in Table 2.

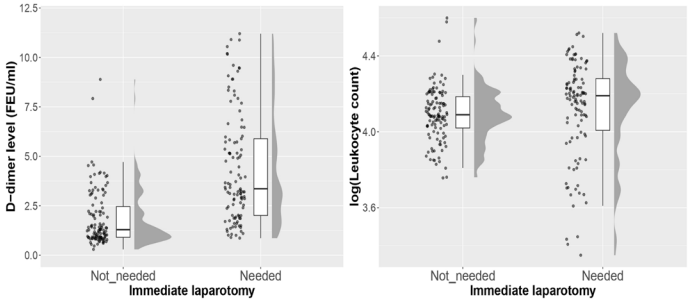

Figure 1. Raincloud plots displaying the distribution of D-dimer levels and leukocyte counts on a logarithmic scale in patients with and without the need for immediate laparotomy

Table 1. Comparison of leukocyte count and D-dimer levels in patients with or without need of immediate laparotomy

Marker	Immediate laparotomy		p-value
	Not needed (n=115)	Needed (n=110)	
Leukocyte count	12200 (10400-15400)	15500 (9900-19100)	0.003
D-dimer level (µg FEU/mL)	1.29 (0.90-2.53)	3.39 (2.01-5.98)	<0.001

Values are expressed as median(1st-3rd quartiles). Significant p values are shown in bold characters

Table 2. The optimal parameters were detected with the linear combination models

Linear combination models	Parameters
Logistic regression	β_0 = -15.069, β_1= 0.563, β_2 = 3.195
Scoring based on logistic regression	β_1 = 0.640, β_2 = 2.690
Pepe & Thompson	λ = 0.651
Pepe, Cai & Langton	λ = 0.382
Su & Liu	α = 0.225, β = 1.382
Minimax procedure	b_1 = 0.558, b_2 = 1.627
Min-max	λ = 0.772
Todor & Saplacan	sin(θ)= 0.330, cos(θ) = 0.944

The area under ROC curves for D-dimer levels and leukocyte counts on the logarithmic scale were 0.706 and 0.699, respectively. The area under the ROC curves for linear combination models was between 0.770 and 0.810 (Table 3). The highest performance was achieved using the min-max method. The ROC curve generated with the combination score from the min-max model, D-dimer levels, and leukocyte count markers were given in Figure 2. It is seen that the min-max method made a significant improvement between 14.7% and 15.9% in AUC statistics as compared to D-dimer level and leukocyte counts, respectively.

The optimal cut-off values for both markers and linear combination models are identified, and classification tables are generated for each marker and model. These cut-off values and the observed frequencies in each table are given in Table 4. For each table, statistical diagnostic measures are calculated with 95% confidence intervals and given in Table 5.

Table 3. Area under the curves of markers and combined score

Markers and models	AUC (95% CI)	z	p-value
Markers			
D-dimer level	0.706 (0.582-0.831)	3.24	<0.001
log (Leukocyte count)	0.699 (0.561-0.838)	2.82	<0.001
Linear combination models			
Logistic regression	0.781 (0.666-0.895)	4.80	<0.001
Scoring based on logistic regression	0.779 (0.665-0.893)	4.78	<0.001
Pepe & Thompson	0.782 (0.666-0.898)	4.76	<0.001
Pepe, Cai & Langton	0.770 (0.655-0.885)	4.61	<0.001
Su & Liu	0.786 (0.671-0.902)	4.86	<0.001
Minimax procedure	0.773 (0.661-0.884)	4.79	<0.001
Min-max	0.810 (0.698-0.922)	5.43	<0.001
Todor & Saplacan	0.772 (0.658-0.886)	4.68	<0.001

AUC: Area under the ROC curve. Significant p values are shown in bold characters

Table 4. Classification tables obtained for each marker and linear combination model

Markers and models	TP	TN	FP	FN
Markers				
D-dimer level (>1.74 µg FEU/mL)	27	19	15	6
log (Leukocyte count) (>4.27)	17	32	2	16
Linear combination models				
Logistic regression (>0.38)	27	23	11	6
Scoring based on logistic regression (>12.72)	26	23	11	7
Pepe & Thompson (>0.33)	26	25	9	7
Pepe, Cai & Langton (>0.29)	23	26	8	10
Su & Liu (>6.32)	27	25	9	6
Minimax procedure (>7.56)	24	24	10	9
Min-max (>0.42)	26	27	7	7
Todor & Saplacan (>4.93)	23	26	8	10

TP: True positive, TN: True negative, FP: False positive, FN: False negative

Table 5. Statistical diagnostic measures with 95% confidence intervals calculated for each marker and linear combination model

Variable	SEN (95% CI)	SPE (95% CI)	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95% CI)	ACC (95% CI)
Markers and models							
D-dimer level (>1.74 µg FEU/mL)	0.82 (0.65-0.93)	0.56 (0.38-0.73)	0.64 (0.48-0.78)	0.76 (0.55-0.91)	1.85 (1.23-2.80)	0.33 (0.15-0.71)	0.69 (0.56-0.79)
log (Leukocyte count) (>4.27)	0.52 (0.34-0.62)	0.94 (0.80-0.99)	0.89 (0.67-0.99)	0.67 (0.52-0.80)	8.76 (2.19-34.97)	0.52 (0.36-0.74)	0.73 (0.61-0.83)
Linear combination models							
Logistic regression (>0.38)	0.82 (0.65-0.93)	0.68 (0.49-0.83)	0.71 (0.54-0.85)	0.79 (0.60-0.92)	2.53 (1.52-4.22)	0.27(0.13-0.57)	0.75 (0.63-0.84)
Scoring based on LR (>12.72)	0.79 (0.61-0.91)	0.68 (0.49-0.83)	0.70 (0.53-0.84)	0.77 (0.58-0.90)	2.44 (1.45-4.09)	0.31 (0.16-0.63)	0.73 (0.61-0.83)
Pepe & Thompson (>0.33)	0.79 (0.61-0.91)	0.74 (0.56-0.87)	0.74 (0.57-0.88)	0.78 (0.60-0.91)	2.98 (1.65-5.36)	0.29 (0.15-0.57)	0.76 (0.65-0.86)
Pepe, Cai & Langton (>0.29)	0.70 (0.51-0.84)	0.76 (0.59-0.89)	0.74 (0.55-0.88)	0.72 (0.55-0.86)	2.96 (1.55-5.65)	0.40 (0.23-0.69)	0.73 (0.61-0.83)
Su & Liu (>6.32)	0.82 (0.65-0.93)	0.74 (0.56-0.87)	0.75 (0.58-0.88)	0.81 (0.63-0.93)	3.09 (1.73-5.54)	0.25 (0.12-0.52)	0.78 (0.66-0.87)
Minimax procedure (>7.56)	0.73 (0.54-0.87)	0.71(0.53-0.85)	0.71 (0.53-0.85)	0.73 (0.54-0.87)	2.47 (1.41-4.33)	0.39 (0.21-0.70)	0.72 (0.59-0.82)
Min-max (>0.42)	0.79 (0.61-0.91)	0.79 (0.62-0.91)	0.79 (0.61-0.91)	0.79 (0.62-0.91)	3.83 (1.93-7.58)	0.27 (0.14-0.53)	0.79 (0.67-0.88)
Todor & Saplacan (>4.93)	0.70 (0.51-0.84)	0.76 (0.59-0.89)	0.74 (0.55-0.88)	0.72 (0.55-0.86)	2.96 (1.55-5.65)	0.40 (0.23-0.69)	0.73 (0.61-0.83)

SEN: Sensitivity, SPE: Specificity, PPV: Positive predictive value, NPV: Negative predictive value, PLR: Positive likelihood ratio, NLR: Negative likelihood ratio, ACC: Accuracy rate, LR: Logistic regression

The sensitivity of the D-dimer level (0.82) was found to be higher than the sensitivity of log-transformed leukocyte counts (0.52). On the other hand, the specificity statistic was higher for log-transformed leukocyte counts (0.94) as compared to the D-dimer level (0.56). The diagnostic accuracy of log-transformed leukocyte counts was 0.73 and was higher than the accuracy of the D-dimer level, which was 0.69. After combining these markers with linear combination models, the accuracy of these models was obtained between 0.72 and 0.79. Higher accuracies were obtained with min-max, Su & Liu, Pepe & Thomson, and logistic regression models. There was no improvement in terms of accuracy statistics for scoring based on logistic regression; Pepe, Cai & Langton; minimax and Todor & Saplacan models. The best performance was achieved with the min-max model with a diagnostic accuracy of 0.79. For this best-performed model, sensitivity and specificity statistics were obtained as 0.79 as well. The final decision rule for the min-max model suggests an immediate laparotomy for patients with acute abdominal pain if the following condition is true:

$$_{max}(Ddimer, log_{10}(WBC)) + 0.772 *_{min}(Ddimer, log_{10}(WBC)) > 0.42$$

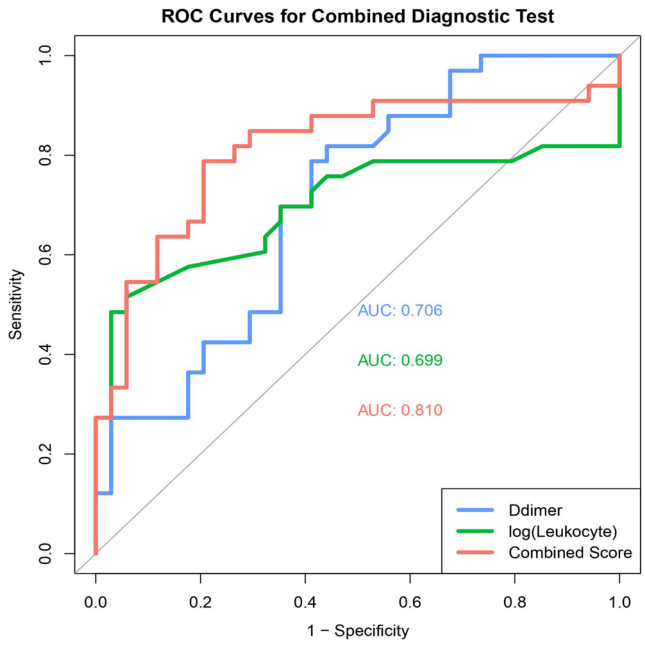

Figure 2. ROC curves for D-dimer levels, leukocyte counts on a logarithmic scale and, Min-max combined scores in diagnosing patients with nontraumatic acute abdomen

DISCUSSION

Rapid intervention is important in non-traumatic acute abdomen patients admitted to the emergency department. Increasing the diagnostic accuracy even at the level of 1% in clinical evaluation is vital for patients admitted to the emergency department with this symptom. In this study, we increased the diagnostic accuracy by combining leukocyte count and D-dimer levels with linear combination methods. The diagnostic accuracy of 79%, obtained with the best-performing min-max method, was found to be 8.2% higher than the leukocyte count marker and 14.5% higher than the D-dimer levels. In addition, the sensitivity of the D-dimer level marker and the specificity of the leukocyte count marker was found to be high. Using the min-max linear combination method, we obtained a high estimation of both sensitivity and specificity.

In the literature, there are studies in which the diagnostic test performance is increased using linear combination methods for different medical problems. Kyurkchiyan et al. (2021) combined the performance of miR-31-3p and miR-196a-5p transcripts using linear combination methods to diagnose advanced laryngeal squamous cell carcinoma (15). The AUC statistics for miR-31-3p and miR-196a-5p transcripts were 0.934 and 0.877, respectively, while the calculated AUC statistics for the combined test were 0.978. Huang et al. (2022) linearly combined ultrasound score and liver stiffness measurement of sound touch elastography in diagnosing liver fibrosis staging in patients with chronic hepatitis B (16). The combined score had the highest AUC in all fibrosis stages. Zhang et al. (2019) used three linear combination models and combined cerebrospinal fluid procalcitonin, lactate, interleukin-8, and interleukin-10 markers in differentiating between post-neurosurgical bacterial meningitis and aseptic meningitis (17). The AUC statistic was improved to 0.954 with the four marker model. Han et al. (2008) combined matrix metalloproteinase-9, N-acetyl-b-D-glucosaminidase, and kidney injury molecule-1 urinary biomarkers linearly in identifying acute kidney injury (18). The highest diagnostic performance was observed with the linear combination.

In a study using the same data in our study and combining D-dimer levels and leukocyte count markers with machine learning methods, the best diagnostic performance was obtained with the Naive-Bayes method with an accuracy of 78.57%. The diagnostic accuracy of the boosted and bagged logistic regression models was 78.12% (4). When performance comparisons are made, it can be said that the diagnostic accuracy of the min-max linear combination model was slightly better than machine-learning models. However, the sensitivity of the combined test was higher than the Naive-Bayes model, and the specificity was higher than the boosted and bagged logistic regression models. It is seen that high diagnostic accuracies are obtained with linear combination methods.

Moreover, the models obtained by linear combination methods are simple and clinically interpretable. In cases where more than one marker is evaluated in health studies, a common procedure is to compare the diagnostic performance of markers, and combining diagnostic tests is ignored in many studies. In the case of a single diagnostic marker, medical decisions can be made simply based on cut-off values. In many cases, combining multiple diagnostic tests with machine learning methods provides high diagnostic accuracies due to their strong mathematical infrastructures. The advantage of linear combination methods is that they may both increase diagnostic accuracies compared to single markers and provide simple and medically interpretable results. Therefore, clinicians may consider applying linear combination methods first and making a comparative evaluation based on the results, especially when making medical decisions based on a small number of diagnostic tests. In cases where similar accuracy performance is obtained with machine learning methods, linear combination methods can be used due to their simplicity and interpretability.

Although linear combination methods have many advantages, their absence in most statistical software limits their use. A further aspect of this work is developing a user-friendly web application in which linear combination methods can be applied.

CONCLUSION

In conclusion, the min-max linear combination model improved the diagnostic performance in differentiating between surgical and non-surgical pathologies in non-traumatic acute abdominal pain patients. Therefore, we suggest that D-dimer level and leukocyte counts should be evaluated together with the min-max procedure because of its higher diagnostic performance and simple interpretation.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Ethics committee approval is not required in this study.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Contribution of Türkiye to the Field of Endodontology: A Visualized Bibliometric Analysis Based on Web of Science

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Abstract

Aim: To determine the current status and trend of endodontic research in Türkiye.

Materials and Methods: A comprehensive literature survey was conducted using the Clarivate search engine. Keyword selection was as follows: "Endodontics" and "Türkiye". All publications until May 2022 were included. The search was restricted to endodontics. Title, first author, country/institute, journal name, publication year, citation, abstracts, and keywords were recorded. VOSviewer version 1.6.10 software was used to map the bibliometric network of the exported data that has an automatic term identification algorithm.

Results: A total of 672 articles between 1985-2022 years were included. The scientific contribution rate of Türkiye was found 4.12% in the field of Endodontology. The highest number of publications is in the category of 'Dentistry Oral Surgery Medicine' with 588 articles and 384 (73%) published by Journal of Endodontics.

Conclusion: This bibliometric analysis highlights Türkiye's contribution to the field of Endodontology. Research articles have been contributed by various authors and institutions.

Keywords: Bibliometric analysis, citation, endodontics

INTRODUCTION

The field of Endodontics involves a variety of clinical interventions that covers a variety of research areas including clinical, mechanical, biological, and materials science subjects (1,2). Endodontology is a field of dentistry that deals with pathologies and injuries of the pulp and periradicular tissue and their relationship to systemic conditions (3). The specialty covers both clinical and basic sciences, including the diagnosis, etiology, treatment, and prevention of pulpal and periradicular pathologies (1). New progress in materials and clinical techniques target better outcomes in endodontic treatment practice.

The visualization analysis of the articles using bibliometrics not only presents a historical overview of scientific progress but also reveals trends in ongoing research (4). Bibliometrics uses quantitative measures to evaluate academic productivity. The field is growing rapidly with the production of new parameters, assessment tools, and normative data (5). Bibliometric visualization is an important type of analysis in evaluating published articles, authors, journals, and publishing institutions and revealing data such as the scientific activity of countries and international co-authorship (3,6-10).

In the field of endodontics, such an analysis was first published in 2011 by Fardi et al by Journal of Endodontics, one of the journals with the highest impact factor (10). In the study, the 100 most cited articles were determined by the bibliographic data of various endodontic journals. Data from these publications showed that the most common research subfield was endodontic microbiology, the most widely published type of article was basic science, and the clinical studies were mostly observational. Similar to Fardi et al., it was stated that endodontic microbiology is the most studied subject according to the analysis carried out by Yılmaz et al (4). In addition, the researchers reported for the first time that regenerative endodontics is the second most studied topic. They stated that the number of basic science articles and reviews is higher than in clinical studies, and there is a greater need for randomized clinical trials and studies with high levels of evidence, such as meta-analysis, in the literature.

Türkiye also has a significant contribution to the scientific field of endodontics and thus this study aimed to highlight Türkiye's contribution by utilizing a bibliometric analysis

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and determining the current status and trends of research.

MATERIAL AND METHOD

A comprehensive systematic literature survey was conducted to identify the related researches in the field through online database, including Thomson Reuters Web of Science, by using the Clarivate search engine. The search strategy was as follows in all fields including the database: "Endodontics" and "Turkey". Keywords selection was conducted with the purpose of search optimization to locate every related publication. All publications until May 2022 were included. The search was restricted to mainly focusing on endodontics. Proceeding papers, conference papers, editorial materials or letters, corrections, notes, and early access papers were excluded from the study. The data including the full record and cited references were exported using the 'tab-delimited file' tool. Title, first author, country/institute, journal name, publication year, citation, abstracts, and keywords were recorded. VOSviewer software 1.6.10 version (Centre for Science and Technology Studies, Leiden University, Netherlands) was used to mapping the bibliometric network of the exported data that has an automatic term identification algorithm (downloadable at www.vosviewer.com).

Questions to be answered in line with the purpose of this study:

- **Q1.** What is the distribution of articles published from Türkiye in the field of Endodontics by years?
- **Q2.** Which are the journals that publish articles from Türkiye in the field of Endodontics?
- **Q3.** What is the distribution of the most contributing researchers and institutions?
- **Q4.** Who are the most cited authors and which are the most cited publications from Türkiye?
- **Q5.** What is the international scientific contribution rate of Türkiye?

RESULTS

A total of 672 articles from 16,328 published by indexed journals (Web of Science Index, SCI-E, ESCI) between 1985-2022 years were included. The distribution of publications by year was presented in Figure 1 (Q1). The highest number of publications is in the category of 'Dentistry Oral Surgery Medicine' with 588 articles and 384 (73%) published by the Journal of Endodontics (Figure 2) (Q2). Hacettepe University in Ankara had the most contribution rate with the highest number of 105 articles (Figure 3) (Q3). Figure 4 presented the authors with the highest contribution by document count and total citations. Co-authorship, international collaboration, and research area according to the keywords presented in Figures 5,6,7 respectively. The 20 top-cited articles were presented in Table 1.

The oldest article available on the Web of Science, written by Alaçam & Yılmaz was published by the Journal of Endodontics in 1985. The number of authors that contributed to the area was 200 and, the most prolific author was Arslan H with 47 published articles (Q3). An evaluation of the root canal configurations published by Sert and Bayırlı in 2004 was the most cited publication (Table 1) (Q4).

The scientific contribution rate of Türkiye was found at 4.12% in the fields of Endodontics (Q5).

Table 1. 20 top-cited articles published from Türkiye

Rank	First Author	Publication Date	Type of Article	Subject Area	Citation
1	Sert & Bayırlı[11]	2004	Original Article	Root canal anatomy	269
2	Calt & Serper[12]	2002	Original Article	Root canal disinfection	251
3	Calıskan et al.[13]	1995	Original Article	Root canal anatomy	179
4	Gursoy et al.[14]	2013	Review Article	Root canal disinfection	163
5	Sen et al. [15]	1995	Original Article	Endodontic microbiology	147
6	Capar et al.[16]	2014	Original Article	Ni-Ti instruments	127
7	Eskitascioglu et al.[17]	2002	Original Article	Post-endodontic restoration	126
8	Cehreli et al.[18]	2011	Case Report	Regenerative endodontics	121
9	Erdemir et al.[19]	2004	Original Article	Root canal disinfection	115
10	Yoldas et al.[20]	2012	Original Article	Ni-Ti instruments	110
11	Ercan et al.[21]	2004	Original Article	Root canal disinfection	106
12	Nagas et al.[22]	2012	Original Article	Endodontic materials	105
13	Ari et al.[23]	2003	Original Article	Root canal disinfection	105
14	Yaltirik et al.[24]	2004	Original Article	Root canal sealers / cements	104
15	Calt & Serper[25]	1999	Original Article	Root canal sealers / cements	103
16	Dogan & Calt[26]	2001	Original Article	Root canal disinfection	100
17	Guneser et al.[27]	2013	Original Article	Root canal disinfection	92
18	Tinaz et al.[28]	2005	Original Article	Root canal anatomy	89
19	Ari et al.[29]	2004	Original Article	Root canal disinfection	89
20	Hakki et al.[30]	2009	Original Article	Root canal sealers / cements	88

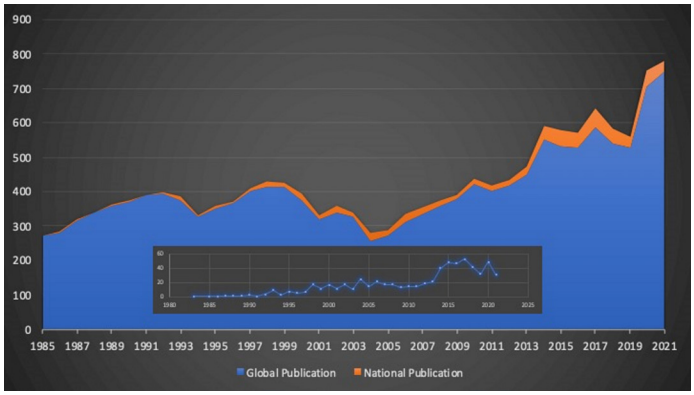

Figure 1. Distribution of publications from Türkiye in the field of endodontics concerning count and citation ranks by year

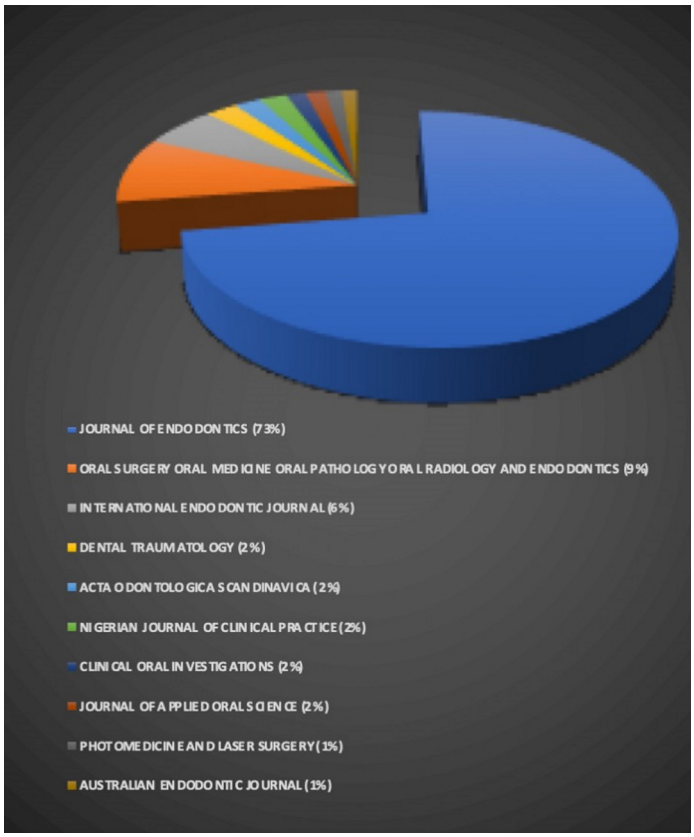

Figure 2. Distribution by journal which published maximum numbers of article from Türkiye in the field of endodontics (top 10)

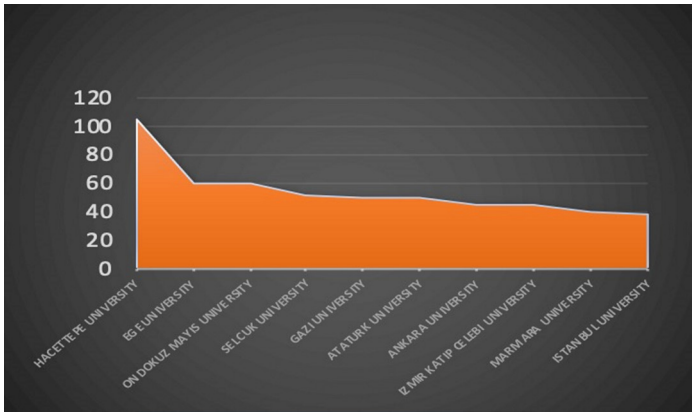

Figure 3. Institutional contribution rates of articles in the field of endodontics (top 10)

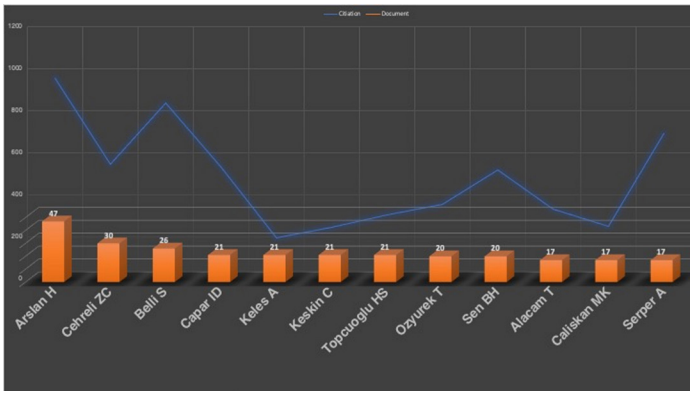

Figure 4. Author contribution by document with total citations in the field of endodontics (top 10)

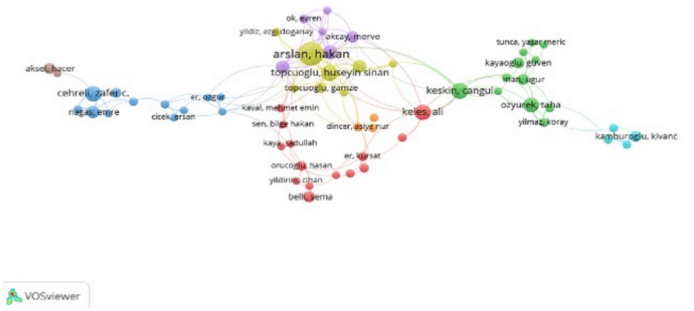

Figure 5. Co-authorship mapping of the researchers by VOSviewer analyzing

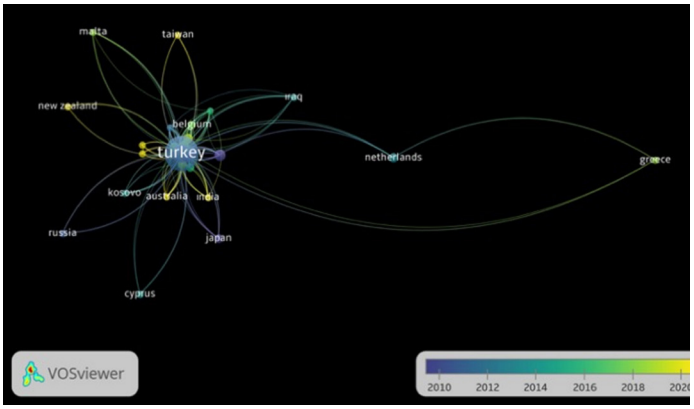

Figure 6. International collaboration of authors by year

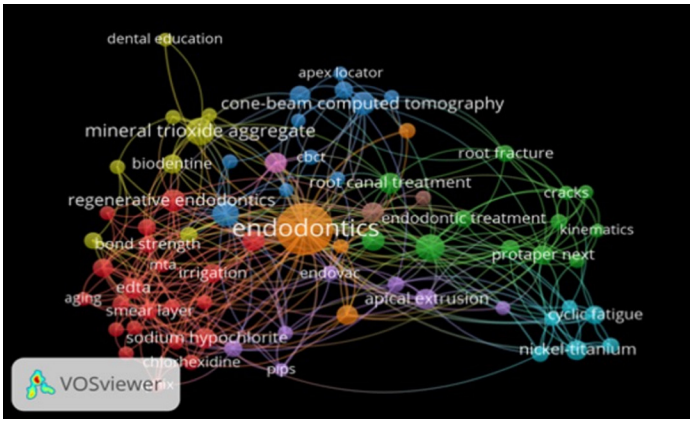

Figure 7. Research interest of publications from Turkiye in the field of endodontics by keywords

DISCUSSION

This study aimed to evaluate the impact of Türkiye's international contribution to the field of endodontics. The research outputs presented that the nationwide contribution to the international literature was 4.12%. Not only in endodontics, but Türkiye is also one of the most productive countries in terms of academic contribution to dentistry as a whole (31). In this study, an inconsistent increase in the number of publications is noticeable. The authors of the study believe this result is likely to be due to increase in the number of researchers, increase in the number of supported projects due to the increase in the number of academic institutions, academic promotion criteria, more accessible international databases, and national and international collaborations.

The main limitation of this study is that the database is limited to the Web of Science. Why Web of Science is not the only platform used for academic search. The fact that the articles in other international databases were not identified may have caused to observe a lower result than the current effect. However, for bibliometric analysis Web of Science is the most popular database so for this study methodology, it was preferred with analyzing with VOSviewer.

Hacettepe University had the most contribution with the highest number of articles. Tonta reported that Hacettepe ranked first in the contribution of Turkish universities and produced almost one-fourth of all biomedical publications worldwide between 1988-1997 (32). Hacettepe University's longstanding success might result from the earlier establishment of the university and well-established study groups.

The highest number of publications by Turkish researchers were published in the Journal of Endodontics. Considering the high impact factor and recognition of the journal, a higher density of manuscript submissions from Türkiye to such a leading journal is quite predictable.

The most used keywords in the studies were cone-beam computed tomography, retreatment, and mineral trioxide aggregate in descending order. It can be interpreted as the use of advanced imaging techniques amongst Turkish researchers has become common. It is also observed that core topics of endodontics such as irrigation and root canal preparation to more recent concepts such as lasers and micro-computed tomography are also studied by Turkish researchers (33,34).

Özbay & Özdemir reported that the most prolific country for laser-activated irrigation studies is Türkiye with the highest contribution rates (35). Our study results presented that almost half of the most cited studies were related to root canal disinfection and irrigation. For example, Calt & Serper presented important findings regarding EDTA flushing times and highlighted the drawbacks of using EDTA for more than 1 minute (12).

As imaging technologies improve, more and more information about root canal anatomies is emerging. However, in the past Çalışkan et al. and Sert & Bayırlı had studied by decalcification and root canal system staining and provided very comprehensive data to the literature on anatomical configurations (11,13). On the other hand, based on the current technology point of view, it was reported that Türkiye is among the most productive countries, especially in root canal anatomy studies.

Among the most cited studies, Şen et al. visualized the micron-level placement of microorganisms into the dentinal tubules using scanning electron microscopy. After fungi observation on the sections, the researchers suggested that it should be considered especially as a factor for persistent infections and in the disinfection protocol (15).

CONCLUSION

The present analysis was based on bibliometrics of Türkiye's contribution to the field of Endodontology. The analysis highlighted the productivity in the field to date and provided insight into the under-explored areas that can be improved to further the country's research output.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *For this type of study, ethical approval is not applicable, because any part of the research does not involve human participants, tissue, data, or animal subjects.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Surgical Fixation with Cannulated Screws in the Adult Femoral Neck Fractures

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Abstract

Aim: Femoral neck fractures are common injuries in orthopedic practice and result in significant morbidity and mortality. They are fractures in the intra-capsule area of the proximal femur. They usually occur in the elderly patient group. However, younger age groups could also experience femoral neck fractures as a result of high-energy traumas. The aim of the present study was to compare with the literature the outcomes in adult patients for whom surgical fixation was performed after femoral neck fracture using cannulated screws in our clinic.

Materials and Methods: The study included patients for whom surgical fixation was performed for femoral neck fractures using cannulated screws between August 2010 and August 2012. Fracture non-union, delayed union, avascular necrosis and arthrosis were evaluated in patients. Avascular necrosis evaluation was performed using Ficat and Arlet classification. Functional outcomes were evaluated using Harris hip score.

Results: The average follow-up period of our patients was 32 months (range: 24-48 months). Follow-ups indicated that 16 patients recovered without problems, walked with a double wand starting from about the third month with respect to the bone union status and full union was achieved in an average of six months. Non-union was observed in four patients. Ficat and Arlet avascular necrosis classification showed that four patients had avascular necrosis. The average time for these patients to be admitted to surgery was seven days. Five of our patients developed superficial wound infections. Antibiotic treatment and wound care were applied to our patients. When the patients were evaluated based on Harris hip score numerical rating chart, it was found that excellent outcomes were obtained in five patients, very good results in eight patients, good results in four patients, moderate results in two patients and poor results in one patient.

Conclusion: Femoral neck fractures are a common type of injury in orthopedic practice and they result in significant morbidity and mortality when treated inappropriately. In order to reduce the rate of bone non-union, avascular necrosis or other complications that could be observed in patients who underwent surgical fixation after femoral neck fractures, and appropriate and acceptable reduction of femoral neck fracture should be realized as soon as possible, and stable fixation should be achieved.

Keywords: Femoral neck fracture, internal fixation, garden classification, ficat and arlet classification, harris hip score

INTRODUCTION

Femoral neck fractures are common orthopedic injuries and associated with considerable morbidity and mortality. Femoral neck fractures, which make up majority of the fractures in the capsular area in femoral proximal, predominantly develop due to osteoporosis in older age. They can also occur in younger age groups as a result of high energy traumas (1-3). Femoral neck fractures constitute about half of the fractures in femur intracapsular area. Although they can occur at any age, as high as 97% of them are observed in patients over 50 years of age (4,5).

The main aim in the treatment for these fractures depends on pre-fracture mobilization status and osteoporosis level of the patient, the amount of displacement in the fracture, if any, and the time to the surgical operation. Femoral neck fractures are predominantly treated surgically. In the treatment of femoral neck fractures in young adults with a physiological age of less than 65, non-surgical treatment is not applied except for the presence of serious risk factors or life-threatening multiple traumas. However, conservative treatment can be considered in patients who do not have a displacement preventing surgical intervention, who have comorbid diseases, who are stable and who have valgus-impacted femoral neck fractures. In elderly patients, on the other hand, non-surgical treatment indication of displaced

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femoral neck fractures is extremely rare (6).

Non-displaced or valgus-impacted femoral neck fractures should be surgically fixed before displacing take place, regardless of the age of the patient. Cannulated spongious screws, nails and sliding hip plaques can be used for surgical fixation. In surgical treatment of these fractures in many trauma centers abroad or in Turkey, fixation with three cannulated spongious screws are preferred, and adequate fixation and stabilization is achieved with this surgical treatment.

In the present study, we aimed to compare with literature the functional and clinical outcomes of adult inpatients who were surgically treated for hip fracture in our clinic using cannulated screws and internal fixation in surgical intervention.

MATERIAL AND METHOD

Functional and clinical results of adult patients surgically treated using canulated screws and internal fixation in our clinic for hip fracture between August 2010 and August 2012 were evaluated in comparison with the literature.

For the classification of fractures that occur after in-vehicle and pedestrian traffic accidents, high fall and firearm injury were considered fractures due to high-energy traumas while fractures after slipping in the bathroom or ice, stumbling while walking, simple falls at home or fractures of the patients who could not remember how the fracture occurred were considered low-energy trauma-related fractures. The classification of fractures was made according to Pauwels and Garden classification in adult patients.

Patients with multiple fractures, patients with associated chest, abdominal or head injuries, simultaneous systemic diseases such as chronic renal failure, rheumatoid arthritis, systemic lupus erythematosus and malignancy were excluded.

Clinical research ethics committee of Fırat University approved the study (Approval No: 17.12.2013/09-05).

Statistical Analyses

The IBM SPSS 19 software (IBM SPSS Statistics 19, SPSS Inc., an IBM Co., Somers, NY) was used for statistical analyses. The continuous variables (age, length of stay, duration of surgery, etc.) were expressed as mean± standard errors. One-way analysis of variance (One-way ANOVA) was used to determine whether there was a difference between the relevant categorical variable groups for continuous variables. Categorized variables were expressed as percentages and numbers. To determine the relationship between categorized variables, the Chi-square test was used. The significance level was taken as 5 and 1%.

Clinic Evaluation

Clinical results and radiographs were evaluated at 1, 3, 6, 12, and 24 months postoperatively. Femur proximal anterior, posterior and side radiographies were taken for patients during the follow-ups and evaluated to see whether there was fracture union or delay in union, and to detect whether avascular necrosis and arthrosis developed. Avascular necrosis evaluation of the patients was performed based on Ficat and Arlet classification. Broker classification was used for the evaluation of ectopic ossification. Pain, function, deformity and mobility were evaluated using Harris hip score numerical rating chart (7,8). According to this chart, 86-100 points were considered excellent, 71-85 points very good, 61-70 points good, 41-60 points moderate, and 40 points or less poor.

Surgical Technique

Patients were operated upon receiving the approval from the Anesthesia and Reanimation department that they could be operated. Patients were taken to the surgical table in supine position. Then, general or regional anesthesia was applied by the anesthesia team. The fractured extremity was washed and painted with Povidone-iodine solution. The patient was covered with sterile green and the preparation was completed. First, a closed reduction was applied to fracture using the Leadbetter maneuver. In this maneuver, while the hip joint was at 90 degrees flexion, the femur was brought to internal rotation, and the external rotation deformity was corrected. Then, the shortness formed was corrected by straight traction along the thigh plane. While maintaining the internal rotation, the hip joint was first taken to abduction then to full extension at table level. Reduction control was achieved with scopy. If anatomic reduction was achieved after scopy, closed surgical intervention was continued. If the reduction was not sufficient, open reduction was indicated. Later, a skin incision was made starting from 3 cm posterior and distal of anterior superior spina iliaca to trochanter major, and from trochanter major to 5 cm distal. After passing under the skin, entrance was made between tensor fascia lata and gluteus medius, and at the distal, vastus lateralis muscle was cut off at the point of proximal end muscle insertion site. The superior gluteal nerve was preserved and the capsule was reached. The capsule was cut off and the fracture line was reached. After the fracture fixation, the incision was washed with serum physiological, bleeding control was applied, and the incision was sewn up.

RESULTS

Demographic data of patients are given in Table 1. Five of the patients with femoral neck fractures who were admitted to our clinic after high-energy trauma had additional accompanying fractures. Only one patient had additional fractures after low-energy trauma. The average age of these patients was 30.76 years, and the average age of patients without additional fractures was 53.83 years. There was a significant difference between these averages ($p<0.01$). High-energy traumas such as in-vehicle traffic accidents in two patients (33.3%), pedestrian traffic

accidents in two patients (33.3%) and falling from a height in one patient (16.6%) were involved in additional fractures while one patient with additional fractures (16.6%) had low-energy trauma. This patient developed a fracture at the radius distal end as a result of a simple domestic fall. Accompanying fractures had significant associations with the etiology of fracture ($p<0.01$). Accompanying fractures involved femur in two patients, humerus in one patient, radius distal tip in one patient, forearm double bone in one patient, ischium pubis arm in two patients and fibula in one patient. We had three patients with other organ injuries after the trauma. They were all injured by high-energy trauma. Car accident was the cause in two patients and falling from height in another. Other accompanying organ injuries included liver laceration, kidney laceration, femoral artery and saphenous vein injuries.

Fifteen of our admitted and examined patients (67.5%) had one or more comorbid diseases (such as chronic obstructive pulmonary disease, hypertension, diabetes mellitus, hyperthyroidism). The mean age of these patients was 54.1 years. Ten of them (66.7%) were female and 5 (33.3%) were male. Presence of comorbid disease was significantly associated with older age and female gender ($p<0.01$). In the patients with comorbid disease, the cause of fracture was low-energy trauma in eight patients (53.3%) and high-energy trauma in seven (46.7%). This association was significant ($p<0.01$).

The mean time for the patients to apply to our post-traumatic emergency department was 48 hours (range: 1 hour-24 days). Patients were operated within an average of two days. The earliest operation was performed four hours later while the latest was eight days later for a patient with comorbid diseases. The patient who underwent surgery after eight days had been transferred to the intensive care unit as a result of additional diseases and due to the fact that the operation was not allowed. Those with any existing systemic diseases were operated within an average of four days after applying to the emergency outpatient clinic. There was a significant difference between the patients with and without systemic disease for the time to surgery ($p<0.01$).

All patients who underwent surgical treatment were operated under regional or general anesthesia. Average operation time was 60 minutes, the shortest being 20 minutes and the longest 140 minutes. Blood transfusion was performed in postoperative period for five of our patients (20.8%) whom we treated with surgical intervention. These patients were the ones with additional fractures who were admitted to our clinic as a result of high-energy trauma.

In 5 of the 24 patients (20.8%) for whom we performed surgical treatment, superficial wound infection developed. All of these patients were those we applied open reduction. These patients responded to antibiotic and wound care treatment, and no additional treatment was required. While the superficial infection rate was 27.7% in the patients whose fractures were fixed with open reduction, this rate was 15% in those whose fractures were fixed with closed reduction. Of the 24 patients treated surgically, two (8.3%) developed deep infections. Both of these patients were the ones whose fractures were fixed with open reduction. In one of these patients, infection was detected in the third postoperative month. The infection was treated by removing the debridement and implants, and after the infection healed completely, the patient was given a total hip prothesis. The other patient developed an infection in the third week after surgery. The infection was treated with wound site debridement and washing. The mean duration of surgery was 133 minutes for patients with deep infection, which was significantly different compared to the duration of surgery for patients without deep infection ($p<0.01$).

Deep vein thrombosis developed in one patient (4.2%). This patient also had a femoral shaft fracture on the same side. This patient was treated with low molecular weight heparin, elevation and anti-thromboembolic socks. Pulmonary thromboembolism developed in two of the patients (8.4%) we treated surgically. One of them died of thromboembolism, and the other patient responded to treatment and recovered.

Decubitus wound developed in three patients (12.5%). The mean age of these patients was 62.4 years and there was a significant difference between the mean age of these patients and that of the patients who did not develop a pressure wound ($p<0.01$). The average time for these three patients to be admitted to surgery was six days. There was a significant relationship between the duration of the patients' admittance to surgery and the development of decubitus wounds ($p<0.01$). These three patients had accompanying systemic disease.

Union was observed in 4 of the 24 patients (16.7%) for whom fixation was performed for fracture treatment. The average age of these patients was 50 years, and one of them was male and three were female. The fracture type was Garden type 2 in two patients and Garden type 4 in two patients. All patients had inadequate fixation and reduction. One of the four patients who were observed to have bone union had open reduction, bone grafting and fixation for the non-union which was detected 1.5 years later during follow-up examinations. For another case, fixation with bone grafting was performed. We managed to achieve union in both fracture areas, but serious avascular necrosis occurred in our patients. Total hip prothesis was applied to two other patients who did not have union.

An average of 0.53 cm (1-3 cm) shortness was found in our patients who were examined and measured for shortness. An average of 1.4 cm of atrophy was detected in patients who were measured for atrophy 10 cm above the knee joint line.

A total of 20 patients who had complete follow-ups and whose files were sufficient were evaluated according to the Ficat and Arlet avascular necrosis classification. Avascular necrosis was observed in four patients (20%). One of these patients was evaluated as Ficat and Arlet Type 2, two as Ficat and Arlet Type 3 and another one as

Ficat and Arlet type 4. The average time for these patients to be admitted to operation was seven days. There was a significant relationship between the duration of the patients' admittance to surgery and the formation of avascular necrosis (p<0.01).

The 20 patients who had complete follow-ups and whose files were sufficient were evaluated with Harris hip score rating chart, and five patients (25%) had excellent outcomes, while eight had (40%) very good, four (20%) good, two (10%) moderate and one (5%) poor outcome.

Table 1. Demographic data	
Gender (Male/Female)	13 (52.2%) / 11 (45.8%)
Side (Right/Left/Bilateral)	10 (41.7%) / 12 (50%) / 2 (8.3%)
Age	49.21±17.6
Fracture etiology	
High energy trauma	45.8%
In-vehicle traffic accident	3 (12.5%) patients
Pedestrian traffic accident	1 (4.2%) patient
Falling from height	6 (25.0%) patients
Firearm injury	1 (4.2%) patient
Low energy trauma	54.2%
Fracture type	
Garden type-1	1 (4.2%) patient
Garden type-2	4 (16.6%) patients
Garden type-3	10 (41.6%) patients
Garden type-4	9 (37.6%) patients
Pauwels type-2	18 (75%) patients
Pauwels type-3	6 (25%) patients
Treatment modality	
Internal fixation with closed reduction	20 (76.9%) patients
Internal fixation with open reduction	6 (23.1%) patients
Harris hip score	
Excellent outcome	5 (25%) patients
Very good outcome	8 (40%) patients
Good outcome	4 (20%) patients
Moderate outcome	2 (10%) patients
Poor outcome	1 (5%) patient
Avascular necrosis (4 (20%) patients)	
Type 2	1 patient
Type 3	2 patients
Type 4	1 patient
Moderate outcome	2 (10%) patients
Poor outcome	1 (5%) patient

DISCUSSION

Fractures of the femoral neck are a common injury in orthopedic practice and result in significant morbidity and mortality as a result of inappropriate treatment. Appropriate and acceptable reduction and stable fixation of the femoral neck fracture should be achieved as soon as possible in order to reduce the rate of development of non-union, avascular necrosis or other complications seen in patients undergoing surgical fixation.

The 20-40% postoperative failure and revision rates has prompted orthopedic surgeons to investigate the factors that influence the failure of internal fixation. Gurusamy et al. (9) found that non-anatomical reduction was the most important factor for failure in femoral neck fracture. In this case, the quality of the reduction, fracture of calcar, small femoral head, varus angling over 30 degrees in femoral neck, the vertical status of the fracture line in relation to the ground plane and the fragmentation of the posterior cortex of the femoral neck are important factors. Slobogean et al. (10) showed that the only factor to be controlled by the orthopedic surgeon for a successful result is the quality of the reduction. Ozturkmen et al. (11) stated that the high failure rate was mainly due to the patients for whom adequate and stable reduction was not achieved. They stated that the surgical technique

was not applied appropriately in 35% of patients and that inappropriate and inadequate implants were used in 8.8% of patients. Han et al. (12) found that 14 (21%) of the 67 patients who underwent surgery with the diagnosis of femoral neck fracture developed reduction loss. The author reported that avascular necrosis developed as a complication in 42% of patients with reduced reduction loss, and that non-union was observed in the fracture in 28% of patients with reduction loss. Karslı et al. (13) evaluated the risk of developing avascular necrosis depending on the operation and anatomic reduction of the patients in their study. According to this study, no difference was found in the risk of developing avascular necrosis between the patient groups who underwent late surgery with poor reduction and who underwent early surgery and anatomically reduced. In the present study, we observed that the reduction was not anatomical in 7 of 26 hips (26.8%) and that there was no acceptable reduction in 3 hips (11%) based on Garden alignment index. Other patients had acceptable reduction. A total of nine patients (34%) were found to have technical defects or inadequate fixation. In these patients, lack of parallelism of the screws in relation to each other, positioning of the grooved part of the screw in the fracture line and inappropriate screw orientation in relation to femoral neck were some of the fixation problems. In addition, appropriate anatomical reduction was not achieved in seven of these patients. We are of the opinion that a proper reduction is essential for a perfect fixation. Both avascular necrosis and non-union were observed in 45% of our patients with inadequate and inappropriate fracture reduction and unstable fixation.

During the development of a femoral neck fracture, damage to the retinacular vessels responsible for supplying the femoral head region causes a high rate of non-union and avascular necrosis (14). Slobogean et al. (10) stated that the time factor played an important role in the results, that the blood supply to the femoral head was impaired in displaced fractures, and that operating the patient within six hours after trauma helped to reduce the rate of avascular necrosis. In addition, the author stated that intra-articular hematoma was an important factor affecting the blood supply to the head by increasing intra-articular pressure, and that while drainage of the hematoma and depressurization affected the outcome of non-displaced fractures, this was not taken into account in displaced fractures. It was shown that suitability of the reduction was an important factor for development of avascular necrosis in the surgical treatment of displaced femoral neck fractures. It was shown that in femoral neck fractures the rate of avascular necrosis could increase between 7 and 65% in cases where the neck angle is less than 155 degrees after reduction or greater than 180 degrees based on the Garden alignment index (15). All of our patients who developed avascular necrosis after surgical fixation had fixation failure or insufficient reduction. We think that the quality of fixation and fracture reduction may be important factors in the formation of avascular necrosis. Lee et al. (16) studied 116 adult patients diagnosed with femoral neck fracture and treated with cannulated screw fixation and reported that 17% of 12 patients with displaced fractures developed avascular necrosis while only 4% of 104 patients with non-displaced fractures developed avascular necrosis. In the present study, avascular necrosis was observed in four patients (20%). One of these patients was concluded as Ficat and Arlet Type 2, two patients as Ficat and Arlet Type 3 and the other patient as Ficat and Arlet type 4. The mean duration to surgery was seven days for our patients with avascular necrosis. While 25% of our cases had non-displaced fractures, 75% had displaced fractures.

Another important complication that may occur in patients who undergo surgical intervention due to hip fracture is the development of hematoma and infection. Studies in the literature revealed that infection development rate could vary between 2 and 20% in patients who undergo surgery (15). Lee et al. (16) reported that infection development rate was 6.3% in patients for whom drainage was used and 28% when drainage was not used. Petersen et al. (17) reported the development of deep wound infection in 1.4% of the patients and superficial infection in 0.3%. Kınık et al. (18) observed deep infection in 2.4% of patients who underwent surgery with a cannulated screw due to a fracture of the femoral neck. Rogmark et al. (19) observed superficial infections in 2.8% of patients when they applied internal fixation and reported no deep infections. In the present study, of the 24 patients who had surgical intervention, five (20.8%) developed superficial wound infection and two (8.3%) developed deep infection. These findings were consistent with the literature.

Deep vein thrombosis is one of the most serious complications that can be observed after fractures of the femoral neck. Previous thromboembolism attack, orthopedic operations, venous surgery and presence of varicose veins, malignancy, advanced age, congestive heart failure, immobilization, obesity, excessive blood loss and blood transfusion, oral contraceptive or hormone therapy use are factors contributing to the development of deep vein thrombosis. It mostly (80-90%) occurs in the fractured extremity. It develops in veins in legs and thighs, and extends towards proximal by 30%. Grosso et al. (20) reported that 2.2% of patients treated for hip fracture developed deep vein thrombosis and 1.1% developed pulmonary embolism. Kumar et al. (21), on the other hand, observed deep vein thrombosis in 3.5% of the patients and pulmonary embolism in 2%. It was reported in the literature that the patients who underwent surgical treatment after a femoral neck fracture had a deep vein thrombosis rate of 58% and fatal pulmonary embolism rate of 7.5%. Deep vein thrombosis developed in one of our patients (4.2%) while pulmonary thromboembolism developed in two patients (8.4%). One patient died whereas the other responded to treatment and recovered. The incidence of deep vein thrombosis and pulmonary embolism in our cases was consistent with the literature.

Although developments in the follow-up and treatment

of femoral neck fractures and improvements in implant quality have considerably reduced the incidence of non-union, it can still occur at a rate of 10-37%. The factors that cause non-union are delayed and poor reduction, blood flow disorders and failure to obtain a stable fixation. The persistence of pain in patients loading on their extremities after surgical fixation suggests that there may be a problem of non-union or delayed union. On average, union takes place in 6-12 months in femoral neck fractures. In their study on femoral neck fractures, Patterson et al. (22) reported that 36% of the patients had non-union and mentioned that infection accompanied non-union in 23% of these patients and that the 38% of the patients had non-anatomical reduction. Lee (16) reported that 9% of the 116 patients with femoral neck fractures who were fixed with cancellous screws had non-union. Kınık (18) reported a non-union rate of 3% among their cases for whom they performed fixation with multiple cancellous screws. In our study, non-union was observed in 4 of 24 patients (16.7%) who underwent fixation for fracture treatment. The mean age of these patients, one male and three female, was 50 years. Two of them had Garden-2 type fracture and other two had Garden-4. These patients had inadequate fixation and reduction. One of our four patients underwent open reduction, bone grafting and fixation for non-union, which was detected 1.5 years later in their follow-ups. For another patient, fixation was performed along with bone grafting due to non-union. Although union was achieved in these two patients, severe avascular necrosis developed after union. Total hip prosthesis was applied to two patients who did not have union. The rate of non-union in our cases was consistent with the literature. The factors stated in the literature as the cause of non-union were also present in our cases.

Rogmark et al. (19) reported success in 64% of the patients who underwent surgical fixation for a femoral neck fracture. Slobogean (10) obtained poor outcomes in 57% of patients for whom internal fixation was applied. The author mentioned that osteosynthesis has the risk of avascular necrosis and nonunion, and hemiarthroplasty involves the risks of arthroplasty loosening and acetabular wear while total hip arthroplasty has disadvantages of high cost and high mortality rate. However, the author reported that the best result was achieved in cases who had osteosynthesis. Öner (4) evaluated internal fixation for femoral neck fractures and reported that when multiple screws were used 36.5% of the cases had very good outcomes while 45.5% had good, 9% had moderate and 9% had poor outcomes. Kınık (18), on the other hand, reported that 28.6% of the patients had very good outcomes, 38.1% good, 14.3% moderate and 19% had poor outcomes with multiple screw fixation. In the present study, 20 patients were followed up for an average of 48 months. An evaluation of our patients based on Harris hip score rating chart indicated that excellent results were achieved in five patients (25%), very good results in eight patients (40%), good results in four patients (20%), moderate results in two patients (10%) and poor results in one patient (5%). Thus, our findings were consistent with the literature. Based on our findings, it could be stated that fracture type and intervention time are important factors for the outcomes of patients.

CONCLUSION

Femoral neck fractures are a common type of injury in orthopedic practice and could result in significant morbidity and mortality as a result of inappropriate treatment. In order to minimize the rates of non-union, avascular necrosis or other complications in patients who underwent surgical fixation, appropriate and acceptable reduction of femoral neck fracture should be achieved as soon as possible and a stable fixation should be performed. A good reduction and stable fixation could improve the outcomes in patients with femoral neck fractures.

LIMITATIONS

Retrospective study design, limited number of patients and short follow-up periods were among the limitations of the study. Studies with higher efficiency levels and larger patient populations are needed.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Clinical research ethics committee of Fırat University approved the study (Approval No: 17.12.2013/09-05).*

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Research Article

Phenformin Inhibits the Proliferation of MCF-7 and MDA-MB-231 Human Breast Cancer Cell Lines

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Abstract

Aim: Breast cancer is the most common malignancy in women. This disease is a critical public health problem and further research at the molecular level would define its prognosis and specific treatment. Phenformin is an antidiabetic agent. Recent studies showed that it should be considered as a potential agent for the prevention and treatment of cancer cell lines. This study aimed to investigate the antiproliferative effect of phenformin on MDA-MB-231 and MCF-7 breast cancer cell lines.

Materials and Methods: MDA-MB-231 and MCF-7 breast cancer cell lines were used in the study. Experimental groups; control group, 1 µM, 2.5 µM, and 5 µM phenformin administered groups were planned. WST-1 analysis was performed to evaluate the viability of cells 24 h after the treatments. Statistical analysis was carried out using the SPSS 17.0 statistical program. $p<0.05$ was considered to indicate a statistically significant difference.

Results: In the MDA-MB-231 breast cancer cell line, there were statistically significant differences among all groups ($p<0.05$, for all), except between the 1 µM phenformin-treated and control groups ($p>0.05$). In the MCF-7 breast cancer cell line; there were statistically significant differences between the control group and 5 µM phenformin-treated, and between 1 µM phenformin-treated and 5 µM phenformin-treated ($p<0.05$, for all) groups.

Conclusion: Phenformin seems to exert antiproliferative effects on MDA-MB-231 and MCF-7 breast cancer cell lines. It was observed that the antiproliferative effect was stronger in the MDA-MB-231 cell line. It showed a stronger antiproliferative effect in the MDA-MB-231 cell line of antiproliferative effect.

Keywords: Breast cancer, MDA-MB 231, MCF-7, phenformin

INTRODUCTION

Cancer is a chronic life-threatening disease that greatly impacts all spheres of life, so we can mention that breast cancer is one of the vicious diseases which changes millions of women's life (1). Breast cancer is classified according to the type of tissue from which it is originated and according to the extent. Breast cancer can start in the mammary glands, mammary ducts, adipose, or connective tissue (2). It has been explained that it occurs in epithelial cells of ducts (85%), or lobules (15%) in the breast tissue (3). Some grow very slowly and spread to other body parts only when they become significant. Others are more aggressive, grow and spread quickly (2). The reason why women die from breast cancer is mainly due to widespread metastasis. According to the World Health Organization (WHO), in 2020 alone, 2.3 million women were diagnosed with breast cancer, with approximately 685,000 deaths (3).

However, recent advances in medicine, and technology, have led to significant advances, improvements in the early detection and treatment of cancer through radiation and targeted chemotherapy, surgery, and drugs such as hormone therapy or targeted biologic therapy. This type of treatment can prevent the growth and spread of cancer and thus save lives (3, 4). Phenformin is an antidiabetic drug of the biguanide group. Phenformin (phenylethyl biguanide; an antidiabetic agent) shows that it is successful in combined cancer treatments (5). Phenformin has been recognized as a drug with antiproliferative potential. It has been observed the introduction of -Cl and -OCF3 substituents significantly increase the antiproliferative activity of phenformin (6). Phenformin has been shown to have an antitumor effect, not only through AMPK (AMP-

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activated protein kinase) but also as a blocker of the mTOR regulatory complex. Moreover, phenformin abolishes resistance to antiangiogenic tyrosine kinase inhibitors (TKI), which prevents uncontrolled glucose metabolism in tumor cells (7). Studies on various tumor types (eg, breast, lung, prostate, and colon cancer) indicate that phenformin is more potent than metformin (biguanide; an antidiabetic agent) in inhibiting cell proliferation and tumor growth both in vitro and in vivo (8). Therefore, phenformin should be considered as a potential agent for the prevention and treatment of breast cancer in cell line research (9).

The aim of the present study was to investigate the antiproliferative effect of phenformin on MDA-MB-231 and MCF-7 breast cancer cell lines and also to compare effectiveness of phenformin in different concentrations.

MATERIAL AND METHOD

MDA-MB-231 and MCF-7 breast cancer cells (ATCC, Manassas, VA, USA) were cultured in RPMI 1640 culture medium containing 10% heat-inactivated fetal bovine serum, streptomycin, and 1% penicillin. The cells were cultured in 75 cm^2 polystyrene flasks and maintained in an incubator at 37°C in 5% CO^2. Their morphology and proliferation were checked microscopically. The cells were split-passaged when they had reached a confluency of approximately 90%. Cells in semi-confluent flasks were harvested using 0.05% trypsin and centrifuged after the addition of RPMI 1640 for trypsin inactivation. After centrifugation, they were resuspended in a culture medium. Phenformin (CAS Number: 834-28-6, Sigma-Aldrich, St Louis, MO, USA) was prepared as a 5 µM stock solution.

WST-1 cell viability assay

The viability of the cells was evaluated with the WST-1 solution (Water-soluble tetrazolium salt) assay. Briefly, the cells were seeded in triplicate in 96-well plates at a density of 1×10^5 cells/well. The experimental groups are planned as follows for both cell lines: control group, 1 µM, 2.5 µM, and 5 µM phenformin administered groups. After 24 h of incubation, each cell line was exposed to increasing concentrations of phenformin (0 µM, 1 µM, 2.5 µM, 5 µM). Then, the plates were incubated at 37°C in a 5% CO^2 incubator for 24 h. A solution of WST-1 (Water-soluble tetrazolium salt) equal to 1:10 of the medium it contained was added to each well of the culture dish, and the plates were incubated at 37°C for 3 h. At the end of the incubation, absorbance was measured on an ELISA plate reader with the wavelength of 450 nm and a reference wavelength of 650 nm (10).

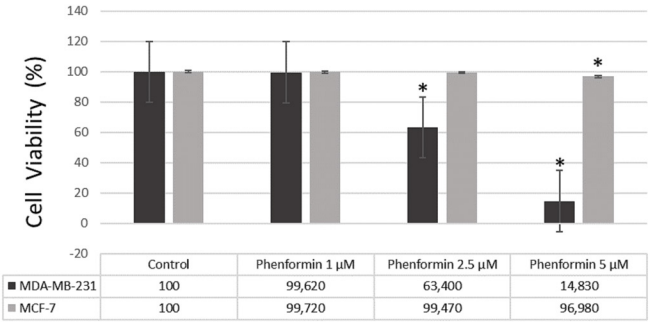
Statistical analysis

Statistical analysis was carried out using the SPSS 17.0 statistical program (SPSS Inc., Chicago, Ill., USA). All experiments were carried out in triplicate and presented as mean±SEM. Statistical analysis was performed by using a one-way analysis of variance, followed by Tukey's or Dunnett's post hoc test. $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

The percentage of MDA-MB-231 and MCF-7 breast cancer cells in the control group was accepted as 100%, and the percentages of cells in the other groups were proportional accordingly. According to the results of the WST-1 analysis, it was found that the percentage of cell viability in MDA-MB-231 cells was 100% (control), 99.62% (1 µM phenformin), 63.40% (2.5 µM phenformin), 14.83% (5 µM phenformin), while according to the results of WST-1 analysis, it was found that the percentage of cell viability in MCF-7 cells was 100% (control), 99.72% (1 µM phenformin), 99.47% (2.5 µM phenformin), 96.98% (5 µM phenformin). Cell viability was reduced in a dose-dependent manner in the MDA-MB-231 and MCF-7 breast cancer cell lines.

In the MDA-MB-231 breast cancer cell line, there were statistically significant differences among all groups ($p<0.05$, for all), except between 1 µM phenformin-treated and control groups ($p>0.05$). In the MCF-7 breast cancer cell line; there were statistically significant differences between the control group and 5 µM phenformin-treated, and between 1 µM phenformin-treated and 5 µM phenformin-treated groups ($p<0.05$, for all) (Figure 1).



	Control	Phenformin 1 µM	Phenformin 2.5 µM	Phenformin 5 µM
MDA-MB-231	100	99,620	63,400	14,830
MCF-7	100	99,720	99,470	96,980

Figure 1. Effects of phenformin on cell viability in the MDMB-231 and MCF-7 breast cancer cell lines after 24h. * Significant difference when compared with control $p<0.05$.

For MDA-MB-231; Control vs 2.5 µM Phenformin and 5 µM Phenformin $p<0.05$, 1 µM Phenformin vs 2.5 µM Phenformin and 5 µM Phenformin $p<0.05$, 2.5 µM Phenformin vs 5 µM Phenformin $p<0.05$. For MCF-7; Control vs 5 µM Phenformin $p<0.05$, 1 µM Phenformin vs 5 µM Phenformin $p<0.05$.

DISCUSSION

The antidiabetic drug phenformin has been considered as a potential agent for treatment in various in vitro cancer studies (7). Coperchini et al. (2019) 's TPC-1 (Human Papillary Thyroid Carcinoma) and 8505C (Anaplastic thyroid carcinoma) cell lines after phenformin applications, cell viability was evaluated with the WST-1 assay performed on the 7th, 14th, and 24th hours; For both cell lines, administration of 10 mM phenformin significantly decreased cell viability at all hours (11). In addition, in

one study, phenformin showed higher cytotoxicity than metformin. The EC50 of metformin was 25 to 15,200,000 times higher than phenformin in B16F10 cells (melanoma), MCF7 cells (breast cancer), CT26 cells (colon cancer), A549 cells (lung cancer), and DU145 cells (prostate cancer) (12).

In a study examining miR-27a-mediated antiproliferative effects of metformin on the breast cancer cell line MCF-7, they reported that metformin inhibits the proliferation of MCF-7 cells at concentrations of 1, 2, 5, 10, and 20 mmol/l (13). In the present study we also investigated the effects of phenformin in different concentrations. We applied 1μM, 2.5μM, and 5μM phenformin and found that the antiproliferative effect of phenformin is increased in a dose dependent manner. Phenformin is a drug in the same antidiabetic group as metformin, and this study supports our data. In a study comparing the efficacy of phenformin and metformin in an in vivo experimental breast cancer model, it was shown that phenformin significantly inhibited both the growth and development of MCF-7 and MDA-MB-231 tumors with greater efficacy than metformin in mice, thus phenformin has clinical potential as an antineoplastic agent opinion has been reached (14).

It has been reported that phenformin reduces cell proliferation and impairs cell cycle progression in SKBR3 and 78617 breast cancer cell lines. This has been shown to occur by inhibiting the growth and epithelial-mesenchymal transition of ErbB2-overexpressing breast cancer cells by targeting phenformin to the IGF1R pathway (15).

Liu et al. (2015) used an intracardiac MDA-MB-231 cancer cells injection model to evaluate the role of phenformin in regulating breast cancer metastasis. They showed that phenformin inhibits the metastasis of MDA-MB-231 cancer cells in nude mice. The same study reported that the respective IC50 values of phenformin in MCF7 and MDA-MB-231 cancer cells were 1.184±0.045 mM and 2.347±0.010 mM. MDA-MB-231 cell line and MCF-7 cell line were used to test the antiproliferative effect of phenformin, so this study shows the efficacy of phenformin, due to its in vitro antitumor activity in preventing the proliferation of cancer cells (9). MDA-MB-231 cells are the most advanced type of breast cancer. In our study we investigated the antiproliferative effect of phenformin on MDA-MB-231 and MCF-7 breast cancer cell lines and observed that phenformin has a more potent antiproliferative effect in the MDA-MB-231 cell line than in the MCF-7 cell line.

CONCLUSION

In this study, it was revealed that phenformin showed strong antiproliferative effect on MDA-MB-231 and MCF-7 breast cancer cell lines and its effect was increased in higher doses. In addition, MDA-MB-231 cancer cells were found to be more sensitive to phenformin compared to MCF-7 cancer cells. In line with data from the literature indicating a controlling inhibiting activity of phenformin on tumor proliferation in vivo, we provide evidence that using a smaller concentration of phenformin exerts a direct lowering effect on tumor proliferation at the cellular level. More studies are certainly required to reveal the reasons behind the difference occurring among breast cancer subtypes. From a future perspective analysis, we can understand that even valuable information about the lower concentrations of phenformin helps us to look in front for solving future problems of our diseases.

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Research Article

Evaluation of Relationship between Modified ATRIA Risk Score and Mortality in Hospitalized Patients with COVID-19

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Abstract

Aim: The ATRIA score was developed to assess the probability of an ischemic stroke in patients with atrial fibrillation (AF). The modified ATRIA (M-ATRIA) risk score incorporates predictive risk variables for coronavirus disease 2019 (COVID-19). As a result, we looked into the association between the M-ATRIA risk score and the risk of in-hospital death in COVID-19 patients.

Materials and Methods: The data of 595 inpatients in the COVID-19 research were evaluated retrospectively and separated into three groups based on the M-ATRIA scoring system. The M-ATRIA score used the troponin I level as a parameter in place of the proteinuria criterion in the ATRIA score. Those with a score between 0 and 5 were classified as group 1 (n = 269), those with a score of 6 as group 2 (n = 64), and those with a score of 7 and above were classified as group 3 (n = 162). In-hospital death, mechanical ventilation, and admission to the critical-care unit were all considered adverse clinical events.

Results: The M-ATRIA risk score associated with adverse clinical events (all, $p < 0.001$). An M-ATRIA score of 6, an M-ATRIA score greater than 7, procalcitonin, and C- reactive protein were found to be independent predictors of in-hospital mortality in the multivariate logistic regression analysis. In the ROC analysis, an M-ATRIA score of 4.5 or above predicted in-hospital mortality with a sensitivity of 90.2% and a specificity of 58.9%.

Conclusion: Regardless of the status of AF, the M-ATRIA risk score computed at admission may be a valuable tool for predicting in-hospital mortality in COVID-19 patients.

Keywords: Coronavirus disease 2019, M-ATRIA risk score, intensive care unit, in-hospital mortality

INTRODUCTION

Even though the vaccine has been available for roughly two years, intensive care unit (ICU) admissions and significant fatality rates are still linked with coronavirus disease 2019 (COVID-19) (1). Most significantly, it affects the respiratory system and causes severe acute respiratory distress syndrome (ARDS). However, arterial thrombosis and venous thromboembolism may also occur during COVID-19 infection secondary to endothelial injury and hypercoagulability (2).

In order to predict the risk of thromboembolism and guide the start of anticoagulant medication in patients with atrial fibrillation (AF), the risk scores CHADS2, CHA_2DS_2-VASc, and ATRIA were developed. In two grand population-based cohort researches, the ATRIA stroke risk score was shown to predict thromboembolic events better than the CHA_2DS_2-VASc and $CHADS_2$ risk scores in non-COVID-19 populations (3,4). Hypertension (HT), diabetes mellitus (DM), older age, chronic heart failure (CHF), and prior ischemic stroke are all components of these three risk scores. In hospitalized COVID-19 patients, these comorbidities raise death and morbidity rates (5). Higher CHA_2DS_2-VASc risk scores at the time of hospitalization were observed to predict in-hospital death in COVID-19 patients (5,6).

Prognostic COVID-19 risk indicators are included in the ATRIA risk score. There are very few studies carried out the association between in-hospital mortality and ATRIA risk score in COVID-19 patients. Proteinuria is a component of the ATRIA risk score, but now that it is not routinely obtained on admission in COVID-19 patients, we excluded this variable from the score calculation. Because greater troponin I levels are linked to an increased mortality rate in COVID-19 patients (7), we developed a modified ATRIA (M-ATRIA) risk score by replacing the proteinuria criterion with troponin I. There is no data in the literature to our knowledge about the value of the newly generated M-ATRIA

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score in predicting the prognosis of COVID-19 patients when the proteinuria criterion in the ATRIA risk score is altered with troponin I. The study's goal was to examine the relation between death and the M-ATRIA risk score in hospitalized COVID-19 patients, irrespective of AF status.

MATERIAL AND METHOD

Study population

The medical data of 555 individuals who presented to our tertiary hospital's emergency department and were hospitalized with COVID-19 between December 1, 2020 and January 1, 2021 were reviewed retrospectively. Data from the physical examination, clinical assessment, laboratory workup, and chest computed tomography (CT) imaging were obtained for all patients. Nasopharyngeal swab samples were collected from all patients by healthcare professionals at the time of hospital admission. Those with a positive PCR (Polymerase Chain Reaction) test result were considered as confirmed COVID-19 cases. Those with a negative PCR test result received treatment for COVID-19 and were comprised in the study if chest CT scans showed typical signs of COVID-19 infection. These included unilateral or bilateral sub-segmental, multiple patchy, or segmental/lobar ground-glass opacities within the lung that could not be explained by another cause or condition (8). All patients were diagnosed with COVID-19 as per the WHO interim guidance on COVID-19, and the decision to admit the patient was in accordance with national guidelines (8).

COVID-19 patients with dyspnea, a respiratory rate > 30/min, hypotension, a heart rate of >100 bpm, PaO_2<70 mmHg, PaO_2/FiO_2<300, immunosuppression, acute organ dysfunction, sepsis, increased troponin level, septic shock, need for mechanical ventilation (MV), acute bleeding diathesis, and arrhythmia were admitted to the ICU. Comorbidities (prior cerebrovascular diseases, CHF, HT, DM, AF, chronic obstructive lung disease, status renal disease, cardiovascular diseases, and prior malignancy), demographic characteristics, treatment protocols, mortality data, radiographic findings, and laboratory parameters were retrieved from the hospital electronic medical record. Laboratory parameters, obtained within the first 24 hours of admission, were also used in the study. Patients who had missing or incomplete laboratory data and terminal cancer patients were excluded. Data from the initial admission were used in the study for the 12 patients who had undergone several hospitalizations in the previous month.

Patients with lung CT findings and PCR tests that did not suggest Covid-19 (n=35), incomplete laboratory data (n=20), and patients with terminal cancer (n=5) were excluded from the study. This retrospective cohort analysis included 495 patients (55.6% males, n=275), with an average age of 67.6±14.7 years.

Patients who presented with neurological symptoms that persisted for more than 24 hours and imaging techniques revealed ischemia or bleeding in the brain, were diagnosed as stroke. Transient ischemic attack was characterized as a temporary neurological dysfunction lasting less than 24 h with associated symptoms. Hypertension was regarded as having a blood pressure of ≥ 140/90 mmHg or being treated for HT. Having DM was based on having a fasting blood glucose level of ≥126 mg/dL or having DM by diagnosis. CHF was described by a left ventricular ejection fraction of <40%. eGFR was calculated as follows:

$186 * (age - ^{0.203}) * (plasma\ creatinine\ ^{-1.154}) * (0.742\ if\ female)$

Ethics statement: Approval was acquired from Clinical Research Ethics Committee for the current study. (Date: 16/02/2021, Decision no: 2021/02-11). The research adheres to the Declaration of Helsinki Principles

Scoring system

Prior ischemic stroke and older age are the two most significant risk variables for the ATRIA scoring algorithm. This scoring system was adapted in this study (Table 1). For patients without prior stroke, scoring according to age is done as follows; ≥85: 6 points, 75-84: 5 points, 65-74: 3 points, <65: 0 points. For patients with a prior stroke, scoring according to age is done as follows; 85: 9 points, 65-84:7 points, <65: 8 points. In addition, 1 point each was assigned for female gender, DM, CHF, HT and eGFR< 45 mL/min/1.73 m^2 irrespective of prior stroke (3).

Table 1. Risk factors used in Modified ATRIA Risk Score

Risk factors	Points without prior stroke (points)	Points with prior stroke (points)
Age, years		
>85	6	9
75–84	5	7
65-74	3	7
<65	0	0
Female sex	1	1
Chronic heart failure	1	1
Diabetes mellitus	1	1
Hypertension	1	1
eGFR <45 mL/min/1.73 m^2 or ESRD	1	1
*Troponin I	1	1

ESRD: end-stage renal disease
* Elevated troponin I (> 0.023 ng/mL) was assigned a score of 1

The proteinuria feature of the original ATRIA score was purposefully replaced with troponin I to improve the prediction of death in COVID-19 patients because increased troponin I is known to be a significant predictor of mortality in hospitalized patients due to COVID-19 (7). Elevated troponin I (> 0.023 ng/mL) was assigned a score of 1. This new score was designated as the modified ATRIA (M-ATRIA) score.

The study population was divided into three groups based on the M-ATRIA risk score due to the stratification of the ATRIA score into high (7 to 15 points), moderate (6 points), and low (0 to 5 points) risk categories (3). Group 1 had scores ranging from 0 to 5 (n = 269), Group 2 had scores ranging from 6 to 64, and Group 3 had scores ranging from

7 to 162. As adverse clinical outcomes, admission to the ICU, invasive MV, and in-hospital mortality were identified.

Statistical analysis

In the study, the minimum total sample size was determined according to a power of 0.85 with moderate effect size. An additional 15% more of power analysis was included in the research. Numerical data distribution was determined by the Kolmogorov-Smirnov test. Baseline parameters were displayed as median (quartile deviation) or mean ± standard deviation, and categorical variables were offered as numbers and percentages. The Kruskal-Wallis test with post-hoc analysis (Dunn's test) was used to evaluate continuous variables, while Pearson's chi-squared test was used to analyze categorical variables. In the univariate binary logistic regression analysis, predictors were those thought to be closely associated to in-hospital mortality. To exclude statistically non-significant predictors and avoid a multicollinearity problem, forward variable selection was applied to the predictors in the multivariate binary logistic regression analysis.

The model's fit was evaluated using the Hosmer-Lemeshow test. Each independent variable's odds ratio (OR) and 95% CI were calculated. Kaplan-Meier curves were also plotted. To explore the relation between in-hospital survival and M-ATRIA score groups, the log-rank test was used. Finally, using the Youden's Index to assess in-hospital mortality, the analysis of receiver operating characteristic (ROC) curve was used to determine the ideal cutoff value for the M-ATRIA scores, troponin I, and C-reactive protein (CRP) levels. Statistically significant difference was regarded as a p value less than 0.05. Statistical computations were carried out using SPSS (v23.0, IBM Corp., Chicago, IL, USA) and R software (version 4.0.5 R Core Team).

RESULTS

In the study population, the mortality rate was 24.6% and the likelihood of admission to an ICU was 28.5%. Table 2 lists the demographic characteristics and comorbidities of the study sample, stratified by M-ATRIA risk scores. There were no significant differences in malignancy, length of hospital stay, or chronic lung disease across the groups (all p>0.05). Patients who have the high M-ATRIA score were older with a higher frequency of CHF, DM, HT, cerebrovascular disease, chronic kidney disease, elevated troponin I, reduced eGFR, coronary artery disease (all, p<0.001), and AF (p=0.005). From a low M-ATRIA tertile or score to a high M-ATRIA tertile or score, in-hospital mortality, ICU admission, and MV rose progressively (all, p<0.001). Group I had an in-hospital mortality rate of 9.7%, Group 2 had a rate of 29.7%, and Group 3 had a rate of 47.5%. Except for favipravir treatment (p=0.021), inpatient treatments were comparable among the three groups.

Table 2. Comorbidities and demographic features of the study population

	Group 1: M-ATRIA= 0-5 (n=269)	Group 2: M-ATRIA= 6 (n=64)	Group 3: M-ATRIA ≥7 (n=162)	p-value
Age (years)	59 (8)	75.7 ± 6.4	80 (6.0)	**<0.001***
M-ATRIA score components				
Age ≥85, n (%)	0 (0 %)	4 (6.3 %)	50 (30.9 %)	**<0.001**
Age 75-84, n (%)	11 (4.1 %)	38 (59.4 %)	84 (51.9 %)	**<0.001**
Age 65-74, n (%)	77 (28.6 %)	20 (31.3 %)	22 (13.6 %)	**0.001**
Age <65, n (%)	181 (67.3 %)	2 (3.1 %)	6 (3.7 %)	**<0.001**
Female, n(%)	99 (36.8 %)	24 (37.5 %)	97 (59.9 %)	**<0.001**
Chronic heart failure, n (%)	4 (1.5 %)	11 (17.2 %)	37 (22.8 %)	**<0.001**
Diabetes mellitus, n (%)	65 (24.2 %)	22 (34.4 %)	68 (42.0 %)	**<0.001**
Serebrovascular disease, n (%)	2 (0.7 %)	2 (3.1 %)	39 (24.1 %)	**<0.001**
Hypertension, n (%)	90 (33.5 %)	26 (40.6 %)	124 (76.5 %)	**<0.001**
Increase in troponin I, n (%)	25 (9.3 %)	14 (21.9 %)	55 (34.0 %)	**<0.001**
eGFR<45, n (%)	15 (5.6 %)	17 (26.6 %)	46 (28.4 %)	**<0.001**
Chronic lung disease, n (%)**	60 (22.3 %)	20 (31.3 %)	45 (27.8 %)	0.223
Chronic kidney disease, n (%)	16 (5.9 %)	13 (20.3 %)	34 (21.0 %)	**<0.001**
Coronary artery disease, n (%)	50 (18.6 %)	16 (25 %)	94 (58.0 %)	**<0.001**
Malignancy, n (%)	19 (7.1 %)	10 (15.6 %)	14 (8.6 %)	0.092
Atrialfibrillation, n (%)	9 (3.3 %)	6 (9.4 %)	18 (11.1 %)	**0.005**
Length of hospitalstay, days	8 (3.5)	10.5 (5.5)	9 (5.0)	0.050
Treatments, n (%)				
Favipiravir	241 (89.6 %)	58 (90.6 %)	157 (96.9 %)	**0.021**
Antibiotics	253 (94.1 %)	60 (93.8 %)	152 (93.8 %)	0.993
Glucocorticoids	269 (100 %)	64 (100 %)	162 (100 %)	-
Mechanicalventilation, n (%)	20 (7.4 %)	14 (21.9 %)	47 (29 %)	**<0.001**
Admissionto ICU, n (%)	53 (19.7 %)	21 (32.8 %)	67 (41.4 %)	**<0.001**
In-hospitalmortality, n (%)	26 (9.7 %)	19 (29.7 %)	77 (47.5 %)	**<0.001**

* p<0.001 between Group 1 and 2, p<0.001 between Group 1 and 3, p= 0.102 between Group 2 and 3; **Chronic lung disease was defined as choronic obstructive pulmonary disease, chronic bronchitis or asthma; eGFR; estimated glomerular filtration rate, ICU; intensive care unit.

Table 3 displays the laboratory results of the patients enrolled in the study. Urea, creatinine, CRP, procalcitonin and fibrinogen levels increased as the M-ATRIA score or tertile increased (all, p<0.05). On the other hand, the levels of hemoglobin, eGFR, and albumin declined from a higher to a lower M-ATRIA tertile or score (all, p<0.001). Patients with low M-ATRIA tertiles or scores had lower levels of troponin I and D-dimer than patients with high and intermediate M-ATRIA tertiles or scores (all, p<0.001).

Table 3. Risk factors used in Modified ATRIA Risk Score

	Group 1: M-ATRIA= 0-5 (n=269)	Group 2: M-ATRIA= 6 (n=64)	Group 3: M-ATRIA ≥7 (n=162)	p-value	Post-Hoc Analysis p-value
Hæmoglobin, g/dL	13.9 (1.1)	13.2 ± 2.4	12.8 ± 1.2	**<0.001***	**Group 1 vs 2:** 0.092 **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 0.562
Platelet count, (×10³/μL)	217.0 (55.5)	185.0 (60)	202.5 (59.6)	**0.021***	**Group 1 vs 2: 0.025*** **Group 1 vs 3:** 0.372 **Group 2 vs 3:** 0.444
White blood cell count, (×10³/μL)	7.6 (2.2)	7.7 (3.9)	8.7 (3.2)	0.130	
Procalcitonin, ug/L	0.2 (0.1)	0.2 (0.1)	0.3 (0.3)	**0.001***	**Group 1 vs 2:** 0.092 **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 0.562
Serum creatinine, mg/dl	0.9 (0.2)	1 (0.4)	1.1 (0.4)	**<0.001***	**Group 1 vs 2:** 1.000 **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 0.233
eGFR, mL/min/1.73 m²	83.1 (21.9)	74.7 (21.0)	60.4 (17.6)	**<0.001***	**Group 1 vs 2: <0.001*** **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 0.060
Urea, mg/dL	31.0 (10.0)	50.5 (26.2)	53.0 (25.5)	**<0.001***	**Group 1 vs 2: <0.001*** **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 0.699
Alanine aminotransferase, U/L	27.0 (11.0)	22.5 (10.3)	22.0 (9.6)	**0.003***	**Group 1 vs 2:** 0.101 **Group 1 vs 3: 0.005*** **Group 2 vs 3:** 1.000
Aspartat aminotransferase, U/L	34.0 (11.5)	33.5 (14.8)	37.5 (14.3)	0.709	
Albumin, g/dL	3.3 (0.4)	2.9 (0.4)	3.0 (0.3)	**<0.001***	**Group 1 vs 2: 0.007*** **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 1.000
Fibrinogen, g/L	495.4 (94.0)	496.7 (92.5)	538 (116.3)	**0.037***	**Group 1 vs 2:** 0.092 **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 0.562
C-reactive protein, mg/dL	7.0 (4.4)	8.8 (3.7)	8.9 (5.5)	**0.004***	**Group 1 vs 2:** 1.000 **Group 1 vs 3: 0.045*** **Group 2 vs 3:** 0.227
D-Dimer, μg/L	986.0 (421.3)	1425 (654.2)	1350 (670.5)	**<0.001***	**Group 1 vs 2: 0.007*** **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 1.000
Troponin I, μg/L	0.01 (0.00)	0.01 (0.00)	0.01 (0.03)	**<0.001***	**Group 1 vs 2: 0.031*** **Group 1 vs 3: <0.001*** **Group 2 vs 3:** 0.319

*P value <0.05; eGFR: Estimated glomerular filtration rate

In order to evaluate the independent determinants of in-hospital mortality, logistic regression analysis was utilized. According to the results of the multivariate logistic regression analyses, the COVID-19 patients' M-ATRIA score of 6 (OR, 3.598; 95%CI, 1.748–7.404; p=0.001), M-ATRIA score of 7 (OR, 6.825; 95%CI, 3.977–11.883; p<0.001), procalcitonin (OR,1.957; 95%CI, 1.370–2.94; p<0.001), and the CRP level (OR,1.100; 95% CI, 1.061–1.141; p<0.001) were all independently predictive factors for in-hospital death (Table 4).

Table 4. Univarite and Multivariate Binary Logistic Regression Analysis to Identify the Predictors of in Hospital Mortality

Univarite	OR	95% Confidence interval	p-value	Multivariate	OR	95% Confidence interval	p-value
M-ATRIA score groups			<0.001	M-ATRIA score groups			<0.001
M-ATRIA 6	3.946	(2.016-7.724)	<0.001	M-ATRIA 6	3.598	(1.748-7.404)	0.001
M-ATRIA ≥7	8.467	(5.091-14.079)	<0.001	M-ATRIA ≥7	6.875	(3.977-11.883)	<0.001
Age (years)	1.060	(1.041-1.079)	<0.001		1.100	(1.061-1.141)	<0.001
Sex (male)	1.380	(0.909-2.096)	0.130	Procalsitonin	1.957	(1.370-2.794)	<0.001
Troponin I	27.470	(8.960-84.214)	<0.001	Constant	0.032	-	<0.001
C-reactive protein	1.120	(1.084-1.157)	<0.001				
Procalsitonin	2.710	(1.909-3.848)	<0.001				

The ROC curve analysis showing the predictive accuracy of CRP, troponin I, and the M-ATRIA risk score for in-hospital mortality is displayed in Figure 1. ROC curve analysis showed that a CRP level of 11.8 mg/dL had a sensitivity of 56.6% and a specificity of 78.2% in predicting in-hospital mortality, whereas a troponin I value of 0.019 µg/L had a sensitivity of 58.2% and a specificity of 93%. An M-ATRIA score of 4.5 and over had a sensitivity of 90.2% and a specificity of 58.9% for the prediction of in-hospital mortality (area under curve [AUC] 0.70, 0.76, 0.80, respectively). Figure 2 displays the Kaplan-Meier survival curves based on M-ATRIA scores. Mortality significantly increased in patients with a higher M-ATRIA score (p<0.001 by the log-rank test). Significantly higher survival rates were seen in patients with M-ATRIA risk scores under 6.

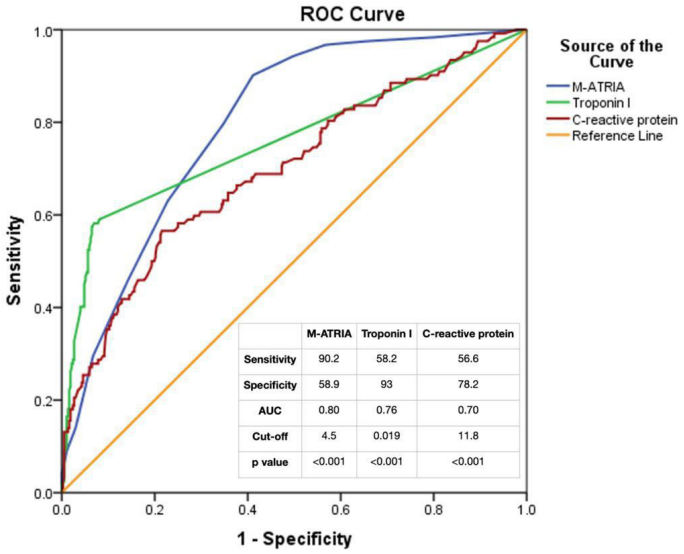
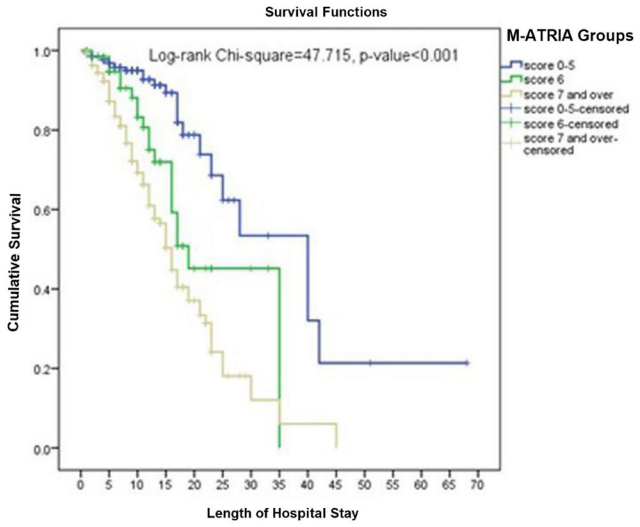


Figure 1. ROC analysis showing the predictive accuracy of Troponin-I, M-ATRIA score, and C-reactive protein for in-hospital mortality.
AUC: Area under the curve



Means and Medians for Survival Time

M-ATRIA GROUPS	Mean Estimate	Mean Std. Error	Mean 95% Confidence Interval Lower Bound	Mean 95% Confidence Interval Upper Bound	Median Estimate	Median Std. Error	Median 95% Confidence Interval Lower Bound	Median 95% Confidence Interval Upper Bound
score 0-5	36.644	4.590	27.648	45.640	40.000	6.761	26.749	53.251
score 6	22.914	2.130	18.740	27.089	19.000	3.176	12.775	25.225
score7 and over	17.682	1.362	15.012	20.352	16.000	0.876	14.284	17.716
Overall	25.923	2.048	21.910	29.937	22.000	1.530	19.000	25.000

Figure 1. ROC analysis showing the predictive accuracy of Troponin-I, M-ATRIA score, and C-reactive protein for in-hospital mortality.

DISCUSSION

The results of the study showed that, independent of AF status, the M-ATRIA score predicted in-hospital mortality in COVID-19 hospitalized patients. Patients with a higher M-ATRIA score also had increased rates of adverse events. In addition, the ROC curve analysis affirmed the prognostic performance of the M-ATRIA score.

COVID-19 primarily impresses the respiratory tract, but can also lead to multi-organ dysfunction and is related to high mortality rates and ICU admission. Although the in-hospital

mortality among patients in ICU is lower compared to the initial stages of the pandemic, it remains high (35.5%) (9). As a result, predicting in-hospital mortality of COVID-19 patients on admission is crucial for devising a treatment strategy and reducing adverse events. Elevated troponin I levels, an indicator of myocardial injury, have been linked to higher in-hospital mortality in this population (7). Similarly, elevated troponin I was an independent and strong indicator of in-hospital mortality in this study. The risk scoring prediction of M-ATRIA for in-hospital mortality was improved by substituting the troponin I levels measured at admission for the evidence of proteinuria in the ATRIA risk score.

Advanced age, a factor in the M-ATRIA score, is linked to higher rates of morbidity and mortality in COVID-19 patients. Age-related increases in COVID-19-related hospital mortality and ICU admissions (10). In high-income countries, individuals under 65 years of age have a 16 to 100-fold lower risk of death from COVID-19 compared to those over 65 years of age (11). In a meta-analysis of COVID-19 reports published until May 7, 2020, mortality rates of 9.5% in patients aged 60 to 69 years, 22.8% in patients aged 70 to 79 years, and 29.6% in patients over the age of 80 were reported (12). A separate study reported a mortality rate of 38.8% among hospitalized individuals with COVID-19 over the age of 85 (13). Thus, risk scores that include particularly high-risk elderly individuals can be used to predict mortality while managing the pandemic.

In a study involving 349 COVID-19 patients, non-survivor patients had a higher CHA_2DS_2-VASc score versus the survivor group. It has been shown to be an independent predictor of death to have a score of three or higher (6). Cetinkal ve ark. (5) examined the link between the CHA_2DS_2-VASc score on admission and mortality in patients with COVID-19. The researchers arbitrarily altered the sex category from female to male and showed that their modified CHA_2DS_2-VASc score predicted in-hospital mortality independently. Ruocco et al. (13) discovered that the CHA_2DS_2-VASc score was an independent predictor of in-hospital mortality in a study of 864 COVID-19 patients.

Similar variables are included in the ATRIA and CHA_2DS_2-VASc risk score models. The ATRIA risk score system is more detailed than the CHA_2DS_2-VASc risk score system in terms of age. For example, when calculating the CHA_2DS_2-VASc score, all age groups over the age of 75 are assigned the same score, whereas in the ATRIA risk scoring, individuals aged ≥ 85 years are assigned a higher score than those aged ≥ 75 years. This may increase the power of the ATRIA score to predict mortality of COVID-19 patients. Additionally, renal dysfunction, a variable linked to a higher mortality in COVID-19 patients, is also included in the ATRIA score (14). This confers greater power to the score for predicting mortality. Aciksari et al. (15) reported in their study that the M-ATRIA risk score predicted in-hospital death in individuals hospitalized due to COVID-19 (AUC:0.74). In the current study, the M-ATRIA score predicted in-hospital mortality (AUC: 0.80). In contrast to their work, adding the troponin value to the M-ATRIA score increased test discriminative power in our study.

Other components of the M-ATRIA score, such as DM, heart failure, prior stroke, and low eGFR, have been linked to higher in-hospital mortality in COVID-19 patients, according to studies (5-7). Likewise, comorbidities were more common in patients with a higher M-ATRIA score in our study. Although the underlying mechanism for increased severity and mortality observed in patients with comorbidities has not been elucidated, a number of factors were implicated, such as an impaired immune system, low-grade inflammation, and an elevated level of angiotensin converting enzyme 2 (ACE-2) receptors (16-18).

Arterial and venous thrombotic events (i.e, pulmonary embolism, acute limb ischemia, deep venous thrombosis, acute mesenteric ischemia, acute myocardial infarction, ischemic stroke) due to immobilization, hypoxia, hypercoagulability, and endotheliosis are a prominent reason of increased mortality and morbidity in patients with COVID-19 (19). Patients experience poor outcomes despite the administration of adequate anticoagulant therapy (20). Thrombotic complications were observed more frequently in elderly patients with comorbidities (21). A meta-analysis of 20 studies involving 1.988 patients reported a venous thromboembolism prevalence of 31% (22). In a meta-analysis of 27 studies examining arterial thrombotic events in COVID-19 patients, about 4.4% of critically ill COVID-19 patients admitted to ICU developed arterial thrombosis (21). In a recent meta-analysis of 42 studies, Malas et al. (23) reported that both venous and arterial thromboembolism rates were high in COVID-19 patients and thromboebolism was associated with a high risk of mortality. Caro-Codón et al. (20) reported that the modified CHA_2DS_2-VASc and CHA_2DS_2-VASc scores anticipate all-cause mortality in COVID-19 patients. The researchers reported that 3.8% of patients presented with a definite thrombotic event, and these scores do not predict thromboembolic events. Therefore, according to the findings of our study, it may be speculated that there is a relationship between M-ATRIA score and COVID-19 mortality.

The ATRIA score is a validated risk score model that has been improved to foretell the thromboembolism risk for AF patients. In non-valvular AF, endothelial dysfunction, local/systemic inflammation, and hypercoagulability play a role in thrombus formation (3,4). Although thrombosis in COVID-19 patients occurs through the same mechanisms, the main underlying pathophysiology is thrombo-inflammation induced by excessive immune activation and cytokine storm (2).

Risk prediction models have been developed for disease progression, ICU admission, and death in COVID-19 patients. Most models are web-based risk scores or nomogram models consisting of clinical, laboratory and radiological components (24,25). When externally validated, some of the risk prediction models were found to be weak in predicting mortality. El-Solh et al.

(26) performed external validation of four risk prediction models to anticipate mortality among COVID-19 patients in a large cohort. The authors noted that all of the prognostic models showed poor performance and a high risk of bias. In COVID-19 patients, the M-ATRIA score of admission time may be utilized as a practical and simple tool to predict in-hospital mortality. The variables that are incorporated into the score also predict mortality, and thromboembolic complications increase mortality.

Limitations of the Study

Now that the study had a retrospective design, the comorbidity status of the participants may not have been fully captured. Adequate imaging studies for ischemic/embolic events may be lacking because of the prioritization of isolation protocols and to prevent the spread of infection. Since we could not determine the exact number of patients experiencing ischemic/embolic events, we were not able to peruse the association between the M-ATRIA score and ischemic/embolic events. In addition, the CHA_2DS_2-VASc scores of the patients were not calculated, and therefore we could not compare the two scores. Also, in contrast to the CHA_2DS_2-VASc, the ATRIA score model does not include vascular disease as a parameter, which is familiar to be associated with mortality.

CONCLUSION

We determined that the M-ATRIA score may be utilized to recognize patients with a high mortality risk on admission. During the COVID-19 pandemic, making simple, practical scores available to clinicians may be helpful in decision-making and reducing adverse events for high-risk patients requiring ICU monitoring in rapidly deteriorating COVID-19 patients. Prospective studies on larger patient populations are warranted to corroborate our findings.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Approval was acquired from Clinical Research Ethics Committee for the current study. (Date: 16/02/2021, Decision no: 2021/02-11). The research adheres to the Declaration of Helsinki Principles.*

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MEDICAL RECORDS-International Medical Journal

Research Article

A Morphometric and Morphological Analysis of Superior Border of Dry Scapulae

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Abstract

Aim: The recognition of the scapula anatomy and visible variations is important in surgical treatments and arthroscopic procedures in case of any diseases of the shoulder. The morphological and morphometric characteristics of the scapular notch on the superior margin are very important. Because compression of the suprascapular nerve extending inside the scapular notch causes entrapment neuropathy. Therefore, the present study was planned in order to contribute to us as well as practitioners about morphometric and morphological characteristics of the superior margin of the scapula.

Materials and Methods: Morphometric measurements (SL: scapula length; SW: scapula width; SI: scapula index; STD: superior transverse diameter of the scapular notch; MTD: medial transverse diameter of the scapular notch; VD: vertical diameter) were performed on 90 (50 left; 40 right) scapulae of Turkish population without unknown age and gender. Furthermore, the superior scapular margin and the scapular notch were categorized through observational classification as well as measurement.

Results: The mean scapular width was detected 98.87±7.71 mm on the right and 94.38±13.01 mm on the left. The scapula index was measured 67.51±4.40 mm on the right and 63.80±8.29 mm on the left. The SL, STD, and VT was larger on the left; the SW, SI, and MTD were larger on the right. The difference between right and left for the SW and SI measurements was statistically significant ($p<0.005$). The most common scapular notch appearance, the U shape (64.4%) was the most common scapular notch shape as VD>MTD (%48.9). The most common superior margin type was moderately oblique (41.1%).

Conclusion: We believe that the data obtained would be helpful for orthopedic surgeons in intramedullary nailing and radiologists in the differential diagnosis of some osteolytic lesions in that region. Furthermore, scapula measurements would help to identify the gender and race in forensic medicine and anthropology.

Keywords: Scapular notch, scapulae, variation, morphometry, anatomy

INTRODUCTION

The shoulder joint is one of the structures that performs important functions in the human body. Bone, joint, and muscle support the shoulder joint. The scapula is the basic bone of the shoulder. The scapula is a flat bone with three margins, three angles, and two surfaces at the level of the 2nd and 7th ribs (1). Recognition of the detailed anatomical information in order to understand the scapula, glenohumeral dislocation, rotator cuff injuries, arthritis, tumors and developmental anomalies on the shoulder region will facilitate the surgical and arthroscopic procedures performed on this region (1,2).

The thinnest and shortest margin of the scapula is the superior margin (SM). Important muscles such as the levator scapula and the supraspinatus for initiation of upper extremity movements attach to this margin.

A notch called the scapular notch scapulae exist on the superior margin. A ligament called the trasverse scapular ligament exist on this notch extending like a bridge and converts the notch into a foramen. The suprascapular nerve extends inside this foramen; the subscapular artery and vein exist over this nerve. The suprascapular nerve may be compressed through extension as a result of the structure of the foramen and compression of some structures in the region, and suprascapular entrapment

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neuropathy may develop (3). The orthopedic surgeons suggest that the suprascapular nerve entrapment and traction injuries are mostly caused by the scapular notch (4). The depth and size of the scapular notch varies among the individuals. Changing the width and depth of the notch causes varying degrees of compression of the nerve (3). The superior transverse scapular ligament ossificates in some cases and the foramen may completely convert into a formation surrounded by osseous formation.

In addition to investigating the morphometric feature of dry bone scapulae, determination of the differences between ethnic groups in the classification of bones according to their morphological features has been tried in recent years. The aim of the present study was to assess morphometric and morphological characteristics of the scapulae of Turkish population in Middle Anatolia region. Furthermore, another aim was to reveal the correlation between scapula measurements and the scapular notch.

MATERIAL AND METHOD

The present study was conducted on 90 (40 right, 50 left) scapula with unknown age and gender in the bone collection of Anatomy Departments of Meram Faculty of Medicine within Necmettin Erbakan University and Faculty of Medicine within KTO Karatay University. The permits required for the study were obtained by 0017 numbered decision of Research Ethics Committee of KTO Karatay University in 2019. The scapulae with variational and fracture deformity were excluded.

The scapulae of our study were examined under two titles including morphometric measurements and morphological classifications of the superior margin (SM) and the scapular notch (inc.). Morphometric measurements were performed by a digital caliper (Mitutoyo Dial caliper gauge, Tokyo, Japan, sensitivity 0.01 mm) and osteometric board (sensitivity 0.1 mm). The measurements were taken by the same person three times and the average of these measurements was obtained in order to have the highest level of measurement reliability in morphometric measurements. Morphological evaluations were performed by photographing from the same distance with a micrometer scale and by a single person both on the photograph and on the bone.

1. Morphometric Measurements (Figures 1A, B)

a) Scapula measurements

Scapula length (SL): The widest upper-lower distance between superior and inferior angles.

Scapula width (SW): The widest medio-lateral distance between the lateral angle and lower margin of the scapular trigon (Figure1A).

Scapula index (SI): It was calculated by SI:SW*100/ SL (5) formula.

a) Measurements of the scapular notch

Superior transverse diameter of the scapular notch (STD): The upper transverse diameter of the scapular notch.

Medial transverse diameter of the scapular notch (MTD): The medial transverse diameter of the scapular notch.

Vertical diameter of the scapular notch (VD): The vertical diameter of the scapular notch was measured (Figure 1B).

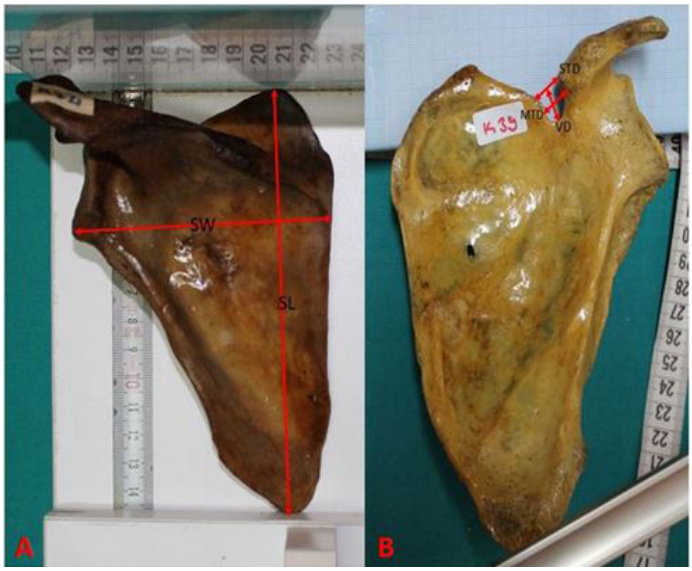

Figure 1. Morphometric evaluation of the scapula and scapular notch (SW: The width of scapula, SL: The length of scapula; STD: Upper transverse diameter of scapular notch, MTD: Middle transverse diamater of scapular notch, VD: Vertical diameter of scapular notch)

2. Morphological Classification (Figures 2, 3, 4)

a) Observational classification of the scapular notch: The classification of the scapular notch was performed by revising the classifications suggested by Rengachary et al. (6) and Okeke et al. (7).

Observational classification of the scapular notch (Figure 2);

Type 1: Arc-shaped or no scapular notch

Type 2: Blunt or large V shaped

Type 3: Deep U-shaped (superior transverse diameter of the scapular notch is smaller than vertical diameter of the scapular notch)

Type 4: Small v-shaped

Type 5: J-shaped which appears to be closed by a ledge

Type 6: Foramen-shaped

b) Measurement-based classification of the scapular notch: The classification was suggested under 5 groups through the classification made by Polguj et al. (8) according to the measurement data (Figure 3).

Type 1: VD>STD (maximum vertical depth)

Type 2: VD=STD (the vertical depth equals to the transverse diameter)

Type 3: STD>VD (the transverse diameter is larger than the vertical diameter)

Type 4: Foramen

Type 5: No notch

c) Shape-based superior margin typing: The superior margin of the scapula was revised and reclassified according to Singroha et al. (9).

Typing according to the superior margin (Figure 4)

Type 1: Moderately oblique

Type 2: Significantly oblique

Type 3: Saddle-shaped

Type 4: Corrugated

Type 5: Flat and on the medial

Type 6: Flat, saddle-shaped, oblique

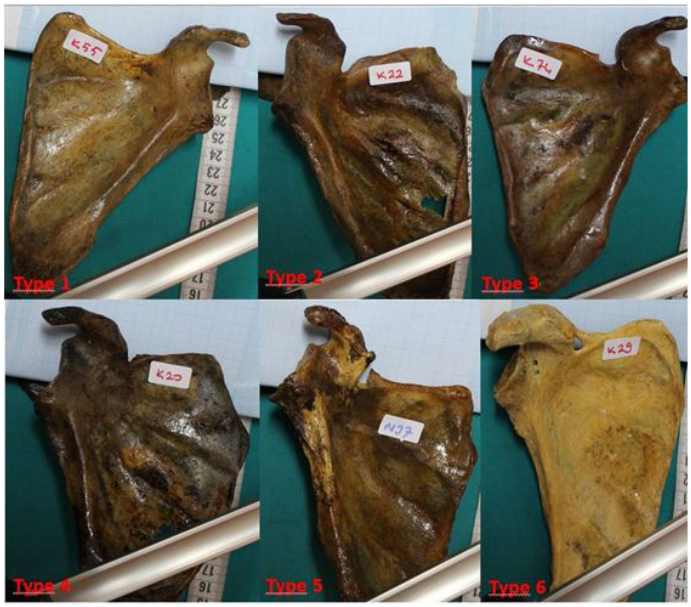

Figure 2. Types of scapular notch according to the morphological evaluation (**Type 1:** Absence of scapular notch; **Type 2:** V shaped; **Type 3:** Deep"U" shaped; **Type 4:** Small"V" shaped; **Type 5:** J shaped; **Type 6:** Hole-shaped)

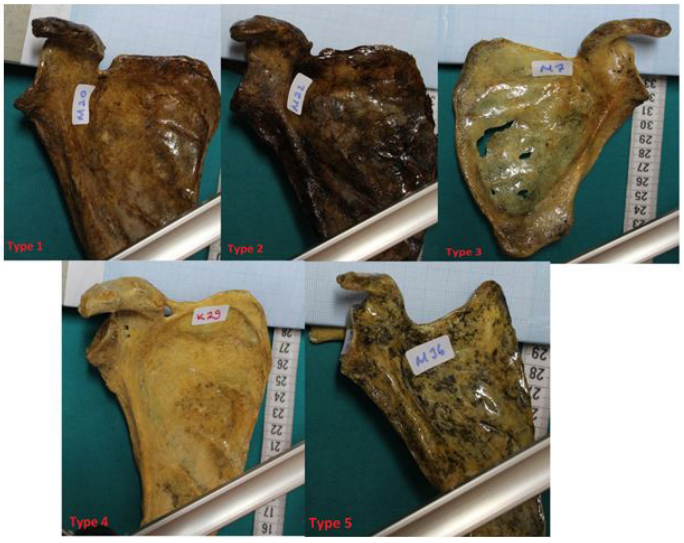

Figure 3. Types of scapular notch according to the scapular notch measurements (**Type 1:** VD>STD; **Type 2:** STD=MTD **Type 3:** STD>VD; **Type 4:** Foramen; **Type 5:** Absence of notch)

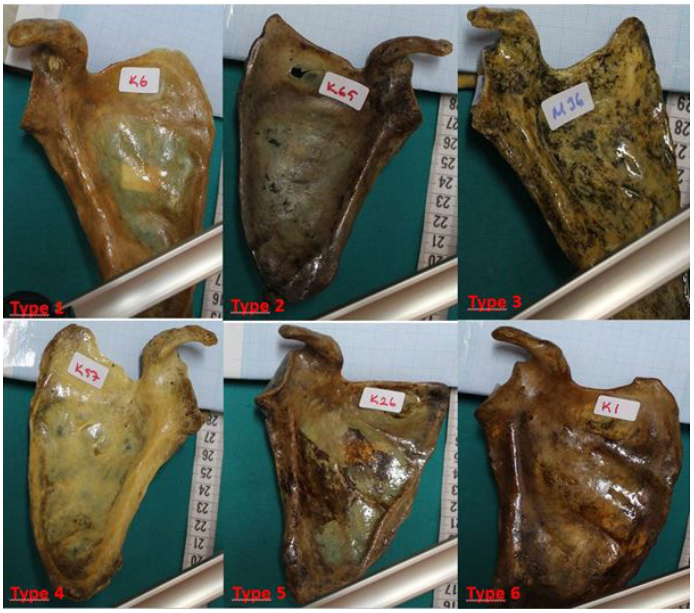

Figure 4. Morphological evaluation of the superior margin of the scapulae. (**Type 1:** Moderately oblique, **Type 2:** Markedly oblique, **Type 3:** Saddle shaped, **Type 4:** Wavy, **Type 5:** Straight, **Type 6:** Straight medially, saddle-shaped laterally)

Statistical analyses

Measurement parameters were evaluated statistically through SPSS 21.0 (IBM, New York, USA) program. The mean±SD (standard deviation), minimum (min.) and maximum (max.) values of all morphometric measurements were given. The association between the right and left parameters was evaluated by independent t test. The association between measurement parameters was analyzed by Pearson's Correlation test. Any p value below 0.05 ($p<0.05$) was accepted as statistically significant.

RESULTS

The present study was conducted on 90 human scapulae including 39.9 (44.4%) right and 50.04 (55.6%) right scapulae. The minimum, maximum, mean and standard deviation (Mean±SD) values of the total measurements of the scapulae and the scapular notch were given; however, the mean and standard deviation values were given for the measurement data of the right and left sides only (Table 1).

Total scapula width (SW) was 96.37±11.15 mm in our study. The scapular width was detected 98.87±7.71 mm on the right and 94.38±13.01 mm on the left. The scapula length (SL) was measured 146.92±13.63 mm on the left, 148.15±13.35 mm on the right, and 147.6±13.41 mm in total. The scapula index (SI) was 146.92±13.63mm on the right, 148.15±13.35 mm on the left, and 63.80±8.29 mm in total. The SW and SI were significantly larger on the right then the left. The SL measurements on the left were larger than the right; however, the difference was not statistically significant ($p>0.05$) (Table 1).

Mean values of STD, MTD, and VD parameters of all scapular notches were 9.90±3,38mm, 8.81±2.51 mm and

10.32±3.85 mm, respectively. Furthermore, results of such measurement data were similar for the right and left sides (Table 1) (p>0.05).

The percentage of distribution between the total, right and left sides of the scapular notch according to the observation and measurement values, and their comparison within the group according to the sides are presented in Table 2. In the observational classification of incisura scapulae, the most common shape was the 'U' shape (n=58, 64.4%); the others were arc-shaped (n=10, 11.1%), large 'V'-shaped (n=9, 10%), 'J'-shaped notch (n=9, 10%), respectively. The foramen-shaped (1%) type formed as a result of ossification of the superior transverse scapular ligament was determined as the least common type.

The most common type in the measurement-based classification of the scapular notch was Type 1 (n=44, 48.9%; VD>STD) the least common type was Type 5 as (n=1, 1.1%). The morphometric classification of the superior margin revealed that the most common type was moderately oblique (n=37, 41.1%) type; however, the saddle-shaped oblique type on the lateral side (1%) was observed as the least common (Table 2). The difference between the right and left sides was not statistically significant in the morphological classification of incisura scapuale and superior margin based on observation and measurement (p>0.05) (Table 2).

Table 1. Measurement values of scapula and scapular notch (mm)

		Total				Right	Left	
		n	Min.	Max.	Mean±SD	Mean±SD	Mean±SD	p
S	SW	90	19.69	119.94	96.37±11.15	98.87±7.71	94.39±13.02	0.046*
	SL	90	117.3	175.5	147.6±13.41	146.92±13.63	148.15±13.35	0.670
	SI	90	15.15	78.39	65.45±7.05	67.51±4.40	63.80±8.29	0.008*
Inc.	STD	76	4.63	18.97	9.90±3.38	9.81±3.58	9.98±3.26	0.832
	MTD	76	3.88	14.98	8.81±2.51	8.87±2.13	8.76±2.80	0.854
		76	2.19	19.95	10.32±3.85	10.19±3.57	10.42±4.11	0.790

(* Significant at the 0.05 level; Independent t-test; S; Measurements of scapulae, Inc.; Measurements of scapular notch, SW: Scapula width, SL: Scapula length, SI: Scapula index, STD: Scapular notch upper of transverse diameter, MTD: Middle transverse diameter of scapular notch, VD: Vertical diameter of scapular notch)

Table 2. Morphological classification distribution percentage of scapular notch and margo superior by sides (%)

	Variable	Combined N(%)	Right N(%)	Left N(%)	χ2	p
Scapular notch of the based on observation classification	Type 1	10 (11.1)	5 (12.5%)	5 (10%)	8.372	0.137
	Type 2	9 (10)	3 (7.5)	6 (12.2)		
	Type 3	58 (64.4)	22 (55)	36 (72)		
	Type 4	3 (3.3)	3 (7.5)	0		
	Type 5	9 (10)	6 (15)	3 (6)		
	Type 6	1 (1.1)	1 (2.5)	0		
Scapular notch of the based on measurement classification	Type 1	44 (48.9)	26 (43.3)	28 (45.9)	3.740	0.809
	Type 2	2 (2.2)	1 (1.8)	1(1.6)		
	Type 3	31 (34.4)	16 (29.1)	23 (37.7)		
	Type 4	1 (1.1)	1 (1.8)	-		
	Type 5	13 (14.4)	11 (20)	9 (14.8)		
Superor margin of the based on observation classification	Type 1	37 (41.1)	20 (50)	17 (34)	3.740	0.809
	Type 2	30 (33.3)	10 (25)	20 (40)		
	Type 3	7 (7.8)	3 (7.5)	4(8)		
	Type 4	5 (5.6)	1 (2.5)	4(8)		
	Type 5	10 (11.1)	5 (12.5)	5 (10)		
	Type 6	1 (1.1)	1 (2.5)			

(*Significant at 0.05 level; Chi-square analysis, STD: Upper transverse diameter of scapular notch, MTD: Mid-transverse diameter of scapular notch, VD: Vertical diameter of scapular notch)

Mean values of measurement data of observational and measurement-based scapular notch and SM were provided in Table 3. The SW and SL data in Type 6 were observed with the highest average. In the typing of the observational scapular notch, the STD and MTD data with the largest mean were found in Type 3, while the data with the smallest mean of measurement was found in Type 4.

The measurement-based incisura scapulae typing revealed that the group with the highest SW average was determined as Type 2, and the group with the lowest average was determined as Type 1. The SI with highest average was detected in Type 2; however, the SI with lowest average was detected in Type 4 (Table 3).

It was detected in the observational classification of the superior margin that the SW with the highest average was found in Type 4 whereas the SL with highest average was detected in Type 3. The SI data with highest average was observed in Type 6 (Table 3).

The association between parameters of the scapulae was evaluated through Pearson's Correlation test. A significant association was observed between many parameters (Table 4). The highest positive correlation was observed between SW and SI parameters on the left (r=0.767). The highest negative correlation was detected between SL and SI parameters on the right (r=-0.548). Although there was a negative association between STD and scapulae measurements (SW, SL, SI) both on the right and on the left, such association was not statistically significant (Table 4).

Table 3. Morphometric measurements of scapular notch and margo superior by sides (mm)

		N	SW	SL	SI	N	STD	MTD	VD
			Mean±SD	Mean±SD	Mean±SD		Mean±SD	Mean±SD	Mean±SD
Scapular notch of the based on observation classification	Type 1	10	97.19±7.88	146.3±14.12	66.71±5.66				
	Type 2	9	87.1±25.87	144.09±12.46	60.15±17.54	9	10.25±2.65	8.78±3.39	12.23±4.57
	Type 3	58	96.79±8.19	147.44±13.63	65.85±4.63	58	10.27±3.4	8.86±2.32	10.21±3.7
	Type 4	3	98.25±7.12	139.13±13.13	70.71±1.71	3	6.32±0.82	8.48±2.82	10.92±1.18
	Type 5	9	100.81±5.26	154.47±10.92	65.4±3.25	9	8.58±3.81	8.71±3.02	8.86±4.39
	Type 6	1	102.55	165.5	61.96				
Scapular notch of the based on measurement classification	Type 1	43	95.44±14.57	147.25±14.34	64.85±8.71	43	8.43±2.16	8.88±2.63	12.64±3.16
	Type 2	2	102.97±2.98	150.5±17.39	68.76±5.96	2	10.07±6.24	9.47±7.09	10.02±6.31
	Type 3	31	97.36±6.21	147.87±12.26	66.13±5.18	31	11.95±3.67	8.67±2.08	7.12±1.91
	Type 4	1	102.55±0	165.5	61.96				
	Type 5	13	95.64±8.09	146.32±13.55	65.59±5.36				
Superor margin of the based on observation classification	Type 1	37	97.19±7.82	145.52±13.07	66.95±3.9	35	9.83±3.71	8.71±2.78	10±3.73
	Type 2	30	94.33±15.98	148.37±14.06	63.59±10.17	30	9.76±2.84	8.89±2.3	10.82±3.98
	Type 3	7	97.41±7.19	153.8±15.6	63.76±6.67	2	10.07±6.95	7.31±4.12	6.92±1.51
	Type 4	5	98.67±8.02	150.6±9.24	65.54±4.02	2	15.55±0.81	11.19±1.37	11.42±4.84
	Type 5	10	97.12±8.65	148.95±12.98	65.33±4.53	6	9.61±2.79	8.82±2.03	11±4.68
	Type 6	1	101.98±0	130.1±0	78.39	1	7.21	8.44	7

(SW: Scapula width, SL: Scapula length, SI: Scapula index, STD: Scapular notch upper of transverse diameter, MTD: Middle transverse diameter of scapular notch, VD: Vertical diameter of scapular notch)

Table 4. Right and left measurement data of correlation relationship (%)

			LEFT					
			SW	SL	SI	STD	MTD	VD
RIGHT	SW	r	1	.534**	.767**	-.156	-.204	-.413**
		p		.000	.000	.322	.195	.007
	SL	r	.735**	1	-.127	-.184	-.069	-.223
		p	.000		.378	.244	.664	.156
	SI	r	.161	-.548**	1	-.049	-.199	-.327*
		p	.320	.000		.757	.206	.035
	STD	r	-.182	-.132	-.068	1	.425**	.033
		p	.302	.458	.700		.005	.838
	MTD	r	.276	.122	.184	.409*	1	.473**
		p	.114	.492	.296	.016		.002
	VD	r	.217	.220	-.053	-.070	.226	1
			.218	.211	.767	.693	.200	

(SW: Scapula width; SL: Scapula length; SI: Scapula index; STD: Scapular notch upper of transverse diameter; MTD: Middle transverse diameter of scapular notch; VD: Vertical diameter of scapular notch)

DISCUSSION

Shoulder pain is an important condition that is frequently encountered in the elderly and young population, restricting quality of life and daily activities. The incidence of this pain varies between 15% and 30% in adults. Shoulder pain is caused by degenerative diseases affecting the glenohumeral joint, the acromiocalavicular joint, and supporting soft tissue structures, inflammatory diseases such as rheumatoid arthritis, and suprascapular neuropathy which is the most important and most common entrapment of the suprascapular nerve (10).

Total scapula length (SL)

The total scapula length was detected as 147.06±13.41 mm in our study (Tables 1-5). Such length measurement was reviewed by different researchers on different populations. The longest scapula length was detected as 156+12.9 mm on German population by Prescher and Klümpen (11). Following the study above, the longest scapula length was detected as 151.16±10.32 mm on Egyptian population by El-din and Ali (12) (Table 5).Taser and Başaloğlu (13) who studied on dry scapulae of the Turkish population found an average SL of 141.5+14.2 mm; however, Coskun et al. (14) detected the average as 98.8±7 mm whereas Aydemir et al. (15) determined it as 147 mm. The SL average was detected as 148.08±13.61 mm in our study. When the length measurement data of the scapulae on dry bones of Turkish population are compared with each other, it is detected that the measurement data made by other researchers are close to our study, except for the studies conducted by Coskun et al. (14).

Total scapula width (SW)

Total scapula width (SW) was 96.37±11.15 mm in our study (Table 1-5). El-din and Ali (12) detected the mean SW as 107.22±9.74 mm in the Egyptian population; however, Kavita et al. (16) determined the mean SW on the Indian population as 105.5±7.6 mm. In Turkish populations, Taser and Basaloglu (13) detected 97.7±7.8 mm; Coskun et al. (14) as 99.29±7.6 mm, and Aydemir et al. (15) as 105 mm. The scapula width is observed as the least value among the studies.

Total scapula index

Limited number of researchers evaluated the SI parameter in the literature. In our study, total scapular notch index was found 65.45±7.05 (Tables 1-5). The following SI values were obtained in the following studies; average 70.93 mm in average on Egyptian population by El-din and Ali (13); 73.32±4.80 mm in average on Indian population by Chhabra et al. (17); 72±11.41mm in average on Northern India by Nazir et al. (18); 71.24±3.1 mm in average on Southern India by Rajeswari and Ramalingam (19); and 75.56±17.67 mm in average on Eastern India by Biswas et al. (5) (Table 5). Our results were found lower than other results.

Lateralization of the scapulae

There are limited studies in the literature that reveal the association between the scapula length and the sides (5,12,15).

Aydemir et al. (15) conducted a research on 40 scapulae of the Turkish population, and measured mean SL as 145 cm on the right side, 148 cm on the left side, and mean SW as 104 cm on the right side and 106 cm on the left side. Furthermore, Aydemir et al. (15) observed that mean SL and SW values were higher on the right scapulae. The mean SL was detected 147.6 mm (146.92±13.63 mm on the right; 148.15±13.35 mm on the left; p=0.670) and mean SW was detected 96.37 mm (98.87±7.71 mm on the right; 94.39±13.02 on the left; p=0.046) in this study (Table 1).

Table 5. The values obtained from the researchers' studies of the length, width and index parameters of the scapulae (mm)

			SL	SW	SI	N	STD	MTD	VD				
	N	Population	Total	N	Right	N	Left	Total	Right	Left	Total	Right	Left
Prescher and Klümpen (12)	214	Germany	156+12.9					104+12.9					
Taşer and Başaloğlu (13)	52	Turkish	141.5+14.2					97.7±7.8					
Coskun et al. (14)	90	Turkish	98.8 ± 7	44		46		99.29±7.6					
Kavita et al. (16)	129	India	145.1±11.7	67	144.6±12.2	62	145.7±11.3	105.5±7.6	104. ±7.7	106.5±7.5			
Singh et al. (20)	129	West India	141.7± 8.9	67	144.60±12.20	62	145.70±11.30	96.4 ± 7	104.60±7.70	106.50±7.50	68.5 ± 4		
El-dın and ali (12)	160	Egypt	151.16±10.32	80	151.05±8.42	80	151.20±9.47	107.22±9.74	107.43±8.07	107.01±9.00	70.93	71.12	70.77
Chhabra et al.(17)	126	India	141.94 ± 12.76	55	141.93±12.88	71	141.94±12.76	103.65 ± 6.82	103.64±6.41	103.67±7.16	73.32 ± 4.80		
Nazir et al.(18)	120	North India	137 ± 20.09					98.16 ± 11.60			72 ± 11.41		
Rajeswari and Ramalingam (19)	100	South India	141.34± 8.5					103.3 ± 6.9			71.24 ± 3.1		
Aydemir et al. (16)	40	Turkish	147	16	145	24	148	105	104	106			
Biswas et al. (5)	200	East Indıa	132.97± 18.00	100	130.61±21.51	100	135.40±13.05	97.96 ± 9.38	98.56±9.48	97.45±9.40	75.56 ± 17.67	78.91±23.5	72.28±6.53
Present study	99	Turkish	147.06±13.41		146.92±13.63		148.15±13.35	96.37±11.15	98.87±7.71	94.39±13.02	65.45±7.05	67.51±4.40	63.80±8.29

N: number of individuals, SW: Scapula width; SL: Scapula length; SI: Scapula index)

Table 6. Comparison of various studies on the suprascapular notch on Rengachary et al. (6) classification (%)

Student	Population	N	Type I (None)	Type II(V shape)	Type III (U shape)	Type IV (v shape)	Type V (J shape)	Type VI (Foramen shape)
Rengachary et al. (6)	Americans	211	8	31	48	3	6	4
Coskun et al. (14)	Turkish	100	5	23	38	13	11	6
Natsis et al.(21)	Greece	423	6	24	40	13	11	6
Sinkeet at al. (22)	Kenya	138	22.5	21	29.6	5.18	18	4
Wang et al. (23)	Chinese	295	9.5	58.16	28.23	-	-	4.08
Albino et al. (24)	Italy	500	12.4	19.8	22.8	31.1	10.2	3.6
Vandana and Patil (25)	India	134	4.5	4.5	35	5.2	34.3	12.5
Gopal ve at. (26)	India	120	15.83	41.66	25	12.5	1.67	3.33
Chhabra et al (17)	India	126	0.79		46	24.6	21.3	2.4
Boyan et al. (27)	Turkish	73	28.8	23.3	13.7	20.5	2.7	5.5
Adewale et al. (28)	Uganda	50	16.3	12.2	51	4	4	10
Okeke et al. (7)	Nigeria	193	3	22	71	1	3	0
Present study	Turkish	100	11.1	9	64.4	3.3	10	1.1

N: number of individuals

Despite the studies conducted by Aydemir et al. (15), the mean values of these parameters were found larger on the right side scapulae in our study.

Biswas et al. (5) carried out a study on 100 right and 100 left scapulae of Indian population and could not find a statistically significant association between SW (98.56 ±9.48 mm on the right; 97.45 ±9.40 mm on the left; p=0.059) and SL (130.61±21,51 mm on the right; 135.40±13.05 mm on the left; p=0.366). However, they detected a significance between sides for SI (78.91±23.5 on the right; 72.28±6.53 on the left; p=0.007) El-Din and Ali (12) detected SI average as 71.12 on the right, and 70.77 on the left. The SI value was detected 67.51±4.40 on the right, and 63.80±8.29 on the left in our study. Although the SI data obtained from our study were observed lower than other studies, we believe that this may be due to racial difference (Table 5).

The scapular notch

The scapular notch is the name of the notch existing on the base of the coracoid process on the superior margin. The suprascapular nerve may be commonly compressed and entrapped on STSL. Differences in the shape and size of the notch, congenital changes such as ossification and comminuted ossification of the STSL, and external mechanical effects are also important factors causing entrapment. Depending on the entrapment, individuals may experience shoulder pain and muscle atrophy on the supraspinatus and infraspinatus muscles (4, 33) (Table 6).

Natsis et al. (21) reported that the size and shape of the scapular notch were the most important factors in the etiopathology of suprascapular entrapment neuropathy. Rengachary et al. (6) reported that a narrow scapular notch may cause predisposition for entrapment neuropathy. Antoniadis et al. (34) stated that individuals with V-shaped scapular notch have higher incidence for entrapment neuropathy. Rengachary et al. (6) stated in their study that the shape of the scapular notch should be considered as the first factor that causes the entrapment of the suprascapular nerve to be supported. They reported that individuals with narrow and sharp incisura scapulae may be exposed to pressure during the passage of the nerve during upper extremity movements, and this may cause microtrauma as a result of injury to the nerve during bending. Albino et al. (24) also reported like Antoniadis et al. (34) that deep and narrow scapular notch may cause the injury of suprascapular nerve.

- **Upper and medial transverse diameter and vertical diameter pf the scapular notch**

Sharma et al. (31) determined the mean upper transverse diameter of the scapular notch as 5.55 mm (5.96 mm on the right; 6.36 mm on the left) on 100 dry scapulae from the Indian population. Similarly, Chhabra et al. (17) also found this length as 7.78±3.09 mm in average on Indian population. We believe that the difference in the results of the studies conducted on the same population may be caused by the difference in the age of the bone. Zhang et al. (35) determined the upper transverse diameter of 308 dry scapulae on 308 Chinese individuals as an average of 10.70 mm on the right side and 10.66 mm on the left side. Khattab et al. (36) determined the upper transverse diameter of the scapular notch as 4.4 mm on 100 dry scapulae from 100 Egyptians. Kastamonu et al. (37) found the mean upper transverse diameter of the scapula as 8.81 mm on dry scapulae of Turkish population. In our study, the mean upper transverse diameter of the scapular notch was determined as 9.90±3.38 mm (9.81±3.58 mm on the right, 9.98±3.26 mm on the left). We observed that the data obtained from this study were close to those obtained by Kastomoni et al. (37). However, the values were quite higher when compared to the researchers working in the Indian population, and lower than the Chinese and Egyptian populations.

Unlike the studies of other researchers, the median transverse diameter of the total scapular notch was determined as 8.81±2.49 mm (8.86±2.10 mm on the right side and 8.76±2.80 mm on the left side) in the present study. Khattab et al. (36) measured the mean transversus scapulae measurements as 2.69 mm on the Egyptian population. The median scapulae diameter data obtained in our study were found higher than the studies of Khattab et al (36). Furthermore, Polquj et al. (8), Polquj et al. (30), Gopal et al. (26), Ahmet et al. (32), provided mean values of measurement-based classification types rather than total mean values of median transverse diameter measurements. Ahmet et al. detected median transverse diameter in Type 1 determined as VD>STD as 3.69 ±0.97; however, they found the median transverse diameter in Type 3 determined as VD<STD 6.46 ± 1.79 mm. In our study, Gopal et al. (26)'s measurements of the scapular notch in Type 1 and Type 3 groups presented close results, while our data on Type 2 was found larger (Table 7).

In our study, the mean vertical diameter of the total scapular notch was found 10.3±3.85 mm (10.19±3.57 mm on the right, 10.42 ±4.11 mm on the left) (Table 1). Kastomoni et al. (37) found the mean upper transverse diameter of the scapula as 5.58 mm on Turkish population. Kale et al. (38) found the vertical diameter of the scapula as 5.5 mm. Sharma et al. (31) determined the mean value as 5.8mm (5.9 mm on the right; 5.9 mm on the left). Chhabra et al. (17) found the mean VD data as 6.39±2.65 mm in their study. Zhang et al. (35) detected scapula depth of 308 dry scapulae on 308 Chinese individuals as 5.63 mm on the right side and 6.28 mm on the left side. Kale et al. (38) found the vertical diameter of the scapula as 9.6 mm. It was determined that the data obtained in our study was larger than the studies conducted.

- **Classification according to the scapular notch**

The scapular notch has been morphologically classified differently by many researchers (8,14,21,39). The scapular notch is often classified under 6 groups in the literature (Table 8). The shape of the scapular notch which is frequently observed by most researchers has been determined as Type 3 ("U"-shaped) (Table 8). Coşgun et al. (14) and Boyan et al. (27) who have conducted researches

on the Turkish population in particular detected shape of the scapulae as Type 3 (38%) and Type 1 (28.8%), respectively. The scapular notch shapes were classified under six groups according to Rengahary (6). The most common scapular notch shape was detected as Type 3 (64.4%). However, the secondly most common scapular notch shape following Type 3 was detected as Type 3 (11.1%). Although there are racial differences, the prevalence of the "U"-shaped scapular notch shape is higher than many researchers.

Table 7. Comparison of studies of scapulae nothc according to measurement-based classification (%)

Student	Ethnicity	N	Type I	Type II	Type III	Type IV	Type V	Type VI
Natsis et al. (21)	German	423	41.85		41.85	7.3	8.3	0.75
Sinkeet et al. (22)	Kenya	135	29		21	4	22	2.9
Polquj et al. (8)	Poland	86	24.40	2.30	54.70	7.00	11.60	
Wang et al. (23)	Chine	295	58.16		28.23	3.00	28.00	
Mady and Shehab (29)	Egypt	132	43.93		45.45	3.03	6.06	1.5
Polquj et al. (30)	Poland	616	24.18	1.95	56.16	4.72	12.99	
Gopal et al. (26)	India	120	20	3.33	55.58	4.16	17.5	
Sharma et al. (31)	India	100	34		39	5	20	2
Ahmet et al. (32)	Egypt	65	39.99	1.54	47.68	3.08	7.69	
Vandana and Patil (25)	India	134	8	3.2	70.1	13.7	4.8	0
Present study	Turkish	100	48.9	2.2	34.4	1.1	0	0

N: number of individuals

Table 8. Mean STD, MTD and VD values according to measurement-based classification (mm)

	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Polguj et al. (8)	6.92±1.72	8.10±1.56	8.83±3.26	8.58±3.94	8.10±1.56	7.12±2.46	9.94±2.49	8.10±1.56	5.46±2.04
Polguj et al. (30)	7.01±3.19	8.02±2.28	12.46±3.66	7.68±2.34	7.98±2.29	9.17±2.63	10.33±2.74	8.0±2.29	7.02±2.71
Gopal et al. (26)	8.98 ± 2.76	5.92 ± 2.69	8.50 ± 1.98	9.08 ± 2.99	3.82 ± 1.50	8.06 ± 1.78	12.55 ± 4.07	5.92 ± 2.69	7.31 ± 2.02
Ahmet et al. (32)	5.79 ± 0.98		8.56 ± 1.80	3.69 ±0.97		6.46 ± 1.79	8.7 ± 1.43		5.86 ± 1.27
Present study	8.43±2.16	10.07±6.24	11.95±3.67	8.88±2.63	9.47±7.09	8.67±2.08	12.64±3.16	10.02±6.31	7.12±1.91

(STD: Scapular notch upper of transverse diameter; MTD: Middle transverse diameter of scapular notch; VD: Vertical diameter of scapular notch)

It was stated by Singh (20) that the most common variant of suprascapular entrapment neuropathy is the ossification of the scapular notch caused by the ossification of the scapulae. It was reported in the literature that the calcification incidence of the superior transverse scapular ligament varies between 0% and 12.79% (Table 8). The incidence of such variant was detected as 1.1% in our study. Similarly, Coskun et al. (14), Boyan et al. (27) and Bayramoglu et al. (39) who conducted research on Turkish population determined this rate as 6%, 5.5% and 12.5%, respectively.

Adewale et al. (28) examined the variation of SL and SW according to types in the classification of the scapular notch based on observation and detected the SL as 15.14 cm and the SW is 10.39 cm in Type 1; SL as 11.90 cm and SW as 8.18 cm in Type 2; SL as 14.37 cm and SW as 9.97 cm in Type 3, and SL as 15.55 cm and SW as 10.78 cm in Type 4. In this study, we detected the SL as 14.63 cm and the SW is 9.71 cm in Type 1; SL as 14.4 cm and SW as 8.7 cm in Type 2; SL as 14.7 cm and SW as 9.67 cm in Type 3, and SL as 13.9 cm and SW as 9.82 cm in Type 4.

The scapular notch has been classified based on observation as well as measurement by many researchers (8,21-23,25,29,32). In our study, the scapular notch was examined morphometrically and classified under 5 groups like in the study of Polquj et al. (31). In this study; the rate of Type 1 (VD>STD) was 48.9%, Type 2 (VD=STD) was 2.2%, Type 3 (VD<STD) was 34.4%, Type 4 (Foramen-shaped) was 1.1%, Type 5 (no notch) was 14.4% (Table 2).

Type 3 was detected as the commonly observed type by researchers in the literature (VD<STD) (8, 25,26,30-33). The incidence of Type 3 and Type 1 were determined equally by Natsis et al. (21).

Sinkeet et al. (22) stated that they frequently detected Type 1 on Kenyan population (29%) like Wang et al. (23) on Chinese population (58.16).

Classification of the superior margin

Singroha et al. (9) classified the superior margin in five types as follows; horizontal type (42%), moderately curved type (27%), significantly curved type (15%), saddle-shaped (12%)

and corrugated (6%). They reported that the most common superior margin shape in their study was the horizontal type (40%). In our study, the typing performed by Singroha et al. (9) was revised and classified under 6 groups. The most common superior margin shape on the right side was moderately oblique (50%) whereas significantly oblique (40%) on the left. There are a limited number of studies in the literature in which scapulae typing is done according to the shape of the superior margin. Therefore, we believe that further researches would support racial differences.

The association between parameters

Polquj et al. (3) reported that they have deeper notches in longer scapulae in their study. A negative but insignificant correlation was found between scapulae measurements and scapular notch measurements in this study (Table 4). A strong correlation (r=0.735 on the right; r=0.534 on the left) was found between SL and SW detected in our study.

Limitations of the Study

This study has two important limitations. The first of these is that our study was carried out only on dry scapulae. The second limitation is that genders of the scapulae is not known. Comparison of morphometric measurements by gender may be a guide for sexual dimorphism in future studies.

CONCLUSION

Recognition of the morphological structure of the scapulae is very important for the treatment of shoulder joint diseases, the design of suitable shoulder implants, understanding the shoulder pathologies that may occur in this region, for determining the insertion sites in shoulder arthroscopy, and surgical procedures planned to be performed on the region. In our study, morphometric and morphological examinations were carried out on scapulae of Turkish population. We believe that the data obtained would contribute to the literature.

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MEDICAL RECORDS-International Medical Journal

Research Article

Comparation with Spectrophotometric and Liquid Chromatographic Methods of Pharmaceutical Forms of Ivermectin

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Abstract

Aim: Ivermectin is a semi-synthetic parasiticide that is used to treat parasitic disorders. Herein, In this study, LC chromatographic and UV spectrophotometric methods were developed and validated for the determination of ivermectin in different ivermectin preparations.

Materials and Methods: In the LC chromatografic method, chromatographic separation was performed using an Agilent Extend-C18 column. Acetonitrile (20/80, v/v) and ultra-pure water was used as mobile phase at a flow rate of 1.2 mL/min. Eluents were determined at a wavelength of 245 nm and the values of ivermectin solutions were detected by spectrophotometric technique at the same wavelength. Lambert–Beer plots showed linear relationships at 6 different concentrations in the range of 10 to 60 µg/mL. Both methods adhered to the protocols published by ICH guidelines Q2(R1) to validate analytical methods.

Results: The developed analytic methods were statistically validated. As a result of the analyzes performed with spectrophotometric and liquid chromatographic methods, it was determined that both methods were precise, accurate and robust with a RSD < 1% result. Recovery values were within the normal range (98-100%). Statistical comparison of both analytical methods was made and there was no statistical significance between them.

Conclusion: These developed methods have been found to be reliable, fast, accurate and simple for tablet and injectable ivermectin forms and can be used for quality control tests. HPLC and UV spectrophotometric methods have shown that they are both adequate to determine the amount of ivermectin in raw materials, tablets and injectable solutions. These methods can be applied in a short time and easily. They can be used successfully in quality control analyzes to quantify and identify ivermectin in marketed formulations.

Keywords: Ivermectin, HPLC, UV, method, validation

INTRODUCTION

Few treatment protocols exist to reduce morbidity and mortality from COVID-19. Although corticosteroids have been shown to reduce mortality in severe diseases caused by COVID-19, no precise treatment data can prevent the disease and reduce hospitalizations and deaths (1). Ivermectin is used to treat various internal and external parasite infestations. In addition to its antimalarial activity, it has anti-amoebic, anti-inflammatory, and antiviral effects (2-5). Ivermectin's another mechanism of action, inhibiting the in vitro replication of some positive single-stranded RNA viruses, has been the focus of attention (6-9). Various analytical methods, such as HPLC (10-14), electrospray ionization mass spectrometry (15), and various liquid or mass spectrometric or chromatographic methods, were used to determine ivermectin (16-19). A European Pharmacopoeia monograph was also made to analyze and identify several substances in samples of active pharmaceutical ingredients (20). In addition, an injectable product was analyzed with only one HPLC method for ivermectin (21). The method specified in the United States Pharmacopoeia is not preferred by quality control laboratories due to the long chromatographic study period. Therefore, there is still a great need for a sensitive, efficient and robust analytical method for the routine analysis of ivermectin in injectables and various end products.

Herein, we developed and validated new liquid chromatographic and spectrophotometric methods for ivermectin assay, including determining and identifying ivermectin in pharmaceutical products. These analytical

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methods are precise, accurate, linear, selective, and robust. Therefore, these newly developed and validated methods would suit quality control laboratories to analyze ivermectin in pharmaceutical products. Analysis of variance was used to compare the results of these analytical methods and their rest reliability was assessed by routine quality control analysis.

MATERIAL AND METHOD

Reagents

The reagents used in the research and the places they were supplied were as follows; Ivermectin from Sigma-Aldrich Chemie Gmb (St Louis, MO, USA); Bomectin® injection 500 mL (10 µg mL-1, ivermectin) from Elanco Australasia Pty Ltd (North Ryde, New South Wales, Australia); Stromectol (3 mg ivermectin) was from Merck Sharp & Dohme and Acetonitrile (HPLC grade) was from Sigma-Aldrich Chemie GmbH. Merck Millipore (Bedford, MA, USA) was used for ultrapure water.

Analytical Instruments and Conditions

HPLC analyzes were performed with Agilent 1260 system (Palo Alto, CA, USA) using a C18 (4.6 mm x 250 mm, 5.0 µm) column at 25°C room temperature. This system consisted of software, quad pump, UV detector and sample collector. Chromatographic detection was performed at 245 nm. The mobile phase measurements were performed at a flow rate of 1.2 mL/min and an injection volume of 20 µL, and the solution used for the measurements contained acetonitrile (20/80, v/v) and ultrapure water.

A Shimadzu UV 1800 dual-beam (Shimadzu, Kyoto, Japan) spectrophotometer with UV-Probe software and a 1 cm quartz cuvette was used for ultraviolet spectrophotometric analysis. Standard solutions were scanned in a UV spectrophotometer to determine the λmax value in the 200-400 nm range, and measurements were obtained against methanol in a blinded manner. The amount of ivermectin was determined and the absorbance values of the standard solution were measured at a wavelength of 245 nm and recorded. It has been shown that ivermectin absorbance values at this wavelength are proportional to standard mixture concentrations.

Preparation of Sample and Standard Solutions

Ivermectin standard solutions: It was dissolved by adding 25 mg of ivermectin reference standard and 20 mL of methanol to dissolve into a 50 mL volumetric tube. Ultrapure water was added to the tube until the total volume was 50 mL. This 500 µg/mL stock standard mix was then diluted with ultrapure water in ten increments to obtain six different concentrations of 10-60 µg / mL standard mix.

Ivermectin sample solutions: Powder tablets equivalent to 25 mg ivermectin raw material and/or 25 mg ivermectin were added to a 50 mL measuring bottle by adding 20 mL methanol and dissolved. The total volume was made up to 50 mL using methanol. Four milliliters of this solution were taken and diluted with methanol in a 50 mL measuring flask to yield 40 µg/mL ivermectin solution.

Validation

The optimized chromatographic and spectrophotometric methods are fully validated following protocols specified in ICH guidelines Q2(R1) to validate analytical methods (22). Parameters such as linearity, stability, sensitivity and system suitability tests were measured for validation purposes.

Linearity: Calibration curves of ivermectin prepared in 6 different densities of 10-60 ug/mL were plotted concentration versus peak area in triplicate (the chromatographic method and concentration versus absorbance value) for the spectrophotometric method. The evaluation of the linearity of the samples was made by regression analysis using the least squares method.

Precision: For six tablet samples (n = 6) at a concentration of 40 µg/mL, intraday precision analysis was performed by UV and HPLC methods. To determine the between-day precision values, measurements were made on three sequential days (n = 18) and the ivermectin content and relative standard deviations (RSD) values were calculated.

Accuracy: The accuracy of the methods was determined by recovery studies at three levels (80%, 100% and 120% of test concentration). This was done by analyzing a sample of known concentration and comparing the measured value with the "real" value. A well-characterized sample solution (40 µg/mL of ivermectin) was used. Samples were prepared in triplicate for each concentration, analyzed by UV and HPLC methods, and recovery percentages were calculated.

Specificity: 40 µg/mL ivermectin sample solution prepared according to the procedure was injected into the chromatographic system to identify possible interfering peaks.

The wavelength range of 200-400 nm was used for spectrophotometric analysis of ivermectin samples. As a result of the analyzes, the interference bands were observed at a wavelength of 245 nm. The spectral peak purity of ivermectin obtained as a result of the analyzes was measured with a diode array detector and ultraviolet spectra were evaluated.

Limits of Quantitation and Limits of Detection: The LOQ and LOD were used to evaluate the sensitivity of both chromatographic and spectrophotometric methods. LOD and LOQ values were calculated according to the formulation in the 1st and 2nd equations. The slope of the calibration curve and the standard deviation of the y-intercept were used in the calculation.

LOD = 3.3xσ/S (1)
LOQ = 10xσ/S (2)

S: the slope of the calibration curve
σ: standard deviation of y-intercept

Analysis of Marketed Formulations

Stromectol® tablet samples containing ivermectin and Bomectin injectable solution were prepared as described in the previous sections and analyzed using HPLC and UV methods. Ivermectin contents were determined using these two methods. ANOVA-Tukey test was used to determine the significance levels and P<0.05 was considered significant.

Comparative Analysis

Both analytical methods were used in these formulations. Evaluation of recovery percentages was done using F and t-test.

Stability of Solutions

The results of the reference standard solutions over 24 hours were used for stability assessment. Standard solutions were used for stabilization analyzes at room temperature (25°C) protected from light.

RESULTS

Method Development

Various preliminary studies were carried out to optimize the conditions for ivermectin quantification for chromatographic method development. First, only ultrapure water was tested without the use of organic modifiers and the results were recorded. Different ratios of acetonitrile solutions were used for optimum conditions. A good ivermectin peak and symmetry were determined at 80% acetonitrile ultrapure ratio.

Finally, it was determined that the mobile phase consisting of ultra-pure water and acetonitrile (20:80, v/v) provides more powerful theoretical plates (>7.000) and a peak queuing factor (<1.0). Mobile phases between at different flow rates (0.8–1.5 mL min-1) and containing organic solvents and ultrapure water at different pH ranges were tested. The best chromatographic conditions were achieved using an isocratic mobile phase comprising ultra-pure water (pH = 7.0)-acetonitrile (20/80, v/v) at a flow rate of 1.2 mL min−1 on an Agilent Extend-C18 (4.6 mm × 250 mm, 5.0 µm) that was kept at 25°C. In addition to being economical, the analysis was carried out at 25°C, which offers advantages such as increased column efficiency, low column pressure, and good chromatographic peak shape.

The eluent was monitored using a UV detector set to 245 nm. The ivermectin retention time in these chromatographic conditions was 12.54 minutes (Figure 1). Tablet samples and injection forms were analyzed for 1 hour to ensure that no matrix components remained in the column at the specified conditions. However, analyzing more than 15 minutes will both prolong the analysis time and increase the cost. No overlapping peaks were observed in the analysis of the samples during consecutive 15-minute analyzes. Therefore, the analysis time was set as 15 minutes.

The UV spectrum of ivermectin in the 200-400 nm range was evaluated (Figure 2). Ivermectin has shown sufficient molar absorptivity at a wavelength of 245 nm. The 245 nm wavelength showed higher selectivity for potantial interfering composites in the samples and therefore the 245 nm wavelength was chosen for detection.

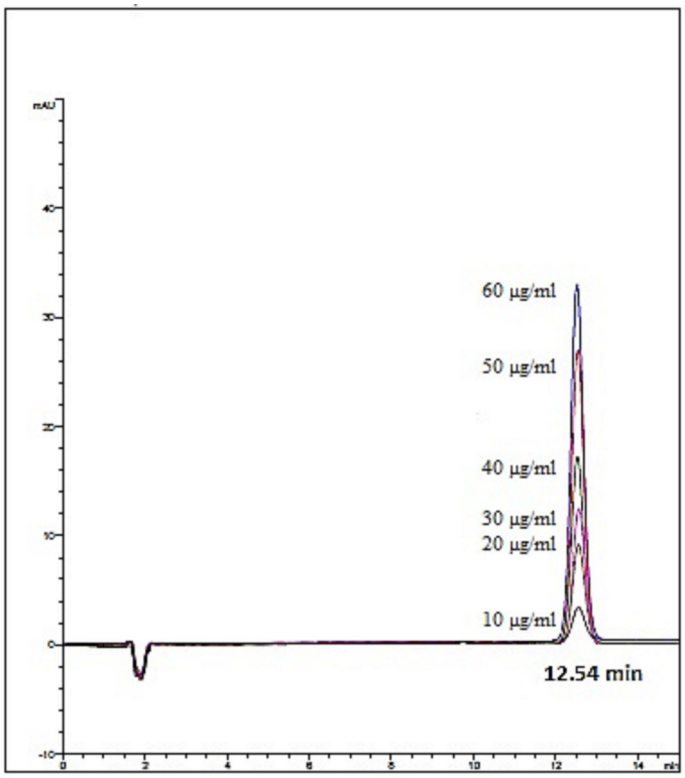

Figure 1. Overlay chromatogram obtained for ivermectin standard solutions (10-60 µg/ml, Retention time: 12.54 min.)

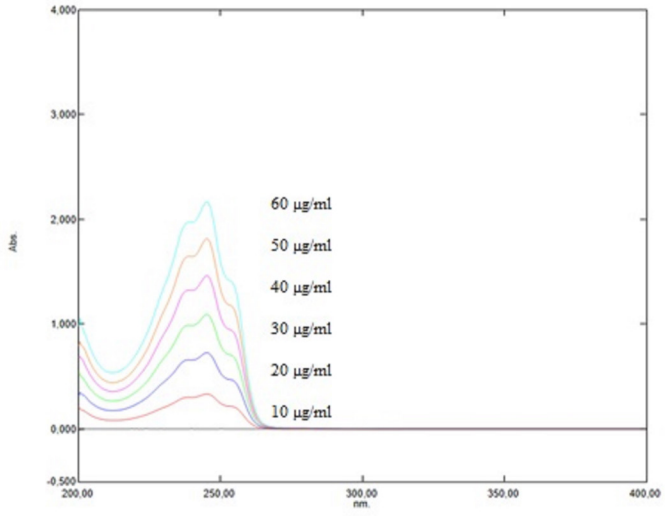

Figure 2. Overlay spectrum of ivermectin standard solutions (10-60 µg/ml)

Analytical Validation

A linear relationship was observed between ivermectin concentrations and the responses of both UV and HPLC methods (Table 1). The regression coefficient (R2) was high in both methods (>0.999) and there was no important different in linearity in the analyzed range.

As a result of the analyses, % RSD values for both analytical methods were lower than 2.0 (table 2). This low is an indication that the sensitivity of the methods is good. Accuracy was investigated using the standard addition method. Both analytical methods of recovery were close to 100% and showed sufficient accuracy (Table 1).

Table 1. The linearity data obtained for ivermectin by both methods

Regression parameters	HPLC	UV
Regression coefficient (R^2)	0.9999	0.9997
Slope±standard error	11.6040±0.1300	0.0366±0.0003
Intercept±standard error	-6.1067±1.078	−0.0153±0.0133
Relative standard error (%)	1.1582	0.55
Concentration range (µg/mL)	10-60	10-60
Number of points	6	6

Table 2. Validation parameters of the evaluated methods

Validation parameters	HPLC	UV
Intra-day precision, (RSD %, n = 6)	0.46	0.66
Inter-day precision, (RSD %, n = 18)	0.67	0.62
Accuracy, (mean recovery, %, n = 9)	99.62	98.95
LOD (µg/mL)/LOQ (µg/mL)	0.80/2.30	2.90/8.90
Concentration range (µg/mL)	10-60	10-60
Number of points	6	6

In the specificity analyzes performed by HPLC method, the ivermectin peak purity was greater than 99% in the sample mixture chromatograms. These results showed that the other compounds diverged from the main peak. No interference peaks were detected in the chromatogram obtained from the tablet excipient mixture used in the analysis of the retention time of ivermectin.

Absorption bands at 245 nm were not observed in the absorbance spectrum obtained from the tablet excipient mixture in methanol in quantifying the UV method. Therefore, this wavelength was shown to be selective for ivermectin in the UV method.

LOD and LOQ values for both methods were calculated as 0.80 and 2.30 µg/mL, respectively (Table 2). In the UV analysis at a wavelength of 245 nm, the absorbance value of the ivermectin standard solution at a concentration of 2.90 µg/mL was found to be 0.0908. Therefore, this concentration is set as the detection limit. The absorbance value of ivermectin standard solution at a concentration of 8.90 µg/mL at a wavelength of 245 nm was measured as 0.3104 (Table 2). Therefore, this concentration is set as the quantitation limit. According to the results obtained, it is proved that the HPLC method is a more sensitive method that allows determining the amount of ivermectin at concentrations about four times lower than the UV.

Marketed Formulations Analysis

The validated chromatographic and spectrophotometric methods were applied to the analysis of ivermectin in raw material, Stromectol tablets, and Bomectin injection solution (Table 3). As a result of the statistical analysis, no significant difference was observed between the methods in the ANOVA test for tablets and injectable solutions of ivermectin. Tukey's multiple comparison test showed that the means obtained by these methods for raw material analysis were statistically equivalent ($p<0.05$). Tukey test for analysis of tablets revealed statistical equivalence ($p<0.05$) between HPLC and UV averages.

Table 3. Ivermectin contents in raw material, tablet sample, and the injectable solution obtained by HPLC, and UV (n=12)

Samples	Ivermectin content (%) ± SD	
	HPLC	UV
Raw material	99.82±0.27	99.47±0.42
Tablet	98.94±0.53	98.03±0.65
Injectable solution	99.22±0.36	99.17±0.51
F-testi	0.31/0.52	
$F_{calculation}/F_{table}$		
t-testi	1.58/2.81	
$t_{calculation}/t_{table}$		

[a]SD = Standard deviation

Even though spectrophotometric analysis assessed degradation products or related chemicals with comparable chemical structures, potential interactions in raw material studies were not determined in any of the evaluated methods. It was observed that the chromatographic method is the most sensitive and selective method. This method can be successfully utilized for the determination of the amount of ivermectin. However, the time and expense of analysis cannot be overlooked. The spectrophotometric approach is more cost-effective and needs less analytical time while also being simple to apply. Because ivermectin is such a widely used antiparasitic drug, it is critical to develop and validate, reliable and straightforward procedures to verify the quality of raw materials and pharmaceutical formulations on the market today.

Statistical Comparison of Methods

As a result of the analyzes performed, the data obtained from both methods were statistically evaluated at the 95% confidence interval using F and t tests, and no significant difference was observed in either method (Table 3).

Table 4. Standard solution stability (n=3, 50 µg/mL)

Time period, h	Peak area	Avg. peak area	SD	RSD, %	Retention time, min	Avg. retention time, min	SD	RSD, %
0	573.0	573.2	0.20	0.027	12.553	12.547	0.018	0.142
	573.3				12.561			
	573.2				12.527			
24	573.5	572.8	0.60	0.109	12.536	12.535	0.018	0.144
	572.3				12.517			
	572.6				12.553			
48	572.4	572.7	0.30	0.053	12.532	12.534	0.006	0.045
	572.6				12.529			
	573.0				12.540			

It is obvious that both methods can be used for the determination of ivermectin in different pharmaceutical forms.

Stability of Standard Solutions

Standard solution injections were made into the HPLC system at eight-hour intervals, stability measurements of the standard solutions to be used as reference for 24 hours were made, and the retention times and peak areas were recorded. RSD % values were measured as 0.144% for the retention time and 0.109 for the peak area (Table 4). In addition, no significant change was detected in the active substance in the standard solution.

DISCUSSION

Analysis of different pharmaceutical forms of ivermectin was evaluated by two different methods (HPLC, UV spectrophotometric).

The results obtained from the developed analytical methods were statistically verified. The results showed that the spectrophotometric and liquid chromatographic methods were linear, precise, accurate, robust and durable with RSD < 1.00%, and the recovery percentage was within the specified limits (98-102%). A statistical comparison of these analytical methods was then made and the results of both methods showed no significant difference. At the 95% confidence interval, there was no statistically significant difference between the two techniques. The linearity data obtained for ivermectin by both methods, the validation parameters of the evaluated methods, the ivermectin contents in the raw material, tablet sample and injectable solution obtained by HPLC and UV, statistical comparison and standard solution stability results are given in Tables (I-V). In addition, the overlay chromatogram obtained for ivermectin standard solutions and the overlay spectrum results of ivermectin standard solutions are shown in Figures (1-2). The results of the analysis were explained in detail in the results section. The UV spectrophotometric method is more advantageous than the LC chromatographic method, as it is inexpensive, takes less time, and does not require detailed procedures and processes. However, according to the statistical comparisons, the results of the LC chromatographic method are more precise and accurate than the UV spectrophotometric method.

CONCLUSION

HPLC and UV spectrophotometric methods have shown that they are both adequate to determine the amount of ivermectin in raw materials, tablets and injectable solutions, and reliable results were obtained. No interfering absorption bands were observed in the UV spectrophotometric method at a wavelength of 245 nm, and no interference peaks were detected during the retention time of ivermectin in the LC chromatographic method. These methods are quick and simple. It can be used successfully in quality control analyzes to measure and identify the amount of ivermectin in commercially available preparations.

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Research Article

The Relationship between *Helicobacter Pylori* and Intestinal Parasites in Patients with Peptic Ulcer

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Abstract

Aim: This study aimed to evaluate the frequency of *Helicobacter pylori* (*H. pylori*), risk factors, and co-infection with intestinal parasites in adult patients presenting gastrointestinal complaints.

Materials and Methods: The working group of the study consisted of 385 patients with gastrointestinal complaints. A questionnaire including questions aiming to canvass the socio-demographic features, lifestyles, and complaints of the patients was administered to the study population. Cellophane slide method, native-lugol, sedimentation and Modified kinyoun acid-fast methods were used for the diagnosis of parasites in stool, under microscope. The *H. pylori* antigen was studied in the stool sample taken for the diagnosis of *H. pylori*.

Results: *H. pylori* positivity was found to be 27.79% in the patients included in the study. 76.6% of those who are positive for *H. pylori* are women, and the positivity rate was found to be higher at the age of 40 and over (75.7%). The majority of patients with *H. pylori* positivity expressed being married (73.8%), having middle / low-income (89.7%), having a low educational background (82.2%), living in a village (55.1%), and in a nuclear family (72.2%) ($p<0.001$). *H. pylori* positivity was higher in those who used tap water (40.2%) and those who had a vegetable-based diet (75.7%) ($p<0.001$). The study found a statistically significant correlation between *Entamoeba histoloytica* and *Enterobius vermicularis* positivity and *H. pylori* positivity ($p<0.05$ $p<0.001$, respectively). The calculated odds ratio showed that *H. pylori* positivity was 1.19 times higher in *Entamoeba histoloytica* positivity and 11.27 times higher in *Enterobius vermicularis* positivity.

Conclusion: Larger and more comprehensive studies should be performed to understand better the epidemiology, clinical effects, treatment, and control of *H. pylori* co-infection.

Keywords: Peptic Ulcer, *Helicobacter pylori*, Intestinal Parasites

INTRODUCTION

Helicobacter pylori (H. pylori) is a well-known stomach bacterium for its role that triggers the development of peptic ulceration and chronic gastroenteritis predisposing to gastric cancer. It is assumed that about half of the world's population is infected with *H. pylori*, mostly in developing countries (1,2). Although the actual transmission route is not known precisely, it has been reported in several publications that it is transmitted by the fecal-oral or oral-oral route (2,3). However, it is controversial whether the human-to-human transmission route of *H. pylori* is oral-oral, fecal-oral, or otherwise (3,4). Some researchers have reported that human-to-human transmission can occur through the fecal-oral route and oral-oral route (2,3,5). Due to the uncertainties about the transmission route of *H.pylori*, transmission cannot be prevented, and its prevalence is relatively high (3).

Similarly, intestinal parasites affect millions of people worldwide and can cause general gastrointestinal symptoms such as abdominal pain, nausea, and vomiting, as well as species-specific symptoms. *Entamoeba histoloytica* is a species of the genus Entamoeba and

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anaerobic protozoa. Mature cysts of the environmentally resistant *Entamoeba histolytica* are ingested with infected food or water. A protozoa coming into the large intestine turns into trophozoites in the intestine, invades the colon, and presents with different clinical pictures ranging from asymptomatic carriage to amoebic colitis (6). *Enterobius vermicularis* is a common parasite. It is a nematode commonly found in temperate regions with tropical climates. As with *H. pylori*, its contagiousness depends on many factors such as hygiene, living environment, and socio-economic status. It is more common in places with many human-to-human contacts, such as nurseries, boarding schools, and barracks. Transmission often occurs through the fecal-oral route of egg retrieval. The parasite can also cause symptoms related to the digestive system, nervous system, urogenital system (7). All three pathogens are transmitted by the fecal-oral route and are very common in underdeveloped countries with poor hygienic conditions (2,6,7).

The prevalences of intestinal parasites and *H. pylori* infection are very close to each other (8). This suggests a strong possibility of co-infection. This study aims to evaluate the frequency of *H. pylori*, risk factors, and co-infection with intestinal parasites in adult patients presenting to a doctor with gastrointestinal complaints.

MATERIAL AND METHOD

Ethical approval

Ordu University Clinical Research Ethics Committee approved this study with date 05/02/2016 and decision number 2016/4.

Patients and study design

The working group of the study consisted of 385 patients who presented to the internal medicine outpatient clinic with digestive system complaints and agreed to bring samples to the Parasitology Department were included in the study. The data about the patients were canvassed through a questionnaire including questions about socio-demographic characteristics of the patients such as age, gender, educational background, economic status, marital status, drinking water, feeding, and companion animals people keep, lifestyles, and their complaints. The patients were informed that if parasites were not found in the first stool examination, the examination should be repeated three times at different times within ten days.

Data Collection and Examination

Stool collection containers were given to the patients, and they were informed that 3-4 tables poons of stool samples for those with diarrhea and a walnut-sized sample for those without diarrhea should be placed in a container, and the container should be tightly closed and delivered to the parasitology laboratory within 1 hour. In addition, the cellophane band method used in the diagnosis of *Enterobius vermicularis*, whose eggs are not usually seen in the stool, was administered before the toilet or bath. The cellophane tape was cut in 10-15 cm length, placed on a sticky side on a stick or a pen, and touched around the patient's anus to allow the eggs to adhere. The tape was flatly attached to a clean slide and examined under a microscope. In terms of the patients' privacy, those who want to make the application themselves were explained in detail. To say that the patient was not infected with the parasite, are-examination was performed every 3-4 days. Cellophane tape method, native-Lugol, sedimentation, and modified Kinyoun's acid-fast methods were used to diagnose parasites in stool (9-11). After the samples were prepared and stained, they were examined under a microscope.

H. pylori stool antigen was performed with the one-step *H. pylori* antigen rapid diagnostic kit (IHP-602, Acon Laboratories Inc, San Diego, USA) with 99.0% sensitivity and 98.9% specificity. The test was performed following the manufacturer's recommendations. Approximately 50 mg of stool samples were taken from at least three different stool samples with the special applicator included in the kit. Two-three drops of this mixture were dropped on the window part of the test cassette. The test result was evaluated after incubation for 10 minutes at room temperature. In cases where the control line did not appear at the end of incubation, the test was repeated for the same patient.

Data Analysis

The descriptive statistics were reported as the frequencies (n and %). Two-way contingency tables were generated, and the two-way chi-square test was used to examine the association between two categorical variables. Phi or Cramer's V coefficients were used to measure the degree of association between qualitative variables depending on the level of measurement. In the *H. pylori*-positive group, a one-way chi-square test was used to compare categorical variable frequencies. In the chi-square tests, if a cell had an expected frequency below 5, the likelihood ratio chi-square value was used instead of the Pearson chi-square value. $p<0.05$ value is accepted as significant. All statistical analyses were performed using SPSS v25.0 (IBM, Armonk, NY, USA).

RESULTS

Method Development

H.pylori positivity rate was 27.79% (n=107). Of the patients, 73% (n=281) were women and 27% (n=104) were men. The distribution of the patients by age groups was 8.1% (n=31) under the age of 25, 17.4% (67) aged 25-39 and 74.4% (n=287) aged 40 and over, respectively. While 22.6% (n=87) of the patients were single, 77.4% (n=298) were married.

The change of *H. pylori* status according to the demographic

characteristics of the patients was analyzed using the two-way chi-square test (Table 1). It was observed that the presence or absence of *H. pylori* did not differ according to the demographic characteristics of the patients (p>0.05). In addition, the correlation coefficients calculated between the presence of *H. pylori* and demographic variables were also quite weak and statistically insignificant (p>0.05).

A one-way chi-square test was used to analyze whether there was a difference between the frequencies of the categories of demographic variables in *H. pylori* positive patients (Table 1). The proportion of *H. pylori*-positive women (76.6%) was significantly higher than that of men (23.4%) (p<0.05). A significant change was also found according to age groups (p<0.05). *H. pylori* positivity rate was higher in patients aged 40 and over (75.7%). The study results indicate that *H. pylori*-positive patients showed a significant change according to demographic variables such as marital status, economic status, educational background, and place of residence (village, district, city, etc.) (p<0.05). The vast majority of patients with *H. pylori* positivity expressed being married (73.8%), having middle / low income (85.7%), being illiterate or primary school graduates (89.7%), living in a village (55.1%), and living in a nuclear family (72.2%).

Table 1. *H. pylori* positivity broken down by demographic variables

		H. pylori			
		Positive	p†	Negative	p‡
Age	<25	13 (12.1%)	0.001	18 (6.5%)	0.065
	25-39	13 (12.1%)		54 (19.4%)	
	Aged 40 and over	81 (75.7%)		206 (74.1%)	
Gender	Women	82 (76.6%)	0.001	199 (71.6%)	0.317
	Men	25 (23.4%)		79 (28.4%)	
Civil Status	Single	28 (26.2%)	0.001	59 (21.2%)	0.299
	Married	79 (73.8%)		219 (78.8%)	
Economic Condititon	Good	11 (10.3%)	0.001	18 (6.5%)	0.152
	Middle and low level	96 (89.7%)		260 (93.5%)	
Educational Background	Illiterate	38 (35.5%)	0.001	79 (28.4%)	0.361
	Elementary school	50 (46.7%)		133 (47.8%)	
	High school	10 (9.3%)		42 (15.1%)	
	Higher education	9 (8.4%)		24 (8.6%)	
Residence Place	Village	59 (55.1%)	0.001	182 (65.5%)	0.147
	District / town	14 (13.1%)		24 (8.6%)	
	City	34 (31.8%)		72 (25.9%)	
Employment Status	Unemployed / trades people	88 (82.2%)	0.001	215 (77.3%)	0.292
	Worker / civil servant / retired	19 (17.8%)		63 (22.7%)	
Employment Status of Spouse	No spouse	20 (18.7%)	0.001	48 (17.3%)	0.919
	Unemployed / trades people	47 (43.9%)		116 (41.7%)	
	Worker / civil servant / retired	38 (35.5%)		107 (38.5%)	
	Private sector	2 (1.9%)		7 (2.5%)	
Life Style	Living alone	8 (7.5%)	0.001	11 (4.0%)	0.178
	With friends / dormitory	2 (1.9%)		1 (0.4%)	
	Nuclear family	77 (72.0%)		220 (79.1%)	
	Extended family	20 (18.7%)		46 (16.5%)	

†: One-way chi-square test, ‡: Two-way chi-square test

According to the patients' lifestyles, the variation of the presence or absence of *H. pylori* was analysed with the two-way chi-square test (Table 2). It was observed that the presence of *H. pylori* in the patients showed a significant change only according to the type of water used (p<0.05) but did not show a significant change according to the other lifestyles (p>0.05). While *H. pylori* was negative in most tap water users, *H. pylori*-positive rate was higher in those using spring water/water in bottles or dispensers available in the market. The correlation coefficient between *H. pylori*-positive or negative status and the type of water used was calculated as 16.7%. In addition, the correlation coefficients calculated between the presence of *H. pylori* and other lifestyles were also quite weak and statistically insignificant (p>0.05).

A one-way chi-square test was used to analyze whether there was a difference between the frequencies of lifestyle categories in *H. pylori*-positive patients (Table 2). A significant difference was found between the water types used, pets kept, feeding patterns, and knowledge about infectious diseases (p<0.05). While the rate of those using tap water was 40.2%, the rate of those who did not keep pets at home was 57.1%. While, on the other hand, the rate of the patients who ate mainly vegetables was 75.7%, and the rate of those who did not know about infectious diseases was 53.3%.

Table 2. *H. pylori* positivity broken down by patients' lifestyle

		H. pylori			
		Positive	p†	Negative	p‡
Water Type Used	Tap water	43 (40.2%)	0.001	156 (56.1%)	0.016* (r=0.167)
	Water from a well / creek / source	41 (38.3%)		89 (32%)	
	Water in bottles available in the market	23 (21.5%)		33 (11.9%)	
Keeping a Pet at Home	Yes	10 (9.3%)	0.001	31 (11.2%)	0.607
	No	97 (90.7%)		247 (88.8%)	
Type of Animals	Keeping no animals	97 (90.7%)	0.001	246 (88.5%)	0.529
	Cows	7 (6.5%)		22 (7.9%)	
	Others	3 (2.8%)		7 (2.5%)	
	Chicken	0 (0.0%)		3 (1.1%)	
Feeding Style	Meat-based diet	9 (8.4%)	0.001	13 (4.7%)	0.157
	Vegetable-sized diet	98 (91.6%)		265 (95.3%)	
Knowledge About Contagious Diseases	Good	12 (11.2%)	0.001	19 (6.8%)	0.165
	Medium	7 (6.5%)		40 (14.4%)	
	Low	31 (29.0%)		74 (26.6%)	
	No knowledge	57 (53.3%)		145 (52.2%)	

†: One-way chi-square test, ‡: Two-way chi-square test, *: p<0.05, r: Cramer's V coefficient

According to some symptoms observed in the patients, the variation of the presence or absence of *H. pylori* was analyzed with the two-way chi-square test (Table 3). *H. pylori* positivity showed a significant relationship only depending on the presence of salivation and joint pain (p<0.05). However, the degree of these relationships was measured very weakly (r=13.2% ; r=10.5%, respectively).

A one-way chi-square test was used to analyze whether or not there was a difference between the frequencies of some symptoms in *H. pylori*-positive patients (Table 3). In *H. pylori*-positive patients, there was no significant difference between the rates of decreased appetite, nausea-vomiting, and constipation symptoms (p>0.05). For other symptoms questioned, the results indicate that the rates of those with and without symptoms were significantly different (p<0.05). The proportion of *H. pylori*-positive patients with symptoms of fatigue, abdominal/stomach pain, gas pain, indigestion, and joint pain was significantly higher than the proportion of patients without symptoms. The proportion of patients with allergies, rectal itching, drooling, increased appetite, diarrhea, weight loss, and frequent urinary tract infections was significantly lower than those without these disorders. The study found no significant difference between the rates of being chronically ill or not (p>0.05).

A two-way chi-square test was performed to examine the relationship between *H. pylori* positivity and parasite positivity (Table 4). The results indicate that *H. pylori* positivity did not show a significant change compared to parasite positivity (p>0.05).

Table 3. *H. pylori* positivity broken down the symptoms observed

		H. pylori			
		Positive	p†	Negative	p‡
Allergy	Yes	24 (22.4%)		77 (27.7%)	0.293
	No	83 (77.6%)		201 (72.3%)	
Weakness	Yes	84 (78.5%)	0.001	208 (74.8%)	0.449
	No	23 (21.5%)		70 (25.2%)	
Rectal Itching	Yes	39 (36.4%)	0.001	79 (28.4%)	0.126
	No	68 (63.6%)		199 (71.6%)	
Saliva	Yes	39 (36.4%)	0.005**	65 (23.4%)	0.010* r=0.132
	No	68 (63.6%)		213 (76.6%)	
Decreased Appetite	Yes	44 (41.1%)	0.005**	98 (35.3%)	0.285
	No	63 (58.9%)		180 (64.7%)	
Increased Appetite	Yes	33 (30.8%)	0.066	66 (23.7%)	0.153
	No	74 (69.2%)		212 (76.3%)	
Diarrhoea	Yes	25 (23.4%)	0.001	57 (20.5%)	0.539
	No	82 (76.6%)		221 (79.5%)	
Abdominal Pain (Stomach Ache)	Yes	67 (62.6%)	0.001	160 (57.6%)	0.366
	No	40 (37.4%)		118 (42.4%)	
Fever	Yes	23 (21.5%)	0.009**	66 (23.7%)	0.640
	No	84 (78.5%)		212 (76.3%)	
Gas Pain	Yes	72 (67.3%)	0.001	183 (65.8%)	0.786
	No	35 (32.7%)		95 (34.2%)	
Nausea and Vomiting	Yes	56 (52.3%)	0.001	127 (45.7%)	0.242
	No	51 (47.7%)		151 (54.3%)	
Indigestion	Yes	72 (67.3%)	0.629	188 (67.6%)	0.950
	No	35 (32.7%)		90 (32.4%)	
Constipation	Yes	51 (47.7%)	0.001	111 (39.9%)	0.168
	No	56 (52.3%)		167 (60.1%)	
Weight Loss	Yes	22 (20.6%)	0.629	39 (14.0%)	0.116
	No	85 (79.4%)		239 (86.0%)	
Joint Pain	Yes	76 (71.0%)	0.001	166 (59.7%)	0.040* r=0.105
	No	31 (29.0%)		112 (40.3%)	
Urinary Tract Infection	Yes	23 (21.5%)	0.001	57 (20.5%)	0.830
	No	84 (78.5%)		221 (79.5%)	
	Yes	86 (80.4%)		231 (83.1%)	
Chronic Disease	No	44 (41.1%)	0.066	133 (47.8%)	0.236
	Yes	63 (58.9%)		145 (52.2%)	

-: Not calculated, †: One-way chi-square test, ‡: Two-way chi-square test, r: Phi coefficient, *: p<0.05, **: p<0.01

Table 4. The relationship between *H. pylori* positivity and parasite positivity

Helicobacter	Parasite		Total	p‡
	Negative	Positive		
Negative	103 (26.8)	175 (45.5)	278 (72.2)	
Positive	39 (10.1)	68 (17.7)	107 (27.8)	0.913
Total	142 (36.9)	243 (63.2)	385 (100.0)	

‡: Two-way chi-square test

Relationships between *H. pylori* positivity and parasite species were investigated by a two-way chi-square test (Table 5). The two-way chi-square test results showed a statistically significant relationship between *Entamoeba histoloytica* / dispar positivity and *H. pylori* positivity (p<0.05).

The degree of this relationship was calculated as 11.6%. The calculated odds ratio showed that *H. pylori* positivity was 1.19 times higher in *Entamoeba histoloytica* / dispar positivity. Similarly, *Enterobius vermicularis* positivity showed a significant correlation with *H. pylori* positivity (p<0.05). The correlation coefficient between *Enterobius vermicularis* positivity and *H. pylori* positivity was calculated as 19.2%. The odds ratio calculated showed that *H. pylori* positivity was 11.27 times higher in *Enterobius vermicularis* positivity. On the other hand, the other parasite species could not be statistically significantly associated with *H. pylori* positivity (p> 0.05).

Table 5. The relationship between *H. pylori* positivity and parasite species

		Helicobacter		Total	p‡
		Negative	Positive		
Blastocystis hominis	-	183 (47. %5)	73 (19.0%)	25 (66.5%)	0.655
	+	95 (24.7%)	34 (8.8%)	129 (33.5%)	
Iodamoeba buetschlii	-	270 (70.1%)	106 (27.5%)	376 (97.7%)	0.217
	+	8 (2.1%)	1 (0.3%)	9 (2.3%)	
Entamoeba coli	-	233 (60.5%)	92 (23.9%)	325 (84.4%)	0.599
	+	45 (11.7%)	15 (3.9%)	60 (15.6%)	
Entamoeba histoloytica / dispar	-	278 (72.2%)	105 (27.3%)	383 (99.5%)	0.023* r=0.116 OR=1.19
	+	0 (0.0%)	2 (0.5%)	2 (0.5%)	
Dientamoeba fragilis	-	266 (69.1%)	103 (26.8%)	369 (95.8%)	0.797
	+	12 (3.1%)	4 (1.1%)	16 (4.2%)	
Giardia intestinalis	-	262 (68.1%)	100 (26.0%)	362 (94.0%)	0.770
	+	16 (4.2%)	7 (1.8%)	23 (6.0%)	
Chilomastix mesnili	-	278 (72.4%)	105 (27.3%)	383 (99.7%)	0.108
	+	0 (0.0%)	1 (0.3%)	1 (0.3%)	
Enterobius vermicularis	-	276 (71.9%)	98 (25.5%)	374 (97.4%)	0.001 r=0.192 OR=11.27
	+	2 (0.5%)	8 (2.1%)	10 (2.6%)	
Hymenolepis nana	-	277 (72.1%)	106 (27.6%)	383 (99.7%)	0.421
	+	1 (0.3%)	0 (0.0%)	1 (0.3%)	
Cryptosporidium spp.	-	193 (50.3%)	67 (17.4%)	260 (67.7%)	0.244
	+	85 (22.1%)	39 (10.2%)	124 (32.3%)	
Cyclospora spp.	-	272 (70.8%)	103 (26.8%)	375 (97.7%)	0.703
	+	6 (1.6%)	3 (0.8%)	9 (2.3%)	
Endolimax nana	-	274 (71.4%)	104 (27.1%)	378 (98.4%)	0.756
	+	4 (1.0%)	2 (0.5%)	6 (1.6%)	
Entamoeba hartmanni	-	275 (71.6%)	106 (27.6%)	381 (99.2%)	0.163
	+	3 (0.8%)	0 (0.0%)	3 (0.8%)	
Ascaris lumbriocoides	-	277 (72.1%)	27.3%	382 (99.5%)	0.502
	+	1 (0.3%)	1 (0.3%)	2 (0.5%)	
Tænia spp.	-	276 (71.9%)	106 (27.6%)	99.5%	0.255
	+	2 (0.5%)	0 (0.0%)	2 (0.5%)	

-: not calculated, †: One-way chi-square test, ‡: two-way chi-square test, r: Phi coefficient, *: p<0.05, **: p<0.01

DISCUSSION

The study's working group consisted of 385 patients presented to the internal medicine outpatient clinic with digestive system complaints. As a result of the antigen test, the rate of *H.pylori* positivity was 27.79% (n=107). A review of the available literature on the subject showed that different findings were observed in similar studies. There may be many reasons for this. The literature review shows that the prevalence of *H. pylori* was found to be different based on several factors such as the country where the study was conducted, the study group (healthy, pregnant, general population, those with dyspeptic complaints, routine health screening, etc.), and the method used (serology, histology, urease test, PCR, culture, urea breath test, etc.). It was found to be 28.3% in a study conducted with serological analyzes in healthy individuals in Saudi Arabia (12), 93.6% in a study performed with serological analyzes in those with dyspeptic complaints in Nigeria (13), 37.9% in a study conducted with histological analyzes in indigenous people in Canada (14), and 63.4% (15) in a study performed with urea breath test in healthy individuals in China (16). In Turkey, in a study on the general population using the urea breath test, Özaydın et al. found an *H. pylori* positivity rate of 82.5% (17). In another study performed in Turkey on patients who presented for the urea breath test, Korkmaz et al. observed an *H. pylori* positivity rate of 49.5% (18). The present study performed in the province of Ordu in Turkey to investigate the *H. pylori* antigen in the stool of patients presented to the clinic involved in the study found a prevalence of 27.79%, a lower rate than expected when compared with other studies, and this may be attributed to the fact that studies' samples are composed of people from different regions and study methods.

Reviewing the socio-demographic characteristics of several past studies, we see that past research has reported different *H. pylori* prevalence rates. While some studies report no difference between women and men (19-22), some report having found no difference between young people and adults (23-25). Some studies have reported lower prevalence in young people than in adults (16). In this study, *H. pylori* positivity was found to be higher (75.7%) in patients aged 40 and over. Unlike studies in the literature, it found a higher rate in women (76.6%). It has been reported in the publications that it is more common in people with low socio-economic and educational levels, living in rural areas, living in crowded houses, and using contaminated drinking water sources (16). Korkmaz et al. (18) found no correlation between civil status and keeping pets at home. In this study, the majority of patients with higher *H. pylori* positivity were married (%73,8), had a middle/low income (85.7%), had a low educational background (89.7%), and lived in a village (55.1%) ($p<0.05$). At the same time, the rate of the *H. pylori*-positive patients using tap water was 40.2%, the rate of those who did not keep pets at home was 57.1%, the rate of those who ate mainly vegetables 75.7%, and the rate of those who did not know about infectious diseases 53.3%. The patient group in this study mostly lived in rural areas and fed farm animals such as cows at home. Therefore, unlike the results observed in previous studies, it is thought that *H. pylori* positivity may be significantly higher in those who keep pets at home.

The present study also investigated the correlation between *H. pylori* and intestinal parasites. Our study found a statistically significant relationship between the prevalence of *Entamoeba histoloytica* / dispar and *Enterobius vermicularis* parasites and *H. pylori* but found no relationship with other parasites. Similar risk factors are involved in the pathogenesis of *H. pylori* infection and parasitic infections (26-28). Many studies have been conducted in different countries and different age groups regarding the association of *H. pylori* and other protozoas, in which different results have been observed. In a study they conducted with children, Ibrahim et al. found that *H. pylori* were found together with Giardia intestinalis and Cryptosporidium (29). Seid et al. found that the prevalence of Giardia intestinalis was significantly higher in *H. pylori*-infected participants but not significantly different in *Entamoeba histoloytica* / dispar infection (1). El-Badry AA et al. found that Giardia intestinalis and H pylori co-infection is common in school-aged children (30). In a study performed with individuals receiving mental rehabilitation, Hassanein et al. showed that *H. pylori*-positive individuals were more likely to be infected with Giardia intestinalis than *H. pylori*-negative individuals (31). In their study on children with chronic abdominal pain, Goksen et al. (32) found that the incidence of Giardia intestinalis was statistically higher in the *H. pylori*-positive group (14.8%) than in the *H. pylori*-negative group (1.6%). In a study conducted on patients with chronic diarrhea, Yakoop et al. (33) showed a significant relationship between H pylori infection and Blastocystis sp. and *Entamoeba histoloytica*/dispar infection. The present study found a significant relationship between *H. pylori* and *Entamoeba histoloytica* / dispar in patients with dyspeptic complaints, and it further showed that *H. pylori* positivity was 1.19 times higher in *Entamoeba histoloytica* / dispar positivity. In the literature review, no publication was found showing a relationship between *Enterobius vermicularis* positivity and *H. pylori* positivity. In our study, *Enterobius vermicularis* positivity was also shown to be associated with *H. pylori* positivity. The correlation coefficient between *Enterobius vermicularis* positivity and *H. pylori* positivity was calculated as 19.2%. The calculated odds ratio showed that *H. pylori* positivity was 11.27 times higher in *Enterobius vermicularis* positivity.

Limitations

As it is a cross-sectional study, it does not reflect the whole universe.

CONCLUSION

Protozoas can phagocytize many bacteria and body cells and contribute to their increased pathogenicity and serve as a reservoir for potentially disease-causing bacteria.

The association of intestinal parasitism and *H. pylori* is quite complex. Different results were observed in studies conducted in different patient groups. To better understand

the epidemiology, clinical effects, treatment, and control of co-infection with *H. pylori*, much more extensive and more comprehensive studies are needed than those available in the literature. The high prevalence of intestinal parasites and *H. pylori* shows that this situation should not be ignored.

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Research Article

Investigation of the Effect of Acute to Chronic Glycemic Ratio on the Development of Postoperative Pneumonia After Stanford Type A Acute Aortic Dissection Surgery

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Abstract

Aim: A critical clinical condition called acute Type A aortic dissection (ATAAD) necessitates quick surgical action. Other significant issues include the emergence of postoperative pneumonia and various organ failures. We sought to determine how well the ratio of admission blood glucose (ABG) to estimated average glucose (eAG) might be used to anticipate postoperative pneumonia following ATAAD surgery.

Materials and Methods: The study comprised patients who underwent ATAAD surgery between January 2016 and January 2022. In the postoperative phase, patients were divided into two groups: Group 1 for those who did not acquire pneumonia and Group 2 for those who did.

Results: The study involved 124 patients in total. Group 1 [N = 92, median age = 51 (32 to 80) years] consisted of those who did not acquire postoperative pneumonia, but Group 2 [N = 32, median age = 53 (30 to 77)] did. ABG/eAG ratio and ventilation time were found to be independent predictors of postoperative pneumonia by multivariate analysis [(OR: 0.886, CI 95%: 0.695-0.990, P=0.009) and (OR: 1.114, 1.030-1.542, P=0.023)].

Conclusion: We demonstrated that ABG/eAG ratio, calculated at admission time, is a significant predictor of the development of postoperative pneumonia.

Keywords: Pneumonia, risk factor, type A acute aortic dissection, glucose

INTRODUCTION

Acute Type A aortic dissection (ATAAD) is an important clinical condition that requires urgent surgical intervention. Even with modern technology, death and morbidity rates remain high (1). Mortality after these operations is the most catastrophic outcome. In addition, various organ failures and the development of postoperative pneumonia are also important problems (2).

Cardiovascular illnesses have higher mortality and morbidity due to disturbances in glucose metabolism. In addition, prothrombotic events and an increase in oxidative stress brought on by acute hyperglycemia result in endothelial dysfunction. Whether or not someone has diabetes mellitus (DM), this circumstance has a negative impact on the prognosis following acute cardiovascular events. Recent research in this area has demonstrated the significance of admission blood glucose (ABG)/estimated average glucose (eAG) values in the prognosis of acutely developing cardiovascular events (3,4). Another finding from a study was the connection between admission hyperglycemia and morbid outcomes following dissection surgery (5).

We sought to determine how well the ABG/eAG ratio might be used to anticipate postoperative pneumonia following ATAAD surgery.

MATERIAL AND METHOD

This retrospective study comprised patients who underwent ATAAD surgery between January 2016 and January 2022. The study was launched following the

CITATION
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local ethics committee's clearance, and it was carried out in conformity with the Helsinki Declaration. Within 12 hours of receiving their diagnoses, every patient participating in the study underwent surgery. Patients with preoperative infection, multiple organ failure, and patients who experienced mortality within the first 24 hours following surgery were disqualified from the research. The study contained 124 consecutive patients as a result of exclusion criteria. Information about the patient's preoperative conditions (smoking, demographic features, and laboratory results including neutrophil, lymphocyte, hemoglobin, platelet count, urea, and creatinine), intraoperative procedures (blood transfusions, cardiopulmonary bypass (CPB) time, and surgery type), and postoperative conditions (hospital stay length, intensive care unit (ICU) were recorded. Group 1 patients were those who did not acquire pneumonia following surgery, while Group 2 patients were those who did.

Surgical Technique and Perioperative Blood Glucose Management

All operations were performed under general anesthesia with a median sternotomy approach. Right axillary artery was used for arterial cannulation. Femoral artery cannulation was used in unsuitable patients. In these patients, antegrade cerebral perfusion was performed with a direct approach from the surgical field. Operations were performed in moderate hypothermia. Cardiopulmonary bypass was applied with a roller pump equipped with a membrane oxygenator and arterial line filter (Maquet, Getinge group, Germany).

In all patients, necessary adjustments were made in the crystallized insulin infusion by measuring blood glucose before CPB, at 15-minute intervals during CPB, and at the end of CPB. Hourly insulin requirement was met by dividing the blood glucose level in mg/dl unit by 100. In intensive care follow-ups, treatment attempts were made to keep blood glucose values between 120-180 mg/dl.

Diagnosis of Pneumonia

The diagnosis of pneumonia was made according to the Society of Thoracic Surgeons criteria. Accordingly, findings such as laboratory (positive sputum culture results from transtracheal fluid and/or bronchial washings), radiological findings (pulmonary infiltrates), and fever were included in the evaluation (6).

ABG/aAG ratio

During the patients' stay, peripheral venous blood samples were taken and their blood parameters were assessed. The following formula was used to determine the ABG/eAG value (4).

Admission levels of blood glucose (mg/dl) / [(28.7 x HbA1c %)- 46.7].

Statistical analysis

The SPSS 21.0 program (IBM Statistical Package for the Social Sciences Statistic Inc. Version21.0, Chicago, IL, USA) was used for the statistical analysis. In the case of numerical values having a normal distribution, the Student's t-test was applied. The Mann-Whitney U test was applied in the case of numerical data without normal distribution. The mean (standard deviation) or mean was used to express numerical numbers (minimum-maximum). The chi-square test was used to compare categorical variables. Statistics were judged significant at $P<0.05$. To assess key factors in the univariate study for predicting the development of pneumonia, a multivariate logistic regression analysis was conducted. The area under the curve (AUC) was computed after performing a ROC curve analysis to assess the predictive usefulness of the ABG/eAG ratio for the development of pneumonia.

RESULTS

The study involved 124 patients in total. Group 1 [N = 92, median age = 51 (32 to 80) years] consisted of those who did not acquire postoperative pneumonia, but Group 2 [N = 32, median age = 53 (30 to 77)] did. Age, gender, smoking, blood pressure, previous history of cardiac surgery, ejection fraction, and rates of chronic obstructive pulmonary disease (COPD) did not statistically differ between the two groups (Table 1).

Table 1. Demographic data and preoperative features of the patients

Variables	Group 1 (N= 92)	Group 2 (N= 32)	P value
Age (years)	51 (32- 80)	53 (30- 77)	0.209‡
Male gender, n (%)	65 (70.7%)	21 (65.6%)	0.595*
Hypertension, n (%)	71 (77.2%)	26 (81.3%)	0.630*
COPD, n (%)	9 (9.8%)	5 (15.6%)	0.368*
Diabetes mellitus, n (%)	14 (15.2%)	8 (25%)	0.212*
Current smoker, n (%)	29 (31.5%)	12 (37.5%)	0.536*
Previous cardiac surgery, n (%)	4 (4.3%)	3 (9.4%)	0.289*
Marfan'syndrome, n (%)	3 (3.3%)	2 (6.3%)	0.459*
Duration of pain (hours)	4 (1- 24)	4 (1- 24)	0.196‡
Ejection fraction (%)	50 (30- 65)	45 (30- 65)	0.219‡
Malperfusion, n (%)	6 (6.5%)	3 (9.4%)	0.592*

* Chi-square test, ‡Mann Whitney U test (Data is expressed as median (interquartile range)). Malperfusion: Coroner artery and/or visceral organ artery and/or preoperative neurological sequelae, COPD: Chronic obstructive pulmonary disease

Table 2 displays the patients' perioperative characteristics and preoperative blood values. White blood cell, hemoglobin, platelet, lymphocyte, creatinine, hemoglobin A1c, and C-reactive protein readings were comparable across the two groups. Group 2 had considerably higher ABG and ABG/eAG ratios ($P<0.001$ for two variables). Antegrade cerebral perfusion times, operating periods, and types of surgery did not statistically differ between the

two groups. Total perfusion and ventilation time in Group 2 were substantially longer (P <0.001 for two variables).

To assess the factors that may lead to the development of postoperative pneumonia, logistic regression analysis was used (Table 3). In univariate analysis, it was discovered that the following variables were significantly correlated with the development of postoperative pneumonia: ventilation time (OR [odds ratio]: 1.210, 95% CI [confidence interval]: 1.079-1.690, P<0.001), blood product use (OR: 0.895, 95% CI: 0.790-0.964, P<0.001), total perfusion time (OR: 0.780, 95% CI: 0.550-0.872, P<0.001), ABG (OR: 0.775, 95% CI: 0.554-0.869, P< 0.001), and ABG/eAG ratio (OR: 1.080, 95% CI: 1.016-1.270, P< 0.001).

ABG/eAG ratio and ventilation time were found to be independent predictors of postoperative pneumonia by multivariate analysis [(OR: 0.886, CI 95%: 0.695-0.990, P=0.009) and ventilation time (OR: 1.114, 1.030-1.542, P=0.023)].

To evaluate the ABG/eAG ratio's performance in predicting the emergence of postoperative pneumonia, receiver operating characteristic curve analysis was carried out. The cut-off value of the ABG/eAG ratio was 1.6 (Area under the curve (AUC): 0.776, 95% Confidence interval (CI): 0.686-0.866, P< 0.001, with 78.4% sensitivity and 66.9% specificity) (Figure 1).

Table 2. Preoperative blood parameters and perioperative features of the patients

Variables	Group 1 (N= 92)	Group 2 (N= 32)	P value
White blood Cell (10^3/µL)	10.1 (7.2- 15.7)	9.8 (8.1- 17.3)	0.326‡
Hemoglobin (g/dL)	12.6 (11.8- 15.5)	13.4 (10.6- 16.4)	0.504‡
Platelet (10^3/µL)	185(100- 395)	192 (89- 378)	0.431‡
Neutrophil (10^3/µL)	6.8 (2.7- 11.7)	7 (2.4- 10.6)	0.374‡
Lymphocyte (10^3/µL)	1.7 (0.7- 4.1)	1.5 (0.8- 4.3)	0.198‡
Creatinine, mg/dL	1.2 (0.7- 2.3)	1.1 (0.8- 2.5)	0.119‡
Urea, mg/dL	18 (14- 50)	16 (12- 55)	0.226‡
C Reactive protein, (mg/dL)	10.9 (3.2- 66)	11.3 (2.6- 59)	0.118‡
ABG, mg/dl	124 (99- 258)	166 (128- 339)	<0.001‡
HbA1c, %	5.9 (5.2- 10.1)	6.1 (5.4- 9.7)	0.184‡
eAG, mg/dl	132.6 (122- 147)	129.8 (119- 151)	0.154‡
ABG/eAG	0.96 (0.91- 1.75)	1.84 (1.3- 3.12)	<0.001‡
Total perfusion time, min	129 (110- 249)	134 (120- 295)	<0.001‡
ACP time, min	26 (18- 40)	29 (22- 36)	0.197‡
Operation time, hours	6.2±1.4	6.4±1.3	0.291†
Ventilator time, hours	6 (4- 96)	12 (8- 240)	<0.001
Surgery types, n			0.491*
Ascending aorta replacement	45	17	
Hemiarch replacement	38	10	
Total Arch replacement	9	5	
Concomitant AVR	11	4	0.935*
Concomitant CABG	14	3	0.408*
Packed blood products (units)	6 (4- 9)	10 (5- 15)	<0.001‡
Total ICU stay (days)	3 (2- 18)	6 (4- 34)	<0.001‡
Total hospital stay (days)	7 (6- 28)	10 (8- 60)	<0.001‡

* Chi-square test, ‡Mann Whitney U test, †Student's t-test, ABG: Admission blood glucose, HbA1c: Hemoglobin A1c, eAG: estimated average glucose, ABG/eAG: Admission blood glucose to estimated average glucose ratio, ACP: Antegrade cerebral perfusion, AVR: Aortic valve replacement, CABG: Coronary artery bypass graft

Table 3. Logistic regression analysis to identify predictors of postoperative pneumonia after aortic dissection surgery

	Univariate analysis			Multivariate analysis		
Variables	**P**	**Exp(B) Odds Ratio**	**95% C.I. Lower Upper**	**P**	**Exp(B) Odds Ratio**	**95% C.I. Lower Upper**
Age, years	0.232	1.110	0.850- 1.270	--	--	--
Hypertension, n	0.659	0.690	0.592- 1.090	--	--	--
Ventilation time, hours	<0.001	1.210	1.079- 1.690	0.023	1.114	1.030- 1.542
Blood product use, units	<0.001	0.895	0.790- 0.964	0.109	0.969	0.756-1.135
Total perfusion time, minutes	<0.001	0.780	0.550- 0.872	0.079	1.190	0.938-1.665
ABG, mg/dl	<0.001	0.775	0.554- 0.869	--	--	--
HbA1c, %	0.230	1.166	0.819- 1.259	--	--	--
eAG, mg/dl	0.194	1.044	0.890- 1.154	--	--	--
ABG/eAG	<0.001	1.080	1.016- 1.270	0.009	0.886	0.695- 0.990

ABG: Admission blood glucose, HbA1c: Hemoglobin A1c, eAG: Estimated average glucose, ABG/eAG: Admission blood glucose to estimated average glucose ratio

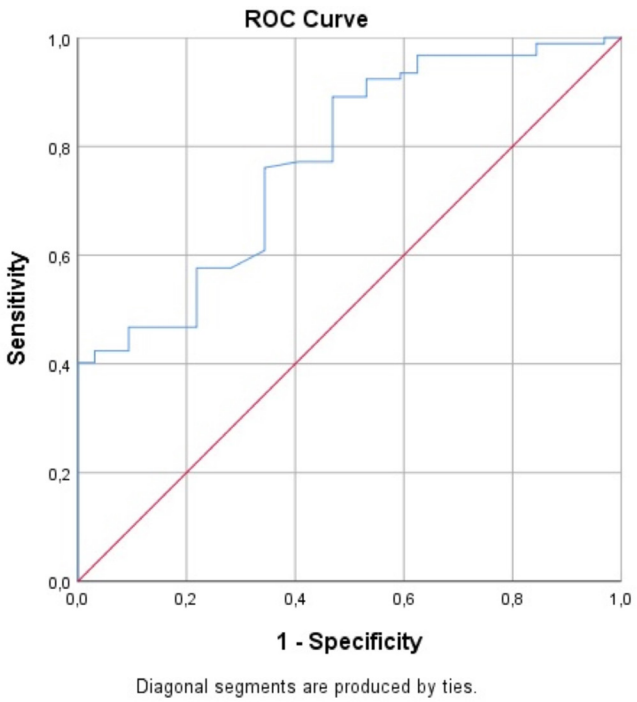

Figure 1. Receiver operating characteristic curve analysis for acute to chronic glycemic ratio to predict the development of postoperative pneumonia

DISCUSSION

Acute Type A aortic dissection is a serious clinical condition that requires urgent surgical intervention. As time passes after the diagnosis, the risk of true lumen collapse increases, so end-organ damage may occur (7). Postoperative pneumonia is a serious condition that can occur after cardiac surgical procedures. This situation is more common after ATTAD surgery, which is an emergency operation. In the present study, we demonstrated that ABG/eAG ratio can be used to predict the risk of pneumonia after ATAAD surgery.

In acute clinical conditions, hyperglycemia also occurs. This has a negative impact on clinical outcomes, regardless of whether patients have diabetes (8). This hyperglycemic condition causes endothelial dysfunction, activates prothrombotic pathways, and raises oxidative stress. As a result, microvascular circulation is also compromised (9). Whether or not someone has DM this circumstance body has a negative impact on the prognosis following acute cardiovascular events (10).

In a study conducted by Lin et al., the effect of high ABG on postoperative results in ATAAD patients was investigated and 734 patients were evaluated retrospectively. The patients were split into two groups with a blood glucose level of 140 mg/dl those with high blood glucose levels and those with normal blood glucose levels. Although there was no difference between the in-hospital mortality rates between the two groups, prolonged ventilation rates were found to be significantly higher in the patient group with hyperglycemia. In addition, in a multivariate analysis investigating the risk factors for prolonged ventilation, hyperglycemia was shown as an independent predictor of prolonged mechanical ventilation in addition to age and body mass index (5). In our investigation, the patient group with pneumonia had significantly higher blood glucose readings upon admission. In a different study, patients who received primary percutaneous coronary intervention with the diagnosis of ST-elevation myocardial infarction showed an association between ABG value and reperfusion failure (11). In another study conducted on orthopedic surgery

patients, an ABG value of over 200mg/dl was found to be associated with deep surgical-site infections (12).

In addition to these unfavorable effects of acute hyperglycemia, people without DM have also shown that HbA1c has worse effects (13). In light of this information, ABG/eAG ratio emerges as an important prognostic marker. The predictive value of the ABG/eAG ratio in patients with acute myocardial infarction was examined in a study by Gao et al. The scientists concluded that ABG/eAG value was more useful than the ABG value in predicting morbid and fatal occurrences (14). In another study by Mondal et al., the prognostic role of the ABG/eAG ratio was investigated in COVID-19 patients with moderate and severe clinical findings. At the end of their study, the usefulness of ABG/eAG value over ABG value in predicting fatal and morbid outcomes was discovered by the authors (15). Chen et al. investigated the clinical significance of the ABG/eAG ratio in patients over 75 years of age with acute myocardial infarction which included 341 patients retrospectively and the ABG/eAG ratio was shown as an important parameter in predicting in-hospital adverse events (16). In our study, we showed the ABG/eAG ratio as an independent predictor in predicting the development of pneumonia after ATAAD surgery.

Postoperative pneumonia is an important infectious pathology that can occur after cardiac surgical operations. This situation not only includes mortal and morbid risks, but also increases the cost of treatment by prolonging hospitalizations. Risk variables affecting the emergence of pneumonia following heart surgery were looked into in a study by Wang et al. in 2021 which included 5,323 patients, postoperative pneumonia was developed in 530 patients. Renal failure, age>60, DM, history of cardiac surgery, prolonged perfusion times, and increased blood transfusion were shown as important parameters affecting the development of pneumonia (17). In another study of 492 ATAAD patients, 34.6% of them developed postoperative pneumonia. Advanced age, COPD, low platelet counts, and increased blood product transfusion were shown as important parameters affecting the development of pneumonia (18). The impact of low platelet counts on the emergence of pneumonia following ATAAD surgery was examined in another study by Yao et al. 268 patients were included in this retrospective investigation, and postoperative pneumonia developed in 36.9% of the patients. In addition to preoperative low platelet counts, long ventilation times, and increased blood product transfusion were found to be significant parameters affecting the development of pneumonia (19). In our study, we found the postoperative pneumonia rate to be 25.8%, and in addition to the ABG/eAG ratio, prolonged perfusion times, prolonged ventilation times, and increased blood product use were significantly correlated with the development of pneumonia.

There are certain limitations to our investigation. The fact that it is a retrospective study with a limited number of patients is the most significant of them.

CONCLUSION

ATAAD is an important cardiovascular disease that requires urgent surgical intervention. Today, it has higher mortality and morbidity compared to other cardiac surgical operations. Pneumonia is an important morbidity that occurs in these patients in the postoperative period. We demonstrated that ABG/eAG ratio, calculated at admission time, is a significant predictor of the development of postoperative pneumonia. Multicenter prospective studies must be used to support our study.

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Research Article

The Relationship Between AKR1B1 rs759853 (C-106T) Polymorphism and the Diabetic Retinopathy Severity in Turkish Type 2 Diabetes Mellitus Patients

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Abstract

Aim: Diabetes mellitus (DM) is an important health problem with an increasing incidence worldwide and causes many complications. Diabetic retinopathy (DR) is one of the most serious complications of DM. Polymorphisms of the AKR1B1 gene, which encodes an aldose reductase enzyme, have been associated with development of DM and DR in some studies. The current study aims to investigate the relationship of AKR1B1 rs759853 polymorphism with type 2 DM (T2DM), DR and DR severity in the Turkish population.
Materials and Methods: A total of 437 individuals, including 141 T2DM patients without DR, 125 T2DM patients with DR, and 171 healthy controls, were included in the study. Genotyping was performed using PCR-RFLP method.
Results: An association between T allele / TT genotype and increased risk of proliferative diabetic retinopathy (PDR) was detected. In the logistic regression analysis in which other risk factors were included, rs759853 polymorphism and diabetes duration were found to be associated with the PDR development. There was no significant relationship between the AKR1B1 rs759853 variation and the development of T2DM and DR.
Conclusion: Obtained data showed that AKR1B1 rs759853 polymorphism is not associated with the development of T2DM and DR in the Turkish patients, but TT genotype and diabetes duration are independent risk factors for the development of PDR.

Keywords: Type 2 diabetes mellitus, diabetic retinopathy, AKR1B1, aldose reductase, rs759853, polymorphism

INTRODUCTION

The prevalence of Diabetes mellitus (DM) is rising worldwide. Turkey has the highest DM prevalence with a rate of 11.1% among European countries according to the International Diabetes Federation 2019 data (1). Diabetic retinopathy (DR), an important microvascular complication of both type 2 DM (T2DM) and type 1 DM (T1DM), is a neurovascular disease characterized by progressive structural and functional disorders in the retina. It causes health problems such as vision loss and blindness in approximately 75% of patients who have had diabetes for at least 15 years (2,3). The early phase of DR is called nonproliferative DR (NPDR) when the late phase is called proliferative DR (PDR). While progressive microvascular changes are observed in the retina in NPDR, these changes are also observed outside the retina in PDR. PDR is also characterized by the growth of newly formed vessels in the retina and optic disc. Diabetic maculopathy (DMP), which can be seen in both NPDR and PDR causes decreased vision in patients with DM. Diabetic macular edema (DME), the most common form of DMP, is a thickening of the posterior pole of the retina (4). Diabetes duration, uncontrolled glucose level of blood and high blood pressure have influence on the development and progression of DR. Other risk factors include dyslipidemia, smoking and high body mass index (BMI). However, there are fundamental differences between individuals in terms

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of DR risk and disease severity, and these differences cannot be explained only by the risk factors listed above (5). Studies have shown that variations in many different genes are among the reasons for this difference between individuals. Vascular endothelial growth factor-A (VEGF-A), erythropoietin (EPO), protein kinase C-β (PKC-β), methylenetetrahydrofolatereductase (MTHFR), angiotensin-converting enzyme 1 (ACE-1), intercellular adhesion molecule 1 (ICAM-1) and aldo-keto reductase family 1, member B1 (AKR1B1) are counted among the genes associated with susceptibility to DR development (2,6).

AKR1B1, a NADPH-dependent aldo-keto reductase, is expressed in many tissues, such as retinal capillary pericytes (6). It is one of the best known enzymes of the polyol pathway and catalyzes the reduction of glucose to sorbitol using NADPH as a cofactor (7). Under normal glycemic conditions, AKR1B1 has low affinity for glucose and less than 3% of glucose is converted to sorbitol by AKR1B1. In the case of chronic hyperglycemia, glucose affinity of AKR1B1 increases (about 30% of glucose is converted to sorbitol) and this results with sorbitol accumulation as well as increased NADPH use. Sorbitol accumulation leads changes in cell membrane osmotic pressure, diffusion of water into the cell, electrolyte imbalance, and ultimately cell damage and oxidative stress. On the other hand, the increased use of NADPH by AKR1B1 causes a decrease in the amount of NADPH to be used for other metabolic processes such as nitric oxide synthesis. Decreased nitric oxide synthesis leads to vasoconstriction, ischemia, and slowing of nerve conduction. NADPH also acts as a cofactor of antioxidant enzymes such as glutathione reductase, and a decrease in its amount again causes increased oxidative stress. Oxidative stress is one of the main causes of DM and DM complications such as DR (7,8).

The AKR1B1 gene is on the 7q35 locus and contains 10 exons. The rs759853 (C-106T) polymorphism is in the AKR1B1 promoter region (6,7). In the literature, various polymorphisms of AKR1B1, including rs759853, have been associated with micro and macro complications of diabetes, such as DR, diabetic neuropathy, diabetic nephropathy, and stroke in some populations (6,7,9–12). To the best of our knowledge, the association between AKR1B1 polymorphisms and susceptibility to T2DM and DR has not been explored in the Turkish population. The present study aims to explore the relationship between the AKR1B1 rs759853 polymorphism and the susceptibility to T2DM and DR and the clinical features of the disease.

MATERIAL AND METHOD

Patients

The approval of the current study was provided by Giresun University's Faculty of Medicine Clinical Trials Ethics Committee (Approval No:23.12.2021-08). All participants gave their signed informed consent before recruitment. Power analysis revealed that a total of 421 patients should be included in the study for an analysis with an effect size of 0.3 (medium) and a power of 90%. A total of 437 individuals (141 T2DM cases without DR, 125 T2DM cases with DR, and 171 healthy subjects) were included in the current study. The patient group was between the ages of 44-84, and the control group was between the ages of 38-84. American Diabetes Association (ADA) criteria were used for the diagnosis of T2DM. All patients underwent a comprehensive eye examination with evaluation of visual acuity, fundoscopic examination, and fundus photography for the diagnosis of DR. Afterwards, staging was performed. Patients with one of the signs of hard exude, cotton wool spot, hemorrhage and intraretinal microvascular anomalies or venous segmentation in addition to microaneurysms but without PDR findings were staged as NPDR. Cases with at least one of the signs of neovascularization, preretinal hemorrhage, vitreous hemorrhage, or fibrovascular proliferation in the disc or elsewhere were staged as PDR. Individuals with other types of diabetes, malignant or inflammatory diseases did not include the study. Healthy individuals without diabetes and any eye disease were selected for the control group. Fasting venous blood samples were taken from all participants for genomic DNA extraction and biochemical analysis. In addition to the age, gender, height, weight and hypertension status of all participants, the duration of diabetes, insulin use, DME and DMP presence in the patient group were recorded. Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), glycated hemoglobin (HbA1c) and fasting plasma glucose (FPG) were measured using blood samples. BMI was calculated as weight/height2 (kg/m2).

DNA extraction and genotyping

DNA isolation was carried out by the High pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method was used for genotyping. PCR reaction was conducted using following primers; F 5' TTCGCTTTCCCACCAGATAC 3'; R 5' CGC CGT TGT TGA GCA GGA GAC 3'. PCR protocol was 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 65°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 5 min. The size of the PCR amplification product was 326 bp and all PCR amplicons are run on a 2% agarose gel. The BfaI enzyme was used to digest the PCR amplicons and the resulted amplicons were run on 3% agarose gel electrophoresis. Two bands of 234bp and 92bp for the CC genotype, 4 bands of 234bp, 175bp, 92bp and 59bp for the CT genotype, 3 bands of 175bp, 92bp and 59bp for the TT genotype were observed.

Statistical analysis

A statistical software package (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA) was used to perform statistical analyses. Shapiro-Wilk Normality test was used to evaluate whether the data were normally distributed. Continuous variables were expressed as

mean ± SD. Categorical data were presented as numbers and percentages. The genotype distribution of rs759853 among groups was tested for the Hardy–Weinberg equilibrium (HWE) using the χ2 test. Genotypes and allele frequencies among the groups were compared using χ2 tests. Mann–Whitney U and Kruskal Wallis tests were used to determine difference between the groups in terms of continuous variables. Binary logistic regression analysis was used to test the factors affecting the development of PDR. P values below 0.05 were considered as statistically significant.

RESULTS

Demographic and clinical characteristics of the patient and control groups are summarized in Table 1. As expected, BMI (p=0.046), presence of hypertension (p<0.001), lipid profiles (p<0.001 for total cholesterol and LDL, p=0.002 for HDL and triglyceride), HbA1c (p<0.001) and fasting blood glucose (p<0.001) were significantly different between DM (with and without DR) and control groups (Table 1). In addition, a difference was observed in terms of gender distribution (p=0.046). The frequency of male gender was found to be lower in the T2DM group compared to control group (Table 1). There was a significant difference between the patients with and without DR in case of BMI (p=0.013), duration of diabetes (p<0.001), total cholesterol (p=0.001), LDL (p=0.022), HbA1c (p<0.001), fasting blood glucose (p=0.027), presence of DME (p<0.001) and DMP (p<0.001) (Table 1). Genotype frequency of AKR1B1 rs759853 polymorphism was found to be compatible with HWE in all three groups (Tables 2 and 3). Genotype and allele frequencies did not differ between the T2DM group and the control group (table 2), and between T2DM patients with and without DR (table 3). As shown in Table 4, the total T2DM group and the T2DM group with DR were divided into groups according to rs759853 genotypes and it was evaluated whether there was a difference in terms of clinical parameters. Accordingly, no significant difference was found between individuals with different genotypes in case of BMI, duration of diabetes, presence of hypertension, total cholesterol, fasting blood sugar, LDL, triglyceride, HDL, DME and DMP presence in both the total T2DM group and the T2DM group with DR (Table 4). On the other hand, a significant relationship was found between the presence of PDR and genotype (p=0.017) and allele frequencies (p=0.003) in the T2DM group with DR (Table 5). According to logistic regression analysis including AKR1B1 rs759853 polymorphism, BMI, duration of diabetes, presence of hypertension, total cholesterol, LDL, HDL, triglyceride, HbA1c, and fasting blood glucose, TT genotype (p=0.019, OR=5.204, 95% CI=1.307- 20.718) and duration of diabetes (p=0.038, OR=1.108, 95% CI=1.006-1.222) were associated with the development of PDR (Table 6).

Table 1. Demographic and clinical characteristics of study groups

Variables	T2DM (without DR)[a] N=141	T2DM (with DR)[b] N=125	Controls [c] N=171	P [a+b/ c] value	P [a / b] value
Sex (% male)	41.8	44	52.6	0.046	0.723
Age at study (years)	62.9 ± 8.4	62.7 ± 7.5	62.4 ± 11.8	0.783	0.980
BMI (kg/m^2)	29.8 ± 3.6	31.3 ± 4.8	29.5 ± 4.5	0.045	0.013
Diabetes duration (years)	8.4 ± 3.7	15.6 ± 5.1	-		<0.001
Presence of hypertension (%)	37.6	47.2	11.7	<0.001	0.113
Total cholesterol (mg/dl)	192.9 ± 34.5	213.7 ± 56.5	154 ± 30.5	<0.001	0.001
LDL (mg/dL)	109.2 ± 29.4	119 ± 36.2	102.9 ± 25.4	<0.001	0.022
HDL (mg/dL)	50.3 ± 12.4	47.1 ± 10.6	50.6 ± 8	0.002	0.070
Triglyceride (mg/dL)	181.2 ± 132.9	171.4 ± 131.3	131.3 ± 18.6	0.002	0.656
HbA1c (%)	7.3 ± 1.2	8.1 ± 1.5	4.7 ± 0.6	<0.001	<0.001
Fasting glucose level (mg/dL)	163 ± 51	187.3 ± 87.8	85.6 ± 9.9	<0.001	0.027
DME (%)	3.5	72.8	-		<0.001
DMP (%)	2.1	88	-		<0.001
PDR (%)	-	32	-		

T2DM: Type II Diabetes Mellitus, DR: Diabetic retinopathy, BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, DME: Diabetic macular edema, DMP: Diabetic maculopathy, PDR: Proliferative diabetic retinopathy

Table 2. Comparison of AKR1B1 genotype and allele frequencies between total T2DM patients and controls

AKR1B1 rs759853 Genotypes and alleles		Control N (%)	T2DM patients N (%)	OR (95% CI)	p value
Multiplicaptive	CC	84 (49.1)	127 (47.7)	Ref	-
	CT	69 (40.4)	109 (41)	1.045 (0.694-1.572)	0.833
	TT	18 (10.5)	30 (11.3)	1.102 (0.578-2.103)	0.767
Dominant	CC	84 (49.1)	127 (47.7)	Ref	0.778
	CT+TT	87 (50.9)	139 (52.3)	1.057 (0.719-1.552)	
Resesive	CT+CC	153 (89.5)	236 (88.7)	Ref	0.806
	TT	18 (10.5)	30 (11.3)	1.081 (0.582-2.006)	
Over dominant	TT+CC	102 (59.6)	157 (59)	Ref	0.896
	CT	69 (40.4)	109 (41)	1.026 (0.694-1.518)	
Alleles	C	237 (69.3)	363 (68.2)	Ref	0.740
	T	105 (30.7)	169 (31.8)	1.051 (0.784-1.409)	
		HWE: 0.489	HWE: 0.372		

T2DM: Type II Diabetes Mellitus, OR: Odds ratio, CI: Confidence interval, HWE: Hardy-Weinberg equilibrium

Table 3. Comparison of AKR1B1 genotype and allele frequencies between T2DM patients with and without DR

AKR1B1 rs759853 Genotypes and alleles		Control N (%)	T2DM patients N (%)	OR (95% CI)	p value
Multiplicaptive	CC	66 (46.8)	61 (48.8)	Ref	-
	CT	59 (41.8)	50 (40)	0.917 (0.549-1.531)	0.740
	TT	16 (11.3)	14 (11.2)	0.947 (0.427-2.101)	0.893
Dominant	CC	66 (46.8)	61 (48.8)	Ref	0.746
	CT+TT	75 (53.2)	64 (51.2)	0.923 (0.570-1.495)	
Resesive	CT+CC	125 (88.7)	111 (88.8)	Ref	0.970
	TT	16 (11.3)	14 (11.2)	0.985 (0.460-2.110)	
Over dominant	TT+CC	82 (58.2)	75 (60)	Ref	0.760
	CT	59 (41.8)	50 (40)	0.927 (0.568-1.512)	
Alleles	C	191 (67.7)	172 (68.8)	Ref	0.791
	T	91 (32.3)	78 (31.2)	0.952 (0.660-1.372)	
		HWE:0.612	HWE:0.445		

T2DM: Type II Diabetes Mellitus, DR: Diabetic retinopathy, OR: Odds ratio, CI: Confidence interval, HWE: Hardy-Weinberg equilibrium

Table 4. Comparison of clinical characteristics stratified by genotypes of AKR1B1 rs759853 polymorphism among total T2DM patients and T2DM patients with DR

Parameters	Total T2DM patients				T2DM patients (with DR)			
	CC	CT	TT	p	CC	CT	TT	*P* value
BMI (kg/m²)	30.3±4.3	30.4±4.2	31.6±4.2	0.117	31.8±4.9	31.8±4.9	32.2±4.8	0.262
Diabetes duration (years)	11.4±5.3	12±5.7	12.9±7	0.638	16.2±5	16.2±5	16.8±5.8	0.502
Hypertension N(%)								
No	68 (44.2)	66 (42.9)	20 (13)	0.325	29 (43.9)	29 (43.9)	7 (10.6)	0.635
Yes	59 (52.7)	43 (38.4)	10 (8.9)		21 (35.6)	21 (35.6)	7 (11.9)	
Total cholesterol (mg/dl)	200.2±46.2	204.6±47.7	206.4±51	0.652	216.6±54.8	216.6±54.8	221.1±63.3	0.276
LDL (mg/dL)	115±36.1	111.9±28.7	115.8±35.2	0.844	117.1±28.4	117.1±28.4	122.4±37	0.899
HDL (mg/dL)	48.5 ± 11.2	48.9 ± 12.8	49.8 ± 9.4	0.532	47.4±12.7	47.4±12.7	47.4±9.3	0.800
Triglyceride (mg/dL)	176.7 ±136.4	170.7 ±103.2	193.2±195.7	0.990	156.8±65	156.8±65	244.8±275.3	0.691
HbA1c (%)	7.7±1.4	7.7±1.5	7.7±1.1	0.625	8±1.7	8±1.7	8.2±1.2	0.358
Fasting blood glucose level (mg/dL)	172.6±83.1	175.3±59.4	178.8±61.1	0.149	179.9±66	179.9±66	195.9±60.9	0.308
DME N (%)								
No	83 (48.8)	69 (40.6)	18 (10.6)	0.847	13 (38.2)	13 (38.2)	3 (8.8)	0.804
Yes	44 (45.8)	40 (41.7)	12 (12.5)		37 (40.7)	37 (40.7)	11 (12.1)	
DMP N (%)								
No	74 (48.4)	62 (40.5)	17 (11.1)	0.972	5 (33.3)	5 (33.3)	1 (6.7)	0.625
Yes	53 (46.9)	47 (41.6)	13 (11.5)		45 (40.9)	45 (40.9)	13 (11.8)	

T2DM: Type II Diabetes Mellitus, DR: Diabetic retinopathy, BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, DME: Diabetic macular edema, DMP: Diabetic maculopathy

Table 5. Comparison of AKR1B1 genotype and allele frequencies between PDR and NPDR subgroups

Groups	Genotypes			*p* value	Alleles		*p* value
	CC	CT	TT		C	T	
NPDR N(%)	48 (56.5)	31 (36.5)	6 (7.1)	**0.017**	127 (73.8)	45 (26.2)	**0.003**
PDR N(%)	13 (32.5)	19 (47.5)	8 (20)		43 (55.1)	35 (44.9)	

PDR: Proliferative diabetic retinopathy, NPDR: Nonproliferative diabetic retinopathy

Table 6. Risk factors for PDR using logistic regression analysis

Variables	Odds ratio	95% CI	P value
BMI	1.063	0.969 - 1.167	0.194
Diabetes duration	1.108	1.006 - 1.222	**0.038**
Presence of hypertension	1.429	0.590 - 3.465	0.429
Total cholesterol	1.001	0.987 - 1.015	0.933
LDL	1.004	0.983 - 1.025	0.734
HDL	0.993	0.951 - 1.037	0.756
Triglyceride	0.999	0.995 - 1.003	0.628
HbA1c	0.788	0.535 - 1.161	0.228
Fasting glucose level	1.006	0.999 - 1.012	0.075
AKR1B1 CT genotype	2.281	0.900 - 5.780	0.082
AKR1B1 TT genotype	5.204	1.307 - 20.718	**0.019**

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein

DISCUSSION

Acute Type A aortic dissection is a serious clinical even if the blood glucose level is effectively controlled, some DM patients develop DR, while others do not. The data obtained from the studies showed that in addition to other risk factors such as long-term exposure to hyperglycemia and the presence of hypertension, genetic factors also involved in the development of DR (6,13). In the past, the relationship of AKR1B1 polymorphisms with the development and severity of DR in DM patients has been investigated, in various populations (12–17). To the best of our knowledge, present study is the first study exploring the association between AKR1B1 variations and the development of T2DM and DR in the Turkish population. In our study, there were three groups: T2DM patients with and without DR, and the control group. A relationship was detected between the AKR1B1 rs759853 variation and the severity of DR. When DR patients were divided into two groups as NPDR and PDR, it was observed that frequencies of TT genotype and T allele was statistically significantly higher in the PDR group. As a result of the logistic regression analysis, which included other risk factors, it was determined that diabetes duration and rs759853 TT genotype were risk factors for PDR. Our findings seem to be compatible with the data of a study conducted in Han Chinese patients (18) that reports patients with CT+TT genotype to have an increased risk for PDR. On the other hand, in a study performed in Brazilian patients, contrary to our study, it was reported that CC carriers had an increased risk of PDR development (19). Another study showed that, there was no relationship between DR severity and AKR1B1 rs759853 variation in Jordanian population (20).

In our study, no significant association was found between AKR1B1 rs759853 polymorphism and T2DM and DR susceptibility. There was also no relationship between rs759853 polymorphism and other clinical features except the presence of PDR. Similar to our study, no relationship was found between rs759853 polymorphism and T2DM susceptibility in studies performed by Abu Hassan et al. and Watari et al. in Jordanian and Japanese populations (10,20). On the other hand, Shawki et al. (21) found TT genotype to be associated with increased T2DM risk in the Egyptians. Opposite of this study, Sivenius et al. reported a relationship between C allele and T2DM susceptibility in the Finnish population (22).

In the literature, studies investigating the rs759853 polymorphism and DR susceptibility have also reported different results in different populations. For example, in 3 different studies conducted in Chinese and Brazilian populations, it was revealed that there is no significant relationship between rs759853 polymorphism and DR susceptibility, similar to our results (19,23,24). In addition, two different meta-analyses reported no association between rs759853 polymorphism and DR susceptibility (6,25). However, Cao et al. performed a subgroup analysis depending on the type of diabetes and they found that this polymorphism has a protective effect on the development of DR in T1DM patients (6,25). In another meta-analysis conducted in recent years, including 4313 DR and 5218 diabetes patients from 23 different studies, the T allele and CT+TT genotype were associated with a lower risk of DR (26). Conversely, studies performed in Jordanian, Chinese and Indian populations showed that TT+CT genotypes or T allele were associated with increased DR risk (12,18,20).

In one of the studies evaluating the effects of the rs759853 polymorphism on AKR1B1 expression, it was reported that the AKR1B1 protein content in erythrocytes was higher in TT and CT carriers compared to the CC carriers (10). High expression of AKR1B1 causes an increase in conversion from glucose to sorbitol and sorbitol accumulation. It is known that the sorbitol accumulation in the retina can cause osmotic stress and ultimately retinopathy (18). Moreover, it has been reported that inhibition of AKR1B1 suppresses the expression of genes involved in angiogenesis and reduces neovascularization, which is one of the characteristic features of PDR (27). The higher frequency of TT genotype and T allele in patients with PDR compared to NPDR patients in our study may be partly due to the high AKR1B1 expression and higher sorbitol accumulation in these individuals. However, in our study, similar to many studies in the literature, no relationship was found between AKR1B1 genotype and allele frequency and the development of DR. Therefore, we think that the effect of AKR1B1 rs759853 in Turkish T2DM patients may not be very important at the onset of DR but may be effective in the increase of retinal vessel anomalies and the development of PDR once the disease has started. In another study on the effect of rs759853 on AKR1B1, it was claimed that the C allele showed higher transcriptional activity (28). Such a scenario suggests that different mechanisms may be dominant in the regulation of glucose conversion and sorbitol accumulation in Turkish patients.

Different results in various populations regarding the relationship between the AKR1B1 rs759853 variation and the development of T2DM, DR and PDR may be due to different ethnicity, the number of individuals included in the study, differences in environmental factors, and differences or errors associated with the statistical methods used.

To conclude, in this study, we revealed that there is no relationship between the AKR1B1 rs759853 variation and the development of T2DM and DR in Turkish individuals, but that the rs759853 variation and diabetes duration are independent risk factors for the PDR development. The relatively small number of patients and the inability to study other polymorphisms of AKR1B1 can be counted among the limitations of the current study. Therefore, future studies with more patients in the Turkish population are important to support our data.

CONCLUSION

ATAAD is an important cardiovascular disease that requires urgent surgical intervention. Today, it has higher mortality and morbidity compared to other cardiac surgical operations. Pneumonia is an important morbidity that occurs in these patients in the postoperative period. We demonstrated that ABG/eAG ratio, calculated at admission time, is a significant predictor of the development of postoperative pneumonia. Multicenter prospective studies must be used to support our study.

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Research Article

Evaluation of Gastrocnemius Spasticity With Shear-Wave Elastography in Children with Cerebral Palsy after Botulinum Toxin Injection: Defining A Proper Position for Measurement

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Abstract

Aim: Cerebral Palsy (CP) is the most common neuromuscular disorder in children and it is characterized by a dysfunction in movement and posture. Botulinum toxin injection is a treatment method used for muscle spasticity in patients with CP. Elastography is a new method which is used for measuring muscle stiffness. This study aims to evaluate the gastrocnemius muscle stiffness in cerebral palsy patients before and after botulinum toxin injection by using the elastography method and contribute to the establishment of a treatment algorithm with a proper position for measurement.

Materials and Method: The participants of this study were chosen from the patients of our hospital's orthopaedics and traumatology department. Among the patients on whom botulinum injection to gastrocnemius muscle was planned, 30 patients were randomly selected. Elastography of both gastrocnemius muscles were taken before the injection of botulinum toxin, in the third week and third month after the injection. Simultaneously with the elastography, Modified Ashworth Scale (MAS) values were noted. In hemiparetic patients, contralateral legs were taken as the control group.

Results: The elastographic values of the medial head of gastrocnemius when the knee is in extension and ankle in passive dorsiflexion, were found to be statistically significantly related ($p<0.05$) to the MAS values before botulinum toxin injection, third week and third month post-injection.

Conclusion: Stiffness due to spasticity in gastrocnemius muscle in CP patients was demonstrated through elastographic evaluation. A correlation was found between clinical MAS values. The most proper position was in which the knee is fully extended and the ankle is passively dorsiflexed. Elastographic measurements may be able to be used in these patients as a method of diagnosis in the future and it will help to assess the effectiveness of the treatment after the injection of botulinum toxin.

Keywords: Elastography, sonoelastography, cerebral palsy, gastrocnemius spasticity

INTRODUCTION

Cerebral palsy (CP) is the most common neuromuscular disorder causing disability in children. The prevalence is estimated to be 2-3 per 1000 live births in the literature (1-5). The most frequent type of this condition is spastic CP which constitutes almost 80% of these children. Spasticity has been defined as a motor disorder which is characterized by a velocity-dependent increase in muscle tone, thus, causing abnormal active and passive muscle stiffness (2). Spasticity can be evaluated clinically by scales such as Modified Ashworth Scale (MAS); however, this examination is both a subjective method and may still lack reliability between physicians (3). While searching for a more reliable, objective and quantitative method in measuring spasticity, advances in the ultrasound techniques have led clinicians to find a solution to this problem in recent imaging modalities. Shear-wave elastography (SWE) is a new, cost-effective and noninvasive ultrasound modality, which provides a quantitative measurement of the passive stiffness of the muscles (6,7). There has been an increasing trend towards objective attempts trying to measure the spasticity of the muscles with SWE in children with CP, as an increased muscle stiffness has been found in this population than in healthy children (8-10).

Intramuscular botulinum toxin A (BoNT-A) injection to spastic muscles in CP has been widely used in the

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treatment of the spasticity and is found to be effective for these children in the literature (12). Some characteristics of BoNT-A injection such as the mechanism of action, application and lethal doses are well-known, while there is still no consensus in some of the characteristics such as application techniques and time to repeat the injection (13-16). Measuring the effect of BoNT-A in a quantitative way in CP patients is another important parameter in order to provide a suitable treatment plan and check the efficacy of the treatment. SWE could also be used in this area as a quantitative tool for both diagnosis and follow-up after treatment.

The motivation that prompted us to perform this study was to find out whether we could achieve a technique which could measure the effect of BoNT-A in children with CP and have a significant correlation with MAS. It would be ideal to combine the clinical and radiological means, providing a better understanding of the spasticity in CP patients, therefore the technique be used as a reliable tool in both diagnosis and checking the efficacy of spasticity treatments in this population. Our aim is to try to find the most appropriate position for measuring spasticity of gastrocnemius muscle before and after BoNT-A injection.

MATERIALS AND METHOD

A total of 30 patients, 15 boys and 15 girls were included in this study. These patients were randomly selected from the patients who were admitted to our hospital's department of orthopaedics and traumatology, diagnosed with CP, had lower extremity spasticity on physical examination, and were scheduled for BoNT-A injection for the gastrocnemius muscle. Parents were informed about the study and their consent was obtained. Ethical approval was also obtained from the faculty, before starting the study (Approval No: 83045809-604.01/02-77203). GMFCS IV and V patients who did not have the ability to sit and walk independently, or patients who had previously undergone surgical intervention for their lower extremities were not included in the study. Patients who had static deformity or joint contracture on the ankle were also excluded. In addition, patients who had difficulty in elastographic examination because they were agitated were also excluded from the study, with the prediction that the measurements could be affected due to the possible increase in spasticity. Families who did not want to participate in the study or come to the follow-up were also excluded.

All of the elastographic measurements were made by the same radiologist who had ten years of musculoskeletal radiology and three years of elastography experience and did not know the severity of the disease of children, while the physical examination and BoNT-A injections of the children were all performed by the same orthopedic and traumatology surgeon.

Clinical Evaluation

The patients were evaluated clinically and elastographically preoperatively, in the third postoperative week and in the third postoperative month. GMFCS and MAS were noted in the clinical examination.

Elastographic Evaluation

Elastography was performed on both lower extremities of all patients participating in the study; 14 days prior to botulinum toxin injection, in the third week and third month after the injection. SWE USG (Aixplorer; Supersonic Imagine, Ltd., Aix-en-provence, France) and 4-15-MHz transducer (Supersonic Imagine, Ltd.) were used for elastographic examination. After the ultrasound was set to the musculoskeletal mode, measurements were made separately on the medial and lateral heads of the gastrocnemius muscle. Ten measurements were taken for each leg at different positions of the ankle and knee joint and the values were recorded as kilopascal (kPa). Measurement positions are given in Table 1. Each measurement was taken twice. In cases where inconsistency was detected between two measurements, a new measurement was made and the average of the two measurements that were consistent with each other was taken. The contralateral leg in hemiplegic patients was considered as the control group, and elastographic measurements were also performed on the healthy legs.

Table 1. Elastographic Positions and Numbers

NO	PATIENT POSITION	KNEE	ANKLE	GASTROCNEMIUS HEAD
1	PRONE	EXTENSION	45° PLANTAR FLEXION	MEDIAL
2	PRONE	EXTENSION	45° PLANTAR FLEXION	LATERAL
3	PRONE	EXTENSION	30° DORSIFLEXION	MEDIAL
4	PRONE	EXTENSION	30° DORSIFLEXION	LATERAL
5	PRONE	FLEXION	45° PLANTAR FLEXION	MEDIAL
6	PRONE	FLEXION	45o PLANTAR FLEXION	LATERAL
7	PRONE	FLEXION	30° DORSIFLEXION	MEDIAL
8	PRONE	FLEXION	30° DORSIFLEXION	LATERAL
9	STANDING	EXTENSION	NEUTRAL	MEDIAL
10	STANDING	EXTENSION	NEUTRAL	LATERAL

Elastographic evaluation was performed while the ultrasound probe was in the transverse position, the myotendinous junction of the gastrocnemius muscle was identified and the probe was ascended from distal to proximal. After identifying the medial and lateral heads of the muscle posteriorly, the ultrasound probe was adjusted to the longitudinal axis, parallel to the muscle fibers and the measurement was taken when the clearest image was obtained. Knee flexion and ankle dorsiflexion movements were performed passively by the same physician. While the knee was in 90 degrees of flexion, the ankle was passively supported and fixed at the point where it reached maximum dorsiflexion. While recording the spasticity values, the measurements were made by taking the region between the superficial and deep fascia of the muscle, which is considered to be the most homogeneous ultrasonographically by the radiologist, in a rectangle (Q-Box) created by the software of the elastography device. A circle with a diameter of 5mm (ROI, range of interest) was created in the region of the color scale of the different spasticity values in the formed rectangle, and the average of the spasticity calculated by the software in this circle was recorded (Figure 1). Care was taken that the circle did not touch the hyperechoic muscle fascia. According to the color scale created by the software, the softest and ost loose part of the muscle appears blue, while as the spasticity increases, the color turns into green and yellow; and red color is formed in the most spastic areas.

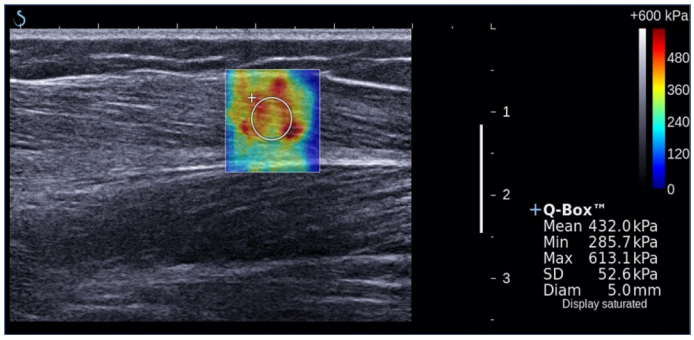

Figure 1. Elastographic appearance and the calculation of the increased spasticity in the gastrocnemius muscle

Botulinum Toxin A (BoNT-A) Injection

BoNT-A was injected into one or both gastrocnemius muscles of the patients whose preoperative clinical and elastographic evaluations were completed, under operating room conditions. Injections were administered (onabotulinum toxin A, Botox, Allergan, Inc. CA, USA) at 5 IU (international unit) per kilogram (kg) after the necessary sterility conditions were met while the patients were under sedation. The injection was technically made from two medial points, namely the medial head of the gastrocnemius muscle and the muscle body. After crossing the muscle fascia with a black tip injector, dorsiflexion and plantar flexion movements of the ankle were made and checking that the injector moves (inside the muscle), 1/3 of the total dose is transferred to the medial head, and the remaining part to the proximal, middle and distal of the muscle body from the second and third points (Figure 2).

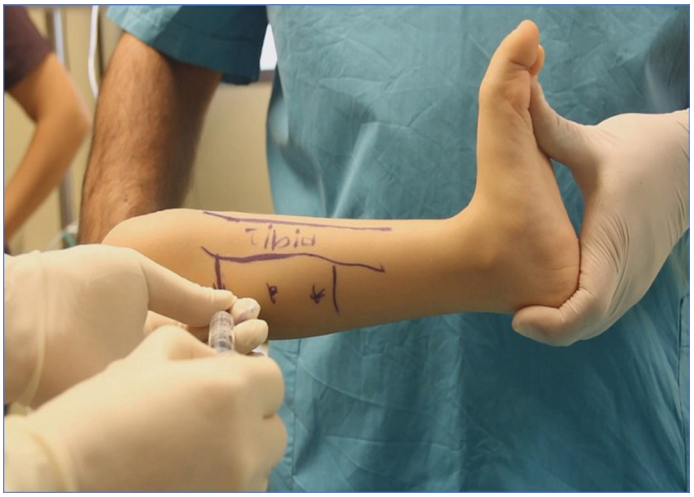

Figure 2. Application technique of botulinum toxin A (BoNT-A) injection to the medial head of the gastrocnemius muscle

Post-injection Care

The patients who were discharged from the hospital at the fourth hour after the injection were kept nonweightbearing for a maximum of one day to relieve their pain and agitation, then they were mobilized on the second day with full weight-bearing, and they were allowed to continue their routine physiotherapy programs.

Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) as well as the conformity of quantitative data to normal distribution were tested with the Shapiro-Wilk test and graphical examinations. One-way ANOVA was used for comparisons between groups of more than two normally distributed quantitative variables, and Tukey test was used for post-hoc pairwise evaluations. The Kruskal Wallis test was used for comparisons between groups of more than two quantitative variables that did not show normal distribution, and the Mann-Whitney U test was used for post-hoc pairwise evaluations. Student's t test was used for two-group comparisons of normally distributed quantitative variables, and the Mann Whitney U test was used for two-group comparisons of non-normally-distributed quantitative variables. Paired t test was used for the in-group pairwise evaluations of normally distributed quantitative variables. Wilcoxon Signed Ranks test was used for the pairwise evaluation of quantitative variables that did not show normal distribution. Spearman correlation analysis was used to evaluate the relationships between quantitative variables. Statistical significance was accepted as $p<0.05$.

RESULTS

The mean age was 4.87±1.63 years. The demographics of the patients and characteristics of the disease were described in Table 2. There were no patients with any side effects or complications related to the injection. The change in the decrease in the postoperative (post-injection) third week values according to the preoperative MAS values of the cases was found to be statistically significant ($p<0.001$). It was determined that the change in the direction of decrease observed in the postoperative third month values according to the preoperative MAS values of the cases was statistically significant ($p<0.001$). In addition, it was determined that the change in the increase in the postoperative third month values compared to the postoperative third week MAS values was statistically significant ($p=0.023$) (Figure 3).

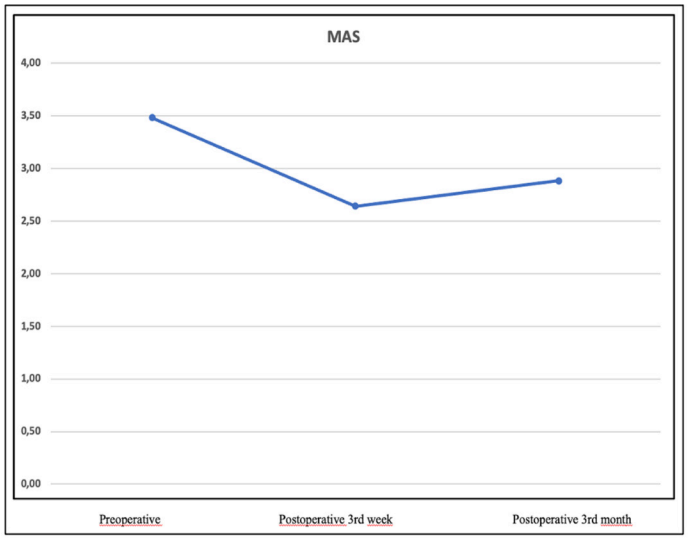

Figure 3. The distribution of Modified Ashworth Scales (MAS) preoperative and postoperatively

Fifty legs of thirty patients in total were evaluated elastographically and clinically in ten different positions. The relationship between these evaluations and MAS values in the preoperative period, postoperative third week, and postoperative third month was examined. As a result of the statistical analysis, the MAS value was found to be correlated with positions 1, 3, 4, 5, 7, 8, 9, and 10, preoperatively. In the evaluations after botulinum toxin application, there was a significant correlation between the 3rd, 9th and 10th positions of these positions and MAS in the postoperative third week ($p<0.001$, $p<0.003$, $p<0.007$); 3rd, 4th, 7th, 8th and 9th positions were found to have a significant correlation with MAS at the postoperative third month ($p<0.001$, $p<0.006$, $p<0.002$, $p<0.007$, $p<0.015$, respectively). When the effect of BoNT-A injection on elastographic measurements was evaluated, there were two positions (3 and 7) out of a total of 10 positions, with statistically significant results both in the third week and third month after the injection.

Table 2. Demographics of the patients and characteristics of the disease

(n=30)		Min-Max	Mean±ss
Age (years)		2-8	4.87±1.63
		n	**%**
Sex	Female	15	50.0
	Male	15	50.0
Diagnosis	Hemiparetic	10	33.3
	Diparetic	20	66.7
MAS	1	1	3.3
	1+	3	10.0
	2	9	30.0
	3	17	56.7
GMFCS	1	10	33.3
	2	16	53.3
	3	4	13.3
Side	Right	5	16.7
	Left	5	16.7
	Bilateral	20	66.7
History of BoNT-A injection	-	18	60.0
	+	6	20.0
	++	5	16.7
	+++	1	3.3

Two positions (3 and 7) with statistical significance were determined in terms of the relationship between the pre-injection period and the elastography values in the third week and third month after the injection. The change observed in the postoperative third week values compared to the preoperative position 3 elastographic values was found to be statistically significant ($p=0.001$). When compared to the preoperative position 3 elastographic values of the cases, the change observed in the postoperative third month values was found to be statistically significant ($p=0.001$). It was determined that the change observed in the postoperative 3rd month values according to the 3rd week position 3 elastographic values of the cases was not statistically significant ($p>0.05$).

There was no statistically significant difference between the cases with and without a history of BoNT-A injection in terms of the changes observed in the postoperative third week values according to the preoperative MAS values ($p>0.05$). Neither was there a statistically significant difference between the cases with and without a history of injection in terms of the changes observed in the postoperative third month values, when compared to the preoperative MAS values ($p>0.05$). The increase observed in the postoperative third month values compared to the preoperative MAS values in cases with a history of injection was found to be statistically significantly larger than the increase observed in this period in cases without a history of injection ($p=0.018$).

When hemiparetic cases were considered as a separate group, statistically significant results were obtained in positions 3, 4, 9 and 10 from the elastographic evaluations made in ten different positions compared with the opposite leg (control group).

To summarize the statistical analysis, only one position (3) achieved statistically significant meaning both in hemiplegic and diplejic patients, in terms of the changes in the MAS, elastographic values and the effect of BoNT-A injection preoperatively, in the third week and third month postoperatively.

DISCUSSION

The main finding of our study claims that spasticity of the gastrocnemius muscle could be measured consistently with sonoelastographic examination in children with cerebral palsy. While the knee is fully extended and the ankle is positioned in passive dorsiflexion, the changes on the medial head of the gastrocnemius muscle are measurable with a statistically significant difference preoperatively and postoperatively. This position has been also correlated with MAS values and the effect of botulinum toxin injection.

In a study conducted by Vasilescu et al. in 2010, the use of sonoelastography was examined in 7 patients with cerebral palsy who received botulinum toxin injection indication due to muscle spasticity, and elastographic examination was performed (9). In the control measurements made after botulinum toxin injection in these patients, it was stated that the stiffness and contraction of the injected muscles decreased in five out of seven children. The small number of patients studied and the lack of homogenization in terms of CP types were the limitations of that study. The use of elastography for gastrocnemius spasticity in CP patients was also approved by the study of Maisetti et al. in 2012 and it was stated that elastography can provide an indirect calculation of passive muscle strength (10).

In a study by Kwon et al. in 27 legs of 15 CP patients in 2012, 12 healthy children were evaluated as the control group and shear wave velocities at the medial gastrocnemius head in CP patients were found to be faster than the control group (11). In addition, it was stated that the results obtained were correlated with the MAS scores in the clinic. In a letter to the editor of this study, the work was appreciated, but some parts of it were criticized (17). The first of these criticisms was about elastography positions. In the study where it was stated that the patients were placed in the prone position and the ankles were hung from the examination bed, the study was criticized for not providing information about the ankle position. Since the gastrocnemius is a double-jointed muscle, muscle stiffness may increase while the ankle is in dorsiflexion and may decrease while in plantar flexion and it was stated that this could affect the measurements. Considering this fact in our study, it was thought that the measurements could change with the knee position as well as that of ankle.

Other studies have shown that the stiffness of the gastrocnemius muscle changes in certain positions of the ankle. In the study published by Chino et al. in 2012, the stiffness in the gastrocnemius decreased in plantar flexion; in the study of Akagi et al., it was reported that this stiffness increased with ankle dorsiflexion (18,19). In our study, the stiffness in the gastrocnemius muscle decreased when the ankle was plantar flexed; and increased when the ankle was dorsiflexed. The majority of elastographic examinations that resulted in statistically significant results in our study were the measurements made in positions where the ankle was passively dorsiflexed.

In a study by Gi-Young Park et al., BoNT-A was injected into the gastrocnemius muscle of 17 CP patients and physiotherapy was performed after the injection (20). The results were evaluated elastographically in the preoperative period and postoperative fourth week. At the end of the study, it was determined that there was a decrease in muscle spasticity in the short-term in patients who received physiotherapy with botulinum toxin injection and MAS scores decreased significantly, both of which could be demonstrated by elastographic methods. In our study, the correlation of elastographic values with clinical MAS at baseline could be demonstrated in eight out of ten different positions. However, considering the values in the third week and third month after BoNT-A injection, this correlation disappeared in most of the positions. There were two positions (3 and 9) where the correlation could be shown. A high correlation was found between elastographic measurements in these two positions and clinical MAS values. A statistically significant relationship was also found between the elastography values in the period before BoNT-A injection and the values in the third week and third month after the injection in two positions (3 and 7). One and only position that has statistically significant changes in terms all MAS and botulinum toxin effect preoperatively and postoperatively was the position 3, where the stiffness of the medial gastrocnemius head is measured when the knee is fully extended and the ankle is in passive dorsiflexion.

Healthy children were not included in our study, but the healthy sides of the hemiparetic patients were examined as the control group. It was observed that the affected leg was statistically significantly stiffer than the healthy leg in the preoperative period, this stiffness statistically approached the stiffness in the intact leg in the third week after BoNT-A injection, and this effect continued in the postoperative third month.

In a study published by Arda et al. in 2011, the elasticity values of normal soft tissues were tried to be defined by elastography method and a standardization established (21). In this study, regardless of age, the average elasticity of the gastrocnemius muscle was 11.4±4.1 kPa in men and 11.0±4 in women. Since all of the patients in our study were hemiplegic or diplegic a value range could not be determined in terms of gastrocnemius muscle stiffness in normal individuals. Although elastographic examination

has been performed on the intact legs of hemiplegic patients, it is known that the legs that are considered to be healthy in hemiplegic type CP patients may also be slightly affected by spasticity. In this respect, these values should not be perceived as the values of a normal individual.

In our study, patients who had botulinum toxin injections before and those who did not have a history of injection were also compared in terms of MAS values. In the examinations, it was observed that the increase in the MAS values in the postoperative third month was higher in patients who had previously received botulinum toxin injection compared to the MAS values in the preoperative period compared to the patients who did not have a previous injection history. This can be interpreted as an indication of the fact that the effect of botulinum toxin may disappear faster in patients with a previous injection history. This effect has been described in the literature as the development of resistance to repeated botulinum toxin injections (22).

There are some restricting factors and limitations of our study. Although it is the largest patient group, as far as we can determine, in which CP and elastography methods have been studied together in the literature, the number of legs that can be taken as a control group has been limited, especially due to the small number of hemiparetic patients. At this point, it should be noted that in hemiparetic patients, both lower extremities are affected by spasticity, and the other leg is usually assumed to be normal since the effect on one leg is dominant in the clinic. Therefore, conducting similar studies in the future with healthy groups will provide useful information to the literature. In addition, the fact that our patient follow-up period is limited to three months is another limiting factor. Long-term follow-up and elastographic examinations of these patients are important, especially in order to investigate the long-term effects of botulinum toxin injection. One of the limitations of our study is that during the elastographic evaluations, it was not detected by the superficial EMG method whether the patients actually were calm with the correct muscles relaxed and measured. Another limiting factor is the amount of passive dorsiflexion applied especially to the ankles of the patients during the extractions. Although passive dorsiflexion is performed by the same physician each time, it is obvious that there may be variations in this amount from time to time. Adjusting the dorsiflexion force with the help of a device to apply the same force to each patient each time would have contributed significantly to the standardization of the study.

Another limitation regarding the botulinum toxin injection we applied in our study is that we did not receive ultrasound support during the BoNT-A injections. However, in a study conducted by Chin et al. in 2005, it was reported that manual injections had given satisfactory results (over 75%) only in gastro-soleus applications after a total of 1372 separate botulinum toxin injections made manually in the upper and lower extremities of 226 CP patients (22). In our study, the applications were directed only to the gastrocnemius muscle. In addition, all of the injections were made by the same orthopedic surgeon who was experienced in botulinum toxin injection and the limitation of this factor was tried to be eliminated.

CONCLUSION

In conclusion, shear wave elastography is a non-invasive, inexpensive, easy-to-apply and effective imaging method that has been increasingly used in the musculoskeletal area in recent years. In addition to the diagnosis of many diseases, studies are ongoing to control and follow-up the efficacy of treatment with elastography. CP is a pathology that elastographic examination may be used due to the spasticity and muscle stiffness. Since the evaluation of muscle spasticity with MAS in clinical examination differs from physician to physician, an effort has been made to demonstrate spasticity with imaging methods in order to provide standardization in this regard. Increased spasticity in the gastrocnemius muscle can be objectively demonstrated by elastographic examination. Elastography can be used as a diagnostic method in this context, as well as to evaluate the effectiveness of botulinum toxin and similar spasticity reduction interventions. In our study, one position was found to be statistically significant and clinically correlated with MAS across the measurements. This was the measurement of the medial head of the gastrocnemius, where the knee is fully extended and the ankle is passively dorsiflexed.

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MEDICAL RECORDS-International Medical Journal

Research Article

Identification of Neutrophil/Lymphocyte Ratio as a Unique Biomarker for Migraine Follow-up

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Abstract

Aim: As in many chronic pain syndromes, self-reported pain is the main criterion used by clinicians assessing patients with migraine. However, it is subjective, and there is no reliable marker for follow-up, and it hinders adequate follow-up and treatment. Therefore, in this study, we aimed to investigate the correlation of the NLR (neutrophil-lymphocyte ratio) level, which is increased due to the neuroinflammatory process and oxidative stress, with the clinical and demographic characteristics of the patients, and to investigate its feasibility as an objective marker during follow-ups.

Materials and Methods: Our study included one hundred migraine patients without aura who met the "The International Classification of Headache Disorders 3rd edition" (ICHD-3) criteria. The demographic characteristics of all patients and the clinical features of migraine pain were obtained. In addition, blood NLR level and its correlation to these features were examined.

Results: There was a significant and positive correlation between NLR level with disease duration and pain severity ($r=0.43$ $p<0.001$, $r=0.76$ $p<0.001$, respectively), however, it was independent of age, gender, and migraine subtypes (episodic/chronic) ($p=0.48$, $p=0.14$, $p=0.13$, respectively).

Conclusions: Our study found a significant correlation between the NLR level and the clinical features of migraine patients, indicating that this easily accessible parameter may be a promising marker that can be used during the follow-up period.

Keywords: Biomarker, migraine, neutrophil lymphocyte ratio

INTRODUCTION

Migraine is one of the most common diseases in the world, and if the attacks are not controlled, it seriously affects the quality of life (1-3). Although there are many alternative therapies for migraine currently, it is still insufficient for many patients. In many chronic pain syndromes, self-reported pain by the patients is the primary criterion used by clinicians assessing pain. However, it is subjective, and multiple intrinsic and extrinsic factors can affect pain levels, and this prevents effective treatment and follow-up. Therefore, it is essential to investigate a reliable and objective marker for better treatments.

The neuroinflammatory process, vasomotor changes, some cytokines, neuropeptides, and inflammation due to increased oxidative stress and the pathophysiological process in the vessels have been suggested as responsible for the underlying mechanism (4,5). In systemic inflammation and oxidative stress, even if the total white blood cell count is within normal limits, increased neutrophil and decreased lymphocyte counts occur. Thus, neutrophil/lymphocyte ratio (NLR) becomes a more reliable marker in this case (6,7). Studies have shown that NLR can predict prognosis and mortality in many conditions such as coronary artery disease, malignancies, and rheumatological and neurological diseases (8-11). In addition, since it is an easily accessible and inexpensive parameter, we hypothesized that NLR can be used as a useful and practical biomarker to show the inflammation and oxidative processes, and follow up the patients.

In previous studies, NLR was found to be significantly higher in migraine patients compared to the control group during the attack, but there was no difference between them in the attack-free period (12,13). While these studies have concluded that NLR is an increasing parameter during the attack period, it is still unknown whether it can be used as biomarker in the patient's follow-up period.

CITATION

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In this study, we aimed to investigate the NLR level in migraine patients and its correlation with clinical and demographic characteristics, thereby its usability as a reliable and objective biomarker in the follow-up of patients.

MATERIAL AND METHOD

Study Design

We conducted a cross-sectional study with 100 subjects with migraine without aura examined at our Neurology Outpatient Clinic. Diagnosis of all patients was made by a neurologist according to International Headache Disorders Classification-III (beta version) (ICHD-III) (14). This study was approved by the X Ethics Committee (Date: 16.6.2022, Decision No: 12/14) and was conducted following the principles of the Declaration of Helsinki.

Participants

Inclusion Criteria: Adult patients who were between 18–65 years old and have certain diagnosis of migraine without aura.

Exclusion Criteria: Presence of any additional disease or condition that may lead to elevated inflammation markers; presence of any infection (sinusitis, otitis, urinary tract infection, etc.), concomitant chronic inflammatory disease, cardiovascular or pulmonary disease, smoking, alcohol use >20g/day, hypertension, diabetes, obesity (BMI ≥30), corticosteroid or use of immunosuppressant medication, and migraine treatment in the last six months or currently having a migraine attack.

Demographic and Clinical Characteristics

As a primary outcome, we investigated the correlation between demographic and clinical characteristisc (age, gender, disease duration, number and severity of attacks) and NLR level. Headache severity was scored with VAS (visual analog scale). NLR level which is calculated by dividing the neutrophil count by the lymphocyte count was evaluated with blood samples obtained during morning fasting.

Pain Assessment

VAS was used for evaluation of pain. VAS is a validated, unidimensional scale for acute and chronic pain (15). It is commonly used in clinics related to pain. Pain severity is rated with a simple 10- point scale (0 = "no pain", 10 = "pain as bad as you can imagine"). Pain severity is considered mild if it was less than 3.5, moderate if it was between 3.5 and 6.5, and severe if it was above 6.5 (16).

Laboratory Assessment

Blood samples were obtained after an overnight fasting period. Blood lipid profiles, including total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG), high-density lipoprotein (HDL) were measured in mg/dl units. TC, TG, and HDL concentrations were measured using an enzymatic method. LDL concentrations were calculated using Friedewald's formula (17).

Statistical Analysis

Statistical Package for the Social Sciences package program version 28.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Descriptive analysis of assessment results was evaluated with mean and standard deviation for continuous variables, and frequency and percentage for categorical variables. Histogram and Shapiro-Wilk tests were used for normality distribution. Unpaired t-test and Mann-Whitney U tests were used for continuous variables according to normality distribution, and Fisher Exact test was used for categorical variables in comparing groups. Pearson or Spearman tests were used for correlation analysis according to normality distribution. The significance value was accepted as $p<0.05$.

RESULTS

A total of 100 migraine patients without aura were included in the study. None of the patients were using anti-migraine treatment during the attack or prophylactically. Further clinical data and the laboratory parameters were provided in Table 1.

Table 1. Descriptive and clinical characteristics of patients

	Patients
Age (years)	34.9 (±11.4)
Gender (F/M)	86/14
Disease duration (month)	28.4 (±33.7)
Episodic/Chronic	58/42
Average of pain severity (0-10)	5.35 (±2.16)
Average of NLO level	1.89(±0.77)

* F: Female, M: Male, NLO: Neutrophil-lymphocyte ratio, Mean± SD; n (%)

The mean pain intensity was 5.35 (±2.16) out of 10 which is considered as the moderate severity. There was a significant and positive correlation between the duration of the disease, and the severity of the pain with the NLR level ($r=0.43$ $p<0.001$, $r=0.76$ $p<0.001$, respectively) (Figures 1 and 2).

Also, NLR level was found to be independent of age, gender, and migraine subtypes (episodic/chronic) ($p=0.48$, $p=0.14$, $p=0.13$, respectively).

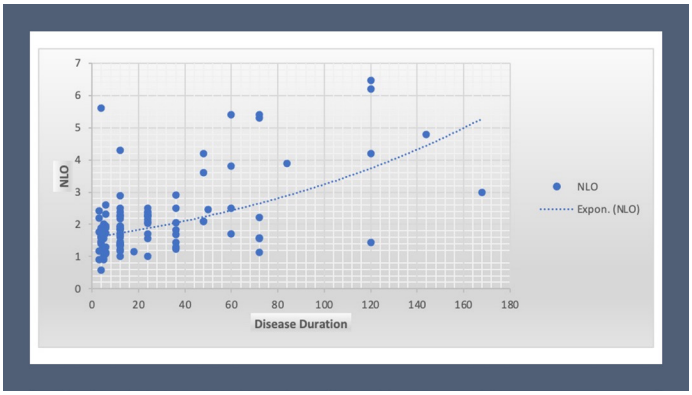

Figure 1. Positive correlation between NLO and disease duration

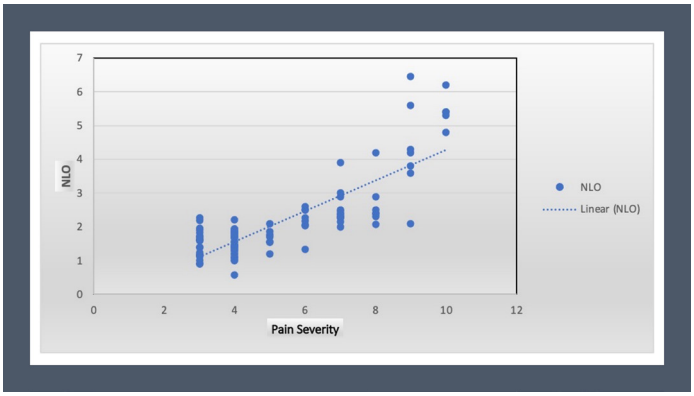

Figure 2. Positive correlation between pain severity and NLO level

DISCUSSION

Our study showed that NLR was positively correlated with the pain severity and disease duration, also independent of age and gender in migraine patients. This result supports our hypothesis that NLR is not only a parameter increasing during the migraine attack period but can also be used as a prognostic marker related with clinical features of the disease. Thus, we did not prefer to obtain only the level of NLR in migraine patients, so we searched the correlation between these parameters to find an easy applicable prognostic tool.

Recent studies have shown that the NLR level, as an indicator of systemic inflammation, is more reliable than the neutrophil or lymphocyte count alone or the total white blood cell count. Furthermore, this parameter is in parallel with the progression of the disease (18-20). Along with studies showing that increased NLR level can be an independent prognostic marker in cerebrovascular, cardiovascular diseases, vasculitis, and cancer. Promising results were also reported in a meta-analysis investigating its usability on sepsis progression, and it was suggested as a reliable marker in this direction (8,9,21-23).

To date, studies on NLR level in migraine patients have usually been conducted to investigate whether NLR level increases during the attack or not (12-24). For example, Karabulut et al. showed that the NLR level in migraine patients was higher during the attack than in healthy controls (24), and Ateş et al. showed that the NLR level in migraine patients with aura was higher during the attack than in the attack-free period, and the control group (13). In addition, Ocak et al. did not find any differences between migraine and other headaches for the NLR level. They suggested that it cannot play a role in the differential diagnosis (25).

In this study, we found a positive correlation between NLR level with migraine attack severity and disease duration. Since there is no reliable biomarker for follow-up of chronic pain diseases, an objective evaluation is often difficult. The fact that NLR is positively correlated with pain severity saves us from this difficulty and offers an objective evaluation opportunity. In addition, the positive correlation between NLR level and the disease duration suggests that patients are constantly exposed to neurogenic inflammation even if they are not in the attack period. This increased exposure time may be related to the NLR level. However, Simsek et al. found that NLR level is not related to parameters such as age, disease duration, attack number and duration (12). This result is likely to be associated with the time of the NLR level evaluation, which is during the attack; the increased NLR level during the attack may not have provided objective information about the clinical features of the disease. Surprisingly, our study found no difference between episodic and chronic subtypes, suggesting that the NLR level may be independent of the number of attacks.

Our study has several limitations. First, only migraine patients without aura were evaluated, so future studies with larger numbers of patients, including migraine patients with aura, will help to understand the value of this parameter as a prognostic marker. Also, we conducted a cross-sectional study, which does not allow us to assess the direction of the association.

CONCLUSION

In conclusion, our study will help to individualize the follow-up and treatment of each patient, thanks to this objective biomarker. In addition, the fact that NLR is an easily accessible, inexpensive, and reliable parameter which is supposed to be a promising biomarker that may be used in the follow-up of chronic pain syndromes such as migraine.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This study was approved by the Antalya Kepez State Hospital Ethics Committee (Date: 16.6.2022, Decision No: 12/14) and was conducted following the principles of the Declaration of Helsinki.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Characterization of Apelin/APJ Axis Expression in Normal Testicular Tissue, Germ Cell Neoplasia in Situ, and Testicular Seminoma

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Abstract

Aim: A testicular germ cell tumour is not observed widely, but its incidence and mortality rates have increased in recent years. One of the most common forms of this tumour is seminoma. Germ cell neoplasia *in situ* (GCNIS) is the precursor of seminoma. The apelin/APJ axis is increased in many cancers and is a pathway that plays an active role in angiogenesis, lymphangiogenesis, tumour growth, and migration. This study investigated the cellular distributions of apelin and APJ protein expressions in normal testicular tissue (TT), GCNIS, and seminoma.

Material and Methods: Tissues from 18 patients who had undergone orchiectomy were used in this study. These tissues include areas of normal TT, GCNIS, and seminoma. Immunolocalisation of apelin and APJ were identified through the immunohistochemical method.

Results: Apelin expression was significantly increased in seminoma and GCNIS compared to normal. Apelin expression were the same in GCNIS and seminoma. APJ expression was significantly increased in seminoma compared to normal and GCNIS. Normal and GCNIS APJ expressions were similar.

Conclusion: Expressions of apelin and APJ proteins were significantly increased in seminoma in our study. Our findings were consistent with the results of relevant studies as increased expression of apelin/APJ has been observed in many different cancers. It can be predicted that the increase of this pathway in seminoma may support angiogenesis, lymphangiogenesis, migration, and metastasis. Therefore, the increase in mortality rates in seminoma patients may be related to apelin/APJ axis. Ultimately, the use of inhibitors of this pathway in these patients may reduce their mortality rate. New studies are needed before these inhibitors can be used clinically.

Keywords: Apelin, APJ, GCNIS, seminoma, testis

INTRODUCTION

Testicular germ cell tumour (TGCT) is a rare tumour seen mostly in developed countries (1). TGCT is common in young men, and 60% of these patients are seminoma. Germ cell neoplasia *in situ* (GCNIS) is the noninvasive precursor of type II TGCT. The pathogenesis of TGCT is an arrest that occurs during the maturation of primordial germ cells (PGCs) or gonocytes in the embryonic period. The reason for the PGCs/gonocytes' arrest remains unclear (2). At puberty, GCNIS transforms into a seminoma with the same gene expression and histology as PGC (2,3) or is remodelled into embryonal carcinoma (EC) (2). In the United States, it is expected that there will be 9910 new testicular cancer diagnoses and 460 deaths from testicular cancer in 2022 (4). Although TGCT is not commonly observed, the incidence rate has increased by 70% in the last 20 years (5). Despite the increase in the incidence of the disease, the mortality rate of the disease has decreased considerably with the new generation of surgical methods accompanied by radiotherapy and chemotherapy (6). Almost all TGCT patients in stage 1 can be treated. However, 30% of TGCT patients are diagnosed in the metastatic stage, which reduces the cure rates of the disease. The 5-year survival rates in patients with metastatic seminoma range from 72% to 86% (7). However, these patients may resist chemotherapy or relapse in the future (8). This situation causes the treatment to fail. One study found that 16.8%

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of the more than 1500 unselected TGCT patients had a recurrence (9). Due to the increase in incidence day by day, its resistance to chemotherapy, and the possibility of recurrence of TGCT, the development of new specific therapeutic agents is needed to increase the success of the treatment of this disease. In order to develop these therapeutics, molecular pathways in seminoma cells need to be elucidated in more detail.

Apelin is the ligand for APJ. This endogenous ligand is encoded by the APELIN gene (10). APJ is a G protein-coupled receptor with 7 transmembrane domains, first identified in 1993 (11). Apelin and APJ proteins are very common in the body. Apelin and APJ play a protective role in cells by regulating blood pressure, food intake, endoplasmic reticulum stress, and cardiac metabolism in the body (12,13). The apelin/APJ axis is expressed in many tissues, including testis (14,15). Apelin and APJ play an important role in angiogenesis and vessel morphogenesis (16). In addition, they are expressed in many cancer tissues. Apelin is an important factor for angiogenesis in muscle-invasive bladder cancer tissues (17). The expression of apelin/APJ is significantly increased in brain tumour microvascular proliferation (18). On the APELIN gene in endothelial cells, there is a binding site for HIF-1α, which is released due to hypoxia, and HIF-1α binding to this region enables the expression of apelin (19). Hypoxia is also clearly observed in solid tumours. Hypoxia in solid tumours increases invasion and metastasis and may cause angiogenesis by activating proangiogenic factors (20,21). Apelin stimulates lymph node metastasis and lymphangiogenesis by activating ERK and PI3K pathways (10). Negative correlations were found between high apelin expression and survival in many different cancers and adenocarcinomas (16,17,22-32). The apelin/APJ axis expression is higher in colorectal cancer patients' tissues (30). Significant correlations have also been found between APJ expression level and the aggressiveness of renal cell carcinoma (33). These cancer studies performed in different organs and tissues showed that the apelin/APJ axis can be used as a marker to predict the patient's prognosis.

The cellular expression distributions of apelin and APJ in TGCT and GCNIS have not been investigated before when the literature was searched. It is essential to elucidate the molecular mechanisms of the apelin/APJ pathway in cancer cells to treat TGCT patients, whose incidence has increased in recent years. In this study, we investigated the expression pattern of apelin and APJ in tissues obtained from patients who undergoned orchiectomy.

MATERIAL AND METHOD

Clinical assessment of patients with prostate cancer

Tissues of patients who had undergone orchiectomy were used in this study. Paraffin-embedded tissues of 18 patients diagnosed with seminoma in postoperative pathology were used. These samples contain areas of normal TT, GCNIS, and seminoma tissue. The study was performed in accordance with the Declaration of Helsinki. Ethical approval was obtained from Akdeniz University Clinical Research Ethics Committee (KAEK-511).

Immunohistochemistry

Paraffin blocks were cut into 5 µm thick sections. After deparaffinisation with xylene, rehydration was performed in decreasing-grade alcohols (100% -90% -80% -70% -dH_2O). Antigen retrieval was made by boiling with pH:6.0 citrate buffer and allowed to cool at room temperature. Afterwards, the sections were washed with phosphate-buffered saline (PBS). Endogenous peroxidase activity was inhibited with 3% H_2O_2. Sections were washed with PBS and blocked for 7 minutes at room temperature with UV block solution (TA-125UB; Lab Vision). Then, apelin rabbit polyclonal primary antibodies (1:200 dilution, Novus Biologicals LLC; NBP10730) and APJ rabbit polyclonal primary antibodies (1:400 dilution, Bioss; bs2430R) were incubated overnight at +4°C. The next day, the sections were washed with PBS and incubated with biotin-conjugated anti-rabbit secondary antibodies (1:500 dilution, Vector Lab.; BA-1000) for 40 minutes in a humidified chamber at room temperature. Sections were washed with PBS and incubated with HRP-conjugated streptavidin (TA-125HR; Lab Vision) for 20 min. Signals were developed with diaminobenzidine (DAB) (D4293; Sigma-Aldrich) by washing with PBS and counterstaining with hematoxylin (Merck, MHS32). The sections were dehydrated, taken into xylene, and then covered with entellan (Merck, MHS32).

ImageJ Analysis

Ten photographs were taken from each immunohistochemically stained section with an Olympus CX31 microscope. The staining intensities of the photographs were evaluated with the ImageJ (http://imagej.nih.gov/ij/) program. The immunostaining intensity in each region was calculated as a percentage by proportioning the immunostained area in each photograph to the total area and multiplying it by 100. The same processes were applied in our previous study (34).

Semi-Quantitative Evaluations

Cells were immunostained with apelin and APJ, and the staining intensity was measured semi-quantitatively. The scoring was performed in the following manner: 0 = negative; (+) = weak positive; + = positive; ++ = dense positive; +++= very dense positive.

Statistical Analysis

All data were analysed with GraphPad Prism 9.0 (GraphPad Software) for statistical significance and are reported as mean±s.e.m. A one-way ANOVA and Tukey method were used for multiple comparisons to evaluate differences. Differences with $p<0.05$, compared to the normal seminiferous tubule, were considered statistically significant.

RESULTS

Apelin immunolocalisation on normal TT, GCNIS, and the seminoma of the human testis

In normal TT, GCNIS, and seminoma, apelin immunolocalisation was cytoplasmic. Apelin immunostaining was negative in normal TT, germ, Sertoli,

and endothelial cells. However, apelin immunostaining in Leydig cells was very dense positive (Figure 1A, Table 1). Apelin expression was significantly increased in GCNIS compared to normal TT ($p<0.05$, Figure 1B, E). The apelin expression in GCNIS was densely positive in germ cells, weakly positive in Sertoli and endothelial cells, and very dense positive in Leydig cells (Figure 1B, Table 1). The expression of apelin in seminoma was significantly increased compared to normal seminiferous tubule ($p<0.05$), while it was the same as in GCNIS ($p>0.05$, Figure 1C, E). Apelin expression was the same in seminoma and GCNIS tissues ($p<0.05$, Figure 1B-C, E). Seminoma apelin expression was very dense positive in tumour cells and weakly positive in endothelial cells (Figure 1C, Table 1). Seminoma areas contain tumour cells and infiltrated leukocytes.

APJ immunolocalisation on normal TT, GCNIS, and seminoma of human testis

APJ immunolocalisation was cytoplasmic and nuclear in normal tissue, GCNIS, and seminoma (Figures 2A-C). Expression of APJ in normal tissue was weakly positive in germ cells and very dense positive in Sertoli, Leydig, and endothelial cells (Figure 2A, Table 1). GCNIS APJ expression was very dense positive in germ, Leydig, and endothelial cells and dense positive in Sertoli cells (Figure 2B, Table 1). Normal TT and GCNIS APJ expression were similar ($p>0.05$, Figure 2E). APJ expression was significantly higher in seminoma than in normal TT and GCNIS ($p<0.05$, Figure 2E). Seminoma APJ expression was very dense positive in tumour cells and positive in endothelial cells (Figure 2C, Table 1).

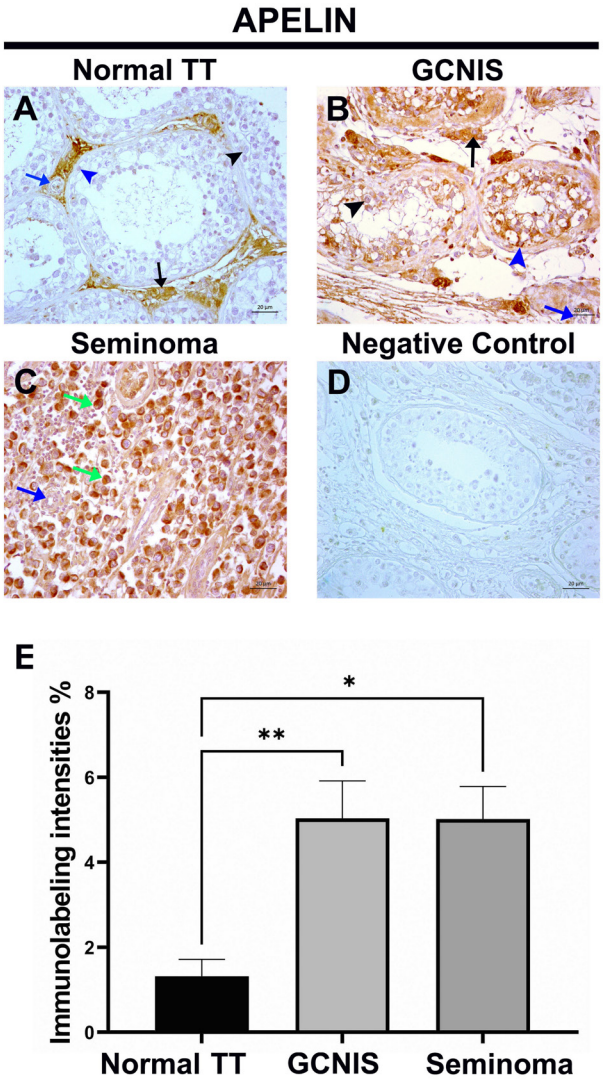

Figure 1. Apelin immunostaining. Apelin's immunohistochemical reaction was significantly increased in GCNIS and seminoma compared to normal TT. A, Normal TT (testicular tissue); B, GCNIS (germ cell neoplasia in situ); C, Seminoma; D, Negative control. Germ cells (blue arrowhead), Sertoli cells (black arrowhead), Leydig cells (black arrow), endothelial cells (blue arrow), and germ cell tumours (green arrow). E, Semi-quantitative analysis of apelin immunolabeling intensity, '*' indicates the significance between normal TT and seminoma ($P<0.05$) '**' denotes the significance of normal TT versus GCNIS ($P<0.05$)

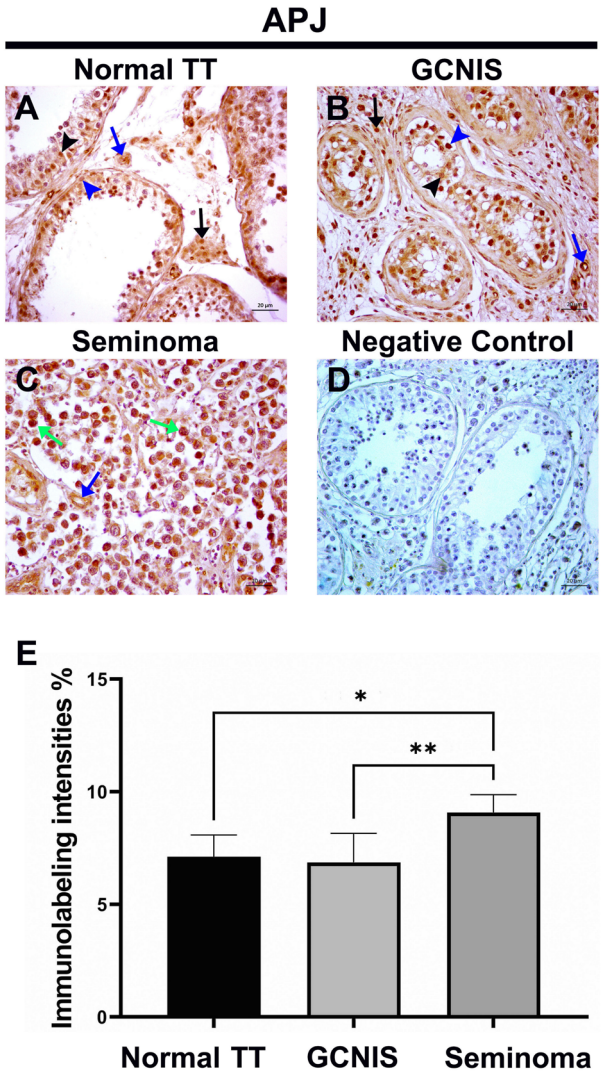

Figure 2. APJ immunostaining. It was observed that APJ immunohistochemical reactivity was significantly increased in seminoma compared to normal TT and GCNIS. A, Normal TT; B, GCNIS (germ cell neoplasia in situ); C, Seminoma; D, Negative control. Germ cells (blue arrowhead), Sertoli cells (black arrowhead), Leydig cells (black arrow), endothelial cells (blue arrow), and germ cell tumour cells (green arrow). E, Semi-quantitative analysis of APJ immunolabeling intensity. The symbol "*" denotes the significance of the difference between normal TT and seminoma ($P<0.05$).'**' indicates the significance between GCNIS and seminoma ($P<0.05$)

Table 1. Semi-quantitative distribution of Apelin and APJ labeling in normal testicular tissue, germ cell neoplasia in-situ(GCNIS) and seminoma of human testis

Cells	Normal TT		GCNIS		Seminoma	
	Apelin	APJ	Apelin	APJ	Apelin	APJ
Germ cells	0	(+)	++	+++	-	-
Sertoli cells	0	+++	(+)	++	-	-
Tumor cells	-	-	-	-	+++	+++
Leydig cells	+++	+++	+++	+++	-	-
Endothelial cells	0	+++	(+)	+++	(+)	+

0: negative; (+): weak positive; +: positive; ++: dense positive; +++: very dense positive

DISCUSSION

Although testicular cancer deaths are low compared to other cancers, patient mortality rates rise yearly. While 360 patients died from testicular cancer in 2012, it is estimated that 460 people will die in 2022 in the United States (4, 35). The expected death rate in the last 10 years has increased by approximately 28% according to these data. TGCT constitutes the majority of testicular cancers. More than half of TGCT patients have seminoma (2). The cure rates of seminoma patients are quite high. However, resistance to treatment and relapse that occurs in patients in the following process reduce the success of the treatment of the disease and even cause death (8). The Apelin/APJ axis is a pathway expressed in many tissues in the body and plays a role in different physiological processes (13,15). This axis has important roles in angiogenesis and lymphangiogenesis (16), and it is also expressed in cancer tissues. This pathway supports angiogenesis (17), proliferation (18), invasion, metastasis (20,21), and lymphangiogenesis (10), which are observed especially in aggressive cancers. In this study, we investigated seminoma, which constitutes most of TGCT and its precursor GCNIS, and the expression changes of apelin and APJ proteins in normal testes.

Previous studies determined that apelin and APJ are important for angiogenesis in cancer tissue (17), and their expression increased microvascular proliferation in brain tumours (18). In this study, we determined that apelin expression was increased in GCNIS and seminoma compared to normal TT. Hypoxia, commonly observed in cancer tissues, increases apelin expression, which supports angiogenesis, invasion, and metastasis in tumour tissue (20,21). In addition, the increased apelin expression has been shown to support lymphangiogenesis and lymph node metastasis (10). High apelin expression in cancer patients reduces their total survival (16,17,22-32). It can be concluded that a significant increase in apelin in seminoma carcinogenesis may cause angiogenesis, lymphangiogenesis, invasion, migration, and lymph node metastasis, which causes a poor prognosis in patients.

APJ contributes to the aggressiveness of cancer cells which was demonstrated by the previous studies. In this study, we found that APJ protein expression in seminoma was increased compared to normal TT and GCNIS. Apelin and APJ expressions were higher in colorectal cancer tissues compared to healthy tissues (30). APJ promotes angiogenesis in muscle-invasive bladder cancer (17). High APJ expression in patients with renal cell carcinoma has been directly associated with the aggressiveness of the disease (33). In mouse models of hepatocellular (37), glioblastoma (38), cholangiocarcinoma (22) and breast cancer (39) cancers, APJ inhibitors reduced tumour growth, angiogenic factors and vascular density (36). It is clear that APJ is a good target for inhibiting angiogenesis, growth, migration, and metastasis in cancer when all these studies are evaluated together. If agents targeting the APJ are used in treating seminoma, it can be predicted that angiogenesis, tumour growth, migration, and metastasis may be reduced. As a result, mortality may decrease, and relapse-free survival times may be prolonged.

CONCLUSION

In conclusion, the apelin/APJ axis is a pathway whose expression is increased in cancer patients. This increased expression promotes angiogenesis, lymphangiogenesis, migration, tumour growth, and metastasis. In different cancer mouse models that directly target the apelin and APJ proteins for therapeutic purposes, it has been shown that tumour growth was slowed, microvascular density and the expression of angiogenic factors were decreased (36). The use of treatments targeting apelin and APJ proteins in seminoma may be a new hope to prevent the increasing incidence and mortality of the disease in recent years. Further studies are needed before apelin/APJ inhibitors can be used clinically.

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Conflict of Interest: *The authors declare that they have no competing interest.*

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Research Article

CRP and LDH Levels Can Be Used for Support the COVID-19 Diagnose in Intensive Care Unit Patients

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Abstract

Aim: The coronavirus disease (COVID-19) has been a public health problem that causes severe acute respiratory syndrome affected all over the word since 2019. The most commonly used parameters as inflammatory response in the clinic are leukocytes, neutrophils, erythrocyte amount and serum C-reactive protein (CRP). In recent years, it has been reported that serum PCT (procalcitonin) level may be useful in the diagnosis of bacterial and viral infections. The aim of our study is to compare blood parameters that may play a supportive role to diagnose of COVID-19 in healthy control and critically COVID-19 patient groups.

Material and Methods: This retrospective research was carried out in Malatya Turgut Ozal University Training and Research Hospital, Malatya, Türkiye. Total 88 critically ill patients and 90 healthy people accepted to the study and electronic medical records of patients and control group has been collected from hospital information system (HIS). COVID-19 diagnose has been confirmed by real-time polymerase chain reaction (RT-PCR) results.

Results: No statistically significant difference was found between the patient and control groups according to gender in the participants included in the study. A statistically significant increase was observed in CRP, LDH, PCT, D-dimer, urea, sediment, lympocyte and neutrophil levels in COVID-19 patients. According to logistic regression analysis CRP, LDH and sediment values were found to be statistically effective in estimating the COVID-19 infection. These results also supported by ROC analysis, CRP, neutrophil, LDH, PCT and D-dimer results were determined to be distinguishing parameters for COVID-19 patients.

Conclusion: We found that CRP, PCT and LDH levels higher in the COVID-19 patients and these parameters can be used to diagnose and estimate the prognose of COVID-19 infection in intensive care patients.

Keywords: COVID-19, CRP, LDH, Intensive care

INTRODUCTION

The coronavirus disease (COVID-19) has been a public health problem that causes severe acute respiratory syndrome affected all over the word since 2019 and it is originated in Wuhan, China (1). The disease spread from the Asian Continent to Europe and America in a short period of two months, and the World Health Organization (WHO) declared the COVID-19 disease as a "Pandemic" on March 11, 2020. Coronaviruses are single-stranded, positively charged viruses; have extensions on their surface. For this reason, these viruses are named as Coronavirus (crowned virus) (2). As a result of the examination of the samples taken from COVID-19 patients, it was understood that the virus causing the disease was from the coronavirus family as well as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (3,4). There is no treatment yet whose efficacy and safety have been proven by scientific studies. COVID-19 is transmitted mainly through droplets. Respiratory symptoms, fever, cough, and dyspnea are the most prevalent symptoms of the disease (5). Efforts are being carried out intensively for effective treatment to control the pandemic (6). COVID-19 disease is a potent disease that leads to multi-organ failure. Studies have shown that oxidative damage, coagulation problems and some metabolic changes occur due to hyperinflammation in COVID-19 disease. These events lead to pathological conditions, especially in critically ill patients (1). Inflammatory cytokines can manifest themselves for a long time in acute kidney injury (7). COVID-19 diagnostic tests are an important part of the epidemic (8). In addition to the evaluation of inflammatory

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markers, it is critical to closely monitor potential indicators of organ failure in intensive care units. The most used parameters in clinical acute phase response are leukocytes, absolute neutrophils, erythrocyte amount, serum C-reactive protein (CRP). In recent years, it has been reported that serum procalsitonin (PCT) levels may be useful in the diagnosis of bacterial and viral infections (2). Procalcitonin and ferritin tests are other parameters used in the evaluation of the inflammatory process and "cytokine storm". Liver and kidney function tests, creatine kinase (CK) and D-dimer test, which is a fibrin breakdown product, are widely used in the evaluation of complications of the disease (9).

In the light of these information, the aim of our study is to compare blood parameters that may play a supportive role to diagnose of COVID-19 in healthy control and critically COVID-19 patient groups.

MATERIAL AND METHOD

Selecting participants and Data Collecting

This retrospective research was carried out in Malatya Turgut Ozal University Training and Research Hospital, Malatya, Turkey. Intensive care specialists were in charge for all the patients in the intensive care unit. Electronic medical records of patients and control group has been collected from hospital information system (HIS) and screening period was between 09.04.2020 and 09.04.2022. COVID-19 diagnose has been confirmed by real-time polymerase chain reaction (RT-PCR) results in the HIS. Participants forming control group have been selected from the same health center and attention was paid to have a parallel characteristic with the critically ill group in terms of age and gender. Total 88 critically ill patients and 90 healthy people accepted to the study as COVID-19 and Control group respectively. Seconder comorbidities such as diabetes mellitus, cardiovascular disease, hypertension, chronic obstructive pulmonary disease, cerebrovascular disease, and chronic kidney disease were determined as exclusion criteria.

Biochemistry, hemogram and coagulation analysis were performed by Abbott Architect c16000, Sysmex Corporation XN-10 and Diagon Coag XL medical devices respectively.

Statistical Analysis

The analysis of the data was carried out with the SPSS (Statistical Program in Social Sciences) 25 program. In order to check whether the data show the normal distribution, Shapiro Wilk Test was used (10). The significance level (p) for comparison tests was taken as 0,05. Since the variables did not have a normal distribution (p>0.05), the analysis was continued with non-parametric test methods. Mann Whitney U test was used to comparisons in independent pairs. ROC analysis was performed to determine the cut-off point (11).

RESULTS

Comparison of the Demographic Characteristics

The mean age of 88 COVID-19 patients was 72.63 (IQR, 22-98). The number of women was 41 (46.6%) and the number of men was 47 (53.4%). The mean age of 90 healthy controls was 69.97 (IQR, 24-95) and the number of women and men were equal and 45.

No statistically significant difference was found between the patient and control groups according to gender in the participants included in the study (p>0.05, Table 1). The groups show a homogeneous distribution according to the gender and age variable.

Table 1. Comparison of groups by gender and age

Parameter	Group		Group Covid-19 (n/%)	Control	Total	Test Value[a]	p Value
Gender	Male		47 (53.4%)	45 (50.0%)	92 (51.7%)	0.207	0.649
	Female		41(46.6%)	45 (50.0%)	86 (43.7%)		
	Total		88 (100.0%)	90 (100.0%)	178 (100.0%)		
Parameter	**Group**	**n**	**Mean ± SD**	**Min-Max**		**Test Value[b]**	**p Value**
Age	Covid-19	88	72.63±14.92	(22-98)		1.212	0.227
	Control	90	69.97±14.34	(24-95)			

Test value[a]; Ki-square test value (X^2), p value; statistical significance, SD; standart deviation, Min; minimum value, Max; maximum value, Test Value[b]; Mann Whitney U Test value

Laboratory Parameters of Participants

Measurements of serum biochemical parameters are given in Table 2. A statistically significant increase was observed in blood sediment, CRP, PCT, LDH, D-dimer, urea, lympocyte and neutrophil levels in COVID-19 patients compared to the healthy control group (p=0.001). On the contrary, significant decrease was found between the patient and control groups according to the calcium (Ca) and magnesium (Mg) levels (p<0.05, Table 2). Apart from that there was no statistically significant difference between the patient and control groups according to the variables of sodium (Na), potassium (K), chlorine (Cl), phosphorus (P) and creatinine in the participants included in the study.

Table 2. Comparations of the COVID positive and control groups blood parameters

Parameter	GROUPS		Test Value	p Value
	Covid-19 (Mean±SD)	Control (Mean±SD)		
CRP (mg/dL)	8.91±8.5	2.7±2.88	1864.500	0.001*
Neutrophil (10^3/µL)	10.46±5.9	7.83±5.65	2640.000	0.001*
LDH (U/L)	532.94±672.13	261.7±117.08	1572.500	0.001*
Lymphocyte (10^3/µL)	1.71±8	1.84±1.01	1397.000	0.001*
PCT (ng/mL)	2.5±7.26	0.47±0.89	2743.500	0.001*
D-dimer (µgr FEU/mL)	2.63±2.69	1.68±2.01	2703.000	0.001*
Urea (mg/dL)	88.23±60.05	63.91±44.03	2785.000	0.001*
Creatinine (mg/dL)	1.47±1.61	1.17±1.03	3327.000	0.066
Ca (mg/dL)	7.96±0.94	8.49±0.86	2458.000	0.001*
Mg (mg/dL)	2.24±0.76	6.16±29.49	3225.500	0.033*
Na (mmol/L)	139.55±5.74	139.11±5.37	3859.500	0.769
K (mmol/L)	4.17±0.96	4.17±0.63	3725.000	0.494
Cl (mmol/L)	103.44±7.19	104.18±5.56	3505.500	0.185
P (mg/dL)	3.57±1.78	3.6±1.37	3592.000	0.284
Blood sediment (mm/hour)	45.3±27.92	23.83±18.31	660.000	0.001*

SD; Standart deviation, Test Value; Mann Whitney Test value; *Shows the statistically significant difference between the groups (p<0.05)

Logistic Regression Analysis for Groups

Logistic regression analysis of the variables are given in Table 3. CRP, LDH and sediment values were found to be statistically effective in estimating the groups (patient – control) (p<0.05). Neutrophil, lymphocyte, procalcitonin, Ca, D-dimer, Mg values were not found to be statistically effective in estimating the groups (patient – control) (p>0.05).

Regression analyzes for CRP, LDH and sediment values are given below.

CRP value is 0.849 times more effective in estimating groups correctly (OR=0.849, 95% CI 0.730-0.988). 1 unit increase in CRP value, increases the risk of COVID-19 infection 0.849 times.

LDH value is 0.985 times more effective in estimating groups correctly (OR=0.849, 95% CI 0.976-0.993). 1 unit increase in LDH value, increases the risk of COVID-19 infection 0.985 times.

Sediment value is 0.961 times more effective in estimating groups correctly (OR=0.961, 95% CI 0.935-0.988). 1 unit increase in sediment value increases the risk of COVID-19 infection 0.961 times.

Table 3. Estimated values of the parameters in the model

Parameters	β	S.E.	W	sd	p Value (sig)	Exp(β)	95% CI for EXP(β)	
							Lower Limit	Upper Limit
CRP	**-0.163**	**0.077**	**4.489**	**1**	**0.034***	**0.849**	**0.730**	**0.988**
Neutrophil	0.014	0.057	0.062	1	0.803	1.014	0.908	1.134
LDH	**-0.015**	**0.004**	**12.078**	**1**	**0.001***	**0.985**	**0.976**	**0.993**
Lymphoside	0.011	0.046	0.062	1	0.803	1.012	0.924	1.107
PCT	-0.016	0.145	0.012	1	0.913	0.984	0.740	1.309
Ca	0.378	0.326	1.344	1	0.246	1.460	0.770	2.767
D-dimer	0.067	0.201	0.112	1	0.738	1.070	0.721	1.587
Mg	-0.851	1.251	0.463	1	0.496	0.427	0.037	4.962
Blood sediment	**-0.040**	**0.014**	**7.958**	**1**	**0.005***	**0.961**	**0.935**	**0.988**
Constant	4.569	4.097	1.244	1	0.265	96.410		

β; parameter estimates, S.E.; standard error, W; Wald statistics, sd; degrees of freedom, EXP (β); odds ratio, 95% CI; confidence intervals

ROC Analysis

ROC analysis results are given in Table 4. According to ROC analysis results, CRP, neutrophil, LDH, PCT and D-dimer results were determined to be distinguishing parameters for COVID-19 patients. It was observed that the parameter with the highest AUC value among these parameters was LDH and the lowest AUC value belonged to the D-dimer parameter. As a result of the ROC analysis for LDH, 274.5 points, which corresponds to the point with the highest sensitivity and the lowest specificity, were determined as the cut-off point, at this point the sensitivity of the scale was found to be 0.778 and the specificity as 0.762. The highest AUC value after LDH belongs to the CRP parameter and as a result of the ROC analysis, it was determined as the cut-off point of 3.45 points, which corresponds to the point with the highest sensitivity and the lowest specificity. At this point, the sensitivity of the scale was found to be 0.698 and the specificity to be 0.810. ROC curves of all analyzed parameters are given in Figure 1.

Table 4. Result of ROC analysis

Parameters	Cutting Point	Sensitivity	Specificity	p	AUC	95% C.I.	
						Lower Limit	Upper Limit
CRP	**3.455**	**0.698**	**0.810**	**0.001***	**0.804**	**0.722**	**0.886**
Neutrophil	**7.205**	**0.730**	**0.714**	**0.001***	**0.734**	**0.635**	**0.833**
LDH	**274.500**	**0.778**	**0.762**	**0.001***	**0.843**	**0.770**	**0.916**
Lymphocyte	0.165	0.968	0.001	0.053	0.186	0.099	0.272
PCT	**0.134**	**0.762**	**0.690**	**0.001***	**0.773**	**0.680**	**0.866**
Ca	6.950	0.905	0.024	0.051	0.246	0.153	0.338
Na	138.500	0.651	0.524	0.113	0.592	0.483	0.701
K	3.675	0.762	0.190	0.291	0.439	0.329	0.549
Cl	104.500	0.460	0.571	0.419	0.453	0.343	0.564
P	5.200	0.127	0.929	0.353	0.446	0.332	0.560
D-dimer	**1.315**	**0.635**	**0.619**	**0.004***	**0.666**	**0.560**	**0.772**
Mg	1.715	0.905	0.095	0.095	0.596	0.488	0.704
Blood sediment	**26.500**	**0.698**	**0.786**	**0.001***	**0.751**	**0.654**	**0.847**

*Shows statistical significant difference (p<0,05), AUC; area under the curve, %95 CI; confidence interval

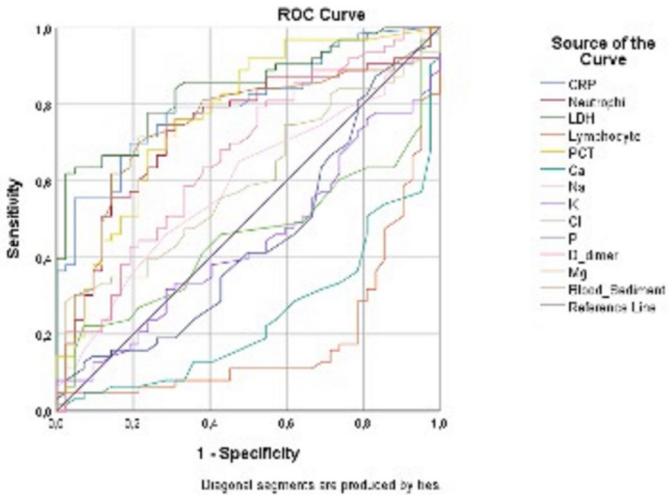

Figure 1. Cutting points according to ROC analysis

DISCUSSION

COVID-19 disease is a severe disease that leads to multi-organ failure. Virus infections can affect various organs, including kidney damage. Studies have shown that oxidative damage, coagulation problems and some metabolic changes occur due to hyperinflammation in COVID-19 disease. These events lead to pathological conditions, especially in critically ill patients (1). Inflammatory cytokines can manifest themselves for a long time in acute kidney injury (7). PCT levels is associated with hyper-inflammation seen in COVID-19 infection. In our research, we analysed the level of procalcitonin in intensive care patients and found a significant association between disturbances in PCT and COVID-19 disease. Serum PCT levels increase not only in severe and life-threatening systemic inflammatory conditions, but also return to normal soon after the septic state has resolved (12). During infection, this value rises above 0.5ng/mL. (3). PCT levels have been investigated during the COVID-19 process in many studies due to inflammation in COVID-19 disease, and high PCT levels in kidney patients lead to pathology (13,14).

CRP is used to distinguish between viral and bacterial infections and to identify developing complications (15).

CRP levels increases significantly after surgery, infections, tumors, autoimmune diseases, and chronic inflammatory diseases (12). Elevated CRP is considered as a risk factor. The normal limit values of CRP are 0-5 mg/L. CRP is slightly higher in women than in men (16). CRP value increases in patients with COVID-19 (17). Studies show that the amount of CRP in plasma increases due to inflammation in COVID-19 patients (18). We observed that CRP were significantly different between the two groups. CRP value groups (patient-control) are 0.849 times more effective in estimating correctly (OR=0.849, 95% CI 0.730-0.988). 1 unit increase in CRP value increases the risk of COVID-19 infection 0.849 times.

Lactate dehydrogenase, a hydrogen transfer enzyme, catalyzes the oxidation of L-lactate to pyruvate. The enzyme enters the circulation in case of inflammation and tissue damage and there is a marked increase in serum levels (19). It is thought that high LDH levels in severe COVID-19 patients may be a potential marker of lung injury and tissue destruction (20). We found a significant difference between the COVID-19 infected and healthy control group in terms of LDH levels.

D-dimer; It is a fibrin degradation product formed by the breakdown of thrombus by the endogenous fibrinolytic system. D-dimer levels increase with inflammation in COVID-19 patients (21). In a study, it was found that some of the COVID-19 patients with high D-dimer (>5000 µg/L) levels had an elevation associated with sepsis (22). In our study, we found that D-dimer levels increased in patients with critical COVID-19 compared with healthy people.

Careful control of kidney functions during COVID-19 infection is vital (23). Various studies have examined the relationship between COVID-19 infection and acute kidney injury, and the risk of death increases with kidney damage in COVID-19 patients (24-26). In COVID-19 disease; fluid and electrolyte imbalance causes many complications. Studies have reported that the risk of death due to kidney damage increases in COVID-19 patients due to pH and electrolyte imbalances in biological fluids. Electrolyte imbalance leads to conditions such as hyponatremia, hypernatremia, hypokalemia, hypocalcemia, and hypochloremia in patients. Hypokalemia (decreased potassium level) in COVID-19 infection is important in various pathophysiological mechanisms (27). According to another study; sodium decreased significantly in COVID-19 patients, similarly, there was a decrease in potassium and calcium levels, but no statistically significant difference was observed for chlorine level (28,29). In our study, no significant difference was observed between the patient and control groups for chlorine levels. There was no statistically significant difference between the patient and control groups according to the variables of sodium, potassium, chlorine, phosphorus and Ca in the participants included in the study.

Structural analysis of COVID-19 is important in terms of activation of calcium-dependent mechanisms and interaction of this ion with the target cell membrane (29). Calcium ions; It is one of the substances involved in signal transmission in every cell. Regulation of the activity of nuclear transcription factors, intracellular and extracellular enzymes, transmembrane channels, ion channels, membrane receptors, various calcium sensitive components; it depends on Ca signals in the cell. Ca is involved in many steps such as the post-translation process of viral proteins, viral gene expression, entry of the virus into the cell, and the viral life cycle. In addition, very rapidly rising creatinine values can be detected, serum calcium levels are decreased in COVID-19 patients (21). In our study, we found that calcium and magnesium levels decreased significantly in COVID-19 infected people.

Serum creatinine and urea levels are the most commonly used tests to evaluate kidney functions. Acute kidney injury (AKI) is one of the major complications of COVID-19 infection and is also associated with increased morbidity and mortality. It is vital to carefully control kidney functions, especially during COVID-19 infection (23). A statistically significant difference was found between the patient and control groups according to the variables of urea in the participants included in the study ($p<0.05$, Table 2).

It is a group of peripheral blood cells consisting of neutrophils, monocytes, eosinophils, basophils and lymphocytes. Their main function is to clear pathogens, dead or aged cells and foreign materials from the body (30). Approximately 75% of leukocytes consist of neutrophils. They play a role in body immunity in inflammation with phagocytosis and degranulation (31). Platelets play a role in the realization of primary hemostasis by initiating coagulation and wound healing at the injury site. In addition, they may exert a direct antimicrobial activity, similar to neutrophils and monocytes, and have a significant effect on the acute inflammatory response (32). In healthy individuals, the total lymphocyte count is over 1500/mm^3. There are also publications that conclude that lymphopenia can indicate the severity of COVID-19 disease (33).

CONCLUSION

Significant differences were found in some routine biomarkers between patients diagnosed with COVID-19 and control group. We found that CRP, PCT and LDH levels higher in the COVID-19 patients and these parameters can be use to diagnose and estimate the prognose of COVID-19 infection in intensive care patients. We also believe that the elevated procalcitonin values, low calcium and magnesium levels of COVID-19 patients may show the risk of multiple organ damage but new researches are needed to test this idea.

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Conflict of Interest: *The authors declare that they have no competing interest.*

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Research Article

Morphometry and Variation in Os Sacrum

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Abstract

Aim: The merger of the fifth lumbar vertebrae with the first sacral vertebrae is called the sacralisation of the lumbar vertebrae. The purpose of this study, changes in the os sacrum with sacralisation were detected, and morphometric measurements were made.
Materials and Methods: Measurements on 30 sacrum bones in the laboratory from the Department of Anatomy were performed using a digital caliper with a sensitivity of 0.01 millimeters (mm). Os sacrum measurements were taken.
Results: In one of the examined sacrums, it was found that tuberositas ossis sacri had the form of a pit, and on the underside of it, there was a pronounced groove. The processus transverse of the last lumbar vertebra fused with the os sacrum was not noticeably fused. Partial sacralisation was detected in the linea transversa at the anterior junction of the os sacrum and the last lumbar vertebra. It was determined that 21 of the sacrums had four foramina sacralia, and 9 of them had five variational foramina sacralia.
Conclusion: It was determined that structural changes might occur in the sacrum and that the number of foramina sacralia may be different. We believe that knowing the morphometric measurements of the sacrum will guide clinicians in the analysis of sacrum fractures and sacrum.

Keywords: Sacrum, sacralisation, morphometry

INTRODUCTION

The sacrum is formed by the fusion of five sacral vertebras. It is a wedge-shaped bone present between the two hip bones and takes part in forming the bony pelvis. It is triangular with its upper end or base, which articulates with the fifth lumbar vertebra, lower end or apex, which articulates with the coccyx, and its auricular surfaces articulating with the two hip bones in an adult individual (1-3). The anterior surface of this bone is concave, and the posterior surface is convex (1). The process of basis ossis sacri to the front is called promontorium. On the sides of the base, there are joint protrusions called Processus articularis superior. The canal in the os sacrum is called canalis sacralis, and the openness under this canal is called hiatus sacralis (4).

The protrusions on the sides at the apex are called cornu sacrale (5). On its outer side, called the pars lateralis, is a joint face called facies auricularis making a joint with ilium in place. It is ear-shaped. The rough area on the posterior inner side of these articular surfaces, where the ligaments are attached, is tuberositas ossis sacri. On the front of the os sacrum, there are four pairs of holes called foramina sacralia anterior, through which the front branches of the spinal nerves pass. Between these holes are lines called linea transversae, which match the fusion places of the vertebral bodies (4). The os sacrum has three crista structures on the back of the convect. Crista sacralis mediana is formed by the merger of processus spinosus, crista sacralis medialis by the merger of processus articularis, and crista sacralis lateralis by the merger of processus transversus (5). There are four pairs of holes on the back called foramina sacralia posterior (4). Above the bases, part of the basis ossis sacri makes joints with the last lumbar vertebrae. The top part, called apex ossis sacri, joints with os coccygeus. The os sacrum gives strength and stability to the pelvic skeleton (6). The os sacrum, which transmits body weight to the lower extremities,

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plays a vital role in the upright posture (7). The merging of the fifth lumbar vertebrae with the first sacral vertebrae is called the sacralisation of the lumbar vertebrae. The combination of the rarer 1st sacral vertebrae with the 5th lumbal vertebra is called the lumbalization of the sacral vertebrae (7, 8). Lumbosacral transitive vertebrae formed in this way are a congenital problem (7). The fusion of the Os sacrum 5th vertebrae and the 1st coccygeal vertebrae is called sacralisation of the coccygeal vertebrae. Lumbar vertebral sacralisation results in the 5th double foramina in the sacrum (8). The vertebral combination in sacralisation can be complete or partial. Sacralisation is more easily distinguishable since the articulation line will be pronounced in partial fusion (9).

Since the lumbar vertebrae will be included in the 5th lumbar vertebrae on the sacrum, more work will fall on the remaining lumbal vertebra joints as the 4th lumbal vertebrae will not behave like the 5th (10). This condition can sometimes cause pain (8-10). Sacralisation can cause spinal radicular pain due to the narrowing of the intervertebral foramina and spinal nerve compression (7). This condition is called Bertolotti syndrome (11). Our aim in our work; The aim is to determine the variation by making morphometric measurements of the sacrum bone and to evaluate the sacrum bone morphometrically by analyzing these measurements. In addition, it is to contribute to both sacral region operations, anatomists, and the literature.

MATERIAL AND METHOD

In this study, measurements on 30 sacrum bones, in the laboratory the from the Department of Anatomy, were carried out using a digital caliper with a sensitivity of 0.01 millimeters (mm). Measurements of the following parameters were taken in the os sacrum.

Parameters for measurements are shown in Fig. 1-4 [10].

1. MVKL: Midventral straight length: Sloping distance between promontorium and anteroinferior sacral border.
2. STW: Superior base width; Maximum transverse length between the dorsocranial corners of facies auricularis.
3. VTW: Ventral base width; Maximum transverse width between the junction points of facies auricularis superior and inferior.
4. DTW: Dorsal base width; The minimum transverse width of the dorsal part of the basis ossis sacri.
5. SVCW: Sacral vertebral corpus width; The first sacral segment is the maximum transverse width.
6. SVCD: Sacral vertebral corpus depth; The first sacral vertebrae is the maximum anteroposterior depth.
7. SVCL: Sacral vertebral corpus length; The length of the first sacral vertebrae sagittal corpus.
8. FADW: Flat width of Facies auricularis; The maximum width of the auricular faces.
9. FADL: Flat length of Facies auricularis. Auricular faces maximum vertical length.
10. PPY: Posterior pediculus height. Distance between the first sacral vertebra superior boundary and the upper limit of the first Sacral foramen.
11. IFAD-IFPD: Distance between anterior boundaries of Processus articularis superiors-Distance between posterior boundaries of Processus articularis superiors.
12. AMFD, PLFD: Anteromedial and posterolateral facet depth: Vertical distances between the first sacral vertebral corpus rear boundary and the frontal planes that pass through the anteromedial and posterolateral boundaries of processus articularis superiors.
13. IPD: Interpedinculus distance, the maximum transverse distance between the first sacral vertebrae pediculus.
14. CSAD: Transverse distance between cornu sacrales.
15. FSPW: Foramina sacralia pelvina width: The maximum transverse widths of the foramen located anterior to the os sacrum.
16. FSPL: Foramina sacralia pelvis length: Maximum longitudinal lengths of foramina in the anterior of Os sacrum.
17. FSDW: Foramina sacralia dorsalia width: Maximum transverse widths of a foramen in the posterior of Os sacrum.
18. FSDL: Foramina sacralia dorsalia length: Maximum longitudinal lengths of a foramen in the posterior of Os sacrum.
19. MLL-MTL: Maximum longitudinal length-Maximum transverse length (Values measured in Foramina Sacralia Dorsalia).

The following indices were calculated to determine the morpho-structural features of the os sacrum and its base.

1. LSI: Longitudinal slope index: Midventral straight length/midventral inclined length x 100.
2. VBI: Ventral base index: Ventral base width/superior base width x 100.
3. DBI: Dorsal base index: Dorsal base width/ superior base width x 100.
4. SHI: Sacral height index: Ventral base width/ midventral straight length x 100.

RESULTS

In one of the sacrums, tuberositas ossis sacri, which is the rough area where the ligaments are attached, was observed to be in the form of a pit on the posterior-inner side of the facies auricularis in the pars lateralis. A prominent groove was detected on the underside of this pit area. The last lumbar vertebrae fused with the os sacrum were found to be noticeably unmapped by the processus transversus (Figure 5) (Figure 6). It was observed that the foramina sacralia formed by the combination of the

os sacrum and the last lumbar vertebrae were different from other foramina (Figure 6). It was determined that 21 of the examined sacrums had four foramina sacralia, and 9 of them had variation and five foramina sacraliae (Figure 6). Measurements of right and left foramina were taken. Variated and normal sacrums and MLL and MTL values were not statistically significant (p>0.05) (Table 1). In sacrum measurements, 21 normal sacrum and nine variation sacrum values MVIL, DBW, SBW, SVCW, IFPD, AMFD, PLFD, IPD, IFPD, and DBI values were found to be statistically significant (p<0.05) (Table 1).

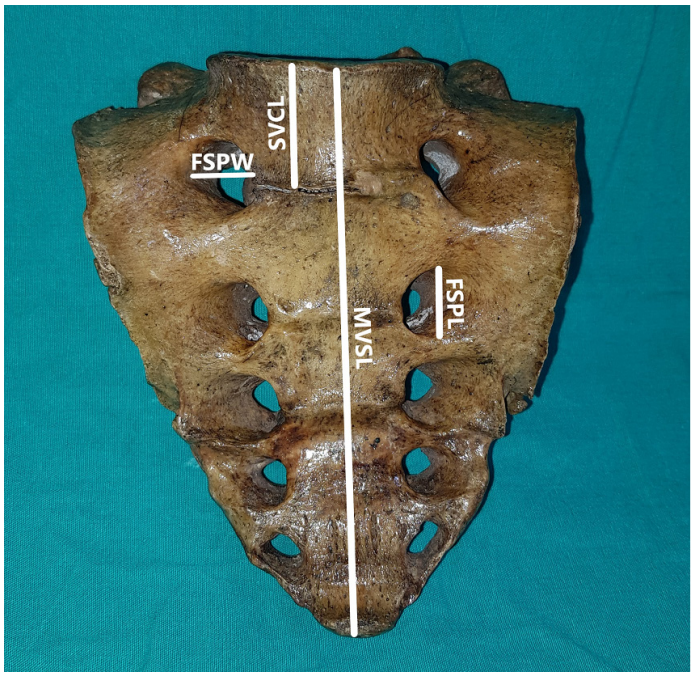

Figure 1. Anterior (facies pelvina) view of the os sacrum

MVSL: Midventral straight length, SVCL: Sacral vertebral corpus length, FSPW: Foramina sacralia pelvina width, FSPL: Foramina sacralia pelvina length

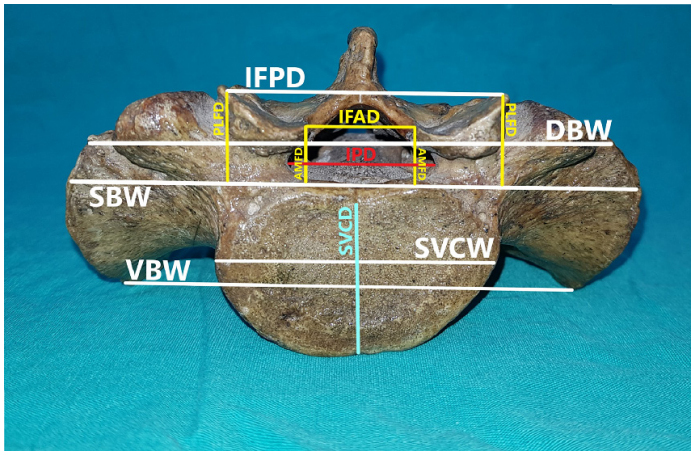

Figure 2. MSuperior view of the os sacrum

VBW: Ventral base width, SVCW: Sacral vertebral corpus width, SBW: Sacral base width, AMFD: Anteromedial facet depth, PLFD: Posterolateral facet depth, DBW: Dorsal base width, IFAD: Interfacet anterior distance, IFPD: Interfacet posterior distance, IPD: Interpedinculus distance

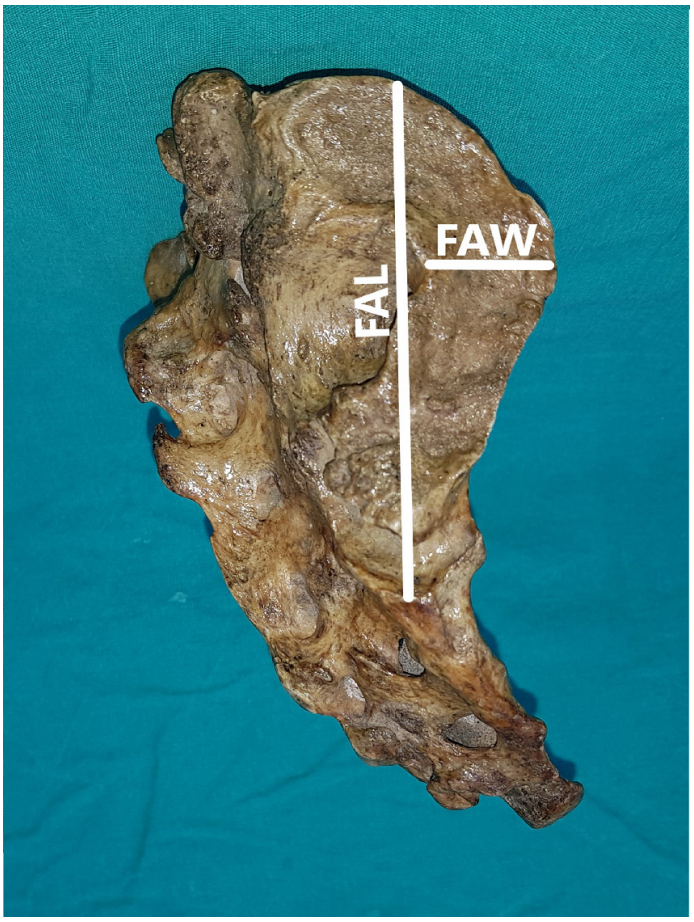

Figure 3. MLateral view of the os sacrum

FAL: Facies auricularis length, FAW: Facies auricularis width

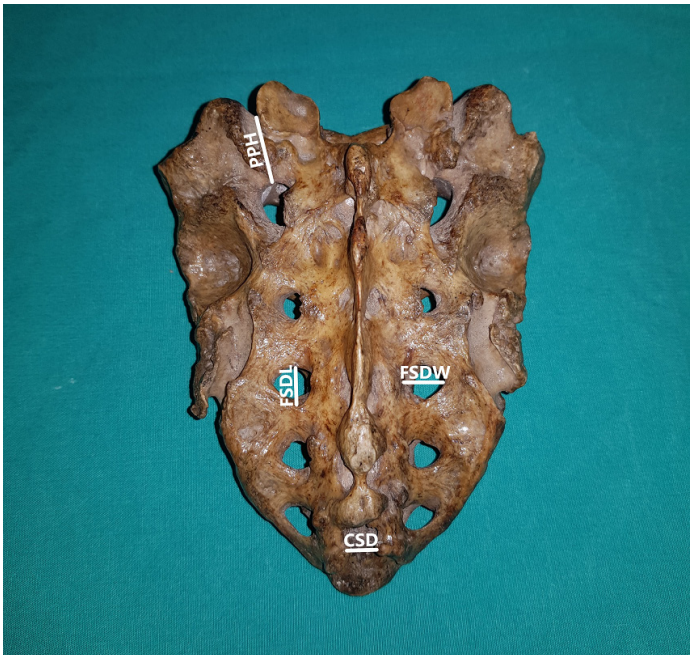

Figure 4. Dorsal (facies dorsalia) view of the os sacrum

CSD: Cornu sacrale distance, PPH: Posterior pediculus height, FSDL: Foramina sacralia dorsalia length, FSDW: Foramina sacralia dorsalia width

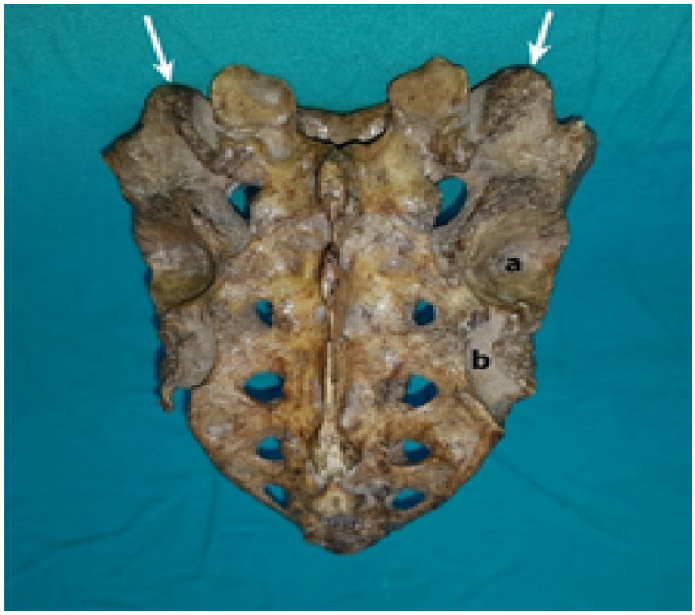

Figure 5. The view of os sacrum from facies posterior (facies dorsalia). a: Tuberositas ossis sacri, b: The prominent sulcus formation, the structures indicated by the white arrows show the processus transversus of the first sacral vertebra, which is not fully fused with the sacrum

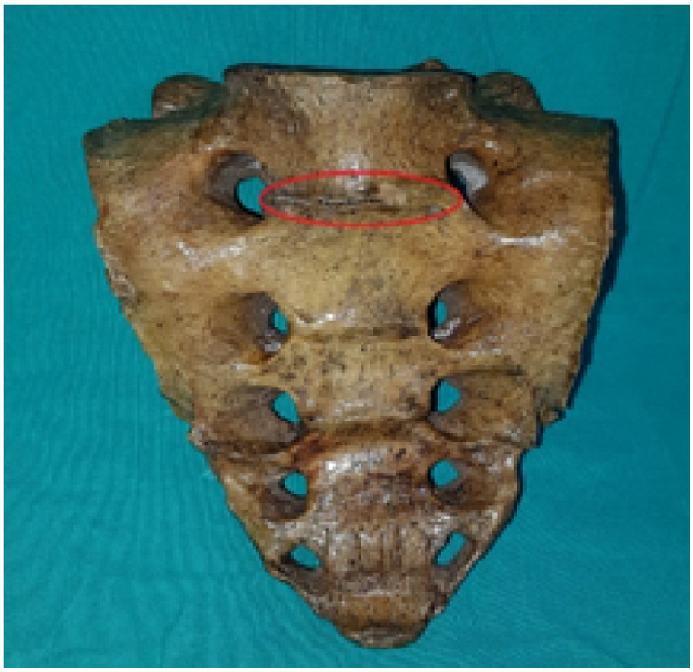

Figure 6. The view of the os sacrum from the facies pelvina (facies anterior), five foramina sacraia anterior (arrow) and partial sacralisation is seen in the structure called linea transversa in the circle

Table 1. Normal and variation values on the sacrum bone

Variable	G	N	Mean	Median	SD	Min	Max	25%	50%	75%	T	p
MVSL	N	21	105.95	106.38	13.81	75.9	131.19	95.45	106.38	116.8	-2.64	0.007
	V	9	123.37	117	14.83	112.78	150.34	114.12	117	134.41		
MVIL	N	21	107.01	107.8	12.99	81.9	130.9	97.18	107.8	116.99	-2.87	0.003
	V	9	123.27	124.2	11.33	102.26	135.6	115.71	124.2	132.76		
SBW	N	21	112.88	114.44	9.99	78.77	127.45	109.02	114.44	118.35	-0.973	0.349
	V	9	105.92	112	19.85	55.03	118.3	104.94	112	116.85		
VBW	N	21	107.77	109.9	8.49	84.35	118.7	102.08	109.9	113.66	-0.475	0.657
	V	9	107.26	107.6	6.43	98.14	116.96	102.05	107.6	113.06		
DBW	N	21	90.38	90.9	10.9	71.01	109.9	81.22	90.9	100.78	-3.23	0.001
	V	9	105.62	105.2	7.41	93.05	115.67	100.31	105.2	112.83		
SBW	N	21	112.88	114.44	9.99	78.77	127.45	109.02	114.44	118.35	-4.27	0.000
	V	9	52.36	54.42	6.88	43.1	64.29	45.9	54.42	56.84		
SVCW	N	21	50.39	51.69	7.23	25.26	60.8	48.06	51.69	53.85	-3.91	0.000
	V	9	30.48	29.8	4.91	20.69	36.6	28.59	29.8	35.39		
FAW	N	21	24.54	23.69	5.31	11.92	32.83	21.48	23.69	28.3	-0.068	0.965
	V	9	38.57	19.2	25.79	15.2	77.8	16.84	19.2	61.4		
FAL	N	21	45.81	54.2	20.53	8.25	69.62	22.73	54.2	62.05	-3.91	0.965
	V	9	24.54	19.7	12.49	15.71	55.48	16.43	19.7	27.29		
PPH	N	21	22.86	22.75	2.62	18.8	28.56	21.02	22.75	24.27	-1.06	0.304
	V	9	28.26	23.25	15.47	4.31	56.23	22.56	23.25	39.2		
IFAD	N	21	25.9	25.16	10.65	11.34	57.26	19.45	25.16	30.75	-1.47	0.150
	V	9	37.44	31.44	19.02	13.25	60	19.76	31.44	57.3		

IFPD	N	21	51.83	53.91	12.56	22.03	69.6	45.15	53.91	60.54	-3.91	0.000
	V	9	14.85	7.9	14.45	5.25	47.62	6.5	7.9	21.61		
AMFD	N	21	7.96	7	3.25	3.36	16.71	5.47	7	10.01	-4.09	0.000
	V	9	22.85	20.9	11.09	11.25	46.66	14.89	20.9	28.18		
PLFD	N	21	18.19	17.55	2.82	12.9	24	16.24	17.55	20.6	-3.91	0.000
	V	9	28.61	29.1	4.6	19.06	34.4	26.41	29.1	31.87		
IPD	N	21	33.1	31.4	7.21	25.1	58.24	28.7	31.4	34.56	-3.41	0.000
	V	9	18.05	15.99	8.75	9.3	40	14.1	15.99	18.83		
CSD	N	21	14.06	14.4	4.19	4.1	19.43	10.95	14.4	18.1	-1.69	0.094
	V	9	17.38	17.1	3.71	11.16	22.45	14.63	17.1	20.85		
FSPW	N	21	14.55	15.5	3.8	5.3	18.84	12.6	15.5	17.75	-1.92	0.056
	V	9	21.88	17.15	13.51	14	57.3	15.76	17.15	21.02		
FSPL	N	21	14.38	15.3	3.27	8.67	20.1	11.79	15.3	16.61	-0.18	0.859
	V	9	89.72	14.3	228.87	8.9	700	11.11	14.3	16.63		
FSDW	N	21	12.25	10.5	5.78	6.3	30.09	8.86	10.5	13	-2.35	0.017
	V	9	158.74	18.2	427.99	8.98	1300	11.95	18.2	20.23		
FADL	N	21	45.81	54.2	20.53	8.25	69.62	22.73	54.2	62.05	-2.24	0.025
	V	9	24.54	19.7	12.49	15.71	55.48	16.43	19.7	27.29		
PPH	N	21	22.86	22.75	2.62	18.8	28.56	21.02	22.75	24.27	-1.06	0.304
	V	9	28.26	23.25	15.47	4.31	56.23	22.56	23.25	39.2		
IFAD	N	21	25.9	25.16	10.65	11.34	57.26	19.45	25.16	30.75	-1.47	0.150
	V	9	37.44	31.44	19.02	13.25	60	19.76	31.44	57.3		
IFPD	N	21	51.83	53.91	12.56	22.03	69.6	45.15	53.91	60.54	-3.91	0.000
	V	9	14.85	7.9	14.45	5.25	47.62	6.5	7.9	21.61		
AMFD	N	21	7.96	7	3.25	3.36	16.71	5.47	7	10.01	-4.09	0.000
	V	9	22.85	20.9	11.09	11.25	46.66	14.89	20.9	28.18		
PLFD	N	21	18.19	17.55	2.82	12.9	24	16.24	17.55	20.6	-3.91	0,000
	V	9	28.61	29.1	4.6	19.06	34.4	26.41	29.1	31.87		
IPD	N	21	33.1	31.4	7.21	25.1	58.24	28.7	31.4	34.56	0	0.084
	V	9	18.05	15.99	8.75	9.3	40	14.1	15.99	18.83		
CSD	N	21	14.06	14.4	4.19	4.1	19.43	10.95	14.4	18.1	-1.69	0.094
	V	9	17.38	17.1	3.71	11.16	22.45	14.63	17.1	20.85		
FSPW	N	21	14.55	15.5	3.8	5.3	18.84	12.6	15.5	17.75	-1.92	0.056
	V	9	21.88	17.15	13.51	14	57.3	15.76	17.15	21.02		
FSPL	N	21	14.38	15.3	3.27	8.67	20.1	11.79	15.3	16.61	-0.18	0.859
	V	9	89.72	14.3	228.87	8.9	700	11.11	14.3	16.63		
FSDW	N	21	12.25	10.5	5.789	6.3	30.09	8.86	10.5	13	-2.35	0.017
	V	9	158.74	18.2	427.99	8.98	1300	11.95	18.2	20.23		
FSDL	N	0	12.3	11.86	3.27	7.16	19.9	9.9	11.86	13.79	-1.99	-1.99
	V	0	15.74	17.91	4.290	8.98	20.5	11.95	17.91	19.58		
LSI	N	0	99.46	95.73	11.44	87.39	128.48	91.64	95.73	105.11	-0.24	0.824
	V	0	100.62	97.81	12.97	84.07	121.91	89.28	97.81	110.67		
VBI	N	0	95.77	95.74	6.19	80.04	107.08	92.21	95.74	99.79	-0.38	0.722
	V	0	107.6	95.5	39.45	89.29	212.54	92.27	95.5	97.96		
DBI	N	0	80.59	79.75	10.97	55.72	98.87	71.5	79.75	89.9	-3.28	0.001
	V	0	104.43	95.04	29.64	87	182.12	90.58	95.04	102.25		
SHI	N	0	86.629	87.72	14.24	56.92	104.47	76.32	87.72	99.89	-0.29	0.790
	V	0	86.48	88.07	10.17	66.66	102.46	79.79	88.07	92.29		

G: Group, Min: Minimum, Max: Maximum, SD: Standard Deviation D: Variable N: number of bones, T: Test value; Mann Whitney-U Test Value, p: p<0.05; (statistically significant difference between groups)

DISCUSSION

Os sacrum is a vital bone included in the field of fusion and stabilization in the treatment of sacral, lumbosacral, and sacroiliac deformities or injuries. The development of the sacrum resembles the boning of a typical spinal cord. The secondary centers of ossification appear after puberty, and all sacral vertebrae begin to fuse. The secondary centers of ossification appear after puberty, and all sacral vertebrae start to fuse. Any defect in the formation of primary centers causes the missing formation of the sacral canal and incomplete ossification of the layer (5,12). The convergence of the lumbal sacral vertebrae occurs as an innate anomaly. The incidence of this anomaly is between 3.6% and 18% in humans and is usually bilateral. This anomaly is caused by defects that occur during the development of lumbosacral segmentation. This is indicated as an anatomical variation (3). The sacrum bone is a critical bone in the determination of sex in death cases and is used in morphometric measurements of the bone (13). In a study, it was found that the width of the sacrum S1 corpus was lower in women than in men (14). In addition, accurate determination of the anatomical position of vertebrae in spinal surgery is essential for the prevention of errors (3, 15).

In their study of 52 sacrum bones, Yilmaz et al., (2018) calculated the average distance between the cornu sacrale and the standard deviation value as 12.63±3.02mm. In our study of the os sacrum bone, CSD (21 normal bones) / (9 bone variations) was calculated as 14.06±4.19/ 17.38±3.71mm. The CSD value was average and the variation values were not statistically significant. This value was found to be higher than the average values of Yilmaz et al. (12).

Matveeva et al., (2016) measured 72 sacral dry bones by dividing them into 3 groups. While the bones in the first group showed normal articulation, the joints were fused with L5 in the second group and completely ossified with L5 in the third group. They calculated the mean MVSL value as (mm) 107.52±9.18 in normal sacrums, 100.16±11.03 in those that fused from the joints, and 116.67±15.1 in those that completely ossified (10). In the Mahato, (2010) study, the MVSL value was estimated at 102.79±10.09 in the normal sacrum, 94.02±15.63 in part lumbarisation of S1, and 101.53± 69.02 in the full sacralisation of S1 (15). In our study, the MVSL value was measured as 106.38±13.81-117±14.83 mm in normal and variable sacrums. This was statistically significant in the comparison of the two values ($p<0.007$). Basaloglu et al. (2005) calculated the MVSL value (mm) as 10.43±10.20±1.02 for men and 1.24 for women (14). In our study, the MVSL value in normal sacrums was found to be similar to the value in these studies. Our variation sacrum value was found to be close to the sacrum values in the third group of Matveeva et al.

Matveeva at al., (2016) studies put the MVIL value at an average of 118.77±9.69 mm, SBW value: 112.58±7.45 mm (first group), 112.7±5.83 mm (second group), 114.61±9.75 mm (third group), DBW averaged 88.78±6.88 mm (first group), 91.5±7.2 mm (second group), 86.31±8.74 mm (third group), VBW on average (mm) 106.82±7.67 (first group), 109.37±5.88 (second group), 107.55±7.99 mm (third group), LSI on average, 90.6±4.48 mm (first group), 86.009±6.87 mm (second group), 89.85±4.38 mm (third group), DBI on average (mm), 79.79.08±5.53 mm (first group), 79.84±6.56 mm (second group), 75.45±5.62 mm (third group), SHI on average , 94.96 ± 4.51 mm (first group), 97.09±3.31 mm (second group), 93.98 ± 3.69 mm (third group) (10). Naksuwan et al., (2021), measured SBW value 95.68±119.71 mm in men, 99.29±115.46 mm in women, VBW value ±78.10-10.00 mm in men, and 80.77±109.19 mm in women. In our study, sacrum also found MVIL value statistically significant in comparison with normal (21 bones)-variation (9 bones) 107.8±12.99-124.2±11.33 mm. ($p<0.05$), SHI: 86.62±14.24 mm (21 bones), 86.48±10.17 mm (9 bones), a comparison was made on bones, and SHI value was statistically significant ($p<0.05$) (16) Our values were found to be close to third group of Matveeva et al. Additionally, our other values are SBW: 112.88±9.99 (22 bones), 105.92±19.85 (9 bones), DBW: 90.38±10.9 mm (22 bone), 105.62±7.41 mm (9 bones), VTW: 107.77±8.49 mm (22 bones), 107.26±6.43 mm (9 bone), LEI: 99.46±11.44 mm (22 bones), 100.62±12.97 mm (9 bone), calculated.

Naksuwan et al., (2021) and Matveeva et al., (2016) worked on many bones but did not compare with each other (10, 16). In our study, many values were compared with each other in our varied (9 bones), regular (22 bones) study and whether they were statistically significant.

The IPD value measured in sacrum has been studied by many researchers. El Rakhawy et al., (2010), the IPD value was calculated as 25.1. Aly et al., (2013), 43.41 mm. Kapoor et al., (2014), 21.5 mm, Sethi et al., (2015), 29.25 mm. Singh and Jafar, (2018) 25.1 mm. Basaloglu et al., (2005), in their study of 30 men and 30 women, they found a statistically significant difference between 2.4-29.7±2.5 mm men and women ± 31 in men and women ($p<0.05$) (14,17-20). In our study ±, 33.1±7.21 mm (22 bones), and 18.05±8.75 mm (9 bones) were measured. Singh and Jafar, in the study, the closest to our work, were found close to his work.

Sacralisation can change the biomechanical structure of the body (10). Foramina sacralia dorsalia measurements were taken in the study of Koç et al., (2014). They calculated the highest MTL (FSPL) at 38.68±4.03 mm and did not compare right or left. In our study, the highest MTL values of right and left were looked at and averaged 14.85±2.25-14.082.49 mm, right-left statistically compared and found no significant ($p>0.05$) (21).

Foramina sacralia dorsalia measurements were taken in the study of Koç et al., (2014). They calculated the highest MTL (FSDL) at 38.68±4.03 mm and did not compare right or left. In our study, the highest MTL values of right and left were looked at and averaged 14.85±2.25-14.082.49 mm, right-left statistically compared and found no significant ($p>0.05$) (21).

Basaloglu et al. (2005), in their study of 30 men and

30 women on the sacrum: SVCW, measured 52.7±6.1 5-52.6±7.9 mm, finding no statistically significant difference between men and women (p>0.05). Koç et al. (2014) calculated the SVCW value at 49.33±6.74 in their study of 30 sacrums. In our study± 50.39 23 mm (22 bones), and 30.48±4.91 mm (9 bones) were measured. Our value has been found close to the studies carried out (21).

CONCLUSION

As a result, measurements were made on dry bone in our study. Twenty-two normal sacrum measurements and nine variation bone measurements were performed. The varying values were found to be lower than normal sacrum measurements, and there was no statistically significant difference between them in the right-left measurements on the foramina. The sacrums we examined also match the data in the studies. We believe that morphometric measurements related to the sacrum and the detection and diagnosis of variances may benefit clinicians and researchers related to the sacrum.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *In our country, the ethics committee was not taken because the approval of the waste board was not required in the study on dry bone.*

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MEDICAL RECORDS-International Medical Journal

Case Report

A Case of Transverse Colon Located Pneumatosis Cystoides Intestinalis Requiring Surgical Resection

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Abstract

Pneumatosis cystoides intestinalis (PCI) is a rare disease characterised by numerous gas-filled cysts in the intestinal wall. This case report is aimed to present the diagnosis and treatment process of a case of PCI treated with intestinal resection. A 56-year-old female was admitted to the emergency department of a tertiary health centre with complaints of abdominal pain and vomiting for about two days. In the abdominal examination of the patient, there was widespread tenderness and defence in all abdominal quadrants with a rebound. Laboratory parameters of the patient were unremarkable. The plain abdominal X-ray observed no free air in the right subdiaphragmatic area. Computed tomography showed dilatation, increased wall thickness and multiple free airs in the small bowel loops, predominantly localised in the left upper quadrant. The patient underwent a diagnostic laparotomy. Diffuse air bubbles were observed along the transverse colon wall at laparotomy. The patient underwent transverse colon resection and colocolic anastomosis with a linear stapler. The patient was discharged on the 7th postoperative day. The pathological evaluation of the surgical specimen was suitable with PCI.

Keywords: Pneumatosis cystoides intestinalis, intestinal perforation, large bowel resection

INTRODUCTION

Pneumatosis cystoides intestinalis (PCI), first described by the pathologist DuVernoi, is a rare disease characterised by numerous gas-filled cysts in the intestinal wall (1). The condition, classified in two forms as idiopathic and secondary, may present different clinical findings (2). Air cysts in the idiopathic or primary form are located in the mucosa and submucosa. The air cysts are primarily found in the intestinal wall in the secondary form. The most common form of this disease is the secondary form. There are uncertain and varied hypotheses for the aetiology of PCI. It possibly occurs due to an underlying cause such as trauma, inflammation, drug use, immunosuppression, neoplasia, and autoimmune or pulmonary diseases (3).

PCI clinics range from asymptomatic disease to severe peritoneal disease. Imaging tools such as plain radiography and computed tomography, endoscopy, surgery and sometimes pathological examinations are used in diagnosis. While hyperbaric oxygen therapy, antibiotics and bowel rest are used to treat asymptomatic patients, surgical treatment is recommended in patients with peritonitis symptoms (4). In surgical treatment, a biopsy from pathologic areas should be taken. Resection is not required in cases without significant perforation and gastrointestinal system content in the abdominal cavity. In contrast, resection with or without anastomosis (with a diverting ostomy) should be performed in cases with suspicious or obvious perforation and gastrointestinal tract infection, depending on the infection amount of the abdominal cavity (5).

This case report is aimed to present the diagnosis and treatment process of a case of pneumatosis cystoides intestinalis treated with intestinal resection.

CASE REPORT

A 56-year-old female was admitted to the emergency department of Erzurum Regional Education and Research Hospital with complaints of abdominal pain and vomiting for about two days in May 2022. She did not have a similar episode of abdominal pain before. She had no other disease, and she had only a history of laparoscopic cholecystectomy ten years ago.

CITATION

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At the time of admission to the hospital, the patient's arterial blood pressure was 142/80 mmHg, oxygen saturation on room air was 95%, pulse rate was 105 beats/min, and body temperature was 37.6°C. In the abdominal examination of the patient, there was widespread tenderness and defence in all abdominal quadrants with a rebound. Other system examinations, including rectal examination, were normal.

Laboratory parameters of the patient were unremarkable. The plain abdominal X-ray observed no free air in the right subdiaphragmatic area. Since she had severe abdominal pain, abdominal computed tomography (CT) with contrast was performed to evaluate the patient. Computed tomography showed dilatation and increased wall thickness in the small bowel loops, predominantly localised in the left upper quadrant (Figure 1). In addition, there were numerous air bubbles on the transverse colon wall (Figure 2).

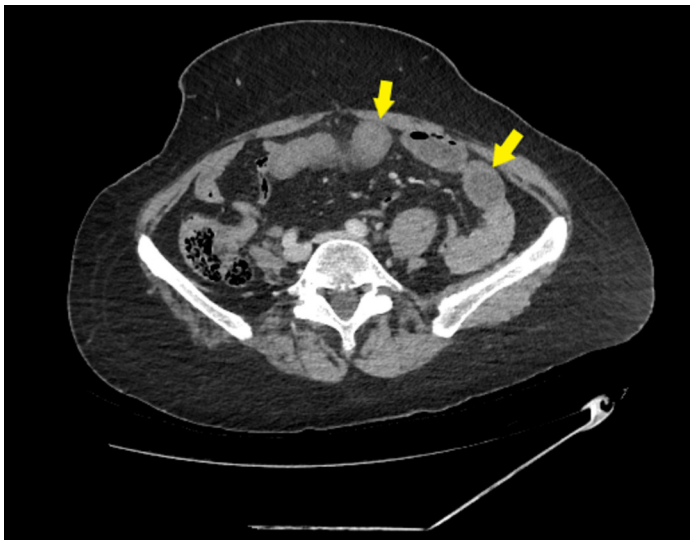

Figure 1. On computed tomography scan, yellow arrows indicate dilated intestinal segments

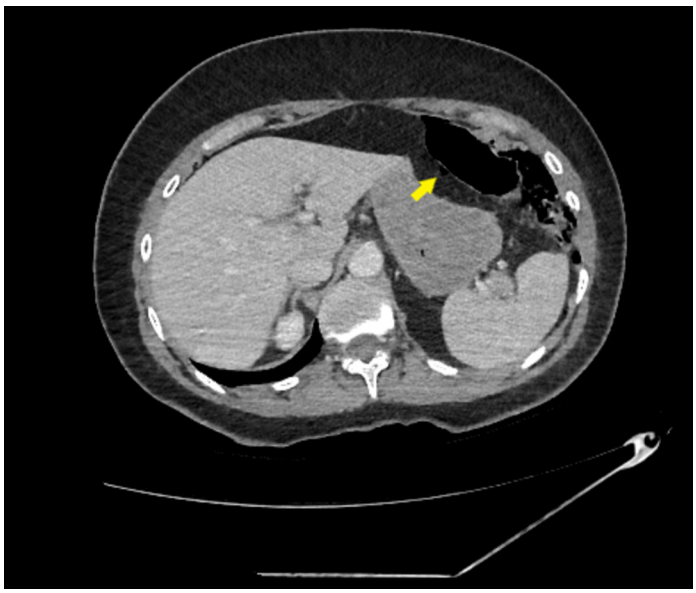

Figure 2. A yellow arrow indicates the free air inside the transverse colon wall on a computed tomography scan

The patient underwent a diagnostic laparotomy. At laparotomy, diffuse air bubbles were observed along the transverse colon wall (Figure 3). No additional gastrointestinal organ perforation was observed. No ischemic bowel loop was observed. Resection anastomosis was planned because the PCI area was extended, and the intact colon wall was low. The patient underwent transverse colon resection with a colocolic anastomosis via a linear stapler. One drain was inserted from the right side to the edge of anastomosis, and the other was inserted into Douglas's pouch. She was followed up in the service during the postoperative period and was started on ceftriaxone 1 gr vial (intravenously every 12 hours) and metronidazole 500 mg/100 ml (intravenously every 8 hours). Oral feeding of the patient, whose complaints regressed in the postoperative period, was opened on the 3rd postoperative day. The drain of the patient who tolerated oral feeding was removed on the 6th postoperative day, the patient's current antibiotherapy was completed for seven days, and the patient was discharged on the 7th postoperative day. The pathological evaluation of the surgical specimen was suitable with PCI.

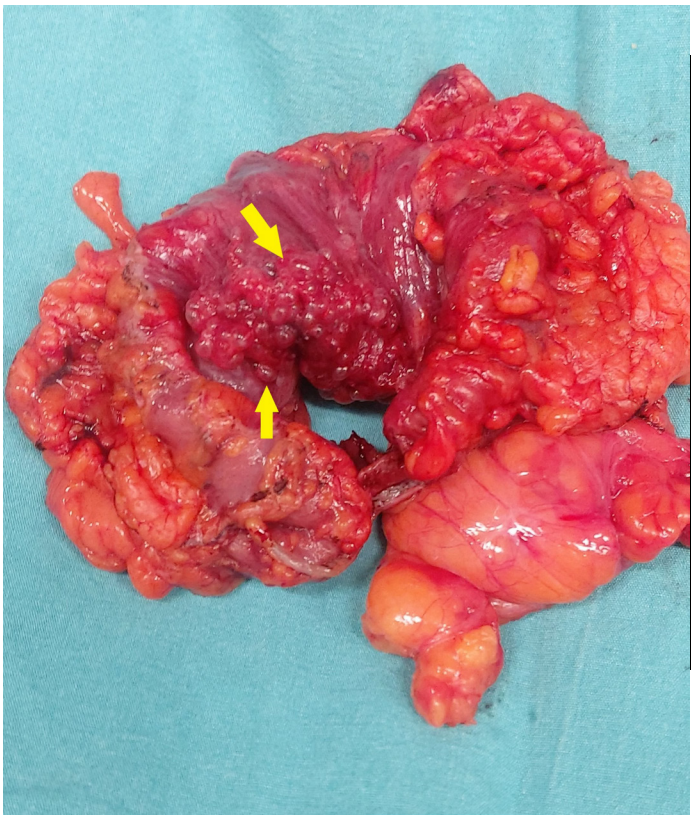

Figure 3. Perioperative image of the resection material (Yellow arrows indicate multiple air bubbles along the transverse colon wall)

DISCUSSION

Pneumatosis cystoides intestinalis (PCI) is a rare pathology of unknown aetiology, characterised by multiple gas-filled cysts in the intestinal subserosa and submucosa. The cyst content consists of nitrogen and hydrogen and various amounts of oxygen, carbon dioxide, butane, propane, methane, ethane, and argon gases (6). In

the autopsy series of 6553 cases performed at Edinburgh Hospital, PCI was detected in only two patients (0.03%) (7). PCI occurs equally in men and women and is most common in the 6th decade (8). In the present case, the patient was a female, and she was 56 years old, which was compatible with the age at which PCI was most common.

Most PCI cases involve the jejunum and ileum; in 6% of the cases, the colon is affected. Many gastrointestinal system diseases include appendicitis, Crohn's disease, pyloric stenosis, ulcerative colitis, diverticular disease, necrotising enterocolitis, gastroduodenal ulcer, and sigmoid volvulus may accompany PCI. In addition, PCI is unknown in cases unrelated to the gastrointestinal system, such as chronic obstructive pulmonary disease, collagen tissue diseases, AIDS, and glucocorticoid use. Pathology accompanying PCI could not be detected in approximately 20% of the cases, and the disease was accepted as primary (9). There was no underlying disease in this case, and the case was evaluated as primary PCI.

Although the pathogenesis of PCI is unknown, some theories have been proposed. As a result of causes that increase intrathoracic pressure such as emphysema, asthma, mechanical ventilation, vomiting and vomiting, the alveoli are fragmented, and the gas content passes from the mediastinum retroperitoneum and mesentery to the intestinal wall (6). In addition, it has been suggested that the disease may also occur due to the gas-forming bacteria (*Clostridium Difficile*, *Clostridium perfringens*, etc.) releasing hydrogen through carbohydrate fermentation and these gases invading the lymphatic vessels and the intestinal wall (10).

Direct abdominal X-ray and abdominal computed tomography (CT) are valuable imaging modalities in diagnosing PCI. CT is the ideal method for diagnosis, although plain abdominal radiography gives positive findings in 2/3 of the cases (11). However, radiological diagnosis is difficult if the patient has a good prognosis and pneumoperitoneum is not detected. In addition, air appearances outside the intestinal lumen were seen in the vicinity of the small intestine wall, where the distinction between free air and PCI could not be made clear (12). In the present case, there was no free air in the right subdiaphragmatic area. On CT scan, in some small bowel loops, localised dilatation, increased wall thickness and multiple free air densities in the abdomen, especially in the left upper quadrant, were observed.

Since spontaneous remission is observed up to 50% in the treatment of the disease and gas cysts can reoccur after surgery, no specific treatment is applied in asymptomatic cases. Nonoperative oxygen therapy is used first if there is no perforation, peritonitis, or sepsis in symptomatic patients (11). According to a study published by EAST (Eastern Association for the Surgery of Trauma) in 2013, various risk factors have been defined in making the surgical decision (13). Hypotension, use of vasopressor drugs, peritonitis, lactate level above two mmol/L, signs of acute renal failure, and mechanical respiratory support were accepted risk factors for pathological PCI. Apart from those mentioned in this study, age 60 and over, nausea, bicarbonate level below 20 mmol/L and white blood cell value above 12x109/L are the clinic-laboratory factors. Gas in the portal vein, free air in the abdomen, enlargement of the intestine, and presence of ascites have also been defined as CT factors (13). In surgical treatment, a biopsy from pathologic areas should be taken. Resection is not required in cases without significant perforation and gastrointestinal system content in the abdominal cavity. In contrast, resection with or without anastomosis (with a diverting ostomy) should be performed in cases with suspicious or obvious perforation and gastrointestinal tract infection, depending on the infection amount of the abdominal cavity (5). Segmental resection with anastomosis was performed in the present case due to suspicious colon wall perforation.

CONCLUSION

While pneumatosis intestinalis may develop due to a disease that can be treated on an outpatient basis in some cases, it can sometimes be a sign of conditions that can be fatal if surgical intervention is delayed. It is essential to evaluate this finding with the patient's complaints, history, comorbidities, physical examination findings, and laboratory and other radiological examinations to delay the diagnosis and apply the proper treatment.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Informed Consent: *The family provided consent for publication.*

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