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Research Article

Determining the Relationship Between Compassion Fatigue and Job Satisfaction and Burnout Levels in Physiotherapists

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Abstract

Aim: The aim of the study is to examine the relationship between compassion fatigue, job satisfaction and burnout levels in physiotherapists.

Material and Methods: A total of 147 physiotherapists were included in this cross-sectional study. Demographic information (age and gender), marital status, working year, field of study (neurological rehabilitation (pediatric rehabilitation, cardiopulmonary rehabilitation) and orthopedic rehabilitation (sports rehabilitation, prosthesis-orthotics rehabilitation)) and institution information (public, private) of the individuals were recorded. Compassion fatigue was evaluated with the Compassion Scale, job satisfaction was evaluated with the Job Satisfaction Scale, and the level of burnout was evaluated with the Maslach Burnout Inventory. Data were filled in by individuals online.

Results: A total of 147 physiotherapists, 40 male and 107 female, were included in the study. A positive significant correlation was found between the compassion scale and the job satisfaction scale (r=0.261, p=0.001). On the Maslach burnout inventory, there was a negative correlation between the Compassion Scale with emotional exhaustion (r=-0.195, p=0.018) and personal achievement score (r=-0.270, p=0.001), and a positive correlation with depersonalization (r=0.413, p=0.000). In addition, a significant difference was found in the Compassion Scale in terms of gender (p=0.008) and field of study (p=0.044).

Conclusion: In this study, in which compassion fatigue was evaluated for the first time in physiotherapists, it was determined that as compassion fatigue increased, job satisfaction decreased and exhaustion increased. At the same time, compassion fatigue is more common in male physiotherapists and physiotherapists working in neurology and pediatrics rehabilitation.

Keywords: Burnout, compassion fatigue, job satisfaction, physiotherapist

INTRODUCTION

Compassion is the feeling of sadness and pity that arises as a result of the individual being affected by bad and painful events (1). The individual puts himself in his place, adopts the same pain and dedicates himself to it, in order to help another individual for whom he is sad and pitying. When the literature is examined, it is seen that the motivation and job satisfaction of health workers who have an intense sense of compassion are also affected in the same way.

Motivation is the desire of the employee to do work. In order to be willing to work, the goals and objectives of the individual and the goals of the institution where he/she works must be in the same direction. Job satisfaction, on the other hand, is the individual's sense of satisfaction with the institution he works for and the positive attitude that the individual develops towards his/her own job.

The health sector is a type of business that provides services to people who have lost their health and who want to protect themselves from the disease, by health workers. For health professionals working in the health sector, compassion is an indispensable feeling for the success of care and treatment of patients (2).

Compassion fatigue studies in the health sector were mostly carried out with nurses, and the scope was expanded by adding other health professionals to these studies (3). When the literature was searched, it was

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determined that emergency room nurses, intensive care and oncology nurses also participated in compassion fatigue studies (4,5). According to the results of the studies, nurses affected by compassion fatigue face many problems in terms of mental, emotional and physical aspects.

In this study, the concept of compassion and compassion fatigue and its symptoms were mentioned, and the concepts related to compassion fatigue were discussed. Then, the definition, importance and techniques of motivation and the concept and importance of job satisfaction are mentioned. The relationship between job satisfaction and the concept of motivation is emphasized.

Although compassion fatigue has been examined in different occupational groups working in the health sector, compassion fatigue of physiotherapists working in many different fields such as disabled individuals, intensive care patients, and elderly patients has not been evaluated. In addition, the relationship between physiotherapists' compassion fatigue and burnout level is unknown. The aim of the study is to examine the relationship between compassion fatigue and job satisfaction and burnout levels in physiotherapists.

MATERIAL AND METHOD

A total of 147 physiotherapists, including 40 men and 107 women, were included in this cross-sectional descriptive study. The inclusion criteria of the individuals were working physiotherapist, being between the ages of 18-65 and volunteering to participate in the study. Those who gave incomplete answers to the questionnaires were excluded from the study. The demographic information (age and gender) of the individuals were recorded. At the same time, the year of work, field of study (neurological rehabilitation (pediatric rehabilitation, cardiopulmonary rehabilitation) and orthopedic rehabilitation (sports rehabilitation, prosthesis-orthotics rehabilitation), marital status and institution (public, private) were recorded. Compassion fatigue was evaluated with the Compassion Scale, job satisfaction was evaluated with the Job Satisfaction Scale, and the level of burnout was evaluated with the Maslach Burnout Inventory. Demographic information form and questionnaires were filled online by individuals via online platforms. Ethics committee approval of the study was obtained from Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee (dated 06.12.2022, decision no: 2022/36-15).

Compassion Scale: The compassion scale was developed by Pommier in 2011 (6). A Turkish adaptation consisting of 24 items was made by Akdeniz and Deniz in 2016 (7). The scale included disengagement (items 1, 7, 19, 23), indifference (items 2, 12, 14, 18), separation (items 3, 5, 10, 22), mindfulness (items 4, 9, 13, 21).), kindness (items 6, 8, 16, 24) common humanity (items 11, 15, 17, 20) with a total of 24 questions and 6 sub-dimensions. The sub-dimensions of indifference, disconnection and separation in the original of the scale were calculated by reversing. With this method, the scores are calculated and the total score average is obtained. It is assumed that when the total score from the scale increases, the level of compassion also increases.

Job Satisfaction Scale: Job satisfaction scale is a subscale of job descriptive scale. It was developed by Hackman and Oldham to determine the level of job satisfaction individuals get from their jobs (8). The scale consists of 14 positive and unfinished statements. Individuals are asked to complete these statements by choosing the most appropriate one from the given options. The Turkish adaptation and validity study of the scale was carried out by Güler (9) (Güler M. 1990). Scoring is between 0 and 5 (1) it does not satisfy me at all, (2) it does not satisfy me enough, (3) I am undecided, (4) very satisfying to me, (5) very satisfying to me). The highest score that can be taken from the scale is “70” and the lowest possible score is “14”. A score between 14-32 is low; It is normal to be between 33-52; A score between 53-70 indicates high job satisfaction.

Maslach burnout inventory; It was developed by Maslach and Jackson in 1981 (10). Burnout is evaluated in 3 dimensions as emotional exhaustion (DT), depersonalization (D) and decreased personal achievement (PD). The scale was adapted into Turkish by Ergin in 1992 (11). The scale consists of 22 statements. A 5-point Likert scale is used for scoring the scale. ((0) never, (1) rarely, (2) sometimes, (3) often, (4) always). Volunteers were asked to mark the appropriate option for each jurisdiction. The scores for each subscale were calculated by summing separately. The scores that can be obtained from the subscales range from 0-36 for emotional exhaustion, 0-20 for depersonalization, and 0-32 for personal achievement. According to Maslach and Jackson, burnout cannot be expressed with a single score, so the results of the subscales should be evaluated together. High emotional exhaustion and depersonalization scores and low personal achievement scores indicate exhaustion.

Considering the correlation coefficient (r=0.784) between compassion fatigue and job satisfaction in the article titled 'The effect of compassion fatigue level on employee motivation and job satisfaction: an application on health workers' (12), when alpha=0.05, power=0.80, the sample size must be at least 100. According to the relationship between Compassion Scale and Job Satisfaction Scale, which is one of the data of our study, the power of the study was found to be 0.99 when post-hoc power analysis was performed (G*Power 3.1, Düsseldorf, Germany). Mean (X) and standard deviation (SD) values for continuous variables as descriptive statistics; Number (n) and percentage (%) values were given for categorical variables. Whether the data were suitable for normal distribution was evaluated with the Kolmogorov Smirnov test. Independent samples t-test was used to compare normally distributed data. The relationship between two continuous variables was evaluated with the Pearson correlation coefficient. The results were considered significant at the $p<0.05$ level.

RESULTS

Socio-demographic information and personal information of the included individuals are given in Table 1.

Table 1. Socio-demographic and personal information of individuals

Variables (n=147)		X±SD (min-max)
Age (year)		29.82±6.61 (22-55)
Gender	Male n (%)	40 (27.2)
	Female n (%)	107 (72.8)
Marital status	Single n (%)	77 (52.4)
	Married n (%)	70 (47.6)
Working time as a physiotherapist (year)		6.51±6.18 (0-32)
Working time at current workplace (years)		3.42±4.76 (0-30)
Field of study	Neurological rehabilitation n (%)	70 (47.6)
	Orthopedic rehabilitation n (%)	77 (52.3)
Institution of work	Public n (%)	46 (31.3)
	Private n (%)	101 (68.7)
Compassion scale		3.99±0.43 (2.67-5)
Job satisfaction scale		41.37±11.79 (15-69)
Maslach Burnout Inventory	Emotional exhaustion	17.37±8.61 (0-36)
	Depersonalization	13.16±3.66 (1-20)
	Decreased personal achievement	13.34±3.85 (3-25)

When the relationship between the Compassion Scale and age, working time as a physiotherapist, working time at the current institution, Job Satisfaction and Maslach Burnout Inventory was examined, a significant positive correlation was found between the Compassion Scale and the job satisfaction scale (r=0.261, p=0.001). On the Maslach burnout inventory, there was a significant negative correlation between the Compassion scale and emotional exhaustion (r=-0.195, p=0.018) and decreased personal achievement score (r=-0.270, p=0.001), and a positive correlation with depersonalization (r=0.413, p=0.000) (Table 2).

When the compassion scale was compared according to gender, marital status, field of study and institution, a significant difference was found in terms of gender (p=0.008) and field of study (p=0.044) (Table 3).

When the relationship between Compassion Scale and age, working time as a physiotherapist, working time in the current institution, Job Satisfaction Scale and Maslach Burnout Inventory according to gender, in male a significant positive correlation was found between the Compassion Scale and the job satisfaction scale (r=0397, p=0.011.). On the Maslach burnout inventory, there was a significant negative correlation between the Compassion scale and emotional exhaustion (r=-0.382, p=0.015) and and a positive correlation with depersonalization (r=0.458, p=0.003). In female, there was a negatice correlation in terms of age (r=-0.207, p=0.032) and working time as a physiotherapist (r=-0.191, p=0.049). A significant positive correlation was found between the Compassion Scale and the job satisfaction scale (r=0.255, p=0.008). On the Maslach burnout inventory, there was a significant negative correlation between the Compassion scale and decreased personal achievement score (r=-0.213, p=0.027), and a positive correlation with depersonalization (r=0.406, p=0.000) (Table 4).

Table 2. The relationship between compassion scale and age, working time as a physiotherapist, working time in the current institution, Job Satisfaction Scale and Maslach Burnout Inventory

Variables		Age	Working time as a physiotherapist (year)	Working time in the current institution (year)	Job satisfaction scale	Maslach Burnout Inventory		
						Emotional exhaustion	Depersonalization	Decreased personal achievement
Compassion scale	r	-0.138	-0.149	-0.080	0.261	-0.195	0.413	-0.270
	p	0.095	0.071	0.337	0.001*	0.018*	0.000*	0.001*

*Pearson Correlation Analysis

Table 3. Comparison of Compassion Scale by Gender, Marital Status, Field of Study and Institution of Work

Variables		Compassion scale X±SD (min-max)	p
Gender	Male	3.83±0.45 (2.67-4.67)	**0.008**
	Female	4.04±0.41 (2.96-5)	
Marital status	Single	4.05±0.45 (3-5)	0.052
	Married	3.91±0.40 (2.67-4.58)	
Field of study	Neurological rehabilitation	3.95±0.35 (2.67-4.63)	**0.044**
	Orthopedic rehabilitation	4.06±0.32 (3.08-5)	
Institution of work	Public	95.13±9.66 (64-120)	0.115
	Private	96.33±10.73 (70-119)	

*Independent sample t test

Table 4. The relationship between compassion scale and age, working time as a physiotherapist, working time in the current institution, job satisfaction scale and Maslach Burnout Inventory according to gender

Variables		Age	Working time as a physiotherapist (year)	Working time in the current institution (year)	Job satisfaction scale	Maslach Burnout Inventory		
						Emotional exhaustion	Depersonalization	Decreased personal achievement
Male compassion scale	**r**	0.187	0.049	0.176	**0.397**	**-0.382**	**0.458**	-0.309
	p	0.248	0.764	0.277	**0.011**	**0.015**	**0.003**	0.052
Female compassion scale	**r**	**-0.207**	**-0.191**	-0.160	**0.255**	-0.157	**0.406**	**-0.213**
	p	**0.032**	**0.049**	0.100	**0.008**	0.106	**0.000**	**0.027**

DISCUSSION

In this study, in which the relationship between compassion fatigue job satisfaction and burnout level in physiotherapists was examined for the first time, it was determined that as compassion fatigue increased, job satisfaction decreased and burnout increased. At the same time, compassion fatigue is more common in male physiotherapists and physiotherapists working in the field of neurological rehabilitation.

Compassion is an important concept in the provision of health care, and it is reported that health professionals are or should be compassionate. Compassion is expressed as “a deep awareness of the suffering of another and the combination of this awareness with the desire to alleviate the pain” (13). Compassionate health care can provide significant benefits, such as increasing trust, patient satisfaction and quality of life among patient-health professionals, and aiding recovery (13,14). At the literature, compassion fatigue and related factors of many health professionals have been examined (12), and compassion fatigue of physiotherapists who work in the rehabilitation field and spend a long time with patients has not been examined to the best of our knowledge.

Job satisfaction is an important tool in determining the feelings and thoughts that employees feel about the organization they are responsible for (15). The physical and mental happiness of individuals significantly affects their job satisfaction. From an organizational point of view, employers who make an effort to keep their employees in the organization and intend to attract talented employees to the organization are significantly effective in ensuring job satisfaction. In organizations where there is no job satisfaction, an increase is observed in the absenteeism of the employees, their complaints about the work and accordingly the turnover of the workforce (16).

There have been studies examining the job satisfaction and related factors of physiotherapists serving patients in many different rehabilitation areas. In a study conducted in 2011, it was determined that the parameters that most affect the job satisfaction of physiotherapists are employers' support for participation in congresses and seminars, a decrease in personal achievement, and emotional exhaustion (17). In the study conducted by Kişmir et al. in 2020, the relationship between compassion fatigue and job satisfaction of healthcare workers was examined (12). In the study, most of which consisted of nurses and health officials, no relationship was found between compassion fatigue and job satisfaction. In our study, all data were obtained from physiotherapists, and it was found that as compassion increased in physiotherapists, job satisfaction increased. Compassion fatigue in healthcare professionals is defined as the gradual decrease in compassion over time to patients in severe emotional or physical pain (18). We think that it is necessary to develop personal, institutional and professional strategies in the prevention and treatment of compassion fatigue in physiotherapists, since high job satisfaction will also affect patient communication and treatment success.

Burnout is a syndrome of emotional fatigue and pessimism that is frequently seen among individuals who work with people (10). In another aspect, it is 'a feeling of emotional fatigue, apathy and low self-esteem in individuals serving a large population' (19).

There are studies on the determination of burnout and job satisfaction especially in nurses and doctors in the field of health in Türkiye. Sünter et al. compared burnout and job satisfaction in general practitioners (20), Özyurt et al. determined the factors affecting burnout and job satisfaction in doctors (21). Ebrinç et al. evaluated these parameters in comparison with burn center nurses, internal medicine intensive care unit and general surgery nurses (22). Erbil et al. investigated job satisfaction, self-esteem and influencing factors in midwives and nurses (23). Ünal et al. investigated the relationship between burnout and job satisfaction levels and life satisfaction levels in physicians (24).

In our study, the relationship between burnout and compassion fatigue was examined. Burnout was examined under 3 headings. Emotional exhaustion is defined as 'the feeling of being unable to make self-sacrifice as an individual's emotional resources, such as the ability to cope with events, decrease'. desensitization; It is the conflict of the person with the individuals he serves and the effort to get away from them by neglecting their personal characteristics. decline in personal achievement; It is the

tendency of the person to evaluate himself negatively, especially towards his/her job. According to the results of the study, as compassion increases, emotional exhaustion decreases, depersonalization increases and personal achievement score decreases. According to these results, physiotherapists working in different specialization areas feel depersonalization towards work and decrease in professional success, although emotional motivation is higher as compassion increases. Since the time spent with the patient in physiotherapy specialization areas and the prognosis of the patients differ, we think that compassion fatigue should be examined separately in each area.

When the factors affecting compassion fatigue were also examined, it was determined that female physiotherapists had higher compassion. Polat et al. In their study conducted in 2017, the relationship between the level of compassion fatigue and quality of life was examined (25). Similar to our study, it was stated that compassion was higher in female nurses. It was also found that marital status did not affect compassion fatigue. Again, in our study, compassion fatigue was compared according to the physiotherapy specialization area. The areas with the lowest compassion score were found to be neurological rehabilitation. We think that depersonalization and compassion fatigue over time are high in physiotherapists working in these fields due to the long time spent with the patient groups in both fields and the slower prognosis.

The limitations of our study are that our study data consists of a young population and the short duration of the study. One of the limitations of the study is that individuals were not questioned about their use of medications that would cause their emotional states to change. In addition, the lack of diversity in field of study may also be related to compassion fatigue.

CONCLUSION

As a result, we think that it is important to evaluate compassion fatigue, which is associated with physiotherapists' job satisfaction and burnout. We think that personal and institutional measures should be taken to reduce the compassion fatigue seen in male physiotherapists and physiotherapists working in the field of neurological rehabilitation. The concept of compassion and compassion fatigue and relationship factors in undergraduate education will contribute to the field. In addition, there is a need for special studies to be carried out in areas of privatization.

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

Ethical approval: *Ethics committee approval of the study was obtained from Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee (dated 06.12.2022, decision no: 2022/36-15).*

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Research Article

Prevalence, Number and Localization of Wormian Bones in Anatolian Adult Dry Skulls

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Abstract

Aim: The locations of the Wormian bones (WBs) are critical knowledge for physicians, anatomists, forensic scientists, and anthropologists. The purpose of the study is to identify the number and location of WBs in the Anatolian population.

Material and Methods: The study included 29 adult skulls from anatomy departments at two universities in Türkiye. The skulls' gender and age were unknown. The prevalence, quantity, and location of WBs were assessed.

Results: The greatest number of WBs (mean 1.79) were found in the lambdoid suture, which was followed by the coronal suture (0.43). Among the fontanelles, the Asterion had the highest number of WBs (0.42).

Conclusion: According to our findings, the Lambdoid suture has the highest number of WBs. There is still a great deal of mystery around the causes of the occurrence and localization of WBs. Radiologists, neurosurgeons, forensic scientists, anatomists, and anthropologists must conduct more research in this area.

Keywords: Wormian bones, Lambdoid suture, coronal suture, Asterion, Pterion.

INTRODUCTION

The bones in the human skull are joined together to form the skull. Wormian bones (WBs) are irregularly shaped bones that develop from independent ossification centers, located on or at the junction of sutures between cranial bones (1). The origin of the name is that Olaus Wormius, a Danish physician, is supposed to have made the initial discovery of these bones (2). These structures are also called sutural bones, supernumerary bones, or ossicles. These ossicles are isolated from other bones by a special suture surrounding them. WBs vary in size, shape, number, and location (3). Even though some studies have suggested that the formation of WBs is genetic, others have suggested that it is physical, and some have proposed a combination of the two, the precise mechanism by which they are formed are still unknown (4-7).

The distribution of WBs in cranial sutures is not homogeneous. They are most frequently seen, respectively, in the lambdoidal and coronal sutures (8). Among the fontanelles, WBs are most common in the asterion, followed by the anterior, posterior, and orbital fontanelles (2). Although WBs can be found in healthy people, they are also associated with diseases, syndromes, or congenital disorders such as pyknodysostosis, osteogenesis imperfecta, rickets, kinky hair syndrome, cleidocranial dysplasia, hypothyroidism, hypophosphatasia, otopalatodigital syndrome, Hadju-Cheney pachydermoperiostosis, Down syndrome, hydrocephalus, cleidocranial dysplasia (2,3,9,10). Because of these associations, WBs can also be used as diagnostic tools in some diseases (11).

Localization of the WBs is important not only for anatomists but also for anthropologists, radiologists, and causality medical officers. Moreover, in autopsy surgery, knowledge of WBs is essential for the correct identification of traumatic fractures of the skull or

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fractures caused by gunshot wounds (12). Despite their clinical significance, limited studies have been done on the number and topographic distribution of WBs in the Anatolian population.

The aim of the present study is to provide a detailed report on the number and topographic distribution of WBs in the Anatolian population.

MATERIAL AND METHOD

In this study, 29 Anatolian dry adult skulls from the anatomy laboratories of Eskişehir Osmangazi University, Faculty of Medicine and Harran University, Faculty of Medicine were used. The specimens' ages and gender were unknown. Since the study was conducted on dry skulls, ethics committee approval is not required. The skulls used in the investigation had no previous surgical procedures and were trauma-free. Only WBs on intact sutures were taken into account. Sutures with even minor damage were eliminated from the study.

The WBs on sutures and bones have been determined individually for the right and left halves of the cranium. The sutures were carefully examined to establish the topographic distribution of WBs.

Statistical Analyses

The number of WBs in each suture was determined separately. Sutures or fontanelles on both sides of the cranium were assessed independently. It was calculated which percentage of skulls contain WBs. The average number of WBs per suture was also calculated numerically. Since the data set exhibited non-parametric characteristics, the Mann-Whitney U test was used to compare the number of WBs on either side of the skull.

RESULTS

24 (82.8%) of the 29 adult dry skulls had at least one WB. In 18 (62.1%) of the adult dry skulls, WBs were found on multiple sutures. The highest number of WBs found in one cranium was 15. There were at least one WB in 15 (53.6%) of the intact Lambdoid sutures. The lambdoid suture had the greatest number of WBs per suture (mean 1.79). A total of 12 WBs were found in a single suture (Figure 1). Of the coronal sutures, at least one WB was found in 6 (21.4%) of them. In general, 0.43 WBs were present per coronal suture. Table 1 lists the major sutures and WBs found in these sutures.

An average of 0.42 WBs was detected in an Asterion. With this number, Asterion contained the highest percentage of WBs among the fontanelles (Figure 2). In Pterion, the average number of WBs was 0.09 (Figure 3). Fontanelles and WBs found in fontanelles are presented in Table 2.

When the minor sutures of the specimens were examined, a total of 6 WBs were detected in the frontozygomatic sutures. A total of 5 WBs were found in sphenozygomatic sutures and 4 WBs were found in parietomastoid sutures. Metopic sutures were found in 3 of the specimens examined. WBs were detected in one of these metopic sutures. All examined minor sutures, and the number of WBs detected are presented in Table 3.

There was no significant difference in the number of WBs between the right and left halves of the cranium for the lambdoid and coronal sutures and for the asterion ($p>0.05$).

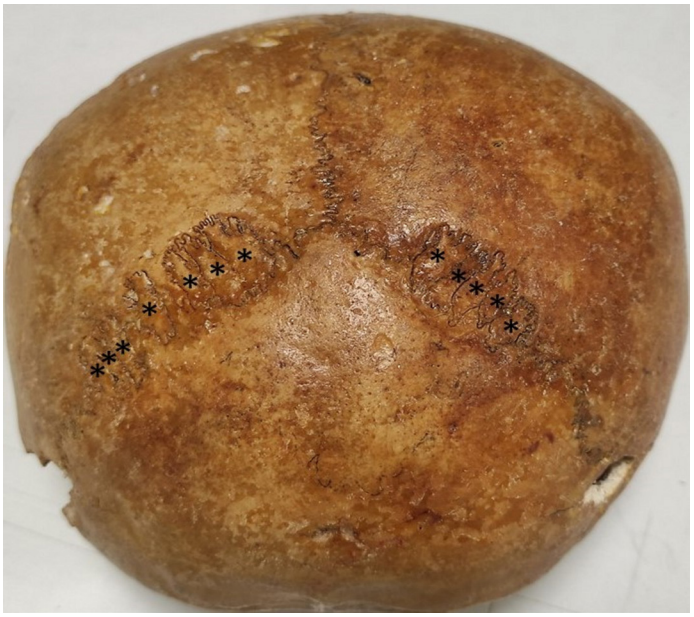

Figure 1. Wormian bones located in the Lambdoid suture. 12 Wormian bones (asterisks) in one Lambdoid suture on the skull

Table 1. Major sutures and WBs located in these sutures.

Suture	n	Number of WBs		WBs per suture	
		R	L	R	L
Lambdoid (b)	28	22	28	0.77	1
Coronal (b)	28	5	7	0.18	0.25
Sagittal (m)	29	3		0.1	
Squamosal (b)	20	0	0	0	0

WBs: Wormian bones, R: right, L: left, b: bilateral, m: median

Figure 2. Skull with Wormian bone localized in the Asterion (asterisk)

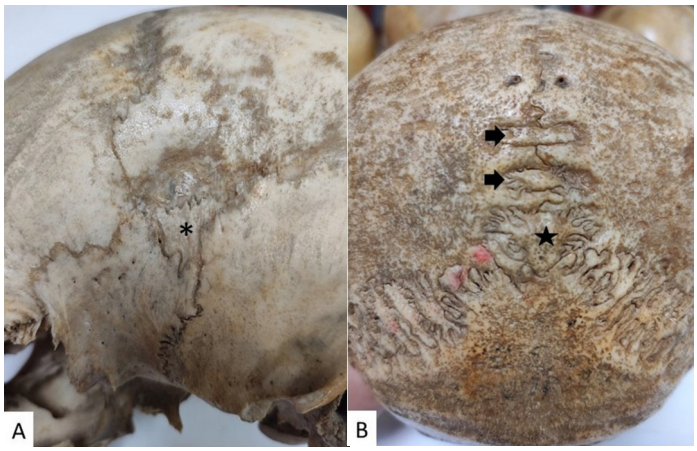

Figure 3. Skull with Wormian bones localized in the Pterion (asterisk), posterior fontanelle (star), and sagittal suture (arrows)

Tablo 2. Major fontanelles and WBs located in these fontanelles

Suture	n	Number of WBs		WBs per suture	
		R	L	R	L
Asterion (b)	26	12	10	0.46	0.38
Pterion (b)	22	1	3	0.04	0.13
Anterior fontanelle (Bregma) (m)	28	0		0	
Posterior fontanelle (Lambda) (m)	29	3		0.10	

WBs: Wormian bones, R: right, L: left, b: bilateral, m: median

Tablo 3. Minor sutures and WBs located in these sutures

Suture	n	Number of WBs		WBs per suture	
		R	L	R	L
Parietomastoid (b)	20	1	3	0.05	0.015
Frontozygomatic (b)	19	5	1	0.26	0.05
Sphenozygomatic (b)	20	3	2	0.15	0.10
Occipitomastoid (b)	20	1	1	0.05	0.05
Zygomatomaxillary (b)	19	1	1	0.05	0.05
Metopic (m)	3	1		0.33	

WBs: Wormian bones, R: right, L: left, b: bilateral, m: median

DISCUSSION

WBs are ossicles located on the sutures. These ossicles are irregular in shape and size. There are different hypotheses that these ossicles are mechanically induced and that they are associated with genetic defects (3). Because of their unusual appearance, these ossicles can be misdiagnosed as fractures during radiographic imaging, which is one of the reasons why these ossicles are clinically important (12,13). Although WBs can also be observed in healthy individuals, they are known to be associated with some cranial or central nervous system disorders (14). Due to the aforementioned important features, it is important to provide information on the early diagnosis of the presence of WBs, as well as the frequency and localization of WBs.

According to the data obtained in the present study, at least one WBs was observed in 82.8 percent of adult skulls. Among the sutures, WBs were most common in the Lambdoid, and among the fontanelles, most common in the Asterion. There was no significant difference in the number of WBs in the right and left halves of the cranium. WB localization was detected in one of the three metopic sutures among our specimens.

In the current study, the rate of adult dry skulls with at least one WB was 82.8%. In the literature, this rate is observed on a very wide scale. In a study conducted in northern India, this rate was found to be 35.3%, while this rate was found to be 80.3% in Chinese (8,12). This wide range of scales may be due to racial differences or physical exposures. In addition, differences in assessment methods may also have contributed to these discrepancies. In the study conducted in the North Indian region, examinations were performed during routine autopsy, whereas we used dry skulls in our study. The changes that occur during drying may have made the sutures more prominent and facilitated the detection of WBs.

WBs were most commonly identified in the lambdoid suture, according to research conducted by Natsis et al. in 2019 on adult dry skulls from Greece (7). In our study, adult dry skulls from Anatolia, a region geographically near to Greece, were examined. In both populations, the lambdoid suture was consistently and remarkably the most prevalent suture where WBs were observed. Many studies have similarly demonstrated that WBs are most commonly observed in the lambdoid suture (12,15). In 2008, ten WBs on the lambdoid suture were described in an Indian case report (16). Similarly, twelve WBs were found on the lambdoid suture in one of the specimens in our investigation. In a study published by Al Kaissi et al. in 2023, it was stated that the Lambdoid suture is part of the brain that bears the most weight (17). However, it is noteworthy that studies are needed to explain why the Lambdoid suture has more WBs than other sutures.

In parallel with the studies in the literature, the fontanella with the highest number of WBs detected in our study was Asterion, followed by Pterion (7,12). Similar to the study by Cirpan et al, no significant difference was found in the number of WBs detected in the right and left asterions in our study (15). The fact that Asterion is the fontanelle with the highest abundance of WBs, consistent with previous studies, is clinically valuable data. However, no valid hypothesis has been proposed as to why Asterion has more WBs than other fontanelles.

Among the minor sutures examined in our study, frontozygomatic, sphenozygomatic, and parietomastoid sutures stand out in terms of the number of localized WBs. The results of the studies examining minor sutures in the literature are similar to the results obtained in our study (7,15). Although only three metopic sutures were observed in the specimens in the current study, one of them had a WB. Although this is a very high rate, the insufficient number of samples prevents this result from being reliable data. Further studies with a large number of metopic suture specimens should be designed to more accurately detect WBs numbers on metopic sutures.

The present study also has some limitations. The most important of these is that the age and sex of our specimens are unknown. Studies on samples with known age and sex will be able to reveal the effect of such variables on the formation and localization of WBs more clearly. In addition, studies in which the number of samples is increased and the region is restricted may provide more effective findings in order to show regional differences in WBs.

CONCLUSION

WBs are crucial for both clinicians like radiologists and emergency physicians as well as researchers including archaeologists and anatomists. Studies that focus on specific regions are more valuable because there is substantial diversity in the prevalence and location of WBs among populations. There isn't enough research in the literature at this point to describe the characteristics of WBs in the Anatolian population. Studies analyzing WBs not only topographically but also developmentally in the Anatolian population will aid in determining a relationship between cause and effect regarding the incidence of WBs.

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

Ethical approval: *Since the study was conducted on dry skulls, ethics committee approval is not required.*

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Research Article

Morphometric Analysis of the Left Main Coronary Truncus, Left Anterior Descending Artery, Circumflex Artery, and Intermediate Artery: Measurements of Length, Angle, and Diameter

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Abstract

Aim: The aim of our study was to group the left main coronary truncus (LMCT) according to its branching structure and to determine its length, angle and diameter measurements together with LMCT's main branches which are left anterior descending artery (LAD), circumflex artery (Cx) and intermediate artery (IA).

Material and Methods: Between June 2019 and June 2021, coronary angiographies of 150 (female-39%, male-61%) patients were analysed by digital subtraction angiography. For each patient, the measurements of the length and diameter of the LMCT, LAD (proximal-middle-distal parts), Cx (proximal-middle-distal parts), and IA were calculated. Measurements were performed with 2-dimensional measurement technique.

Results: The LMCT showed bifurcation pattern in 90.7% and trifurcation pattern in 9.3% of cases. The mean LMCA length and diameter were 15.9±5.7 mm and 6.0±0.9 mm, respectively. The LAD-CX angle defined as the bifurcation angle was 75.8±25.5°. The results that differed significantly between the sexes were the LMCT-LAD angle (159.2±17.8°) and the LAD-distal diameter (2.5±0.5 mm) ($p<0.05$).

Conclusion: In our study, the length-angle-diameter measurements of the LMCT and its main branches (LAD, Cx, IA) were determined in detail. These results are important anatomical data that may contribute to the diagnosis and treatment procedures, especially in cardiology, cardiovascular surgery, and radiology.

Keywords: Left main coronary truncus; left anterior descending artery; circumflex artery; intermediate artery; length-angle-diameter measurements; morphometric analysis

INTRODUCTION

The left coronary artery (LCA), which is the main source of supply to the heart, starts from the left aortic sinus, passes behind the pulmonary truncus and proceeds along the atrioventricular sulcus, before dividing into its main branches, this part is called the left main coronary truncus (LMCT). In classical morphology, the LMCT bifurcates into two main branches, the left anterior descending artery (LAD) and circumflex artery (Cx). In some hearts, there may be a third artery called intermediate artery (IA) between the LAD and Cx origins. In this case, LCA trifurcation is referred. The LCA is very important as it supplies most of the myocardium and interventricular septum, especially the left heart (1-5).

Various studies have shown that there is an important relationship between coronary artery morphology and the diagnosis and treatment of coronary artery diseases. Coronary stenoses of various degrees of stenosis, which require advanced by-pass surgery, are usually seen in the branching regions of coronary arteries. There are several studies showing that there is a significant relationship between the diameters and bifurcation angles of coronary arteries and coronary artery diseases (6-10). There are also studies showing a relationship between the diameter of coronary arteries and long-term graft patency in coronary artery bypass surgery (11).

Currently, stenting is the most preferred non-surgical percutaneous coronary intervention (PCI) to keep the arterial lumen open. For successful stenting, the compatibility between vessel diameter and stent size is very important. If the stent size is larger or smaller than it

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should be, it may lead to undesirable complications such as rupture, restenosis and thrombosis. For this reason, diameter, length and angle information of coronary arteries are very valuable anatomical information for determining coronary artery surgical or percutaneous treatment procedures and preventing complications (12-14).

Coronary angiography, which has been used in the detection, evaluation and treatment of coronary artery diseases from past to present, is one of the most widely used radiological techniques today, especially in determining the location and degree of coronary stenosis (6,15-17).

The aim of this study was to determine the morphological characteristics as length-diameter-angle measurements of LMCT, LAD, Cx and IA by digital subtraction angiography technique and to find out whether there is a significant difference according to gender.

MATERIAL AND METHOD

This study was approved by the Scientific Research Ethics Committee (Decision No/Date: 15.12.2020/258) and was conducted by applying the principles of the Helsinki Declaration at every stage.

Our study was a retrospective study of coronary angiographies. Angiograpy was performed with digital subtraction angiography (DSA) at the Batman Training-Research Hospital between June 2019 and June 2021. For DSA (Angiostar and Axiom Artis units, Siemens, Erlangen, Germany), angiographic catheters were used and a nonionic contrast medium was delivered to the arteries via the catheter. We included 150 patients (58 females-39%, 92 males-61%) aged between 29 and 85 years. The mean age was 58.40±12.38 years. Patients who underwent coronary angiography and had normal coronary arteries were included in the study. Patients with a history of coronary artery by-pass surgery, coronary artery stenosis, coronary artery balloon angioplasty and/or stenting, coronary artery anomalies, heart failure, cardiomyopathy and valvular heart disease were excluded from the study.

In the study, firstly, the LMCT was divided into two groups according to the branching structure. The patients with classical branching that they had the LAD-Cx were grouped as bifurcation pattern and patients with triple branching they had the Cx-LAD-IA were grouped as trifurcation pattern and the length-diameter measurement of the LMCT, angle measurements (LMCT-LAD, LAD-Cx (bifurcation angle), Cx-LMCT) was performed for each patient (Figures 1 and 2). Afterwards, the length-diameter-angle measurements of the Cx, LAD, and IA for both groups were performed separately according to the criteria described below:

For Cx; Cx-proximal part [between the centre of the Cx ostium and the ostium level of the first obtuse marginal branch (OM1)], Cx-middle part [between the ostium level of OM1 and the ostium level of second obtuse marginal branch (OM2)], Cx-distal part [OM2 ostium level and beyond] were determined (Figure 3).

For LAD; LAD-proximal piece [between the LAD ostium centre and the first diagonal branch (D1) ostium level], LAD-middle piece [between the D1 ostium level and the second diagonal branch (D2) ostium level], LAD-distal piece [D2 ostium level and beyond] were determined (Figure 4).

In addition to the measurements described above, the length and diameter measurements of the IA were performed.

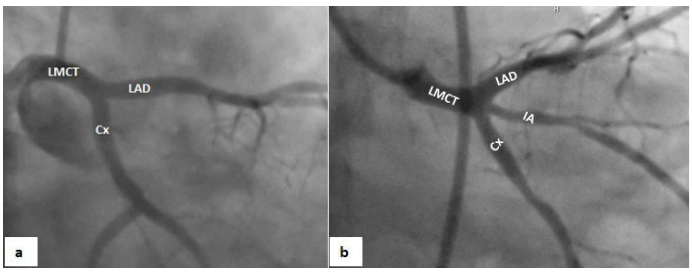

Figure 1. The bifurcation pattern (a) and trifurcation pattern (b) of the LMCT (LMCT: Left main coronary truncus, LAD: Left anterior descending artery, Cx: Circumflex artery, IA: Intermediate artery)

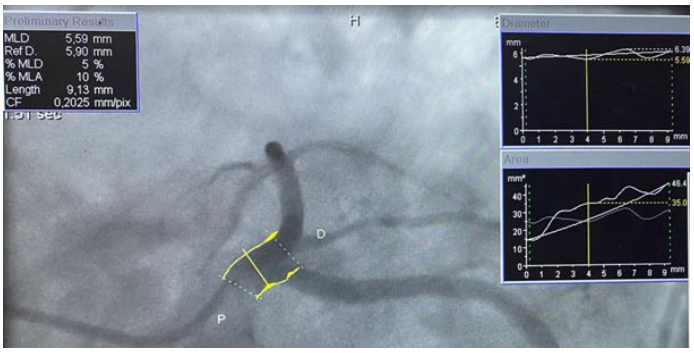

Figure 2. Length and diameter measurements of the left main coronary truncus (yellow lined part) (Ref D: Diameter measurement, Length: Length measurement)

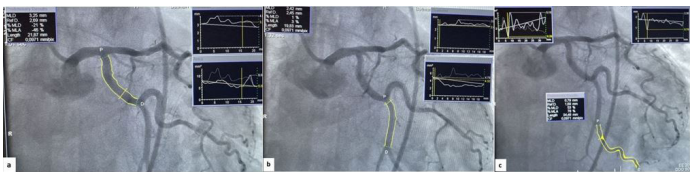

Figure 3. The proximal, middle, and distal parts of Cx (yellow lined parts) (a) The Cx-proximal part [between the centre of the Cx ostium and the level of the OM1 ostium centre]; (b) The Cx-middle part [between the OM1 ostium level and the OM2 ostium level]; (c) The Cx-distal part (OM2 ostium level and beyond] (Cx: Circumflex artery, OM1:First obtuse marginal branch, OM2: Second obtuse marginal branch, Ref D: Diameter measurement, Length: Length measurement)

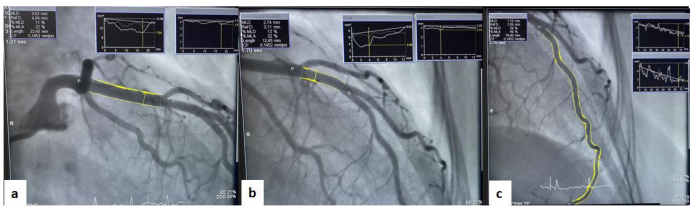

Figure 4. The proximal, middle, and distal parts of the LAD (yellow lined parts) (a) The LAD-proximal part [between the centre of the LAD ostium and the level of the D1 ostium centre]; (b) The LAD-middle part [between the D1 ostium level and the D2 ostium level]; (c) the LAD-distal part [the D2 ostium level and beyond] (LAD: Left anterior descending artery, D1: First diagonal branch, D2: Second diagonal branch, Ref D: Diameter measurement, Length: Length measurement)

Statistical Analyses

SPSS-21 programme (IBM SPSS-Statistics for Windows, New York/USA) was used for statistical analysis of the data recorded in the study. Independent Samples t test was used for comparison between genders and P<0.05 was considered significant. Data were summarised as percentage, mean±standard deviation.

RESULTS

LMCT showed bifurcation pattern in 136 (90.7%) and trifurcation pattern in 14 (9.3%) of 150 hearts (Table 1). Bifurcation pattern was observed in 86.96% of the while trifurcation pattern was observed in 13.04% in a total 92 male cases. In 58 female cases, bifurcation pattern was detected in 96.55% and trifurcation pattern in 3.45%. Table 2 shows the LMCT, Cx, and LAD length-diameter-angle measurements. Among the results presented in this table, in bifurcated hearts, the mean length of the left coronary artery truncus was 15.9±5.7 mm and the mean diameter was 6.0±0.9 mm. In the angle measurements, the angle between the LMCT and the LAD was 159.2±17.8°, this was statistically significant (p=0.000). The LMCT-Cx and LAD-Cx angles are 124.5±21.9°, 75.8±25.5°, respectively. In the LAD measurements, the distal diameter of the LAD was 2.5±0.5 mm (p=0.033).

Table 1. LMCT branching patterns

LMCT branching patterns	Female	Male	Total
Bifurcation	56	80	136 (90.7%)
Trifurcation	2	12	14 (9.3%)
Total	58 (39%)	92 (61%)	150 (100%)

LMCT: left main coronary truncus

In LMCT-trifurcated hearts, the mean length and diameter of IA, LMCT length and diameter were 121.7±28.2 mm, 2.8±0.5 mm, 19.4±6.7 mm, and 6.2±0.8 mm, respectively (Table 3). The results in these hearts were not statistically significant (p>0.05).

Table 2. LMCT (with bifurcation pattern) and Cx-LAD length-diameter-angle measurements and gender distribution (n: 136)

LMCA-Cx-LAD measurements		Gender	Mean±SD	General Mean±SD	t	P
LMCT measurements						
LMCT	**Length (mm)**	Female	14.8±4.9	15.9±5.7	-1.810	0.072
		Male	16.6±6.1			
	Diameter (mm)	Female	6.0±0.9	6.0±0.9	0.074	0.941
		Male	6.0±0.9			
LMCT-LAD-Cx angles	**LMCT-LAD (°)**	Female	165.5±14.5	159.2±17.8	3.662	0.000*
		Male	155.00±18.6			
	LAD-Cx (bifurcation angle) (°)	Female	73.6±22.0	75.8±25.5	-0.842	0.401
		Male	77.3±27.6			
	Cx-LMCT (°)	Female	121.0±19.6	124.5±21.9	-1.575	0.117
		Male	126.8±23.1			
Cx measurements						
Cx-proximal	**Length (mm)**	Female	24.7±18.7	26.3±18.3	-0.884	0.378
		Male	27.4±18.0			
	Diameter (mm)	Female	4.3±0.7	4.2±0.7	0.483	0.630
		Male	4.2±0.8			
Cx-middle	**Length (mm)**	Female	40.7±25.1	39.7±22.9	0.402	0.688
		Male	39.0±21.4			
	Diameter (mm)	Female	3.7±0.7	3.7±0.7	0.495	0.621
		Male	3.7±0.7			
Cx-distal	**Length (mm)**	Female	67.8±29.2	70.8±29.6	-0.988	0.325
		Male	72.8±29.8			
	Diameter (mm)	Female	2.6±0.7	2.5±0.6	0.662	0.509
		Male	2.5±0.5			
LAD measurements						
LAD-proximal	**Length (mm)**	Female	27.6±14.4	29.1±16.9	-0.858	0.392
		Male	30.0±18.4			
	Diameter (mm)	Female	4.4±0.7	4.3±0.6	1.675	0.096
		Male	4.2±0.6			
LAD-middle	**Length (mm)**	Female	42.3±23.6	39.8±24.0	0.915	0.362
		Male	38.3±24.3			
	Diameter (mm)	Female	3.5±0.6	3.5±0.6	0.081	0.936
		Male	3.5±0.6			
LAD-distal	**Length (mm)**	Female	100.1±33.7	99.4±33.3	0.191	0.849
		Male	99.0±33.2			
	Diameter (mm)	Female	2.4±0.4	2.5±0.5	-2.154	0.033*
		Male	2.6±0.5			

LMCT: left main coronary truncus, LAD: left anterior descending artery, Cx: circumflex artery, IA: intermediate artery, *p<0.05

Table 3. LMCT (with trifurcation pattern), IA length-diameter measurements and distribution according to gender (n: 14)

LMCT-IA Measurements		Gender	Mean±SD	General Mean±SD	t	P
LMCT	Length (mm)	Female	16.2±2.3	19.4±6.7	-0.721	0.488
		Male	20.1±7.2			
	Diameter (mm)	Female	5.4±0.5	6.2±0.8	-1.515	0.161
		Male	6.3±0.8			
IA	Length (mm)	Female	137.4±30.5	121.7±28.2	0.849	0.416
		Male	118.5±28.4			
	Diameter (mm)	Female	2.6±0.4	2.8±0.5	-0.599	0.562
		Male	2.8±0.6			

LMCT; left main coronary truncus, IA; intermediate artery

DISCUSSION

Cardiovascular diseases are one of the leading health problems with a mortality rate of 25.1% in the world. There are several studies showing that there is a significant relationship between the occurrence of coronary artery diseases, which constitute an important part of these diseases, determination of treatment procedures and coronary artery morphology, especially bifurcation angles (18-21).

In our study, the average values of the LMCT were 6.0±0.9 mm for its diameter and 15.9±5.7 mm for its length and its angles were found as 159±17°, 75±25°, 124±21°, respectively. Gracia et al. found the mean diameter of the LCA to be 3.5±0.8 mm, the length to be 10.5±5.3 mm, and the LCA-Cx, Cx-LAD (bifurcation angle), and LCA-LAD angles to be 126±21°, 75±23°, and 138±20, respectively, in their study on 3D images obtained from coronary angiographies (22). Kawasaki et al. found the above-mentioned angles as 121±21° (LMCT-Cx), 72±22° (LAD-Cx), 143±13° (LMCT-LAD) in their angle investigations using 3D measurement method in 209 patients with multislice computed tomography technique (23). Gazetopoulos et al. found the LCA length to be 16.8±4.13 mm in their study on 43 angiograms and reported a significant relationship between coronary atherosclerosis and left coronary artery length (24). Pereira da Costa Sobrinho et al. determined the length of the left main coronary artery as 6.44 mm for patients with bifurcation branching pattern and 9.77 mm for the group with trifurcation pattern in their dissection study in 63 cadavers and mentioned the clinical significance of these results (25). In our study, LMCA length was found to be 15.9±5.7 mm in the group with classical branching and 19.4±6.7 mm in the group with triple branching. This difference between the studies may be related to the fact that the DSA technique provides more and more detailed examination.

Raut et al. reported left main coronary artery diameter as 4.08±0.44 mm, LAD-proximal diameter as 3.27±0.23 mm, and Cx-proximal diameter as 2.97±0.37 mm in 229 patients who underwent coronary angiography (26). Zhang et al. determined the mean length and diameter of LAD (origin-distal), mean length and diameter of Cx (origin-distal) as 130 mm, 3.92 mm (origin diameter)-2.10 mm (distal diameter), 130 mm, 3.57 mm (origin diameter)-2.10 mm (distal diameter), respectively, in 526 cases using CT coronary angiography (27). In our study, these values were 168.3 mm (LAD proximal-middle-distal mean sum), 4.3 mm (proximal diameter), 2.5 mm (distal diameter), 136.8 mm (Cx proximal-middle-distal mean sum), 4.2 mm (proximal diameter), 2.5 mm (distal diameter), respectively.

Our study differs from the other studies we discussed, as this study provides detailed results in male and female cases. The studies mentioned above are mostly based on average values. In our study, we evaluated male and female cases separately.

Limitations in our study, unequal female (39%) and male (61%) populations, limited number of patients (150 patients).

CONCLUSION

In conclusion, in this study, the length, diameter and angle of the left coronary artery and its main branches were determined. We believe that this anatomical information will improve the interpretation of diagnostic cardiac imaging and contribute to the success of interventional cardiac procedures, as well as contribute to the development of new therapeutic interventional treatment techniques.

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MEDICAL RECORDS-International Medical Journal

Research Article

Association between Anesthesia Management and Preoperative Magnetic Resonance Image Quality in Patients Scheduled for Deep Brain Stimulation Surgery

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Abstract

Aim: To investigate magnetic resonance image quality and the number of motion artifact-related repeated sequences based on anesthesia or sedation management during preoperative MR imaging for DBS surgery.

Material and Methods: The medical records of patients who underwent DBS procedures at the hospital of Ondokuz Mayis University, between April 2011 and October 2021 were retrospectively analyzed. Age, gender, and diagnosis information were recorded for each case. Patients were grouped into general anesthesia, sedation, no sedation groups. The evaluation of magnetic resonance images was performed by a specialized in neuroradiology. The radiologist classified the image quality as good, moderate, or poor based on artifacts resulting from unwanted motion.

Results: A total of 127 patients, out of 190 patients, were included in the study. There were no significant differences in image quality based on anesthesia/sedation method and airway management ($p>0.05$). No significant differences were observed in the number of repeated sequences when compared based on anesthesia/sedation method and airway management ($p>0.05$).

Conclusion: General anesthesia, sedation, or no sedation during preoperative magnetic resonance imaging in patients with movement disorders did not result in significant differences in image quality and the number of sequences requiring repetition.

Keywords: Deep brain stimulation, magnetic resonance imaging, movement disorders, Parkinson's disease, anesthesia, sedation

INTRODUCTION

Deep brain stimulation (DBS) is a surgical treatment option used in movement disorders such as Parkinson's disease (PD), essential tremor (ET), and dystonia. The surgical method involves the placement of stimulator electrodes into target nuclei located in the basal ganglia, followed by connection to a pacemaker implanted in the infraclavicular or abdominal region (1). Precise determination of the target nuclei's exact location is of utmost importance, not only to ensure the clinical effectiveness of the treatment but also to minimize the occurrence of unintended complications. Preoperative magnetic resonance (MR) imaging is one of the methods used in targeting the anatomical localization (2). However, the presence of artifacts during imaging in patients with movement disorders can pose difficulties in determining the target and may require sequence repetition, leading to prolonged scan duration.

In adult patients, anxiety, claustrophobia, mental retardation, and movement disorders necessitate the administration of sedation or general anesthesia during MR imaging. Although sedation and general anesthesia procedures are considered relatively safe in the MR imaging suite (3), they require detailed preparation beforehand and a competent team trained in monitoring and airway interventions during imaging. The entry of the head into the magnet and the placement of receiver coils around it make airway access difficult during brain imaging. It is known that decreased ventilatory functions,

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weakened cough reflex, and inadequate clearance of secretions in PD patients increase the frequency of post-anesthetic laryngospasm and aspiration pneumonia (4). When making the decision to apply sedation or general anesthesia during MR imaging, healthcare providers should consider the benefits it will offer, potential side effects, and costs involved.

There is limited data regarding whether the administration of sedation or general anesthesia during MR imaging in patients with movement disorders provides higher-quality images (5).

The aim of this retrospective study is to evaluate and compare the image quality and the frequency of motion artifact-related repeated sequences, in patients who underwent preoperative MR imaging for DBS surgery based on their anesthesia or sedation management.

MATERIAL AND METHOD

Study Population

This retrospective study was approved by the Ondokuz Mayıs University Medical Ethics Committee (approval number: 2022/157). The medical records of patients who underwent DBS procedures at the Department of Neurosurgery of our University, between April 2011 and October 2021 were retrospectively reviewed. The study included patients with accessible preoperative MR images and information regarding the administration of sedation or anesthesia during the imaging. Patients lacking accessible MR images, preoperative evaluations, and intraoperative monitoring forms, despite undergoing sedation or anesthesia administration, were excluded from the study.

Data Collection

The records of patients who underwent DBS surgery were obtained from the hospital information management system, and the MR images were obtained from the radiological imaging archives. Preoperative evaluation and intraoperative monitoring forms were used to gather information on sedation and anesthesia management. Age, gender, and diagnosis information were recorded for each case. Data on airway management and medications used were collected for patients who received sedation or anesthesia.

Evaluation of the MRI Records and MRI Protocol

The evaluation of MR images was performed by a radiologist specialized in neuroradiology. A randomized list of included patients was provided to the radiologist. The radiologist was asked to classify the image quality as good, fair, or poor based on artifacts resulting from unvoluntary motion. Additionally, the number of sequences requiring repetition during imaging was recorded for each patient.

MR images were obtained according to our institutional protocol with a 3T scanner (Ingenia, Philips Healthcare, Best, The Netherlands) using a 32-channel head coil. All examinations included a 3D T1-weighted conventional gradient echo (3D T1-TFE) sequence with and without gadolinium (160 contiguous sagittal slices with an in-plane voxel resolution: 1x1x1 mm; repetition time [TR]/ echo time [TE], 7.9/3.5 millisecond [ms], number of excitations [NEX], 1), 3D T2 fuid attenuation inversion recovery (FLAIR) sagittal (TR/TE/TI, 4800/381/1650 ms; section thickness, 3 mm; NEX, 2); T2W sagittal turbo spin-echo sequence (TR/TE, 3000/80 ms; section thickness, 5 mm; matrix, 261 x 384; NEX, 3); T2W axial turbo spin-echo sequence (TR/TE, 3000/80 ms; section thickness, 5 mm; matrix, 261 x 384; NEX, 3); and contrast-enhanced T1W axial conventional spinecho sequence (TR/TE, 606/17 ms; section thickness, 5 mm; FOV, 230; matrix, 230 x 384; NEX, 2).

Anesthesia, Sedation and Related Side Effects

According to the obtained records, patients were classified into three groups: those who received general anesthesia, those who received sedation, and those who did not receive sedation. The general anesthesia procedure consisted of iv propofol induction (1-2.5 mg/kg bolus), rocuronium (0.6 mg/kg iv), endotracheal intubation, and mechanical ventilation, followed by propofol infusion (4-10 mg/kg/h). Sedation was defined as the administration of intravenous sedative agents while maintaining spontaneous respiration without the need for airway management. Patients who did not receive sedation were either not given any sedative agent or only received oral premedication. Respiratory, hemodynamic, and allergic side effects that could occur during anesthesia were recorded from the patients' anesthesia records.

Statistical Analysis

The statistical analyses were performed using the NCSS (Number Cruncher Statistical System) 2007 software program (Kaysville, Utah, USA). Descriptive statistical methods such as mean, standard deviation, median, frequency, ratio, minimum, and maximum were used to evaluate the study data. The distribution of the data was assessed using the Shapiro-Wilk test. The Kruskal-Wallis test was used for comparing three or more groups of quantitative data, while the Mann-Whitney U test was used for comparing two groups. Chi-square analysis was employed to determine the relationship between qualitative variables. The significance level was evaluated at $p<0.05$.

RESULTS

A total of 127 patients, out of 190 patients identified as having undergone DBS surgery, were included in the study due to the availability of sufficient data. The age of the evaluated patients ranged from 18 to 77 years, with a mean age of 55.23±13.78. Among the study participants, 39.4% (n=50) were female, and 60.6% (n=77) were male. Parkinson's disease was the most common preoperative diagnosis, accounting for 74% (n=94) of the patients. The demographic characteristics and diagnostic information of the patients are presented in Table 1.

Among the patients, it was determined that no sedation was administered during MR imaging in 103 cases, general anesthesia with endotracheal intubation was performed in

4 cases, and sedation was administered in 20 cases (Figure 1). Out of the patients who did not receive sedation, 27 of them received oral diazepam premedication on the night before the scan. The most commonly preferred drugs for sedation were found to be propofol and midazolam. The distribution of patients according to the sedation method, drugs used, and airway management is shown in Table 2.

According to the assessment performed by the radiologist, 61.4% (n=78) of the patients' MR images were classified as good quality, 29.1% (n=37) as fair quality, and 9.4% (n=12) as poor quality. The number of repeated sequences ranged from 0 to 6, with a mean of 2.32±1.33. When comparing image quality based on anesthesia/sedation method and airway management, no significant differences were found (p>0.05) (Table 3). Similarly, no significant differences were observed in the number of repeated sequences when compared based on anesthesia/sedation method and airway management (p>0.05) (Table 4). Except for two cases where mild allergic reaction was successfully resolved with antihistamine administration, no other side effects or complications were observed.

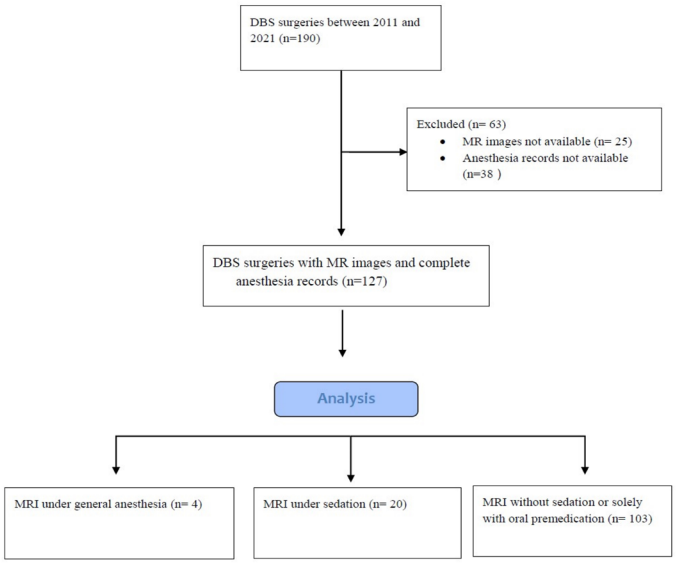

Figure 1. Flow chart detailing the exclusion criteria applied to all DBS surgeries with preoperative MR imaging. DBS, deep brain stimulation; MR imaging, magnetic resonance imaging

Table 1. Demographic and diagnostic characteristics of the patients

Age (years), (mean±sd)		55.23±13.78
Sex (n), (%)	Female/male	50 (39.4)/77 (60.6)
Diagnosis (n) (%)	Dyskinesia	1 (0.8)
	Dystonia	21 (16.5)
	Essential tremor	7 (5.5)
	Huntington's disease	2 (1.6)
	Multiple sclerosis	1 (0.8)
	Parkinson's disease	94 (74)
	Tourette syndrome	1 (0.8)

Table 2. Airway and Anesthesa/Sedation management information of the patients

Drug used (n)(%)	Diazepam (oral premedication)	27 (21.3)
	Midazolam	9 (7.1)
	Ketofol	2 (1.6)
	Ketamine	1 (0.8)
	Thiopental	3 (2.4)
	Propofol	10 (7.9)
	No drug administered	76 (59.1)
Airway management (n) (%)	Intubation	4 (3.1)
	Spontaneous breathing	123 (96.9)
Anesthesia/sedation management n (%)	No sedation	103 (81.1)
	General anesthesia	4 (3.1)
	Sedation	20 (15.7)

Table 3. Image quality according to anesthesia/sedation management and airway management

		Image quality n (%)			p
		Good	**Moderate**	**Poor**	
Anesthesia/sedation management	No sedation	64 (62.1)	29 (28.2)	10 (9.7)	0.92
	General anesthesia	3 (75)	1 (25)	0 (0)	
	Sedation	11 (55)	7 (35)	2 (10)	
Airway management	Intubation	3 (75)	1 (25)	0 (0)	0.76
	Spontaneous breathing	75 (61)	36 (29.3)	12 (9.8)	

Table 4. Number of repeated sequences according to anesthesia/sedation management and airway management

			n	Mean±sd	Min-Max (Median)	p
Number of repeated sequences	Anesthesia/ sedation management	No sedation	103	2.36±1.34	0-6 (3)	0.72
		General anesthesia	4	2±1.16	1-3 (2)	
		Sedation	20	2.15±1.35	0-5 (2)	
	Airway management	Intubation	4	2±1.16	1-3 (2)	0.65
		Spontaneous breathing	123	2.33±1.34	0-6 (2)	

DISCUSSION

In this retrospective study, which examined the records related to preoperative MR imaging of patients who underwent DBS procedures between April 2011 and October 2021 in our hospital, it was observed that the administration of general anesthesia or sedation during MR imaging did not result in better MR image quality compared to no sedation. Furthermore, when comparing the frequency of repeated sequences due to motion artifacts among the three groups, no significant difference was found. Although MR imaging is frequently performed for mapping prior to DBS surgery, data on the relationship between sedation or general anesthesia during MR imaging and image quality in patients with movement disorders are limited.

Contrary to our research, a recent study reported that the administration of general anesthesia during preoperative MR imaging of patients with movement disorders resulted in higher-quality images compared to intravenous sedation and no sedation (5). However, in the same study, the use of sedation did not demonstrate any superiority over no sedation. There are data in the literature indicating that sedation provides better image quality in pediatric patients and adults with anxiety disorders (6,7). The lack of any difference in image quality related to anesthesia management in our study could be attributed to several reasons. The severity of the movement disorder may have been taken into account while determining whether anesthesia or sedation should be administered to the patients for the MR imaging scan. The limited number of patients who received general anesthesia, only four patients in total, suggests that this option may have been preferred for patients with unvoluntary movements that did not improve even during rest or sleep. In patients who received sedation, the fact that the underlying cause necessitating sedation was the movement disorder rather than an anxiety disorder may have contributed to achieving similar results as in patients without sedation who had milder movement disorder.

Indeed, in our study, the rates of good quality images for patients without sedation and those with sedation were found to be 62.1% and 55%, respectively. In the previously mentioned study, these rates were reported as 65%, similar to our study (5). The attainment of a considerable number of good quality images even without sedation highlights the importance of patient selection when deciding on the necessity of anesthesia or sedation, considering the increased costs and potential risks. The administration of sedation necessitates the use of medication, preparation procedures, monitoring, and dedicated personnel, thereby contributing to elevated costs through the extension of MR imaging room occupancy, recovery periods, and hospital stays (8). One study reported that sedation-requiring MR images accounted for one-third of all outpatient costs (9). Furthermore, although sedation and anesthesia are considered relatively safe when the necessary conditions are met, procedural sedation carries risks such as aspiration, respiratory depression, and laryngospasm that can lead to serious hypoxia (10). The use of coils in brain imaging during MR imaging and the positioning of the patient's head within the tunnel can further hinder access and increase these risks. However, no serious complications were encountered in this study.

While the rate of images classified as poor was approximately 10% in both the sedation and no-sedation groups, no patients in the general anesthesia group were found to have poor-quality images. In cases without sedation, this may be attributed to both the presence of movement disorders and the occasional inability to maintain stillness during the long imaging duration. The need for prolonged immobility is one of the main reasons that necessitate sedation during MR imaging (11). On the other hand, the inability to achieve sufficient image quality despite sedation may be attributed to decreased oropharyngeal tone and a tendency for obstruction, leading to artifact formation during spontaneous respiration.

Undesired movements and respiratory artifacts during MR imaging examinations can necessitate the repetition of specific sequences (12). This can lead to prolonged scan durations, disruptions in scheduled appointments, and increased costs. The purpose of anesthesia or sedation is to achieve immobility and obtain the best possible images. When comparing our patients in terms of the number of sequences requiring repetition, no significant differences were found. These findings suggest that the experience of the sedation team and patient selection play a role in determining the anesthesia management during MR imaging.

In our study, it was observed that the most commonly preferred drugs for sedation management were propofol and midazolam. Although propofol has been reported to trigger dyskinesia (13), it can still be used in patients with movement disorders (14) and is one of the frequently preferred agents for sedation in MR imaging units (15).

The use of dexmedetomidine has been reported for preventing the dyskinesia triggered by propofol (13). However, one patient who received dexmedetomidine in our study was excluded from the analysis due to unavailability of MR images. Whilst midazolam can cause dystonic extrapyramidal side effects (16), it still is used in Parkinson's patients and during the DBS procedure. Ketamine has been presented as an alternative in a recent case report for a DBS patient with severe dyskinesia who could not be adequately sedated with other drugs (17). It was observed that two of our patients received a combination of propofol and ketamine, and one patient received ketamine alone. Concerns regarding extended recovery, increased secretion, and the possibility of aspiration may have contributed to our sedation teams' choice for alternative medications over ketamine.

This study has certain limitations due to its retrospective design. The unavailability of specific information during the data collection process prevented us from determining how the anesthesia management selection was made and which criteria were considered. Similarly, it was not possible to compare the severity of patients' movement disorder symptoms for the same reason. Furthermore, evaluating the image quality in conjunction with the surgical targeting success and outcomes could have provided valuable information. Lastly, using computer-calculated objective image quality measures instead of manual scoring for image quality assessment could have yielded more unprejudiced results. However, due to the technical constraints and lengthy computational durations required for these methods (18), our study utilized the subjective interpretation of an experienced radiologist for the evaluation.

CONCLUSION

In this retrospective study, the application of general anesthesia, sedation, or no sedation during preoperative MR imaging in patients with movement disorders did not result in significant differences in image quality and the number of sequences requiring repetition. Prospective studies evaluating the obtained images in conjunction with targeting accuracy and surgical outcomes are needed.

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MEDICAL RECORDS-International Medical Journal

Research Article

Assessing the Correlation between Helicobacter Pylori Infection and Increased Incidence of Colorectal Cancer, Gastric Atrophy, and Intestinal Metaplasia

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Abstract

Aim: Our retrospective study was designed to explore the potential link between *Helicobacter pylori* (*H. pylori*) infection and the occurrence of colorectal cancer (CRC), gastric atrophy, and intestinal metaplasia.

Material and Methods: We assembled two groups of 150 patients each, categorized based on their endoscopic biopsy results for *H. pylori* - one group testing positive (study group), the other negative (control group). All subjects underwent upper and lower gastrointestinal endoscopies, and we assessed their endoscopic gastric biopsy results as well as any indications of colorectal malignancies.

Results: Our investigation established a noteworthy association between the presence of *H. pylori* infection and the incidences of CRC, gastric atrophy, and intestinal metaplasia ($p<0.05$). Our data suggests that individuals testing positive for *H. pylori* faced a 64% greater likelihood of developing CRC compared to those who tested negative. Among patients diagnosed with gastric atrophy, a remarkable 73.9% tested positive for *H. pylori*. Similarly, 78.9% of patients with intestinal metaplasia were also *H. pylori* positive.

Conclusion: Our results underscore a meaningful correlation between *H. pylori* infection and a heightened incidence of CRC, gastric atrophy, and intestinal metaplasia. Further, prospective studies will be instrumental in deciphering the underlying causative mechanisms and assessing the feasibility of *H. pylori* eradication as a preventive measure against these conditions.

Keywords: Helicobacter pylori, colorectal cancer, gastric atrophy, intestinal metaplasia, endoscopic biopsy

INTRODUCTION

Helicobacter pylori (*H. pylori*), a type of Gram-negative bacteria primarily found in the stomach, has been associated with several gastrointestinal diseases, such as peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (1,2). More recent studies have expanded our understanding of *H. pylori* infection, demonstrating its associations with extra-gastric disorders such as cardiovascular, metabolic, and neurological diseases (3-5). However, the link between *H. pylori* infection and colorectal neoplasia remains a subject of dispute, with varying conclusions drawn from different studies (6). Moreover, it's been determined that *H. pylori* can induce precancerous changes like atrophy and intestinal metaplasia in the stomach (7), conditions that have been linked with an escalated risk for CRC (8). Yet, the exact relationship between *H. pylori*-induced atrophic gastritis and intestinal metaplasia and CRC risk is still in need of thorough clarification. Hence, our study aims to evaluate the association between *H. pylori* infection and the increased incidence of CRC, gastric atrophy, and intestinal metaplasia. In light of the possible connection, we believe it's crucial to delve further into the multifaceted roles *H. pylori* might play in the pathogenesis of gastrointestinal disorders, as the implications of such research could transform diagnostic strategies and therapeutic interventions for these prevalent conditions. By retrospectively examining a patient cohort presenting with dyspeptic symptoms, we aim to contribute to the current understanding of the role *H. pylori* plays in the development of gastrointestinal malignancies.

CITATION

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MATERIAL AND METHOD

This retrospective study was given ethical approval by our hospital's local committee, as confirmed by the dated decision (17.07.2023, no. 134). Given the retrospective nature of this study, obtaining informed consent from patients was not deemed necessary. In compliance with the 2013 revision of the Declaration of Helsinki, our study encompassed a total of 300 participants. These patients had sought medical consultation at our clinic between January 2010 and December 2022, presenting with various symptoms, including gastrointestinal bleeding, weight loss, and irregular bowel habits such as constipation and diarrhoea. All included patients had undergone upper and lower gastrointestinal endoscopy within this period. Following the endoscopic biopsy results, patients were divided into two distinct groups: those with *H. pylori* infection (n: 150) and those without (n: 150).

To be included in the study, patients had to have:

- Undergone an endoscopic examination of the upper and lower gastrointestinal tract at our institution due to dyspeptic symptoms,
- *H. pylori* infection status (either positive or negative) confirmed through histopathological evaluation,
- Available and current medical records along with follow-up data.

On the other hand, we excluded:

- Any patients with a history of CRC, other malignancies, or inflammatory bowel disease before the endoscopic biopsy,
- Patients lacking necessary medical records or follow-up data,
- Patients who had been previously treated for *H. pylori* infection.

A comparative analysis was undertaken between the two groups on demographic data, tobacco and alcohol use history, NSAID consumption, family history of CRC, body mass indices, gastrointestinal complaints, and findings from the endoscopic gastric biopsy. We also evaluated the endoscopic biopsy results that led to the diagnosis of CRC, focusing on the differences in the number and location of CRC between the two groups.

Statistical Analysis

The Shapiro-Wilk test was employed to determine if the variables conformed to a normal distribution. Variables under the normal distribution were presented as mean ± standard deviation, whereas those not under normal distribution were expressed as median values (ranging from minimum to maximum). Categorical variables were given as n (%).

In line with the results of the normality test, comparisons between groups were done using a T-test for those following a normal distribution, and a Mann-Whitney U test for those not conforming to the normal distribution. The differences among categorical variables were ascertained using either a Continuity Correction Chi-Square test or a Pearson's Chi-Square test.

To evaluate the likelihood of colon cancer occurrence in *H. pylori* positive patients, we used logistic regression analysis. IBM SPSS Statistics 25.0 (SPSS Inc, Chicago, USA) was our tool of choice for all statistical analyses. The accepted threshold for statistical significance in the data analysis was established at $p<0.05$.

RESULTS

The study encompassed 300 participants, evenly divided into 150 *H. pylori* positive and 150 *H. pylori* negative patients. These patients comprised of 134 females (44.7%) and 166 males (55.3%), aged between 37 and 76 years with a mean age of 57.15±7.814 years. Looking at their lifestyle factors, we found that of 94 smokers, 54 (57.4%) tested positive for *H. pylori*, and 40 (42.6%) were negative. Among the 46 alcohol consumers, 28 (60.9%) were *H. pylori* positive, and 18 (39.1%) were negative. For the 128 patients using NSAIDs, *H. pylori* was found positive in 63 (49.2%) and negative in 65 (50.8%). Additionally, out of 33 patients with a familial cancer history, 19 (57.6%) were *H. pylori* positive and 14 (42.4%) were negative. However, statistical analysis revealed no significant correlation between these patient characteristics and their *H. pylori* infection status ($p>0.05$).

In the investigation, we found that of the 138 patients presenting with abdominal pain, 76 (55.1%) tested positive for *H. pylori* while 62 (44.9%) were negative. Among the 79 patients who experienced rectal bleeding, 40 (50.6%) were *H. pylori* positive, and 39 (49.4%) were negative. For patients with diarrhoea, numbering 112, 55 (49.1%) tested positive for *H. pylori*, and 57 (50.9%) were negative. Furthermore, out of the 216 patients who presented with other symptoms, 102 (47.2%) were *H. pylori* positive and 114 (52.6%) were negative. Upon statistical analysis, it was determined that there was no significant correlation between the presence of *H. pylori* and the specific symptoms in either group ($p>0.05$).

The demographic and clinical attributes of the patients across the two groups are encapsulated in Table 1.

Table 2 shows the comparison of endoscopic gastric biopsy results between the two groups.

Out of the 150 patients who tested positive for *H. pylori*, colorectal tumours were found in 14 cases (9.3%), while amongst the 150 patients who were *H. pylori* negative, only 5 cases (3.3%) showed the presence of colorectal tumours. It was thus concluded that a statistically significant association exists between *H. pylori* infection status and the occurrence of CRC ($p<0.05$) (Refer to Table 3).

Our analysis established a significant impact of *H. pylori* positivity on the prevalence of CRC ($p<0.05$). The likelihood

of CRC in patients with *H. pylori* infection is shown to be 64% greater when compared to those without the infection. The odds ratio reflecting the status of CRC in patients who tested positive for *H. pylori* is detailed in Table 3.

Out of 19 patients diagnosed with CRC, 14 tested positive for *H. pylori*, while five were negative for the bacteria. Among the patients positive for *H. pylori*, one patient (7.1%) had a tumour in the caecum, two (14.2%) in the hepatic flexure, three (21.4%) in the splenic flexure, two (14.2%) in the descending colon, and three each (21.4%) in the sigmoid colon and rectum. On the other hand, in the *H. pylori* negative group, the tumour was found in the splenic flexure in one patient (20%), the descending colon in one patient (20%), and the sigmoid colon in three patients (60%).

The majority of tumours identified in both cohorts were classified as adenocarcinomas, accounting for 92.9% in the *H. pylori* positive group and 80% in the *H. pylori* negative group. Meanwhile, mucinous adenocarcinoma was observed in a single patient from each group. The average size of the tumours was slightly larger in the *H. pylori* positive group at 4.5 cm compared to 4.2 cm in the *H. pylori* negative group. When the tumour stages were assessed based on the TNM classification, a total of 15 patients were found to be at stage I and four were at stage II.

Table 1. Demographic and clinical characteristics of patients

	H. pylori (+) (n=150)	*H. pylori* (-) (n=150)	T/U/χ^2	p*
Age (years)*	57.09±8.268	57.20±7.359	-0.118	0.906
Gender			0.337	0.561
Male	86 (57.3%)	70 (46.6%)		
Female	64 (42.7%)	80 (53.4%)		
Habits of patients				
Smoking	54 (36%)	40 (26%)	2.618	0.106
Alcohol consumption	28 (18.6%)	18 (12%)	2.080	0.149
NSAID use	63 (42%)	65 (43.3%)	0.014	0.907
Family history of colorectal cancer	19 (12.6%)	14 (9.3%)	0.545	0.460
BMI (kg/m²)	29 (20:41)	29 (21:41)	11393.5	0.848
Symptoms				
Abdominal pain	76 (50.6%)	62 (41.3%)	2.268	0.132
Rectal bleeding	40 (26.6%)	39 (26%)	0.00	1.00
Diarrhoea	55 (36.6%)	57 (38%)	0.014	0.905
Constipation	76 (50.6%)	69 (46%)	0.481	0.488
Others	102 (68%)	114 (76%)	1.620	0.203

Variables are expressed as mean±standard deviation, median (minimum:maximum), and n(%). T: Student's t-test, U: Mann-Whitney U-test, Continuity Correction χ^2 test; *p<0.05

Table 2. Endoscopic gastric biopsy results between the two groups

Biopsy findings	*H. pylori* (+) (n=150)	*H. pylori* (-) (n=150)	T/U/χ^2	p*
Atrophy	17 (11.3%)	6 (4%)	4.709	**0.030**
Intestinal metaplasia	30 (20%)	8 (5.3%)	13.288	**<0.001**

Variables are expressed as n(%). T: Student's t-test; U: Mann-Whitney U-test; Continuity Correction χ^2 test; *p<0.05

Table 3. Examination of H. pylori positivity and negativity in patients diagnosed with CRC

H. pylori	CRC (+) (n=19)	CRC (-) (n=281)	p*
Positive	14 (73.6%)	136 (48.3%)	**0.033**
Negative	5 (26.4%)	145 (51.7%)	
Total	19 (100%)	281 (100%)	

n: number of patients; %: percentage of number of patients; Pearson χ^2 test; *p<0.05

DISCUSSION

This retrospective analysis provides robust evidence associating *H. pylori* infection with an increased prevalence of CRC, gastric atrophy, and intestinal metaplasia. Our results highlight the far-reaching effects of *H. pylori*, extending beyond its established influence on peptic ulcer disease and gastric cancer, thereby underlining its systemic implications on gastrointestinal wellbeing. Our findings, indicating a 64% greater risk of CRC in individuals positive for *H. pylori*, coincide with a burgeoning agreement in contemporary studies suggesting a linkage between such infections and CRC (9-12). In terms of biological mechanics, the exact pathways remain to be clearly identified, but a theory suggests that the chronic inflammation within gastric mucosa caused by *H. pylori* could incite a systemic inflammatory condition, creating a favourable environment for CRC development (13). Another intriguing hypothesis involves the disruption of gut microbiota by *H. pylori*, which could affect the intestinal environment and thus promote CRC progression (14). The notable association established in our research between *H. pylori* infection and precancerous states such as gastric atrophy and intestinal metaplasia underscores previous research linking *H. pylori* with these premalignant gastric conditions (15,16). The mechanisms contributing to these observations possibly involve inflammation triggered by *H. pylori* and the generation of virulence factors, potentially causing damage and transformation in gastric epithelial cells (17-19).

Study Limitations

Despite the valuable insights gleaned, our study has inherent limitations due to its retrospective design, limited population scope, and inability to establish causation, alongside potential uncontrolled confounding factors, lack of *H. pylori* strain differentiation, and a relatively modest sample size. Future investigations should seek to overcome these constraints for a more comprehensive and robust elucidation of the associations observed in this study.

CONCLUSION

In conclusion, this study strongly links *H. pylori* infection to an increased risk of CRC, gastric atrophy, and intestinal metaplasia, highlighting its systemic effects on gastrointestinal health. It underlines the importance of early detection and management of *H. pylori*, suggesting it as a key preventive measure against these conditions. Regardless of the constraints inherent to this study, the results provide a foundational basis for more in-depth investigations into the connection between *H. pylori* and CRC. The ultimate goal is to refine preventive and therapeutic approaches for these prevalent conditions.

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

Ethical approval: *This retrospective study was given ethical approval by Giresun Training and Research Hospital local committee, as confirmed by the dated decision (17.07.2023, no. 134).*

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Research Article

The Efficacy of Medical Check-up Programs in Screening Healthy, Asymptomatic Individuals: A Cross-Sectional Study

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Abstract

Aim: Healthy asymptomatic individuals can be screened for various diseases using medical check-up programs. On the other hand, the prevalence of diabetes mellitus (DM) and prediabetes (PD), which are common metabolic disorders, are increasing worldwide. In this context, this study aims to determine the prevalence of DM and PD among the asymptomatic participants of a medical check-up program.

Material and Methods: The population of this cross-sectional study consisted of 440 healthy, asymptomatic volunteers aged 40 years or older who attended a medical check-up program conducted between January and August 2021. Patients with confirmed morbidities or regular medication use and pregnant women were excluded from the study. All study participants underwent physical examination, laboratory test, and sonographic assessment. The study participants were grouped according to their glycemic states, i.e., DM, PD, and normoglycemia (NG). The study's primary outcomes were the prevalence of DM and PD.

Results: The prevalence of DM and PD among the study participants was 12.5% (n=55) and 58.9% (n=259), respectively. The number of males was significantly higher in Group DM than in Groups PD and NG (p=0.014). Additionally, Group DM was significantly older than Groups PD and NG (p<0.001). Furthermore, there were significantly more participants in Group DM with high urea, creatinine, uric acid, triglyceride, and very low-density lipoprotein (VLDL) levels (p>0.05 for all cases).

Conclusion: The prevalence of DM and PD in presumed healthy asymptomatic participants was 12.5% and 58.9%, respectively, indicating unusually high prevalence in this population. In conclusion, the results of this study demonstrate that it is imperative that presumed healthy individuals are screened within the scope of medical check-up programs and followed closely afterward.

Keywords: Diagnostic screening programs, healthy subjects, diabetes mellitus, prediabetes, prevalence.

INTRODUCTION

Medical check-up programs featuring various laboratory and imaging investigations are commonly used to screen asymptomatic individuals to diagnose insidious diseases early (1). Different terminology is used to refer to medical check-up programs in different countries. Accordingly, medical check-up programs are also referred to as, among other things, check-up scans, periodic health examinations, periodic health checks, and routine health checks (1-7).

In addition to screening programs specifically designed to screen individuals with hematuria, chronic kidney disease, hyperuricemia, chronic diarrhea, fatty liver disease, and polyneuropathy (1-3,8-12), volunteer-based routine health examinations have become widespread in recent years (6). Such check-up programs allow for obtaining data about the epidemiological characteristics of chronic diseases (5). Additionally, volunteers who opt-in for medical check-up programs have unique characteristics compared to the patients admitted to hospitals to obtain health care (2). Increasing health awareness via health check-up programs reduces morbidity and mortality at an earlier stage (K).

Diabetes mellitus (DM) and prediabetes (PD) are among

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the most common metabolic disorders worldwide, with progressively increasing global prevalence (13,14). Early detection of PD is vital in preventing its progression to type 2 DM (T2DM). Therefore, timely recognition and introduction of simple lifestyle changes in voluntary people without known DM who underwent any check-up program might be beneficial in preventing any undesired consequences of DM in the future (14).

The number of studies covering the whole spectrum of medical check-up programs explicitly focusing on the prevalence of DM and PD in Türkiye is limited (1,15). In this context, this study was carried out to evaluate the asymptomatic participants attending a medical check-up program regarding the prevalence of DM and PD.

MATERIAL AND METHOD

Study Design

This study was designed as a cross-sectional study. The local ethical committee approved the study protocol (No: September 01, 2021-45). The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Verbal informed consent was obtained from all participants.

Population and Sample

This study's population consisted of voluntary participants who attended a medical check-up program aimed at presumed healthy asymptomatic individuals conducted at the Family Medicine Outpatient Clinics of Hürrem Sultan Hospital located in İstanbul, Türkiye. Hürrem Sultan Hospital is a private hospital located in the centrum of Üsküdar city with 9000 monthly outpatient patient admissions. The records of the patients presented with various complaints to the family medicine outpatient clinics during the last eight months of 2021 were evaluated within the scope of the medical check-up program. Accordingly, individuals aged 40 years or older, without known morbidity, were informed about a newly purchased medical check-up program aimed at healthy, asymptomatic individuals over the phone between 10:00 AM and 5:00 PM on weekdays during the period between January and August 2021 and the individuals who opted in the program were scheduled to have the check-up.

In this context, 498 healthy, asymptomatic individuals were scheduled for a standard check-up between January and August 2021. Based on the interviews conducted by trained receptionists with these individuals during admission to the check-up unit, 58 individuals aged below 40 years, pregnant, or with confirmed morbidities or regular medication use were excluded from the study. Ultimately, the study sample consisted of 440 healthy, asymptomatic individuals.

Content of the Check-up Program

All participants who fasted overnight gave blood samples during the morning hours of the day they were scheduled to have the check-up. Afterward, they underwent an ultrasonographic examination of the abdominal organs. All imaging modalities were performed or interpreted by experienced radiologists. Depending on their gender, the participants were examined by a urologist or gynecologist at the hospital and then by a family physician with at least 28 years of experience. The family physician has taken anamnesis, performed a detailed physical examination, and interpreted the results of all clinical investigations. The family physician consulted with other departments at their discretion based on the results of the laboratory tests and imaging modalities.

Laboratory Tests

The hematological, biochemical, and hormonal tests performed in the context of the medical check-up program included the complete blood count test and measurements of the fasting plasma glucose, glycated hemoglobin (HbA1c), urea, creatinine, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) levels. The results of the laboratory parameters were categorized as low, normal, or high based on the predefined limits for each parameter.

Definitions

HbA1c values of ≥6.5% and/or fasting glucose levels of ≥126 mg/dL were considered to indicate the diagnosis of DM (3). On the other hand, HbA1c values between 5.7% and 6.4% and fasting glucose levels between 100 mg/dL and 125 mg/dL were considered to indicate the diagnosis of PD (16).

Variables and Groups

Demographic characteristics, including age, gender, smoking history, laboratory characteristics, and imaging findings, were prospectively recorded. The participants were stratified into subgroups based on whether they had DM (Group DM) or PD (Group PD). The remaining participants with fasting glucose levels of <100 mg/dL or HbA1c <5.7% were included in the normoglycemia group (Group NG).

Statistical Analysis

The study's primary outcomes were the prevalence of DM and PD in the study group. In contrast, the secondary outcomes were the differences between the participants with and without DM and PD.

Descriptive statistics obtained from the collected data were expressed as mean±standard deviation values in the case of continuous variables determined to conform to the normal distribution, as median with minimum-maximum values in the case of continuous variables determined not to conform to the normal distribution, and as numbers and

percentage values in the case of categorical variables. The Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests were used to analyze the normal distribution characteristics of the numerical variables.

In comparing the differences between categorical variables, Pearson's chi-square and Fisher's exact tests were used in 2x2 tables, and the Fisher-Freeman Halton test was used in RxC tables.

The one-way analysis of variance (ANOVA) and Kruskal-Wallis tests were used to compare more than two independent groups where numerical variables were determined to conform and not to conform to the normal distribution, respectively. The differences between the groups were evaluated with Games-Howell or Tukey tests depending on the heterogeneity of the data if parametric tests were applied and with the Dwass-Steel-Critchlow-Fligner test if non-parametric tests were applied.

Jamovi project 2.3.24.0 (Jamovi, version 2.3.24.0, 2023, retrieved from https://www.jamovi.org) and JASP 0.17.1 (Jeffreys' Amazing Statistics Program, version 0.17.1, 2023, retrieved from https://jasp-stats.org) software packages were used in the statistical analyses. The probability (p) statistics of ≤0.05 indicated statistical significance.

RESULTS

There were 55 and 259 participants in Groups DM and PD, respectively. The prevalence of DM and PD were 12.5% and 58.9%, respectively. Significant differences in demographic characteristics were detected between the groups (p<0.05). Accordingly, the participants in Group NG were significantly younger than those in Groups PD and DM (p<0.001). In addition, the participants in Group DM were significantly older than Group PD participants (p<0.001). The rate of male participants was significantly higher in Group DM than in other groups (p=0.01). There was no significant difference between the groups in smoking status (p=0.35) (Table 1).

There were significant differences between the groups in the laboratory parameters related to the glycemic profile of the participants (p<0.05) (Table 2). The median fasting blood glucose and HbA1c values in Groups DM, PD, and NG were 138 mg/dL and 7.1%, 104 mg/dL and 5.8%, and 93 mg/dL and 5.4%, respectively (Table 2).

The comparison of the groups in terms of laboratory results indicating pathology revealed significant differences between the groups (p<0.05) (Table 3). Accordingly, there were significantly more participants in Group DM with high urea, creatinine, uric acid, triglyceride, and VLDL levels than in other groups (Table 3).

The sonographic assessment of the groups regarding hepatosteatosis revealed significant differences (p=0.03). Accordingly, the number of participants with Grade 1 hepatosteatosis was significantly lower in Group DM than in the other groups. Additionally, the number of participants with Grade 2 hepatosteatosis was significantly higher in Group DM than in Group NG (41.2% vs. 16.7%) (Table 4).

Table 1. Demographic characteristics and smoking history of the study groups

	Groups			
	Group DM (n=55)	Group PD (n=259)	Group NG (n=126)	p
Age (year) †	58.0±12.6	47.1±11.2	39.7±12.0	**<0.001**, *****
Sex ‡				
Female	23 (41.8) a	158 (61.0) b	81 (64.3) b	**0.014***
Male	32 (58.2) a	101 (39.0) b	45 (35.7) b	
Current smoker ‡	9 (16.4)	50 (19.3)	31 (24.6)	0.348*

‡: n (%), †: mean±standard deviation; a, b: different letters in each row show statistical differences; DM: diabetes mellitus, PD: prediabetes, NG: normoglycemia; *. Pearson Chi-Square test, **. One-way ANOVA test, ***. Tukey test.

Table 2. Laboratory parameters related to the glycemic profile of the participants in the groups

	Groups			
	Group DM (n=55)	Group PD (n=259)	Group NG (n=126)	p
Fasting blood glucose (mg/dL) §	138.0 [85.0–372.0]	104.0 [81.0–125.0]	93.0 [67.0–99.0]	**<0.001*, ****
HbA1c (%) §	7.1 [5.5–11.7]	5.8 [5.0–6.4]	5.4 [4.7–5.6]	**<0.001*, ****

§: median [min-max]; DM: diabetes mellitus, PD: prediabetes, NG: normoglycemia; *. Kruskal Wallis-H test; **. Dwass-Steel-Critchlow-Fligner test for pairwise comparisons

Table 3. Comparison of the participants with pathological laboratory results between the groups

		Groups			
		Group DM (n=55)	Group PD (n=259)	Group NG (n=126)	p*
Hemoglobin ‡	Low ‡	5 (9.1)	28 (10.8)	15 (11.9)	0.853
Leukocyte count	Leukopenia ‡	0 (0.0)	1 (0.4)	1 (0.8)	0.704
	Leukocytosis ‡	1 (1.8)	10 (3.9)	2 (1.6)	
Platelet count	Thrombocytopenia ‡	2 (3.6)	2 (0.8)	1 (0.8)	0.268
	Thrombocytosis ‡	0 (0.0)	3 (1.2)	0 (0.0)	
Urea	High ‡	10 (18.2) a	21 (8.1) b	5 (4.0) b	**0.012**
Creatinine	High ‡	7 (12.7) a	10 (3.9) b	2 (1.6) b	**0.011**
Uric acid	High ‡	9 (16.4) a	15 (5.8) b	8 (6.3) b	**0.039**
Alanine aminotransferase	High ‡	5 (9.1)	12 (4.6)	5 (4.0)	0.306
Aspartate aminotransferase	High ‡	4 (7.3) a	3 (1.2) b	4 (3.2) a b	**0.023**
Triglyceride	High ‡	29 (52.7) a	87 (33.6) b	33 (26.2) b	**0.002**
Cholesterol	High ‡	34 (61.8)	178 (68.7)	74 (58.7)	0.135
Low-density lipoprotein	High ‡	30 (54.5) a	144 (55.6) b	52 (41.3) a	**0.027**
High-density lipoprotein	High ‡	16 (29.1)	103 (39.8)	58 (46.0)	0.099
Very low-density lipoprotein	High ‡	29 (52.7) a	83 (32.0) b	32 (25.4) b	**0.001**

‡: n (%); a, b: different letters in each row show statistical differences; DM: diabetes mellitus, PD: prediabetes, NG: normoglycemia; *. Pearson Chi-Square/Fisher Freeman Halton test

Table 4. Distribution of hepatosteatosis grades according to the sonographic examination

	Groups			
	Group DM (n=55)	Group PD (n=259)	Group NG (n=126)	p
Hepatosteatosis grades ‡				
1	17 (50.0) a	91 (71.1) b	30 (83.3) b	
2	14 (41.2) a	34 (26.6) a, b	6 (16.7) b	**0.026***
3	3 (8.8) a	3 (2.3) a	0 (0.0) a	

‡: n (%); a, b: different letters in each row show statistical differences; DM: diabetes mellitus, PD: prediabetes, NG: normoglycemia; *. Fisher Freeman Halton test

DISCUSSION

This study's results indicated that the prevalence of DM and PD among the presumed healthy participants who attended a medical check-up program was 12.5% and 58.9%, respectively. In addition, it was determined that patients with DM were more likely to have increased urea, creatinine, triglyceride, and VLDL levels than patients with PD and normoglycemic individuals.

Varying prevalence rates have been reported in the literature for DM and PD in presumed healthy individuals participating in medical check-up programs. In a study by Tangjittipokin conducted in Thailand (17), the prevalence of DM and PD in the said population was reported as 1.07% and 54.3%, respectively. In two studies conducted in India, the prevalence of DM and PD in the population was reported as 17.18% and 12.3%, and 35.93% and 37.7%, respectively (6). In a multicenter study conducted in Croatia, hemoglobin A1c-based screening revealed that the prevalence of DM and PD in presumed healthy Croatian adults ranged from 3.3% to 7.3% and 14.2% to 20.5%, depending on the hospital setting (13). The lower PD prevalence found in the study compared to the studies mentioned above may be attributed to a Mediterranean diet. A real-world study from China reported that the overall prevalence rates for DM and PD were 8.0% and 27.6%. This study included 15.8 million adults screened in 519 Meinian health check-up centers across 243 cities (18). The authors thought that the absence of postprandial glucose measurements in the health check-

up assessments might be a factor for the differences in the prevalence rates of PD. The current study used fasting blood glucose measurements and HbA1c levels. In another study conducted in China, impaired fasting glucose levels were detected in 8.97% of the individuals who underwent a health check-up during the follow-up period (14). They concluded that assessing impaired fasting glucose levels using a predictive model could help control DM and its cardiovascular complications. Degertekin et al. (15) reported that 8.1% of the 113239 presumed healthy subjects who underwent check-ups across Türkiye had DM. The varying prevalence between these studies may be attributed to the differences in methodologies, demographic characteristics, and participants' lifestyles. Different inclusion and diagnostic criteria also influenced the generalizability of these findings. Therefore, future studies must be adjusted for such confounding variables to obtain more reliable results.

Non-alcoholic fatty liver and steatohepatitis impose significant health burdens on diabetic and prediabetic individuals (5,19). It has been shown that changes in a fatty liver were a risk factor for the development of diabetes in prediabetic people (10). Studies from different parts of the World reported varying prevalence for fatty liver and steatohepatitis in participants who participated in general health check-ups. In one of these studies conducted in India, Kumar et al. (18) determined that almost one-third of diabetic and prediabetic individuals had elevated transaminase levels. In addition, the prevalence of sonographically detected fatty liver in individuals diagnosed with DM and PD due to a medical check-up program was 57.6% and 46.4%, respectively. Morinaga et al. (20) found an association between increased serum ALT levels and the future development of DM in the general Japanese population. To screen the general population for the prevalence rates of liver steatosis and fibrosis, Man et al. (21) detected a rate of 44.39% for steatosis among 5.7 million Chinese adult people. They also found that elevated transaminases were significantly associated with steatosis and fibrosis. In a study conducted in Türkiye analyzing 10-year data of presumed healthy individuals who participated in a check-up program in a private hospital group, the prevalence of non-alcoholic fatty liver disease was found as 48.3% (15). In comparison, in this study, 9.1% and 8.8% of the participants, who were found to be diabetic due to the check-up program, had elevated ALT levels and grade 3 hepatosteatosis, respectively. There was no significant difference between the groups in the rate of the participants with elevated liver transaminases or grade 3 hepatosteatosis. It may be that the number of participants with laboratory and imaging findings confirming their morbidity was too low to reach statistical significance. Kitazawa et al. (10) recommended the fatty liver index as an effective tool to predict the development of DM in individuals with PD. Thus, increased serum transaminases might be considered the risk factor for developing future glycemic problems during medical check-up programs. Consideration of such laboratory results and sonographic imaging findings seems to be a beneficial approach in shaping future pathways for screening and risk stratification (21). Large-scale studies are needed to evaluate further the relationship between the glycemic status and fatty changes in the liver among presumed healthy people participating in check-up programs.

Limitations of the Study

This cross-sectional study was conducted at a family medicine clinic of a private hospital. Therefore, the prevalence reported in this study may be different from other populations. A large-scale, multicenter study is needed to obtain more reliable findings. The lack of detailed clinical characteristics of the participants may be a study limitation. Additionally, the fact that impaired fasting glucose or glucose tolerance statuses were not addressed may be another limitation of the study.

CONCLUSION

The prevalence of DM and PD found in presumed healthy asymptomatic participants included in this study indicated an unusually high prevalence in this population. In conclusion, the results of this study demonstrate that it is imperative that the presumed healthy individuals are screened within the scope of medical check-up programs and followed closely afterward.

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MEDICAL RECORDS-International Medical Journal

Research Article

Burnout and Turnover Intentions of Emergency Department Staff

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Abstract

Aim: In this study, it was aimed to determine the level of burnout and turnover intentions of emergency service staff and to examine the relationship between these two variables.

Material and Methods: This cross-sectional study was conducted in four different training and research hospitals in Ankara. The study was conducted with a total of 414 people, including 130 physicians, 215 nurses and 69 health techs/technicians who agreed to participate. The participation rate was 80%. Questionnaire method was used in the study. The questionnaire included "Sociodemographic Characteristics Form", "Maslach Burnout Inventory (MBI)" and "Turnover Intention Scale (TIS)". The analysis of the research data was performed with SPSS 23.0 statistical program.

Results: The age range of the participants was 18-55 years and the mean age was 32.7±6.1 years. 65% of the participants were female, 52% were nurses, and 55.1% were married. The mean scale scores were (61.6±11.9) for MBI and (2.9±1.7) for TIS. There was a positive, moderate, statistically significant relationship between MBI and TIS ($r=0.623$; $p<0.01$). It was found that emergency department staff had high levels of burnout and moderate levels of turnover intentions. Women had higher levels of emotional exhaustion than men, and physicians had higher turnover intentions than nurses and health techs/technicians.

Conclusion: It was observed that as burnout increased, turnover intention also increased. It is considered that health policy makers and administrators should make structural and functional reforms to reduce burnout and turnover intention in emergency department staff.

Keywords: Burnout, turnover intention, emergency department

INTRODUCTION

Burnout, which is a new dimension of stress in healthcare workers, has emerged in many countries due to the decrease in the level of welfare and the restriction of health care expenditures. Healthcare workers are experiencing hopelessness due to work intensity, low wages, increase in the number of patients and unfavorable working conditions. Therefore, empowering healthcare workers and increasing their working power is a fundamental problem in many parts of the world, especially in developing countries (1). Burnout is defined as employees' distancing from the meaning and purpose of the profession, no longer being genuinely interested in the patients, experiencing excessive stress and psychologically withdrawing themselves from the profession (2). Burnout in healthcare professionals is quite common and is an important problem in terms of its consequences (3). Burnout in healthcare professionals who provide patient-oriented services under difficult conditions causes a decrease in their job success and emotional fatigue. Therefore, the concept of burnout and effective factors are becoming increasingly important for healthcare professionals (4).

Burnout negatively affects the quality and efficiency of the service provided. The quality of life of people experiencing burnout decreases, marriage and family life are negatively affected, inability to fulfill their duties occurs, insomnia and physical fatigue occur, drug, narcotic and excessive alcohol use increases (5). Burnout has negative consequences not only on individuals but also on health institutions in the form of decreased performance, increased absenteeism and turnover (6).

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Turnover intention is a person's willingness to leave the current institution and position (7). An increase in turnover intention is not a desirable situation for the health institution. Because the most valuable assets of organizations are qualified and educated healthcare professionals. Considering that the institutions make various investments for the development and experience of the personnel and that the employees will suffer serious losses by leaving the job, preventing this is among the primary responsibilities of the institution (8). After the COVID-19 pandemic, it was found that approximately 500 thousand healthcare workers quit their jobs in the United States in 2021 and excessive workload was found to be effective on this (9). Studies in Taiwan and Türkiye have also found that burnout due to job stress, workload and job insecurity increases the turnover intention of healthcare staff (10,11).

Emergency departments are easily accessible health units that provide 24-hour and free service to all urgent patients. In Türkiye, emergency departments are the most common unit to which patients apply (12). The increasing number of patients and workload, staff shortage, shift work, patient violence, stress caused by serving serious cases (cardiopulmonary arrest, trauma, bleeding, etc.) and patients presenting with very different complaints have a greater impact on emergency healthcare workers compared to other branches (13). This situation causes burnout and turnover in emergency health workers (14).

In this study, it was aimed to determine the level of burnout and turnover intentions of emergency department staff and to examine the relationship between these two variables.

MATERIAL AND METHOD

Study population

This cross-sectional study was conducted in four different training and research hospitals in Ankara between May 16-25, 2023. Healthcare personnel working in the emergency department constituted the universe of the study. There are 523 personnel in total, including 169 physicians, 276 nurses, and 78 health techs/ technicians, and it was tried to reach the entire universe by not making a sampling calculation. The study was conducted with a total of 414 people, including 130 physicians, 215 nurses and 69 health techs/technicians who agreed to participate in the study. The rate of participation in the study was 80%. The study was conducted with those who voluntarily participated.

Data collection method

Questionnaire method was used in the study. The data were collected either by face-to-face or electronically via e-mail/WhatsApp (WhatsApp Inc. Menlo Park, CA) depending on the availability of the participants. The questionnaire consists of three parts. The first part included "Sociodemographic Characteristics Form", the second part included "Maslach Burnout Inventory (MBI)", and the third part included "Turnover Intention Scale (TIS)".

Sociodemographic characteristics form

This section includes 6 questions prepared by the researchers: age, gender, marital status, status, income and duration of employment.

Maslach Burnout Inventory (MBI)

The MBI was developed by Maslach and Jackson in 1981 to measure the level of burnout of individuals. The MBI consists of 22 items and 3 sub-dimensions (emotional exhaustion, depersonalization and decreased sense of personal accomplishment). The Turkish adaptation study of the scale was conducted by Ergin in 1992. In the original study, Cronbach's alpha value of the scale was 0.89 for emotional exhaustion, 0.77 for depersonalization and 0.74 for decreased sense of personal accomplishment. Emotional exhaustion and depersonalization sub-dimensions consist of negative statements, whereas decreased sense of personal accomplishment sub-dimension consists of positive statements. During the analysis, the responses in the decreased sense of personal accomplishment sub-dimension were reverse coded and analyzed. In the 5-point Likert-type scale, 1 point is scored for Strongly Disagree and 5 points are scored for Strongly Agree. A high score on the scale indicates a high level of burnout (15,16).

Turnover Intention Scale (TIS)

The TIS was developed by Rosin and Korabik in 1991 to determine the turnover intentions of employees. TIS consists of 4 items and one dimension. The Turkish adaptation study of the scale was conducted by Tanrıover in 2005. The Cronbach's alpha value of the scale was 0.82 in the original study and 0.93 in the Turkish adaptation study. In the 5-point Likert-type scale, 1 point is obtained for Strongly Disagree and 5 points are obtained for Strongly Agree. Scores between 1 and 5 can be obtained from the scale. A high score on the scale indicates a high level of turnover intention (17,18).

Statistical analysis

The analysis of the study data was performed with SPSS 23.0 statistical program. Normality of the data was evaluated by Kolmogorov-Smirnov test. Due to the normal distribution of the data, Independent T test was used to compare two independent groups, One Way ANOVA and Post Hoc Tukey tests were used to compare three or more groups, and Pearson Correlation analysis was used to determine the relationships between variables. A value of $p<0.05$ was accepted as statistically significant.

Ethical Issues

Ethics committee permission was obtained from Lokman Hekim University Scientific Research Ethics Committee (Code No: 2023067). The necessary permissions to use the scales were obtained via e-mail from the responsible authors who developed the scales and conducted the Turkish adaptation studies. The study was conducted in accordance with publication and research ethical rules.

RESULTS

The sociodemographic characteristics of the participants in our study are presented in Table 1. The age range of the participants was 18-55 years and the mean age was 32.7±6.1 years.

Table 1. Sociodemographic characteristics of the participants

Sociodemographic characteristics		n	%
Age	≤27	121	29.2
	28-40	177	42.7
	≥41	116	28.1
Gender	Male	145	35.0
	Female	269	65.0
Marital status	Married	228	55.1
	Single	186	44.9
Monthly income	Income less than expenditure	266	64.2
	Income equal to expenditure	103	24.9
	Income more than expenditure	45	10.9
Profession	Physician	130	31.4
	Nurse	215	52.0
	Health technician/technician	69	16.6
Duration of employment	≤5 years	73	17.7
	6-10 years	182	43.9
	≥11 years	159	38.4

Descriptive information about the responses of the participants to the scales is presented in Table 2. The mean scores were (61.6±11.9) for MBI and (2.9±1.7) for TIS. In the reliability analysis of the scales, Cronbach Alpha value was found to be 0.89 for MBI and 0.81 for TIS.

MBI and its sub-dimensions were compared according to sociodemographic characteristics and it was found that females (29.7±7.5) had a significantly higher average value than males (25.9±8.3) in the emotional exhaustion sub-dimension (p=0.03). There was no statistically significant difference between the groups in the MBI scale and its sub-dimensions according to other sociodemographic characteristics (p>0.05).

When TIS scores were analyzed according to sociodemographic characteristics, it was found that physicians (3.2±0.9) had a statistically significantly higher mean score than nurses (2.9±1.1) and health techs/technicians (2.8±1.6) (p<0.001). No statistically significant difference was found between the groups in TIS scores according to other sociodemographic characteristics (p>0.05).

The correlation analysis results between MBI and TIS are shown in Table 3.

In our study, positive, high-level, statistically significant relationships were found between MBI total score and emotional exhaustion (r=0.752; p<0.01), depersonalization (r=0.836; p<0.01), and decreased sense of personal accomplishment (r=0.704; p<0.01) sub-dimensions. Positive, high-level, statistically significant relationships were also found between the MBI scale sub-dimensions (p<0.01). There was a positive, moderate, statistically significant relationship between MBI and TIS (r=0.623; p<0.01).

Table 2. Descriptive findings for MBI and TIS

Scale	Number of items	Min	Max	Mean	SD	Cronbach Alpha
MBI	22	22	101	61.6	11.9	0.89
Emotional exhaustion	9	9	45	27.7	8.6	0.91
Depersonalization	8	5	21	16.6	6.5	0.83
Decreased sense of personal accomplishment	8	8	37	21.8	7.3	0.87
TIS	4	1	5	2.9	1.7	0.81

Min: minimum, Max: maximum, SD: standart deviation, MBI: Maslach Burnout Inventory, TIS: Turnover Intention Scale

Table 3. Correlation analysis between MBI and TIS

	1	2	3	4	5
1.MBI	1				
2. Emotional exhaustion	0.752*	1			
3.Depersonalization	0.836*	0.756*	1		
4.Decreased sense of personal accomplishment	0.704*	0.712*	0.720*	1	
5. TIS	0.623*	0.706*	0.742*	0.680*	1

MBI: Maslach Burnout Inventory, TIS: Turnover Intention Scale, * significant at p<0.01 level

DISCUSSION

In this study, it was aimed to determine the level of burnout and turnover intentions of healthcare workers in emergency departments and to examine the relationship between these two variables.

It is seen that the level of burnout of the healthcare staff in emergency departments is high in our study. While some previous studies found that the burnout of healthcare workers was at a moderate level (4,19), some studies conducted with emergency department staff found that it was at a high level (20-22). It is considered that burnout syndrome is more common in healthcare professionals who are the first responders to the patient, especially in emergency departments, and that these units are more at risk (23). In a study conducted with emergency physicians, it was stated that the level of burnout after the COVID-19 pandemic was higher than before the pandemic, and that workload and working conditions had an impact on the level of burnout (24). Factors such as long working hours, inability to spare time for sports and hobbies, fear of malpractice, financial difficulties and therefore taking additional shifts, patient load, witnessing death, violence, mobbing, lack of infrastructure and personnel, frequent change of duty location are thought to cause high levels of burnout in emergency department staff (20,25,26).

In our study, it was observed that emotional burnout levels of females were higher than males. While some studies in the literature found no difference in the level of burnout according to gender (4,27); some studies found similar results to our research (22,28,29). Emotional exhaustion is the first and most important dimension of burnout. The reluctance and fatigue felt at the beginning of the day are the characteristics of individuals in this dimension. It may occur due to intense life activity (29). It is thought that emotional burnout in females is higher than males due to the fact that women are emotional and have more responsibilities in home and childcare in addition to business life. No significant difference was found in the level of burnout of the participants according to other sociodemographic characteristics. In this respect, it is similar to some previous studies (4,21).

In some studies conducted with healthcare workers, turnover intention was found to be low (30,31), while in some studies it was found to be at a moderate level (32,33). In our study, it was observed that the participants' turnover intention was at a moderate level. It was found that there were significant differences in the studies conducted before and after the COVID-19 pandemic and that emergency healthcare workers, like all healthcare workers, experienced higher work stress, depression, anxiety, insomnia, stayed away from their families and missed, had increased workload due to the increasing number of patients and new care protocols developed, experienced burnout, decreased quality of life, experienced more health problems, and all these factors led to an increase in turnover intentions. It has also been reported that these effects are long term and persist after the pandemic (34,35).

In our study, the turnover intention of physicians was higher than that of nurses and health techs/technicians. In Türkiye, there has been a significant increase in the number of physicians resigning from their jobs and going abroad in recent years. Between 2020 and 2022, a total of 13,557 physicians, including 6,592 specialists and 6,965 general practitioners, resigned from their positions in health institutions and organizations affiliated to the Ministry of Health. In the last 10 years, the number of physicians going abroad, such as to European countries and the USA, has increased 40 times. In 2012, the number of physicians going abroad was 59, while in 2022 it was approximately 2,500. There is a remarkable increase in the number of physicians going abroad from critical branches such as emergency medicine, neurosurgery, anesthesiology and reanimation, general surgery, pediatrics, gynecology and obstetrics (36). It is seen that many factors such as insufficient wages, difficult working conditions, patient burden, exposure to violence by patients and their relatives are effective in physicians' resignation and going abroad (37). In addition, although these difficult working conditions are also valid for nurses and health technicians/technicians, it is considered that physicians' higher opportunity to work in the private sector or abroad is effective in their quitting and intentions. No significant difference was found in the turnover intentions of the participants according to other sociodemographic characteristics. In this respect, it is similar to some previous studies (30,38).

In our study, a moderately significant positive correlation was found between burnout and turnover intention. In other words, the increase in burnout in healthcare workers increases turnover intention. Similar results were found in previous studies (39,40).

The limitation of the study is that the study was carried out only in the city of Ankara and with health workers in the emergency departments of four hospitals.

CONCLUSION

In conclusion, it was found that the level of burnout of emergency health workers was high and their turnover intention was at a moderate level. Females had higher levels of emotional exhaustion than males, and physicians had higher turnover intentions than nurses and health technicians/technicians. It was found that there was a positive and moderately significant correlation between burnout and turnover intention, and as burnout increased, turnover intention also increased. It is considered that health policy makers and administrators should make structural and functional reforms to reduce burnout and turnover intention in emergency health workers. It is suggested that future studies should be planned to compare public and private hospitals, emergency departments and other departments, and between big and small cities.

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

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MEDICAL RECORDS-International Medical Journal

Research Article

The Effect of Mesh Fixation Methods on Pain Sensation After Laparoscopic Inguinal Hernia Repair

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Abstract

Aim: Groin hernia repair is one of the most common surgical procedure in general surgery. The use of prosthetic meshs has reduced the recurrence rate after inguinal hernia repair to less than 5%. Chronic pain is thought to be multi-factorial. Among these, surgical-related factors are the types of mesh used and fixation methods. It was aimed to evaluate the effect of the use of absorbable and non-absorbable screws for mesh fixation on post-operative pain during TEP repair.

Material and Methods: The data of patients who were operated on by a single surgeon and who underwent TEP repair for unilateral or bilateral inguinal hernia were reviewed retrospectively. Post-operative first-week pain sensations were compared between patient groups in which absorbable and non-absorbable screws were used for patch fixation.

Results: A total of 35 patients who were operated by a single surgeon and who underwent laparoscopic total extraperitoneal (TEP) repair for unilateral or bilateral inguinal hernia were included in the study. It was found that patients in the absorbable screw group felt statistically significantly less pain [VAS: 3 (2-7) vs. 4 (2-8); p=0.03].

Conclusion: Chronic pain after patchy hernia repair is also seen as a late complication that can last up to 6 months. This study, which proves that the use of absorbable stabilizers leads to less pain in the early period, reveals that this method may also be a suitable method for preventing the development of possible chronic pain.

Keywords: Hernias, inguinal hernias, surgical mesh, surgical fixation, Laparoscopy, TEP repair

INTRODUCTION

Groin hernia repair is one of the most common surgical procedure in general surgery. More than 20 million groin hernia surgery are performed yearly in the world (1).

In the surgical history, conventional repair techniques were applied at the beginning. These techniques, which have a high recurrence rate and related with severe post-operative pain, have been replaced by tension-free mesh repair techniques over time (2). The use of prosthetic meshs has reduced the recurrence rate after inguinal hernia repair to less than 5% (3). However, chronic pain still remains a major complication. Chronic pain and its effect on quality of life is nowadays one of the most important problem after hernia surgery. The etiology of chronic pain is thought to be multi-factorial. Among these, surgical-related factors are the types of mesh used and fixation methods (4).

Sutures, screws, staples, self-adhesive meshes, fibrin or other tissue adhesives are used to fix the mesh. Although there are studies on the superiority of the different fixation methods used over each other, there is no consensus on this issue (5).

In this study, it was aimed to compare absorbable and non-absorbable fixation techniques in terms of post-operative pain.

MATERIAL AND METHOD

The study was designed retrospectively. A total of 35 patients who were operated by a single surgeon and who underwent laparoscopic total extraperitoneal (TEP) repair

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for unilateral or bilateral inguinal hernia were included in the study. The patients were divided into two groups according to the fixation method of the mesh used during the surgery. Pain sensations of patients at postoperative 1 week follow-up were evaluated using a standardized visual analog pain scale (VAS). Descriptive statistics are given as frequency (percentage) for categorical data and as median (minimum-maximum) value for numerical data. Chi-square or Fisher's exact test was used to compare categorical data between groups, and Mann-Whitney U test was used to compare numerical data. The study received ethics committee approval with the number TCTR20230427008.

RESULTS

The median age of all patients included in the study was 42 (19-72), and the patients were predominantly male (except for two female patients). In total, 23 patients had bilateral inguinal hernias. There was no significant difference between the groups in terms of demographic data (Table 1).

When the pain scores of the patients were evaluated, it was found that the patients in the absorbable screw group felt statistically significantly less pain [VAS: 3 (2-7) versus 4 (2-8); p=0.03] (Table 2).

Table 1. Demographic datas

Variable	Total N: 35	Absorbable N: 14	Non-absorbable N: 21	P Value
Age*	42 (19-72)	36 (19-72)	46 (25-68)	0.23
Gender				0.77
Female	2 (%5.7)	1 (%0.7)	1 (%0.4)	
Male	33 (%94.3)	13 (%99.3)	20 (%99.6)	
Sides				0.76
Unilateral	12 (%34.2)	6 (%42.8)	6 (%28.5)	
Bilateral	23 (%65.8)	8 (%57.2)	15 (%71.5)	

*Median (min-max), ŝFrequency (percent)

Table 2. Comparison of pain scores between groups

	Total N=35	Absorbable n=14	Non-absorbable n=21	P value
VAS*	4 (2-8)	3 (2-7)	4 (2-8)	0.03

* Median (min-max)

DISCUSSION

After decreasing recurrence rates with the use of prosthetic meshs, inguinal pain is one of the most important problem developing after surgery (4). Approximately 16% of patients complain of post-operative pain (6). Multiple hypotheses regarding the mechanism of pain have been put forward (6) and no consensus has been found (6–8). This uncertainty also complicates the postoperative pain management, therefore it is important to reduce the factors that may cause pain in hernia surgery.

Fixation methods used during hernia repair have often been blamed as the cause of pain, and many studies with conflicting results have been conducted on this subject (9–12). However, studies on whether there is a relationship between patients' perception of early pain and the possibility of late pain have revealed that pain sensation in the first week is an important predictor of the development of chronic pain (13). There are three methods for mesh fixation including suture, self-gripping and glue (14). Recurrence rates and risk of chronic pain are similar for both techniques (14). The main factors for chronic pain are tissue trauma and nerve injury during surgery (14). With comparing surgical techniques, laparoscopic techniques including totally extraperitoneal (TEP) and Transabdominal preperitoneal (TAPP) are superior than open techniques for chronic pain and cosmetic results (15). Some studies show that there is no need for mesh fixation in TEP technique but most preferred method is fibrin sealant (15). Because of high incidence of chronic inguinal pain, some studies especially investigate heavyweight and lightweight meshes versus non-fixation technique (16). Early results showed that there is no increased risk for recurrence in non-fixation technique (16). In this study, we examined the effect of fixation methods on early period pain in patient group. It was shown that the patient group in which absorbable screws were used for fixation had statistically significantly less pain sensation in the early period pain assessments made on the VAS (p=0.03). However, the limitation of this study is that it evaluated pain sensation only in the early period and in addition to this, the study has low-volume patient. Chronic pain after patchy hernia repair is also seen as a late complication that can last up to 6 months (6).

CONCLUSION

In conclusion, this study, which proves that the use of absorbable screws causes less pain in the early period, reveals that be a suitable method for preventing possible chronic pain development.

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MEDICAL RECORDS-International Medical Journal

Research Article

Aortic Arch Angle and Aortic Arch Morphometry in COVID 19 Patients: A Radioanatomical Study

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Abstract

Aim: In endovascular surgery, knowing the morphometry of the aortic arch increases the success of surgery. The aim of this study was to examine the angle and morphometry of aortic arch in COVID 19 patients and to compare these with healthy individuals to find out the effect of the disease on the vessel.

Material and Methods: A total of 120 individuals - 60 COVID 19 (30 females, 30 males) patients and 60 healthy (30 females, 30 males) individuals participated in the study. In the study, the parameters of aortic arch angle (AAA), aortic arch diameter (AAD), aortic arch (AA) branches of brachiocephalic trunk diameter (BTD), left common carotid artery diameter (LCCAD), left subclavian artery diameter (LSAD), transverse superior thoracic aperture length (TR-STAL) and anteroposterior superior thoracic aperture length (AP-STAL), transverse inferior thoracic aperture length (TR-ITAL) and anteroposterior inferior thoracic aperture length (AP-ITAL) were measured from thoracic computed tomography images.

Results: As a result of the study, when female and male patients with COVID-19 were compared, LCCAD, LSAD, AP-ITAL, TR-ITAL values were found to be higher in favour of male patients. While Proximal AAD, BTD, LCCAD and LSAD values were higher in female patients with COVID 19 when compared with control group female patients, Proximal AAD, BTD, LCCAD, LSAD, AP-STAL, TR-STAL, AP-ITAL, TR-ITAL values were higher in male patients with COVID 19 when compared with control group male patients. When the measurements of COVID 19 and control group individuals were compared, Proximal AAD, BTD, ACCS, LSAD, TR-STAL, AP-ITAL and TR-ITAL values were found to be higher in favour of COVID 19 patients.

Conclusion: COVID 19 is an important disease that causes dilatation of the AA and its branches. We think that diseases that can change oxygen saturation such as COVID19 can change aortic morphology.

Keywords: COVID 19, aortic arch, superior-inferior thoracic aperture, morphometry, thoracic computed tomography

INTRODUCTION

Aortic arch (AA) and its branches develop with a complicated process in the first few weeks of the foetal life. Anatomically, AA is the second part of the aorta and has three branches as brachiocephalic trunk (BT), left common carotid artery (LCCA) and left subclavian artery (LSA). It is responsible for the blood supply of the head, neck and upper extremity (1). Differences in aortic morphology have been shown in some studies conducted among various patient populations; however, these differences should be well-known. Morphology of the aortic arch is also very important to choose the appropriate graft/stent sizes and identify the appropriate devices, especially in cases when stent/graft application is required (2). Knowing the morphology of aortic arch will be of great help in correct pre-procedural planning and determining the treatment of aortic pathologies in the next stage (4). It is also very important to know the anatomical formations of this artery, to know its adjacent structures and to consider the possible anomalies of these structures in terms of preventing unpredictable complications (5). Acute and chronic pathologies involving the aortic arch are vitally important. The effects of a disease like coronavirus (COVID 19), which affected large masses, on the morphology of aortic arch still remains unclear (6).

CITATION

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COVID 19 is a viral respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2); it was first identified in Wuhan, China and later declared as pandemic by World Health Organization (7). Although the findings related to Covid-19 are related to the respiratory system, cardiovascular features of the disease have also started to be taken into account since it has caused cardiac problems in a significant number of patients (6, 8, 9).

Understanding the morphology of the aortic arch and its branches is important in terms of planning surgical procedures. Radiological imaging methods are the gold standard in identifying the morphology of this artery (10). Although aortic arch morphology is examined with methods such as magnetic resonance (MR) imaging and echocardiography (ECO), we can reach the most critical images with contrasted thoracic computed tomography (CT) (11). Imaging methods have a very important place in detecting these anomalies and pre-operative evaluation (12).

To date, the effects of COVID 19 on most veins have been examined, while its effect on the aortic arch has remained unclear. Knowing the morphology of aortic arch is crucial especially in terms of determining brain and upper extremity vascular pathologies (8,13). The aim of our study was to examine the angle and morphometry of aortic arch in COVID 19 patients, to compare these with healthy individuals to find out the effect of the disease on the vessel.

MATERIAL AND METHOD

In the power analysis performed to determine the research sample, when the analysis was made as Type I error (α) 0.05, power (1-β) 0.80, effect size 1.3, it was found that there should be at least 40 participants, 20 COVID 19 patients and 20 healthy individuals, in the study (9). COVID 19 patients aged 40 and older who did not have a cardiovascular disease diagnosis were included in our study. Those who had a lung mass or who had received lung surgery were excluded. As a result, a total of 120 individuals - 60 COVID 19 (30 Females, 30 Males) patients and 60 healthy (30 Females, 30 Males) individuals participated in the study.

In this retrospective study, ethics committee approval was taken from Malatya Turgut Özal University Non-interventional clinical research ethics committee with 2021/22 protocol number. The examinations were carried out by an expert radiologist with 12 years of experience in the field by using thoracic CT.

CT examination

Radiological images of patients who received COVID 19 treatment in our hospital between March 2019 and December 2019 were examined from Picture Archiving and Communication Systems (PACS). 60 patients who received COVID 19 diagnosis and who had contrast-enhanced thoracic computed tomography (CT) (Somatom Definition Flash, Siemens Healthcare, Forchheim, Germany) were included in the study. The other group consisted of 60 patients who had received contrast-enhanced CT but did not have a COVID 19 history. CT scans in the measurements were made in 128 slices, 0.5 mm thickness and 0.5 mm increments in the axial plane.

The parameters used in the measurements were age, gender, height, weight, body mass index, aortic arch angle (AAA), aortic arch diameter (AAD), aortic arch branches of brachiocephalic trunk diameter (BTD), left common carotid artery diameter (LCCAD), left subclavian artery diameter (LSAD), transverse superior thoracic aperture length (TR-STAL) and anteroposterior superior thoracic aperture length (AP-STAL), transverse inferior thoracic aperture length (TR-ITAL) and anteroposterior inferior thoracic aperture length (AP-ITAL)

CT Scanning: Proximal aortic arch diameter and diameters of aortic arch branches (BT, LCCA, ASS) in axial reformat images and AA coronal plane (CD) angle (AA-CD angle) were measured. ATSD and ATID measurements were made from axial sections. Multiplanar images were used in determining the vertebral levels of some anatomical structures. Proximal AAD was measured from the right 2nd sternocoastal joint level and from the outer wall to the outer wall (Figure 1A). AA branches were measured inwardly from the level of the arch originating from the aorta on the proximal (Figure 1B). AAA was measured between the horizontal line drawn to the anterior of the vertebral corpus and the line drawn parallel to the arcus aorta in axial sections. (Figure 2). TR-STAL was measured from the axial section in the bone window. It was measured anteroposteriorly between the sternum and T1 vertebral body at the level of the upper edge of the manubrium of sternum. It was measured transversely between the inner edges of the first rib (Figure 2).

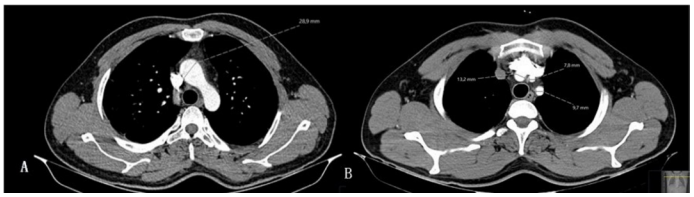

Figure 1A. Diameter measurement proximal to the aortic arch, **1B.** Measurement of aortic arch branches. Brachiocephalic trunk, left CCA, and left subclavian artery from the right to the left

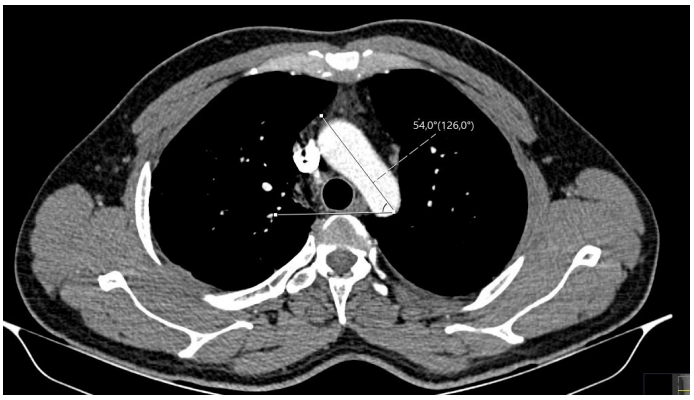

Figure 2. Angle measurement of the aortic arch with the coronal plane

TR-ITAL measurements were made at T12 vertebral level. In the multiplanar reformat images, the location of the sternum xiphoid part was found and a transverse line was drawn to this level at the T12 level. AP measurement was made between this line and the vertebra. Transverse measurement was made by measuring the widest diameter in the same section. (Figure 3A-B).

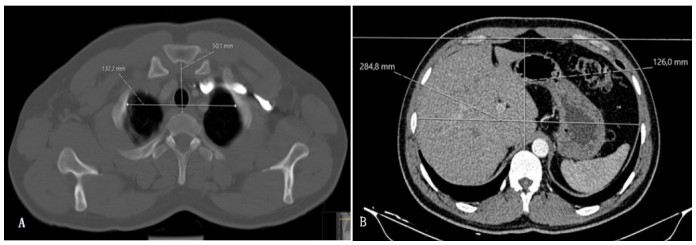

Figure 3A: Anteroposterior superior thoracic aperture and transverse diameter measurement, **3B.** Anteroposterior inferior thoracic aperture and transverse diameter measurement

Statistical analysis

For statistical analysis of the data, IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA) package program was used. The conformity of the data to the normal distribution was tested with Kolmogorov Smirnov test. Mean and standard deviation were used for numerical data, number and percentage values were used for categorical data, and independent t test and Mann Whitney U test were used for group comparisons. In evaluating the level of significance in the analysis, a p-value of ≤0.05 was considered statistically significant.

RESULTS

In the COVID 19 group, mean age of women was 50.76±12.73 years, while the mean age of men was 47.65±12.91 years and the difference between was not statistically significant (p=0.379). Statistically significant difference was found between women and men in terms of LCCAD, LSAD, AP-ITAL, TR-ITAL lengths (p<0.05). The results of morphological measurements obtained from COVID 19 patients are shown in Table 1.

Mean age of women in COVID 19 group was 50.76±12.73 years, while the mean age of women in the control group was 42.60±11.89 years and there was a statistically significant difference between the two groups (p=0.023). Mean age of men in COVID 19 group was 47.65±12.91 years, while the mean age of men in the control group was 38.56±12.74 years and the difference between was statistically significant (p=0.012).

Proximal AAD, BTD, LCCAD and LSAD were found to be statistically higher in female patients with COVID 19 compared to women in the control group (p<0.05). Proximal AAD, BTD, LCCAD, LSAD, AP-STAL, TR-STAL, AP-ITAL, TR-ITAL lengths were found to be statistically significantly higher in male patients with COVID 19 compared to men in the control group (p<0.05) (Table 2).

When the morphometric measurements of AA and its branches were compared in COVID 19 and Control group individuals, a statistically significant difference was found in Proximal AAD, BTD, LCCA, LSAD, TR-STAL, AP-ITAL and TR-ITAL values in favour of the COVID 19 group (p<0.05) (Table 3).

In the correlation analysis, we found a moderate correlation between BTD and Proximal AAD and between LCCAD and BTD. In addition, we found a weak correlation between LCCAD and proximal AAD; between LSAD and proximal AAD; between LSAD and BTD. While we found a good level of correlation between AP-STAL and AAA, we found a weak correlation between AP-ITAL and BTD and AP-STAL. While there was a weak correlation between TR-ITAL and AAA, we found a good level of positive correlation between TR-ITAL and BTD, TR-STAL and an excellent positive correlation between TR-ITAL and AP-ITAL (Table 4).

Table 1. Distribution of morphological measurements of COVID 19 patients by gender

	COVID 19 group		
	Women	Men	p
Age	50.76±12.73	47.65±12.91	0.379
AAA	63.74±6.72	60.03±7.82	0.070
Proximal AAD	30.28±3.38	31.13±3.29	0.353
BTD	11.6±1.42	12.56±1.43	0.17
LCCAD	7.48±0.85	8.68±1.28	0.001
LSAD	8.64±1.59	10.37±1.33	0.001
AP-STAL	52.36±5.32	70.44±78.05	0.253
TR-STAL	109.00±9.87	113.51±10.17	0.105
AP-ITAL	115.60±13.03	140.34±19.42	0.001
TR-ITAL	252.88±10.51	289.17±13.73	0.001

AAA: aortic arch angle, Proximal AAD: aortic arch proximal diameter, BTD: brachiocephalic trunk diameter, LCCAD: left common carotid artery diameter, LSAD: left subclavian artery diameter, AP-STAL: antero-posterior superior thoracic aperture length, TR-STAL: transverse superior thoracic aperture length, AP-ITAL: antero-posterior inferior thoracic aperture length, TR-ITAL: transverse inferior thoracic aperture length

Table 2. Comparison of morphometric measurements of AA and its branches in COVID 19 and Control group

	Female			Male		
	COVID 19 group 1	Control group 2	p	COVID 19 group	Control group	p
Age	50.76±12.73	42.60±11.89	0.023	47.65±12.91	38.56±12.74	0.012
AAA	63.74±6.72	62.20±7.47	0.447	60.03±7.82	62.66±8.11	0.233
Proximal AAD	30.28±3.38	27.82±3.57	0.016	31.13±3.27	26.38±3.43	0.001
BTD	11.60±1.42	10.14±1.41	0.001	12.56±1.43	10.40±1.53	0.001
LCCAD	7.48±0.85	6.70±0.99	0.001	8.68±1.28	7.02±1.29	0.001
LSAD	8.64±1.59	7.76±1.20	0.032	10.37±1.33	8.70±1.86	0.001
AP-STAL	52.36±5.32	51.44±6.99	0.603	70.44±8.05	53.88±5.93	0.295
TR-STAL	109.00±9.87	106.52±8.85	0.348	113.51±10.17	107.00±9.5	0.019
AP-ITAL	115.60±13.03	111.92±17.24	0.399	140.34±19.42	124.52±20.36	0.005
TR-ITAL	252.88±10.51	250.76±16.36	0.588	289.17±13.73	267.24±23.11	0.001

AAA: aortic arch angle, Proximal AAD: aortic arch proximal diameter, BTD: brachiocephalic trunk diameter, LCCAD: left common carotid artery diameter, LSAD: left subclavian artery diameter, AP-STAL: antero-posterior superior thoracic aperture length, TR-STAL: transverse superior thoracic aperture length, AP-ITAL: antero-posterior inferior thoracic aperture length, TR-ITAL: transverse inferior thoracic aperture length

Table 3. Comparison of morphometric measurements of AA and its branches in all individuals of COVID 19 and Control group

	COVID 19 group	Control group	p
AAA	61.75±7.50	62.43±7.72	0.651
Proximal AAD	30.73±3.33	27.10±3.54	0.001
BTD	12.12±1.50	10.27±1.46	0.001
LCCAD	8.12±1.25	6.86±1.04	0.001
LSAD	9.57±1.69	8.23±1.62	0.001
AP-STAL	62.07±7.57	52.66±6.53	0.253
TR-STAL	111.42±10.19	106.76±10.17	0.015
AP-ITAL	128.88±20.76	118.22±19.73	0.009
TR-ITAL	272.37±21.98	259.00±21.49	0.001

AAA: aortic arch angle, Proximal AAD: aortic arch proximal diameter, BTD: brachiocephalic trunk diameter, LCCAD: left common carotid artery diameter, LSAD: left subclavian artery diameter, AP-STAL: antero-posterior superior thoracic aperture length, TR-STAL: transverse superior thoracic aperture length, AP-ITAL: antero-posterior inferior thoracic aperture length, TR-ITAL: transverse inferior thoracic aperture length

Table 4. Correlation analysis of morphometric measurements of AA and its branches in all individuals in COVID 19 and control group

Parameters	Test	AAA	Proximal AAD	BTD	LCCAD	LSAD	AP-STAL	TR-STAL	AP-ITAL	TR-ITAL
Proximal AAD	r	-.100								
	p	.449								
BTD	r	.032	.664							
	p	.810	.000**							
LCCAD	r	.045	.306	.440						
	p	.733	.018*	.000**						
LSAD	r	.155	.366	.297	.600					
	p	.237	.004*	.021*	.000**					
AP-STAL	r	.501	-.049	.114	.232	.219				
	p	.000**	.709	.384	.074	.093				
TR-STAL	r	-.043	.090	.162	.030	.068	.115			
	p	.743	.496	.217	.820	.605	.381			
AP-ITAL	r	.252	.229	.343	.142	.135	.487	.189		
	p	.052	.079	.007*	.279	.305	000**	148		
TR-ITAL	r	.288	.347	.521	.275	.387	.319	.518	.655	
	p	.026*	.007*	000**	.034*	002**	.013*	.000**	.000**	

AAA: aortic arch angle, Proximal AAD: aortic arch proximal diameter, BTD: brachiocephalic trunk diameter, LCCAD: left common carotid artery diameter, LSAD: left subclavian artery diameter, AP-STAL: antero-posterior superior thoracic aperture length, TR-STAL: transverse superior thoracic aperture length, AP-ITAL: antero-posterior inferior thoracic aperture length, TR-ITAL: transverse inferior thoracic aperture length

DISCUSSION

In this study which aimed to examine aortic arch angle and aortic arch morphometry in COVID 19 patients, to compare with healthy individuals and to determine the effects of the disease on the vessel, when female and male patients with COVID-19 were compared, LCCAD, LSAD, AP-ITAL, TR-ITAL values were higher in favour of male patients. While Proximal AAD, BTD, LCCAD and LSAD values were higher in female patients with COVID 19 when compared with females in the control group; Proximal AAD, BTD, LCCAD, LSAD, AP-STAL, TR-STAL, AP-ITAL, TR-ITAL values were higher in male patients with COVID 19 when compared with males in the control group. When the measurements of COVID 19 and control group individuals were compared, Proximal AAD, BTD, LCCA, LSAD, TR-STAL, AP-ITAL and TR-ITAL values were higher in favour of COVID 19 patients.

In our study, we found that Proximal AAD increased with the increase in BTD, and there was an excellent correlation between LSAD and LCCAD and between TR-ITAL and AP-ITAL.

Although there is evidence that COVID-19 can be considered a systemic inflammatory disease resulting in multi-organ failure, there still no strong data about aortic pathologies as a possible expression of this infection (14). The present study provides data to show the damage of COVID 19 on AA and its branches in COVID 19 patients.

In one study, AAA was examined in patients with aortic dissection and healthy individuals and the angle was found to be wider in patients with dissection when compared with healthy individuals (15). In another study, AAA was reported to be wider in men (59.8±5.5 degrees) when compared with women (55.9±3.8 degrees) (16). In our study, the angle was found to be wider than the literature in COVID 19 patients. At the same time, AAA was wider in female COVID 19 patients when compared with male COVID 19 patients. Our results showed that COVID 19 affected the vessel more than aortic dissection. In addition to this, COVID 19 caused more enlargement in AAA in women.

In a study conducted on cadavers in literature, Proximal AAD, BTD, LCCAD measurements were found to be 18.3 mm, 9.5 mm and 10.6 mm, respectively (3). In another study, AAD, BTD, LCCAD were examined in patients with trauma, dissection and aneurism and these arteries were found to be 17.7 mm, 11.7 mm, 14.1 mm, respectively in trauma patients and higher values were found in dissection patients (2). In our study, diameter measurements of these arteries were found to be wider in COVID 19 patients. The measurements were in parallel with the literature. As a result of these studies, we can say that COVID 19 virus causes great destruction on the artery diameter.

Aortic chemoreceptor tissue is found along the aorta, pulmonary artery trunk, and subclavian artery (17). The partial recovery of chemoreflex function has been attributed to augmented aortic body responses to hypoxia (18). Like the carotid body, hypercapnia increases the aortic body chemoreceptor activity, albeit of lesser magnitude, and the effects of CO2 are augmented by hypoxia (19, 20). Whereas metabolic acidosis stimulates the aortic chemoreceptor response, metabolic alkalosis reduces the response to hypoxia and CO2 (21). Carbondioxide increased and oxygen decreased in the blood gas of our COVID 19 patients who were treated in the hospital. We also thought that the increase in aortic diameter in COVID 19 patients was due to hypoxia and hypercapnia due to the mentioned literature.

LSA is an important artery that originates from the AA and is responsible for feeding the left upper extremity. In a study conducted on healthy individuals, LSAD was reported to be larger in women than in men (22). In the present study, LSAD measurement was higher in favour of women in both COVID 19 patients and control group individuals. In the control group, in parallel with the literature, while it was larger in females than males, it was narrower in both genders in COVID 19 patients.

ATSD, which is known as the thoracic inlet, connects the neck root to the rib cage and has great importance on the vessels in this region. In the reviewed literature, it has been reported that the size of TR-STAL is 10 cm and the size of AP-STAL is 5 cm (23,24). In our study, while results parallel to the literature were found in control group individuals, it was found that the size of this structure tended to increase in COVID 19 patients.

ATID, also known as thoracic outlet, connects the thorax and the abdomen. In studies conducted, while TR diameter of ATID is larger than its AP diameter, it is wider than ATSD (25,26). In our study, we found it to be wider in COVID 19 patients when compared with control group patients.

CONCLUSION

COVID 19 is a major disease that causes enlargement of the aorta, the main artery of the body. Knowing the AA morphology in COVID 19 patients, especially in vascular surgery, will positively affect the success of the surgery and will provide vital data to the clinician, especially in procedures such as stent placement. Future studies are needed to examine the effects of different pathologies on AA morphology.

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Research Article

Prevalence of Adrenal Incidentaloma by Chest Computed Tomography in Patients with a Preliminary Diagnosis of COVID-19 Pneumonia

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Abstract

Aim: Adrenal incidentalomas are typically discovered as an incidental finding during routine computed tomography (CT) or magnetic resonance imaging (MRI) scans conducted for unrelated purposes. Our objective was to examine the frequency of adrenal incidentaloma, in individuals who underwent thoracic CT scan as a result of Covid-19 infection.

Material and Methods: In the retrospective descriptive study, 808 patients who applied to the emergency radiology department with the suspicion of covid 19 and underwent thoracic CT between March 2020 and July 2020 were included. The presence of incidentaloma in the CT images of the patients was evaluated.

Results: Adrenal incidentaloma was detected in 78 (9.7%) of the 808 patients, and of those 78 adrenal incidentalomas, 70 (8.7%) were unilateral and 8 (1%) were bilateral. The mean diameter of the adrenal incidentaloma was 20 mm, and it was 18.5 mm in females and 21.5 mm in males. A total of 808 patients, 351 (43.4%) females and 457 (56.6%) males, were included in the study.

Conclusion: Adrenal incidentaloma have become more common radiological findings with the recent COVID-19 pandemic due to the increased frequency of CT scans, and it is important to appropriately manage these patients.

Keywords: Adrenal incidentaloma, COVID-19 infection, chest CT

INTRODUCTION

An adrenal incidentaloma (AI) refers to the presence of an asymptomatic adrenal mass with a diameter exceeding 1 cm, which is fortuitously detected during imaging procedures unrelated to suspected adrenal disease (1). Upon detection, these tumors necessitate radiological and biochemical evaluation to determine their potential for malignancy and whether they secrete excessive hormones (2).

In December 2019, a novel coronavirus was identified during the examination of lower respiratory tract samples obtained from several individuals exhibiting viral pneumonia in Wuhan, Hubei, China (3). This virus, known as novel coronavirus disease 2019 (COVID-19), rapidly spread worldwide, causing a pandemic and persisting in its impact (3). The severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) are previously identified viral infections with similar features to COVID-19 (4,5). These zoonotic viruses give rise to significant respiratory tract complications (6).

In the present era, computed tomography (CT) has become widely available in nearly all hospitals, and chest CT imaging has gained popularity due to its ease of accessibility in facilitating early diagnosis of these diseases (7).

Since the 1980s, the extensive use of high-resolution imaging techniques like CT and magnetic resonance

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imaging (MRI) has posed a notable clinical challenge in identifying incidentally detected adrenal gland lesions, such as adrenal incidentalomas (8). Furthermore, the increased utilization of chest CT examinations across all age groups for the diagnosis of COVID-19 pneumonia has resulted in a higher frequency of incidental adrenal lesions being detected and encountered by radiologists.

The primary objective of this study was to assess the incidence of adrenal incidentalomas in patients admitted to our hospital who underwent chest CT scan as part of the initial diagnostic workup for COVID-19.

MATERIAL AND METHOD

This study protocol received approval from both the local ethics committee of Trakya University School of Medicine (TUTF-BAEK 2020/295) and the COVID-19 Scientific Research Committee of the Republic of Turkey Ministry of Health. Between March 2020 and July 2020, a total of 948 chest CTs were performed in our hospital for the preliminary diagnosis of COVID-19 pneumonia.

The selection and description of the participants

In our retrospective study we included 808 patients. 128 patients due to known history of primary cancer and 12 patients due to poor image quality in lung CT were excluded from the study.

Technical information

Patient with suspected COVID-19 infection were scanned on an eight-channel multislice Toshiba Aquilion 64 CT scanner (Toshiba Medical Systems, Tokyo, Japan). The imaging procedure involved the patient lying supine and holding their breath in a suspended state, while axial images were acquired from the thoracic inlet down to the mid-abdominal region. The images were produced on a multi-detector CT using 120 kVp, 240–400 mA (according to patient body weight and sex), 80 mm × 0.5 mm collimation, and reconstructed at 1 mm slice thickness. The gantry rotation time was 0.5 s per rotation. The field of view was maximum 500 mm and an acquisition matrix of 512×512 was used. Contrast agent was not administered in the CT examinations. Sagittal and coronal images were used at the workstation (Sectra PACS Linköping, Sweden) to confirm the diagnosis after the CT data was sent there electronically.

Image analysis

The research covered all patients with adrenal incidentaloma. Both adrenal glands were assessed in each patient utilizing axial images on an abdomen soft tissue setup (window level 40 HU; width 400 HU). Measurements were taken by blinded radiologists with 10 years of experience in radiology. While measuring for adrenal incidentalomas, only the long axis of the lesion was measured and evaluated in the axial plane (Figure 1).

At the same time, the densities of the detected lesions were examined, and lesions with a density less than 10 Hounsfield Unit (HU) were considered as lipid-rich adenoma, and those with a higher density were considered as high-density lesion.

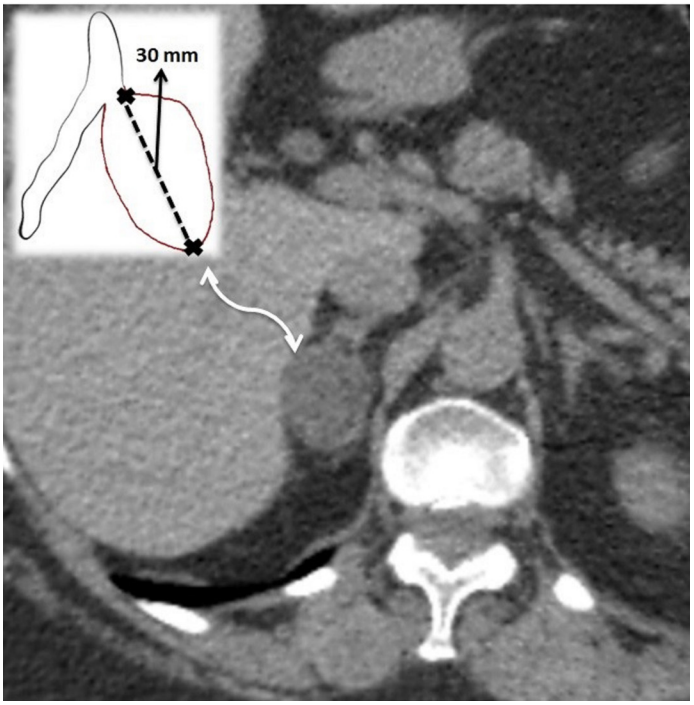

Figure 1. Long axis measurement of adrenal incidentalomas in the axial plane

Statistics

Statistical analyses were carried out using the SPSS 22.0 (IBM, Armonk, NY, USA) software package. While categorical data distribution information is given as a percentage, numerical data distribution information is expressed as the mean±standard deviation (SD). To analyze the normal distribution of numerical data, the Kolmogorov Smirnov test was applied. An independent sample t-test was used to compare the two independent groups. To compare categorical variables, the Pearson-chi-square test was used. The significance level was considered as $p<0.05$.

RESULTS

A total of 808 patients, 351 (43.4%) females and 457 (56.6%) males, were included in the study. The median patient age in our study was 62 (18–97) years. Incidentaloma rates by age are given in Table 1. While the mean age of the patients with incidentaloma was 69.5±13.9, the mean age of the patients who were not detected incidentaloma was 57.6±20.4 years. It was found that the mean age of cases with incidentaloma was significantly higher than those without ($p<0.001$). There was no significant difference in terms of gender distribution between cases with and without incidentaloma ($p=0.338$).

Adrenal incidentaloma was detected in 78 (9.7%) of the 808 patients, and of those 78 adrenal incidentalomas, 70 (8.7%) were unilateral and 8 (1%) were bilateral. The rate of insidentolama was 10.8% in women and 8.8% in men. No significant difference was observed between genders in terms of incidentolama rate ($p=0.323$).

Table 1. Adrenal incidentaloma distributions by age

Age (years)	Incidentaloma n (%)	No incidentaloma n (%)	Total n (%)
18-19	-	13 (1.6)	13 (1.6)
20-29	1 (0.2)	82 (10.1)	83 (10.3)
30-39	1 (0.1)	75 (9.3)	76 (9.4)
40-49	5 (0.7)	82 (10.1)	87 (10.8)
50-59	10 (1.3)	110 (13.6)	120 (14.9)
60-69	17 (2.1)	113 (14)	130 (16.1)
70-79	23 (2.7)	125 (15.5)	148 (18.2)
80-89	17 (2.1)	112 (13.9)	129 (16)
90-97	4 (0.5)	18 (2.2)	22 (2.7)
Total	78 (9.7)	730 (90.3)	808 (100)

Values are expressed as n (%), n: number

The distribution of the lesions by gender are given in Table 2. The mean diameter of the adrenal incidentaloma was 20 mm, and it was 18.5 mm in females and 21.5 mm in males. There was no statistically significant difference between genders in terms of lesion diameter (p=0.088)

Table 2. Adrenal incidentaloma distributions by gender

Gender	Adrenal incidentaloma			
	Absent n (%)	Unilateral n (%)	Bilateral n (%)	Total n (%)
Female	313 (89.2)	33 (9.4)	5 (1.4)	351 (100)
		38 (10.8)		
Male	417 (91.2)	37 (8.1)	3 (0.7)	457 (100)
		40 (8.8)		
Total	730 (90.3)	70 (8.7)	8 (1)	808 (100)
P value	0.323*			

Values are expressed as n (%), n: number, *Chi square test

The radiological findings of incidentaloma lesions observed on non-contrast computed tomography are summarized in Table 3 and in Figure 2. When we examined the average HU among different groups, it was determined that in the lipid-rich adenoma group, the mean value was 2.51±16.56 HU (ranging from -34 to 70). For high-density lesions, the mean value was 39.4±16.44 HU (ranging from 13 to 69). In cases of adrenal hemorrhage, the mean value was 55.0±25.40 HU (ranging from 37 to 73). Lastly, in the myelolipoma group, the mean value was -17.50±84.10 HU (ranging from -77 to 42).

Tablo 3. Non-contrast CT Findings of Incidentalomas

	Adrenal incidentaloma n (%)
Lipid-rich adenoma	43 (55.1)
High density lesion	31 (39.7)
Adrenal hemorrhage	2 (2.6)
Myelolipoma	2 (2.6)
Total	78 (100)

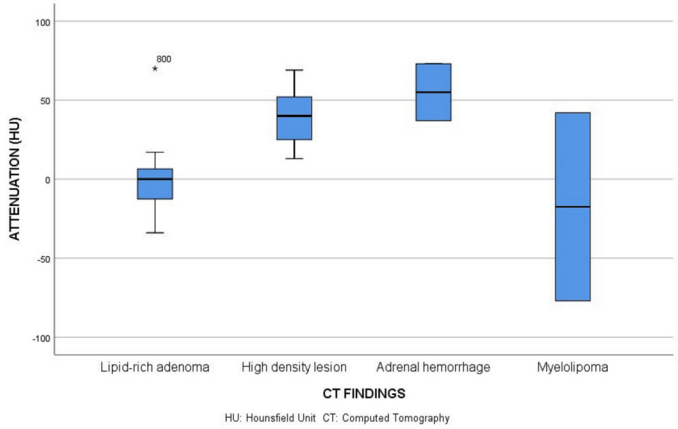

Figure 2. Attenuation distributions of incidentalomas on CT

The most common lesion localization was the left body, and the localizations of incidentaloma lesions are shown in Table 4.

Table 4. Localizations of lesions

	Adrenal incidentaloma	
	Unilateral n (%)	Bilateral n (%)
RB	18 (25.7)	7 (43.8)
LB	26 (37.1)	7 (43.8)
RML	2 (2.9)	1 (6.2)
LML	11 (15.7)	-
RLL	3 (4.3)	-
LLL	10 (14.3)	1 (6.2)
Total	70 (100)	16 (100)

RB: right body, LB: left body, RML: right medial limb, LML: left medial limb, RLL: right lateral limb, LLL: left lateral limb

DISCUSSION

The prevalence of adrenal incidentalomas can vary depending on the nature of the study, whether it is based on autopsy or radiological series, and whether the patients are drawn from the general population or specific cohorts (9). Increased frequency of imaging has led to a higher detection rate of incidental adrenal lesions, which is estimated to be around 4–6% in the population (10). The proportion of incidentally detected lesions is expected to rise with advancements in imaging technologies (11), thereby highlighting the crucial role of radiologists in diagnosing incidental adrenal lesions.

In our study, we observed a prevalence of 9.7% for adrenal incidentalomas. This finding aligns closely with the prevalence reported in autopsy series, some of which have reported rates as high as 9% (12). The relatively smaller sample size in our study may have contributed to a higher incidence of adrenal lesions, as the imaging was specifically focused on detecting adrenal abnormalities. Earlier studies conducted before 2000 have reported a relatively lower prevalence of incidental adrenal masses, approximately around 1% (13). However, with the

evolution and widespread use of imaging techniques, the prevalence of adrenal masses has shown a shift over time. By excluding patients with lung cancer metastasis from the study conducted by S. Bovio et al. (14), which is one of the studies used to estimate the prevalence of adrenal incidentaloma, the prevalence of adrenal lesions was found to be 4.2%. Another study by EV Ferreira et al. (15) reported a 2.5% prevalence of incidentalomas in the adrenal glands on CT imaging. Our study revealed a higher prevalence of 9.7% compared to previous studies. It is anticipated that the prevalence of adrenal incidentaloma on CT imaging will continue to increase with the advancement of high-resolution CT techniques.

Incidental tumors are frequently encountered in clinical practice, and their incidence tends to rise with age. Age has been shown to have a positive correlation with the occurrence of incidentalomas, suggesting that the incidence of these lesions increases as individuals get older (1). Studies by Bovio et al. (14) and Mantero et al. (16) have demonstrated that the prevalence of incidentalomas rises with age, and older patients are at a higher risk of developing adrenal tumors. Our findings align with these studies, as we also observed an increased incidence of incidental tumors with age, particularly after the age of 60. These results support the notion that age should be considered an important factor in the evaluation and monitoring of patients with adrenal incidentalomas.

Several studies have found no statistically significant difference in the frequency of adrenal incidentalomas between male and female patients (2,17). Our data support these findings since we found no significant difference in the frequency of adrenal incidentalomas between male and female individuals. This finding is consistent with previous research and supports the assumption that gender is not a significant risk factor for the development of adrenal incidentalomas.

Adrenal incidentalomas are commonly observed in imaging studies, typically exhibiting a mean diameter of 20 mm. This finding is in line with previous studies reporting mean diameters ranging from 15 mm to 30 mm. As in the literature, we observed that the diameter of the lesion and the prevalence of incidentaloma were slightly higher in men than in women in our study. However, this difference was not statistically significant.

The most frequently observed adrenal incidentaloma on non-contrast computed tomography (NCCT) is an adenoma, characterized by well-defined margins and a round or oval-shaped mass. The presence of calcifications or fat within the mass serves as a strong indicator of an adrenal adenoma. In rare cases, adrenal adenomas may exhibit increased attenuation values on NCCT, which could suggest a lipid-poor adenoma or malignant transformation (17,18). Our study confirmed the predominance of lipid-rich adenomas as the most common subtype of adrenal incidentaloma, consistent with existing literature (19). Additionally, we identified lesions demonstrating high density, adrenal hemorrhage, and imaging features characteristic of myelolipoma, aligning with established findings. However, due to the absence of contrast enhancement in our imaging protocol, additional imaging with an adrenal-specific CT protocol was required to evaluate non-fat adenomas.

Kulalı et al. investigated the prevalence of adrenal incidentaloma on CT scan in the geriatric population and found a high prevalence of 47.2% in this age group (20). In our own study, we found that the prevalence of incidentaloma increased as the age population increased, in accordance with this study. In addition, our study is the first current study in Turkey, covering all age categories.

It is important to acknowledge several limitations of our study. Being retrospective in nature, our study may have reported higher prevalence values compared to routine clinical practice, as CT images were reviewed specifically to identify adrenal lesions. Another limitation pertained to the diagnostic evaluation of lesions other than fat-rich adenomas, which necessitated the use of a CT protocol specific to the adrenal glands due to the absence of contrast enhancement in our imaging modality.

CONCLUSION

Adrenal incidentaloma have become more common radiological findings with the recent COVID-19 pandemic and the increased frequency of CT scans, and appropriate management of these patients is crucial . Radiologists and clinicians who properly interpret the results can spare these patients from needless tests and expenses.

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Ethical approval: *This study protocol received approval from both the local ethics committee of Trakya University School of Medicine (TUTF-BAEK 2020/295) and the COVID-19 Scientific Research Committee of the Republic of Turkey Ministry of Health.*

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Research Article

Nucleolus Scoring May Increase the Objectivity of Pathological Evaluation of Endometrial Cancers

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Abstract

Aim: Nucleolus has been shown as a prognostic parameter in many cancer types. In this study, we showed that the nucleolus score may provide more objective FIGO grading in endometrial cancers (ECs). We examined the role of nucleolus score in predicting treatment and prognosis in ECs.

Material and Methods: The presence of nucleoli were scored as 1, 2 and 3 in curettage materials. The relationship between this value and clinicopathological parameters was examined.

Results: Nucleolus score was associated with FIGO grade, myometrial invasion and lymphovascular invasion. The nucleolus score, which has the potential for objective evaluation,was found as an independent risk factor for disease-free survival (DFS) as well as there was an association between nucleolus score and DFS.

Conclusion: Nucleolus score was associated with better prognosis in ECs. It can be evaluated objectively with no need of requiring extra cost and time. Thus, adding nucleolus score in FIGO grading can provide more reliable prediction in prognosis and choice of the treatment method.

Keywords: Endometrial carcinoma, nucleolus score, FIGO grade, prognosis

INTRODUCTION

Endometrial carcinoma is the most common gynecological malignancy among women in worldwide (1-3).

Endometrioid type endometrial cancers (ECs) are are grading according to Federation Internationale des Gynaecologistes et Obstetristes (FIGO) grade based on the glandular differentiation (4,5). Assesment of tumor grading together with the depth of tumor invasion and cervical involvement plays an important role in curettage specimens, since the need for a complete surgical procedure including lymph node dissection rely on these. Grade 1 ECs have a better prognosis compared to Grade 3. However, there has not been enough data in the literature for Grade 2 ECs (6) and this group of tumors remain in the gray area. FIGO ternary grading system comprises a combination of two different parameters includingarchitectural and nuclear grading (4,6,7). According to this system, features for architectural grading have been adopted from the well-defined criteria of the Gynecologic Oncology Group (GOG) pathology committee (4,8), Grading is based on the percentage of the solid component within the tumor. If the tumor has solid component less than 5%, 6-50% and more than 50%, it is graded as 1, 2 and 3 respectivelyThe overall grade is increased by one for tumors with nuclear atypia (6,9).

In FIGO grading, quantitative values are used for architecture grading. However, there is no quantitative

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criteria for nuclear atypia. Therefore, the lack of rigorous criteria for evaluating nuclear atypia makes grading subjective (6,10). The absence of a defined criterion causes confusion for pathologists and clinicians (7,8) Nucleolus evaluation in tumor cells can be performed using silver staining of argyrophilic nucleolar organiser regions (AgNOR), and digital techniques (11,12). A morphological nucleolus grading method was firstly defined by Helpap for renal cell cancer (13). In ECs there is no optimal method for nucleolus prominence and only used as a component of nuclear atypia.

In this study, we evaluated the nucleolus of tumor cells in the endometrial curettage samples with the Modified Helpap method, in order to find an objective parameter that can be used in grading. Additionally, we examined the relationship of nucleolus score with prognostic variables and other clinicopathological parameters.

MATERIAL AND METHOD

Patients and Tissue Samples

Our retrospectively planned study included patients with a tissue diagnosis of EC from curettage sample in our hospital, between January 2010-2020. Hematoxylin-eosin (HE) stained slides were retrieved from the pathology archive. Cases whom paraffin-embedded blocks, HE slides or clinical data could not be obtained, as well as patients with a second primary or who died within the first month after surgery were excluded. As a result, a total of 101 patients were included.

Clinical Data

The age, distant organ metastasis, adjuvant treatment status, and survival data were obtained from the hospital database, while tumor size, FIGO grade, nuclear grade, pathological stage, were obtained from pathology reports.

FIGO grade, nuclear grade, and nucleolus prominence were reevaluated from HE stained slides. The patients were classified as EC according to the World Health Organization criteria (14).

Outcomes

Overall survival (OS) and disease-free survival (DFS) were defined as the time from diagnosis to death, and the time from diagnosis to the first recurrence, respectively.

Histopathological Evaluation of Nucleolus Score

In this study, HE-stained slides (N=101) were evaluated by two pathologists (SDO, ÇO). Nucleolus prominence was assessed with a conventional light microscope (Olympus, BX-51, ocular 22mm) with Modified Helpap method (13). We divided the nucleolus scores into three subgroups. Nucleolus that was not evident in any way or were difficult to see at 20x magnification were given 1 point. A nucleolus was scored as three if there was a prominent nucleolus or dysmorphic nucleolus or it was easily visible at 10x magnification and was identified in at least 20% of the tumor. Nucleolus not rated as 1 or 3 was scored as 2 (Figure 1).

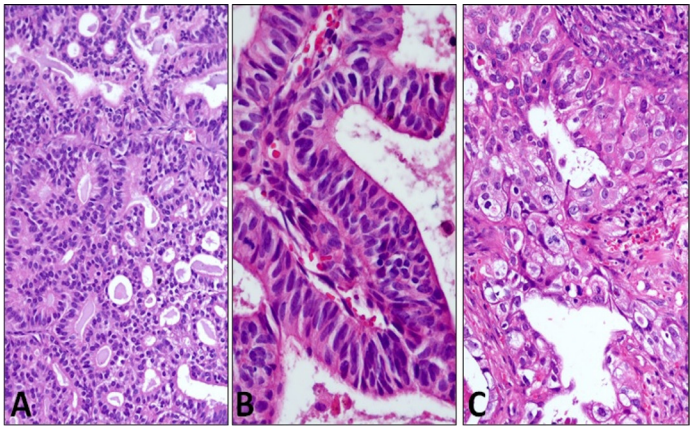

Figure 1: Examples of nucleolus scores 1, 2 and 3 in. Hematoxylin&Eosin x400. (A: score 1 . B: score 2. C: score 3)

Statistical Analysis

Statistical analyses were performed with IBM SPSS Statistics, Version 22.0 (SPSS Inc., Chicago, USA). The Chi-square test (Pearson Chi-square) or Fisher's Exact Test, where appropriate was used to compare the association of categorical variables. The possible prognostic factors identified with univariate analyses were further entered into the Cox regression analysis with backward selection to specify independent predictors of overall and disease-free survival, and a hazard ratio (HR) with 95% CI is presented. The Kaplan-Meier method was used for survival analysis and was evaluated with the log-rank test to identify the effect of nucleolus prominence on overall and disease-free survival. For statistical significance, a 5% type-1 error level was used.

Ethical Approval

Ethics committee confirmation for our study was obtained from the ethics committee of University Faculty of Medicine, non-interventional clinical research. The study was managed under the Declaration of Helsinki and the ethical standards of the institutional research committee, and the Reporting recommendations for tumor marker prognostic studies (REMARK) guidelines (15).

RESULTS

Clinical Features

A total of 101 cases with a tissue diagnosis of EC were included. The median age was 59 years (range, 41 to 87 years) for the entire cohort. Tumor diameter was less than 3 cm in 51 (51%) of the cases, and 3 cm or more in 50 (49%) cases. 66 (61%) cases were classified as FIGO Grade 1, while 33 (32%) were FIGO Grade 2, and 6 (6%) were FIGO Grade 3.

The number of cases with nuclear grade 1, 2 and 3 were 26 (26%), 68 (67%), and 7 (7%) respectively. Myometrial invasion was found less than 50% in 81 (80%), while equal to or more than 50% in 20 (20%) cases. The cervical invasion was detected in only 10 (10%) cases. Lymph node dissection was performed in 81 of 101 patients, and lymph node metastasis was observed only in 6 (7%) (Table 1).

Table 1. Clinicopathological data

		n	%
Nucleolus score	1	29	28.7
	2	46	45.5
	3	26	25.7
Tumor size	<3cm	51	50.5
	≥3 cm	50	49.5
FIGO grade	1	62	61.4
	2+3	39	38.6
Nuclear grade	1	26	25.7
	2+3	75	74.3
Pathologic stage	0	76	75.2
	1+2	25	24.8
Myometrial invasion ratio	<50%	81	80.2
	≥50%	20	19.8
Lymph node status	Negative	75	92.6
	Positive	6	7.4
Lymphovascular invasion	Negative	79	78.2
	Positive	22	21.8
Cervical invasion	Negative	91	90.1
	Positive	10	9.9
Paracentesis cytology	Benign	60	92.3
	Malign	5	7.7
Adjuvant chemotherapy	0	86	85.1
	1	15	14.9
Follow-up	0	91	90.1
	1	10	9.9
Distant metastasis	0	91	90.1
	1	10	9.9

Table 2. Nucleolus score and clinichopathologic criteria

		Nucleolus Score			
		1	2	3	
		n (%)	n (%)	n (%)	p
Tumor size	<3cm	20 (69)	20 (43.5)	11 (42.3)	0.062
	≥3 cm	9 (31)	26 (56.5)	15 (57.7)	
FIGO grade	1	28 (96.6)	29 (63)	5 (19.2)	<0.001
	2+3	1 (3.4)	17 (37)	21 (80.8)	
Nuclear grade	1	15 (51.7)	10 (21.7)	1 (3.8)	<0.001
	2+3	14 (48.3)	36 (78.3)	25 (96.2)	
Pathologic stage	0	25 (86.2)	36 (78.3)	15 (57.7)	0.041
	1+2	4 (13.8)	10 (21.7)	11 (42.3)	
Myometrial invasion ratio	<50%	26 (89.7)	39 (84.8)	16 (61.5)	0.019
	≥50%	3 (10.3)	7 (15.2)	10 (38.5)	
Lymph node status	Negative	18 (94.7)	40 (97.6)	17 (81)	0.056
	Positive	1 (5.3)	1 (2.4)	4 (19)	
Lymphovascular invasion	Negative	27 (93.1)	37 (80.4)	15 (57.7)	0.006
	Positive	2 (6.9)	9 (19.6)	11 (42.3)	
Cervical invasion	Negative	27 (93.1)	42 (91.3)	22 (84.6)	0.537
	Positive	2 (6.9)	4 (8.7)	4 (15.4)	
Paracentesis cytology	Benign	14 (93.3)	30 (96.8)	16 (84.2)	0.266
	Malign	1 (6.7)	1 (3.2)	3 (15.8)	
Adjuvant chemotherapy	0	27 (93.1)	40 (87)	19 (73.1)	0.102
	1	2 (6.9)	6 (13)	7 (26.9)	
Follow-up	0	29 (100)	43 (93.5)	19 (73.1)	0.002
	1	0 (0)	3 (6.5)	7 (26.9)	
Distant metastasis	0	27 (93.1)	45 (97.8)	19 (73.1)	0.003
	1	2 (6.9)	1 (2.2)	7 (26.9)	

Nucleolus Prominence and Clinicopathological Criteria

According to evaluation with a light microscope at 200x magnification, the number of patients with nucleolus score 1, 2 and 3 were 29 (29%), 46 (45%) and 26 (26%) respectively. There was a significant relationship between nucleolus scores and FIGO grade, as well as nuclear grade, myometrial invasion ratio, and lymphovascular invasion (p<0.001, p<0.001, p=0.019, p=0.006, respectively) (Table 2).

The number of cases with nuclear grade 1, 2 and 3 were 26 (26%), 68 (67%) and 7 (7%) respectively. When the nucleolus is evaluated according to the Modified Helpap method, which is known as an optimal method; nucleolus scores were significantly related with nuclear grades (p<0.001).

Relationship Between Nucleolus Score and Prognosis

The median follow up for entire-cohort was 60 months (range, 18 to 120 months). Ten (10%) were died of disease, and 7 (27%) of them had a nucleolus score of 3. The outcome was significantly poor as the nucleolus score increased (p=0.002). Distant organ metastases was observed in 10 (10%) cases . A significant correlation was found between the nucleolus score and disease-free survival (DFS) (Log rank p:0.001) (Figures 2 and 3). In Cox regression analysis, nucleolus score was found to be an independent prognostic factor for DFS (Hazard ratio (HR) 8.045; 95% CI 2.064-31.355; p: 0.003) (Table 3). In Cox regression analysis, nucleolus score, FIGO grade, lymphovascular invasion, and distant metastasis were found as prognostic factors in univariate analysis, but only FIGO grade was an independent variable for OS in multivariate analysis (Table 4).

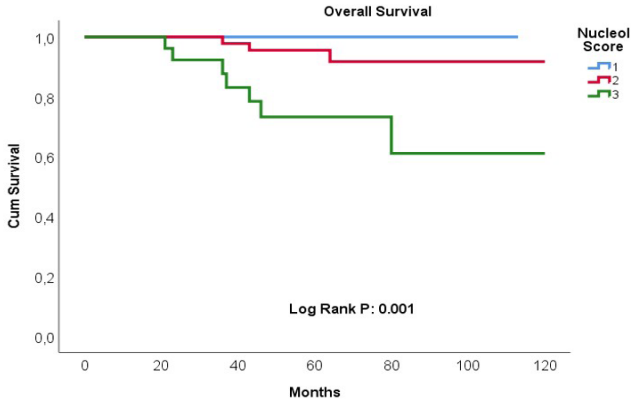

Figure 2. The relationship of nucleolus scores with overall survival by Kaplan Meier analysis

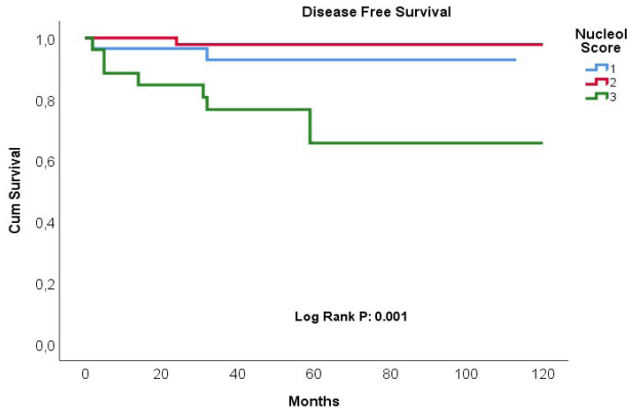

Figure 3: The relationship of nucleolus scores with disease free survival by Kaplan Meier analysis

Table 3. Disease free survival

	p	HR (95% CI)	p	HR (95% CI)
Nucleolus Score	0.003	8.045 (2.064-31.355)	0.003	8.045 (2.064-31.355)
Tumor size	0.216	2.35 (0.607-9.104)		
FIGO grade	0.016	6.668 (1.416-31.407)		
Nuclear grade	0.249	32.865 (0.087-12467.469)		
Pathologic stage	0.080	3.038 (0.877-10.525)		
Myometrial invasion ratio	0.023	4.221 (1.22-14.601)		
Mucin	0.544	0.044 (0-1088.848)		
Lymphovascular invasion	0.032	3.887 (1.124-13.436)		
Necrosis	0.910	1.094 (0.232-5.15)		
Squamous metaplasia	0.070	3.228 (0.91-11.449)		
Cervical invasion	0.318	2.207 (0.467-10.443)		

Nucleolus score, FIGO Grade, lymphovascular invasion, pathologic stage were selected as covariate

Table 4. Overall survival

	p	HR (95% CI)	p	HR (95% CI)
Nucleolus Score				
Tumor size	0.641	1.353 (0.38-4.813)		
FIGO grade	0.010	15.432 (1.951-122.087)	0.024	11.432 (1.380-94.724)
Nuclear grade	0.241	33.956 (0.094-12237.687)		
Pathologic stage	0.358	1.812 (0.51-6.439)		
Myometrial invasion ratio	0.138	2.621 (0.735-9.352)		
Mucin	0.523	0.043 (0-670.927)		
Lymphovascular invasion	0.034	3.829 (1.108-13.235)		
Necrosis	0.479	0.474 (0.06-3.747)		
Squamous metaplasia	0.931	0.934 (0.198-4.416)		
Cervical invasion	0.108	3.054 (0.782-11.921)		
Distant metastasis	0.002	7.362 (2.07-26.188)	0.053	3.593 (0.982-13.149)

Nucleolus score, FIGO Grade, lymphovascular invasion, distant metastasis was selected as covariate

DISCUSSION

The FIGO ternary grading system has been widely used in ECs all over the world (6). While the FIGO grading has significant criteria rely on tumor architecture, it does not have rigorous criteria for nuclear atypia (4). This grading, which is directly related to the prognosis of the patients, also plays a critical role in additional lymph node dissection during the surgical procedure. The lack of distinct criteria for nuclear grading in endometrial curettage specimens is confusing for pathologists, and most tumors are graded as nuclear Grade 2 (4). There has not been enough data in the literature regarding the prognosis of cases evaluated as FIGO Grade 2 (7). Our original proposal was to evaluate the nucleolus more objectively as a part of nuclear atypia, and add it to the FIGO grading system. Although, there are different methods in the literature for nucleolus evaluation, our idea was to evaluate the nucleolus with the Modified Helpap method, since it is a practical method and can be used for grading in endometrial curettages.

In our study, we found that nucleolus prominence was associated with FIGO grade, nuclear grade, myometrial invasion, lymphovascular invasion, OS, and DFS. Adding nucleolus score to FIGO grading, which is directly related to the prognosis, shows that pathologists can grade more easily, practically, and objectively.

Although the importance of the nucleolus in cancer cells has been konwn , its importance has begun to be realized again. In the literature, prominent nucleolus in cancer cells have been associated with poor prognosis (16,17). For example, the nucleolus, which can be evaluated morphologically, predict metastasis and recurrence in prostate cancer. In addition, prominent nucleolus are associated with poor clinical outcomes in breast cancer in different studies (12,17).

Recently, there have been studies showing that nucleolus can also be used as a target in cancer therapy (18-20). Helpap used nucleolus grading first in renal cell carcinoma and subsequently in prostate cancer (13,21). He showed that nucleolus status can be used in grading and predicting prognosis in renal cell carcinomas (13). Elshrawy, KA et al. evaluated nucleolus in invasive breast carcinomas using the Modified Helpap method, and found nucleolar prominence was associated with prognosis (12). Similarly, in this study, we evaluated the nucleolus using the Modified Helpap method in grading of ECs.

Qianhan Lin et al. showed that heterogeneous nucleolin staining is a potential prognostic marker in endometrial cancers. Besides, in the same study, they argued that the nucleolin immunohistochemistry staining, which was defined for the content of the nucleus, would be used as a therapeutic target (2).

The importance of nucleol in FIGO grading was emphasised in different studies For example, Zaino et al. and Takeshima et al. reported that FIGO grade should be increased in tumors with high nuclear grade (8,22). Later, in the study of Ayhan et al., triple grading was used for nuclear grading, and the architectural grade was increased by one in tumors with high nuclear grade (23). Although grading in this way was important in terms of prognosis, no quantitative value was presented for nuclear grading. In our study, we tried to provide quantitative criteria in grading of ECsby using the nucleolus Modified Helpap method, which is an important indicator of nuclear atypia.

To the best of our knowledge, our study is the first morphological study showing that the nucleolus may be useful in grading of ECs grading. We believe that nucleolus prominence can be evaluated more objectively than nuclear atypia. Thus, tumor grading can be done more objectively.

In the surgical treatment of ECs, it is still controversial whether lymph node dissection should be added to surgery (24,25). Although it is routinely performed in some centers, it has been shown hat performing lymphadenectomy in low-risk ECs does not contribute to survival (26-28). In addition, routine lymphadenectomy has several complication after surgery (26,29). By adding nucleolus score to FIGO grading, the surgical procedure may be directed more accurately. There may be no need to add unnecessary lymph node dissection to the surgical procedure and the morbidity of the complications may decrease. In our study, the nucleolus score increased as lymphovascular invasion increased. However,no relationship was found between nucleolus score and lymph node metastasis. This may be due to the small number of cases. Nucleolus prominence in curettage materials, the only material belonging to pre-surgery patients, can be used as a parameter before adding lymph node dissection to surgical treatment.

In addition, there are many controversial aspects of treatment, including patient selection for adjuvant radiation and chemotherapy. In our study, we observed that more patients receive adjuvant treatment in cases with high nucleolus score. Rebecca A. Brooks et al. argue that there have been recent significant advances in understanding EC biology (30). In their research, they stated that pembrolizumab, one of the current recommendations in endometrial cancer, can be used in immunotherapy with FDA approval in microsatellite unstable and metastatic cases (30). Qianhan Lin et al. suggested the nucleolin immunohistochemistry stain showing nuclear properties for target therapy in EC (2). We think that the nucleolus grading, which is an important component of the nucleus, can also be used as a parameter in choice of treatment method.

Digital pathology is gaining importance day by day. However, this method is not cost effective. In the current study, the nucleolus was evaluated microscopically, not digitally, Therefore, it is practical and low-cost for pathologists. Nucleolus evaluation in larger patient groups in different studies can also be done in combination with digital measurements or histochemical stains.

In our pioneering study, evaluation of the nucleolus prominence in curettage specimens morphologically; a high nucleolus score significantly predicted poor survival. Nucleolus scoring using the Modified Helpap method can be used in FIGO grading alongside architectural and nuclear grades. The current study is a pioneering study in terms of both prognosis and treatment approach in ECs patients. Integrating the nucleolus grading system into FIGO grading can make a significant difference in the clinical approach and prognosis of the cases.

CONCLUSION

Nucleolus score was associated with better prognosis in ECs. It can be evaluated objectively with no need of requiring extra cost and time. Thus, adding nucleolus score in FIGO grading can provide more reliable prediction in prognosis and choice of the treatment method.

Financial disclosures: *The authors declared that this study has received no financial support.*

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

Ethical approval: *Ethics committee confirmation for our study was obtained from the ethics committee of Recep Tayyip Erdoğan University Faculty of Medicine, non-interventional clinical research (E-40465587-050.01.04-573).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Comparison of Peripheral Nerve Conventional and Pulse Radiofrequency Applications in Patients with Primary Trigeminal Neuralgia: A Retrospective Analysis

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Abstract

Aim: This study aims to retrospectively investigate the results of continuous radiofrequency (CRF) and pulsed radiofrequency (PRF) applications to the peripheral branches of the trigeminal nerve in patients with trigeminal neuralgia (TN).

Material and Methods: Patients who experienced a significant reduction in pain symptoms after local anesthetic application to the peripheral branches of the trigeminal nerve were divided into two groups. The first group received PRF treatment and the second group received CRF treatment. Pain intensity scores of both groups at 1 and 3 months were compared. The results were analyzed retrospectively.

Results: Among the participants, 10 received PRF treatment and another 10 received CRF treatment. At the 1st month follow-up, both groups demonstrated considerable reductions in pain levels. By the 3rd month, no substantial disparities were noted between the two groups in terms of pain-related disability and pain intensity.

Conclusion: Both PRF and CRF interventions emerge as effective and secure techniques applicable to the peripheral branches of the trigeminal nerve. They should be contemplated as valuable options in cases where conventional medical treatments fall short in delivering adequate pain control.

Keywords: Trigeminal neuralgia, radiofrequency ablation, ultrasound, nerve block

INTRODUCTION

Trigeminal neuralgia (TN) is characterized by unilateral facial pain resulting from the involvement of the fifth cranial nerve. The condition is classified as classic TN (cTN) or secondary TN (sTN). sTN refers to cases where a specific lesion, like multiple sclerosis, tumor, or cerebral aneurysm, occupies a particular location. While TN can manifest at any age or gender, its prevalence is higher among females and increases with age (1). Pain often affects the distribution of the second (maxillary [V2]) or third (mandibular [V3]) branches of the trigeminal nerve (2).

Treatment success for TN is evaluated differently in medical and surgical research. In medical studies, success is generally defined as at least a 50% reduction in pain from baseline, while surgical research defines success as complete pain elimination (3). Treatment options encompass anticonvulsant drugs, antidepressants, and, if inadequate or accompanied by undesirable side effects, alternative interventions like interventional procedures (1,2). Surgical interventions such as neurectomy, alcohol injections, or radiofrequency lesions can be conducted on the trigeminal nerve, aiming to establish an anesthetic zone corresponding to the affected facial area (2).

Radiofrequency ablation (RFA) utilizes radio waves directly applied to the nerve to block pain signals. RFA comprises two main subtypes: continuous radiofrequency (CRF) and pulsed radiofrequency (PRF) (4,5). For TN, RFA of the Gasser ganglion is a minimally invasive procedure, initially providing significant pain relief. Another approach is RFA of the peripheral branches of the trigeminal nerve, proven to be both safe and effective. Peripheral branch RFA is considered secure due to its extra cranial procedure nature, reducing complications such as nerve

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and vessel damage. Although peripheral branch RFA may offer immediate relief post-procedure, its recurrence rate surpasses that of Gasser RFA (6).

The objective of this study is to retrospectively analyze the comparative clinical outcomes of peripheral PRF and CRF applications in patients with TN who exhibited clinically significant reduction in symptoms following diagnostic local anesthesia block under ultrasound guidance to the peripheral branches of the V2 and/or V3 trigeminal nerve, within a short-term period of 3 months.

MATERIAL AND METHOD

Participant Data Collection

This study conducted a retrospective examination of patients who had sought treatment at the pain clinics of Bakırçay University Faculty of Medicine and Bağcılar Training and Research Hospital. These patients met the diagnostic criteria for TN as outlined in the third edition of the international classification of headache disorders (ICHD-3) (7). The data collection period spanned from January 1, 2022, to January 1, 2023 (İzmir Bakırçay University Non-Interventional Ethics Committee, decision no: 1143).

The participants consisted of individuals who had previously undergone evaluations by specialists and received a diagnosis of cTN. This group of patients either experienced insufficient pain control despite undergoing medical treatments or were unable to tolerate medical interventions. As a result, these patients were considered for interventional procedures in the subsequent phase to attain effective pain management.

Demographic information, encompassing factors like age, weight, height, gender, employment status, marital status, educational background, and the presence of any concurrent medical conditions, was meticulously documented for every participant. Comprehensive data concerning the pain experienced, including its precise location, duration since diagnosis, and characteristics, were meticulously recorded. Before undergoing any procedural interventions, the intensity of pain was gauged employing the Numeric Rating Scale (NRS), while the level of pain-related impairment was assessed through the application of the Headache Impact Test-6 (HIT-6).To maintain the study's targeted scope, specific individuals were excluded from participation. This excluded category encompassed patients dealing with cancer, bilateral facial pain, persistent pain attributed to systemic conditions (such as rheumatological disorders), as well as those presenting with non-neuralgiform facial pain (referred to as atypical facial pain).

Ultrasound-Guided Peripheral Nerve Blocks: The procedural steps were carried out utilizing the MyLab 6 ultrasound device (Esaote Europe B.V., Maastricht, Netherlands), and the corresponding sonographic images are presented in Figure 1 and Figure 2. A high-frequency linear transducer with an operational frequency of 10–12 MHz was employed to meticulously scan superficial anatomical structures. The Power Doppler mode was engaged to facilitate the identification of vascular components. For the execution of the superficial nerve block, a 5 cm peripheral nerve block needle was meticulously positioned under the guidance of ultrasound. Subsequently, 1-2 ml of a local anesthetic solution, typically 0.5% lidocaine, was carefully administered.

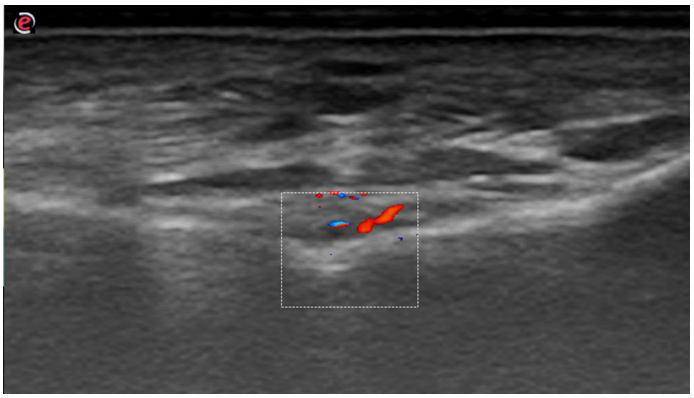

Figure 1. Ultrasonographic identification of infraorbital foramen, artery, and nerve. Images sourced from the archive of Dr. Ilteris Ahmet Senturk

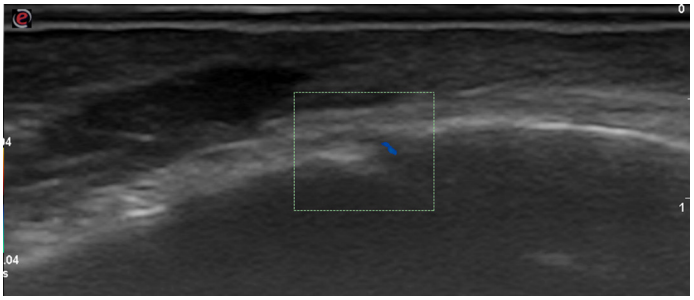

Figure 2. Mental nerve and associated vessels, along with mental foramina. Images sourced from the archive of Dr. Ilteris Ahmet Senturk

Radiofrequency Nerve Ablation: For patients diagnosed with cTN who reported significant, albeit short-term, relief from pain following peripheral nerve blocks, the decision was taken to advance to the subsequent stage, which involved performing RFA. Prior to this, patients were presented with detailed information and their informed consent was obtained.

The core technique of RFA entails the strategic positioning of an electrode in proximity to a nociceptive pathway. This positioning disrupts pain signals through the controlled delivery of radiofrequency currents, facilitated by a catheter-guided approach. In the CRF technique, the current is terminated once the desired temperature is attained, and then reactivated to maintain tissue temperature at a predefined level. This cyclic alternation between open and closed currents sustains the designated tissue temperature. Nerve tissue disruption commences at temperatures surpassing 45 degrees Celsius. During the PRF approach, radiofrequency currents are administered for duration of 120 seconds at a frequency of 2 Hz, with each pulse lasting 20 milliseconds. Voltage adjustment is performed to ensure that the maximum temperature remains below 42 degrees Celsius (8).

All interventions were conducted within a specialized pain unit. The procedures employed a 5 cm peripheral nerve block needle furnished with a 5 mm active tip, a disposable 20-gauge caliber, and an electrode. Additionally, a radiofrequency device (Diros Technology Inc, Markham, Ontario, Canada) was employed as an integral component

of the procedure.

The outcomes were assessed within two distinct groups: individuals who underwent CRF applications and those who underwent PRF applications. Pain intensity and pain-associated disability scores for all patients were meticulously recorded at both the initial and third months.

Statistical Analysis

The data of the study were analyzed by SPSS 25.0 (IBM®, New York, USA). The findings were expressed as frequency and percentages. Normality analysis was carried out using the Shapiro-Wilk test. The variables without normal distribution are presented as the median (min-max). Wilcoxon signed rank, and Friedman tests were used to compare numeric rating scale pain scores over time. Spearman Correlation analysis was performed to determine possible correlations with HIT-6 score. The statistical significance value was set at $p<0.05$ value.

RESULTS

Ultimately, the medical records of twenty (20) patients were subjected to analysis, following the exclusion of records with three missing data points and insufficient information. The mean age of the participants was calculated as 56.55±14.80 years, with an age range spanning from 28 to 75 years. The diagnosis of TN was more frequently established in female patients, with a female-to-male ratio of 7:3. Among the participants, hypertension emerged as the most commonly observed systemic ailment, afflicting 11 individuals (55% of the cohort). Regarding the interval between the onset of symptoms and the point of referral, patients self-reported an average duration of 27.0 months, with a range spanning from 3.0 to 240.0 months. Among the twenty participants, the initial pain intensity before the procedural interventions was assessed at 9.0 (on a scale of 0-10), indicating severe pain (9). Additionally, the baseline pain-related disability was documented to be ≥60 points, signifying a severe impact (10).

Subsequently, the Numeric Rating Scale (NRS) scores were determined to be 3.0 (ranging from 0.0 to 6.0) at the 1-month mark and 5.0 (ranging from 2.0 to 7.0) at the 3-month assessment. This observed alteration in scores between the 1-month and 3-month follow-up points post-treatment exhibited statistical significance ($p<0.001$). Furthermore, substantial and statistically significant distinctions were evident across all paired comparisons ($p<0.001$ for NRS-baseline and NRS-1st month, and NRS-baseline and NRS-3rd month comparisons; $p<0.01$ for NRS-1st month and NRS-3rd month comparison). Notably, there was a marginal increase in pain intensity scores noted during the 3-month evaluation.

Concise summaries of sociodemographic data and pain characteristics are provided in Table 1.

A total of 10 patients received CRF treatment, while another 10 patients underwent PRF treatment. The pain intensity results of these patients were recorded at 1 month and 3 months after the procedures. When comparing the pain intensity results of both groups during these time intervals, no statistically significant differences were observed. The outcomes are presented in Table 2.

Table 1. Sociodemographic characteristics of the patients (n=20)

	N (%)	Mean±SD	Median (min-max)
Age		56.55±14.80	
Gender			
Female	14 (70.0)		
Male	6 (30.0)		
BMI		28.27±5.30	
Smoking			
Yes	8 (40.0)		
No	12 (60.0)		
Working status			
Working	3 (15.0)		
Quitted job	2 (10.0)		
Housewife	7 (35.0)		
Retired	8 (40.0)		
Educational status			
Literacy course	5 (25.0)		
Primary school	5 (25.0)		
Secondary school	4 (20.0)		
High school	3 (15.0)		
University	3 (15.0)		
Marital status			
Married	16 (80.0)		
Widower	4 (20.0)		
Concomitant systemic diseases			
DM	2 (10.0)		
HT	11 (55.0)		
CAD	1 (5.0)		
Thyroid dysfunction	6 (30.0)		
Asthma-COPD	4 (20.0)		
Others	2 (10.0)		
Pain duration (history) (months)			27.0 (3-240.0)
Pain intensity (baseline)			9.0 (4.0-10.0)
Pain intensity (1st. month)			3.0 (0.0-6.0)
Pain intensity (3rd. month)			5.0 (2.0-7.0)
Pain disability (baseline)			61.70± 6.12

N: number, SD: standard deviation, BMI: body mass index, DM: diabetes mellitus, HT: hypertension, CAD: coronary artery disease, COPD: chronic obstructive pulmonary disease
Pain intensity was calculated by the numeric rating scale (NRS)
Pain disability was calculated by the headache impact test (HIT-6)

Table 2. The comparison of numeric rating scale scores of the pulsed and continuous radiofrequency treatment groups

	Pulsed RF (n=10)	Continuous RF (n=10)	P
NRS-baseline	8.5 (4.0-10.0)	9.0 (5.0-10.0)	0.631
NRS-1st month	3.0 (0.0-6.0)	3.0 (2.0-5.0)	0.631
NRS-3rd month	5.0 (2.0-7.0)	5.0 (2.0-7.0)	0.393

n: number, NRS: numeric rating scale, RF: radiofrequency

DISCUSSION

TN presents as a severe and distressing facial pain, typically localized unilaterally within one or more areas of the trigeminal distribution (11). The incidence of TN increases with age, women are more at risk than men, and may be associated with hypertension (12). Our results were consistent with these demographics. In cases where TN patients do not respond to conservative medical approaches or encounter difficulties in tolerating medication side effects, minimally invasive interventional procedures come into play as treatment options (11).

The focus of this study was to compare the results of PRF and CRF treatments applied to the peripheral branches of the trigeminal nerve. We found significant results in both treatment groups in the evaluation of pain intensities in the first and third months after the procedures. There was no difference between the groups in the comparison between the two groups. It is worth noting that relatively few studies have evaluated the efficacy and safety of RFA procedures on peripheral branches of the trigeminal nerve.

In a recent review and meta-analysis published (6), the effectiveness and reliability of CRF treatment on peripheral nerves were compared with CRF treatment on the Gasser ganglion. The study indicated that there were no significant differences in terms of pain scale, and complications. The authors observed that CRF treatment of peripheral branches showed better early results compared to CRF treatment of the Gasser ganglion, but this was associated with a higher recurrence rate. They attributed this discrepancy to the fact that Gasser ganglion contains cell bodies of pseudomonopolar neurons, whereas peripheral nerves contain Schwann cells. In our study, we did not directly compare Gasser's ganglion RFA procedures, but retrospectively analyzed the short-term results of RFA procedures to peripheral nerves. Therefore, we cannot provide information about possible recurrence rates for CRF.

Zeng et al. (13) stated their initial suggestions for the possible reasons of higher recurrence rates with peripheral RFA compared to the study by Wan et al. (14). They mentioned that they performed ablation at a lower temperature (75°C), which might contribute to the higher recurrence rate. They refrained from using higher temperatures due to the potential risk of causing severe mandibular motor dysfunction. Similarly, in our study, we used a temperature of 75°C for CRF treatment.

Luo et al. (15) compared the 1-year outcomes of high-voltage PRF and standard-voltage PRF treatments in patients with refractory infraorbital neuralgia and found that high-voltage PRF was significantly more effective. Similarly, Fang et al. (16) conducted a similar comparison for Gasser ganglion PRF and also found high-voltage PRF to be more effective. The authors have suggested from these results that high-voltage PRF technology is likely to substantially reduce the number of patients requiring ablative procedures. However, there are comparative studies on the Gasser ganglion reporting that PRF is ineffective compared to CRF (17). In a study by Tanyel et al. (18), PRF treatment was applied to the peripheral branches of the trigeminal nerve, highlighting appropriate pain control over approximately one year, and they reported no complications or side effects. In our study, we used standard PRF. Taking into consideration the assessments of Luo and Fang, we believe that a comprehensive and prospective evaluation of the comparison between CRF and high-voltage PRF for peripheral branches, informed by our study, would contribute to scientific knowledge.

CONCLUSION

We believe that PRF and CRF procedures for the peripheral branches of the trigeminal nerve are effective and safe interventional methods in patients with TN. In our study, clinical outcomes were similar in both groups. Based on this, considering that the procedure-related recurrence risk is minimal, PRF treatment could be considered a first-line option for patients who do not respond to medical treatment or cannot undergo medication due to side effects.

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Research Article

Relation of Immune Thrombocytopenia and Blood Group: A Retrospective Single Center Study

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Abstract

Aim: Although blood group antigens were initially identified as erythrocyte surface antigens and their significance was mainly ascribed to serology, it soon became evident that these antigens are widely distributed in human tissues such as platelets. Immunthrombocytopenia (ITP) is a common autoimmune disorder characterized by a low platelet count. In our study, we investigated the relationship between ABO and Rhesus (Rh) blood groups and primary ITP.

Material and Methods: A retrospective study was conducted at our center with 304 patients diagnosed with primary ITP and 491 blood donors.

Results: ABO phenotype in patients group A, B, O, AB were 42.8%, 14.1%, 34.9%, 8.2%, respectively and 51.6%, 8.1%, 27.9%, 12.4% in the control group, respectively. Rh phenotype in patients group positive or negative 87.5%, 12.5 % respectively and 88.8%, 11.2% in the control group, respectively. A total of 304 patients with primary ITP, consisting of 203 (66.8%) females, and 101(33,2%) males were included in this study. The mean age was 49,5 ±18 years. There was no significant difference in the distribution of ABO blood types and Rh factor by gender (respectively p=0.176, p= 0.195).

Conclusion: In our study, no significant difference was found between the blood group distribution in the population and the blood group distribution of patients diagnosed with primary ITP.

Keywords: Blood groups, immunthrombocytopenia, thrombocytopenia, splenectomy

INTRODUCTION

Blood group antigens were initially discovered as erythrocyte surface antigens, and their importance was primarily assigned to serology. However, it quickly became apparent that these antigens are broadly distributed throughout human tissues, including the digestive and respiratory tracts. A, B, O, and Rh are the most prevalent blood groups out of the 330 near blood groupings. The ABO blood group system was first described by Karl Landsteiner in 1900. Despite their conventional association with red blood cells, the carbohydrate structures that make up the ABO blood group system (A, B, and H determinants) are expressed in a range of different cell types, including platelets and endothelial cells. It is well known that the ABO blood group is important for blood transfusion and transplantation (1). Surprisingly, significant links have been found between ABO blood groups and a variety of other diseases (2). Immunthrombocytopenia (ITP) is a common autoimmune disorder with a low platelet count. In primary ITP patients, autoantibodies have been found primarily to the platelet surface glycoproteins glycoprotein (gp) IIb - IIIa and gpIb – IX (3).

ABO antigens have been associated with various diseases. Platelets normally express small amounts of A and B antigens on their surface, which has been known for many years. First, it was discovered that approximately 5% of normal people with blood types A or B had platelets with an unusually high number of A and B antigen sites. In a study using flow cytometry to investigate the presence of blood group antigens on platelet surfaces, major and

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minor blood group antigens were found in low, moderate or high amounts on platelet surfaces. According to this study, blood group antigens were found in varying amounts on the platelet surface (4). Numerous investigations have discovered that in some circumstances, maternal IgG anti-B (and possibly anti-A) antibodies may result in newborn alloimmune thrombocytopenia. As a result, it appears that blood type and primary ITP may be related (5).

According to the study, gangliosides and LKEs have been found on the surfaces of erythrocytes and platelets. In paroxysmal nocturnal hemoglobinuria, gangliosides, which are frequent autoantigens for cold agglutinins in RBCs, may aid in complement-mediated hemolysis. These compounds may draw both alloantibodies and autoantibodies (6). Platelets may also experience cellular fragmentation similar to that seen in erythrocytes.

Hemostasis is significantly influenced by the ABO blood groups. They have a large quantitative effect on factor VIII and von Willebrand factor levels in plasma. Blood types A and AB are more likely to experience myocardial infarction, ischemic stroke, and venous thromboembolism, which may be because these conditions are modulated by functional ABO glycol transferases. Cerebral venous thrombosis is more likely to occur in non-O groups (4-6). Pre-eclampsia prevalence has been shown to be significantly correlated with ABO groups, and the AB group is linked to a 2.1-fold increased risk (7).

Studies are being done to determine how blood type and thrombocytopenia are related. To our knowledge, two investigations have looked into the connection between primary ITP and blood type. The goal of our study was to add to the body of knowledge by examining the association between primary ITP and the ABO and Rhesus blood groups (8,9).

MATERIAL AND METHOD

Our study included 304 patients (203 females) with primary ITP diagnosis and 491 (29 females) healthy blood donors as the control group. The mean age was 49.5 18 years. To determine ABO Rh blood group, the DiaClon ABO/D+Reverse Grouping (BIO-RAD) ID-card method was applied.

The association between the blood types of patients with primary ITP and those of blood donors without a diagnosis of any disease was evaluated using IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp. A mean, standard deviation, and percentage are used to represent the data. The chi-square test was employed to ascertain the association between category variables. A 0.05 p value was regarded as statistically significant. This study (date: December 12, 2019; No: 2019/1272) was authorized by the Adnan Menderes University School of Medicine's Ethics Committee for Non-Invasive Clinical Studies. Since this study was designed retrospectively, informed consent forms were not required.

RESULTS

The ABO phenotype in patients groups A, B, O, and AB were 42.8%, 14.1%, 34.9%, and 8.2%, respectively, compared to 51.6%, 8.1%, 27.9%, and 12.4% in the control group. Rh phenotype was positive or negative in 87.5%, 12.5% of patients and 88.8%, 11.2% of controls, respectively. This study included 304 ITP patients, of whom 203 (66.8%) were female and 101 (33.2%) were male. There was no statistically significant difference in the distribution of ABO blood types and Rh factor by gender (p=0.176, p=0.195, respectively) (Table 1). Splenectomy was performed on 79 patients (26.3%). There was no correlation discovered between blood type and splenectomy.

Table 1. The frequency distribution of ABO. Rh blood group among the ITP patients and controls

	ITP frequency (%)	Controls frequency (%)	Total frequency (%)	P
A	130 (42.8)	253 (51.6)	383 (49.1)	p>0.01
B	43 (14.1)	40 (8.1)	83 (10.4)	p>0.01
O	106 (34.9)	137 (27.9)	243 (30.6)	p>0.01
AB	25 (8.2)	61 (12.4)	86 (10.8)	p>0.01
Rh (+)	266 (87.5)	436 (88.8)	702 (88.3)	p>0.01
RH (-)	38 (12.5)	55 (11.2)	93 (11.7)	p>0.01
TOTAL	304	491	795	

*Chi-square test

DISCUSSION

In our study, there was no significant difference between the blood group distribution in the general population and the blood group distribution in patients with primary ITP. Although the B blood group was more common in ITP patients than in the control group, the difference was not statistically significant. Furthermore, there was no difference in the blood group distribution of patients diagnosed with primary ITP who underwent splenectomy for treatment. There haven't been many studies done on the relationship between primary ITP and blood type. Our study, which included a large number of patients and blood donors, was one of the first studies to investigate the relationship between primary ITP and blood group distribution.

Our study's primary flaw is that it was restricted to a specific

center and geographic area. Studies that are carried out in several centers can produce varying outcomes. Thus, understanding the connection between blood types and ITP may help us better treat ITP patients.

El-Khateeb et al. studied the relationship between blood types and ITP in 25 patients with primary ITP. In this study, there were 34 volunteers in the control group. ITP patients had a higher prevalence of blood group A (64%) than the general population (37.98%). The A phenotype blood group was found to be significantly higher in patients with primary ITP than in the control group. The findings of this study differed from those of ours. However, unlike our study, El-Khateeb et al. had a very small number of patients (8).

Sturgill et al. investigated the relationship between the response to IVIG treatment and blood groups in patients with primary ITP. Twenty-seven O blood type patients, nineteen A blood type patients, four B blood type patients, and two AB blood type patients participated in the study. RH was found to be negative in 12 patients. This study found no significant difference between response to IVIG treatment and blood group. In this study, only pediatric patients with ITP were included. The relationship between ITP and blood groups was not investigated in this study, but blood groups and response to IVIG treatment were. Unlike our study, this study did not compare the patients' blood group distribution to that of the control group (9).

In their study, E. Hussein et al. looked into the connection between TTP and blood types. In this study, the surveillance of 33 patients with severe ADAMTS-13 deficiency with blood group O and other blood groups were compared. It was hypothesized that the distribution of blood group O among TTP patients may be lower than expected due to low VWF levels. Contrary to predictions, the study's findings showed that the death rate for patients was 15.2% and was unaffected by blood type. Patients with idiopathic TTP had a lower (12%) than predicted (30%) distribution of group O. The outcomes could not have been as anticipated due to the study's limited sample size and lack of a control group (10).

Ozbay et al. investigated the relationship between epistaxis and blood type in the Turkish population. In this study, 359 patients who presented to the otolaryngology department from more than one centre and who did not have any other disease or drug use were included. The mean age of the epistaxis group was 43.37 and 23.61 years. Among the epistaxis patients, 43% were female and 57% were male. There were no differences that were statistically significant. While 43.5% of patients in the epistaxis group had O blood group, the rate in the control group was 33.9%. Patients with the O blood group in the epistaxis group were found to be significantly more than the control group (P 0.001). Unlike our study, bleeding was found more in patients with the O blood group in this study (11).

Tanrverdi O. investigated thrombocytopenia-inducedby chemotherapy in 131 stage 3 colon cancer patients, and chemotherapy-induced thrombocytopenia was found in 51 of these patients. When chemotherapy-induced thrombocytopenia patients were classified according to their blood groups, it was found that chemotherapy-induced thrombocytopenia was more common in patients with blood group O. The study discovered that blood group O was a distinct risk factor for chemotherapy-induced thrombocytopenia (P=0.035, OR 3.14, 95% CI 1.16-7.01). Unlike our study, this one lacked a control group, and the majority of the patients had the O blood group, which may have affected the findings (12).

Kumar S. et al. examined 81 patients with hematological malignancies who had been treated in a tertiary hospital for a year in terms of blood type and many other parameters. B blood group was found in 28.4% of acute hematological malignancies and 16.4% of chronic hematological malignancies. There was no statistically significant difference in blood groups between any malignancy and the control group, despite the greater rate of B blood group compared to the control group. Despite the fact that our study's results were similar, the small number of patients and the simultaneous evaluation of several characteristics can be viewed as potential factors that may have an impact on the study's conclusion (13).

Burd J. et al. conducted a retrospective study in which 32023 women who applied to the gynecology department over a seven-year period were evaluated in terms of postpartum hemorrhage and blood type. Even after adjusting for demographic differences (p=0.02), women who underwent cesarean delivery had a significantly greater rate of postpartum hemorrhage (5.2% type O vs 3.8% type A vs 4.4% type B vs 4.2% type AB, p=0.035). Between the vaginal and cesarean birth groups, there was no change in the rates of any of the secondary outcomes, including blood transfusion, hysterectomy, intrapartum dilatation and curettage, and critical care unit admission. The study's findings suggest that having a blood type of O may increase the risk of postpartum bleeding in women who have had cesarean deliveries. In contrast to our study, more bleeding was observed in patients with O blood type. However, because this study only included women, the results may have been different (14).

The prevalence of the O blood group in society is approximately 45%-49%, the prevalence of the A blood group is 27%-40%, the prevalence of the B blood group is 11%-20%, and the prevalence of the AB blood type is approximately 4% (15). Looking at the overall rate in Türkiye, the A, O, B, and AB blood group distributions are 42.84%, 32.67%, 16.46%, and 8.03%, respectively. The percentage of Rh positive people in Türkiye was determined to be 88.54% (16).

CONCLUSION

In conclusion, the findings of studies on bleeding and blood group or thrombocytopenia and blood group are contradictory. The blood groups of patients with primary

ITP in our study were similar to the blood group rates in the general population. Although there were 304 patients in our study, it is recommended to conduct studies involving more patients from different centers. Our research added to the body of knowledge about the relationship between ITP and blood types.

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

Ethical approval: *This study (date: December 12, 2019; No: 2019/1272) was authorized by the Adnan Menderes University School of Medicine's Ethics Committee for Non-Invasive Clinical Studies.*

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Research Article

Use of Tourniquet Under Sedation Anesthesia or the Walant Techniques in Bilateral Carpal Tunnel Surgery: A Comparative Analysis

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Abstract

Aim: The objective of the study was to compare the outcomes and patient satisfaction of two different anesthesia techniques; wide-awake-local anesthesia-no tourniquet (WALANT) and sedation anesthesia with a tourniquet, in open carpal tunnel release surgery for bilateral cases.

Material and Methods: The study included 57 patients (41 female, 16 male) who underwent carpal tunnel release surgery between January 2016 and September 2021. The files were retrospectively evaluated and included in the present study. Patient evaluations were conducted using QuickDASH and Michigan Hand Outcomes Questionnaire scores before surgery, on the 15th day after suture removal, and at six months postoperatively. Surgical duration and complications were also recorded. Statistical analyses were performed to compare the outcomes between the two groups.

Results: Both QDash and MHQ scores were analyzed for anesthesia effects on hands. The study's reliability was ensured by an 85% statistical power, 95% confidence level, and $p<0.05$ significance. The results showed no significant differences in QuickDASH and Michigan Hand Outcomes Questionnaire scores between WALANT and sedation anesthesia Surgical duration was similar for sedation and WALANT groups. Patient preference was inconclusive, with comfort and symptom relief prioritized. Notably, neither group experienced complications like nerve injuries or infections.

Conclusion: The study found that both WALANT and sedation anesthesia with a tourniquet were equally effective and provided similar levels of patient comfort and satisfaction in open carpal tunnel release surgery. The choice between these techniques can be based on individual preferences and considerations.

Keywords: Carpal tunnel syndrome, sedation, tourniquet

INTRODUCTION

Carpal tunnel syndrome (CTS) is the most common compression neuropathy affecting the upper extremity and is frequently managed through surgical intervention, yielding the highest benefit from surgery (1,2). Although various incisions and interventions have been described, such as classic incision, small incision, mini-incision, incision passing the proximal to the flexor wrist crease, and endoscopic approaches, the sole objective remains the release of the hypertrophied or thickened transverse carpal ligament, which causes compression of the nerve (3). The primary importance lies in the patient's comfort during the surgical procedure and their well-being during the postoperative period (4).

Just as important as the patient's comfort is the surgeon's comfort. Optimizing surgical ergonomics, including the attainment of a hemostatic and sterile surgical field, and ensuring optimal visualization, are of paramount importance. These factors serve as critical determinants for facilitating expedited surgical completion and enhancing overall surgical outcomes (5,6).

The wide-awake, local anesthesia, no tourniquet (WALANT) technique, initially described by Bezuhly et al. in 2007, along with surgical techniques performed under

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general or sedation anesthesia either with or without the application of a tourniquet are the anesthesia and surgical techniques employed in contemporary carpal tunnel surgery (1).

In almost all publications in the literature, different cohort groups have been utilized to compare carpal tunnel surgery techniques performed under WALANT and sedation with tourniquet application (7). Comparative assessments have been made by introducing different surgical techniques while comparing patients with bilateral involvement (2).

In the present study, considering that CTS often involves bilateral involvement, we compared cases in which patients were initially diagnosed with bilateral CTS or developed symptomatic CTS in the contralateral side during follow-up after unilateral surgery (6,8). We examined the outcomes of performing surgery on one side using the WALANT technique while using sedation and a tourniquet on the other side, employing the same surgical method performed by the same surgeon.

Our aim was to compare two different anesthesia methods objectively by performing surgery on two different extremities of the same patient using different anesthesia techniques. This comparison aimed to determine which anesthesia method provided greater comfort for the patient. Additionally, we aimed to compare the surgical duration, complications encountered during and after surgery, and surgical comfort in terms of the aspects relevant to the surgeon between the two methods.

MATERIAL AND METHOD

Patients who underwent CTS surgery at Tekirdağ Yaşam Hospital between January 2016 and September 2021 were retrospectively evaluated and included in the present study. The study was carried out with the permission of Kastamonu University Hospital, Noninvasive Clinical Ethics Committee (Date:05.07.2023 Decision No:2023-KAEK-80). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The criteria for participation in the study were that the patients had clinically positive bilateral or unilateral Tinnel and Phalen tests at the time of initial diagnosis, had pain that they felt more especially at night, and had been diagnosed with at least moderate CTS in electromyographic (EMG) tests with evidence of increased median nerve latency or denervation. Mild CTS detected by EMG was an exclusion criterion and those patients were excluded from the study. The development of CTS requiring surgical intervention within an average of 2.5 years (1 year to 4 years) was another criterion for inclusion in the study, with the same criteria provided in the follow-ups of the patients who were diagnosed unilaterally and underwent surgical intervention. Patients with bilateral CTS were operated on at different times, with a minimum interval of 3 months (3 months to 1 year).

The patients were classified based on gender, age, surgical side, and the sequence of anesthesia methods used for each side. QuickDASH (QDash) scoring and Michigan Hand Outcomes Questionnaire (MHQ) scoring which are used for the objective evaluation of the results of interventions in hand surgery clinical practice are utilised for the evaluation of the patients in the preoperative period, on the 15th day after suture removal, and at 6 months postoperatively.

The QuickDASH represents a concise iteration of the initial DASH outcome assessment. In contrast with the comprehensive 30-item DASH assessment, the QuickDASH encompasses just 11 items. This instrument serves as a survey to gauge an individual's capacity to accomplish tasks, absorb physical stresses, and the intensity of symptoms experienced. Utilizing a 5-point Likert scale, the QuickDASH empowers the patient to designate a numerical value that aligns with their degree of severity and functional capability. MHQ is formulated as a standardized tool designed to assess diverse dimensions of health status encountered by individuals with hand-related conditions. Its efficacy resides in its ability to identify and quantify patients' symptoms, functionality, visual appeal, and contentment pertaining to hand-related ailments and issues affecting the upper limb (9,10).

Furthermore, the patients were asked about their perceived comfort levels in relation to the surgical side.

The study employed rigorous exclusion criteria to ensure the integrity and homogeneity of the patient population. Individuals with underlying conditions, such as rheumatoid arthritis, diabetes, gout, and hypothyroidism, were excluded from participation. Additionally, patients with a history of renal dialysis, pregnancy, or the presence of space-occupying lesions (e.g., ganglions) were not included in the study. Moreover, individuals with previous carpal tunnel release surgery or a history of distal radius fracture were excluded. Allergy to local anesthetics, medical conditions that contraindicated the use of sedation, and any other contraindications to sedation were also factors considered for exclusion. Finally, individuals with contraindications for subcutaneous epinephrine use, including a history of digital gangrene, Buerger's disease, previous replantation, Raynaud's disease, or sclerodactyly, were also excluded from the study population. These exclusion criteria were implemented to ensure a well-defined and homogeneous patient cohort for accurate analysis and interpretation of the study findings.

Out of the 98 patients who underwent surgery for CTS between the specified dates, 23 patients were not included because of not meeting the inclusion criteria or because they underwent unilateral surgery and were therefore not eligible for participation in the study, among them 11 patients were refused to participate in the study. Seven of the patients were lost during follow–up period. Finally 57 patients ranging in age from 28 to 85 years were included in the study. The flowchart of the patients is seen in Figure 1. Utilizing a cohort comprising 57 patients, a meticulous a priori power analysis has been conducted, unveiling

an anticipated robust statistical power of around 80% to discern a noteworthy two-fold alteration in anesthesia preference relative to baseline, deploying an eminent significance level of 0.05. To prudently address potential attrition effects within the study populace, an imperative objective was set forth to surpass the requisite minimum of 30 enrolled patients, in accordance with judicious precautionary measures. Eventually, a gratifying total of 57 patients exhibited their unwavering willingness to partake in the investigation, exhibiting commendable commitment to the research endeavor. Based on the findings obtained from this study, considering the sample size, the statistical power of the study was determined to be 85% at a 95% confidence level and a significance level of 0.05.

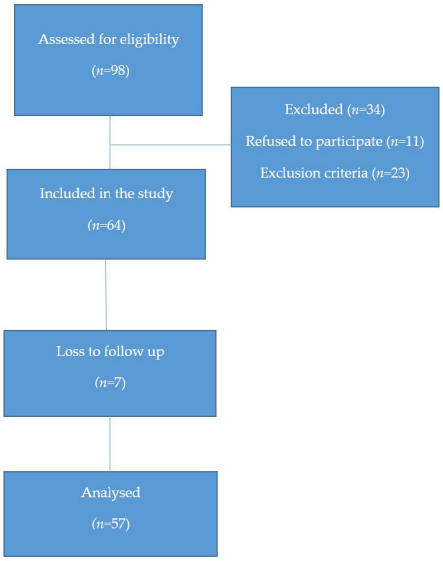

Figure 1. Flowchart of the patients

The patients were fully informed about the potential benefits and drawbacks associated with each anesthesia method. By being provided with detailed information about the anesthesia techniques, the patients were able to make well-informed decisions regarding their participation in the study. This comprehensive informed consent process contributed to the ethical conduct of the research and ensured that the patients had a clear understanding of the potential risks and benefits involved in their anesthesia choices.

In the WALANT technique, a 10 mL subcutaneous injection was administered using a 27G needle. The injection consisted of 1% lidocaine, 8.4% bicarbonate, and buffered 1/100,000 epinephrine.1 Notably, no sedation or tourniquet was employed during the procedure. Conversely, in the sedation technique, monitored anesthesia care performed by an anesthesiologist with the application of a tourniquet was utilized. Midazolam and propofol were used together according to patient's weight. The choice of method to be applied to the extremities that would be operated on first was determined by randomization. The other method was then used for the second surgery in another time interval not before three months.

A longitudinal incision measuring 3–4 cm in length was carefully made along the axis of the 4th digit, precisely located between the thenar and hypothenar eminences. Sharp dissection techniques were employed to meticulously navigate the layers of the skin and subcutaneous tissue. This approach facilitated the thorough release of the transverse carpal ligament using a No. 15 scalpel blade, ensuring comprehensive intervention for the condition. Regardless of the anesthesia method used, this surgical method was applied to all the patients.

The surgical duration was defined as the time elapsed from the initiation of the initial skin incision to the placement of the final suture in both techniques. Prolongation of the surgery already implied insufficient surgical comfort for the surgeon.

Statistical Analysis

The distribution of the data was examined using the Shapiro–Wilk test. Group and within-group comparisons of the dependent variables were conducted using a repeated-measures analysis of variance test. Descriptive statistics for numerical data were presented as mean±standard deviation, while descriptive statistics for categorical data were presented as frequency (percentage). All statistical analyses were performed and reported at a significance level of α=0.05 and a 95% confidence level using IBM SPSS Statistics 26.0 software.

RESULTS

Out of the 98 patients who had surgery for CTS during the specific time frame, 23 patients were not included because they didn't meet the criteria to be part of the study or they had surgery on just one side and weren't able to join the study. Among them, 11 patients chose not to take part in the study. Seven patients were not available for follow-up during the study. In the end, the study included 57 patients who were between 28 and 85 years old. Using a cohort of 57 patients, a thorough analysis was conducted prior to commencing the study. Our analysis revealed a strong likelihood (approximately 80%) of detecting a significant change in anesthesia preference compared to the baseline. This analysis was performed with a confidence level of 0.05, a critical factor in studies of this nature. To ensure the study's robustness even in the face of potential dropouts, we aimed to enroll more than 30 patients initially. It is gratifying to note that eventually, we successfully recruited 57 committed patients who participated in the research. Based on the insights garnered from this study and considering the size of the participant group, we established that the study's findings were credible with an 85% probability, a 95% confidence level, and a significance level of 0.05. These parameters collectively affirm the reliability and validity of the study's results.

A total of 57 patients were included in the study. Of these, 41 (71.9%) were female, and 16 (28.1%) were male. The mean age of the patients was 57.16±18.63 years. The age and gender distribution of the group is seen in Figure 2.

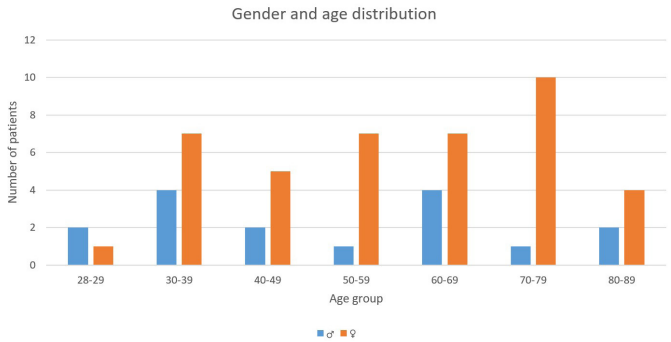

Figure 2. Age and Gender distribution of the group

The statistical analysis results regarding the examination of score improvements in the right and left hands of the patients under the two different anesthesia techniques (WALANT technique and sedation anesthesia) are provided in Tables 1 and 2, respectively.

Table 1 presents the changes over time in the QDash and MHQ scores for the right and left hands under sedation anesthesia. The QDash scores showed significant differences between the scores before surgery, at the 15th day, and at the 6th month for both hands (p<0.001). The scores before surgery were higher than those at the 15th day and 6th month for both hands. The 15th-day scores were also higher than the 6th-month scores for both hands. However, there were no significant differences in the changes in QDash scores between the right and left hands at different times (p=0.936).

Regarding the MHQ scores, significant differences were found between the scores before surgery, at the 15th day, and at the 6th month for both hands (p<0.001). The scores before surgery were higher than those at the 15th day and 6th month for both hands. The 15th-day scores were also higher than the 6th-month scores for both hands. There were significant differences in the changes in MHQ scores between the right and left hands at different times (p=0.045).

Figure 3 illustrates the change in mean QDash scores according to side and time.

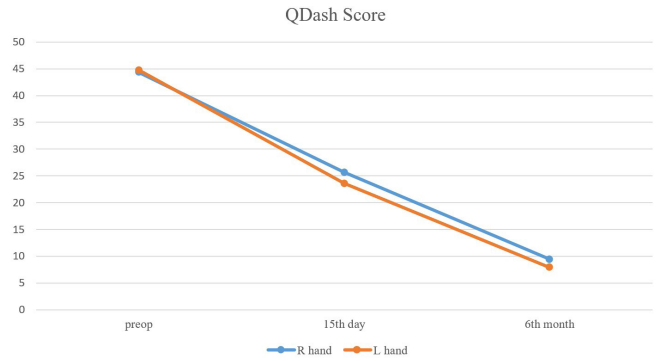

Figure 3. The change in mean values of QDash scores according to side

Table 2 displays the changes over time in the QDash and MHQ scores for the right and left hands under WALANT anesthesia. The QDash scores showed significant differences between the scores before surgery, at the 15th day, and at the 6th month for both hands (p<0.001). The scores before surgery were higher than those at the 15th day and 6th month for both hands. The 15th-day scores were also higher than the 6th-month scores for both hands. However, there were no significant differences in the changes in QDash scores between the right and left hands at different times (p=0.936).

Regarding the MHQ scores, significant differences were found between the scores before surgery, at the 15th day, and at the 6th month for both hands (p<0.001). The scores before surgery were higher than those at the 15th day and 6th month for both hands. The 15th-day scores were also higher than the 6th-month scores for both hands. There were no significant differences in the changes in MHQ scores between the right and left hands at different times (p=0.085). Figure 4 shows the changes over time in the mean MHQ score according to side.

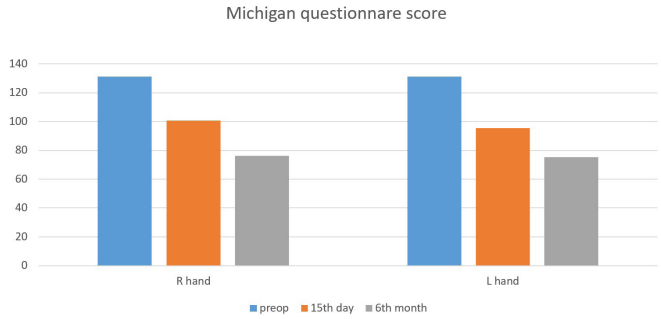

Figure 4. The change in mean values of MHQ scores according to side

Table 3 provides the results of the analysis for each anesthesia technique separately for the right and left hands. Statistically significant differences in the QDash scores were observed for both hands between the preoperative period, 15th day, and 6th month. The 6th-month scores were lower than both the preoperative and 15th-day scores (p<0.001). Similar patterns were found for the MHQ scores for both hands, with significant differences between the preoperative period, 15th day, and 6th month. The 6th-month scores were lower than both the preoperative and 15th-day scores (p<0.001).

The study did not find significant differences in superficial infections, surgical duration, or patient preference for anesthesia methods. Patients reported experiencing similar comfort with both methods and emphasized the importance of symptom resolution regardless of the chosen method. Neuropraxia, nerve injury, peripheral circulatory problems, and deep infections were not observed in either group.

The average surgical durations were 9 minutes and 11 seconds in the sedation anesthesia group and 8 minutes and 55 seconds in the WALANT group. The difference was not statistically significant (p=0.096).

When asked about their preference for anesthesia methods for a hypothetical third limb, patients reported that they experienced sufficient comfort with both methods and could not differentiate between them. They emphasized the resolution of their complaints and how it positively affected their daily lives, particularly their sleep, regardless of the chosen method.

Table 1. Intra-group and inter-group comparisons of QDash and MHQ scores for the right and left hand in case of sedation anesthesia

		Sedation anesthesia			
		Right	Left	Mean diffrence (between groups) (right-left)	Mean diffrence (within groups)
QDash score					
	Preop[1]	43.80±6.70	45.71±6.78	-1.906	1-2: 19.350
	15th day[2]	24.45±7.50	24±3.74	0.450	1-3: 34.750
	6th month[3]	9.05±6.21	7.94±3.47	1.109	2-3: 15.400
MHQ score					
	Preop[1]	131.30±4.64	130.88±3.91	0.418	1-2: 34.540
	15th day[2]	99.75±7.46	93.35±6.01	6.397	1-3: 55.087
	6th month[3]	75.95±4.12	76.06±3.89	-0.109	2-3: 20.547

The p-values correspond to the repeated measures ANOVA test. The data is presented as mean±standard deviation, Post-hoc p-values in between 1, 2 and 3 are <0.001 for QDash and MHQ Scores

Table 2. Intra-group and inter-group comparisons of QDash and MHQ scores for the right and left hand in case of WALANT Technique

		WALANT			
		Right	Left	Mean diffrence (between groups) (right-left)	Mean diffrence (within groups)
QDash score					
	Preop[1]	45±5.99	44.40±6.95	0.600	1-2: 19.496
	15th day[2]	27.06±8.79	23.35±4.20	3.709	1-3: 35.838
	6th month[3]	9.82±7.76	7.90±3.49	1.924	2-3: 16.343
MHQ score					
	Preop[1]	131.46±3.82	132±3.05	-0.538	1-2: 33.108
	15th day[2]	101.31±6.87	95.94±7.56	5.370	1-3: 56.067
	6th month[3]	76.08±4.36	75.25±4.49	0.827	2-3: 22.959

The p-values correspond to the repeated measures ANOVA test. The data is presented as mean±standard deviation, Post-hoc p-values in between 1, 2 and 3 are <0.001 for QDash and MHQ scores

Table 3. The independent evaluation of the measured QDash and MHQ scores for the right and left hand separately

		Pre-op1	15th day2	6th month3	p value
QDash score					
	Right	44.35±6.32	25.65±8.11	9.41±6.88	**<0.001**
	Left	44.73±7.08	23.65±3.96	7.92±3.43	**<0.001**
MHQ score					
	Right	131.11±4.12	100.57±6.95	76.14±4.04	**<0.001**
	Left	131.24±3.39	95.32±7.10	75.30±4.20	**<0.001**

The p-values correspond to the repeated measures ANOVA test. The data is presented as mean±standard deviation, Post-hoc p-values in between 1, 2 and 3 are <0.001 for QDash and MHQ scores

DISCUSSION

CTS is a common condition that can cause significant discomfort and functional limitations for patients. Understanding the optimal techniques and approaches for carpal tunnel release is crucial for achieving successful outcomes and patient satisfaction.

An important consideration in carpal tunnel release is the bilateral nature of the condition. Bagatur et al. investigated the bilateral aspect of CTS and found that it is indeed a bilateral disorder (8). This highlights the importance of considering bilateral involvement when evaluating treatment options and outcomes. Padua et al. explored the incidence of bilateral symptoms in CTS and found that approximately one-third of patients with unilateral symptoms had bilateral electrodiagnostic evidence of median nerve involvement (6). This suggests that bilateral evaluation and management must be considered in patients presenting with unilateral symptoms. The starting point and main theme of the present study was CTS's characteristic feature of bilaterality. It is important to be aware that, whether the initial diagnosis is bilateral or once

one side is affected, even in the absence of any symptoms on the contralateral side, the involvement of the other side is inevitable, and treatment approaches must be planned accordingly.

The use of a tourniquet during carpal tunnel release is a subject of debate. Olaiya et al. conducted a systematic review and meta-analysis, which indicated that carpal tunnel release without a tourniquet was associated with reduced postoperative pain and faster recovery (5). Furthermore, a randomized controlled trial by Saleh et al. compared minor hand procedures performed with or without the use of a tourniquet and found that the use of a tourniquet might not be necessary for certain hand procedures (11). These findings raise questions about the routine use of tourniquets in carpal tunnel release and suggest that individualized approaches must be considered. In the present study, we compared the two sides of our patients by performing surgery on one side with a tourniquet and on the other side without a tourniquet. We organized our results accordingly and found no significant difference between the use and non-use of a tourniquet on either side of the same patient.

The choice of anesthesia technique is another critical factor in carpal tunnel release. The WALANT technique has gained popularity in recent years due to its potential benefits in terms of patient comfort and satisfaction. Gallucci et al. compared the WALANT technique to local anesthesia with a tourniquet for CTS (12). The results indicated that carpal tunnel release performed with the WALANT technique provided better patient comfort and satisfaction. This finding is consistent with that of a previous study by Moscato et al. which also reported improved patient satisfaction with the WALANT technique in an office-based setting (13). Perhaps the most notable aspect of the present study stands out here. In almost all studies in the literature, different cohorts were compared, and few studies focused on bilaterality by attempting different anesthesia methods, but different surgical techniques were applied. In the present study, the same surgical method, performed by the same surgeon, was applied with different anesthesia methods in the surgeries on two different extremities of the same patient. No significant difference was observed between the two groups.

The choice of surgical setting is another important factor in carpal tunnel release. Office-based settings have gained attention as potential alternatives to traditional operating room settings, offering convenience and cost-effectiveness. A study by Moscato et al. compared patient satisfaction between office-based carpal tunnel release and procedures performed in other settings (13). The results showed that carpal tunnel release performed in an office-based setting led to superior patient satisfaction. This finding supports the idea that office-based carpal tunnel release can provide a comfortable and satisfactory experience for patients. All patients in the present study were operated on in operating rooms. Although there was a preparatory period, especially in the sedation group, which included routine preoperative tests (e.g., blood tests, chest X-rays) and the requirement for the patient to wear a surgical gown and enter a sterile environment, which could increase patient stress and discomfort, none of the patients in both groups reported any discomfort related to the preparatory period.

The WALANT technique has also been compared to sedation in endoscopic carpal tunnel release. Wellington et al. compared the two techniques and found that the WALANT technique was associated with shorter operative times and fewer complications, highlighting its potential advantages in endoscopic carpal tunnel release (14). In the present study, an endoscopic technique was not used; however, the surgical duration of the side operated on with the WALANT technique was not found to be shorter. No significant difference was detected between the surgical durations on the two sides.

Sasor et al. examined the effects of tourniquet use during wide-awake CTS surgery (15). They found that tourniquet use might not be necessary in wide-awake carpal tunnel release procedures. This is an important finding as the use of a tourniquet can lead to patient discomfort and may increase the risk of complications, such as nerve injury or hematoma formation. The researchers also concluded that taking a local anesthetic with epinephrine alone is a safe and effective alternative to tourniquet use. By avoiding tourniquet use, surgeons may be able to improve patient comfort and reduce the risk of complications. While the WALANT technique itself does not involve the use of a tourniquet, none of the patients in the present study who received sedation anesthesia reported any discomfort from the tourniquet used.

Tulipan et al. compared the outcomes of open carpal tunnel release performed with sedation versus the WALANT technique in two cohorts of different numbers (2). They found no significant differences in patient-reported outcomes, including pain, satisfaction, or functional improvement. These findings suggest that performing carpal tunnel release surgery without sedation and without a tourniquet is feasible and will not compromise the surgical outcomes. This is important as avoiding sedation can reduce the overall cost and risks associated with anesthesia, while allowing patients to remain awake during the procedure may provide them with a sense of control and improve their overall experience.

In the present study, we also compared the use of a tourniquet under sedation anesthesia with the WALANT technique. However, it is important to note that the groups compared in our study were not two different cohorts but a patient's two extremities. This approach was chosen to obtain more objective responses to subjective symptoms.

Via et al. compared the WALANT technique to sedation without a tourniquet but with local anesthesia in patients undergoing staged bilateral carpal tunnel release (7). Although they applied the same surgical technique to

different extremities of the same patient, it should be noted that there were variations in the surgical techniques used among the patients. The study found no significant differences in pain, patient satisfaction, or complications between the two groups. These findings suggest that local anesthesia alone, without the need for sedation or a tourniquet, can be effective in carpal tunnel release surgery. This is important as local anesthesia is generally safer and less invasive than sedation, and its use may reduce the overall cost and potential risks associated with the procedure. In our study, the same surgical technique was applied both among different patients and when operating on two different extremities of the same patient. This consistency in the surgical technique used was an important factor contributing to the evaluation of the study's results, particularly in assessing postoperative patient comfort, which is another objective measure.

In a retrospective cohort study Carroll et al. concluded that endoscopic carpal tunnel release was associated independently with a 2.96 times greater likelihood of requiring revision carpal tunnel release within one year, compared to open carpal tunnel release (16). Although numerous publications have juxtaposed endoscopic and open methods for the purpose of comparing revision rates, discernible differences in revision outcomes have not been ascertained in many of these studies. Nonetheless, in this very recent investigation, surgeries conducted through endoscopic means were found to exhibit significantly higher rates of revision when contrasted with those performed via open surgery. This notable disparity has given rise to the necessity of reevaluating the preference for endoscopic surgery. In this study, all patients who did not undergo endoscopic techniques were subjected to the same standardized open surgical approach during the operative intervention.

Borekci et al. have elucidated the identification of accessory muscles that may be encountered during carpal tunnel surgery and possess the potential to induce compression neuropathy (17). Their findings have demonstrated that inadequate release of these accessory muscles could lead to unfavorable outcomes in carpal tunnel surgery. In our present investigation, we did not encounter any cases necessitating release of these accessory muscles during the surgical procedure.

Limitations

This study is not without limitations. Firstly the sample size is small and should be increased for improved statistical power and generalizability. Secondly randomization the choice of anesthesia methods for each extremity would help address potential selection bias. Thirdly specific patient characteristics which can influence surgical outcomes were not mentioned. The follow-up period should be extended for a more comprehensive assessment of surgical results and potential long-term differences between anesthesia methods.

CONCLUSION

It can be concluded that the WALANT technique and the sedation technique with a tourniquet have similar levels of effectiveness and lead to similar degrees of satisfaction in patients undergoing open CTS release. The study revealed no statistically significant differences between the two groups in terms of outcome measures, including complications and postoperative pain. These results provide valuable insights for surgeons and patients as they can freely choose between the WALANT and sedation techniques based on their preferences and considerations. Overall, the two techniques can be considered equally effective and satisfactory in achieving successful outcomes and patient comfort in carpal tunnel release procedures. These conclusions were reached mainly through the use of different anesthesia methods on the two different extremities of the same patient during the surgery, which allowed for obtaining more objective results. By employing this approach, the study was able to eliminate potential confounding factors associated with individual variations. The use of different anesthesia methods on separate extremities added an extra layer of objectivity, strengthening the study's conclusions and making them applicable to a broader patient population.

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Research Article

The Systemic Immune-Inflammation Index and its Connection with Maternal Age in Naturally Conceived Pregnancies: A Single-Center Cohort Study

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Abstract

Aim: Maternal age is associated with perinatal outcomes, which include preeclampsia, low birth weight, preterm birth, neonatal mortality, increased cesarean rates, and maternal mortality. This study aimed to investigate the effects of maternal age on hematological parameters and systemic immune-inflammatory indices in pregnant women.

Material and Methods: A retrospective analysis of 400 pregnant women was conducted, divided into four age groups. Hematological parameters, systemic immune-inflammatory indices, and clinical outcomes were compared across these groups.

Results: The mean neutrophil count and monocyte count increased with maternal age, significantly highest in the 40-49 age group ($p<0.001$, $p=0.003$). Age-associated inflammation was reflected by increased NLR, MLR, PLR, and SII in the advanced age groups ($p<0.001$). Positive correlations were found between age, BMI ($r=0.301$, $p<0.001$), and MLR ($r=0.122$, $p=0.015$). The prevalence of chronic diseases, drug usage, and complications such as preeclampsia and gestational diabetes also significantly increased with age ($p<0.001$).

Conclusion: This analysis reveals age-related alterations in immune-inflammatory indices and clinical outcomes in pregnancy and provides insights for better management and monitoring of different age groups during pregnancy, particularly those at advanced maternal age.

Keywords: Maternal age, systemic immune-inflammatory indices, pregnancy outcomes

INTRODUCTION

Maternal age is associated with perinatal outcomes, which include preeclampsia, low birth weight, preterm birth, neonatal mortality, increased cesarean rates, and maternal mortality (1). Adolescent pregnancy, defined as ages 10-19, accounts for 11% of all births globally, with over 90% occurring in developing countries (2). The pregnancies are associated with adverse obstetric results and maternal neonatal mortality (3). Advanced maternal age pregnancies aged 35 and over and very advanced maternal age pregnancies are terms used by the International Federation of Gynecology and Obstetrics (4). The trend towards fertility at older ages has been popularized in the last decade due to increasing marriage age, second marriages, more significant opportunities for education and career development, improving family planning methods, and advances in assisted reproductive techniques (5).

The systemic inflammatory process can inform physicians about pregnancy-related disorders (6). Parameters from blood counts such as neutrophil-lymphocyte, platelet counts, and ratios can predict the systemic inflammatory process (7). The ratio of neutrophil to lymphocyte (NLR), monocyte to lymphocyte (MLR), platelet to lymphocyte (PLR), and the Systemic Immune-Inflammation index (SII) were evaluated in diseases where inflammation is thought to be involved in the pathogenesis, such as preeclampsia, gestational diabetes mellitus, low birth weight, and their relationship with inflammation was investigated (8). The SII, calculated using platelet, neutrophil, and lymphocyte counts together, is a more important indicator of inflammation and immune response than the neutrophil to lymphocyte and platelet to

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lymphocyte ratios (9,10). Examining the interplay between age groups and the Systemic Immune-Inflammation Index (SII) could potentially illuminate predictive insights for clinicians regarding post-pregnancy complications, underscoring the further exploration in this area.

The current research aims to analyze the differences observed in the first trimester of adolescent, adult, and advanced-maternal age pregnant by calculating and comparing the NLR, MLR, PLR, and SII in naturally conceived pregnancies.

MATERIAL AND METHOD

Study Design and Participants

This retrospective single-center cohort evaluated the first-trimester blood count parameters of naturally conceived pregnancies. Out of 532 potential participants, the final sample size was reduced to 400 pregnant women for various reasons, including exclusion criteria, non-compliance, and loss of follow-up. These 400 pregnant women were divided into four groups based on age: adolescent (15-19 years, n=81), adult (20-34 years, n=172), advanced maternal age (35-39 years, n=106), and very advanced maternal age (40-49 years, n=41).

Ethical Statement

The study was initiated with the approval of the Ankara Etlik City Hospital Clinical Researches Ethics Committee (Date: 14/06/2023, Decision No: AEŞH-EK1-2023-247). Every method carried out in the research adhered strictly to the ethical guidelines set by our institutions and national research committee and those established in the 1964-Helsinki Declaration and its subsequent updates or ethical norms.

Data Collection

The cornerstone of this research was a meticulous gathering of data, primarily sourced from hospital records, ensuring accuracy and consistency. To begin with, we extracted demographic details that would provide a holistic understanding of the participant's profile. The maternal age served as a baseline, indicating the pregnant woman's age during her first trimester. Another fundamental parameter was gravida, which revealed the number of times a woman had been pregnant, regardless of the pregnancy's progression or outcome. Complementing this, parity was used to identify the number of pregnancies a woman had carried past the pivotal gestational age of 24 weeks, irrespective of whether they culminated in live births or other outcomes. Recognizing the significance of conception methods in modern times, we documented the mode of fertilization. This allowed us to differentiate between pregnancies resulting from natural processes and those conceived through assisted reproductive technologies. Another vital metric was the Body Mass Index (BMI), which furnished insights into the nutritional and health status of the participants.

As smoking and underlying health conditions can significantly influence pregnancy outcomes, data on smoking status and the presence of other diseases were meticulously logged. Medications, especially during pregnancy, can bear considerable implications. Hence, a comprehensive list of medication use was drawn for each participant. Recognizing the potential challenges some pregnancies face, specific data on complications like gestational diabetes mellitus and hypertensive disorders of pregnancy were diligently recorded. Outcomes of pregnancies, particularly preterm birth, birth weight, and the newborn's health reflected in the APGAR score, were essential components of our data set. We derived specific indices from the first-trimester complete blood count parameters to delve into the hematological aspect. This involved calculating the NLR, MLR, PLR, and SII. For precision, the SII was determined using the formula: Platelet count multiplied by Neutrophil count, divided by Lymphocyte count.

Patients Selection Criteria

Inclusion Criteria: The study prioritized including pregnant women within specified age brackets. The adolescent group consisted of those aged 15-19 years; the adult group, 20-34 years; the advanced maternal age group, 35-39 years; and the advanced maternal age group, 40-49 years. Only participants experiencing a single pregnancy were considered. Furthermore, an essential criterion was the availability of comprehensive medical records necessary for the study. A clear history devoid of recurrent miscarriages and hematological disorders was also pivotal for inclusion.

Exclusion Criteria: Several factors led to the exclusion of potential participants. Pregnant women diagnosed with an active infection were omitted from the study. Similarly, if a participant had an autoimmune disease or a history of any malignancy, they were excluded. The study also refrained from including those on a current or recent steroid regimen. Pregnancies involving multiple births, such as twins or triplets, were not considered. Women undergoing fertility treatments or had a history of severe anemia or other blood-related conditions were also left out. Lastly, the study ensured that pregnant women with any known genetic disorders that might influence them were excluded.

Data Analysis

Using the SPSS software, our first step in the analytical journey was using descriptive statistics. It represented continuous variables as mean values accompanied by their standard deviations, while categorical variables found representation through frequency counts. Once this foundational analysis was established, we progressed to inferential statistics to draw comparisons across the diverse groups. The Chi-square test became our primary tool for categorical variables, allowing us to discern the proportions scattered across the groups. When the Chi-square test faltered due to inadequately populated cells, we used Fisher's exact test as a reliable alternative. On the front of continuous variables, we employed the Analysis of Variance (ANOVA) – but this was contingent on data

behaving in a normally distributed fashion. When this assumption didn't hold, we opted for the Kruskal-Wallis test, a non-parametric counterpart. Following instances where either ANOVA or Kruskal-Wallis yielded significant findings, we conducted post-hoc analyses to pinpoint the groups with pronounced differences. Our statistical litmus for significance was a p-value under 0.05.

RESULTS

In this study, we compared a variety of parameters across four distinct age groups: 15-19 (n=81), 20-34 (n=172), 35-39 (n=106), and ≥40 (n=41) years, as seen in Table 1. The mean age for the respective groups was 18.1±1, 26.4±3.3, 36.5±1.3, and 42.2±2 years (p<0.001). A similar pattern of differences (p<0.001) was observed for BMI, with mean values of 27.1±4.3 kg/m², 29.1±4.8 kg/m², 31.4±4.5 kg/m², and 30.3±5 kg/m² respectively. Although Delta Hemoglobin levels were relatively consistent across groups (p=0.442), the average delivery week differed (p=0.032) with values of 38±3.1, 38±2.3, 38±3.2, and 37±3.3 weeks in respective groups. The mean birth weights were 2808±603, 3058±549, 3035±608, and 2838±705 g, which showed a significant variation (p=0.005).

Regarding health conditions and lifestyle factors, the prevalence of chronic diseases and drug usage increased with age (p=0.027 and 0.041, respectively). The occurrences of preeclampsia and gestational diabetes mellitus also escalated in older age groups (p<0.001), as did the rate of cesarean section deliveries (p=0.026). Conversely, no differences were observed across the age groups for cigarette usage (p=0.872), preterm birth rate (p=0.112), the incidence of intrauterine growth restriction (p=0.077), and the proportion of male babies (p=0.984).

There was a variation in the mean neutrophil counts for the respective groups, which were 7583±1911, 6407±1834, 6686±1828, and 7289±1939 cells/μL (p<0.001). Similarly, as seen in Table 2, monocyte counts varied across the groups with respective counts of 680±192, 621±182, 577±139, and 690±266 cells/μL (p<0.001). In contrast, lymphocyte and platelet counts did not differ between the groups, with lymphocyte at 1909±694, 2101±640, 2043±587, and 2062±871 cells/μL (p=0.187) and platelet counts at 280±58, 276±58, 265±58, and 282±60 cells/μL (p=0.216). The NLR and MLR varied across the groups p<0.001). NLR values for the respective groups were 4.6±2.3, 3.3±2, 3.6±1.6, and 4.1±2.6, and MLR values were 2.9±1.1, 3.6±1.3, 3.6±1.1, and 3.2±1.2. The PLR showed a difference (p=0.015) with respective ratios of 167.4±77.7, 142.9±60.4, 141.2±57.3, and 161.8±97.1. The SII also showed variation with values of 1299.2±733, 911.3±470.9, 947.5±514.2, and 1203±1025.2 respectively (p<0.001). Significant differences were observed in neutrophil and monocyte counts and in the NLR, MLR, PLR, and SII across the different age groups. In contrast, lymphocyte and platelet counts remained consistent.

Our correlation analysis yielded significant associations between age, BMI, NLR, MLR, PLR, and SII variables. Age was positively correlated with BMI (r=0.301, p<0.001), suggesting that an increase in age is associated with a higher BMI. The age factor positively correlated with MLR (r=0.122, p=0.015). Age was not significantly correlated with NLR, PLR, or SII. Despite a slight positive correlation with age, BMI did not show correlations with any of the remaining variables. PLR and SII displayed a strong positive correlation (r=0.875, p<0.001).

Table 1. Comparative analysis of parameters across age groups

Variables	15-19 (n=81)	20-34 (n=172)	35-39 (n=106)	≥40 (n=41)	p value
Age, years	18.1±1	26.4±3.3	36.5±1.3	42.2±2	0.001
BMI, kg/m²	27.1±4.3	29.1±4.8	31.4±4.5	30.3±5	0.001
Delta HB, mg/dL	1.5±0.8	1.5±1	1.5±1.3	1.2±0.9	0.442
Delivery, week	38±3.1	38±2.3	38±3.2	37±3.3	0.032
Baby weight, g	2808±603	3058±549	3035±608	2838±705	0.005
Chronic disease, yes	2 (2.5%)	22 (12.8%)	16 (15.1%)	7 (17.1%)	0.027
Drug usage, yes	2 (2.5%)	22 (12.8%)	16 (15.1%)	5 (12.2%)	0.041
Cigarette usage, yes	15 (18.5%)	29 (16.9%)	21 (19.8%)	6 (14.6%)	0.872
Preeclampsia, yes	0 (0%)	2 (1.2%)	6 (5.7%)	8 (19.5%)	0.001
GDM, yes	0 (0%)	8 (4.7%)	14 (13.2%)	7 (17.1%)	0.001
Preterm birth, yes	19 (23.5%)	22 (12.8%)	19 (17.9%)	10 (24.4%)	0.112
IUGR, yes	24 (29.6%)	29 (16.9%)	19 (17.9%)	6 (14.6%)	0.077
Birth method, C/S	30 (37%)	83 (48.3%)	61 (57.5%)	24 (58.5%)	0.026
Baby gender, male	40 (49.4%)	84 (48.8%)	54 (50.9%)	22 (53.7%)	0.984

BMI: body mass index, HB: hemoglobin, GDM: gestational diabetes mellitus, IUGR: intrauterine growth restriction, C/S: cesarean section, Delta Hemoglobin: change in hemoglobin, the age groups are segmented as under 19, 20-34, 35-39, and above 40 years. The p-value less than 0.05 is considered significant. For continuous variables (like age, BMI, delta hemoglobin, delivery week, and baby weight), means and standard deviations were calculated for each group using Analysis of Variance (ANOVA). For categorical variables, the numbers and percentages of individuals for each category within the groups were computed using a chi-square or Fisher's exact, depending on the data characteristics

Table 2. Comparative analysis of hematological parameters and indices across age groups

Variables	15-19 (n=81)	20-34 (n=172)	35-39 (n=106)	≥40 (n=41)	p value
Neutrophil, cells/μL	7583±1911	6407±1834	6686±1828	7289±1939	0.001
Lymphocyte, cells/μL	1909±694	2101±640	2043±587	2062±871	0.187
Monocyte, cells/μL	680±192	621±182	577±139	690±266	0.001
Platelet, cells/μL	280±58	276±58	265±58	282±60	0.216
NLR, ratio	4.6±2.3	3.3±2.0	3.6±1.6	4.1±2.6	0.001
MLR, ratio	2.9±1.1	3.6±1.3	3.6±1.1	3.2±1.2	0.001
PLR, ratio	167.4±77.7	142.9±60.4	141.2±57.3	161.8±97.1	0.015
SII, ratio	1299.2±733	911.3±470.9	947.5±514.2	1203±1025.2	0.001

NLR: neutrophil to lymphocyte ratio, MLR: monocyte to lymphocyte ratio, PLR: platelet to lymphocyte ratio, SII: systemic immune-inflammation index, The p-value is derived from a statistical test (ANOVA) comparing the means of the age groups. A p-value less than 0.05 indicates the difference

DISCUSSION

The present study analyzed the comparative results of hematological parameters and indices across four age groups. We found several notable trends and correlations in the data, underpinning the intricate dynamics between age, BMI, and systemic immune-inflammatory markers. This study not only expands our knowledge of the relationship between age and systemic inflammation for pregnant but also invites further exploration into the practical implications of this relationship in clinical and public health settings.

Considering the increasing trend of advanced maternal-age pregnancies worldwide, our findings significantly affect public health (11). Recognizing the changes in systemic inflammation with aging, as indicated by SII (12-14), could guide the development of preventive measures and health promotion strategies for older pregnant women. Several studies have suggested that a high SII is linked to unfavorable outcomes in numerous medical conditions, including preeclampsia and gestational diabetes (15,16). Our findings support the hypothesis that age-related variations in SII might influence the risk of these complications in pregnant women. This result aligns with the growing body of evidence suggesting that aging is associated with chronic, low-grade inflammation, a phenomenon termed "inflammaging" for pregnant (17). The aging process, characterized by immunosenescence, is linked with altered immune cell functions and inflammatory markers imbalances, which could explain the noted variations in SII (18). Given the potential clinical utility of SII as an inflammatory marker in various conditions, its relationship with age merits further investigation (19,20). Our study has linked higher SII values with adverse outcomes in different medical conditions, including preeclampsia and gestational diabetes mellitus. Hence, our findings shed light on the influence of age-related changes in SII and how they might impact the risk of these complications in pregnant women. The significant difference we observed in the SII across different age groups suggests a need for age-specific SII cut-offs to enhance the predictive accuracy for pregnancy complications. These key insights contribute towards a more profound understanding of how immune system changes associated with aging might impact pregnancy outcomes, potentially influencing management strategies during pregnancy.

Notably, age positively correlated with BMI, reflecting the well-documented trend of increasing BMI with advancing age (21). This result further supports the broad consensus that aging is associated with changes in body composition, including an increase in adipose tissue (22,23). Although age also demonstrated a positive correlation with MLR, its lack of significant correlations with other systemic immune-inflammatory indices suggests that additional factors, possibly genetic, environmental, or lifestyle-related, might influence these indices. Our findings did, however, expose a disparity between different age groups regarding the prevalence of certain health conditions and lifestyle factors. An increase in the rate of chronic diseases, drug usage, and conditions like preeclampsia and gestational diabetes mellitus in older age groups underscores the influence of aging on health status. These findings align with the well-established understanding that aging is a significant risk factor for numerous chronic diseases and health conditions.

While our study unveils significant insights, it is imperative to recognize its inherent limitations. Foremost among these is our study's cross-sectional retrospective design. Such a design inherently challenges drawing definitive causal links between observed associations. Additionally, selection bias could have been introduced because our sample was derived from a single center. This could impact the generalizability of our findings to a broader population. Instruments used might have varied in calibration, or the personnel recording the data might have interpreted values differently. Given these limitations and potential biases, we must approach our findings with a degree of caution. We emphasize the need for future longitudinal studies.

CONCLUSION

In conclusion, our study presents significant insights into the relationship between age, hematological parameters, and systemic immune-inflammatory indices, especially

the SII. These findings reinforce our understanding of the role of systemic inflammation in aging but also highlight the potential clinical utility of markers like SII in assessing inflammatory status across different age groups. Future research could investigate whether age-specific SII cut-offs might improve the predictive accuracy for these pregnancy-related complications.

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Research Article

Evaluation of the Effect of SARS-COV-2 Infection During Pregnancy on Fetal Doppler Ultrasound Parameters: A Prospective Study

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Abstract

Aim: The fetal effects of severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) infection have been the subject of controversy since the beginning of the pandemic. We aimed to investigate the effect of SARS-COV-2 infection on fetal Doppler parameters.

Material and Methods: This prospective case-control study was conducted at İzmir Tepecik Training and Research Hospital between September 1, 2021, and June 1, 2022, on pregnant women confirmed to have SARS-CoV-2 by RT-PCR testing. Pregnant women who had mild to moderate coronavirus disease 2019 (COVID-19) infection during pregnancy were compared with a control group of pregnant women not infected with the COVID-19 virus. All Doppler and fetal biometry ultrasound assessments between 34 and 37 weeks of gestation were performed in our unit.

Results: Pregnant women infected and not infected with COVID-19 were demographically homogeneous. When the groups were compared in terms of fetal Doppler parameters, including left and right uterine artery (UtA) pulsatility index (PI), middle cerebral artery (MCA) PI, and systolic/diastolic ratio (S/D), as well as umbilical artery (UA) S/D and PI, no statistically significant difference was observed (p>0.05).

Conclusion: We consider that mild to moderate COVID-19 does not affect Doppler ultrasound parameters and fetal well-being during pregnancy.

Keywords: COVID-19, doppler ultrasound, fetal well-being

INTRODUCTION

The coronavirus disease 2019 (COVID-19) infection, which emerged at the beginning of 2020 and caused a pandemic in a short time, has been a major public health problem. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) may cause asymptomatic infection and present with various clinical conditions such as severe cough, fever, multi-organ failure, and respiratory distress (1-4). In the literature, pregnant and control groups of the same age with COVID-19 were compared and observed that pregnancy does not increase the risk of SARS-CoV-2 infection but aggravates clinical outcomes (5). Due to the immune suppression status during pregnancy, more severe complications and death rates are observed as a result of SARS-CoV and MERS-CoV infections in pregnant women (6-8).

Recent data show an increase in obstetric complications with COVID-19, such as preterm birth, fetal distress, and stillbirth (9). It was also found that the rate of hospitalization in the neonatal intensive care unit was higher in newborns born to mothers infected with SARS-

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CoV-2 (10). Considering the hypercoagulation and modulated immune status in pregnancy, the pulmonary and hematological effects of COVID-19 may cause adverse effects on the placental and fetal circulation (11).

Fetal Doppler parameters have been used safely for years to evaluate fetal well-being. Vascular ultrasonographic Doppler measurements such as uterine artery (UtA), umbilical artery (UA), middle cerebral artery (MCA) provide physicians with valuable information about the well-being of the fetus (12). This study aimed to evaluate the effects of COVID-19 infection on neonatal outcomes in the late preterm period, fetal biometry, and fetal Doppler measurements.

MATERIAL AND METHOD

This prospective case-control study was conducted on pregnant women infected with SARS-CoV-2 between September 1, 2021, and June 1, 2022, at Health Sciences University İzmir Tepecik Training and Research Hospital. Ethics committee approval was obtained before starting the study (decision no. 2021/8–14). Pregnant women with mild to moderate COVID-19 infection, regardless of hospitalization status, were compared with pregnant women of the same age with similar clinical characteristics followed for standard antenatal care in the control group. RT-PCR method was used to confirm the infection. Inclusion criteria were gestational age, late preterm, confirmed by head-rump length at 11-14 weeks of screening.

Women known to have multifetal pregnancies, maternal systemic diseases, need for intensive care, uterine anomaly, severe COVID-19 infection, and fetal structural anomalies were excluded. Also, even if the COVID-19 RT-PCR test result is negative, pregnant women with respiratory tract infection symptoms such as fever and cough were not included in the study. All pregnant women diagnosed with SARS-CoV-2 infection had symptoms such as fever, cough, diarrhea, and the positive PCR method obtained from nasopharyngeal swab samples was used for diagnosis.

Doppler measurements were made between 34 and 37 weeks of gestation by the same perinatologist working in the perinatology clinic of the Tepecik Training and Research Hospital, using the Samsung's HS70A with Prime Ultrasound machine CA1-7A convex probe (1.0 - 7.0 MHz) which is made Germany. UA, MCA, and UA Doppler values were recorded in the late preterm period in pregnant women who had COVID-19 infection and in control group patients. Fetal vessels used when evaluating fetal Doppler parameters; UtA, MCA, and UA (13). The recommendations provided by the International Society of Obstetric Ultrasound were followed when evaluating all Doppler parameters and measuring fetal biometry (14).

The statistical analysis was conducted using SPSS, Version 24.0 (SPSS Inc., Chicago, IL). The assumption of normality for variable distributions was assessed using the Shapiro-Wilk test. Variables with a normal distribution were presented as mean ± standard deviation, while those displaying non-normal distribution were characterized by the median and interquartile range (25th - 75th percentiles). When comparing individuals who were diagnosed with COVID-19 to those who were not, we performed an independent sample t-test or Mann-Whitney U test for continuous variables and a Chi-Square test for categorical variables. For the analysis of ultrasound parameters across different COVID-19 exposure trimesters, we employed either the one-way ANOVA or the Kruskal-Wallis test. The threshold for determining statistical significance was set at a two-tailed p-value of less than 0.05.

RESULTS

Our study included 81 pregnant women who had mild to moderate COVID-19 infections during their pregnancy and 70 patients who had no RT-PCR positivity or symptoms during their pregnancy. Table 1 shows the demographic characteristics of COVID-19 patients and the patients in the control group. The two groups were homogeneous and no statistically significant difference was recorded when demographic data were analyzed. UtA, UA, MCA Doppler measurement (pulsatility index [PI], systolic/diastolic ratio [S/D]), cerebroplacental ratio (CPR), amnion fluid index, head circumference, abdominal circumference, and femur length measurements performed in the late preterm period between 34 and 37 weeks, and there was no difference significantly between COVID-19 and control groups (Table 2).

Table 1. General characteristics of the COVID-19 group and the control group

	COVID-19 group (n= 81)	Control group (n=70)	p-value
Maternal age, years	28 (23-32)	31 (26-32)	0.286
Gravidity, n	3 (2-4)	3 (2-3)	0.153
Parity, n	2 (1-3)	2 (1-2)	0.083
Body mass index, kg/m²	27.5 (23.7-31.2)	28.04 (24.8–32.7)	0.066
Gestational week at COVID-19 infection	20 (12-25)	18.5 (17-24)	0.308
Gestational week at ultrasound examination	35.6 (35.2-37.2)	36 (36- 36.2)	0.549
Gestational week at delivery	38.3 (37.1-39.1)	38.1 (36.2-39.1)	0.536
Birth weight, g	3000 (2715-3210)	3050 (2670-3210)	0.931
Apgar score	8 (7-9)	8 (7-9)	0.250
Neonatal intensive care unit admission, n (%)	8 (9.9%)	8 (11.4%)	0.241

The patients were divided into 3 groups according to the trimester in which they had COVID-19 infection. There were 26 patients in the first trimester, 47 patients in the second trimester, and 8 patients in the third trimester. There was no significant difference between these groups in the Doppler parameters measured between 34 and 37 weeks (Table 3).

Table 2. Comparison of fetal Doppler and biometric measurements

	COVID-19 group (n= 81)	Control group (n= 70)	p-value
Umbilical artery S/D	2.76 (2.42-3.25)	2.90 (2.70-2.98)	0.433
Umblical artery PI	0.99 (0.87-1.15)	1.00 (0.90-1.10)	0.502
Middle cerebral artery S/D	6.53 (5.09-8.65)	6.11 (4.93-7.95)	0.130
Middle cerebral artery PI	1.96 (1.74-2.24)	1.90 (1.82-1.98)	0.137
Right uterine artery PI	0.93 (0.75-1.18)	0.99 (0.73-1.00)	0.266
Left uterine artery PI	0.93 (0.73-1.24)	0.96 (0.95-0.97)	0.773
Cerebroplasental ratio	2.30 (2-2.5)	2.31 (2-2.6)	0.331

S/D: Systolic/Diastolic ratio, PI: Pulsatily Index

Table 3. Doppler ultrasound parameters according to the timing of the trimester of the COVID-19 Infection

	1st trimester (n=26)	2nd trimester (n=47)	3rd trimester (n=8)	p-value
Umbilical artery S/D ratio	2.77 (2.36-3.52)	2.83 (2.47-3.21)	2.43 (2.10-2.77)	0.138
Umblical artery PI	1.01 (0.91-1.15)	1.00 (0.87-1.19)	0.86 (0.79-1.11)	0.348
Middle cerebral artery S/D	6.37 (4.49-7.83)	6.53 (5.50-9.20)	6.84 (4.91-8.13)	0.438
Middle cerebral artery PI	1.92±0.46	2.01±0.36	2.11±0.40	0.433
Right uterine artery PI	1.08 (0.86-1.39)	0.92 (0.73-1.16)	0.85 (0.60-1.17)	0.112
Left uterine artery PI	0.97 (0.76-1.25)	0.96 (0.75-1.25)	0.70 (0.57-0.93)	0.190

S/D: Systolic/Diastolic ratio, PI: Pulsatily Index

DISCUSSION

Both cohorts of pregnant women, including those with and without COVID-19 infection, demonstrated demographic homogeneity. In the course of our investigation, we meticulously analyzed several Doppler parameters, encompassing the left-right UtA PI, MCA PI, systolic/diastolic ratio (S/D), as well as UA S/D ratio and PI. Nonetheless, upon meticulous comparison of these parameters in relation to fetal Doppler profiles, no statistically significant discrepancies were ascertained.

SARS-CoV-2, which originated in Wuhan, China, spread rapidly, causing a pandemic in early January 2020 (15,16). Malperfusion and parenchymal infarction were noted in the placentas of pregnant women who had SARS-CoV-2 (17). In the angiogenic phase of placental development, there is an increase in fetal capillary volume and surface area required for fetomaternal gas exchange, and an increase in UA end-diastolic Doppler flow velocity is observed. On the contrary, in pregnancies with developmental delay with defective placentation, umbilical artery blood flow decreases abnormally, resulting in the absence of end-diastolic flow or reverse flow of UA (18,19).

Endothelial damage or hypoxemia causes low blood flow and high vascular resistance, resulting in placental insufficiency. Studies reveal that increased uterine artery flow impedance is associated with the development of preeclampsia, fetal growth restriction, and perinatal mortality. As placental dysfunction progresses, resistance in the villi causes a proportional increase in the UA Doppler indices, while a decrease in oxygen level causes a decrease in the MCA Doppler index. This effect can be easily and quickly measured non-invasively using UtA Doppler ultrasound (20). The negative effects of COVID-19 on the placenta impair uteroplacental circulation by causing pathophysiological hypoxemia, thrombosis, and villitis. In the study of Anuk et al., an increase was observed in umbilical and uterine artery PI and RI in pregnant women who had COVID-19 compared to the control group (21). In this study not found differences in UtA Doppler parameters between the two groups in this study.

MCA Doppler examination is an important tool in the diagnosis and treatment of fetal anemia and intrauterine growth restriction. Investigation of fetal central nervous system hemodynamics and in cases with intrauterine growth restriction, MCA Doppler flow shows early and late changes. When the relationship of MCA flow rate in normal and abnormal pregnancies with perinatal outcomes was evaluated, they concluded that a higher rate of abnormal flow was observed in pregnancies with intrauterine growth retardation and MCA flow rate measurement was reliable in the diagnosis of fetal distress (22). In this study, there was no difference in MCA Doppler parameters between the two groups.

CPR is especially important for the evaluation of fetal well-being in the near-term follow-up of fetuses with intrauterine growth restriction (23). The cerebroplacental ratio is an important parameter to predict adverse perinatal outcomes. No significant difference was observed when CPR was compared in the COVID-19 and control groups. A recent study reported that fetal brain and heart tissues do not express Angiotensin-Converting Enzyme-2 (ACE-2) receptors and that these organs will not be a target for the virus (24). In this study, there was no difference in CPR parameters between the two groups.

There are many publications and unanswered questions in the literature about the effect of SARS-CoV-2 infection on the fetus and placenta. In the early stages of the pandemic, transmission from mother to fetus was reported as negligible. However, in a study that included 936 newborns from mothers affected by COVID-19 according to newer and larger studies, 27 newborns reported that they were RT-PCR positive and the rate of transmission was 3.2% (25). Despite this, the actual risk of vertical transmission and its potential consequences on the fetus is currently largely unknown.

In recent studies, it has been observed that SARS-CoV-2 causes arteriopathy in the placenta, fibrinoid necrosis, and mural hypertrophy of decidual arterioles, but it was not detected in newborns (26). In this study, the placenta was not analyzed. However, like any situation that may cause placental hypoperfusion, it is thought that SARS-CoV-2 infection may be associated with conditions such as intrauterine growth restriction and stillbirth, preeclampsia (27). However, in this study, no significant difference was observed between the two groups in Doppler and biometric measurements, in which we evaluated perfusion and fetal well-being.

The strengths of the study are its prospective design, Doppler and biometric measurements made by the same perinatologist, and numerous parameters investigated. Limitations are examination of mild to moderate COVID-19 patients only, and lack of postnatal fetal and maternal follow-up.

Anxiety symptoms are an independent risk factor for adverse obstetric outcomes during pregnancy (28). Increasing anxiety in the antenatal period may cause mental adverse conditions such as increased postnatal depression, as well as negative obstetric outcomes such as premature birth and fetal growth restriction (29-31).

CONCLUSION

It has been reported that SARS-CoV-2 has negative effects on the placenta in the literature. However, as seen in recent publications, it was observed in this study that pregnant women with mild/moderate COVID infection did not reflect negatively on newborn outcomes, fetal biometry and Doppler measurements (32). It has been reported that anxiety can cause both negative effects on maternal mental health and obstetric complications during pregnancy. It has been reported that pregnancy anxiety increases during the COVID-19 pandemic and that early diagnosis and intervention by a psychiatrist can prevent stress-related pregnancy complications (33). In the literature, it is considered that there is no need for additional visits in pregnancy follow-up for mild to moderate COVID-19 patients (26). In this study, it was observed that there was no deterioration in Doppler and biometric measurements for mild and moderate COVID-19 patients compared to the controls.

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MEDICAL RECORDS-International Medical Journal

Research Article

Effect of Folic Acid in Sepsis-induced Lung Damage in Rats

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Abstract

Aim: Sepsis-induced lung injury remains a critical concern with significant morbidity and mortality. This study aimed to investigate the potential therapeutic role of Folic Acid in mitigating lung injury induced by sepsis while exploring its interaction with the soluble suppression of tumorigenicity 2 (sST2) protein in an experimental rat model.

Material and Methods: Rats were divided into three groups: a normal control group, a group induced with sepsis and treated with saline, and a group induced with sepsis and treated with Folic Acid (5 mg/kg). Biomarkers of oxidative stress, inflammation, respiratory gas exchange, and lung histopathology were assessed.

Results: Folic Acid administration resulted in significantly decreased Malondialdehyde (MDA) levels ($p<0.01$) and reduced soluble ST2 levels ($p<0.05$) compared to the sepsis-induced saline group. Tumor necrosis factor α (TNFα) levels were markedly reduced ($p<0.001$), and lung histopathology demonstrated decreased alveolar inflammation and septal thickness. Additionally, improved oxygenation (PaO_2) was observed following Folic Acid treatment ($p<0.05$), while $PaCO_2$ remained stable.

Conclusion: These findings suggest that Folic Acid protects against sepsis-induced lung injury, ameliorating oxidative stress, inflammation, and histopathological alterations. The modulation of soluble ST2 levels may implicate Folic Acid's role in immune regulation and potential crosstalk with the IL-33/ST2 pathway. Despite promising results, limitations inherent to animal models and the complexities of clinical translation warrant further investigation. This study highlights the potential of Folic Acid as a therapeutic intervention in sepsis-induced lung injury. It underscores the need for mechanistic studies and clinical trials to validate its effectiveness in human patients.

Keywords: Sepsis, acute lung injury, soluble ST2, folic acid

INTRODUCTION

Sepsis is a clinically significant condition with widespread implications, especially in critical care settings. The mortality rate associated with the hyperactive immune response to infections, specifically the occurrence of multiple organ dysfunction, ranges from 25% to 52% (1). In the initial stages of sepsis, the lungs, kidneys, and liver are among the organs that experience a significant impact. The presence of dysfunction in two or three of these factors exhibits a strong correlation with heightened mortality rates among patients diagnosed with sepsis (2). A "cytokine storm" phenomenon results from an exaggerated immune response directed toward combating and containing an infection (3). Cytokines have a substantial impact as pleiotropic regulators in modulating the immune response and are essential in the complex pathophysiology of sepsis. By demonstrating both pro- and anti-inflammatory properties, they can regulate the immune response in the context of an infection.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are pathological states that manifest with the abrupt onset of respiratory failure, leading to significant morbidity and mortality (4). According to empirical evidence, individuals who effectively recuperate from ALI encounter an adverse impact on their long-term quality of life (5). Considerable advancements have been achieved in understanding this malady's epidemiological facets, pathogenic mechanisms, and therapeutic strategies. However, further progress is necessary to reduce the rates of mortality and morbidity related to ALI and ARDS (6).

Folic acid (FA), also known as vitamin B9, is an essential

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nutrient that plays a crucial role in DNA synthesis, cell division, and other cellular processes (7). It is essential during rapid cell growth and division, such as pregnancy. While FA is primarily known for its role in cell division and DNA synthesis, it also has anti-inflammatory properties. It has been investigated for its potential to modulate inflammatory responses (8).

ST2, the interleukin-1 receptor-like 1 (IL1RL1), is a protein receptor involved in immune responses and inflammation. ST2L is expressed on the surface of specific immune cells, such as T-helper 2 (Th2) cells, which play a role in allergic responses and immune regulation. When ST2L binds to its ligand, interleukin-33 (IL-33), it can trigger signaling pathways contributing to immune responses and inflammation (9).

Soluble suppression of tumorigenicity 2 (sST2) has been identified as a promising biomarker for heart failure, with potential applications in inflammatory diseases due to its ability to indicate increased serum sST2 concentrations (10). sST2, on the other hand, is a soluble version of the ST2 receptor that is released into the bloodstream. It acts as a decoy receptor, preventing IL-33 from binding to membrane-bound ST2L (10). This IL-33/ST2 pathway modulation has been implicated in various physiological and pathological processes, including immune responses, inflammation, and tissue repair. ST2 and its soluble form might serve as biomarkers of disease severity (10).

This study examines the potential impact of FA on sepsis-induced lung injury (SILI) through the utilization of histopathological examination, assessment of lipid peroxidation (LPO), and immunohistochemistry in a rat model of sepsis.

MATERIAL AND METHOD

Animals

This study utilized a sample of 30 male Wistar albino mature rats with an average weight of 200 to 250 grams. The experiments conducted in this study were executed by the guidelines outlined in the Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health in the United States. After obtaining approval from the Animal Ethics Committee (Science University, Ethical number: 2023083321), The laboratory rats utilized in the experiment were sourced from the Experimental Animal Laboratory at Science University. The rats were provided with unlimited access to food. They were kept in steel cages under controlled environmental conditions, maintaining a temperature of 22±2°C and a light/dark cycle of 12 hours each.

Experimental Procedures

A study was conducted on a sample of 30 rats. Twenty rats were randomly divided into three groups. The feces intraperitoneal-injection group (FIP) procedure was conducted on these rats to induce a sepsis model. A total of ten rats were divided into two groups: a normal group and a group that did not undergo any experimental procedures. The FIP rat model was established using a methodology previously outlined by Karaali et al. (11). The fecal samples were gathered and subsequently suspended in a saline solution, resulting in the preparation of the fecal saline solution. They were administered intraperitoneal injection at 1 gram per kilogram of body weight. The structure and organization of study groups were devised as follows: Group 1 consisted of 10 individuals who served as the normal control group. These individuals did not undergo any surgical procedures and were orally fed. Group 2, on the other hand, consisted of 10 rats diagnosed with FIP. This group was administered 1 ml/kg of tap water as a placebo via oral gavage. In Group 3, the subjects were administered FIP and 5 mg/kg of FA (specifically, Folbiol tablet 5 mg manufactured by Menarini) via oral gavage. The sample size for this group was 10. All treatments were administered one hour following the FIP procedure. The study was concluded within a 24-hour timeframe. A total of six rats expired within the initial 24-hour period after the procedure, resulting in their exclusion from the study. Specifically, four rats from the placebo group and two from the FA group were among the deceased.

After the study, all animals underwent euthanasia through cervical dislocation, following administration of anesthesia consisting of Ketamine (100 mg/kg, Ketasol, Richterpharma AG Austria) and xylazine (50 mg/kg, Rompun, Bayer, Germany). Subsequently, blood samples were obtained via cardiac puncture for biochemical analysis.

Determination of TNF-a and sST2 in Plasma

Plasma tumor necrosis factor α (TNFα) and sST2 levels were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Biosciences and Abcam. The measurements were conducted following the guidelines provided by the manufacturer.

Measurement of Lipid Peroxidation

Lipid peroxidation was quantified in plasma samples by assessing malondialdehyde (MDA) concentrations as thiobarbituric acid reactive substances. Concisely, the experimental procedure involved the addition of trichloroacetic acid and TBARS reagent to the plasma samples. Subsequently, the mixture was thoroughly combined and incubated at 100°C for 60 minutes. After cooling on ice, the samples underwent centrifugation at a speed of 3000 revolutions per minute for 20 minutes. Subsequently, the absorbance of the resulting supernatant was measured at a wavelength of 535 nanometers.

Histopathological Examination of Lung

To facilitate histological analysis, anesthesia was administered to all animals via intraperitoneal injection of Ketamine and xylazine. Following that, the subjects were subjected to perfusion using a solution comprising 200 milliliters of 4% formaldehyde in a phosphate-buffered saline (PBS) with a concentration of 0.1 M. Lung sections with a thickness of 5 µm, which had been fixed with formalin, were subjected to staining using the hematoxylin and eosin (H&E) technique. The sections were obtained through an Olympus C-5050 digital camera, securely attached to an Olympus BX51 microscope. The primary histopathological lung damage score was calculated per

the methodology described in prior research. In brief, the histopathological assessment of lung injury encompassed the evaluation of several parameters, namely alveolar congestion (AC), hemorrhage (H), leukocyte infiltration or aggregation in air spaces/vessel walls (AL), perivascular/interstitial edema (PE), and the thickness of the alveolar wall/hyaline membrane formation (TA). The severity of each item was evaluated utilizing a grading scale encompassing a range of values from 1 to 4. Each grade was associated with a particular percentage range: 1 (0-25%), 2 (25-50%), 3 (50-75%), and 4 (75-100%) (12).

Arterial Blood Gas Analysis

The carotid artery blood of rats in each group was sampled (0.2 mL) at 24 h after the operation, and PaO2 and PaCO2 in the blood samples were assayed using a blood gas analyzer.

Statistical Analysis

The data are displayed in the format of mean values and the standard error of the mean (SEM). The data analyses were performed using SPSS version 15.0 for the Windows operating system. The data underwent analysis utilizing the non-parametric Mann-Whitney U test. P-values equal to or less than 0.05 were considered to have statistical significance.

RESULTS

Plasma oxidative stress marker malondialdehyde (MDA) levels exhibited significant differences among the experimental groups. The standard control group measured the plasma MDA level at 14.3±1.02 nM/mg protein. Conversely, the group subjected to FIP induction and saline treatment demonstrated a markedly elevated plasma MDA level of 48.3±1.6 nM/mg protein ($p<0.001$). Notably, the administration of 5 mg/kg FA to the FIP-induced rats resulted in a significant reduction in plasma MDA levels, measuring 33.5±0.8 nM/mg protein ($p<0.01$), compared to the FIP and saline group (Table 1).

Regarding the plasma sST2 levels, the normal control group displayed a 0.85±0.1. In contrast, the FIP-induced rats treated with saline exhibited a significantly higher plasma sST2 level of 2.59±0.2 ($p<0.05$). In the FIP-induced group treated with 5 mg/kg FA, the plasma sST2 level was reduced to 1.24±0.1 (#$p < 0.01$#) in comparison to the FIP and saline group (Table 1).

Measurement of plasma tumor necrosis factor alpha (TNF-α) levels revealed substantial variations across the experimental groups. The normal control group registered a TNF-α level of 15.2±1.8 pg/ml. Remarkably, the FIP-induced rats treated with saline displayed a significantly elevated plasma TNF-α level of 374.3±8.2 pg/ml ($p<0.001$). However, administration of 5 mg/kg FA to FIP-induced rats led to a substantial reduction in plasma TNF-α levels, measuring 228.1±5.5 pg/ml (##$p<0.001$##), in comparison to the FIP and saline group (Table 1).

The levels of various biomarkers in different lung tissues exhibited significant variations among the experimental groups. In the normal control group, the levels of AC, H, AL, PE, and TA were measured at 0.3±0.2, 0.2±0.1, 0.3±0.2, 0.5±0.1, and 0.2±0.2, respectively (Table 2).

Upon induction of FIP and subsequent saline treatment, a pronounced increase was observed in all biomarkers' levels. Specifically, the FIP-induced rats treated with saline displayed significantly elevated levels of AC (2.6±0.2, $p<0.001$), H (1.5±0.1, $p<0.01$), AL (2.1±0.2, $p<0.001$), PE (2.9±0.1, $p<0.001$), and TA (2.8±0.3, $p<0.001$), compared to the respective normal control values (Table 2).

However, the administration of 5 mg/kg FA to the FIP-induced rats led to significant mitigation of these elevated biomarker levels. The levels of AC (1.2±0.3, ##$p<0.001$##), H (0.9±0.1, ##$p<0.001$##), AL (1.1±0.2, #$p<0.05$#), PE (1.7±0.3, #$p<0.05$#), and TA (1.5±0.2, #$p<0.05$#) were notably reduced in comparison to the FIP and saline group (Table 2).

Table 1. The results of the biochemical analysis in the three study groups: Normal control, FIP and saline group, and FIP and 5 mg/kg Folic Acid group

	Normal control	FIP and saline	FIP and 5 mg/kg Folic Acid
Plasma MDA (nM/mg protein) level	14.3±1.02	48.3±1.6**	33.5±0.8#
Plasma soluble ST2 level	0.85±0.1	2.59±0.2*	1.24±0.1#
Plasma TNF alfa (pg/ml) level	15.2±1.8	374.3± 8.2**	228.1±5.5##

Results were presented as mean±SEM. Statistical analyses were performed by one- way ANOVA. *$p<0.05$, **$p<0.001$ different from normal groups; #$p<0.01$, ##$p<0.001$ different from FIP and saline group

Table 2. The results of the histopathological examination of the lung in the three study groups: Normal control, FIP and saline group, and FIP and 5 mg/kg Folic Acid group

	Normal control	FIP and saline	FIP and 5 mg/kg Folic Acid
AC	0.3±0.2	2.6±0.2**	1.2±0.3##
H	0.2±0.1	1.5±0.1*	0.9±0.1##
AL	0.3±0.2	2.1±0.2**	1.1±0.2#
PE	0.5±0.1	2.9±0.1**	1.7±0.3#
TA	0.2±0.2	2.8±0.3**	1.5±0.2#

Results were presented as mean±SEM. Statistical analyses were performed by one- way ANOVA. *$p<0.01$, **$p<0.001$ different from normal groups; #$p<0.05$, ##$p<0.001$ different from FIP and saline group

The respiratory gas exchange parameters, including arterial oxygen tension (PaO2) and arterial carbon dioxide tension (PaCO2), exhibited significant variations across the experimental groups. In the normal control group, the PaO2 level was recorded at 99.5±4.4 mmHg, reflecting normal oxygenation levels (Table 3).

In contrast, the FIP-induced rats treated with saline displayed a markedly reduced PaO2 level of 54.6±3.8 mmHg ($p<0.001$), indicating severe impairment in oxygen exchange. Remarkably, the administration of 5 mg/kg FA to FIP-induced rats resulted in a significant improvement in PaO2 levels, measuring 85.1±5.7 mmHg ($p<0.05$), compared to the FIP and saline group (Table 3).

Analysis of arterial carbon dioxide tension (PaCO2) revealed noteworthy differences among the experimental groups. The normal control group exhibited a PaCO2 level of 40.6±2.9 mmHg, which falls within the normal range. In the FIP-induced group treated with saline, the PaCO2 level was reduced to 31.6±1.9 mmHg ($p<0.05$). Similarly, the FIP-induced rats administered with 5 mg/kg FA displayed a PaCO2 level of 30.5±1.3 mmHg, comparable to the FIP and saline group (Table 3).

Table 3. The results of the blood gas analysis in the three study groups: Normal control, FIP and saline group, and FIP and 5 mg/kg Folic Acid group

	Normal control	FIP and saline	FIP and 5 mg/kg Folic Acid
PaO2 (mmHg)	99.5±4.4	54.6±3.8**	85.1±5.7#
PaCO2 (mmHg)	40.6±2.9	31.6±1.9*	30.5±1.3

Results were presented as mean±SEM. Statistical analyses were performed by one- way ANOVA and post- hoc Bonferroni test. *$p<0.05$, **$p<0.001$ different from normal groups; #$p<0.05$ different from FIP and saline group

The histopathological analysis of lung tissues was performed using hematoxylin and eosin (H&E) staining to assess the structural changes and inflammatory responses. Representative micrographs captured at 40x magnification are presented below (Figure 1).

A. Normal Control Group Lung: Figure 1A shows lung tissue from the normal control group. Alveolar structures appear intact and well-maintained.

B. FIP Group with Severe Histopathologic Alterations: Figure 1B displays lung tissue from the FIP group, demonstrating severe histopathological alterations characterized by increased alveolar inflammation (*) and thickened septal structures (arrow).

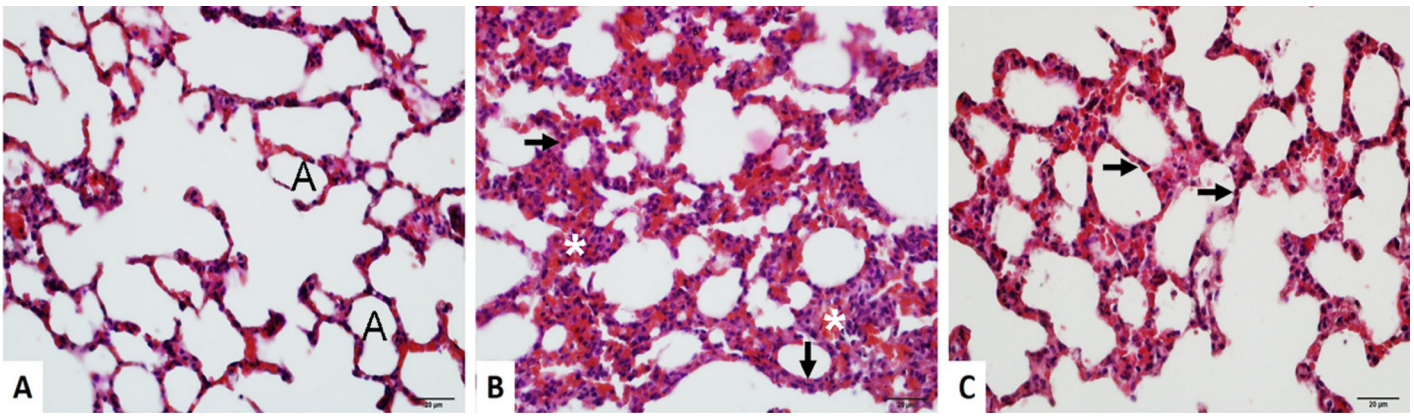

Figure 1. Lung histolopathology x40 magnification H&E staining. A: Normal control group lung, (A) Alvelol, B: FIP groups showed severe histopathologic alteration related to increased alveolar inflammation (*) and septal septal thickness (arrow), C: FIP and 1 ml/kg tap water (plasebo) groups showed severe histopathologic alteration related to increased alveolar inflammation (*) and septal septal thickness (arrow), D: FIP and 5 mg/kg Folic Acid groups showed decreased inflammation and septal septal thickening (arrow). Scale bar=20µm

C. FIP and Placebo Group with Severe Histopathologic Alterations: Figure 1C depicts lung tissue from the FIP group treated with 1 ml/kg tap water (placebo). Like the FIP group, this group exhibits marked alveolar inflammation (*) and septal thickening (arrow).

D. FIP and 5 mg/kg FA Group with Reduced Inflammation: Figure 1D illustrates lung tissue from the FIP group treated with 5 mg/kg FA. Notably, this group reduces inflammation and septal thickening (arrow), suggesting a potential protective effect of FA treatment.

The scale bar in all micrographs corresponds to 20 µm, indicating the relative size of the captured tissue area.

DISCUSSION

Sepsis-induced lung injury remains a critical clinical challenge, often leading to ARDS and compromised respiratory function (13,14). In this study, we aimed to investigate the potential therapeutic effects of FA in mitigating the pathological alterations associated with sepsis-induced lung injury and to explore the potential interplay between FA and the sST2 (sST2) protein, a known inflammation biomarker.

Our results provide compelling evidence for the beneficial effects of FA in the context of sepsis-induced lung injury. FA is a cofactor in one-carbon metabolism that promotes the restoration of methionine from homocysteine-mediated preservation of neuronal integrity and has been shown to have antioxidant activity (15). New data have been added to

the literature to strengthen the idea that FA is an essential micronutrient that plays a novel role in combating ROS. Therefore, it acts as a redox regulator through the proper regulation of GSH biosynthesis, GSH transport system, and mitochondrial GSH recycling through the reorganization of the precipitating proteins of ROS and proper regulation of redox homeostasis and prevention of mitochondrial GSH depletion (16). The profound increase in oxidative stress, as indicated by elevated MDA levels in the FIP and saline group, underscores the magnitude of the oxidative damage inflicted upon lung tissues. However, FA administration was associated with a significant reduction in MDA levels. FA's anti-inflammatory and antioxidant properties likely contribute to these effects, as it regulates GSH biosynthesis and reduces the production of pro-inflammatory mediators (17).

Similarly, Shalby et al. show FA's antioxidant effect in acute lung injury in rats (18). This finding aligns with FA's known antioxidant properties, which could counteract the generation of reactive oxygen species and prevent oxidative stress-induced cellular damage. Similarly to MDA in TNF-α, FA reduces TNF-α levels in the FIP group.

A study conducted by Hoogerwerf et al. 2010 observed that sST2 concentration could predict the severity of sepsis (19). Moreover, Hur et al. 2015 similarly describe that sST2 has a prognostic role in sepsis. In this study, we observed a noteworthy decrease in sST2 levels upon FA treatment. sST2, a decoy receptor for interleukin-33 (IL-33) (20), is associated with inflammation and immune responses. The substantial elevation of sST2 levels in the FIP and saline group likely reflects the exaggerated inflammatory response of sepsis-induced lung injury. FA's ability to attenuate sST2 levels might suggest a regulatory effect on the immune response, potentially via modulation of IL-33 signaling. Although the precise mechanisms underlying this interaction warrant further investigation, our findings suggest that FA might exert its anti-inflammatory effects through modulation of the IL-33/ST2 pathway.

As known, methotrexate is the antagonist of FA and causes lung injury-like side effects (21). Contrary histopathological analysis shows the protective effects of FA. The reduced alveolar inflammation and septal thickening observed in the FIP and 5 mg/kg FA group suggest potential mitigation of lung injury by FA administration.

The intricate relationship between FA and the IL-33/ST2 pathway warrants special attention. IL-33, a cytokine involved in immune responses and tissue repair, binds to its receptor ST2L on immune cells to initiate signaling. The soluble form of ST2 acts as a decoy receptor, competing with ST2L for IL-33 binding. Upon FA treatment, the observed reduction in sST2 levels might reflect potential crosstalk between FA and the IL-33/ST2 axis. Given FA's ability to attenuate the inflammatory response, it is plausible that FA may modulate IL-33 signaling by altering sST2 levels (22).

Limitations

The study utilized a rat model of sepsis-induced lung injury, which may need to fully capture human sepsis's complexity. The study employed a specific dosage of Folic Acid (5mg/kg) for treatment. The optimal dosage and timing of Folic Acid administration for sepsis-induced lung injury in humans might vary and need to be explored. While the study hinted at potential mechanisms underlying the observed effects, mechanistic insights into how Folic Acid interacts with the IL-33/ST2 pathway and modulates inflammatory responses remain speculative. The study focused on histopathological and biomarker changes in a controlled experimental setting. Clinical translation of these findings to actual patient outcomes requires rigorous clinical trials considering factors such as patient heterogeneity, comorbidities, and variations in treatment responses. The study evaluated the effects of Folic Acid alone. The study duration might have yet to capture the long-term effects of Folic Acid treatment or the potential for disease progression beyond the observed timeframe. Longer-term studies provide a more comprehensive understanding of Folic Acid's sustained effects.

CONCLUSION

This study provides valuable insights into the potential therapeutic benefits of FA in sepsis-induced lung injury. FA demonstrated its capacity to alleviate oxidative stress, inflammation, and histopathological alterations, collectively indicating its protective effects on lung tissues. Additionally, the modulation of sST2 levels and its potential interaction with the IL-33/ST2 pathway suggest a complex network of immune regulation that merits further investigation.

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Research Article

Retrospective Assessment of Forensic Facts Under 18 Years of Age in İzmir

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Abstract

Aim: The aim of this study was to examine the cases under the age of 18 who applied to the forensic medicine outpatient clinic of our hospital in İzmir.

Material and Methods: Among the 6,492 reports prepared for the patients who applied to İzmir Katip Çelebi University, Atatürk Training and Research Hospital Forensic Medicine Polyclinic between January 2019 and January 2022, 471 final reports prepared for forensic cases under the age of 18; age, gender, type of incident, area of injury, according to TCK 86 law clause; It was examined retrospectively with parameters such as whether it is life-threatening or not, and whether it can be eliminated with a simple medical intervention or not.

Results: In our study, 471 reports were examined; The most common age was 18, the mean age was 14, the number of girl cases was 203 (43.1%), the number of male cases was 268 (56.9%), the most common type of event was the assault, while traffic accidents were the second most frequent, the most frequently injured body area in cases under 18 years old was the extremities in 154 (32.7%), followed by head injuries in 101 (21.4%) cases, and life-threatening injuries in 19 (4%) cases. It was found that it was seen most frequently in July and least in April.

Conclusion: The data determined in our study were in parallel with similar studies in the literature, while the most common cause of death in autopsy series was determined as traffic accident, it was seen that the most common cause of injury in outpatients who applied to our polyclinic was assault.

Keywords: Forensic reports, child, İzmir, forensic medicine

INTRODUCTION

İzmir is the third most populous city of the Republic of Türkiye in terms of population, which has a coast to the Aegean Sea in the westernmost part of the Republic of Türkiye. It is one of the leading cities of the country in economic, socio-cultural terms. According to the results of the Address Based Population Registration System (ADNKS); As of the end of 2021, Türkiye's population is 84 million 680 thousand 273 people, of which 22 million 738 thousand 300 are children. 51.3% of the child population is boys and 48.7% are girls. According to the estimated data of İzmir for 2022, it is 4,455,294 and its population under the age of 18 is; 783,993 species. When the child population ratios of 27 European Union (EU) member countries are examined; It has been observed that Türkiye's child population rate is 26.9% higher than that of EU member states (1).

According to the Convention on the Rights of the Child, which has been implemented by our country since 1995, "Every person under the age of 18 is considered a child, except for being considered a minor at a younger age by national laws." According to the Turkish Penal Code, a person who has not completed the age of 18 is defined as a child (2).

The World Health Organization defines child abuse as "behaviors involving physical and/or emotional maltreatment, neglect, use of the child for all kinds of commercial interests, which may harm the health, life, development and value of the child in his or her general state of responsibility, trust and ability" (3). Although sexual

CITATION

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abuse comes to mind when child abuse is mentioned in Türkiye, physical abuse, emotional abuse and neglect are other types of abuse that should be emphasized. Although there is no descriptive study to give an idea about the whole of Türkiye, it is estimated that the frequency of physical abuse is 30-35%, and 13% for sexual abuse (4).

Every event that causes deterioration of health, injury or death as a result of will, negligence, carelessness and carelessness of the person himself or another is defined as a judicial case. Another common cause of forensic incidents involving children and adolescents is juvenile delinquency. According to the Child Protection Law, a juvenile delinquent is defined as a juvenile for whom an investigation or prosecution has been made with the allegation that he has committed an act defined as a crime in the law, or for whom a security measure has been decided for the act he has committed (5).

According to TUIK data, the number of incidents involving children who came or were brought to security units in 2020 was 450 thousand 803. In these incidents, 37.9% of the children were accused of committing an act defined as a crime in the law (driving into a crime), 18.5% of them were alleged to have committed a misdemeanor, and 13.0% of them were seeking information. It was determined that 5.0% of them came to the security units because of missing (about which a loss application was made and found later) and 0.2% of them came to the security units for reasons other than these reasons (6).

When the total number of children dragged into crime in criminal courts in 2019 is analyzed on a provincial basis; it was seen that the highest number of juvenile delinquents was in Istanbul with a rate of 17.6%, followed by Ankara with a rate of 5.8%. When the regions are examined in terms of the number of children driven to crime in 2019; It is seen that the number of children dragged into crime in a population of 100000 is in the Aegean Region with the highest 1973 children. When the crime types of the children dragged into crime in İzmir are examined; The crime of theft takes the first place with a rate of 68.6% among the lawsuits filed against the crimes against property. In the cases filed for crimes against public health, the crime of purchasing, accepting or possessing drugs or stimulants or using drugs or stimulants is taken in the first place with a rate of 80.3% (7).

Forensic incidents occurring in childhood are among the leading preventable health problems all over the world. Detection of forensic cases in the region we live in is important in terms of taking appropriate measures. The aim of this study; The aim is to provide data that will provide the basis for the practices that will ensure the elimination of the victimization of the children who are the subject of forensic cases and prevent them from being dragged into crime.

MATERIAL AND METHOD

Before the study, ethical approval was obtained from the İmir Katip Çelebi University Health Sciences Non-Interventional Clinical Research Ethics Committee (No: 2022/0281).

Out of 8,492 reports prepared for the patients who applied to İzmir Katip Çelebi University Atatürk Training and Research Hospital Forensic Medicine Polyclinic between January 2019 and January 2022, 471 reports prepared for forensic cases under the age of 18, according to age, gender, type of incident, injury region, TCK Article 86. according to; It was examined retrospectively with parameters such as whether it is life-threatening or not, and whether it can be eliminated with a simple medical intervention or not.

The obtained data were evaluated statistically. Statistical analyses were conducted using IBM SPSS Statistics for Windows 29.0 software. The Mann Whitney U test was employed to compare non-normally distributed variables across two groups. A p-value less than 0.05 was deemed statistically significant.

RESULTS

Examining 471 final reports prepared for forensic cases under the age of 18, among the 8,492 reports prepared for the patients who applied between January 2019 and January 2022, it was determined that 268 (56.9%) of the patients who applied were male.

In forensic reports, the most common type of incident was found to be assault with 286 (60.7%) cases, and traffic accidents were the second most frequent with 90 (19.1%) cases. The gender and event type distributions of the cases are given in Figure 1. When the injured body parts of the cases were examined, it was seen that the most frequently affected body area was the extremities with 154 (32.7%) cases, and the head was the second most frequently injured with 101 (21.4%) cases. The number of cases with 2 body parts affected was 91 (19.3%), and the number of cases with 3 or more body parts affected was 91 (19.3%).

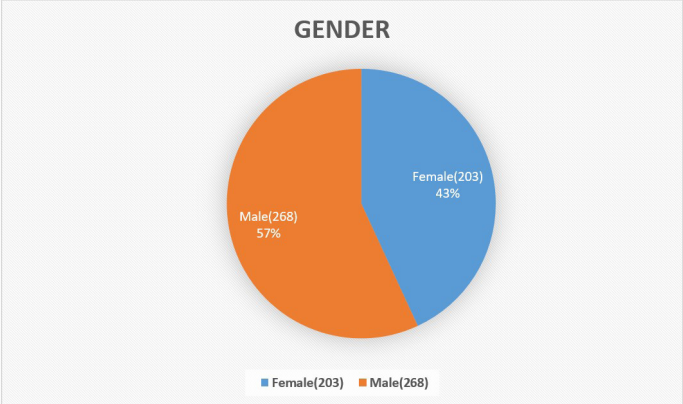

Figure 1. Distribution of cases by genders

When the cases that could be resolved with simple medical intervention were examined, it was found that 346 (73.5%) cases could be resolved with simple medical intervention, and 100 (21.2%) cases could not be cured. When the life-threatening status of the cases was examined, it was found

that there was life-threatening in 19 (4.0%) cases, and no life-threatening situation in 450 (95.6%) cases (Figure 2).

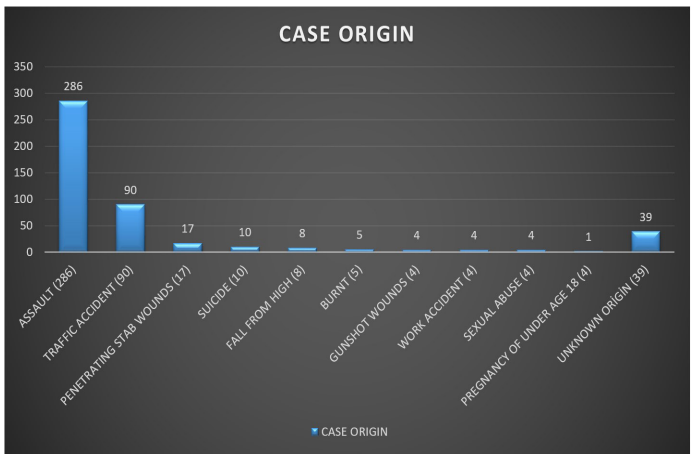

Figure 2. Distribution of event types

The injured body parts and numbers of the cases, their ability to be healed with simple medical intervention, and life-threatening situations are given in Table 1.

Table 1. Injured body parts and numbers of cases, recoverability with simple medical intervention, life-threatening situations

Injured body areas	Female	Male	Total
Extremity	59	95	154 (32.7%)
Head	42	59	101 (21.4%)
Body	7	13	20 (4.3%)
Neck	1	4	5 (1.1%)
Back	0	5	5 (1.1%)
No lesions	32	37	69 (14.6%)
2 areas	68	23	91 (19.3%)
3 and more areas	6	20	26 (5.5%)
Recoverability with simple medical intervention			
Curable	138	208	346 (73.5%)
Incurable	27	73	100 (21.2%)
Unspecified	18	7	25 (5.3%)
Vital endangered status			
Yes	5	14	19 (4.0%)
No	125	325	450 (95.6%)
Unspecified	0	2	2 (0.4%)

The median age of the cases was 15 (interquartile range 6) and the most common age of the cases was 18. The mean age of the cases included in the study was calculated as 13.7. When the cases were grouped according to their age, 33 (7%) between the ages of 0-5, 64 (13.6%) between the ages of 6-10, and 155 (32%) between the ages of 11-15. 9), and 219 (46.5%) between the ages of 15-17. The median age of male and famale subjects was 15 (İnterquartile range 6) for both groups, and no statistical significance was found between the groups (p>0.05, Mann Whitney U test). The distribution of the cases according to their ages is shown in Figure 3.

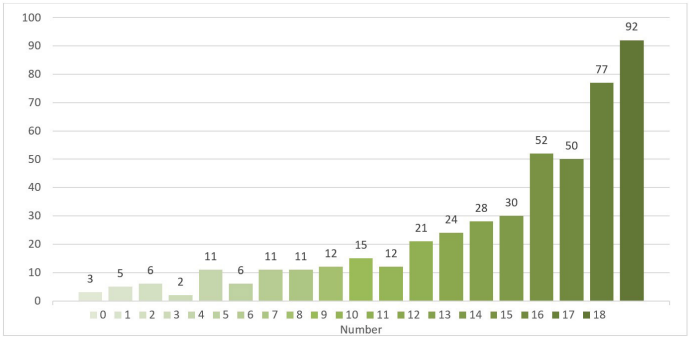

Figure 3. Distribution of cases by age groups

DISCUSSION

Forensic cases have an important place in the applications of health institutions. Evaluation of juvenile cases from these cases and revealing risky situations will prevent juveniles from being dragged into crime and from being harmed, together with the measures to be taken (8).

When the literature is examined, it has been determined that male gender is more common in forensic cases in the pediatric age group. Sever et al. In their study, the rate of male cases was found to be 66.3% (9). Çetinel et al. In the study in which he evaluated pediatric forensic cases, the rate of male cases was determined as 58%. Consistent with the literature, it was determined that 56.9% of the forensic cases in our study were male.

When the cases were evaluated according to age groups, Özdeş et al. In his study evaluating the forensic cases in Kastamonu, it was determined that the most common forensic cases were between the ages of 11-15, followed by the cases between the ages of 15-17 (10). In the same study, it was stated that the highest number of cases were 15 and 16 years old. Esen et al. In his study in which he evaluated the forensic cases who applied to the pediatric emergency clinic, it was determined that 37.94% of the cases were between the ages of 11-18 (11). In our study, the median age of forensic cases was 15 (SCA 6) and it was determined that forensic cases were frequently seen in the 15-17 age group. Consistent with the literature, it is seen that the frequency of forensic cases increases with age. We think that the reason for this is that with the increasing age, the child is involved in social life, and accordingly, he/she may face more situations such as assault and traffic accidents.

When the types of events that the cases were exposed to were examined, Özdeş et al. In the study, assault was found to be the most common with 35.44%, sexual events were the second most common with 24.05%, and traffic accidents were the third most common with 22.78%. Demir et al. In the study, traffic accidents (76%) were the first, falling (12.7%) and battering (6.1%) were the third (12). In our study, it was observed that the most common type of event was assault with 286 (60.7%) cases, and

traffic accidents were found to be the second most frequent with 90 (19.1%) cases. When the literature was examined, it was seen that the frequency of different types of events was found to be high in different studies. We think that this situation develops due to the difference in the type of event in the relevant centers. For this reason, it can be generalized for our region that assault and traffic accidents are common causes of forensic cases.

When examining body injuries encountered in forensic cases, Korkmaz et al. found that the most frequently injured body region was the head and neck region, followed by the upper extremity (13). Sever et al. In the study of, the frequency of head, neck and lower extremity traumas was found to be high (9). In our study, however, the extremities were not evaluated as upper and lower, but were evaluated together. However, in accordance with the literature, extremity and head injuries were found with a high frequency in our study.

CONCLUSION

As a result of our study, we found that male gender and increasing age were high in pediatric forensic cases, consistent with the literature. We determined that assault is the most common cause of forensic cases in our region, followed by traffic accidents. We concluded that the extremity and head region are frequently injured in forensic cases.

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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 the İmir Katip Çelebi University Health Sciences Non Interventional Clinical Research (No: 2022/0281).*

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MEDICAL RECORDS-International Medical Journal

Research Article

Investigation of the Effect of Propolis on Penicillin Induced Epileptiform Activity in Rats

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Abstract

Aim: The aim of this study was to investigate the effects of propolis (PP), which has antioxidant and neuroprotective effects, on penicillin-induced epileptiform activity in rats.

Material and Methods: Forty-two adult male Wistar rats were divided into 6 groups as control (CONT), penicillin (PEN), diazepam (DZM), only propolis (OPP), 50 mg/kg propolis (PP50), and 100 mg/kg propolis (PP100). ECoG recording was taken from rats. At the end of the experiment, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels were determined from serum samples. Moreover, the latency of the first epileptiform activity, spike-wave frequency (SWF), and spike-wave amplitude (SWA) of the epileptiform activity were analyzed.

Results: The latency of the DZM and PP100 groups was found to be longer than the CONT groups. The time-dependent SWF and total SWF of the PP50 and PP100 groups were lower than the CONT group. No significant difference was found between the groups in terms of SWA. SOD, CAT, and GPx levels were found to be higher, but the MDA level was lower in PP50 and PP100.

Conclusion: As a result, propolis may be a potential antiepileptic drug candidate in the future with its antioxidant activity as well as prolonging latency and reducing SWF in epilepsy models.

Keywords: Epilepsy, propolis, oxidative stress, penicillin, electrocorticography

INTRODUCTION

Epilepsy is one of the most common and heterogeneous neurological conditions, and also a brain disorder characterized by a persistent predisposition to generate seizures and the neurobiological, cognitive, psychological, and social consequences of seizure recurrence. Although an etiological agent can be identified, the cause of approximately 50% of cases is still unknown (1). Currently, there are around 70 million people with active epilepsy who require treatment and have ongoing seizures. Of these patients, 30% are resistant to all known antiepileptic drugs (2,3). In the past thirty years, studies on various diseases of the nervous system have focused on the balance between the oxidant and antioxidant systems. The first experimental findings that described the relationship between oxidative stress and epilepsy were presented by Armstead et al. in 1989 (4). The relationship between oxidative stress and epilepsy has been demonstrated in many studies in different experimental models and in epileptic patients (5). In animals, it has been shown that increased reactive oxygen species (ROS) levels lead to a decrease in GABA levels in the brain, which occurs parallel to the onset of convulsions. One of the main reasons for this is that ROS production inactivates the glutamate decarboxylase enzyme (6).

Approximately 70% of epilepsy patients are controlled with monotherapy using current antiepileptic drugs. Herbal products play an important role in the development of new antiepileptic drugs. It is known that many plants have anticonvulsant effects. Various phytochemical, pharmacological, and electrophysiological studies have been carried out on these anticonvulsant plants, and these studies are increasing day by day (7).

Propolis (PP) is a natural, non-toxic, resinous substance collected by bees from various plants to maintain hive homeostasis and provide physical and biochemical protection (8,9).

CITATION

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The bioactive components of PP vary according to geographical origins, plant sources, and bee species. The chemical composition of PP can vary considerably both qualitatively and quantitatively (10). Nevertheless, most unprocessed PP consists of approximately 50-70% resin, 30-50% oils and waxes, 5-10% pollen, along with various minor chemical constituents like amino acids, sugars, vitamins B, C, and E, minerals, as well as flavonoids, phenols, and aromatic compounds such as caffeic acid (CA), caffeic acid 1,1-dimethylallyl ester, and caffeic acid phenethyl ester (CAPE) (11).

The therapeutic effects of PP's bioactive compounds have been extensively researched and continue to be investigated in many fields. Previous studies have shown that PP has antioxidant, antiproliferative, anti-inflammatory, antiparasitic, cytotoxic, and antibacterial properties (12,13). Additionally, it exhibits cardioprotective, hepatoprotective, and neuroprotective activity (14). However, to date, no studies have investigated the antiepileptic or anticonvulsant effects of PP. Only some studies have examined the effectiveness of PP in reducing the side effects of the antiepileptic drug valproate (15).

Based on the information provided, it has been shown that oxidative stress triggers the occurrence and frequency of epileptic seizures, and also increases neuronal loss and cognitive impairments after seizures. The aim of this study is to investigate the effects of PP, which has antioxidant and neuroprotective effects, on epileptiform activity induced by penicillin in rats.

MATERIAL AND METHOD

Animals

Fourty two male Wistar rats (250±30 g) were used. Rats were acquired from Duzce University Experimental Animals Application and Research Center. The rooms where the animals were housed were maintained under optimal conditions (23°C room temperature, 60±5% humidity, and a 12/12 light-dark cycle). Ethical approval was taken from the Animal Research Local Ethics Committee of Duzce University with the code number 2021/3/03. Animal maintenance and applications were conducted following the Health Guide for Animals, as approved by Animal Experiments Center Ethics Committee. Rats were divided into 6 groups control (CONT), Penicillin (PEN), Diazepam (DZM), Only Propolis (OPP), Propolis 50 mg/kg (PP50), and Propolis 100 mg/kg (PP100).

Preparation of Propolis Extraction

PP samples were obtained from honey bee colonies of Düzce University. Raw PP was harvested during the summer season. In previous studies, ethanol extraction of PP was used because the strongest antioxidant activity of PP was in the extractions obtained with ethanol solvent (16,17). Therefore, we have used ethanol as a solvent of the PP. The content of PP used had been determined in previous study (16).

Drugs and Doses

PP was administered orally at the doses of 50 and 100 mg/kg. 5 mg/kg DZM (DIAZEM, DEVA Holding A.Ş. Istanbul, Turkey) was administered intraperitoneally. As an anesthetic, 1.25 g/kg urethane (Sigma-Aldrich Missouri, USA) was applied intraperitoneally to the rats. Penicillin G (IE Ulagay Turkey Pharmaceuticals Inc., Istanbul, Turkey) used to induce epilepsy was applied as 500 IU intracortical (i.c.) in 2 µl volume.

Surgical Procedure and Electrophysiological Recordings

The surgical procedure and ECoG recording were performed as in previous studies (3,18). Briefly, urethane was used to anesthetize animals in all groups. Then rats were fixed in the stereotaxic frame (Harvard Instruments, MA, USA) after being placed in the prone position. After the head area was shaved, the scalp was cut with a scalpel from front to back along the midline. The bone portion over the left cerebral cortex was then carefully removed by slenderized with a drill (FST, KF Technology, Rome, Italy). Two Ag-AgCl ball electrodes were placed in the somatomotor cortex area opened lateral to the bregma line on the left hemisphere. After the electrodes were placed, ECoG recordings were taken with the PowerLab system (PoweLab/8 SP ADInstruments, Australia) throughout the experiment. A five-minute baseline activity recording was taken. PP or DZM application were applied 30 mins before PEN injection. Epileptiform activity was performed as in previous studies (3,18). Briefly, epileptic activity was created by intracortical administration of 500 IU/2 µl PEN with a micro injector (Hamilton Co., USA) to 2 mm laterally, 1 mm in front of the bregma line and 1.2 mm in the cortex depth. A total of 125 minutes of ECoG recording was obtained from each animal. The records were digitized with the help of the PowerLab Chart v.7.0 software program. ECoG recordings from each animal were divided into 5-minute periods. The data obtained were analyzed in terms of the time of onset of epileptiform activity, SWF and SWA, and total SWF.

After the ECoG recording of the animals in the groups, blood was taken from the heart by cardiac puncture method under anesthesia. At the end of the experiment rats were sacrificed with cervical dislocation under urethane anesthesia. The blood samples taken were centrifuged at 4000 rpm for 15 minutes, and serum was removed and stored at -80ºC until analysis. In the study, CAT, GPx, SOD, and MDA levels were measured with ELK (ELK Biotechnology CO., Ltd., Hubei, P.R.C) ELISA kits.

Statistical Analysis

From recordings obtained for each animal, the onset of epileptiform activity, SWF, and SWA were calculated automatically using software (Chart v.7.3.8, ADInstruments Pty Ltd, Australia). Epileptiform activity recordings were analyzed after dividing into five-minute periods. Differences between groups in terms of the onset of epileptiform activity and SWF and SWA measurements in each period were examined with the Kruskal-Wallis

test, and different groups were determined by the post hoc Dunn multiple comparison test. P<0.05 was accepted as the statistical significance level. GraphPad Prism 9 program was used in the analysis.

RESULTS

It was tested whether PP alone had an effect on ongoing basal activity. Accordingly, no epileptiform activity effect was observed on basal activity of only PP application (Figure 1). Similarly, no epileptiform activity was detected in the CONT group that underwent only surgery (Figure 1).

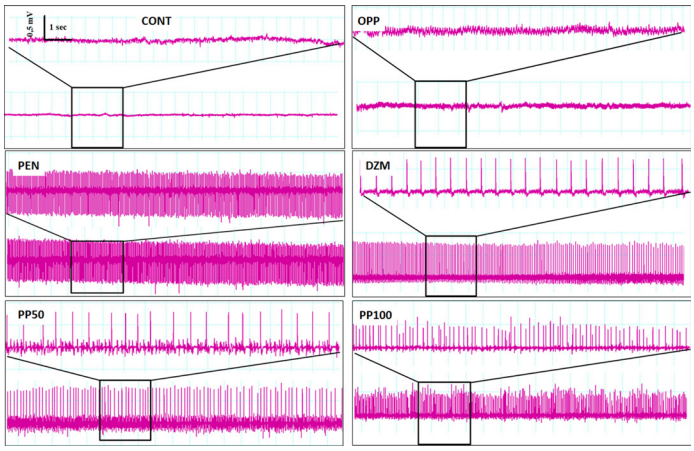

Figure 1. Representative samples of ECoG records from groups

Time of Onset of First Epileptiform Activity

Spike waves of epileptiform activity after PEN administration were observed between 360 and 1200 seconds (Figure 2). A statistically significant difference was identified among the groups when comparing based on the onset time of the first epileptiform activity (P=0.0012). When the groups were analyzed in more detail, it was observed that the means of onset times of the first epileptiform activity of the DZM and PP100 groups were statistically longer than the PEN group (p=0.0126 and p=0.0183, respectively). In addition, the means of onset times of the first epileptiform activity of the DZM and PP100 groups were found to be statistically longer than the PP50 group (p=0.0484 and p=0.0486, respectively).

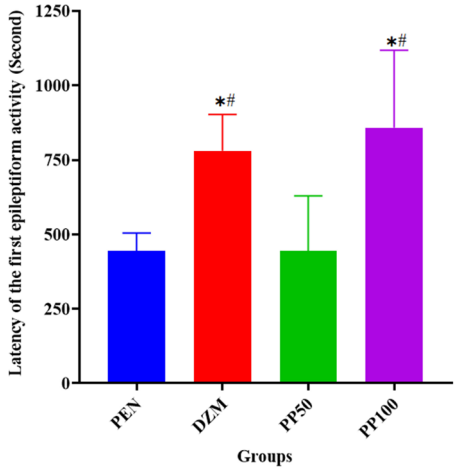

Figure 2. Latency of first epileptiform activity (*significant according to PEN group, #significant according to PP50 group)

The Effect of Propolis on the Time-Dependent Spike-Wave Frequency of Epileptiform Activity

During the 5-minute baseline activity recordings from the groups, any epileptiform activity was not detected in the ECoG recording measurements. SWF values were determined in 24 different measurements taken in five-minute periods after PEN application. Between 0-50th and 96th-120th minutes following PEN administration, a statistically significant difference was observed in the mean spike-wave numbers among all groups (p<0.05). Nevertheless, there was no difference between the groups between 51st-95th minutes (p>0.05). In the periods between 6th-60th and 96th-120th minutes, SWF of the PP50 group was lower than the PEN group (p<0.05). Similarly, in the time periods between 6th-50th minutes (except for the 21-25 time periods) the SWF of the DZM group was less than PEN group (p<0.05). In addition, the spike-wave frequency of the of the PP100 group was statistically less than the PEN group in the time periods between 6th-30th minutes (p<0.05). Data on the spike-wave frequency of epileptiform activity obtained from 120-minute ECoG recordings from the groups were given in Figure 3.

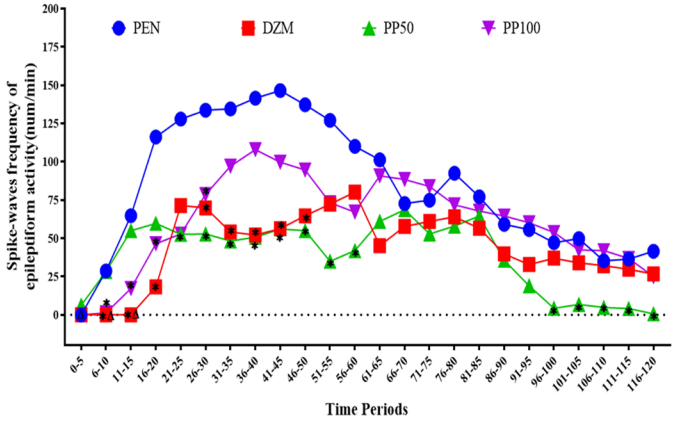

Figure 3. Mean of the time-dependent spike-wave frequency of epileptiform activity (number/min) obtained from recording after penicillin. *Significance compared with the PEN group; Δsignificance compared with the PP50 group)

Effect of Propolis on the Total Spike-Wave Frequency of Epileptiform Activity

After penicillin application in the groups, the mean of the total SWF that occurred during the 120-minute ECoG recording was evaluated. According to the results of the comparison of the means of the total spike wave frequency of the groups, it was found that statistical differences between the groups (P<0.0001), (Figure 4). In terms of total spike wave frequency of epileptiform activity, the means of the DZM, PP50, and PP100 groups were found to be statistically less than the PEN group (p<0.0001). In addition, it was determined that the mean of the DZM and PP50 groups were lower than those of the PP100 groups (p=0.0022 and p<0.0001).

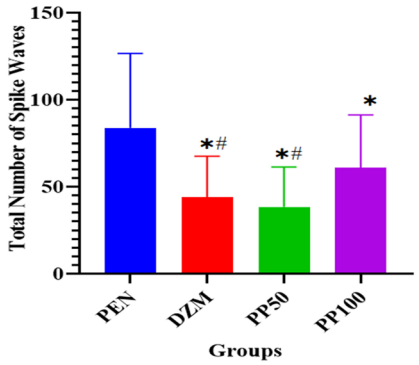

Figure 4. Display of total spike-wave number averages obtained from 120-minute recordings after penicillin (*significant according to PEN group, significant according to #PP100 group)

The Effect of Propolis on the Spike-Wave Amplitude of Epileptiform Activity

There was no statistical difference between the groups in terms of mean SWA obtained from all groups between 0-5th and 21st-120th minutes after penicillin administration (p>0.05) (Figure 5). However, there was a statistically difference in SWA of the groups between 6th-20th minutes (p<0.05). It was found that the SWA mean of the PP100 group was statistically less than the PEN group in the 6th-10th, 11st-15th and 16th-20th time periods (p=0.0389, p=0.0135 and p=0.0497). In the same time period, although the SWA means of the DZM and PP50 groups were less than those of the PEN group, it was not statistically significant (P>0.050).

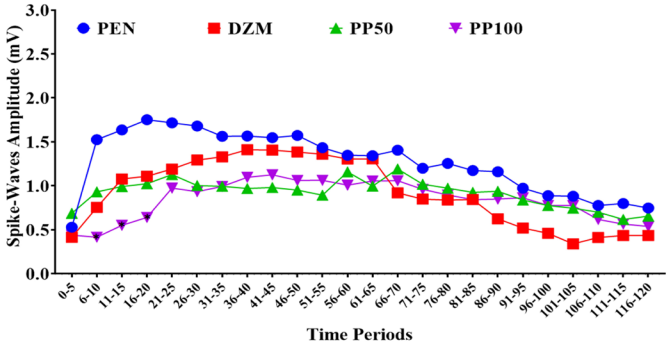

Figure 5. Mean of the time-dependent spike-wave amplitude of epileptiform activity (mV) obtained from recording after penicillin. *Significance compared with the PEN group)

Evaluation of Antioxidant Activity of Propolis

When the groups were compared in terms of SOD level, a statistical difference was found between the groups (P<0.0001) (Figure 6A). As the results were examined in more detail, the mean SOD level of the DZM group was found to be higher than the CONT and PEN groups (p<0.0001 and p<0.0001). Similarly, the mean SOD level of the OPP group was determined to be higher than the CONT and PEN groups (p<0.0015 and p<0.0001). It was determined that the mean SOD level of the PP50 group was higher than the CONT and PEN groups (p=0.0002 and p<0.0001, respectively). In addition, the mean SOD level of the PP100 group was found to be higher than the CONT and PEN groups (p=0.0008 and p=0.0002, respectively). It was no difference between OPP, DZM, PP50, and PP100 groups (p>0.05).

The groups were compared for CAT level, and a statistical difference was found between the groups (P<0.0001) (Figure 6B). Similarly, the mean CAT level of the OPP group was determined to be higher than the CONT and PEN groups (p=0.0002 and p<0.0001). It was determined that the mean CAT level of the PP50 group was higher than the CONT and PEN groups (p=0.0119 and p=0.0041). In addition, the mean CAT level of the PP100 group was found to be higher than the CONT and PEN groups (p=0.0353 and p=0.0127).

A significant difference was found between the groups in terms of GPx levels (P<0.0001) (Figure 6C). While the groups were examined in detail, it was determined that the mean GPx level of the OPP group was higher than the CONT and PEN groups (p=0.0041 and p=0.0041, respectively). The mean GPx level in the DZM group was determined to be elevated compared to the CONT and PEN groups (p=0.0178 and p=0.0178, respectively). The mean GPx level of the PP50 group was determined to be higher than the CONT, PEN and DZM groups (p<0.0001, p<0.0001 and p=0.0214). Likewise, the mean GPx level of the PP100 group was found to be higher than the CONT and PEN groups (p=0.0034 and p=0.0033).

A significant difference was found between the groups in terms of MDA levels (P<0.0001) (Figure 6D). When the groups were examined in detail, it was determined that the mean MDA levels of the PEN, PP50 and PP100 groups were lower than the CONT group (p<0.0001, p=0.0464 and p=0.0138, respectively). Similarly, the mean MDA levels of the PEN, PP50 and PP100 groups were determined to be lower than the OPP group (p<0.0001, p=0.0464 and p=0.0138, respectively). The mean MDA level of the PP50 group was found to be less than the PEN group (p<0.0001). Similarly, the mean MDA level of the PP100 group was found to be lower than the PEN group (p<0.0001).

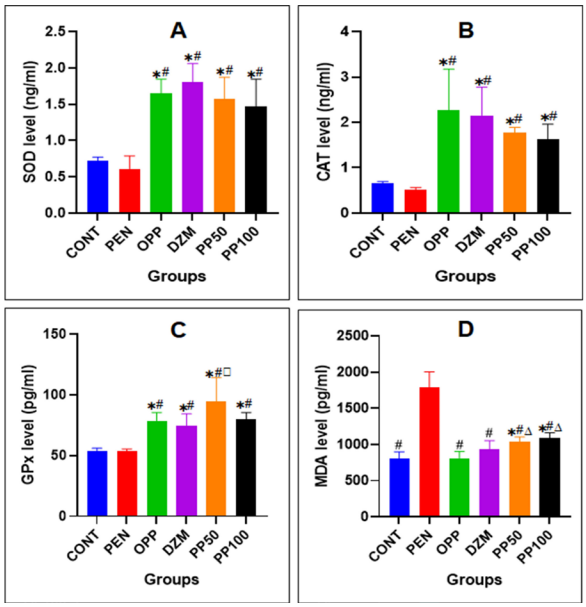

Figure 6. Effect of propolis on SOD (A), CAT (B), GPx (C) and MDA (D) levels (*Significant compared to CONT group; #Significant according to PEN group; □Significant compared to the DZM group; ΔSignificant compared to the OPP group)

DISCUSSION

This study is the first study to examine the effects of PP on PEN-induced epileptiform activity electrophysiologically. In the study, the effect of PP in the doses of 50 and 100 mg/kg, which was acutely applied, on experimentally generated PEN-induced epileptiform activity was investigated in male Wistar rats. The epileptiform activity shapes and patterns spied out in the ECoG recordings are compatible with the literature (3,18).

In the current study there was found no evidence of epileptiform activity during the baseline recording period in any of the groups prior to administration of PEN, and no spike-wave discharges were observed during the entire recording period in the CONT and only PP groups. These data cannot be compared to those of other electrophysiological studies in the literature due to the lack of such studies on the effects of PP. However, the data from the groups suggest that the use of PP does not lead to convulsions. This finding is significant as there are no electrophysiological studies on the effects of PP on epilepsy in the literature.

Intracortical administration of PEN to anesthetized rats generated an epileptiform activity within 360-536 seconds in the PEN group, this value was found within 360-1200 seconds in DZM, PP50, and PP100 groups. It was determined that the onset time of the first epileptiform activity in the PP100 group was grown approximately twice compared to the PEN group. On the contrary, the first epileptiform activity onset time of the PP50 group was found to be similar to the PEN group. In this case, it shows that the efficacy of PP may be dose dependent. Only PP which was administered group without inducing PEN epilepsy did not cause any epileptiform activity in any animal. These data suggest that the use of PP in epileptic or healthy rats will not cause any epileptic effect.

After the application of PEN, a significant difference was found in the mean SWF between all groups during the 0-50th and 96-120th minute periods. This suggests that PP reduces SWF over time. As PP' effects on epilepsy have not been studied electrophysiologically in the literature, this finding is important.

In the present study, the total spike wave frequencies during the 120-minute ECoG recording period after PEN administration were compared between groups, and it was found that both PP50 and PP100 groups had significantly lower mean spike wave frequencies compared to the PEN group. This suggests that PP reduces spike wave activity. As there is no electro-physiological study on the effect of PP on epilepsy in the literature, this finding is important.

In terms of mean epileptiform activity spike wave amplitude obtained from PP groups, similarity was found between the groups at 0-120 time periods (except 06-20 time periods). In addition, since there is no study in the literature that shows changes in epileptiform activity spike wave amplitude over time, the obtained data could not be compared.

There have been many studies on the mechanism of action of PP (19,20). However, due to the fact that PP contains 300 different substances, such as flavonoids and phenolic acids, its mechanism of action has not yet been fully understood. Hence, the potential protective impact of PP may arise through diverse mechanisms. For instance, it might involve the elimination of free oxygen radicals from the surroundings or the mitigation of free oxygen radicals' generation.

In a status epilepticus (SE) model induced by lithium+pilocarpine, it has been reported that PP repairs neuronal damage (19). The researchers emphasized that PP works by clearing free radicals or reducing the production of free radicals that can damage surrounding neuronal cells. The study in question indicated that PP could hold utility in managing SE, demonstrating potential to ameliorate neurological harm both as an antiepileptic medication on its own and when employed alongside an antiepileptic agent.

Epileptic rats induced by pilocarpine were treated with fish liver oil and PP in combination with valproate (21). After pilocarpine administration, it was reported that the lipid peroxidation levels and lactate dehydrogenase activity significantly increased in the hippocampus while the total antioxidant capacity significantly decreased compared to the control group. However, the combined application of PP and valproate improved the effect of lipid peroxidation toward normal levels. This finding is supported by other studies (20). In the present study, both doses of PP (50 and 100 mg/kg) were shown to reduce MDA levels.

Several investigations have highlighted the extensive pharmacological spectrum of CAPE, a key constituent within PP. Its manifold properties encompass antioxidative, anti-inflammatory, antiviral, antifungal, antiproliferative, and antineoplastic effects (22-24). CAPE has been reported to increase SOD, CAT, and GSH-Px levels in brain, while decreasing MDA levels (25). In the current study, it was shown that PP increased serum SOD, CAT, and GPx levels compared to the PEN group.

Cortical pyramidal cells play an active role in the epileptiform activity induced by PEN. In the epilepsy model induced with PEN, the potentials dependent on GABAA and GABAB receptors contribute to the sudden depolarization shifts observed in cells (26). Direct application of PEN to the cortex causes inhibition of GABA receptors, disrupting the brain's inhibitory system and initiating locally but generalized continuing epileptiform activity. Studies show that PEN binds to subunits of GABAA receptors, reducing the intracellular Cl- influx in cells (27). In addition, it has been reported in other studies that PEN also binds to the chloride receptor and blocks the opening of the chloride channel (28). In another study, it was suggested that PEN binds to the binding site of benzodiazepines, causing convulsions (29). The primary target of PEN is the β-subunit of the GABAA receptor to which GABA binds. It is believed that PEN binds to the GABA binding site with its β-lactam ring, preventing GABA from binding to this site.

This finding demonstrates a possible mechanism of PEN in epileptogenesis.

In recent years, many studies have been conducted or planned to clarify the role and impact of oxidative stress in epilepsy. In the early 2000s, oxidative stress was studied in epileptic conditions. The findings showed that oxidative stress is important in the neurological pathology of epilepsy. Particularly, in animal models such as lithium-pilocarpine, kainic acid, PEN, bicuculline, pentylenetetrazole (PTZ), sleep deprivation, and cocaine, an increase in ROS, nitrite levels, and lipid peroxidation products was observed. Since the 2000s, extensive studies have been conducted on the use of antioxidants in the treatment of epilepsy. In various studies, the use of various antioxidants such as plant extracts or flavonoids used in the treatment of epilepsy has been shown to reduce lipid oxidation in the hippocampus, striatum, and cortex and improve SOD, CAT, GSH, and GPx activity (30). In the current study, it was shown that PP increases CAT, SOD, and GPx levels while decreasing MDA levels. The increase in MDA levels in the PEN group in the experimental epilepsy model induced with PEN suggests that PEN triggers the production of reactive oxygen species. The data obtained from the groups are consistent with the literature.

In seizures induced by (PTZ), activation of glutamate receptors and inhibition of GABA are observed. Activation of glutamate receptors increases ROS levels. However, it has been reported that CAPE administration prolongs latency and reduces seizure duration in PTZ-treated mice (31). By virtue of its capability to eliminate ROS, diminish MDA concentration, and elevate antioxidant SOD levels, CAPE has demonstrated its capacity to safeguard brain tissue against oxidative harm.

It has been reported that administration of bee PP (30 and 60 mg/kg/day) to male Wistar rats significantly prolonged the latency of both clonic and tonic seizures induced by PTZ, reduced the duration and frequency of seizures, and decreased mortality (32). In a study on the effects of PP application on oxidative stress in rats with PTZ-induced epilepsy model, it was reported that total antioxidant capacity levels in the PP groups were significantly higher than in other groups and total oxidant status levels were lower (33).

PP has been shown to have agonist effects on the GABA receptor in different studies (34). It is known that GABA receptors are the most important receptors in epilepsy. PP contains flavonoids and their derivatives, and it is known that these substances have antioxidant effects (35). In different studies, it has been found that substances with antioxidant properties reduce epileptic seizures (36).

It has been reported that MDA and NO levels in brain tissue increase in epileptic seizures induced by PTZ in mice, while SOD activity remains unchanged (31,37). In another study, it was shown that in rats with the PTZ seizure model, the MDA level in the brain tissue of seizure animals increased compared to controls, while SOD, CAT, and GSH levels decreased (38). However, there are also studies in the literature that show no significant difference in SOD activity in brain tissue compared to controls after a single dose of PTZ application (39,40).

The current study's findings are consistent with the literature in terms of PP extending latency, reducing seizure frequency, lowering MDA levels, and increasing SOD, CAT, and GPx levels.

CONCLUSION

In conclusion, the protective and reducing effects of PP have been demonstrated in experimental epilepsy models. In the present study, only the electrophysiological response of PP to epileptiform activity and oxidative stress was investigated. Future longer-term and multidisciplinary studies in this field will shed light on the subject.

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

Ethical approval: *Ethical approval was taken from the Animal Research Local Ethics Committee of Düzce University with the code number 2021/3/03.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Examination of Treatment Options According to Clinical Features and Radiological Findings in Wake-up Stroke

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Abstract

Aim: Wake-up stroke patients account for one-fifth of all ischemic stroke patients and they have been deprived of recanalization treatment as the onset of the stroke is not known. It has come into the focus of recent research that this treatment could be applied to particularly patients who are selected on a radiological basis. We aimed to examine clinical and demographic characteristics of wake-up strokes.

Material and Methods: All ischemic stroke patients who presented to the emergency service throughout the night were analyzed. Patients with wake up stroke were recorded. The time of finding these patients, clinical features, treatment options and mortality status of these patients were examined. It was examined whether they received intravenous thrombolysis (IVT), endovascular therapy (EVT), or both as acute reperfusion therapy.

Results: Age, gender, the last time when they were seen healthy, treatment start time, treatment types, clinical classification, comorbid diseases, and discharge status of 72 wake-up stroke patients were analyzed. It was found that the time of discovery of 51.4% (n=37) of the wake-up stroke patients was 06.00 am and afterwards. Of these patients, 15.3% (n=11) received intravenous tissue plasminogen activator and/or endovascular treatment. These patients' hospital stay durations, intracerebral hemorrhage status following the procedure, NIHSS scores, angiography findings, and mortality rates were examined. Here, mortality rate was found to be significantly high especially in patients with high NIHSS score.

Conclusion: Wake-up stroke is more common in the period close to the time of waking up in the morning. Recanylazation therapy should always be considered as an option in these patients.

Keywords: Wake-up stroke, circadian rhythm, intravenous tissue plasminogen activator, endovascular treatment, NIHSS

INTRODUCTION

Cerebrovascular diseases (CVD) occur as a result of an occlusion or rupture in brain vessels. Poor blood flow in the brain tissue results in cell death (1). Cerebrovascular diseases are the leading causes of death in both genders worldwide (2). It is also one of the causes of disability in adults (3). There are some risk factors for stroke. These risk factors can be counted as diabetes mellitus (DM), atrial fibrillation (AF), hypertension (HT), transient ischemic attack and carotid stenosis (4).

According to the World Health Organization (WHO), it is estimated that 5.4 million individuals worldwide in 2000 and approximately 6.2 million individuals worldwide in 2015 lost their lives due to CVDs (2). In the USA, stroke was one of the leading causes of death in both sexes in 2015 (5). Stroke is in second place among the leading causes of death in Europe and accounted for the death of nearly 1 million individuals in 2015. This constitutes approximately 14% of female deaths and 9% of male deaths (6).

Ischemic stroke accounts for 80% of all strokes (7). Wake-up stroke constitutes 25-30% of all ischemic stroke cases (8). Intravenous thrombolysis and thrombectomy are significant treatment methods which have positive effects on mortality and morbidity in ischemic stroke patients (9,10). Unknown time of symptom onset in wake-up stroke patients is important in terms of thrombolysis and thrombectomy treatment opportunity (11). While deciding on the treatment type, rather than depending on time, making a decision on the basis of individual patients may provide more patients with treatment opportunity (12).

In the study, relationship of symptom time of especially wake-up strokes with circadian rhythm and their clinical

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and demographic characteristics, and patient-based treatment options and progression rather than being time-dependent were examined.

MATERIAL AND METHOD

Study Data Collection

Study ethical approval was obtained with the decision numbered 2021/88 and dated 13.10.2021 from Malatya Turgut Özal University Clinical Research Ethics Committee. In the study, patients who were diagnosed with ischemic stroke in the emergency service of Malatya Education and Research Hospital between January 2019-June 2021 were retrospectively examined over the hospital's automation system. Based on their clinical characteristics at admission, the patients were classified according to Oxfordshire Community Stroke Project (OCSP) classification system as Total Anterior Circulation Infarction (TACI), Partial Anterior Circulation Infarction (PACI), Posterior Circulation Infarction (POCI) and Lacunar Infarction (LACI). Among the ischemic stroke patients who presented to the emergency service (door time) between pm 11:00-am 11:00, those with wake-up stroke were included in the study. Patients with ischemic stroke but not wake-up stroke and patients with hemorrhagic stroke were not included in the study. The last time when the patient was seen healthy (witness), time of presentation to the emergency service, radiological imaging time, and treatment start time were recorded. First of all, the wake-up patients' age, gender, clinical classification, comorbid diseases, treatment options, and mortality status were examined. Acute reperfusion treatments have been classified as intravenous thrombolysis (IVT), endovascular treatment (EVT), and combined use of these two treatments. National Institutes of Health Stroke Scale (NIHSS) scores, hospital stay durations, angiography findings, intracerebral hemorrhage status after procedure, and discharge status of the wake-up stroke patients whose computerized tomography findings were normal or close to normal were examined. These patients were divided into subgroups according to their mortality status, and risk factors were analyzed.

Statistical Analysis

SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL) 22 package software was used. Descriptive data were expressed as number (n) and percentage (%) values for categorical variables, while they were presented as mean±standard deviation (mean±SD) for continuous variables. Chi-square analysis (Pearson Chi-square) was used to compare categorical variables. Compliance of continuous variables with normal distribution was evaluated through Shapiro-Wilk test. Student's t test was employed in pairwise comparison of the groups. $p<0.05$ was accepted significant.

RESULTS

72 patients in total, 42 (58.3%) of whom were male and 30 (41.7%) of whom were female, were included in the study. The mean age of the participants was found as 71.8±12.7 years (min-max=36-96/years). Of the patients, 61 (84.7%) received medical treatment, 3 (4.2%) IVT (intravenous thrombolysis), 3 (4.2%) EVT (Endovascular treatment), and 5 (6.9%) IVT+EVT. Regarding the OCSP classification of the patients, 14 (19.4%) were TACI, 29 (40.3%) were PACI, 15 (20.8%) were LACI, and 14 (19.4%) were POCI. 66.7% of the patients had HT, 27.8% had DM, 33.3% had AF, 41.7% had coronary artery diseases, and 30.6% had hyperlipidemia 26.4% of the patients died (Table 1).

Table1. Characteristics of the patients included in the study (n=72)

		Number	%
Gender	Male	42	58.3
	Female	30	41.7
Age, Mean±SD		71.8±12.7	
Treatment	Medical	61	84.7
	IVT	3	4.2
	EVT	3	4.2
	IVT+EVT	5	6.9
OCSP classification	TACI	14	19.4
	PACI	29	40.3
	LACI	15	20.8
	POCI	14	19.4
Comorbid diseases*	HT	48	66.7
	DM	20	27.8
	AF	24	33.3
	CAD	30	41.7
	HL	22	30.6
Status	Discharged	53	73.6
	Dead	19	26.4

*There are patients who had more than one comorbid diseases
EVT: endovascular treatment, IVT: intravenous thrombolysis, OCSP: Oxfordshire Community Stroke Project, TACI: total anterior circulation infarction, PACI: partial anterior circulation infarction, POCI: posterior circulation infarction, LACI: lacunar infarction, HT: hypertension, DM: diabetes mellitus, AF: atrial fibrillation, CAD: coronary artery disease, HL: hyperlipidemia

While 61 (84.7%) of the patients received medical treatment, the remaining 11 (15.3%) patients received acute treatment. The characteristics of the patients who received acute treatment are presented in Table 2.

The last time when the patients were seen healthy, the time of discovery of the patients, and hospital presentation times of the patients were examined. When the discovery times of the patients were divided into three groups according to circadian rhythm as 23:00-03:00, 03:00-06:00, 06:00 and afterwards (n=11 [15.3%], n=24 [33.3%] and n=37 [51.4%], respectively), it was determined that 51.4% (n=37) of the patients were discovered at 06:00 and afterwards. 63.7% (n=7) of the patients receiving acute treatment were discovered after 06.00.

Table 2. Characteristics of the patients who received acute treatment

Case	Gender	Age	NIHSS	OCSP classification	Treatment	CT angiography	Hemorrhage	Hospitalization duration (day)	Mortality
1	Male	66	14	TACI	EVT	LEFT ICA OCCLUSION OVER 80%	No	12	No
2	Female	45	11	TACI	IVT+EVT	RIGHT MCA TOTAL OCCLUSION	No	6	No
3	Female	78	17	PACI	IVT+EVT	NORMAL	Yes	3	Yes
4	Female	80	20	TACI	IVT	LEFT MCA OCCLUSION OVER 90%	No	10	Yes
5	Male	75	13	PACI	IVT+EVT	LEFT MCA TOTAL OCCLUSION	Yes	29	No
6	Male	53	8	LACI	IVT	LEFT ICA NEAR-TOTAL OCCLUSION	No	3	No
7	Male	79	21	PACI	IVT+EVT	RIGHT MCA BRANCH TOTAL OCCLUSION	Yes	106	Yes
8	Male	78	13	TACI	IVT+EVT	RIGHT MCA TOTAL OCCLUSION	Yok	15	Yes
9	Female	68	20	PACI	EVT	LEFT MCA TOTAL OCCLUSION	Yok	56	No
10	Male	48	20	PACI	EVT	LEFT VA OCCLUSION OVER 70%	Yok	61	Yes
11	Male	78	12	POCI	IVT	NONE	Yok	8	No

NIHSS: National Institutes of Health Stroke Scale, OCSP: Oxfordshire Community Stroke Project, EVT: endovascular treatment, IVT: intravenous thrombolysis, CT: computer tomography, ICA: internal carotid artery, MCA: middle cerebral artery, VA: vertebral artery

It was observed that there was no significant gender difference between those who received medical treatment and those who received acute treatment. Who received medical treatment, 57.4% were male. Who received acute treatment, it was seen that 63.6% of them were male (p=0.753). While the mean age of the patients who received medical treatment was 72.5±12.6, the mean age of the patients who received acute treatment was 68.0±13.3, and no significant difference was found in terms of age (p=0.443). When the mortality was examined, it was observed that there was no significant difference between those who received medical treatment and those who received acute treatment (23%, 45.5%, respectively, p=0.145). No statistically significant difference was determined between the treatment groups in terms of other parameters (p>0.05) (Table 3). CT angiography examination was performed on 10 of the wake-up stroke patients who received acute treatment, and major blood vessel occlusion was detected in 9 patients.

Table3. Comparison of the characteristics of the patients according to treatment groups

		Medical treatment (n=61)		Acute treatment (n=11)		p*
		Number	%	Number	%	
Gender	**Male**	35	57.4	7	63.6	0.753
	Female	26	42.6	4	36.4	
Age, Mean±SD		72.5±12.6		68.0±13.3		0.443**
OCSP classification	**TACI**	10	16.4	4	36.4	0.384
	PACI	24	39.3	5	45.5	
	LACI	14	23.0	1	9.1	
	POCI	13	21.3	1	9.1	
Hypertension	**Yes**	41	67.2	7	63.6	0.817
	No	20	32.8	4	36.4	
Diabetes Mellitus	**Var**	18	29.5	2	18.2	0.716
	Yok	43	70.5	9	81.8	
Atrial Fibrillation	**Yes**	21	34.4	3	27.3	0.741
	No	40	65.6	8	72.7	
Coroner artery disease	**Yes**	26	42.6	4	36.4	0.753
	No	35	57.4	7	63.6	
Hyperlipidemia	**Yes**	19	31.1	3	27.3	0.797
	No	42	68.9	8	72.7	
Durum	**Discharged**	47	77.0	6	54.5	0.145
	Dead	14	23.0	5	45.5	

*Chi-square analysis, **Student's t test was applied, OCSP: oxfordshire community stroke project

When various parameters of 11 patients who received acute treatment were examined according to mortality status, NIHSS values of those who died were found to be significantly higher than NIHSS values of the patients who were discharged (p=0.045). 42.9% of the males and 50% of the females died, and no significant difference was found between the genders (p=0.819). Regarding dying, no significant difference was found in terms of age (p=0.238). As regards OCSP classification, while 50 % of those with TACI and 60% of those with PACI died. No patients with LACI and POCI died. No significant difference was found in OCSP classification in terms of mortality (p=0.547). 66.7% of those with hemorrhage and 37.5% of those without hemorrhage died, and no significant difference was determined between the two groups (p=0.545). Regarding dying, no significant difference was found in terms of hospitalization duration (P=0.342) (Table 4).

Table 4. Comparison of various parameters of the patients who received acute treatment according to mortality status

		Dead (n=5)		Discharged (n=6)		p*
		Number	%	Percentage	%	
Gender	Male	3	42.9	4	57.1	0.819
	Female	2	50.0	2	50.0	
Age, Mean±SD		72.6±13.7		64.2±12.7		0.238**
OCSP classification	TACI	2	50.0	2	50.0	0.547
	PACI	3	60.0	2	40.0	
	LACI	0	.0	1	100.0	
	POCI	0	.0	1	100.0	
Hemorrhage	Yes	2	66.7	1	33.3	0.545
	No	3	37.5	5	62.5	
NIHSS, Mean±SD		18.2±3.3		13.0±4.0		**0.045****
Hospitalization Duration, Mean±SD		39.0±43.8		19.0±20.3		0.342**

*Chi-square analysis, **Student's t test was applied, OCSP: oxfordshire community stroke project, NIHSS: National Institutes of Health Stroke Scale

DISCUSSION

Wake-up stroke (WUS), which constitutes a significant portion of ischemic stroke patients, is defined as the stroke in which patients who do not have any anomalies before sleeping present with a newly developing neurological deficit when they wake up (13,14). One out of every five patients present with a wake-up stroke (15). Wake-up strokes have long been excluded from acute recanalization treatments due to their uncertain time of symptom onset. In studies conducted, the onset time of wake-up strokes has been reported to be the morning hours closest to the time of waking up (16,17).

In a study in which they examined circadian changes in acute ischemic stroke patients, it was reported that all stroke subtypes peaked between 06.00 a.m. and noon (18). Chaturvedi et al. also examined stroke subtypes and symptom onset times according to TOAST staging and demonstrated that all types concentrated relatively around early morning hours (19). In addition, when Serena et al. examined the clinical properties of strokes that occur during waking up and wakefulness, they could not find a distinct difference (20). In the present study, only the patients who presented to the hospital between 23:00-11:00 were examined, and especially wake-up stroke patients were tried to be determined. Acute ischemic stroke patients other than wake-up stroke patients were excluded from the study. In addition, the last time when they were seen healthy, discovery time, and hospital presentation time of the WUS patients were recorded. It was seen that especially the time of the patients waking up with a stroke was close to the waking up time of the circadian rhythm. When the patients were classified according to Oxfordshire Community Stroke Project (OCSP) staging which is based on clinical characteristics, there was no difference between those receiving medical treatment and acute treatment (21).

It is seen that acute ischemic stroke (AIS) patients are frequently faced with death, being bedbound, and needing others' help in the following periods (22). However, in the treatment of AIS, intravenous tissue plasminogen activator (IV t-PA), endovascular recanalization treatment, or combined use of these two procedures have significantly changed the course of the disease (23,24). Regarding the use of these treatments, symptom onset time and hospital arrival time in especially WUS patients are one the most important restricting factors (11). Still, it has been arguedin recent years that WUS patients can benefit from IV t-PA or endovascular reperfusion treatments (25,26). Especially in this group of patients, the applicability of these treatments by considering radiological imaging based diffusion-weighted and fluid-attenuated inversion recovery mismatch (Diffusion-FLAIR mismatch) has been evaluated (27-29).

The study conducted by Fink et al. in 2020 demonstrated that both clinical and multimodal MRI (magnetic resonance imaging) characteristics in wake-up strokes were not different from stroke patients with known symptom onset time. It was also recommended to research treatment

methods based on imaging parameters in these patients (30). In another study conducted, Barreto et al. included 40 WUS patients in the age range of 18-80 years, NIHSS ≤25, and based only on non-contrast CT imaging. They obtained 52.6% success modified RANKIN scores of 0 or 1 at the end of three months in these patients (31). In this study, patients aged 45-80 whose NIHSS scores were between 8-21, who did not have hypodensity in CT images, who had normal or close to normal CT findings, who mostly had major branch occlusion in CT, whose clinical and MR findings did not match were chosen.

It has also been stated that CT perfusion examination has an important place for radiological imaging based approach in WUS patients (32). In patients who were examined with CT and CT perfusion, many findings such as perfusion area-hypodensity mismatch, clinic-diffusion mismatch (DAWN criterion), diffusion-FLAIR mismatch (MR-Witness criterion), perfusion-diffusion mismatch (PDM criterion) have been used in the evaluation of wake-up stroke patients (17,33-35). As CT perfusion was not a radiological imaging technique routinely used in our hospital, some of these criteria could not be evaluated.

In 2011, Roveri et al. determined that patients who presented with wakefulness and wake-up strokes had similar disease characteristics and early ischemic changes, and they stated that early period WUS patients can benefit from thrombolytic treatment (36). Barreto et al. (2009) shared the data of 46 WUS patients who received IV t-PA (28 patients), intraarterial thrombolysis (14 patients), and combined treatment (4 patients). As a result of the study, they reported that thrombolysis treatment can be a reliable treatment option for WUS patients (25). In the double-blind, placebo-controlled randomized study they conducted (WAKE-Up Trial) in 2018, Thomalla et al. reported that iv-tPA treatment could be useful in selected patients (37). In this study, there was no significant difference between who received treatment and others in terms of age, gender, stroke subtype, and hospital stay. In addition, there was no significant difference the early mortality rates between the groups (p=0.145).

One of the most fatal complications seen after recanalization treatments is intracranial hemorrhages that may develop following the procedure. In one of the studies, although it had significant benefits in functional recovery outcomes following thrombolysis treatment, there was an increase in the risk of hemorrhagic complication, and there was no evidence showing that the treatment effect of alteplase decreased in ischemic stroke patients with ≥1 CMBs (cerebral microbleeds) (38). National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study Group found the risk of symptomatic intracerebral hemorrhage (SICH) as 6.4% in acute ischemic stroke patients who received IV t-PA (9). They also demonstrated that if National Institutes of Health Stroke Scale (NIHSS) score was >20 in these patients, SICH rate increased by 5-17% (39). SICH risk was found to have increased in wake-up stroke patients who received thrombolysis treatment compared to the control group (3%, 1%, respectively); however, this increase in hemorrhage risk did not have a significant effect on post-treatment functional outcomes (40). In this study, it was observed that there was no significant difference between intracranial hemorrhage rates in patients who received treatment according to their mortality status. However, the NIHSS scores of the patients who received treatment and died were significantly higher than the others (18.2±3.3, 13.0±4.0; p=0.045, respectively). This suggests that high NIHSS score is an important factor in terms of hemorrhage risk in patients after recanalization treatment applied.

In the present study, occurrence times, demographic and clinical properties, treatment choice, and the course of the disease regarding wake-up strokes, which constitute a significant portion of ischemic stroke cases, was reviewed in light of the literature.

Limitations of Study

As the study had a retrospective design, the patients' long-term RANKIN scores and mortality rates could not be determined. Small number of patients, being a single-center study, and CT perfusion or FLAIR magnetic resonance imaging techniques not being in routine use in our emergency service are among the limitations of the study.

CONCLUSION

Wake-up strokes continue to form a significant portion of acute ischemic stroke cases. It is thought that these patients, who constitute a significant group in terms of mortality and disability, should be examined in detail in terms of acute treatment options, and that radiological imaging based treatment options in selected patients should be evaluated. In addition, it is recommended to be careful with especially patients with high NIHSS score in terms of treatment failure.

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Research Article

Cone-Beam Computed Tomographic Evaluation of the Posterior Wall of the Nasopharynx in Turkish Population

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Abstract

Aim: This study aims to evaluate the radio-morphometry of important anatomical structures such as Rosenmüller fossa (RF), pharyngeal bursa (PB), and Eustachian tube (ET) in the posterior wall of the nasopharynx by cone beam computed tomography (CBCT).

Material and Methods: The posterior wall of the nasopharynx was analyzed retrospectively in CBCT images of 110 patients. The depth and width of the Rosenmüller fossa (RF), pharyngeal bursa (PB), and Eustachian tube (ET), their distances to the posterior nasal spina (PNS) and mid-sagittal plane, and the angles between them were measured. RF was categorized into three types. The relationship between the measured values and gender, age groups, and RF types was investigated. The obtained variables were analyzed statistically.

Results: The mean right RF depth was 8.2 and left RF was 8.6 mm. RF widths differed significantly by gender (right p=0.013, left p=0.004). There was a statistically significant positive correlation between RF-PNS distances and age (left r=0.314, p=0.001; right r=0.240, p=0.011). The prevalence of RF types was 31.8%, 19.5%, and 48.6% for type A, type B, and type C, respectively. In individuals with RF Type C, both RF and ET were located more lateral to the midline. The prevalence of PB was 45.5%.

Conclusion: Nasopharyngeal carcinoma (NPC) most commonly occurs in the RF. A good knowledge of the anatomy and variations of the nasopharyngeal region is important in the early diagnosis of NPC. Oral and maxillofacial radiologists must know the anatomy of the nasopharynx to understand and interpret incidental findings in CBCT.

Keywords: Cone-beam computed tomography, Eustachian tube, nasopharynx, nasopharyngeal carcinoma, pharyngeal bursa, Rosenmüller fossa.

INTRODUCTION

The posterior wall of the nasopharynx has important anatomical structures and consists of loose connective tissue (1). Some of these important anatomical structures are the pharyngeal recess (PR), the pharyngeal bursa (PB), and the Eustachian tube (ET). During nasotracheal intubation, if the end of the nasotracheal tube strikes the posterior wall of the nasopharynx, this can lead to retropharyngeal dissection and other serious complications (2). Being aware of the differences in the anatomy of the nasopharyngeal region can reduce the risk of such complications during nasotracheal intubation (1). In addition, it has been reported that calcific formations (3) such as rhinoliths, and structures such as Tornwaldt cysts (4), which are epithelial remnants of embryological origin, can be seen in this region.

Rosenmuller fossa (RF) also called lateral PR, was first reported by Johann Christian Rosenmuller. The RF is located just posterior to the torus tubarius, below the skull base, and extends laterally behind the ET orifice. Its location is important because there is only a thin layer of fibro connective tissue between it and the internal carotid artery (5). RF is lined with nasopharyngeal mucosa and is the most common area of nasopharyngeal carcinoma (NPC). NPC is most commonly seen in the 50-60 age group (6). Early diagnosis and treatment increase the survival rate of patients (7). The first symptom to appear on imaging of NPC is bluntness and asymmetry in RF (8). For this reason, it is very substantial for oral and maxillofacial radiologists to fully understand the anatomy of RF, which is the most common area of NPC (9).

While the posterior wall of the nasopharynx can only be

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seen with endoscopy, computed tomography (CT) allows the investigation of all anatomical features (2). Cone beam computed tomography (CBCT) is often utilized in dentistry to examine bone for dental implants and orthodontic treatment. It also allows the acquisition of noninvasive, accurate measurements and three-dimensional images of anatomical structures and spaces (10). The use of CBCT has many advantages such as low radiation dose, high bone resolution, ease of use, and accessibility (1).

This study aims to investigate the morphometric features of the anatomical structures including RF, PB, and ET in the posterior wall of the nasopharynx using CBCT.

MATERIAL AND METHOD

The study was approved by the Izmir Katip Çelebi University Non-Interventional Clinical Studies Ethics Committee (IRB:26.05.2022,262) in accordance with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from individuals before the CBCT procedure.

Sample size calculation was done using G*Power 3.1.9.7 software with 0.693 effect size, 5% significance level, 0.95 power. CBCT images of 110 random patients taken for assorted reasons (Dental Trauma, implant planning, examination of impacted teeth, orthodontic treatment planning, etc.), at the Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, İzmir Katip Çelebi University were evaluated. Inclusion criteria were the patient's age over 18, high-quality CBCT images, and clear visualization of the nasopharynx. Patients with craniofacial deformity or syndrome, pathological formation in the nasopharynx region, the presence of any artifact that may interfere with the interpretation of the image and a history of surgery and trauma in the relevant region were not included in the study.

All CBCT images used were acquired with a CBCT device (NewTom 5G, Quantitative Radiology, Verona, Italy) shooting at 110 kVp. Images with a voxel size of 0.200 mm and a FOV range of 15x12 cm were used. CBCT images were evaluated in dim light conditions using a 27-inch screen size monitor (Eizo Radiforce MX270W - EIZO Corp.; Ishikawa, Japan) at 2560x1440 resolution and computer software NNT Viewer version 8.0 (NewTom - Quantitative Radiology; Verona, Italy). All measurements were made by one observer - oral and maxillofacial radiologist.

Reference points (posterior nasal spina (PNS) and basion (Ba)), RF, and ET orifice are shown in Figure 1 in the CBCT axial section. For measurements, an axial view with PNS (the posterior most point of the bony hard palate in the mid-sagittal plane) and Ba (The midline point of the anterior border of the foramen magnum) in the same frame was chosen. Reference points (PNS and Ba) in the oropharynx were determined based on another study (10).

In the axial section, the depth, width, length, distance from the PNS, the distance to the mid-sagittal plane and the angle between the sagittal plane of the RF and ET recesses were measured (Figure 2). The presence or absence of PB was noted. If PB was present, its depth and width were measured in the axial section and its length was measured in the sagittal section (Figure 3). RF is classified into three types(2): type A, depth <5 mm; type B, depth ≥5 mm and width <1 mm; and type C, depth ≥5 mm and width ≥1 mm (Figure 4). All measurements were made bilaterally.

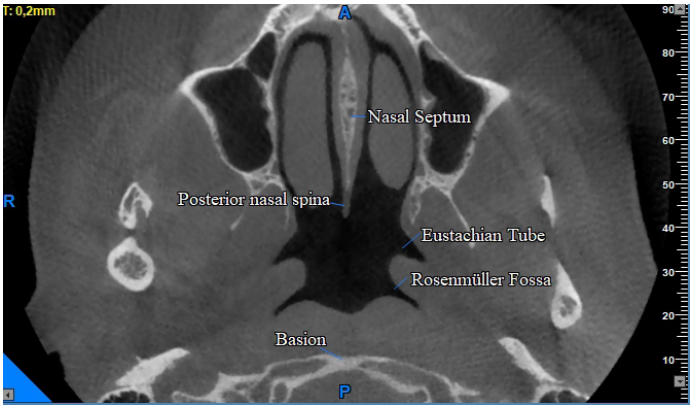

Figure 1. Anatomical structures of the nasopharynx in CBCT axial sections. Position of reference points (Posterior nasal spina and basion), Eustachian tube orifice, and Rosenmüller fossa

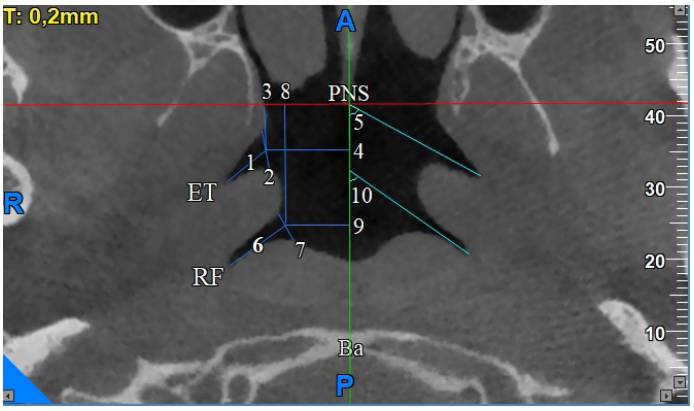

Figure 2. The measurements of Rosenmüller fossa (RF) and Eustachian tube (ET) orifice in axial section. 1, ET depth; 2, ET width; 3, ET-PNS distance; 4, ET-Mid-sagittal plane distance; 5, ET-Mid-sagittal plane angle; 6, RF depth; 7, RF width; 8, RF-PNS distance; 9, RF- Mid-sagittal plane distance; 10, RF-Midsagittal plane angle

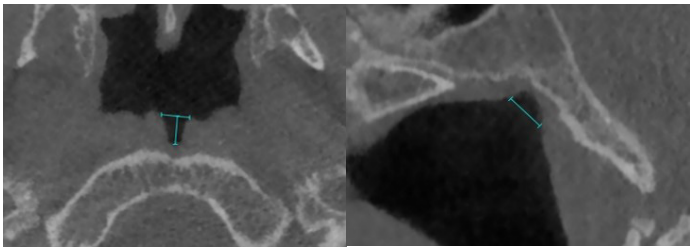

Figure 3. The pharyngeal bursa (PB) measurements on CBCT images: (a) depth and width of PB in axial section, (b) length of PB in sagittal section

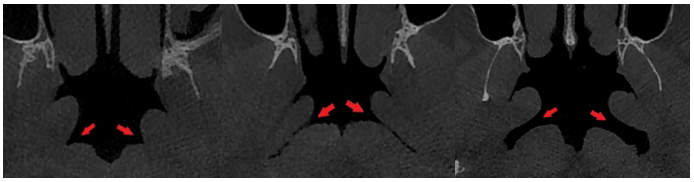

Figure 4. The classification of Rosenmüller fossa in axial section. (a) Type A, shallow fossa (<5 mm deep), (b) Type B, deep fossa (≥5 mm) with <1 mm wide openings, (c) Type C, deep fossa (≥5 mm) with ≥1 mm wide openings

Statistical Analysis

All data were analyzed using the IBM SPSS Statistics Version 26.0 package program (IBM Corp.; Armonk, New York, USA). The conformity of the data to the normal distribution was evaluated with the Shapiro-Wilk test. Differences between normally distributed group means were analyzed using the t-test and one-way ANOVA. Tukey test was used in pairwise comparison tests of normally distributed variables. The relationship between normally distributed variables was defined using the Pearson correlation coefficient. In the comparison of qualitative variables, the number of observations (n) and their ratios (with the % values) were shown and the relationship between them was compared using the Chi-square test. The significance level was accepted as 0.05.

RESULTS

This study was performed retrospectively using CBCT images of 110 patients (57 females, 53 males) with a mean age of 44.68 years.

Measurements related to RF, and comparisons according to gender, age groups, and RF types are given in Table 1. While no statistically significant relationship was found between RF depths and gender ($p>0.05$), there was a significant relationship between RF widths and gender ($p<0.05$). In addition, there was a statistically significant difference between RF-PNS distances and age groups ($p<0.05$). Pairwise comparison results showed that the RF-PNS distance of individuals aged 51 and older increased significantly compared to individuals aged 18-35 ($p<0.05$). In addition, it was observed that the RF-PNS distance of individuals aged 51 and older increased compared to individuals aged 36-50 years, but this increase was not significant ($p>0.05$).

The prevalence of RF types A, type B and type C was 31.8%, 19.5%, and 48.6%, respectively, in a total of 220 RFs. The prevalence of right RF type A, type B and type C was 32.7%, 19.1%, and 48.2%, and the prevalence of left RF was 31%, 20%, and 49%, respectively.

Measurements related to ET and their comparisons according to gender and age groups are presented in Table 2. While the right ET-midsagittal plane distance was statistically significantly higher in men, the left ET-midsagittal plane angle was higher in women ($p<0.05$). Bilateral ET depths and ET-Midsagittal plane distances were statistically significantly higher in individuals with RF type C than in those with type A ($p<0.05$).

The prevalence and dimensions of PD according to gender are given in Table 3. RF-PNS distances were positively correlated with age (Table 4).

Table 1. The Rosenmüller fossa dimensions in axial section according to gender/age groups/RF types

		MEAN±SD									
	N	R-RF depth	R-RF width	R-RF-PNS	R-RF-MID	R-RF-angle	L-RF depth	L-RF width	L-RF-PNS	L-RF-MID	L-RF-angle
Gender											
Female	57	8.3±5.3	2.5±1.5	15.5±3.4	8.2±2	50.4±8.6	9.1±5	2.8±1.5	15.5±3.5	8.2±2.3	50.5±8.9
Male	53	8.1±5.4	1.9±1.2	14.8±3.7	8.7±2.5	51±12.2	8.1±5.6	2.1±1.2	15.2±3.6	7.5±1.7	51.3±12.9
p value		0.829	0.013*	0.299	0.245	0.750	0.331	0.004**	0.713	0.093	0.708
Age											
18-35	36	7.4±5.5	1.8±1.2	13.9±3.1^{a}	8.6±1.7	48.2±11.6	8.1±5.6	2.2±1.3	13.8±3.5^{a}	8.2±2.8	47.8±11.7
36-50	32	9.3±5.7	2.3±1.4	15.1±4.1^{ab}	8±1.8	49.9±10.3	9.5±5.9	2.6±1.3	15.4±3.5^{ab}	7.4±1.8	52.5±11.4
51+	42	8±4.9	2.5±1.6	16.3±3.3^{b}	8.8±2.9	53.3±9.1	8.3±4.5	2.5±1.6	16.7±3^{b}	7.9±1.8	52.3±9.6
p value		0.352	0.138	0.009***	0.288	0.087	0.498	0.437	0.001***	0.274	0.125
Total	110	8.2±5.3	2.2±1.4	15.2±3.6	8.5±2.3	50.7±10.5	8.6±5.3	2.5±1.4	15.4±3.4	7.9±2.1	50.9±11
RF type	N (R-L)										
Type A	36-34	2±2.4^{a}	2.3±1.4^{a}	14.4±3.7	7.2±1.8^{a}	43.6±11.1^{a}	2.3±1.8^{a}	2.7±1.5^{a}	14.7±3.6	6.5±1.5^{a}	42.5±12.2^{a}
Type B	21-22	10.4±3.4^{b}	0.8±0.4^{b}	15.2±3	8.5±1.8^{ab}	53.5±8.9^{b}	10.2±3.3^{b}	0.9±0.6^{b}	16.3±3.5	8.4±1.6^{b}	56.2±9.2^{b}
Type C	53-54	11.5±3.3^{b}	2.7±1.4^{a}	15.7±3.7	9.3±2.4^{b}	54.3±8^{b}	11.9±3.6^{b}	2.9±1.4^{a}	15.4±3.4	8.5±2.1^{b}	53.9±7.6^{b}
p value		0.000***	0.000***	0.257	0.000***	0.000***	0.000***	0.000***	0.224	0.000***	0.000***
Total	220	8.2±5.3	2.2±1.4	15.2±3.6	8.5±2.3	50.7±10.5	8.6±5.3	2.5±1.4	15.4±3.4	7.9±2.1	50.9±11

RF: rosenmuller fossa, PNS: posterior nasal spina, *Statistically significant at level P≤0.05 (independent samples t test), **Statistically significant at level P≤0.01 (independent samples t test), ***Statistically significant at level P≤0.01 (one way ANOVA), The lowercase superscript indicates statistical differences within column

Table 2. The orifice of the Eustachian tube dimensions in axial section according to gender/age groups/RF types

		MEAN±SD									
	N	R-RF depth	R-RF width	R-RF-PNS	R-RF-MID	R-RF-angle	L-RF depth	L-RF width	L-RF-PNS	L-RF-MID	L-RF-angle
Gender											
Female	57	5.2±1.8	4.7±1.1	6.1±2.4	11.3±1.7	53±7.3	5.1±1.7	4.7±1	6.5±2.5	11.2±1.3	51.3±8.2
Male	53	4.8±1.4	4.9±1.2	6.9±2.9	12.2±1.8	51.7±8	4.9±1.4	4.9±1.3	7.1±2.9	11.4±1.4	48.1±8.6
p value		0.168	0.381	0.123	0.012*	0.347	0.380	0.341	0.224	0.401	0.050*
Age											
18-35	36	4.4±1.4	4.5±1.2	6±2.9	11.8±1.8	50.9±9	5±1.6	4.6±1.2	6.3±2.8	11.5±1.3	46.7±8.5[a]
36-50	32	4.9±1.6	4.7±1.2	6.6±2.8	11.7±1.9	53.3±5.3	5±1.4	4.9±1.2	6.8±2.9	11.2±1.4	50.9±7.7[ab]
51+	42	5.5±1.7	5±1.2	6.8±2.4	11.6±1.8	52.9±7.9	5±1.7	4.7±1.1	7.1±2.5	10.8±1.4	51.6±8.6[b]
p value		0.690	0.155	0.432	0.903	0.373	0.998	0.535	0.411	0.658	0.029*
Total	110	5±1.6	4.7±1.2	6.5±2.7	11.7±1.8	52.4±7.6	5±1.6	4.8±1.2	6.8±2.7	11.3±1.4	49.8±8.5
RF type	N (R-L)										
Type A	36-34	4.3±1.4[a]	4.8±1.1	6.4±2.4	11.1±1.7[a]	50±7.8	4.4±1.3[a]	4.3±1.1	7±3	10.8±1.3[a]	48.5±7.8
Type B	21-22	4.9±1.5[ab]	4.7±1.3	6.1±2.7	11.7±1.2[ab]	53.6±7.5	5.3±1.6[ab]	5.2±1.3	7.1±2.7	11.2±1.2[ab]	49.6±10.4
Type C	53-54	5.5±1.6[b]	4.7±1.1	6.5±2.8	12.1±1.9[b]	53.4±7.3	5.2±1.5[b]	4.8±1	6.4±2.4	11.5±1.3[b]	50.6±8.1
p value		0.003***	0.930	0.890	0.038**	0.076	0.022**	0.007*	0.479	0.039**	0.552
Total	220	5±1.6	4.7±1.2	6.5±2.7	11.7±1.8	52.4±7.6	5±1.6	4.8±1.2	6.8±2.7	11.3±1.4	49.8±8.5

RF: rosenmuller fossa, PNS: posterior nasal spina, *Statistically significant at level P ≤ 0.05 (independent samples t test), **Statistically significant at level P≤0.05 (one way ANOVA), ***Statistically significant at level P≤0.01 (one way ANOVA), The lowercase superscript indicates statistical differences within column

Table 3. The relationship between the incidence and dimensions of pharyngeal bursa with gender

	PB Presence N (%)	PB Absence N (%)	Total N (%)	PB depth Mean (SD)	PB width Mean (SD)	PB length Mean (SD)
Female	29 (26.4)	28 (25.4)	57 (51.8)	3.8 (1.4)	4.3 (2.5)	5.7 (1.9)
Male	21 (19.1)	32 (29.1)	53 (48.2)	4.1 (1.1)	4.6 (1.8)	6.8 (2.6)
Total	50 (45.5)	60 (54.5)	110 (100)	3.9 (1.3)	4.4 (2.2)	6.2 (2.2)
p value		0.236		0.400	0.551	0.093

PB: Pharyngeal Bursa

Table 4. The comparison of Rosenmüller fossa and posterior nasal spina distance by age

		R-RF-PNS	L-RF-PNS
Age	**r**	0.240*	0.314**
	p	0.011	0.001
	n	110	110

RF: rosenmuller fossa, PNS: posterior nasal spina, *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

In the literature, the anatomical structures of the posterior wall of the nasopharynx were examined using computed tomography (CT) (2,11-13) and CBCT (1,7-9). CBCT provides accurate and reliable measurements of bone tissue. CBCT has advantages such as shorter scanning times, lower costs, and a lower radiation dose (14). The CBCT technology allows scanning with the patient in an upright position (7). Sutthiprapaporn et al. (10) evaluated RF depth in supine and upright positions using computed CT and CBCT. They argued that when in an upright position, the tissues surrounding the RF may fall under the influence of gravity, causing the RF to appear deeper. They reported that in 83% of cases, the RF was deeper when the position was changed from the supine to the upright position. They measured the right and left RF depths of 9.8±6.6 mm and 6.8±6.5 mm, respectively, in images acquired in the upright position using CBCT. In this study, right and left RF depths were measured as 8.2±5.3 mm and 8.6±5.3 mm, on CBCT images obtained in the supine position. The RF depths measured in this study were like those measured using CBCT by Sutthiprapaporn et al. (10). This contradicts the argument that RF will be imaged less deeply by taking CBCT in the supine position. There is a need for different comparative studies in which the sample size is increased.

NPC is a rare tumor arising from the epithelial lining of the nasopharynx. RF is the most common origin of NPC. Its incidence is higher in men (6). The pathogenesis of NPC is not clear. It is estimated that there are many risk factors such as the Epstein-Barr virus, genetic factors, and nutrition (15). Hoe (13) retrospectively analyzed CT images of 56 patients with biopsy-proven NPC to investigate the origins and extent of NPC. Hoe (13) reported that the first NPC lesion appeared in the RF area and this tumor spread to the adjacent parapharyngeal area in 64% of the cases. In another study, Hoe analyzed CT images of 60 patients with biopsy-proven NPC and reported that levator veli palatini muscle was thickened and RF was blunt in all these patients (12). Therefore, it is important to know the mean measurement values of the anatomical structures in the posterior nasopharyngeal wall and their relationship with age and gender for the early diagnosis of infiltrates and possible pathologies.

Some early symptoms and signs of NPC, such as ear, face, and headache, and trismus, may be confused with symptoms of temporomandibular dysfunction (TMD) (15). Özyar et al. (16) found that 5 percent of patients had trismus at the time of diagnosis of NPC. Zubizarreta et al. (17) declared that trismus was the first sign of NPC in up to 36% of patients. The possibility of NPC should also be evaluated in patients who are diagnosed with TMD. For this reason, RF, where NPC is most common, should be meticulously investigated for bluntness and asymmetries in CBCT images. During these CBCT examinations, oral maxillofacial radiologists may have a substantial role in the early recognition of NPC. In cases where doubts increase, patients should be directed to the necessary medical doctors (8).

Nasotracheal intubation is a potentially traumatic procedure. Takasugi et al. (2) reported that in wide-opened RFs (Type C), the nasotracheal tube may impinge on the posterior wall of the nasopharynx, resulting in possible retropharyngeal laceration and even dissection. In their study, type C was detected in 24% of cases. In this study, 48.6% of the total 220 RFs were type C. Type C with a high rate indicates that the regional anatomy should be well known for each patient to reduce the potential risks. An interesting finding in our study was that both ET and RF were located more lateral to the midline in individuals with RF type C than in those with RF type A. It can be thought that this may reduce the risk of injury or laceration.

Serindere et al. (7) reported that RF depth was statistically significantly higher in women. In our study and other studies (1,8,11), no significant difference was found in RF depth according to gender. In our study, the width of R-RF was 2.5 mm in females and 1.9 mm in males, and the width of L-RF was 2.8 mm in females and 2.1 mm in males. There is a statistically significant difference in RF width by gender. We obtained equivalent results with another study investigating the relationship between RF width and gender (1). While Kaplan et al. (1) did not report a significant difference in L-RF-PNS distance according to age groups, they found a difference between groups in R-RF-PNS distance. In our study, there was a significant difference according to age groups in both L-RF-PNS and R-RF-PNS distances. In the correlation analysis, there was a positive correlation between age and RF-PNS distances.

The PB is a congenital blind sac located in the midline of the nasopharynx near the lower end of the pharyngeal tonsils (18). Takasugi et al. (2) determined the prevalence of PB as 16% in their study and reported that it is very unlikely to cause nasopharyngeal laceration during nasotracheal intubation since their openings are narrow. Kaplan et al. (1) reported the prevalence of PB as 40% in their study of 150 patients. In our study, the prevalence of PB was 45.5%, which is higher than in previous studies.

This study had several limitations. The study was conducted on CBCT data obtained from single center in the supine position. This situation prevents comparisons of both regional and ethnic differences. This study only performed morphometric analysis on CBCT images retrospectively. A clinical examination of the patients was not performed, and demographic information was not obtained. In future studies, demographic information on patients can be obtained, and comparisons can be made using CBCT data from different centers in the supine and upright positions.

CONCLUSION

In conclusion, there are few studies in the literature about the posterior wall of the nasopharynx, which contains important anatomical structures, including the RF, which is the most common site of NPC. Although CBCT is not used in practice for the diagnosis of NPC, it is frequently used for various purposes in dentistry. Dentists, and especially oral and maxillofacial radiologists, should be aware of these structures that can enter the imaging field, albeit incidentally, and have adequate anatomical knowledge to perform their responsibilities. A good knowledge of regional anatomy and its variations is particularly important for distinguishing pathologies. Further studies comparing different imaging modalities and positions with larger sample sizes may provide useful information.

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MEDICAL RECORDS-International Medical Journal

Research Article

The Correlation between Melasma and ABO Blood Type

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Abstract

Aim: Melasma is an irregular brown hyperpigmentation mostly observed in the middle face. Although genetic predisposition, ultraviolet radiation and female sex hormones have been reported as the main causes, inflammatory processes were also considered to play a role in melasma. It has been determined that blood groups play a role in many genetic and inflammatory diseases. Since the genes that encode blood types were associated with inflammation, blood type could play a role in the etiology of melasma, an inflammatory and genetically inherited disease. The present study aimed to investigate the correlation between melasma and ABO/Rh blood types, which has never been investigated before.

Material and Methods: The study was conducted 100 patients with melasma and 1000 healthy controls. The patient and healthy control blood types and Rh factor data were collected from the hospital automation system retrospectively.

Results: Female gender ratio was significantly higher in the patient group compared to the control group ($p<0.05$). The mean patient age was significantly higher when compared to the controls ($p<0.05$). The analysis of the ABO blood type distribution revealed that the AB-type incidence was significantly higher in melasma patients, while the B-type incidence was higher in the control group ($\chi 2:4.512$; $p<0.05$). There was no significant difference between the A and 0 blood types in the patient and control groups. Rh positivity was significantly higher in the patient group ($p<0.05$).

Conclusion: Since this is the first ABO blood type study conducted on melasma patients, multicenter studies with large samples are needed to clarify our findings.

Keywords: Melasma, ABO blood types, hyperpigmentation

INTRODUCTION

Melasma is a melanogenesis dysfunction that leads to chronic and regional hyperpigmentation of the skin (1). Melasma usually appears as irregular brown spots on sun-exposed areas such as the face.It is observed less frequently in the neck and forearms (2-4). Although it could be observed in men, it is more common in women in their thirties and forties and with darker skins. Hyperpigmentation significantly affects the quality of life of the patients due to cosmetic concerns (3-6).

Genetic characteristics, ultraviolet radiation and female sex hormones have been cited as the main factors behind melasma (4). Recently, it was reported that inflammatory processes play a role in the development of melasma (7,8). Recent research indicated a more heterogeneous pathogenesis that involved interaction between keratinocytes, mast cells, gene regulation abnormalities, high vascularization, and basal membrane problems (5,9). Clinicians should be familiar with the pathogenesis development of melasma, since it could help determine successful therapy combinations in melasma, a difficult and recurrent disease.

One of the most important genetic human characteristics is the blood type. ABO blood type antigens are complex carbohydrate molecules and serve as red blood cell surface markers. They are also expressed in bodily fluids, various cell and tissue types, and the skin. The presence of antigens in several tissues suggested that blood type antigens may not only determine the blood type but also play wider roles (10). The correlation between ABO antigene system and various proinflammatory mediators suggested that it could play a role in inflammatory diseases (11).

CITATION

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Possible correlations between dermatological diseases such as acne, rosacea, alopecia areata, lichen planus, skin cancers, vitiligo, urticaria, pemphigus, psoriasis have been investigated (12-18). However, there are no studies where the correlation between melasma and ABO/Rh type was investigated. Since the genes that encode blood types were associated with inflammation, it could be suggested that blood type might play a role in the etiology of melasma, an inflammatory and genetically inherited disease, and we planned the current study to help understand the influence of blood groups on the etiology of melasma.

MATERIAL AND METHOD

The study was started after approval from the local ethics committee (2023/01-12). The study was conducted on hundred over the age of 18 patients who presented to the Elazig Urban Dermatology Center between January 2020 and March 2022 and diagnosed with melasma based on clinical examinations. The control group included thousand over the age of 18 healthy individuals who applied to the hospital to obtain health reports required for applications or routine check-ups. Melasma patients who had no concomitant dermatological disease, systemic diagnose, cardiovascular disease, neurological disease, cancer, and did not abuse alcohol or substances were included in the patient group. Medical data, blood type and Rhesus (Rh) factor data of the patients and healthy controls were collected retrospectively from the hospital automation system. Declaration of Helsinki and good clinical practice standards were considered while conducting the study.

Statistical Analysis

Statistical Package for Social Science for Windows (SPSS) 24.0 software was used to analyze the study data. Frequency and percentage distribution analysis was employed to determine the gender and blood type distributions of the patients. Also, the means and standard deviations were analyzed to determine the mean participant age. Independent samples t-test was used to determine the differences between the mean participant age of the patient and control groups. Chi-square test was employed to determine whether there were significant differences between the gender and blood type distributions of the patient and control groups. $p<0.05$ was considered statistically significant.

RESULTS

Ninety-two percent of the patient group were female. A statistically significant difference was determined between the gender of the melasma patients and the controls (χ^2:97.349; $p<0.05$) (Table 1). The mean patient age (32.73±8.40) was significantly higher than the mean age of the control group (27.22±7.97) (t:6.550; $p<0.05$) (Table 2).

A significant difference was determined between the ABO blood types of the melasma patients and the control group (χ^2:4.512; $p<0.05$). There was no significant difference between the A and 0 blood type melasma and control group members, AB blood group prevalence was significantly higher in the melasma group, and B blood type prevalence was significantly higher in the control group (Table 3).

The analysis of the Rh blood group distribution in the melasma patients revealed that 93% Rh(+), 7% were Rh(-). On the other hand in controls, 88% were Rh(+). A significant difference that favored the melasma patients was determined in the number of Rh(+) individuals between melasma and controls (χ^2:4.176; $p<0.05$). The investigation of the ABO and Rh blood type distribution across the participants is presented in Table 4.

Table 1. Participant gender distribution

			Group		Total
			Patient	Control	
Gender	Female	N	92	405	497
		Gender %	18.5%	81.5%	100.0%
		Group %	92.0%	40.5%	45.2%
		Total %	8.4%	36.8%	45.2%
	Male	N	8	595	603
		Gender %	1.3%	98.7%	100.0%
		Group %	8.0%	59.5%	54.8%
			0.7%	54.1%	54.8%
Total		N	100	1000	1100
		Gender %	9.1%	90.9%	100.0%
		Group %	100.0%	100.0%	100.0%
		Total %	9.1%	90.9%	100.0%
Pearson Chi-Square			97.349		
P			.001<0.05		

Table 2. Participant gender distribution

Variable	Patient (n=100)	Control (n=1000)	p
Age "x̄(±sd)"	32.73 (±8.40)	27.22 (±7.97)	.0001*
Independent samples t-Test, *p<0.05			

Table 3. Participant ABO blood type distribution

	A n (%)	B n (%)	AB n (%)	0 n (%)	Total n (%)
Melasma	39 (39%)	12 (12%)	14 (14%)	35 (35%)	100 (100%)
Control	404 (40.4%)	193 (19.3%)	75 (7.5%)	328 (32.8%)	1000 (100%)

Table 4. Participant ABO/Rh distribution

	Melasma n (%)	Control n (%)	Totaln (%)
A+	36 (36.0%)	359 (35.9%)	395 (35.9%)
A-	3 (3%)	45 (4.5%)	48 (4.4%)
B+	12 (12%)	168 (16.8%)	180 (16.4%)
B-	0 (0%)	25 (2.5%)	25 (2.3%)
AB+	13 (13%)	70 (7.0%)	83 (7.5%)
AB-	1 (1.0%)	5 (5.0%)	6 (0.5%)
0+	32 (32.0%)	283 (28.3%)	315 (28.6%)
0-	3 (3.0%)	45 (4.5%)	48 (4.4%)
Total	100 (100%)	1000 (100%)	1100 (100%)

DISCUSSION

The ABO blood type system was described several years ago. According to, there are Four main blood types were determined based on the presence of A/B antigens (A, B, AB and 0) (19,20). Rhesus system was classified as Rh (-) or Rh (+) based on the presence of Rhesus D antigen on red blood cell surface (21).

ABO antigens are expressed from the endothelium, skin and many organs and tissues in the body (13,22). It is also present in various body secretions (23). ABO antigens are located in the layers of the skin and hair follicles (15,22). Due to the presence of these antigens in several tissues, studies have been conducted on the correlations between blood types and development of certain diseases such as infections, cancer, and coagulation disorders. Most studies reported a correlation between the studied disease and the ABO blood type and in some studies, no relationship was found. (24,25).

Studies have shown that there is a relationship between cancer and the ABO blood group (15). A study conducted by Xie et al. found that people with blood type 0 have a higher risk of developing non-melanoma skin cancers. (26). In another study, it was determined that cutaneous malignant melanoma was more common in people with A blood group (27).

An older study that investigated the correlation between vitiligo and blood type reported that groups A and B were significantly more prevalent in vitiligo patients, and group 0 was less prevalent (16). However, a recent study reported no correlation between vitiligo and ABO blood type (17).

A study conducted on mild acne vulgaris, severe acne vulgaris and healthy controls reported significant findings. Severe acne was found to be higher in type A, while mild acne was significantly higher in blood groups other than type A (12).

Studies where the correlations between rosacea (13), alopecia areata (14), chronic spontaneous urticaria (18), and androgenetic alopecia (28) and ABO blood type did not report significant correlations.

CONCLUSION

In our study, female gender was significantly higher in the melasma group than in the control group. The incidence of AB blood type was significantly higher among melasma group. The incidence of type B was significantly higher in the control group. There was no significant difference between the patient and control groups based on A and 0 blood types. ABO blood group iso-antigens are known to be expressed in various skin layers. In this study, the high presence of AB blood group antigens may contribute to the etiopathogenesis of melasma by causing melanogenesis dysfunction. However, this should be confirmed by future studies. Since this was the first ABO blood type study in melasma patients, multicenter studies with large samples are needed to clarify our findings.

Study Limitations

Since this study is a retrospective study, patient information is limited to the data of the hospital database. Prospective studies with a larger number of patients may be more useful in enlightening this issue.

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Research Article

Assessment of the Relationship between Vitamin D Deficiency and Epin Calcanei

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Abstract

Aim: There is no research exploring the connection between vitamin D insufficiency and epin calcanei, despite strong evidence linking it to a number of health issues, including diabetes, infections, autoimmune disorders, cancer, cardiovascular illnesses, and widespread muscular discomfort. In this study, we examined whether vitamin D insufficiency is linked to epin calcanei.

Material and Methods: 205 patients with foot pain clinically diagnosed with epin calcanei by radiograph and 205 patients without epin calcanei clinically diagnosed by radiograph were evaluated. These patients' data were reviewed retrospectively. Radiographic evaluation was performed on all patients for epin calcanei. Vitamin D levels were evaluated by looking at the 25-hydroxycholecalciferol (25(OH)D) level.

Results: The 25(OH) vitamin D values in the group with epin calcanei showed a significant (p=0.001) difference.

Conclusion: In our research, epin calcanei and vitamin D levels were shown to be significantly correlated. There are research on vitamin D levels in a variety of fields, but none have looked at how it could relate to epin calcanei. More research is required to fully comprehend the possible contribution of vitamin D levels on the etiology of epin calcanei.

Keywords: Epin calcanei, heel pain, vitamin D

INTRODUCTION

Heel pain is a common problem. In an American study, the prevalence of heel pain was found to be 7% (1). German surgeon Plettner used the term epin calcanei anatomically for the first time in 1900. When the plantar fascia inserts, it frequently happens on the medial calcaneal tuberosity. The radiological appearance of epin calcanei has been found between 11% and 16% in studies (2). The radiological appearance of epin calcanei is mostly seen in women, elderly patients, patients with osteoarthritis, and patients with heel pain (2-4). The main complaint is pain felt in the heel which increases over time. Pain is more common when we wake up in the morning and when we start walking. It is stinging and burning, especially in the inner part of the heel. It decreases after walking for a while but increases towards the evening with the load depending on the activity. Rest relieves and reduces pain. However, when we start walking after sitting, it increases again in the first step. The diagnosis is made with clinical and radiological radiography.

There are many factors in the etiology of epin calcanei disease, the exact cause of which is unknown. The most common cause is inflammation caused by repetitive traction at the beginning of the plantar fascia and healing of this inflammation by ossification (5). This is called the longitudinal traction hypothesis. A significant part of the etiology is played by recurrent microtraumas, persistent injury to the tiny foot muscles, and the onset of the plantar aponeurosis. Intrinsic foot muscle weakness is one of the most important factors in the etiology of epin calcanei, but hereditary conditions and metabolic disorders may be just as important by accelerating the inflammatory process (6,7). Chronic injury results from a reduction in the elasticity of the insertional cartilage. Mesenchymal cells in the scar tissue fill the cracks in the damaged cartilage. With the re-formation of blood vessels, the scar gradually ossifies to form a bony spur (8). Another reason is the increasing degeneration of the elastic adipose tissue in

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the heel with aging. Therefore, it is thought to be more common in elderly patients.

Mineral and vitamin supplements are utilized for the skeletal and bone system, joints, and cartilage to develop and regenerate themselves in a healthier and faster way (9,10). Vitamin D is a class of sterols that act as hormone precursors and are classified as hormones since they may be created endogenously. It is also considered one of the fat-soluble vitamins. Its most important effect is on bone mineralization, phosphorus, and calcium metabolism (11,12). Vitamin D deficiency and insufficiency, a general health issue for a wide range of acute and chronic disorders, is a risk factor (13). Vitamin 25(OH)D deficiency leads to secondary hyperparathyroidism. Osteoclasts are responsible for bone resorption. As a result, adult osteopenia and osteoporosis may accelerate and worsen (14). Vitamin D increases osteoblastic activity and supports bone mineralization by keeping parathormone (PTH) levels at normal levels (14,15). Its main function is to affect calcium and bone metabolism in an anabolic direction. Vitamin D has been shown to have many effects in different systems such as neuroprotective, anti-inflammatory, and antiproliferative effects (16).

Recent studies have shown links between vitamin D deficiency and insufficiency and a variety of chronic illnesses, including cancer, immune system disorders, neurodegenerative diseases, psychiatric illnesses, metabolic syndrome, diabetes mellitus, insulin resistance, cardiovascular diseases, and infectious diseases (17,18). Although significant research has linked vitamin D insufficiency to a number of illnesses, including cancer, diabetes, autoimmune disorders, cardiovascular diseases, infections, and general muscular discomfort, no research has looked specifically at the connection with epin calcanei. In this study, our aim investigate whether there is any relationship between epin calcanei and vitamin D deficiency in patients presenting to the physiotherapy outpatient clinic with heel pain.

MATERIAL AND METHOD

Patient Population

With the consent of the local ethics committee (with the decision dated 29.05.2018 and 001), a total of 410 patients (205 epin calcanei +, 205 epin calcanei-) who applied to the Department of Physical Therapy and Rehabilitation between January 2021 and November 2022 with complaints of pain in the foot, clinically diagnosed as epin calcanei, 25(OH)D level was checked and lateral standing with a load direct foot radiography was taken were retrospectively analyzed. 205 individuals without epin calcanei were used as the control group. The socio-demographic characteristics of the patients were recorded.

Measurement of Vitamin D Level

Vitamin D levels measured in venous blood obtained from all patients were evaluated. A total of 25 (OH) vitamin D was quantitatively determined in human serum and plasma (EDTA, lithium heparin, and sodium heparin) using Atellica IM Analyser. 25(OH)D levels below 30 ng/ml were considered as vitamin D deficiency.

Statistical Analysis

The data obtained using the IBM SPSS 26 program were analyzed. Firstly, analyses were performed to determine the normality distribution. Accordingly, Kolmogorov-Smirnov test results were found to be $p<0.05$. Then, skewness kurtosis values were analyzed by considering the literature (19). The data were distributed normally according to these values. Therefore, independent groups T-Test, one of the parametric tests, was used in the analyses. The highest, lowest, median, mean, and standard deviation values were used in the statistics of descriptive data. Analyzing qualitative independent data involved using the chi-square test.

RESULTS

Epin calcanei was present in 205 of the patients included in the study. Of these, 30 were men and 175 were women. The mean age was 48.8±11.5 years. Vitamin D level was 17.5±10.2 (4-62) ng/ml. 23 patients had normal results. 182 patients had vitamin D deficiency. 205 of them did not have epin calcanei. Of these, 65 were men and 140 were women. The mean age was 49.3±16.5 years. Vit- D level was 21.3±11.9 (6.1-72.3) ng/ml. The results of 38 patients were normal. Vitamin D deficiency was found in 167 patients. Socio-demographic characteristics and 25(OH) D levels of all patients are presented in Table 1.

The age of the patients in the epine calcanei (+) group did not differ significantly (p=0.758) from the epine calcanei (-) group. Gender distribution in the epine calcanei (+) and epine calcanei (-) groups differed significantly (p=0.000). Vitamin D levels of patients with epin calcanei (X=17.54, SD=10.2) were lower than vitamin D levels of individuals without epin calcanei (X=21.34, SD=11.90). The significance of the difference was evaluated using the independent groups t-test. According to this, vitamin D level was significantly lower in individuals with epin calcanei [t (408)=-3.47, p=0.001] (Table 2).

Table 1. Descriptive data on gender, age, 25-OH-D* vitamin level and 25-OH-D* vitamin level of the groups

Group	N	Gender		Age					25-OH-D* vitamin level			25-OH-D* vitamin grade	
		Women	Men	Mean	Median	SD	Min	Max	Mean	Median	SD	<30	>30
Epin calcanei (+)	205	175	30	48.80	48.00	11.56	23	84	17.50	15.40	10.20	182	23
Epin calcanei (-)	205	140	65	49.30	50.00	16.50	17	88	21.30	18.90	11.90	167	38

*25-OH-D: 25- hydroxy-cholecalciferol

Table 2. 25-OH-D* vitamin levels of the groups

	Group	N	Mean	SD	t	p
Vit-D level	Epin calcanei (+)	205	17.5430	10.27722	-3.465	.001
	Epin calcanei (-)	205	21.3495	11.90550		

*25-OH-D: 25- hydroxy-cholecalciferol

DISCUSSION

In the present study, we looked for a relationship between vitamin D insufficiency and epin calcanei. In addition, we found that vitamin D deficiency was found at a very high rate of 85% (n=349). Studies have reported that vitamin D deficiency has a high prevalence in developing countries including Turkey (20,21). In our investigation, the ages did not significantly differ. However, research has revealed that vitamin D insufficiency is more prevalent among women (20). The genders differed significantly in our study. This condition is comparable to other studies.

Epin calcanei (calcaneal spur) is a bony protrusion larger than 2 mm, which is seen at the attachment site of the plantar musculofacial structure to the calcaneus starting from the inner projection of the calcaneal tuberosity. It has been shown in the study of Aydogdu et al. (22) that patients with epin calcanei are over middle age and overweight, and women are more affected. Overload causes an increase in regional pressure and tension in the plantar fascia. Chang et al. (23) showed that the thickness of the plantar fascia 1 cm distal to the starting point is directly related to body weight and emphasized that overloading of the plantar fascia is effective in this mechanism. In studies, it has been reported that female gender, age, high body mass index, and plantar fascia thickness, as well as functional disorders such as intrinsic muscles of the foot, soleus, and gastrocnemius weakness, are associated with epin calcanei and plantar fasciitis (20,23).

The etiology of epin calcanei has many causes and these causes are not fully known. There are many mechanical hypotheses related to the formation of epin calcanei. These are vertical compression and longitudinal traction hypotheses. The most common cause is inflammation due to repetitive traction at the beginning of the plantar fascia and healing of this inflammation by ossification (5). This is called the longitudinal traction hypothesis. Repetitive microtraumas and chronic damage to the small muscles of the foot and the beginning of the plantar aponeurosis caused by repetitive injuries play an important role in the pathogenesis. Intrinsic foot muscle weakness is one of the most important factors in the etiology of epin calcanei, but hereditary conditions and metabolic disorders may be just as important by accelerating the inflammatory process (6,7). Chronic damage occurs with a decrease in the elasticity of the insertional cartilage. Mesenchymal cells in the scar tissue fill the cracks in the damaged cartilage. With the re-formation of blood vessels, the scar gradually ossifies to form a bony spur (8). Another cause is the increasing degeneration of the elastic adipose tissue in the heel with aging.

An insufficient consumption of foods containing vitamin D, insufficient sun exposure, or issues with absorption are the causes of vitamin D insufficiency. It has been observed that vitamin D insufficiency should be included in the differential diagnosis of muscle and bone pain, and that correction of deficiency is an essential component in the treatment of these individuals (24). There is controversy about the limited value of vitamin D in studies. Plasma 25-OH-D3 is the best clinical indicator of vitamin D because it includes all dietary and cutaneous synthesized vitamin D (25). It is recommended to assess the 25(OH)D level, which accounts for both endogenous synthesis and vitamin D consumption and has a half-life of 2-3 weeks in an individual. 1,25(OH)2D active biological form is not suitable for ideal measurement. Considering that its half-life is as brief as 4-6 hours and that its circulation levels are 1000 times lower than those of 25(OH)D. There have been studies to identify vitamin D insufficiency and deficiency, as well as the typical range of 25(OH)D values. According to these studies, if the 25(OH)D level is below 20 ng/mL, it is defined as vitamin D insufficiency, and if it is between 21-29 ng/mL, it is defined as vitamin D deficiency. Between 30-60 ng/mL' is defined as normal. Over 150 ng/mL is considered as vitamin D intoxication (26,27). Low vitamin 25(OH)D levels cause secondary hyperparathyroidism. Thus, they may accelerate and worsen osteopenia and osteoporosis in adults because they cause bone resorption via osteoclasts (14). Vitamin D increases osteoblastic activity and supports bone mineralization by keeping PTH levels in the normal range and consequently significantly reduces the likelihood of falls and fractures (14,15). Studies are showing that the probability of fracture is lower in patients with vitamin 25(OH)D levels >30 ng/mL (26). In our study, we accepted the limit value of vitamin D as 30 ng/ml. There is a positive relationship between vitamin 25(OH)D and proximal muscle strength, physical activity, and lower extremity functions. Postural and dynamic balance and muscle strength can be increased by vitamin D supplementation (28,29). The Framingham Study suggested that low vitamin D levels may be associated with the development of cartilage loss and progression of knee osteoarthritis (30). Similarly, moderate evidence has shown that low vitamin D levels are associated with increased progression of radiographic osteoarthritis. In our study, serum vitamin D levels were decreased in epin calcanei formation, suggesting that this may be a predisposing factor.

Vitamin D has a variety of vascular effects. Thrombosis, smooth muscle cell proliferation, and inflammation modulation are some of them. PTH is a hormone that increases vascular remodeling and myocyte hypertrophy. Studies are showing that adequate vitamin D reduces inflammation at the cellular level and has a protective effect on cell functions (31). Low vitamin D may impair bone mineralization and cause pain in muscles and joints associated with diffuse or isolated bone pain. It has been suggested that vitamin D has a cartilage-supporting effect and that osteoarthritis may develop or progress as a result of cartilage thinning due to the loss of this support in vitamin D deficiency (32). Vitamin D has anti-inflammatory effects via macrophages, tumor necrosis factor, and interleukin (31). Muscle tone and muscle strength decrease in low vitamin D levels. As a result of the interaction of vitamin D with its receptors in muscle, protein synthesis increases, and muscle strength and mass increase (33,34). Vitamin D deficiency leads to increased inflammation, thinning of cartilage, and especially weakness of muscles. Thus, we have seen in our study that microtraumas in the bone and impaired mineralization of the newly formed bone due to vitamin D deficiency an etiological factors in the formation of epin calcanei. Since there are no publications on epin calcanei in the literature, we wanted to show its relationship with epin calcanei in our study. This study will allow us to look at patients diagnosed with epin calcanei from a different perspective or to develop a new perspective to prevent epin calcanei when heel pain is present before it occurs. With the discovery of vitamin D receptors in several tissues, interest in vitamin D has recently grown. Several research have been carried out to determine how low vitamin D levels relate to different disorders. In our study, low vitamin D levels were seen in both groups, although the epin calcanei group had lower vitamin D levels than the control group. This suggests that it is important in the pathogenesis of epin calcanei. This conclusion needs to be supported by more research.

Our research has several limitations. The data in the study were collected retrospectively from file records and were not evaluated seasonally. The duration of sun exposure of the patients was also not taken into consideration. Vitamin D insufficiency may be a factor in a number of musculoskeletal diseases.

CONCLUSION

As a result, our study's case group had statistically lower levels of vitamin 25(OH)D than the control group. Low levels of vitamin 25(OH)D, in our opinion, are predisposing factors in the formation of epin calcanei. We believe that vitamin D supplementation may be able to stop the development of epin calcanei in individuals with low vitamin D levels at various points in time. Supporting our data with studies with large patient groups may allow a better understanding of etiology.

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Research Article

Evaluation of the Level of Knowledge and Awareness of Dentists about the Use of Antibiotics in Periodontal Treatment

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Abstract

Aim: Antibiotics are widely used in dentistry and are essential in periodontal treatment. The purpose of the study is to evaluate dentists' knowledge and awareness levels about the use of antibiotics in periodontal therapy and to find out in which cases they prescribe antibiotics to their patients.

Material and Methods: The participants consisted of 150 volunteer dentists. They were asked to fill in the questionnaire that consisted of related questions. Chi-square and Fisher's exact tests determined the relationship between categorical variables. Statistical significance level $p<0.05$ was accepted as significant.

Results: The study included 105 (67.7%) female and 50 (32.3%) male participants. It was revealed that the antibiotics most prescribed by dentists in periodontal treatment procedures were amoxicillin-clavulanic acid (80%) and metronidazole (57%). A statistically significant correlation was observed between the dentists' "expertise/doctoral status" and the question"Prescribing antibiotics" in the tooth surface cleaning and root surface straightening, crown lengthening, crest augmentation, and sinus lifting procedures. After periodontal surgical procedures, they mostly preferred to use antibiotics for 5 (23.4%) and seven days (24%) after the process. The study also argued that most participants prescribed antibiotics in addition to non-surgical mechanical therapy, believing that it improves clinical outcomes (70.5%) and reduces post-operative complications (70.5%).

Conclusion: The findings of the study suggest that dentists need to be more aware of the use of antibiotics in periodontal treatment procedures.

Keywords: Survey study, antibiotic usage, periodontal treatment

INTRODUCTION

The development of periodontal diseases is associated with subgingival bacterial colonization and biofilm formation, which causes chronic inflammation of soft tissues, destruction of tooth-supporting collagen fibers, and resorption of alveolar bone (1). Simultaneously, bacterial plaque is shown as the primary etiological agent of periodontal diseases (2). Today, periodontal therapy emphasizes the suppression or destruction of specific periodontal pathogens in the mouth and the regeneration and maintenance of periodontal health-related microbial flora (3). Conventional non-surgical periodontal treatment involves mechanical debridement, including tooth surface cleaning and root surface straightening. It is associated with clinical recovery, suppression of periodontopathogens, and recolonization of the biofilm with host-compatible beneficial species (4). The findings of the study suggest that dentists need to be more aware of the use of antibiotics in periodontal treatment procedures

Antibiotics are widely used in dentistry, and these drugs have an essential place in periodontal treatment (5,6). Systemic antibiotics are widely used in periodontal therapy in addition to mechanical therapy (scaling-root planning) with their ability to destroy soft tissue-invasive pathogens and inhibit microorganisms in hard-to-reach areas. It is also known that systemic antibiotics and scaling-root planning (SRP) significantly increase clinical attachment gain and contribute to maintaining gained periodontal health for extended periods (7).

There is widespread concern about the overuse of antibiotics and the emergence of resistant strains of

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bacteria. Several features characterize antibiotic use in dentistry. Worldwide, antibiotic use in dentistry is characterized by empirical prescribing based on clinical factors and previously known bacteriological epidemiology (8,9). As a result, broad-spectrum antibiotics are widely used, and resistance development of oral microorganisms has increased (5,10). For example, β-lactamase-producing microorganisms are isolated from patients treated with β-lactam antibiotics (11). A similar pattern of resistance has been reported for Streptococcus viridans (a pathogen commonly isolated in dental infections) to macrolides, penicillin, and clindamycin (12). In addition to the development of resistance, side effects(including gastrointestinal, allergic, and hematological reactions) are other problems of antibiotic use. For this reason, the rational use of antibiotics in dentistry is essential in terms of reducing the development of resistance and side effects in oral pathogens and increasing their effectiveness (13). Conversely, informing patients about the antibiotics prescribed is important. This information should include doses and dose ranges, drug side effects, drug interactions, storage conditions, and the price of prescribed drugs. This avoids misunderstanding of the information provided. On the other hand, the completeness of the information to be given to the patient will increase the success rate of the treatment, the patient's compliance, quality of life, and cost-effectiveness (14).

There have been limited studies of dentists prescribing antibiotics in periodontal treatment procedures, which have varied widely in what is prescribed (5,6). Our study aims to evaluate the knowledge and awareness levels of dentists about the use of antibiotics in periodontal treatment and to learn when they prescribe antibiotics to their patients.

MATERIAL AND METHOD

This research is a descriptive epidemiologic survey study. The study was conducted between January 2022 and February 2022. The study protocol was approved by the Research Ethics Committee of Recep Tayyip Erdoğan University; Date:09.05.2022, Number 2023/116.

A total of 155 voluntary participants, 105 female and 50 male dentists, who were informed about this study in detail and agreed to participate, were included.

Statistical Analyses

The sample size of this study was calculated using the G*Power statistical program (ver.3.1.9.7). The 11-question questionnaire form, which was prepared with Google Forms after reviewing the relevant literature on the subject, was sent to dentists, doctoral/specialty students, and specialist dentists via various social media (WhatsApp, Gmail, etc.) to be filled out online. In the first part, to determine demographic data, questions were asked about professional experience, gender, the institution of employment, and specialty/doctoral education. In the second part, questions were asked to determine the frequency/tendency of antibiotic prescription in periodontal treatment procedures, frequency of antibiotic prescription in periodontal findings/patient types, type of antibiotic prescribed, frequency of use, and reason for prescribing antibiotics.

In addition, questions were asked to find out whether the physicians prescribe antibiotics to patients in non-surgical mechanical treatment, including tooth surface cleaning and root surface smoothing, and if so, with which protocol they prescribe antibiotics, the tendency to prescribe antibiotics in surgical procedures, and the antibiotic prescription protocol. The necessary Institutional Approval Certificate was obtained for the study.

This study determined descriptive variables such as professional experience, gender, education, and institution of employment. In our research, the professional experience of the participants was categorized as 1-5 years, 6-10 years, 11-15 years, and >15 years, and the educational status was categorized as a general dentist, specialty/doctorate in periodontology, specialty/doctorate in another branch, specialty/doctorate in periodontology and specialty/doctorate in another branch. Participants were divided into three groups: private practice/polyclinic, Oral and Dental Health Center, or other public institutions and university employees.

The criteria for inclusion in the study were to be a dentist/specialty student/doctoral student/specialized dentist and to have approved the voluntary consent form for the study.

In calculating the sample size of this study, the power (power of the test) for each variable was at least 80%, and the type 1 error was 5%. Descriptive statistics for categorical variables in our study were expressed as numbers (n) and percentages (%). "Chi-square" and "Fisher's exact" tests determined the relationship between categorical variables. The statistical significance level was taken as 5%, and the SPSS (IBM SPSS for Windows, ver.26) statistical package program was used for calculations.

RESULTS

A total of 155 people participated in the survey. Table 1 shows the general descriptive statistics of the demographic data of the physicians. Accordingly, the majority of the participants (67.7%) were female; the majority (63.2%) had 1-5 years of experience; 56.1% worked in universities; and the majority (30.3%) did not have a specialty/doctorate.

Table 2 shows the relationship and distribution between "specialty/doctoral status" and "the frequency of dentists prescribing antibiotics in procedures". According to this; A statistically significant correlation was observed between the dentists' question of "expertise/doctoral status" and "prescribing antibiotics in the procedure of tooth surface cleaning and root surface straightening" ($p<0.05$). Similarly; A statistically significant correlation was observed between the dentists' "expertise/doctoral status" and the question "Prescribing antibiotics in the crown lengthening procedure" ($p<0.05$). At the same time; a statistically significant correlation was observed

between the dentists' "expertise/doctoral status" and the question "antibiotic prescribing in the crest augmentation procedure" (p<0.05). Finally; A statistically significant correlation was observed between the dentists' question of "expertise/doctoral status" and "prescribing antibiotics in the sinus lift procedure procedure" (p<0.05). Despite that; Except for the questions mentioned above, no statistically significant relationship was observed between the questions "frequency of dentists prescribing other antibiotics" and "expertise/doctoral status".

Table 3 shows the relationship and distribution between "expertise/doctoral status" and "antibiotic prescribing frequency in periodontal findings/patient types". According to this; For all the questions in the table, no statistically significant relationship could be observed between "the frequency of antibiotic prescribing in periodontal findings/ patient types" and "expertise/doctoral status" (p>0.05). In other words; "These antibiotic prescribing frequency questions" do not differ "according to specialty/doctoral status".

Table 4 shows the relationship and distribution between "expertise/doctoral status" and "what type of antibiotics and how often they prefer in periodontal findings/ procedures". According to these results; A statistically significant correlation was observed between the dentists' "specialty/doctoral status" and the "frequency of clindamycin prescription" question (p<0.05).

Similarly; A statistically significant correlation was observed between the dentists' "expertise/doctoral status" and the "frequency cephalosporin prescription" question (p<0.05).

Also; A statistically significant correlation was observed between the dentists' question of "expertise/doctoral status" and "frequency of prescribing Erythromycin" (p<0.05).

Finally; A statistically significant correlation was observed between the dentists' "expertise/doctoral status" and the "frequency of prescribing clarithromycin" (p<0.05).

Despite that; Except for the questions mentioned above, no statistically significant relationship was observed between the questions "frequency of dentists prescribing other antibiotics" and "expertise/doctoral status" (p>0.05).

Table 1. Demographic descriptive data of the participants

		N	%
Gender	M	50	32.3
	F	105	67.7
Professional experience	1-5 years	98	63.2
	6-10 years	37	23.9
	11-15 years	11	7.1
	>15 years	9	5.8
Working place	Oral and Dental Health Centre or other public institutions	45	29.0
	Private practice/polyclinic	23	14.8
	University	87	56.1
Specialty/doctorate status	No	47	30.3
	I received specialization/doctorate training in another branch	20	12.9
	I am studying for a specialty/doctorate in another branch	40	25.8
	I received specialty/doctorate training in periodontology	13	8.4
	I am studying for a specialty/doctorate in periodontology	35	22.6

Table 2. The relationship and distribution between "specialty/doctoral status" and "the frequency of dentists prescribing antibiotics in procedures"

		Specialty/doctoral status										
		None		I received specialist/ doctorate education in another branch		I am studying for a specialist/ doctorate in another branch		I received a specialist/doctorate education in periodontology		I am studying for a specialist/ doctorate in periodontology		*p.
		N	%	N	%	N	%	N	%	N	%	
Tooth surface debridement and root surface straightening	Sometimes	5	26.3	0	0.0	4	21.1	2	10.5	8	42.1	.035
	Never	20	26.3	14	18.4	26	34.2	5	6.6	11	14.5	
	Rarely	22	36.7	6	10.0	10	16.7	6	10.0	16	26.7	
Frenectomy	Sometimes	6	28.6	1	4.8	8	38.1	2	9.5	4	19.0	.717
	Usually	1	14.3	1	14.3	2	28.6	1	14.3	2	28.6	
	Always	3	75.0	1	25.0	0	0.0	0	0.0	0	0.0	
	Never	22	27.5	12	15.0	17	21.3	7	8.8	22	27.5	
	Rarely	15	34.9	5	11.6	13	30.2	3	7.0	7	16.3	

*Chi-square, Fisher's exact tests

Table 2. The relationship and distribution between "specialty/doctoral status" and "the frequency of dentists prescribing antibiotics in procedures"												
	Sometimes	8	42.1	1	5.3	6	31.6	1	5.3	3	15.8	
	Usually	3	25.0	3	25.0	3	25.0	1	8.3	2	16.7	
Gingivectomy	Always	3	75.0	1	25.0	0	0.0	0	0.0	0	0.0	.468
	Never	18	24.0	13	17.3	17	22.7	7	9.3	20	26.7	
	Rarely	15	33.3	2	4.4	14	31.1	4	8.9	10	22.2	
	Sometimes	11	47.8	3	13.0	6	26.1	1	4.3	2	8.7	
	Usually	6	23.1	5	19.2	9	34.6	2	7.7	4	15.4	
Vestibuloplasty	Always	2	25.0	1	12.5	3	37.5	0	0.0	2	25.0	.685
	Never	17	30.9	6	10.9	12	21.8	7	12.7	13	23.6	
	Rarely	11	25.6	5	11.6	10	23.3	3	7.0	14	32.6	
	Sometimes	10	30.3	2	6.1	9	27.3	3	9.1	9	27.3	
	Usually	13	29.5	9	20.5	8	18.2	4	9.1	10	22.7	
Flap surgery	Always	4	25.0	3	18.8	2	12.5	0	0.0	7	43.8	.478
	Never	12	35.3	4	11.8	11	32.4	3	8.8	4	11.8	
	Rarely	8	28.6	2	7.1	10	35.7	3	10.7	5	17.9	
	Sometimes	7	31.8	3	13.6	5	22.7	0	0.0	7	31.8	
	Usually	2	8.7	3	13.0	5	21.7	4	17.4	9	39.1	
Crown lengthening	Always	1	10.0	0	0.0	3	30.0	0	0.0	6	60.0	**.026**
	Never	25	39.7	8	12.7	18	28.6	6	9.5	6	9.5	
	Rarely	12	32.4	6	16.2	9	24.3	3	8.1	7	18.9	
	Sometimes	11	36.7	4	13.3	7	23.3	2	6.7	6	20.0	
Resektive periodontal surgery (hemisection, root amputation)	Usually	10	26.3	5	13.2	9	23.7	3	7.9	11	28.9	
	Always	6	23.1	4	15.4	5	19.2	1	3.8	10	38.5	.684
	Never	9	32.1	3	10.7	11	39.3	2	7.1	3	10.7	
	Rarely	11	33.3	4	12.1	8	24.2	5	15.2	5	15.2	
	Sometimes	12	35.3	4	11.8	8	23.5	2	5.9	8	23.5	
Regenerative periodontal surgery (YDR)	Usually	9	25.0	6	16.7	9	25.0	6	16.7	6	16.7	
	Always	10	25.0	4	10.0	7	17.5	4	10.0	15	37.5	.268
	Never	9	34.6	4	15.4	11	42.3	0	0.0	2	7.7	
	Rarely	7	36.8	2	10.5	5	26.3	1	5.3	4	21.1	
	Sometimes	10	33.3	3	10.0	7	23.3	1	3.3	9	30.0	
	Usually	12	30.8	6	15.4	10	25.6	4	10.3	7	17.9	
Free gingival graft	Always	7	26.9	4	15.4	6	23.1	1	3.8	8	30.8	.851
	Never	10	26.3	5	13.2	12	31.6	6	15.8	5	13.2	
	Rarely	8	36.4	2	9.1	5	22.7	1	4.5	6	27.3	
	Sometimes	10	37.0	1	3.7	6	22.2	1	3.7	9	33.3	
	Usually	11	25.6	7	16.3	12	27.9	4	9.3	9	20.9	
Connective tissue graft	Always	8	27.6	5	17.2	6	20.7	2	6.9	8	27.6	.822
	Never	11	30.6	5	13.9	11	30.6	5	13.9	4	11.1	
	Rarely	7	35.0	2	10.0	5	25.0	1	5.0	5	25.0	
	Sometimes	10	38.5	1	3.8	7	26.9	2	7.7	6	23.1	
	Usually	14	32.6	6	14.0	11	25.6	3	7.0	9	20.9	
Implant surgery	Always	10	19.2	9	17.3	10	19.2	8	15.4	15	28.8	.312
	Never	6	31.6	3	15.8	8	42.1	0	0.0	2	10.5	
	Rarely	7	46.7	1	6.7	4	26.7	0	0.0	3	20.0	
	Sometimes	10	50.0	1	5.0	5	25.0	1	5.0	3	15.0	
	Usually	11	24.4	7	15.6	12	26.7	4	8.9	11	24.4	
Crestal augmentation	Always	11	19.0	9	15.5	11	19.0	7	12.1	20	34.5	**.024**
	Never	7	38.9	2	11.1	9	50.0	0	0.0	0	0.0	
	Rarely	8	57.1	1	7.1	3	21.4	1	7.1	1	7.1	
	Sometimes	10	43.5	2	8.7	7	30.4	1	4.3	3	13.0	
	Usually	13	28.9	7	15.6	11	24.4	4	8.9	10	22.2	
Sinus elevating operation	Always	10	16.9	8	13.6	12	20.3	8	13.6	21	35.6	**.042**
	Never	9	47.4	2	10.5	8	42.1	0	0.0	0	0.0	
	Rarely	5	55.6	1	11.1	2	22.2	0	0.0	1	11.1	

*Chi-square, Fisher's exact tests

Table 3. "The relationship and distribution between "specialty/doctoral status" and "antibiotic prescribing frequency in periodontal findings/patient types"

		Specialty/doctoral status										
		None		I received specialist/ doctorate education another branch		I am studying for a specialist/ doctorate in another branch		I received specialist/doctorate education in periodontology		I am studying for a specialist/ doctorate in periodontology		*p.
		N	%	N	%	N	%	N	%	N	%	
In gingivitis with more than 30% bleeding on probing	Sometimes	6	54.5	1	9.1	2	18.2	1	9.1	1	9.1	.056
	Usually	5	83.3	0	0.0	1	16.7	0	0.0	0	0.0	
	Never	18	20.5	14	15.9	23	26.1	11	12.5	22	25.0	
	Rarely	18	36.0	5	10.0	14	28.0	1	2.0	12	24.0	
If there is mobility	Sometimes	5	38.5	1	7.7	1	7.7	2	15.4	4	30.8	.776
	Usually	2	66.7	0	0.0	1	33.3	0	0.0	0	0.0	
	Never	27	29.3	11	12.0	27	29.3	6	6.5	21	22.8	
	Rarely	13	27.7	8	17.0	11	23.4	5	10.6	10	21.3	
If pocket depth is ≥5 mm and radiographic bone loss has reached the middle and apical third	Sometimes	11	32.4	8	23.5	4	11.8	2	5.9	9	26.5	.188
	Usually	5	33.3	3	20.0	3	20.0	1	6.7	3	20.0	
	Always	3	100.0	0	0.0	0	0.0	0	0.0	0	0.0	
	Never	17	27.9	6	9.8	22	36.1	4	6.6	12	19.7	
	Rarely	11	26.2	3	7.1	11	26.2	6	14.3	11	26.2	
In young patients with periodontitis with molar/ incisional involvement	Sometimes	7	33.3	6	28.6	3	14.3	2	9.5	3	14.3	.123
	Usually	9	22.5	4	10.0	8	20.0	6	15.0	13	32.5	
	Always	4	25.0	2	12.5	4	25.0	2	12.5	4	25.0	
	Never	9	28.1	3	9.4	15	46.9	1	3.1	4	12.5	
	Rarely	18	39.1	5	10.9	10	21.7	2	4.3	11	23.9	
Necrotizing gingivitis/ periodontitis	Sometimes	11	37.9	1	3.4	8	27.6	2	6.9	7	24.1	.969
	Usually	9	22.5	7	17.5	11	27.5	5	12.5	8	20.0	
	Always	9	30.0	4	13.3	7	23.3	3	10.0	7	23.3	
	Never	10	38.5	3	11.5	6	23.1	1	3.8	6	23.1	
	Rarely	8	26.7	5	16.7	8	26.7	2	6.7	7	23.3	
In the presence of a periodontal abscess	Sometimes	9	25.0	4	11.1	12	33.3	4	11.1	7	19.4	.856
	Usually	16	42.1	5	13.2	6	15.8	3	7.9	8	21.1	
	Always	8	32.0	4	16.0	4	16.0	3	12.0	6	24.0	
	Never	3	16.7	3	16.7	7	38.9	1	5.6	4	22.2	
	Rarely	11	28.9	4	10.5	11	28.9	2	5.3	10	26.3	
In the presence of pus draining from the periodontal space	Sometimes	6	20.7	5	17.2	7	24.1	3	10.3	8	27.6	.672
	Usually	11	40.7	4	14.8	5	18.5	2	7.4	5	18.5	
	Always	8	42.1	2	10.5	4	21.1	2	10.5	3	15.8	
	Never	7	18.4	3	7.9	16	42.1	3	7.9	9	23.7	
	Rarely	15	35.7	6	14.3	8	19.0	3	7.1	10	23.8	
Class 2 or 3 furcation defects	Sometimes	9	26.5	6	17.6	4	11.8	3	8.8	12	35.3	.091
	Usually	7	38.9	2	11.1	5	27.8	2	11.1	2	11.1	
	Always	3	60.0	2	40.0	0	0.0	0	0.0	0	0.0	
	Never	16	30.2	7	13.2	20	37.7	2	3.8	8	15.1	
	Rarely	12	26.7	3	6.7	11	24.4	6	13.3	13	28.9	
Diabetic patients	Sometimes	12	33.3	5	13.9	7	19.4	2	5.6	10	27.8	.242
	Usually	11	36.7	6	20.0	5	16.7	1	3.3	7	23.3	
	Always	1	25.0	0	0.0	1	25.0	2	50.0	0	0.0	
	Never	9	31.0	4	13.8	10	34.5	1	3.4	5	17.2	
	Rarely	14	25.0	5	8.9	17	30.4	7	12.5	13	23.2	

*Chi-square, Fisher's exact tests

Table 4. "The relationship and distribution between "specialty/doctoral status" and "which antibiotics and how often they prefer in periodontal findings/procedures"

		Specialty/doctoral status										
		None		I received specialist/ doctorate education another branch.		I am studying for a specialist/ doctorate in another branch		I received specialist/doctorate education in periodontology		I am studying for a specialist/ doctorate in periodontology		*p.
		N	%	N	%	N	%	N	%	N	%	
Amoxicillin (Largopen, Alfoxil)	Sometimes	16	29.6	9	16.7	12	22.2	3	5.6	14	25.9	.934
	Usually	9	34.6	2	7.7	6	23.1	2	7.7	7	26.9	
	Always	1	33.3	0	0.0	1	33.3	0	0.0	1	33.3	
	Never	10	37.0	2	7.4	6	22.2	4	14.8	5	18.5	
	Rarely	11	24.4	7	15.6	15	33.3	4	8.9	8	17.8	
Amoxicillin + clavulanic acid (Klamoks, Augmentin, Croxilex etc.)	Sometimes	8	25.8	6	19.4	9	29.0	2	6.5	6	19.4	.184
	Usually	18	22.5	10	12.5	20	25.0	11	13.8	21	26.3	
	Always	7	43.8	1	6.3	3	18.8	0	0.0	5	31.3	
	Never	4	80.0	0	0.0	1	20.0	0	0.0	0	0.0	
	Rarely	10	43.5	3	13.0	7	30.4	0	0.0	3	13.0	
Metronidazole. ornidazole (Flagyl. Biteral. etc.)	Sometimes	21	34.4	7	11.5	14	23.0	7	11.5	12	19.7	.435
	Usually	11	19.3	9	15.8	14	24.6	5	8.8	18	31.6	
	Always	6	54.5	1	9.1	2	18.2	0	0.0	2	18.2	
	Never	2	28.6	0	0.0	4	57.1	0	0.0	1	14.3	
	Rarely	7	36.8	3	15.8	6	31.6	1	5.3	2	10.5	
Tetracycline (Tetra etc.)	Sometimes	14	33.3	4	9.5	12	28.6	4	9.5	8	19.0	.434
	Usually	5	25.0	4	20.0	7	35.0	1	5.0	3	15.0	
	Always	2	66.7	1	33.3	0	0.0	0	0.0	0	0.0	
	Never	11	26.2	3	7.1	11	26.2	7	16.7	10	23.8	
	Rarely	15	31.3	8	16.7	10	20.8	1	2.1	14	29.2	
Doxycycline (Monodox, Tetradox)	Sometimes	14	34.1	4	9.8	13	31.7	2	4.9	8	19.5	.744
	Usually	2	11.8	4	23.5	3	17.6	3	17.6	5	29.4	
	Always	2	50.0	1	25.0	1	25.0	0	0.0	0	0.0	
	Never	16	34.8	4	8.7	11	23.9	5	10.9	10	21.7	
	Rarely	13	27.7	7	14.9	12	25.5	3	6.4	12	25.5	
Clindamycin (Klindan, Clin, Cleocin)	Sometimes	15	34.1	3	6.8	16	36.4	2	4.5	8	18.2	.049
	Usually	3	16.7	7	38.9	5	27.8	1	5.6	2	11.1	
	Always	1	33.3	1	33.3	1	33.3	0	0.0	0	0.0	
	Never	14	38.9	2	5.6	8	22.2	2	5.6	10	27.8	
	Rarely	14	25.9	7	13.0	10	18.5	8	14.8	15	27.8	
Spiramycin (Rovamycine)	Sometimes	4	19.0	5	23.8	7	33.3	2	9.5	3	14.3	.148
	Usually	4	50.0	2	25.0	2	25.0	0	0.0	0	0.0	
	Never	23	29.1	5	6.3	22	27.8	5	6.3	24	30.4	
	Rarely	16	34.0	8	17.0	9	19.1	6	12.8	8	17.0	
Cephalosporin (Sef etc.)	Sometimes	6	26.1	6	26.1	6	26.1	2	8.7	3	13.0	.001
	Usually	2	18.2	6	54.5	2	18.2	1	9.1	0	0.0	
	Never	18	26.5	4	5.9	16	23.5	7	10.3	23	33.8	
	Rarely	21	39.6	4	7.5	16	30.2	3	5.7	9	17.0	
Erythromycin (Eritro, Erimicin etc.)	Sometimes	4	21.1	6	31.6	5	26.3	0	0.0	4	21.1	.015
	Usually	2	50.0	2	50.0	0	0.0	0	0.0	0	0.0	
	Never	25	29.8	3	3.6	27	32.1	8	9.5	21	25.0	
	Rarely	16	33.3	9	18.8	8	16.7	5	10.4	10	20.8	
Clarithromycin (Klacid. Macrol. etc.)	Sometimes	8	26.7	6	20.0	11	36.7	0	0.0	5	16.7	.006
	Usually	2	20.0	5	50.0	1	10.0	1	10.0	1	10.0	
	Never	20	27.0	4	5.4	21	28.4	7	9.5	22	29.7	
	Rarely	17	41.5	5	12.2	7	17.1	5	12.2	7	17.1	
Azithromycin (Azitro, Zithromax)	Sometimes	7	20.6	9	26.5	11	32.4	2	5.9	5	14.7	.073
	Usually	6	40.0	3	20.0	2	13.3	0	0.0	4	26.7	
	Never	19	32.2	2	3.4	18	30.5	4	6.8	16	27.1	
	Rarely	15	31.9	6	12.8	9	19.1	7	14.9	10	21.3	

*Chi-square. Fisher's exact tests

DISCUSSION

This epidemiological study compares the antibiotics dentists prefer to treat periodontal disease and conditions and the reasons for prescribing and not prescribing antibiotics.

Periodontal disease is an inflammatory disease of bacterial origin that affects the supporting tissues, and its leading cause is the accumulation of microbial dental plaque (15). Although there is strong evidence that mechanical therapy is effective in removing supra and subgingival plaque, it may not be able to destroy pathogens in hard-to-reach areas (16). Therefore, there is a consensus that systemic antibiotics and mechanical therapy may be beneficial in some cases (17,18).

In preventing the progression of periodontal diseases, the priority is to eliminate the factors that cause the onset of the disease and then to observe the regression of symptoms. Therefore, antibiotic use is not necessary in all cases (19). In a clinical study by Mohan et al.(20), while not supporting the routine use of antibiotics after periodontal surgical treatments, they concluded that antibiotics should be used only for medical indications and in case of infection in the postoperative period. The majority of the participants in our study stated that they never prescribed antibiotics for non-surgical mechanical treatment, including tooth surface cleaning and root surface straightening (76%) and for surgical procedures such as frenectomy (80%), gingivectomy (75%) and crown lengthening procedures (63%). Studies have shown that systemic antibiotics are unnecessary in such simple periodontal surgical procedures, and our survey results are consistent with the available literature (21,22). Compared to routine dental surgery, sinus surgery is supported by studies that show higher morbidity due to possible additional infection routes such as bacterial invasion and graft material (23). In our study, most participants (59%) said they preferred to prescribe antibiotics for sinus surgery.

In a study conducted by Mollahaliloğlu et al. (24) in 2013 covering various healthcare institutions in Turkey, it was reported that physicians most frequently prescribed "beta-lactam antibacterials, penicillins" and "amoxicillin+clavulanic acid" and this attitude was similar in 4 different healthcare institutions (primary healthcare institutions, state hospital, private hospital, and university hospital). In a study conducted by Mainjot et al. (25) with 268 dentists, 4.2% of patients were prescribed antibiotics, and 82% of the choices were amoxicillin, amoxicillin/clavulanate, and clindamycin. In a study of 686 dentists investigating antibiotic prescribing practices of members of the American Association of Endodontists, it was reported that the most preferred antibiotics were amoxicillin and penicillin. At the same time, clindamycin was the first choice in cases of medical allergy. It was reported that antibiotics prescribed in 36.89% of patients were unnecessary, and the most common reason for prescribing antibiotics was patient expectations (26). Similarly, in our study, it was observed that amoxicillin-clavulanic acid (80%) and metronidazole (57%) were the most commonly prescribed antibiotics in periodontal treatment procedures.

In a study done in Germany that investigates the accuracy of the prescription and the amount of antibiotics prescribed by clinical guidelines, it was emphasized that dentists prescribe antibiotics with the desire to prevent clinical complications, fear of losing patients, and patient pressure (27). In our study, the majority of the participants stated that they prescribed antibiotics with the idea that antibiotic use improves clinical outcomes (70.5%) and reduces postoperative complications (70.5%) in addition to non-surgical mechanical treatment.

There are different regimens regarding the timing of antibiotic use in surgical procedures. In most studies, there is no standard and definite antibiotic regimen (28,29). In our study, most dentists preferred to use antibiotics for five days (23.4%) and seven days (24%) after periodontal surgical procedures.

CONCLUSION

The use of adjunctive systemic antibiotics in periodontal treatments is a subject that needs to be evaluated very well because systemic antibiotics have disadvantages such as having side effects, causing the development of bacterial resistance, and interactions with other drugs used by the patient. To prevent these side effects, dentists are responsible for choosing the correct indication, the proper dosage, and the right drug. The expertise of physicians affects both the use of antibiotics with the correct indication and the selection of the appropriate group of antibiotics.

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Research Article

The Importance of Perinatal Care Practices in Determining Pregnant Women's Satisfaction with Birth

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Abstract

Aim: Perinatal care services constitute an inseparable structure of the "safe motherhood" approach introduced by the World Health Organization (WHO) in 1987. In the Antenatal Care Guide published by WHO in 2016, they revealed that the woman should perform eight antenatal follow-ups during the pregnancy period. The "Antenatal Care Monitoring Protocol", which was revised by the Ministry of Health in Turkey in 2017, emphasized that at least four antenatal follow-ups should be made for each pregnant woman. The main purpose of perinatal services is; Regular monitoring of the pregnant and fetus by health personnel, having a healthy pregnancy and delivery process, preventing and determining risky situations, and meeting the psychosocial and medical needs of the pregnant. Our study was carried out as a descriptive and relationship seeker in order to determine the satisfaction of mothers with their birth in the perinatal care practices given in a university hospital.

Material and Methods: The sample part of the study was accepted as 60 women who applied to the delivery room of İstanbul University Çapa Medical Faculty to give birth, and 60 women who gave birth by cesarean section. The data obtained are; It was obtained by using the "Descriptive Information Form", "Optimality Index-Turkey (OI-TR) Case Report Form", "Maternal Satisfaction Assessment at Delivery Scale (DAMDÖ) (Normal Birth)" and Maternal Satisfaction Assessment at Birth (Cesarean Section)".

Results: When the results obtained in the research are interpreted; Perinatal CV index (PPI) score of 87.61% and optimality index (OI) score of 79.11% of the women in the research group; PPI score of 90.16% and OI score of 76.11% of women who had vaginal delivery; It was revealed that the PPI score of women who gave birth by cesarean section was 85.07% and the OI score was 82.12%.

Conclusion: When the DAMDÖ scores according to the delivery type were examined, it was determined that the women who gave birth in both ways had a high level of satisfaction with the birth.

Keywords: Perinatal car, optimality, birth satisfaction

INTRODUCTION

The World Health Organization (WHO) states that there should be a valid reason to interfere with the physiological process of the woman during the perinatal period (1). In this respect, every woman and fetus/newborn; have the right to receive optimal care during pregnancy, childbirth and postpartum. (2). One of the main ways to provide optimal care during the perinatal period is to use evidence-based practices (3). Use of evidence-based practice (CDU) in the perinatal field; during the perinatal period, it provides to reduce the harm and unnecessary interventions for the mother and fetus/newborn, to standardize the care and to increase the quality of maternal care (4,5). Today, many non-evidence-based medical interventions are applied during the perinatal period, especially in the intrapartum period. In our country, some interventions without evidence base in the intrapartum period (monitoring with continuous electronic fetal monitoring (SEFM), enema and shaving of the perineum, induction of labor, restriction of oral intake; use of lithotomy position, frequent vaginal tapping, not allowing companion during delivery, restriction of mobilization and episiotomy etc.) has been reported to be widely applied (6). However, it has been shown that applications without evidence base and the increase in technology use increase the cost and do not cause any change in perinatal outcomes, especially neonatal mortality (7).

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Nurses who care for women during the perinatal period; By performing evidence-based perinatal practices, it has a key role in enabling mothers, newborns and family members to spend this period comfortably and healthily, increasing maternal and infant health, and increasing maternal satisfaction (8). In this respect, nurses have important duties in providing optimal care in the perinatal period. Considering the literature review on the subject, although there are studies that reveal the satisfaction of the mother from birth and the affecting factors according to the birth type of women (9-11), no clear study has been found. For this reason, it was carried out in order to determine the perinatal care practices given in a university hospital and the satisfaction of mothers with birth.

The perinatal period covers the period from the 20th week of pregnancy to the first postpartum week (12). The target group of care services provided in this period are healthy/ at risk pregnant women, fetuses, newborns, puerperant women and family members.

In 1998, WHO recommended the principles necessary for optimal perinatal care. These principles (13);

- Providing non-medical perinatal care,
- Use of appropriate technological interventions while giving care,
- Evidence-based delivery of care,
- Based on a health system that includes an effective referral chain,
- Providing care with a multidisciplinary team,
- Giving holistic care,
- Providing family-centered care,
- Considering cultural differences in care,
- Ensuring women's participation in decisions while giving care,
- Paying attention to the privacy and dignity of the woman while giving care.

The perinatal period is a period in which the risk of preventable maternal/fetal/neonatal harm should be minimized and the quality of care should be increased (5). The purpose of the perinatal care given in this period; It is to protect and improve the health of the mother and newborn, and to minimize maternal/fetal/neonatal mortality and morbidity by providing early diagnosis and treatment of existing health problems that may occur during pregnancy. The content of care needed before and during pregnancy to achieve this goal is as follows (14):

- Preparation to parenting,
- Pregnancy planning,
- Physiological, anatomical and psycho-social adaptation to pregnancy and childbirth,
- Pregnancy maintenance and monitoring,
- Providing prenatal diagnosis methods,
- Early diagnosis and treatment of pregnancy-related health problems,
- Providing support in labor and determining the risks that may occur in labor,
- Caring for the newborn,
- Identification of newborn health problems,
- Providing care to the woman in the postpartum period and determining the problems that may occur,
- Adjustment of family members to pregnancy, birth and newborn.

The narrowing of the line between health and disease in the perinatal period requires careful consideration of the specified care needs (14). All mothers and newborns; have the right to safe and qualified care during pregnancy, childbirth and postpartum period. The task of the team working in the field of perinatology is to facilitate this process, rather than control it, and to meet the needs and expectations of the woman with an effective care (5).

Quality indicators of perinatal service such as maternal/ perinatal death, birth trauma and cesarean section rate; It has gradually lost its importance due to the decrease in mortality/morbidity rates and developments in health technologies. These indicators, which have been re-evaluated in recent years, have revealed that quality assessment should be made in accordance with changing conditions and multidimensional (15). For these reasons, the evaluation of satisfaction with the care received by women regarding the perinatal period has become increasingly important in recent years.

Birth experience, which can lead to physiological and psychological changes in a woman's health, and her satisfaction with birth are very important for the health of women and newborns, and positive family relationships (15). Women; may perceive birth as a difficult and negative experience to cope with. Negative perceptions and thoughts about birth; it disturbs the comfort of the woman and the newborn and may adversely affect the labor (16).

There are many factors that affect maternal satisfaction at birth. Factors affecting maternal satisfaction; socio-demographic-obstetric characteristics, organization of maternity services, expectations and birth plan, mode of delivery, prenatal preparation, communication with health personnel and respect for privacy, medical treatment and interventions, nursing care and emotional support, pain control, people participating in the birth, information, participation in decisions, communication with the baby and baby care, postpartum care, continuity of care and early discharge (15). High rates of medical interventions may adversely affect the woman's birth experience (17). Negative birth experience; It can cause postpartum depression in women, PTSD, cesarean section request in subsequent births, insufficiency in mother-newborn attachment, breastfeeding problems, neglect of the

baby, sexual dysfunction, and abortion after unwanted pregnancy (18).

Yaldır et al. (19), in which maternal satisfaction was evaluated in the early postpartum period, it was determined that the women's satisfaction with birth score was 93.25, that is, their satisfaction with the birth was low. Koc et al. (20) in Türkey to determine the relationship between maternal satisfaction at birth and the role of motherhood, it was determined that the maternal satisfaction score at birth was 131.96±21.02 for women who had vaginal delivery, and 141.81±21.32 for women who gave birth by cesarean section.

MATERIAL AND METHOD

Purpose of the research

The research was carried out as a descriptive and relationship seeker in order to determine the perinatal care practices given at İstanbul Çapa Medical Faculty and the satisfaction of mothers with birth. The ethical approval was taken from Diyarbakır Gazi Yaşargil Training and Research Hospital, Health Science University (Date: 16/12/2022 and no:368).

Research hypotheses

In this study, the Optimality Index (OI) was used to evaluate perinatal care practices. However, since it is not recommended to compare Optimality Index-TR scores according to delivery type, the following research questions were created and the scores were evaluated separately according to the delivery type.

- What are the OI scores of women who have given vaginal birth?
- What are the OI scores of women who gave birth by cesarean section?
- What is the satisfaction level of mothers who gave birth vaginally?
- What is the satisfaction level of mothers who gave birth by cesarean section?
- Is there a relationship between the total OI score of women who gave birth by cesarean section and the level of satisfaction with the birth?
- Is there a relationship between the total OI score of women who gave birth vaginally and the level of satisfaction with delivery?

Data collection tools

"Descriptive Information Form", "Optimality Index-Türkiye (OI-TR) Case Report Form", "Maternal Satisfaction Assessment at Delivery Scale (DAMDÖ) (Normal Birth)" and Maternal Satisfaction Assessment at Birth (Cesarean section) scales were applied to the participants.

Women who gave birth by cesarean section and women who did not have any allergic disease were included in the study. Women who gave birth normally or women with chronic diseases were not included in the study.

Statistical analysis

Statistical Package for the Social Sciences version 22.0 (SPSS Inc., Chicago, IL, USA) software was used for the statistical analysis of this study. While descriptive data and frequencies were calculated with the help of computer, the normal distribution of the data was tested with the Shapiro-Wilk test. Continuous variables were expressed as mean±standard deviation and nominal variables as numbers (percentage). Normally distributed data were compared between the two groups using Student's t-test, while those that did not fit normal distribution were tested with the Mann-Whitney U test. For categorical variables, Pearson's chi-square or Fisher's exact test was used as appropriate. P value <0.05 was considered statistically significant.

RESULTS

Findings related to descriptive characteristics of pregnants

Table 1 shows the distribution of some introductory characteristics of women. It was determined that 84% of the women had a high school or higher education level, 92% had a nuclear family type, and 92% did not have consanguineous marriages. It was determined that the mean age of the women included in the study was 27.15±3.28, and the mean body mass index was 23.21±3.20.

Table 1. Properties of women patients

Introductory features	Number	%
Level of education		
Under high school	20	16.6
High school and above	100	83.4
Family type		
Wide	10	8.33
Core	110	91.67
Consanguineous marriage		
There is	10	8.33
No	110	91.67
Age	27.15±3.28	
Body mass index*	23.21±3.20	
Total	120	100

*Data are given as mean±standard deviation

Table 2 shows the distribution of some obstetric characteristics of women. It was determined that 50% of the women gave birth by vaginal delivery and 50.0% by cesarean section. It was determined that 66.7% of the women were multiparous. It was determined that 97.5% of the participants did not have a history of preterm labor, 83.4% had a history of abortion and 88.4% did not have a history of D&C. It was determined that 96.67% of the women did not experience pregnancy complications in their current pregnancy.

Table 2. Distribution of some obstetric characteristics of women		
Obstetric features	**Number**	**%**
Type of birth		
Vaginal birth	60	50
Birth by cesarean section	60	50
Parity		
Primiparous	40	33.3
Multiparous	80	66.7
History of preterm action		
There is	3	2.5
No	117	97.5
Abortion story		
There is	20	16.6
No	100	83.4
D&C story		
There is	14	11.6
No	106	88.4
Current pregnancy complication status		
Lived	4	3.33
Didn't live	116	96.67
Total	120	100

Table 3 shows the distribution of OI-TR scores of women. It was determined that the PAI score of the women was 85.81%, the OI score was 76.51%, and the total OI score was 83.42%.

Table 3. Distribution of OI-TR index scores of women	
Oİ-TR	**Oİ-TR Score***
PÖİ	85.81%±9.11 (55.56%-100.00%)
Oİ	76.51%±5.64 (60.87%-86.96%)
Total Oİ	83.42%±4.12 (66.67%-91.18%)
*Data are given as mean±standard deviation (min-max)	

Table 4 shows the distribution of SEMSB scores of women according to the mode of delivery. It was determined that the mean SEMSB score of women who had vaginal delivery was 151.24±13.96, and the mean score of SEMSB of women who gave birth by cesarean section was 152.29±20.07 (p>0.05).

Table 4. Distribution of the scores of the Maternal Satisfaction Evaluation Scale at Childbirth according to the delivery type of women			
The scale for evaluation of maternal satisfaction at birth	**Vaginal birth (n=62)**	**Birth by cesarean (n=62)**	**Statistical analysis****
Total Score*	151.24±13.96 (106-203)	152.29±20.07 (100-197)	t=-0.10 p=0.91
*Data are given as mean±standard deviation (min-max), **In data analysis, t test was used			

DISCUSSION

In our study, when the OI-TR scores of women who gave birth by cesarean section were examined; It was determined that the PAI score was 85.07% and the OI score was 82.12%. The Optimality Index does not recommend comparing groups by mode of delivery. However, as expected, when compared to the PPI score of women who had vaginal delivery, women who gave birth by cesarean had a lower PPI score, that is, these women were more risky; When the OI score (76.11%) of the women who gave vaginal delivery was compared with the OI score (82.12%) of the women who gave birth by cesarean section, which is an invasive procedure in itself, it can be said that more interventions were made for women who had vaginal delivery.

Birth, which is seen as a very important experience in women's lives, and the woman's satisfaction in the perinatal period are extremely important for the woman's own health, the health of her baby, and positive family relationships (21). For this reason, it is very important for health personnel working in the perinatal area to evaluate the satisfaction of women with the care given and their views on birth, in order to increase the quality of perinatal care. Many factors affecting the level of maternal satisfaction at birth (socio-demographic-obstetric characteristics, organization of maternity services, expectations and birth plan, delivery method, prenatal preparation, communication with health personnel and respect for privacy, medical treatment and interventions, nursing care and emotional support, pain control, people participating in the birth, being informed, participation in decisions, communication with the baby and baby care, postpartum care, continuity of care and early discharge (22). Mode of delivery is one of the factors affecting maternal satisfaction (23). In our study, it was determined that women who gave birth by vaginal delivery and cesarean section had a high level of satisfaction with delivery. In addition, it was determined that the satisfaction levels of women who gave birth both vaginally (151.24±13.96) and cesarean section (152.29±20.07) were similar, but the difference was statistically insignificant (p>0.05).

CONCLUSION

In the literature, different results were obtained from our study in which the satisfaction of mothers who gave birth by vaginal and cesarean section were evaluated. In the cross-sectional study of Özkan and Bal (24) to determine maternal satisfaction in vaginal and cesarean delivery, the satisfaction level of women who gave birth by cesarean section in the early postpartum period was higher (158.1±15.2) than the satisfaction level of women who had vaginal delivery (161.8±16.0) found to be high.

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Research Article

A Case Series of Butane Intoxication Fatalities in the Southeastern Anatolia Region of Türkiye

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Abstract

Aim: Volatile solvent abuse (VSA), is the third most common form of substance abuse after alcohol and cigarettes. The aim of this study was to determine the incidence of fatalities due to butane gas.

Material and Methods: From 8,075 autopsies conducted in our center in Şanlıurfa, Türkiye in a 10-year period, 10 deaths were determined to be due to butane gas intoxication.

Results: All the fatalities were males (mean age: 17.6 years). Friends reported chronic use of lighter gas by the fatalities prior to the incident. In contrast, close relatives stated that it was the first instance of VSA. The preferred inhalation methods were bagging (i.e., inhaling gas from a plastic bag, n=4) and direct inhalation (i.e., spraying the gas directly in the mouth, n=6). The scene of incident findings revealed more than one lighter gas cartridge at the scene in nine cases and many lighters at the scene in one case. The autopsy examinations revealed minimal grazing on the body in three cases and no traumatic findings in seven cases. Butane was detected in blood samples in all 10 cases and in lung tissue samples in eight cases. In all 10 cases, there were areas of intra-alveolar swelling, edema, and bleeding in the lungs.

Conclusion: The actual incidence of VSA-related deaths is likely much higher than the number of reported cases, as our center is located near the Syrian border and has one of the highest populations of children and young people in Türkiye.

Keywords: Forensic science, volatile solvent abuse (VSA), butane, chronic abuse, sudden death, autopsy

INTRODUCTION

Volatile solvent abuse (VSA) is defined as the intentional inhalation of a volatile substance to reach a state of euphoria (1). After alcohol and cigarettes, VSA is the third most common form of substance abuse. The most frequently used VSA substances in Türkiye are toluene (paint thinner), chloride hydrocarbons (commonly found in solvents and pesticides), and lighter gas fluid (2,3). The incidence of VSA among adolescents and young adults in the U.S. has been estimated to vary between 10 and 15%, with a higher incidence among states with large rural populations (4).

Butane (n-butane, butyl hydrate; equivalent to the isomer isobutane) is a gas used in industrial products, personal care products, and cosmetic products. Thus, butane is commonly found in lighter fluid, fuel bottles, paint, hairspray, air fresheners, and deodorants. Butane is obtained by liquefaction and distillation of petroleum and classified as an aliphatic hydrocarbon (1). Typically, the composition of commercial butane gas is butane (60%), isobutane (30%), propane (9%), and ethane (1%) (5-7). Butane is the most widely used substance among children and adolescents with a VSA habit and has the highest death rate (5-7). Its widespread use is primarily due to its low cost, ease of availability, and lack of legislation governing its sale. (2-6). Deaths resulting from butane intoxication are typically due to i) butane causing direct toxicity and cardiac arrhythmia, such as ventricular tachycardia and fibrillation (8,9); ii) indirect toxicity caused by hypoxemia, anoxia, and vagal inhibition due to oxygen in the airway being used up (10); or iii) trauma associated with high-risk behaviors due to confusion while intoxicated (11). A combination of these factors may also be implicated in butane intoxication-related deaths (8-11).

Butane evaporates rapidly from biological samples after death, making it difficult to detect and analyze the gas postmortem. Most forensic publications recommend immediate taking of samples in suspected cases of butane intoxication and sample storage in appropriate closed glass jars, followed by analysis in as short a time as possible after collection (4-8). Despite the rapid clearance

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of inhaled butane, it tends to remain in brain and fat tissues because of high liposolubility (12,13). Sironi et al. investigated the distribution of butane and propane in two cases of fatal butane intoxication and showed that a high concentration in fat (adipose) tissue was consistent with the time interval from gas inhalation to death (12,14).

In this study, scene of incident findings, autopsy findings, and histopathological and biochemical analyses of samples taken postmortem were examined in deaths resulting from butane gas intoxication. The aim of this study was to determine the incidence of fatalities due to butane gas.

MATERIAL AND METHOD

This study was approved by the Training and Scientific Research Committee of the Council of Forensic Medicine Directorate (Date: 26/09/2022, Decision number: 21589509/2022/854). In total, 8,075 forensic autopsy cases were examined. The examinations of all the cases took place at the Forensic Medicine Institute, Şanlıurfa, Türkiye between 1 January 2012 and 31 December 2021. Of the cases examined, 10 cases were identified as deaths resulting from butane intoxication.

We conducted a retrospective examination of the hospital files of the cases, including autopsy reports and photographs, histopathology reports, incident reports prepared by the police or armed forces, witness statements, and expert opinion reports. The cases were examined in respect of sociodemographic data, findings in the area of the incident, information related to substance addiction, autopsy findings, histopathology and toxicology analysis results of bodily fluid and internal organ samples taken during the autopsy, and cause of death.

Headspace Gas Chromatography/Mass Spectroscopy (HS-GC/MS) Analysis of Butane

N-butane gas was detected in this study using a CLARUS 500 Gas Chromatograph (Perkin Elmer) and a Headspace sampler Turbo Matrix 16 device (Perkin Elmer). In the analyses, a C10 column and nitrogen as the carrier gas were used. A flame ionization detector was operated under the following conditions: temperature of 250°C, A-Cap of 120°C, injection section temperature of 60°C, column temperature of 40°C, and carrier gas of 20 psi. From the blood samples taken from the cases, 200 µl was taken using an automatic pipette and placed in a 20 ml gas chromatography/headspace vial. The vial was immediately closed with a silicone cover and metal ring using the crimping method. The butane gas content of a commercial lighter gas cartridge purchased in a local market was used as a reference in the analysis. Using the HS/GC method, the presence of n-butane in femoral blood taken during the autopsy was analyzed.

RESULTS

The study population comprised 10 (0.12%) cases that resulted in death after been admitted to the emergency department with cardiac arrest, where the cause of death was determined to be butane intoxication in an autopsy. All the cases were males, and the mean age was 17.6 years, ranging from 14–23 years. The incidents occurred most often in the summer months. None of the deceaseds had any known chronic disease. In all the cases, arrest occurred either at the scene of the incident or on the way to the hospital. The location of the incident was most often the home of the deceased (n=8). Before the autopsy, information about the incident was obtained from close relatives or friends of the deceased. In anamneses taken from friends of the deceased, chronic use of lighter gas before the incident was described. In contrast, in anamneses taken from close relatives, they reported that to their knowledge, this was the first instance of VSA. According to the findings at the scenes of the incidents and witness statements, the inhalation method was via a plastic bag filled with gas in four cases and spraying the gas directly into the mouth in six cases. The scene of incident findings revealed more than one lighter gas cartridge in nine cases and many lighters in one case.

In the autopsies, no acute traumatic lesions were seen in seven cases. One case had been sitting on a school's garden wall, approximately 1 meter in height, inhaling lighter gas, when he suddenly felt bad and fell to the ground. In this case, grazing was found on the knees and face. One case had inhaled the lighter gas at home and then gone out into the street and collapsed on the pavement in front of his house. In this case, minimal grazing was found. Another case had been released from prison because of the COVID-19 pandemic. In this case, the individual was presumed to have fallen down four or five steps when leaving his house after inhaling lighter gas, and there was minimal grazing on the face and extremities. In another case, the individual had been arguing with friends inside a vehicle when he suddenly became ill. However, no traumatic findings were found in the autopsy.

In addition to blood samples, intraocular fluid, bile fluid, urine, liver, and kidney samples were taken for biochemical analysis in all 10 cases, as routinely done in autopsies in our center. Lung tissue samples were also taken in eight cases. In all 10 cases, butane (n-butane/isobutane) was detected in blood samples. In the blood samples, propane was detected in one case, ethanol in one case, and therapeutic drugs in three cases. Butane (n-butane/isobutane) was detected in all the lung sample tissues taken from the cases. As only a qualitative analysis was performed in our laboratory, the concentration of butane gas could not be determined. In three cases, throat and nasal smear samples were taken, but no volatile substance was detected. Details on all the findings are presented in Table 1.

In the histopathological examination, the brain, heart, lungs, liver, and kidneys were examined. In all the lung samples from all the cases, there were intra-alveolar areas of swelling, edema, and bleeding. In one case, edema and focal subarachnoid bleeding were detected in the brain, in addition to focal perivascular and interstitial fibrosis areas of a mild degree in the heart.

In one case where the anamnesis recorded no history of paternal chronic disease or substance addiction, macrovesicular steatosis was found in the liver and a minimal atrioventricular septal defect in the heart. Contraction band necrosis was found in the heart of one case and early-stage acute ischemic changes in the heart in another case. All the deaths were determined to be as a result of intoxication due to the inhalation of pure butane or derivatives.

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Table 1. Details on the 10 fatalities attributed to volatile substance abuse (VSA)

Case number	1	2	3	4	5	6	7	8	9	10
Gender	M	M	M	M	M	M	M	M	M	M
Age (years)	14	15	17	19	19	19	15	18	17	23
Season	Fall	Winter	Summer	Summer	Summer	Fall	Spring	Fall	Summer	Spring
Scene of incident	Home (his own)	Home (a friend's)	School-yard	In a car	Home (a friend's)	Home (his own)	Home (his own)	Home (his own)	Home (his own)	Home (his own)
First history taken before autopsy	Friend	Friend	Friend	Friend	Uncle	Uncle	Cousin	Uncle	Brother	Father
Chronic usage	- Alcohol - Drugs - Lighter gas	- Alcohol - Drugs - Lighter gas	- Lighter gas	- Lighter gas	- None	- None	- None	- None	- None	- None
Method	Bag inhalation	Bag inhalation	Bag inhalation	Direct inhalation	Bag inhalation	Direct inhalation	Direct inhalation	Direct inhalation	Direct inhalation	Direct inhalation
Source	Lighter gas cartridge	Lighter gas cartridge	Lighter	Lighter gas cartridge	Lighter gas cartridge	Lighter gas cartridge	Lighter gas cartridge	Lighter gas cartridge	Lighter gas cartridge	Lighter gas cartridge
Trauma signs	None	None	Minimal abrasions on the knee and face	None	None	None	None	None	Minimal abrasions on face and extremities	Minimal abrasions on the face
Chemical analysis	Blood: Butane, ethanol (25 mg/dl), and chlorpheniramine	Blood: n-Butane	Blood: n-Butane Lungs: n-Butane	Blood: n-Butane Lungs: n-Butane	Blood: Butane Lungs: Butane, isobutane, and propane	Blood: Butane Lungs: Butane	Blood: Butane, pseudoephedrine/ ephedrine, and chlorpheniramine Lungs: Butane	Blood: Butane and paracetamol Lungs: Butane	Blood: Butane Lungs: Butane and isobutane	Blood: Butane Lungs: Butane and isobutane

Sex (M=male, F=female)

DISCUSSION

Worldwide, the use of sedative and narcotic drugs is a growing public health problem. Volatile substances have been added to the list of substances, such as heroin, cocaine, and synthetic synthetic drugs, that are already widely abused by the younger generation (i.e., children, adolescents, and young adults). According to a report by American Drug Use and Health Research Report on drug use in the U.S., in 2017, 9.3% of those aged ≤12 years, 8.6% of those aged 12–17 years, 9.5% of those aged 18–25 years, and 9.3% of those aged ≥26 years had used a volatile substance at least once (15).

In a meta-analysis that examined the lifetime use of different substances by abandoned children living on the street in low socioeconomic environments, 47% reported at least one instance of use of substance inhalation (16). In another study of high-school students in Türkiye, 8.8% of students reported volatile substance use at least once (17). Although substance abuse is not gender specific, males account for 90% of deaths due to substance abuse (18). In the current study, all the cases were males, and the mean age was 17.6 years, which is consistent with the literature (1-5). Şanlıurfa, Türkiye has a continental climate, with the air temperature reaching up to 50°C in the summer. Most of the 10 deaths (n=4) in the present study occurred during the summer months.

Screening for volatile substances is not performed systematically during autopsies in many countries because of issues relating to cost. Thus, it is highly likely that the number of deaths attributed to VSA is under reported. Testing for butane is only performed when there is evidence suggesting butane intoxication. In addition, VSA-related deaths are frequently not witnessed, and the source of intoxication (e.g., aerosol deodorant) can be easily overlooked. When abuse of illegal toxic substances is suspected, such abuse can only be revealed through a structured, directed investigation (19).

In the current study, more accurate information was obtained about the incident when the informant was a friend of the deceased. When the informant was a close family member (e.g., parent, sibling, or cousin), the informant they tried to cover up the cause of the incident. The accuracy of the information provided by a child or friend of the deceased who had witnessed the incident was likely due to fear of punishment, whereas the family would try to hide the incident for various reasons, such as societal pressure. As shown by an examination of all the statements in the investigation file, family members denied any history of VSA by the deceased. However, closer investigation revealed that the family was aware of the VSA.

To avoid overlooking the use of lighter gas in the deaths of children and young people, sharing of information details amassed by the authorities conducting the investigation with the physician performing the autopsy makes an important contribution to the determination of the cause of death. Given the high prevalence of young people in Şanlıurfa, Türkiye, the incidence of VSA is likely much higher than that recorded in the present study.

Due to the volatility of butane, it can be difficult to amass toxicological evidence in cases of suspected VSA (20). Some analytes may be lost during collection and storage, and this can lead to incorrect quantitation and false negative results (21). In cases of suspected butane intoxication, fat and brain tissue samples, where butane remains for long periods, can be analyzed as alternatives to blood samples (12,14). Fat and brain tissue samples reflect chronic use of volatile substances. In addition, the butane concentration in such tissues is higher than that in lung tissue (22). There are very few cases in the literature in which butane levels in fat (adipose) tissue and lung tissue have been compared (1,10). Generally, butane levels found in fat tissue at autopsy are higher than those found in lung tissue, suggesting that most cases are chronic users (19). In the current series, butane was analyzed in blood samples from all cases and in lung samples from 80% of cases. However, fat and brain tissue samples were not taken in any of the cases. This could have been due to a lack of experience, as butane intoxication is uncommon, or it could have been due to insufficient information being obtained about the incident before the autopsy as family members tried to conceal the VSA.

Methods of substance abuse include blowing (inhaling using a piece of cloth soaked in a volatile substance), sniffing (inhaling directly from a tube), or bagging (inhaling from a plastic bag) (5). The highest gas concentration is generally obtained by inhaling via the sniffing method (6). Although it is not easy to determine the adjuvants in aerosols in toxicology analysis, what method can be used, as this has what advantage as compared to other methods of butane analysis In addition, the detection of substances added to butane to give the gas its distinctive smell can be helpful in determining the cause of death when butane cannot be detected in postmortem samples. Butane gas evaporates rapidly and may be totally eliminated from fluids and organs after death and before samples are taken, which makes the detection of butane intoxication difficult.

A previous report on a suspected case of butane intoxication reported that while nasal smears at autopsy were negative, swabs of the back of the throat were positive for decamethylcyclopentasiloxane, which showed that inhalation was probably through the mouth rather than the nose (19). This hypothesis was supported by a large amount of butane found in the stomach contents. The presence of toxic substances on the clothes and face in this case further suggested that a bag was used for inhalation (19). In the current study, the findings were limited to butane determined in blood and lung samples in a qualitative analysis. In three of the 10 cases in the current series, no volatile substance was detected in throat and nasal smear samples. However, as a result of information about the scene of the incident and witness

statements, butane intoxication most likely occurred via inhalation from a bag or directly from a spray into the mouth.

In fatal cases of butane intoxication, warning symptoms, such as feeling faint, are uncommon, and most cases result in sudden death syndrome. The pathophysiological mechanism responsible for sudden death syndrome due to the abuse of a deadly substance is not clear (23). It has been reported that > 50% of sudden deaths are due to direct toxic effects, especially cardiac effects (23). N-butane can cause death by direct myocardial toxicity or ventricular arrhythmia secondary to global hypoxia due to butane/oxygen competition causing laryngospasms and direct damage to pulmonary tissue (19,23). Alunni et al. showed that butane inhalation directly affected pulmonary tissue (19). The majority of microscopic findings determined in cases of volatile gas inhalation are nonspecific, such as pulmonary and cerebral edema and subendocardial cardiomyocyte necrosis (6,19,24). Novosel et al. (24) observed hemorrhagic pulmonary edema, with macrophage activation following butane inhalation. Arrhythmia has been associated with coronary spasms in sudden sniffing death syndrome and consequently myocardial infarction and ventricular fibrillation in deceaseds with healthy coronary arteries (6,25). According to the literature, the direct effect of hypoxia on the brain and myocardium may lead to arrhythmia and necrosis via a catecholaminergic mechanism (26). Adrenergic activity due to physical effort or stress, together with aerosol inhalation, can increase the sensitivity of the heart to the autonomous effects of butane and render the individual more vulnerable to life-threatening tachyarrhythmias (26).

Several clinical patients reported in the literature have described the development of myocardial infarction, without any changes in coronary arteries (27). In all the cases in the current series, swollen intra-alveolar areas, edema, and bleeding in lung tissues were detected, consistent with the literature (1,2,6-9). Three cases became ill while performing tasks requiring sudden effort (descending steps, walking, and engaging in a violent argument). An atroventricular septal defect was detected in one case. These factors may have contributed to these deaths. In three cases, cardiac findings, including focal mild perivascular and interstitial fibrosis areas, contraction band necrosis, and early-stage acute ischemic changes in the heart, were detected, which were consistent with findings in the literature (25-27). Again, consistent with the literature, myocardial infarction, with early-stage ischemic changes, was detected in one case, despite healthy coronary arteries (6,25,27). These findings support the view that death could have occurred as a result of respiratory depression and asphyxia due to hypoxia and heart inhibition because of vagal nerve stimulation after spraying the gas into the mouth, leading to a vasospasm.

CONCLUSION

In conclusion, the true incidence of deaths due to butane intoxication, which is an increasing public health problem, is unknown. The majority of reports in the literature related to deaths due to butane intoxication are case reports (2,3,7,12-14). Studies including multiple case series are limited, both in Türkiye and worldwide. Based on a search of the literature, the current study seems to one of the largest reported series. One of the most important reasons for this is the insufficent communication with the specialist performing the autopsy of guiding information related to the scene of the incident and consequently, butane intoxication can only be determined by chance. Moreover, in routine autopsy examinations performed in Türkiye, the toxicology examination does not include regions, such as brain and adipose tissue, where butane is absorbed and remains for a long period. In cases of sudden death, especially in the adolescent and young adult age group, butane intoxication should be kept in mind, and appropriate samples for biochemical analysis should be taken.

As our center is located in a city with one of the highest populations of children and young people in Türkiye, it can be estimated that the number of deaths resulting from butane intoxication is actually much greater than official records. The actual incidence of abuse of lighter gas, which is low cost and readily available, among the young population of Şanlıurfa is thought to be much greater than has been reported. There is a need for legislation and regulations governing the availability and sale of products containing butane. Furthermore, educational campaigns should be conducted to inform families, at-risk groups, and educators about the potential outcomes of lighter gas abuse for individuals and society. In addition, we suggest that professionals working with children and adolescents need to to take a more active role in VSA prevention.

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MEDICAL RECORDS-International Medical Journal

Research Article

The Effect of Anti-Inflammatory Drugs on MEFV, PSTPIP1, Siva, and ASC Gene Expression Levels

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Abstract

Aim: Familial Mediterranean Fever (FMF) is the one of the most common autoinflammatory diseases. FMF is characterized by fever attacks and inflammation and colchicine treatment reduces the frequency and severity of FMF attacks. The FMF gene, MEditerranean FeVer (*MEFV*), encodes a protein called Pyrin, which regulates inflammation through its interactions with several proteins. These proteins are; Apoptosis-associated speck like protein with a CARD (*ASC*), Proline serine threonine phosphatase interacting protein 1 (*PSTPIP1*), 14.3.3 proteins and *Siva* proteins. In this study, we aimed to study the effect of anti-inflammatory drugs with different mechanisms of action on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression levels.

Material and Methods: We used differentiated monocytic cell line called THP-1 cells. Cells treated with colchicine, naproxen, prednol-L, acetylsalicylic acid, or azathioprine w and w/o lipopolysaccharide (LPS). After incubation, quantitative RT-PCR (qRT-PCR) was performed to measure *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression levels.

Results: *MEFV* gene expression level was down regulated in colchicine, naproxen, and azathioprine treated cells whereas *PSTPIP1* gene expression level was down regulated in naproxen and azathioprine treated cells with LPS. *Siva* gene expression level was up regulated in all treatments although *ASC* gene expression level was up regulated in only prednol-L treated cells with LPS.

Conclusion: These anti-inflammatory drugs are known to have different mechanisms of action however they are all used to treat pain or inflammation. Since Pyrin, *PSTPIP1*, *Siva*, and *ASC* have pro and anti-inflammatory roles, the results showing an alteration in gene expression levels with specific drugs may indicate the possible mechanisms of therapeutic action.

Keywords: Familial Mediterranean Fever, anti-inflammatory drugs, inflammation, inflammation-related genes

INTRODUCTION

FMF (MIM 249100) is one of the most common hereditary autoinflammatory disorders, which is caused by unprovoked inflammation and tissue destruction (1). FMF is caused by mutations in the *MEFV* (MEditerranean FeVer) (OMIM: 608107) (2) gene which encodes a protein called Pyrin (NP_000234.1). Pyrin is expressed predominantly in neutrophils, monocytes, and dendritic cells (3). It is claimed that through the interactions of several Pyrin interacting proteins, Pyrin may function in regulation of cell death, cytokine secretion, and cytoskeletal signaling (4).

In recent studies for elucidating the pathogenesis of FMF, Xu et al. (5) have shown that pyrin is a specific immune sensor for bacterial modifications of Rho GTPases and responds to Clostridium difficile, the cause of nosocomial diarrhea. In a study by Park et al. (6), it was shown that when pyrin mutated or in response to bacterial modification of RhoA GTPase, RhoA activated pyrin-binding and phosphorylating serine-threonine kinases PKN1 and PKN2. Phosphorylated pyrin has been found to bind 14-3-3 proteins that block pyrin infiltration. The FMF-associated mutant pyrin binding of 14-3-3 and PKN proteins was significantly reduced and IL-1β released from peripheral blood mononuclear cells of FMF patients by activation of PKN1 and PKN2. The same was true for hyperimunoglobulinemia D syndrome (HIDS) as a result of mevalonate kinase (MVK) mutations, and defects in prenylation in HIDS resulted in RhoA inactivation and consequently activation of pyrin infiltration (6). These data suggest that there is a common basic molecular link between two apparently different autoinflammatory disorders, while providing important findings for pyrin function.

CITATION

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Several Pyrin-interacting proteins have been identified. One of the Pyrin-interacting proteins, *PSTPIP1* is a key protein associated with cytoskeleton (7). *PSTPIP1* has a C-terminal SH3 domain that is important for the binding of several PEST phosphatase substrates and also coiled-coiled domain of Pyrin protein (7). *PSTPIP1* is expressed especially in neutrophils and macrophages (8). *PSTPIP1* is involved in regulating inflammation through a complex of proteins termed the inflammasome and also has function in cell migration through actin-based processes. Mutations in the gene encoding *PSTPIP1* leads to the human autoinflammatory disease PAPA (pyogenic sterile arthritis, pyoderma gangrenosum, and acne) syndrome (9). The disease is characterized by recurrent, destructive inflammation of the skin and joints. Treatment can be achieved by using either infliximab or etanercept (10, 11).

Pyrin and *PSTPIP1* proteins are the components of an inflammasome complex that mediates the generation of activated Interleukin (IL)-lβ (12). *PSTPIP1* mutant proteins bind more strongly to the protein Pyrin (7, 13) and leads to more IL-1β production and more inflammation (13). The dysregulated production of IL-1β contributes to many of the symptoms and the recurrent episodes of inflammation occurring in patients. In addition, it is demonstrated that Pyrin and *PSTPIP1* forms branched and reticulated fibrils in Pyrin transfected cells (14). Our group also showed that this reticulated fibril ratio was decreased by colchicine in Pyrin-*PSTPIP1* co-transfected COS-7 cells (15). Furthermore, *PSTPIP1* has shown to be a part in regulation of directed neutrophil migration and leukocyte uropod formation (16) and localized in actin polymerization side with Pyrin during cell migration (17). Pyrin also localized at the leading edge of the cell together with actin and *PSTPIP1*. Based on the studies that have been done so far, it is indicated that, Pyrin and *PSTPIP1* are important proteins for many cellular processes including inflammation and inflammatory cell migration. For understanding of the pathophysiology of FMF and PAPA syndrome, the function of the proteins should be carefully analyzed either alone or when they are together.

The second Pyrin-interacting protein, *ASC* protein plays a crucial role in regulating the immune system and controlling inflammation. The *ASC* gene is primarily expressed in immune cells. *ASC* protein is composed of 195 amino acids and contains Pyrin domain (PyD) at amino terminus and caspase recruitment domain at carboxyl terminus (CARD) (18). The *ASC* protein acts as a bridge between a receptor that senses the presence of the pathogen and an enzyme called caspase. When the receptor detects the pathogen, it signals *ASC* to assemble the inflammasome (3). This assembly triggers a c*ASC*ade of reactions that activate caspase, leading to the release of molecules called cytokines. The interaction between *ASC* and Pyrin occurs when certain triggers, such as pathogenic signals or cellular stress, activate Pyrin. The PYD domain of Pyrin interacts with the PYD domain of *ASC*, leading to the formation of a molecular complex called the *ASC* speck. The formation of the *ASC* speck is a critical step in the activation of the inflammasome pathway (18). The *ASC*-Pyrin interaction and subsequent formation of the *ASC* speck are particularly relevant in autoinflammatory disorders associated with Pyrin mutations.

Siva was identified as Pyrin-associated proteins, as a result of yeast two-hybrid screen experiments (19). The *Siva* gene contains 6490 nucleotides and has two different transcription products called *Siva*-1 and *Siva*-2 proteins (20). *Siva* protein can promote apoptosis through multiple mechanisms. In two different studies, it was observed that Pyrin has no role in *Siva*'s pro-apoptotic activity (21, 22). The *Siva* protein itself acts as a pro-apoptotic factor by promoting cell death through multiple mechanisms, including caspase activation, disruption of mitochondrial function, modulation of cell signaling pathways, and regulation of DNA repair.

In clinical practice, colchicine is highly effective in the treatment of FMF, preventing the development of recurrent attacks and amyloidosis (23). Its effect on the pyrin and its interacting proteins on cellular level and gene expression profile has already been characterized by our group (15). There are some other specific or nonspecific anti-inflammatory agents, but their effects on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression levels have yet to be defined. In this study, we have compared the effects of methylprednisolone, acetylsalicylic acid (ASA), naproxen and azathioprine (AZA) on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression levels. We used THP-1 cells, which naturally express Pyrin, *PSTPIP1*, *Siva*, and *ASC* for this purpose we treat cells with colchicine, naproxen, prednol-L, acetylsalicylic acid, and azathioprine containing mediums, individually. The results of this study provide an outline of the effect of selected drugs on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression levels.

MATERIAL AND METHOD

Cell Culture and Drugs Treatment

THP-1 cells were obtained from ATCC (American Type Culture Collection) and grown in RPMI (Gibco by Invitrogen) supplemented with 10% FBS (vol/vol), 1% Penicillin/Streptomycin (vol/vol) and 1% glutamine (vol/vol). Cells were differentiated with PMA as previously described in Daigneault et al (24). THP-1 cells were treated 100 ng/ml, 5 uM, 50 nmol/L, 600 uM, and 10 uM of colchicine, naproxen, prednol-L, acetylsalicylic acid, and azathioprine containing medium respectively, for 24 h after starting with the 7th day of differentiation. THP-1 cells were also treated with 10 ng/mL LPS (Sigma–Aldrich, Steinheim, Germany) for 2 hours where indicated. All experiments were performed in triplicate.

RNA extraction and quantitative real time PCR (qRT-PCR)

Total RNA was extracted by Qiagen (Valencia, CA) RNeasy Mini kit according to manufacturer's instructions. RNA yield and quality were assessed based on spectrophotometric measurements at wavelengths of 260 and 280 nm at NanoDrop ND 1000 (Thermo Scientific Inc., Waltham,

MA). The reverse transcription was carried out using Qiagen (Valencia, CA) QuantiTect Reverse Transcription kit according to manufacturer's recommendations with 400 ng total RNA.

qPCR was performed with SYBR Green JumpStart Taq Ready Mix kit (Sigma, St. Louis, MO) on Corbett Rotor Gene 6000 Light Cycler. The qPCR conditions were 2 min at 94°C, 5 s at 94°C, and 20 s at 59°C. The specific primers were designed for human ACTB (human beta-actin), *MEFV*, *PSTPIP1*, *Siva*, and *ASC*. The relative amount of mRNA, normalized to an internal control ACTB and relative to a calibrator (normal), was calculated by $2^{-\Delta\Delta CT}$. All samples were run in triplicate.

Statistical Analysis

Student's t test was used for comparison of the means among groups. All statistical analyses were performed using the software GraphPad Prism (version 9.0; GraphPad Software Inc., CA) for the Macintosh. P-values of <0.05 were considered statistically significant.

RESULTS

For determining the possible effect of colchicine, naproxen, prednol-L, acetylsalicylic acid, and azathioprine on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression levels, qRT-PCR analysis was performed. All the experiments were also performed in the presence of 10 ng/mL LPS (bacterial lipopolysaccharide). As shown in Figure 1, LPS treatment induced all of the 4 genes expression levels. Although the expression levels of all the genes were increased, only *PSTPIP1* gene level increased significantly.

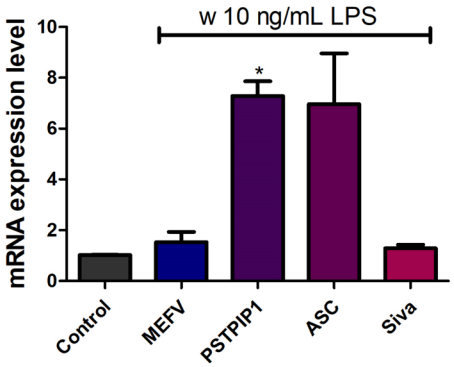

Figure 1. The expression levels *MEFV*, *PSTPIP1*, *Siva*, and *ASC* genes in the presence of LPS (10ng/ml). The qRT-PCR results for *MEFV*, *PSTPIP1*, *Siva*, and *ASC* genes in THP-1 cells that incubated with drugs for 24 h in the absence or presence of LPS (10ng/mL). Gene expressions were normalized to ACTB. Values represent means±SD of three separate experiments. *, P<0.05; **, P<0.01; ***, P<0.001

In colchicine treated cells, *MEFV* gene level was not changed although *PSTPIP1*, *ASC*, and *Siva* genes were down regulated in the absence of LPS (Figure 2). In the presence of LPS, a decrease in *MEFV* mRNA level was observed. Colchicine did not affect the *PSTPIP1* and *ASC* mRNA levels whereas interestingly it caused an increase in *Siva* gene expression level in response to LPS (Figure 2).

After naproxen treatment, in the absence of LPS, *MEFV* and *PSTPIP1* genes were down regulated, *ASC* gene expression level didn't change and *Siva* gene was up regulated. In the presence of LPS, *MEFV* and *PSTPIP1* mRNA levels were also decreased although *ASC* mRNA level was similar and *Siva* mRNA level was increased compared to control (Figure 2).

In prednol-L treated cells, *MEFV* and *ASC* mRNA expression levels were significantly inhibited in the LPS (-) cells. As shown in Figure 2, prednol-L treatment didn't affect *PSTPIP1* and *Siva* mRNA levels in the absence of LPS. In the presence of LPS, *MEFV* and *PSTPIP1* gene expression levels weren't changed whereas *ASC* and *Siva* gene expression levels were increased (Figure 2).

In the absence of LPS, in acetylsalicylic acid treated cells, *MEFV*, *ASC*, and *Siva* mRNA levels were down regulated although *PSTPIP1* gene expression level was shown dramatic increase. *MEFV*, *PSTPIP1*, and *ASC* mRNA levels weren't effected whereas *Siva* mRNA level was increased in the presence of LPS (Figure 2).

After azathioprine treatment, *MEFV* mRNA level was down regulated but the transcription level of *PSTPIP1*, *ASC*, and *Siva* genes weren't affected in LPS (-) cells. In the presence of LPS, *MEFV* and *PSTPIP1* genes were both down regulated whereas *ASC* mRNA level was similar and *Siva* mRNA level was increased compared to control (Figure 2).

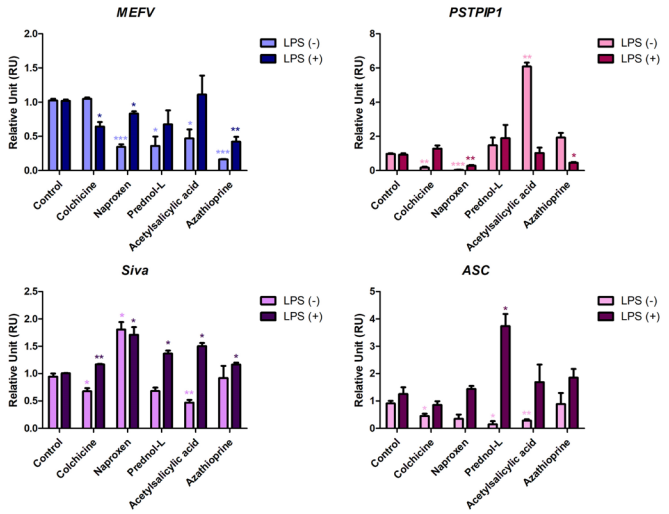

Figure 2. The expression analysis of *MEFV*, *PSTPIP1*, *Siva*, and *ASC* genes in colchicine, naproxen, prednol-L, acetylsalicylic acid, and azathioprine treated THP-1 cells. The qRT-PCR results for *MEFV*, *PSTPIP1*, *Siva*, and *ASC* genes in THP-1 cells that incubated with drugs for 24 h in the absence or presence of LPS (10ng/mL). Gene expressions were normalized to ACTB. Values represent means±SD of three separate experiments. *, P<0.05; **, P<0.01; ***, P<0.001

DISCUSSION

In this study, we have tested the effect of several anti-inflammatory drugs on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression levels. In order to do that, we used a monocytic cell line (THP-1) and treat cells with colchicine, naproxen, prednol-L, acetylsalicylic acid, and azathioprine in the presence or absence of LPS. The results of LPS treated cells

are more valuable because they mimic the inflammation state which causes FMF disease pathogenesis.

Our data showed that in LPS treated cells, *MEFV* gene level was decreased significantly in colchicine, naproxen and AZA treated cells. The effect of colchicine is expected as it is the mainstay therapeutic option in preventing attacks of FMF via effecting the level of *MEFV* expression. The absence of colchicine effect on basal (non-LPS stimulated) conditions may be dose related as our previous work showed that dramatic decreases of *MEFV* was observed at or above 100 ng/ml colchicine concentrations (15). Of additional interest, naproxen and AZA were also found to regulate the *MEFV* gene level both in the presence and absence of inflammation. Naproxen is a member of nonsteroidal anti-inflammatory drugs (NSAIDs) and it may be used to alleviate symptoms during FMF attacks (25). Naproxen has also been used during the attacks of HIDS and several other auto-inflammatory disorders. Its effect on both basal and LPS-induced *MEFV* gene expression may have an additional role in its rather nonspecific anti-inflammatory effect. Azathioprine, which is a cytotoxic and immunosuppressive drug used in organ transplantation and several autoimmune diseases, had also decreased *MEFV* gene levels both in basal and LPS-stimulated conditions. AZA acts as a prodrug for mercaptopurine, which blocks DNA synthesis (26). Thus, it mostly affects proliferating cells, such as the T cells and B cells of the immune system (20). There is anecdotal evidence that AZA treatment had effectively controlled FMF attacks and ameliorated nephrotic range proteinuria in colchicine-resistant patients (27). The effect of AZA on *MEFV* gene mutation may complement its nonspecific anti-inflammatory effect in FMF patients.

In this study, *PSTPIP1* gene level was decreased significantly in colchicine and naproxen treated cells in basal conditions. In the presence of inflammation *PSTPIP1* gene level was significantly decreased only in naproxen and AZA treated cells. Colchicine treatment was not found to be associated with *PSTPIP1* gene level in the presence of inflammation. The therapeutic approaches are different for FMF disease and PAPA syndrome even though related genes have similar roles in inflammatory pathways. In PAPA syndrome, colchicine is less effective and there is need for additional treatments (28). Data in this study support that naproxen may act on *PSTPIP1* gene level as a therapeutic choice. AZA is also effective in various manifestations of the disease such as fever, peritonitis and pleuritis.

In the presence of LPS, expression levels both *Siva* and *ASC* genes were increased. Interestingly, pro-apoptotic protein *Siva* expression level was up-regulated significantly in all treatments. This result indicated that treatments of these drugs triggered the apoptosis pathway regardless of specify to a certain disease type. Apoptosis is one of the important types of the cell death and does not trigger any inflammatory reaction (29). Apoptotic cells can release anti-inflammatory signals which help to reduce the inflammation (30). All these features showed that the increase of *Siva* gene expression level may be related with the apoptosis pathway which is important for silencing the inflammation. *ASC* gene expression level was significantly up-regulated in only prednol-L treatment. This increase may be nonspecific as *ASC* is a component of inflammasome complex and act as pro-inflammatory protein so this anti-inflammatory, immune suppressing drug should have reduced the *ASC* gene expression level. But on the other hand, *ASC* can trigger apoptosis therefore this increase may be for reducing the inflammation. Dual role of *ASC* and *Siva* have also been monitored in cancer. *ASC* can either be increased in tumor cells and overexpressed in the myeloid compartment within the tumor microenvironment, or it can be downregulated in malignancies, primarily by aberrant methylation (31). Therapeutic methods that are already in use or being developed with the goal of increasing *ASC* expression or interfering with inflammasome components. *Siva*, a crucial regulator of apoptosis and metastasis, is abundantly expressed in a variety of malignant tumors, including ovarian cancer, osteosarcoma, non-small cell lung cancer, and gastric cancer, and promotes carcinogenesis (32). *Siva* may also be a promising target to address the main difficulties of therapeutic intervention in cancer patients, including cancer relapse and chemotherapy resistance in addition to treatment in inflammatory diseases.

It is well known that current treatments for many autoinflammatory disorders including FMF and PAPA are innovative biologic agents like recombinant IL-1 receptor antagonist (IL-1Ra), anti-IL-6 receptor antibodies or anti-TNF antibodies. These drugs have beneficial effects by ameliorating disease pathogenesis and progression via their systemic blocking of pro-inflammatory pathways, however they risk the patients by abrogating the host defenses against infectious pathogens. In these conditions, more precise understanding of pathogenic links between several genes (like *MEFV* and *PSTPIP1*) and their interacting proteins may guide us in finding more precise therapeutic targets. The findings of this study show versatility of various mechanisms and effects of anti-inflammatory agents. Further studies are obviously warranted for better identification of similarities and differences linking *MEFV* and *PSTPIP1* genes in FMF and PAPA.

This study has several strengths and limitations. Various anti-inflammatory drugs with different mechanisms of actions on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression patterns were tested first time. This is critically important not only for elucidating the effects of these drugs but also deciphering the precise pathogenic links between *MEFV*, *PSTPIP1*, *Siva*, and *ASC* genes. Testing only one, fixed doses of the drugs was a limitation of this study.

CONCLUSION

To our knowledge, though, this is the first study that has investigated the effects of any of anti-inflammatory drugs on *MEFV*, *PSTPIP1*, *Siva*, and *ASC* gene expression patterns. This is critically important to understanding how these drugs have their effect on FMF and disease related

gene Pyrin and its interacting proteins *PSTPIP1*, *Siva*, and *ASC* by altering their expression levels. In addition, altered expression patterns are also essential for explaining the drugs on action of mechanism.

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

Ethical approval: *This article does not contain any studies with human or animal subjects.*

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MEDICAL RECORDS-International Medical Journal

Research Article

High Salt-Induced Hyperosmolality Reduces in Vitro Survival and Proliferation of Pre-B Cells

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Abstract

Aim: B cells of the adaptive immunity are critical for protection against the vast majority of pathogens through the production of specific antibodies. A number of signaling pathways and transcription factors control B cell development. Environmental factors, including diet, are also important in determining how B cell develop and function. Here, the effects of hyperosmolality induced by elevated salt on the survival, IL-7-induced proliferation and differentiation of pre-B cells were tested in vitro.

Material and Methods: The wk3 pre-B cell line generated from SLP65$^{-/-}$ mice was used. Hyperosmolality in the cell culture medium was created by increasing the salt concentration with the addition of 40 mM NaCl. Wk3 pre-B cells were cultured in standard (normal NaCl) and high salt (+40 mM NaCl) medium, followed by flow cytometric analysis.

Results: It was found that hyperosmolality caused by high salt reduced survival and induced apoptosis in wk3 pre-B cells. In addition, hyperosmolality inhibited IL-7-induced proliferation of pre-B cells. Conversely, pre-B cells treated with high salt were able to differentiate normally into IgM^{+} immature B cells when IL-7 was removed.

Conclusion: These findings suggest that the hyperosmolar microenvironment induced by high salt may play a key role in B cell development in the bone marrow.

Keywords: Hyperosmolality, pre-B cells, salt, B cells

INTRODUCTION

The development of B cells in the mammalian bone marrow is a tightly regulated process controlled by multiple factors, including transcription factors and signaling through the cytokine receptors (1). At the pro-B cell stage, a functional Igμ heavy chain results from successful immunoglobulin (Ig) heavy chain rearrangements (2). The assembly of Igμ with VpreB, lambda5, Igα and Igβ leads to the formation of a pre-BCR complex (3-6). Expression of the pre-BCR is a hallmark of pre-B cell differentiation and serves as a checkpoint (3-6). Functional pre-BCR expression initiates interleukin (IL)-7-dependent proliferation to increase pre-B cell numbers (7). The cessation of proliferation leads to the differentiation of large proliferating pre-B cells into small pre-B cells, which initiates the rearrangement of the Ig light chain gene (3-6). After successfully producing a light chain, these cells express IgM and become immature B cells (8).

In addition to transcription factors and signaling pathways, environmental factors such as diet are thought to play critical roles in the immune system (9). The consumption of table salt (sodium chloride, NaCl) has been increasing in western societies. Salt consists approximately of 40% sodium and 60% chloride, and is an important source for these minerals. Sodium, in particular, is the most abundant cation found in the human body and plays key roles in human physiology. According to the World Health Organization, a healthy individual should consume 5 grams of salt per day. However, people consume much more salt than recommended, which can lead to significant health problems such as hypertension, stroke, cardiovascular and kidney diseases (10). Due to the important biological properties of sodium, low salt intake may also have negative biological effects. Therefore, understanding the positive or negative effects of low/excessive salt intake on different biological systems is critical for public health. This study investigated the effects of hyperosmolality caused by elevated salt on pre-B cells in vitro.

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MATERIAL AND METHOD

Cell Culture

The cell culture and experimental procedures used in this study were performed as previously described (11). The wk3 cell line was originally generated from SLP65$^{-/-}$ mice (12) and cultured in IMDM (Sigma) supplemented with 7.5% premium FBS (Cegrogen), 1% L-Glutamine (Sigma), 1% Penicillin-Streptomycin (Sigma) and 50 µM 2-Mercaptoethanol. Supernatant from IL-7-expressing J558L cells was also added to the medium as an excess source of IL-7 (12,13). For hyperosmolality, the medium was supplemented with an extra 40 mM NaCl (Sigma) to mimic the high salt environment (14-21).

Survival Assay

10^5 wk3 cells were cultured in standard salt (normal) and high salt medium supplemented with excess IL-7 for 24, 48 and 72 h. The cells were then stained with an Apoptosis Detection Kit (Cat: 640914, Biolegend) based on the manufacturer's instructions, followed by flow cytometric analysis.

Carboxyfluorescein Succinimidyl Ester (CFSE) Labelling

For the detection of IL-7-dependent proliferation, 10^5 wk3 cells were labelled with 10 µM CellTrace™ CFSE kit (Invitrogen) in IL-7-free medium for 10 min at RT, followed by three washes with the same medium (22). The cells were then cultivated in standard salt (normal) and high salt in medium with excess IL-7 for 48 and 72 h. A dead cell marker (Cat: S34859, ThermoFisher) was then used for the exclusion of dead cells from the analysis and the remainder cells were tested for CFSE dilution by flow cytometry.

Differentiation Assay

5x10^5 wk3 cells were cultivated in standard salt (normal) and high salt medium without IL-7 for 48 and 72 h. The cells were then labelled with a FITC-conjugated anti-mouse Igκ (Clone: RMK-45, Biolegend) and a PE-conjugated anti-mouse IgM (Clone: eB121-15F9, ThermoFisher), and stained at 4ºC for 30 min. After the incubation period, the cells were resuspended in PBS supplemented with 2% FBS before their analysis on a flow cytometry.

Analysis on Flow Cytometry

An Attune Acoustic Focusing Cytometer (Applied Biosystems) was used for the flow cytometric analysis and FlowJo software (Tree Star) was used for data analysis.

Statistical Analysis

For statistical analysis of the two groups (normal and high salt), Student's t-test was used with the GraphPad Prism 5. When $p<0.05$, differences were considered significant.

RESULTS

Hyperosmolality induced by high salt diminishes pre-B cell survival

In order to test whether hyperosmolality has any effect on pre-B cells, wk3 cells were used. These cells were originated from SLP65$^{-/-}$ mice and are able to proliferate indefinitely when IL-7 is present (12). However, in the absence of IL-7, wk3 cells differentiate into immature B cells (12). In this study, hyperosmolality was created in the cell culture medium by increasing the NaCl concentration with an additional 40 mM NaCl (high salt), as previously reported (19-21,23). Wk3 cells were cultured in standard and high salt medium with excess IL-7 for 24, 48 and 72 h. The cells were labelled with Annexin-V and PI, and analyzed for cell viability and the detection of apoptotic cells by flow cytometry (Figure 1A). The analysis revealed a reduction in the proportion of live (Annexin-V$^-$PI$^-$) cells (Figure 1B) and a rise in the proportion of dead (Annexin-V$^+$PI$^+$) cells (Figure 1D) when wk3 cells were treated with an additional 40 mM salt for 24 h. Apoptosis and cell death were further induced by high salt at 48 and 72 h, as evidenced by an increase in the proportion of early apoptotic (Annexin-V$^+$PI$^-$) cells and dead (Annexin-V$^+$PI$^+$) cells (Figure 1C, D). Concomitantly, there was a more pronounced reduction in the percentage of live (Annexin-V$^-$PI$^-$) cells when cells were cultured in high NaCl medium for 48 and 72 h (Figure 1B). These results suggest that hyperosmolality caused by high salt reduces survival and increases apoptosis in wk3 pre-B cells in vitro.

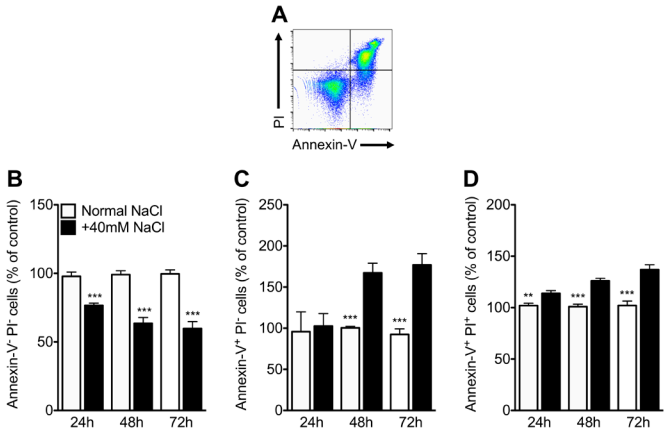

Figure 1. High salt-induced hyperosmolality diminishes survival of wk3 cells. 10^5 wk3 cells were cultivated for 24, 48 and 72 h in IL-7 containing medium supplemented with or without an extra 40 mM NaCl. Cells were labelled with Annexin-V and PI for flow cytometric analysis. **(A)** Representative flow cytometric plot showing the gating strategy for the analysis of Annexin-V and PI in cultured cells, pre-gated on singlets. The graphs show % of **(B)** live cells (Annexin-V$^-$PI$^-$), **(C)** early apoptotic cells (Annexin-V$^+$PI$^-$) and **(D)** late apoptotic/necrotic cells (Annexin-V$^+$PI$^+$) presented as percent of control. Data are representative of at least three independent experiments per group in triplicate. **p<0.01, ***p<0.001

Hyperosmolality Induced by High Salt Reduces IL-7-Induced Pre-B Cell Proliferation

Given that treatment of wk3 cells with high salt resulted in reduced survival, and that wk3 cells are capable of IL-7-induced proliferation (12,13), the impact of hyperosmolality on IL-7-induced pre-B cell proliferation was next tested. To this end, wk3 cells were labelled with CFSE and cultured in normal and high salt medium for 48 and 72 h, followed by analysis (Figure 2A). The

results demonstrated that wk3 cells treated with high salt accumulated in cell division phases 2 and 3 after 48 h of incubation (Figure 2A, B). Conversely, the proportion of proliferating cells in cell division phase 4 was reduced when wk3 cells were cultured in high salt medium for 48 h (Figure 2A, B). Similarly, wk3 cells treated with high salt had a rise in the percentage of proliferating cells in cell division phases 1, 2 and 3 and a reduction in phases 4 and 5 after 72 h of incubation (Figure 2A, C). Collectively, these findings indicate that hyperosmolality induced by high salt in wk3 cells results in a compromised or delayed in vitro response to IL-7.

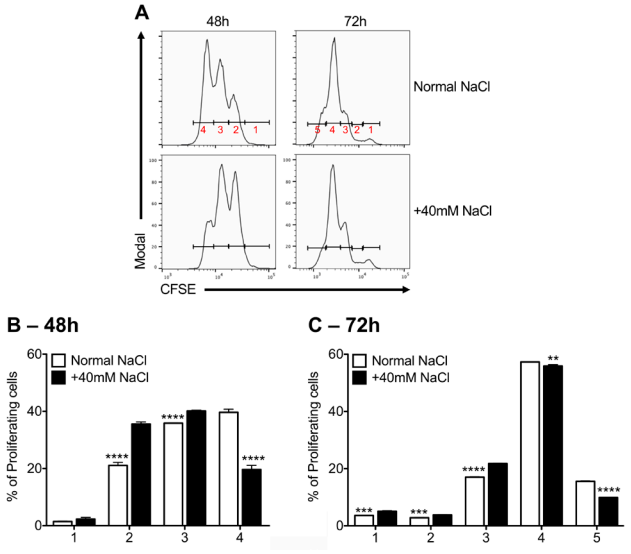

Figure 2. High salt-induced hyperosmolality reduces IL-7-induced wk3 cell proliferation. 10^5 wk3 cells were labelled with 10μM CFSE and cultivated for 48 and 72 h in IL-7 containing medium supplemented with or without an extra 40 mM NaCl and analyzed for CFSE dilution. Doublets and dead cells were excluded from the analysis using the dead cell marker. **(A)** Representative histograms show CFSE dilution on live cells. The graphs show the % of cells that have undergone cell division after **(B)** 48 h and **(C)** 72 h of incubation. Cells were gated as in **(A)**. Data are representative of at least three independent experiments per group in triplicate. **p<0.01, ***p<0.001, ****p<0.0001

Hyperosmolality Induced by High Salt has no Effect on the Differentiation of Pre-B Cells

Loss of IL-7R signaling suppresses pre-B cell proliferation and provokes the differentiation of pre-B cell into IgM⁺ B cells (12,13). In order to test whether hyperosmolality due to elevated salt has any effect on pre-B cell differentiation, wk3 cells were cultured in standard and high salt medium for 48 and 72 h in the absence of IL-7, followed by their Igκ and IgM expression. The results demonstrated that the percentage of Igκ⁺IgM⁺ cells in cells cultured in high salt medium was comparable to those cultured in standard medium after 48 h of incubation (Figure 3). In addition, wk3 cells cultured in high salt medium induced slightly more Igκ⁺IgM⁺ immature B cells after 72 h than cells cultured in medium with standard salt (Figure 3). These data indicate that high salt-induced hyperosmolality has no effect on wk3 cell differentiation into immature B cells when IL-7R signaling was terminated in vitro.

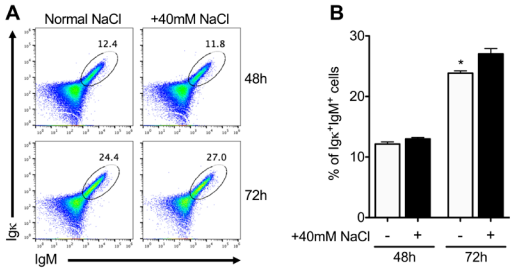

Figure 3. High salt-induced hyperosmolality has no effect on the differentiation of wk3 cells after IL-7 deprivation. $5x10^5$ wk3 cells were cultured for 48 and 72 h in medium containing normal and high salt without IL-7. Cells were labelled with Igκ, IgM and Sytox Red, followed by the flow cytometric analysis. An exclusion of doublets and dead cells from the analysis using the dead cell marker was done. **(A)** Representative flow cytometric plots of Igκ and IgM analysis in cultured cells. **(B)** The graph demonstrates % of Igκ⁺IgM⁺ immature B cells after 48 and 72 h of incubation. Data are representative of at least three independent experiments per group in triplicate. *p<0.05

DISCUSSION

It has long been recognized that there is an important link between the immune system and environmental factors such as diet (9). As salt is one of the most commonly consumed nutrients, this study investigated the impacts of high salt-induced hyperosmolality on pre-B cells in vitro. The results demonstrated a reduction in wk3 cell survival and proliferation caused by the hyperosmolar environment.

Salt is an important source of the sodium and chloride that our bodies need. Because sodium is particularly important for human physiology, through its ability to control osmotic balance and regulate the amount of extracellular fluid, the optimal amount of salt should be consumed, which is 5 grams per day for a healthy person. However, people consume much more than the recommended amount (24), which can cause significant health problems such as hypertension, stroke, cardiovascular diseases and kidney diseases (10). Recent studies have revealed that there may be an important link between salt intake and the immune system (25-27). In 2015, a study showed that macrophages activated in high salt concentrations were more effective against cutaneous Leishmania major than macrophages activated in normal salt (28). Moreover, high salt treatment increased type 1 interferon signaling in macrophages and resistance to vesicle stomatitis virus, and provided protection against vesicle stomatitis virus in mice (29). The tumor growth was also inhibited by high salt (23,30), and high salt prevents autoimmune demyelination through regulation of the permeability of the blood-brain barrier (31).

Despite these beneficial effects of high salt intake, studies have reported that high sodium chloride intake triggers autoimmune development by inducing pathogenic T17 helper cells (TH17) (15,32). In addition, high salt exacerbated central nervous system autoimmunity by activating pro-inflammatory macrophages (33). Moreover, dietary intake of high salt has also been shown to reduce the antibacterial responses of neutrophils against pyelonephritis (18), and to inhibit the suppressive capacity

of regulatory T cells (17).

In B cells, hyperosmolality induced by high level of salt inhibits the survival and LPS-dependent proliferation of peripheral mature B cells isolated from mice (20). Interestingly, hyperosmolality led to a rise in B activation and the generation of more antibody-producing plasma cells (20). Similarly, in this study high salt-induced hyperosmolality also diminished the survival of wk3 cells and induced apoptosis. In addition, wk3 cells showed an impaired or delayed IL-7-induced proliferation. Taken together, these data suggest that the hyperosmolar environment created by elevated salt has a negative effect on the survival and proliferation of pre-B cells as well as LPS-stimulated mouse splenic mature B cells.

Salt has been suggested to control the development and function of immune cells by modulating various signaling pathways (25, 34). Hyperosmolality induced by increased salt has been shown to increase the p38-dependent NFAT5 response in peripheral B cells, T cells and macrophages (15, 20, 28, 35). It is therefore possible that increased salt may have similar mechanistic effects in pre-B cells. Furthermore, since IL-7R signaling is important for pre-B cell survival and proliferation (36, 37), and hyperosmolality caused a block in IL-7-dependent proliferation of wk3 cells, an alternative mechanism responsible for the phenotype reported in this study would be the effects of hyperosmolality induced by increased salt on signaling through the IL-7R. It is plausible that salt could affect IL-7R (IL-7Rα and γc) expression on the cell surface, the phosphorylation of its downstream molecules (such as JAK1, JAK3 and STAT5) and/or the transcription factors regulated by IL-7R signaling. Future studies will help to determine how high salt induced hyperosmolality mechanistically regulates pre-B cell survival and proliferation.

CONCLUSION

In conclusion, this study shows that hyperosmolality induced by increased salt negatively affects pre-B cell survival and proliferation in vitro. Elucidating the impacts of a high salt diet on B cell biology may help to understand the pathophysiology of B cell-related disorders, including autoimmune diseases. As a limitation, it should be noted that this in vitro study used a pre-B cell line derived from SLP65-deficient mice.

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MEDICAL RECORDS-International Medical Journal

Research Article

Placental Histopathological Alterations in COVID-19 Infected Pregnancies

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Abstract

Aim: The ongoing global COVID-19 pandemic, caused by the SARS-CoV-2 virus, has generated significant apprehensions in maternal-fetal medicine. Initially considered to affect the respiratory system primarily, recent findings have indicated that the pandemic has far-reaching implications for various physiological functions, particularly in pregnant individuals. This study focused on examining the influence of COVID-19 on placental histopathology in pregnant women infected with SARS-CoV-2.

Material and Methods: We conducted a comparative study involving two groups of pregnant women with similar demographic characteristics: a group testing positive for COVID-19 (n=31) and a control group of COVID-19-negative pregnant women (n=31). After delivery, placental tissues were collected and subjected to comprehensive histopathological examination to determine any potential alterations in the placenta induced by SARS-CoV-2 infection.

Results: Our study revealed substantial histopathological alterations in pregnant women with COVID-19 placentas. Notably, the COVID-19 group displayed a higher incidence of cesarean deliveries, possibly due to concerns related to maternal-fetal transmission and respiratory complications. Furthermore, neonates born to mothers in the COVID-19 group had significantly lower birth weights. Several placental histopathological changes, including villous fibrin deposits, thrombosis, intervillous hemorrhage, agglutination, avascular fibrotic villi, and syncytial knots, were markedly increased in the COVID-19 group, indicating compromised fetal blood circulation. Although not statistically significant, trends toward elevated villous infarction, fetal vascular malperfusion, and chorioamnionitis were observed.

Conclusion: Our study underscores the potential risks associated with COVID-19 on placental health, maternal well-being, and neonatal outcomes. We must understand the underlying physiological mechanisms behind these pathological changes to provide optimal maternal-fetal care during this ongoing crisis. Comprehensive and multicentric studies are urgently required to confirm and expand our findings.

Keywords: COVID-19, Placental histopathological changes, pregnancy

INTRODUCTION

The emergence of COVID-19, which is linked to the SARS-CoV-2 virus, has caused many public health complications worldwide (1,2). Although it is primarily viewed as a disease affecting the respiratory system, mounting data indicate that its scope is broader, extends to various physiological functions, and notably has repercussions on pregnant individuals (3-5).

Pregnant individuals have been identified as a group requiring special attention in the context of COVID-19 owing to the immediate and potential long-term health considerations for both the mother and the unborn child (6-8).

The placenta is a vital organ during gestation that enables the exchange of essential nutrients and oxygen between the mother and the fetus. Its function is paramount for a successful pregnancy (9,10). Initial studies have pointed toward the susceptibility of the placenta to SARS-CoV-2 due to the presence of ACE-2 receptors on placental cells (11,12). Nevertheless, the exact histological changes occurring in the placenta due to COVID-19 and their impact on fetal health still need to be clearly defined (13).

CITATION

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Given the limited breadth of research focusing on this crucial aspect of maternal and fetal health, our study scrutinized the available scientific literature regarding the histopathological effects of SARS-CoV-2 on the placenta. Therefore, we aim to shed light on this critical area. Our study also provides the groundwork for future research. Future work could lead to better treatment strategies for managing COVID-19 during pregnancy.

MATERIAL AND METHOD

This study involved two groups of pregnant women. The test group comprised 31 women who were COVID-19-positive, as confirmed by PCR tests, but had no chronic diseases. The patients were hospitalized and treated according to standard protocols based on their symptoms and disease severity. Treatments included oxygen support through a nasal cannula, antiretroviral drugs lopinavir/ritonavir (400 mg twice daily for seven days), and hydroxychloroquine (200 mg once daily) when necessary. Prophylactic low-molecular-weight heparin (LMWH) was administered to all COVID-19-positive patients throughout their hospitalization and postpartum period. Antibiotics such as ampicillin or cephalosporins were administered as required.

The control group consisted of 31 pregnant women who were COVID-19-negative, as confirmed by PCR tests and had no chronic diseases or COVID-19 symptoms.

Placentas from both groups were collected immediately post-delivery and underwent routine gross examinations. Tissue specimens were fixed in formalin for 48 h and processed for histological analysis. These were embedded in paraffin, sectioned at 5 microns, and stained with hematoxylin and eosin.

An experienced pathologist evaluated the slides for chorioamnionitis, maternal vascular malperfusion, and fetal malperfusion based on the Amsterdam criteria (13). Definitions for these conditions were as follows:

Chorioamnionitis was identified by neutrophil infiltration into the chorion and amnion.

Maternal vascular malperfusion was marked by lesions such as fibrinoid necrosis, intervillous thrombi and infarcts.

Fetal vascular malperfusion was characterized by chorangioma, villous fibrosis, or villous edema.

Neither the mothers nor the infants were subjected to COVID-19 testing of their placental tissue; only RT-PCR was used for diagnosis.

This study was approved by our institution's Clinical Research Ethics Committee (approval number KAEK/2020.06.66) and the Ministry of Health. All the participants provided written informed consent.

RESULTS

The characteristics of mothers in both the control and COVID-19 groups are outlined in Table 1. The control and COVID-19 groups comprised 31 participants of comparable ages. There were no marked statistical differences in variables like gravidity, parity, history of miscarriage, smoking habits, and BMI between the control and COVID-19 groups ($p>0.05$).

Details of the COVID-19-positive cases are summarized in Table 2. This table shows variables such as gestational age at diagnosis and delivery, severity of the disease, and treatment modalities. Table 3 indicates no statistically significant difference in gestational age at delivery between the two groups ($p=0.096$). Notably, all deliveries in the COVID-19 group were by cesarean section, in contrast to 71% of deliveries in the control group ($p=0.001$). The reasons for cesarean deliveries were multifaceted and included previous cesarean sections and COVID-19-related indications ($p=0.009$).

Regarding neonatal outcomes, the mean newborn weight was 3158±188 g in the control group and 2940±149 g in the COVID-19 group, which was statistically significant ($p=0.037$). No significant difference was observed in the neonatal intensive care unit (NICU) admissions rate between the two groups ($p=0.215$). One fetal death occurred in each group, accounting for a rate of 3.2%, which was not statistically significant.

Although pharmacological treatment was administered in a few cases, most patients did not receive specific drug therapies. Oxygen therapy was primarily administered through a nasal cannula when required. One maternal death occurred 26 days after delivery.

Table 1. Maternal characteristics of the study groups.

	Controls (n=31)	COVID-19 (n=31)	Significance
Age (years)	29.1±5.6	31.4±5.6	0.109
Gravidity	3.0 (1-6)	3.0 (1-9)	0.317
Parity	1.0 (0-4)	2.0 (0-4)	0.273
Miscarriage	0 (0-3)	0 (0-3)	0.361
Smoking	9 (29%)	11 (35%)	0.587
Body mass index (kg/m^2)	23 (20-40)	21 (20-37)	0.312

Continuous variables are represented by the mean value and the standard deviation (SD), derived using descriptive statistics. On the other hand, categorical variables are displayed as frequencies, calculated using frequency distribution, with their corresponding percentages enclosed in parentheses.

Table 2. COVID-19 positive cases features

Features	Values
Gestational age at infection diagnosis (wks)	36 (24-39)
Gestational age at delivery (wks)	38 (26-41)
Severity of the disease	
Mild	29 (93.5%)
Moderate	1 (3.2%)
Severe	1 (3.2%)
Pharmacologic therapy	
None	26 (83.9%)
Lopinavir/Ritonavir	1 (3.2%)
Hydroxychloroquine	4 (12.9%)
Oxygen therapy	
None	24 (77.4%)
Nasal cannulae	7 (22.6%)
Maternal death	1 (3.2%)

Continuous variables are represented by the mean value and the standard deviation (SD), derived using descriptive statistics. On the other hand, categorical variables are displayed as frequencies, calculated using frequency distribution, with their corresponding percentages enclosed in parentheses.

Table 3. Perinatal Outcomes of the study groups.

	Controls (n=31)	COVID-19 (n=31)	Significance
Gestational age at delivery (wks)	38 (26-41)	38 (26-41)	0.096
Delivery mode			
Vaginal delivery	22 (71%)		0.001
Cesarean section	9 (29%)	31 (100%)	
Indications for cesarean section			
Prior cesarean section	17 (68%)	21 (67.7%)	0.009
COVID-19	0	7 (22.6%)	
Non-reassuring fetal status	8 (32%)	2 (6.5%)	
Preeclampsia	0	1 (3.2%)	
Newborn weight (g)	3158±188	2940±149	0.037
NICU admission	6 (19.4)	10 (33.4%)	0.215

Table 4. Placental histopathologic findings of study groups.

Histopathological findings	Controls (n=31)	COVID-19 (n=31)	Significance
Villous infarction	14 (45.2%)	18 (58.1%)	0.31
Fetal Vascular Malperfusion (Chorangiosis)	30 (96.8%)	31 (100%)	0.31
Chorioamnionitis	3 (9.7%)	6 (19.4%)	0.28
Villitis	6 (19.4%)	10 (32.3%)	0.25
Villous fibrin deposits	9 (29%)	27 (87.1)	0.001
Villous thrombus	14 (45.2%)	31 (100%)	0.001
Intervillous hemorrhage	15 (48.4%)	31 (100%)	0.001
Villous agglutination	10 (32.3%)	25 (80.6%)	0.001
Avascular fibrotic villi	7 (22.6%)	17 (54.8%)	0.009
Increased syncytial knots	7 (23.3%)	27 (87.1%)	0.001

The chi-square test was utilized to evaluate the statistical differences indicated in this table. The 'Significance' values are representative of the differences in each histopathological finding between the control and COVID-19 groups. A p-value of <0.05 is considered to be statistically significant.

As illustrated in Table 4, significant increases in specific placental histopathological changes were identified in the COVID-19 group. These included villous fibrin deposits, thrombosis, intervillous hemorrhage, agglutination, avascular fibrotic villi, and syncytial knots ($p<0.05$). Increases in villous infarction, fetal vascular malperfusion, and chorioamnionitis were also noted, but these were not statistically significant.

DISCUSSION

The global reach and complexity of the COVID-19 crisis have made it essential to examine its effects on specific high-risk groups, among which pregnant women stand out. This study contributes to the scant but growing body of literature on placental histopathological changes in SARS-CoV-2-infected pregnancies.

Our study was particularly strengthened by the comparable maternal characteristics between the control and COVID-19-positive groups, enabling a more accurate interpretation of the direct impact of the virus on pregnancy outcomes. Notably, there was a disproportionately high rate of cesarean sections in the COVID-19-positive group. This finding suggests that the pandemic could influence obstetric care decisions, potentially due to maternal-fetal transmission concerns or respiratory complications.

Histologically, the dominant feature in our COVID-19-affected cohort was cholangitis, a finding associated with compromised fetal blood circulation. Previous studies, including those by Umar et al., Shanes et al., and Bobei et al., resonate with our observations, indicating heightened maternal vascular malperfusion, fetal vascular thrombosis, and increased inflammation (14-16). The clinical relevance of these placental changes, seen in the increased risk of adverse outcomes, such as preterm birth and fetal distress, cannot be overstated.

Acknowledging that our findings do not align universally with the broader scientific discourse is essential. A recent multicenter case-control study found no discernible differences in placental histology between COVID-19-positive and -negative groups (17). These inconsistencies necessitate further research to untangle the true extent of COVID-19's impact on pregnancy and fetal outcomes.

While our study offers crucial insights, its limitations are nontrivial. The limited sample size, geographic specificity, and potential methodological constraints suggest that caution should be exercised when generalizing these findings. Future research should strive for a more expansive and multicentric approach to corroborate or challenge our results.

CONCLUSION

In summary, our research, alongside existing studies, signifies the latent risks of COVID-19 on placental health and, by extension, maternal and neonatal well-being (13,14,18,19). The pathologies we identified could be the mechanisms that contribute to adverse pregnancy outcomes, including reduced neonatal weight. This situation necessitates vigilant monitoring and tailored medical interventions for pregnant women with SARS-CoV-2 infection. Further studies are urgently needed to decode the physiological mechanisms behind these pathological changes, paving the way for optimized maternal-fetal care during this ongoing crisis.

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Research Article

Evaluation of Ventricular Arrhythmia Markers in Obstructive Sleep Apnea Syndrome Patients

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Abstract

Aim: Obstructive sleep apnea syndrome (OSAS) is a prevalent sleep condition marked by recurrent upper airway blockages causing intermittent hypoxia, fragmented sleep, and autonomic nervous system issues. Significant emphasis has been paid to the connection between OSAS and the development of ventricular arrhythmias in recent years. The index of cardiac electrophysiological balance (ICEB) represents a new measure designed to predict the likelihood of ventricular arrhythmias.

Material and Methods: Forty OSAS patients and 40 healthy controls were enrolled in the research. Clinical and demographic variables of both groups were evaluated. Electrocardiogram was taken and routine blood values of the patients were studied. The ICEB is computed by dividing the QT interval by the QRS duration (QT/QRS). Apnea-hypopnea index was used to evaluate the severity of OSAS.

Results: The body-mass index value of OSAS patients was significantly higher than HCs (p=0.002). No significant smoking status difference between OSAS patients and HCs (p=0.822). As a result, QT, QTc, and ICEB were substantially greater in OSAS patients than in HCs (p<0.001, for all). According to Pearson correlation analysis, apnea-hypopnea index and body-mass index, QT, QTc, and ICEB were significantly correlated (p=0.020, p=0.009, p=0.010, and p=0.003, respectively). In linear regression analysis where the apnea-hypopnea index was taken as a dependent variable, ICEB predicted the apnea-hypopnea index significantly and positively (F(5.76)=18.451, R2=0.657, adjusted R2=0.715 and p<0.001).

Conclusion: In this study, a positive and significant relationship was found between the AHI value, which is used to evaluate the severity of OSAS, and ICEB. The fact that ICEB, which is used to evaluate the risk of ventricular arrhythmia, is higher in OSAS patients indicates that these patients should be followed more closely in the cardiologic outpatient clinic.

Keywords: Index of cardiac electrophysiological balance, obstructive sleep apnea syndrome, ventricular arrhythmia

INTRODUCTION

The common sleep disease known as obstructive sleep apnea syndrome (OSAS) is defined by recurrent episodes of partial or total upper airway obstruction while asleep, which can result in intermittent hypoxia and interrupted sleep cycles (1). Ventricular arrhythmias, on the other hand, are abnormal heart rhythms that originate in the heart's lower chambers, potentially leading to serious complications including sudden cardiac arrest (2). The relationship between OSAS and the risk of ventricular arrhythmias has garnered significant attention in recent years due to emerging evidence suggesting a complex interplay between these two conditions (3).

OSAS-related sleep fragmentation and intermittent hypoxia can lead to disruptions in the autonomic balance that regulates heart rate variability. A higher risk of abrupt cardiac events and ventricular arrhythmias is linked to decreased heart rate variability. These alterations can affect the heart's electrical conduction system, creating an environment that favors the emergence of arrhythmias (4).

The index of cardiac electrophysiological balance (ICEB) represents a new measure designed to predict the occurrence of ventricular arrhythmias. This index is computed by dividing the QT interval by the QRS duration (QT/QRS). It essentially encapsulates the equilibrium between the ventricular depolarization and repolarization phases of the cardiac cycle. What's noteworthy about ICEB is that it offers a non-invasive and readily quantifiable approach to evaluating this balance (5).

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ICEB carries several advantages over other electrocardiogram (ECG) parameters like QT interval or QT corrected (QTc) interval. By focusing on the ratio between two distinct phases of the cardiac cycle, ICEB captures more comprehensive information related to the prediction of ventricular arrhythmias (6). Remarkably, within the existing literature, there is a gap in employing ICEB to predict ventricular arrhythmias in patients with OSAS. No study has explored the application of ICEB specifically for this purpose. This brings us to the purpose of this study, which aims to fill this gap.

MATERIAL AND METHOD

Study Design

The study was conducted prospectively. Adıyaman University Ethics Committee approved the study protocol for Non-Interventional Clinical Trials (2022/2-7). Guidelines from the Helsinki Declaration were observed throughout the investigation. After exclusions, 40 out of 62 eligible OSAS patients participated. All participants provided informed consent for their participation.

Patients with OSAS who applied to the otorhinolaryngology outpatient clinic made up the study's patient population. The control group consisted of healthy volunteers who applied to the otorhinolaryngology outpatient clinic and had no obstructive pathology. Forty OSAS patients and 40 healthy controls were included in the study. Clinical and demographic characteristics of both groups were evaluated. ECG was taken and routine blood values of the patients were studied. Glucose, thyroid hormones, creatinine, complete blood count, electrolytes were studied from the blood samples taken. Demographic and clinical characteristics such as laboratory results, age, gender, smoking, body mass index (BMI), diastolic and systolic blood pressure measurement, and iCEB calculated by dividing the QT interval in the ECG, the QTc interval, the QT interval by the QRS duration (QT/QRS) group compared.

Those with left or right bundle branch block, extrasinus rhythm, atrioventricular conduction disorder on ECG were not included in the study. Those with a history of rheumatoid heart disease, valvular heart disease, thyroid dysfunction, chronic lung disease, chronic kidney disease, anemia, electrolyte disorder, chronic liver disease, chronic infection, systemic autoimmune disease, diabetes, hypertension, coronary heart disease, those using antidepressants, antipsychotics or antihistamines, were excluded from the study.

Electrocardiogram Examination

Measurements were conducted on the CardioFax S device (Nihon Kohden, Tokyo, Japan) 12-lead ECG. Resting heart rate was calculated as 300 divided by the number of large squares between consecutive R waves. QRS duration was gauged from onset to end. For QT interval, the interval between QRS onset and T wave end was measured. QTc interval was derived as heart-rate adjusted.

Laboratory Examination

During hospital admission, venous blood samples were subjected to comprehensive analysis. Utilizing the CELL-DYN Ruby device from Abbott Diagnostics, we quantified the white blood cell count, including neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts in x 103 cells/mm3. Moreover, measurements encompassed hemoglobin, hematocrit, and thrombocyte counts.

Abbott Diagnostics' biochemistry kits and the Architect c8000 Chemistry System were instrumental in assessing CRP, creatinine, thyroid hormones, and urea levels. Plasma concentrations of fasting blood glucose and electrolytes were determined through a meticulous enzymatic chemical clearing process executed via the Cobas 6000 system from Roche Diagnostics GmbH in Mannheim, Germany. These exhaustive analyses provided crucial insights into participants' physiological status, enhancing the depth and accuracy of the study's findings.

OSAS Severity Evaluation

The Apnea-Hypopnea Index (AHI) is a crucial parameter in sleep medicine, used to quantify the severity of obstructive sleep apnea (OSA) and assess its impact on an individual's sleep quality and overall health. It measures the frequency of respiratory events that occur during sleep, including apneas (complete pauses in breathing) and hypopneas (partial reductions in airflow). The total number of apneas and hypopneas recorded during a sleep study is divided by the total number of hours of sleep to arrive at the AHI. The result is typically expressed as the number of events per hour. The interpretation of AHI values categorizes the severity of OSA as follows. AHI less than 5 occurrences per hour is considered normal. AHI of 5 to 15 incidents per hour is considered mild. AHI that is moderate has 15 to 30 incidents per hour. AHI over 30 occurrences per hour is considered severe (7).

Statistical Analysis

Sample size determined as 40 via G*Power (3.1) (power: 0.8, alpha: 0.05, effect size: 0.58). SPSS 26.0 for Mac (SPSS Inc., Chicago, IL) facilitated the analysis. Numbers and percentages were used to present categorical data. The Kolmogorov-Smirnov test assessed data normality. Normally distributed parameters were presented as mean±standard deviation, while non-normally distributed parameters were shown as median [minimum-maximum]. Independent samples t-test, Mann-Whitney U test, and chi-square test compared ECG and lab data between patients and controls. Pearson correlation and linear regression explored inflammation-ECG links. Significance was set at $p<0.05$, enhancing the rigor and precision of the study's outcomes.

RESULTS

The study included 40 OSAS patients and 40 healthy controls. Age and gender distribution showed no significant differences ($p=0.886$, $p=0.501$, respectively) (Table 1). The BMI value of OSAS patients was significantly higher

than HCs (p=0.002). Smoking status showed no notable difference between OSAS patients and HCs (p=0.822). Table 2 displays the ECG data comparison, indicating significantly higher QT, QTc, and ICEB values in OSAS patients compared to HCs (p<0.001, all).

Table 3 outlines the lab data contrast between OSAS patients and HCs. OSAS patients exhibited notably higher glucose and TSH levels than HCs (p=0.024 and p=0.023, respectively). Additionally, T4 levels were significantly lower in OSAS patients compared to HCs (p=0.044).

According to Pearson correlation analysis, AHI and BMI, QT, QTc, and ICEB were significantly correlated (p=0.020, p=0.009, p=0.010, and p=0.003, respectively) (Table 4). In the linear regression, ICEB significantly and positively predicted AHI (F(5.76)=18.451, R2=0.657, Adjusted R2=0.715, p<0.001) as presented in Table 5.

Table 1. Sociodemographic features of the OSAS patients and HCs

	OSAS Patients n=40 (M+SD) or n (%)	HCs n=40 (M+SD) or n (%)	p value
Gender			
Male	17 (42.5)	20 (50)	0.501[a]
Female	23 (57.5)	20 (50)	
Age	47.98±8.77	46.90±9.67	0.886[b]
Smoking	18 (45)	17 (42.5)	0.822[a]
BMI, kg/m²	29.2±4.56	25.8±5.3	**0.002[b]**

OSAS: Obstructive sleep apnea syndrome, HCs: healthy controls, BMI: body mass index
a: p value based on chi-square analysis, b: p value based on Student's t-test. A significance level of p<0.05 was considered

Table 2. Comparison of electrocardiogram parameters of OSAS patients and HCs

	OSAS Patients n=40 (M+SD) or n (%)	HCs n=40 (M+SD) or n (%)	p value
Heart rate, bpm	82.58±13.64	81.25±14.03	0.729
QRS, msec	89.30±8.39	88.00±8.42	0.491
QT, msec	408.15±30.85	359.90±27.76	**<0.001**
QTc, msec	435.75±25.41	406.58±25.21	**<0.001**
ICEB	4.60±0.51	4.12±0.41	**<0.001**

OSAS: obstructive sleep apnea syndrome, HCs: healthy controls, ICEB: index of cardiac electrophysiological balance
[1]Student's t test was used. p<.05 was accepted as statistical significance value

Table 3. Comparison of lab parameters of OSAS patients and HCs

	OSAS Patients n=40 (M+SD) or n (%)	HCs n=40 (M+SD) or n (%)	p value
Glucose, mg/dL	86.46±10.24	78.21±8.56	**0.024**
Hemoglobin, mg/dL	14.65±1.66	14.39±2.12	0.178
TSH, mU/L	3.91±1.86	3.40±1.35	**0.023**
T4, ng/dL	0.70±0.190	0.82±0.22	**0.044**
Urea, mg/dL	18.11±8.37	16.56±6.77	0.242
Creatinin, mg/dL	0.92±0.32	0.85±0.36	0.356
Na, mEq/L	139±6.51	140±6.44	0.781
K, mEq/L	4.01±0.65	3.81±0.78	0.101
WBC, $10^3/\mu L$	7.56±1.62	6.21±2.27	0.186
Neutrophil, $10^6/\mu L$	4.78±1.66	4.45±1.46	0.174
Lymphocyte, $10^3/\mu L$	2.26±0.56	2.53±0.93	0.102
Monocyte, $10^3/\mu L$	0.52±0.16	0.51±0.15	0.628
Basophil, $10^3/\mu L$	0.08±0.09	0.09±0.10	0.446
Eosinophil, $10^3/\mu L$	0.18±0.17	0.16±0.18	0.166
Platelet, $10^3/\mu L$	255.77±66.73	244.53±53.82	0.229
CRP, mg/dL	0.22±0.06	0.20±0.10	0.648

OSAS: obstructive sleep apnea syndrome, HCs: healthy controls, TSH: thyroid stimulating hormone, T4: thyroxine, WBC: white blood cell, CRP: C-reactive protein
Student's t test was used. p<.05 was accepted as statistical significance value

Table 4. Pearson correlation analyzes of AHI with age, BMI and ECG parameters in OSAS patients

	AHI
Age	r=0.254
	p=0.021
BMI	**r=0.246**
	p=0.020
QT	**r=0.180**
	p=0.009
QTc	**r=0.216**
	p=0.010
ICEB	**r=0.315**
	p=0.003

OSAS: obstructive sleep apnea syndrome, AHI: Apnea-Hypopnea index, QTc: corrected QT interval, ICEB: index of cardiac electrophysiological balance
Pearson correlation test was used. p <.05 was accepted as statistically significant

Table 5. Linear regression analyzes of AHI in OSAS patients

					95% CI	
	B	Beta	t	p	Lower	Upper
Age	1.651	0.212	1.830	0.137	-0.040	4.160
BMI	3.314	0.120	1.142	0.214	-2.503	10.136
QT	1.400	0.132	1.352	0.150	-0.644	3.640
QTc	0.134	0.254	2.753	0.186	0.050	0.218
ICEB	1.712	0.860	2.412	**0.004**	-1.768	4.365
Constant	-22.670		-2.106	0.046		

OSAS: obstructive sleep apnea syndrome, AHI: Apnea-Hypopnea index, QTc: corrected QT interval, ICEB: index of cardiac electrophysiological balance, CI: confidence interval
Lineer regression analyses was used. p<0.05 was accepted as statically significance. F(5.76)=18.451, R2=0.657, Adjusted R2=0.715 and p<0.001

DISCUSSION

This study shows crucial results: i) ICEB, QT, QTc, and BMI values were higher in OSAS patients than HCs. ii.) AHI was positively and significantly correlated with BMI, QT, QTc, and ICEB in OSAS patients. iii.) ICEB was found to be closely related to disease severity in OSAS patients.

An major factor in the pathogenesis of OSAS is BMI. Excess adipose tissue, especially around the neck and upper airway, can lead to airway narrowing and obstruction during sleep. This contributes to the hallmark symptoms of OSAS, including loud snoring, recurrent awakenings, and daytime sleepiness. Research consistently demonstrates a positive correlation between higher BMI levels and an increased risk of developing OSAS. As BMI rises, the risk of OSAS also escalates, underscoring the importance of weight management in OSAS prevention and management (8). The way OSAS is managed and treated is greatly influenced by BMI. Lifestyle modifications, including weight loss through diet and exercise, have been shown to alleviate OSAS symptoms and even lead to resolution in some cases. For individuals with a higher BMI, these interventions can be particularly effective. However, weight loss can be challenging for individuals with OSAS due to the factors mentioned above, making a multidisciplinary approach essential (9).

Several studies have investigated the potential impact of OSAS on the QT interval duration (10). The intermittent hypoxia and sympathetic activation that occur during OSAS episodes can lead to autonomic nervous system dysregulation. This imbalance can affect the duration of the QT interval, potentially leading to QT interval prolongation (11). An increased risk of torsades de pointes, a particular kind of arrhythmia that can result in fatal ventricular arrhythmias, is linked to prolonged QT intervals (12). The underlying mechanisms connecting OSAS and QT interval prolongation are multifaceted. Sympathetic nervous system overactivity triggered by OSAS can alter cardiac repolarization processes, influencing the duration of the QT interval. Additionally, the oxidative stress and inflammation resulting from intermittent hypoxia and reoxygenation in OSAS episodes can impact ion channel function, further affecting the QT interval duration (13).

OSAS, with its physiological disruptions, can significantly influence cardiac electrophysiology, creating an environment conducive to arrhythmias. The underlying mechanisms are complex and multifaceted. OSAS is known to trigger sympathetic overactivity and parasympathetic withdrawal. This imbalance can disrupt the heart's normal rhythm and predispose individuals to arrhythmias. Intermittent hypoxia during OSAS episodes can lead to alterations in the duration of the QT interval, a key parameter reflecting ventricular repolarization (14). OSAS-induced oxidative stress and inflammation can impact ion channel function and cardiac conduction pathways, increasing arrhythmia susceptibility. The intricate relationship between OSAS and the risk of arrhythmias underscores the significance of recognizing and addressing both conditions. The physiological disruptions caused by OSAS create an environment conducive to arrhythmias, necessitating vigilant monitoring and comprehensive patient care. By understanding this intricate connection, healthcare providers can proactively manage both OSAS and arrhythmias, enhancing the cardiovascular well-being and quality of life for individuals affected by these conditions (15).

The connection between autonomic nervous system imbalance and ventricular arrhythmias is established. Sympathetic and parasympathetic dysfunction disrupts cardiac rhythms, affecting ventricular repolarization (16). Elevated ICEB values may result from increased catecholamines linked to rising intracranial pressure (17). Catecholamines, including adrenaline and noradrenaline are released by the adrenal glands in response to stressors, exerting significant effects on the cardiovascular system. In OSAS, the sympathetic nervous system is overactivated, resulting in elevated catecholamine levels (18). This autonomic imbalance contributes to increased

heart rate, blood pressure variability, and altered cardiac electrophysiology. One of the cardinal concerns stemming from heightened catecholamine levels in OSAS is their potential role in the genesis of ventricular arrhythmias. Sympathetic overactivity triggered by OSAS episodes can provoke cardiac electrical instabilities, leading to alterations in ventricular repolarization. These changes, as reflected in parameters like the QT interval, can heighten the risk of ventricular arrhythmias, including life-threatening conditions like ventricular tachycardia (19). However, the correlation between serum catecholamine levels and ECG alterations requires further elucidation.

Although within normal limits, TSH value was higher in OSAS patients and T4 values were lower in OSAS patients. The complex interplay between hypothyroidism, a disorder marked by insufficient thyroid hormone production, and OSAS reveals a fascinating terrain of potential connections that affect people's health and well-being. Investigating this interaction reveals the complex mechanisms that demand careful medical assessment. Sleep patterns can be greatly impacted by hypothyroidism. Individuals are more vulnerable to OSAS-related symptoms such loud snoring, disrupted sleep, and daytime weariness due to slowed metabolism and decreased sympathetic activity (20).

Expanding the research to involve more patients and considering additional ECG markers like Tp-e and Tp-e/QT could yield more comprehensive insights. Our study's drawback includes the lack of electrolyte data, like calcium and magnesium, which can influence QT interval and ventricular repolarization. Since the normal ranges of ICEB are unknown, prospective longitudinal axis studies are needed to predict the risk of ventricular arrhythmias in OSAS patients. The duration of illness in OSAS patients may influence the risk of ventricular arrhythmias. Not recording this information is a limitation.

CONCLUSION

In this study, a relationship between ICEB and the AHI value, which is used to assess the severity of OSAS, was discovered to be both positive and significant. The fact that ICEB, which is used to evaluate the risk of ventricular arrhythmia, is higher in OSAS patients indicates that these patients should be followed more closely in the cardiologic outpatient clinic.

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MEDICAL RECORDS-International Medical Journal

Research Article

Five-Years Intensive Care Percutaneous Tracheostomy Results

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Abstract

Aim: Tracheostomy is an interventional procedure frequently performed on critically ill patients in the intensive care unit (ICU). The purpose of this study is to report the characteristics of patients undergoing percutaneous tracheostomy in intensive care.

Material and Methods: Cases admitted to intensive care between 2018 and 2022 and subjected to percutaneous tracheostomy were included. Cases involving surgical tracheostomy were excluded. We scanned the patients' demographic and clinical characteristics, follow-up characteristics in intensive care, tracheostomy complications, and discharge characteristics. Patients were also divided into surviving and non-surviving groups and compared.

Results: One hundred seven patients were included in the study. Men represented 64.5% of the patients, and the mean age of the entire patient group was 61.5 years. Tracheostomies were most frequently performed due to prolonged endotracheal intubation. Neurological diseases were the most common diagnoses, and the most frequent complication was bleeding. The groups differed in terms of age, comorbidity, presence of tracheostomy at time of discharge from intensive care, and anticoagulant use. The mortality rate was 69.2%.

Conclusion: We think that percutaneous tracheostomy can be employed because it can be performed at the point of care in intensive care, and due its ease of application and low complication rate.

Keywords: Tracheostomy, percutaneous, intensive care

INTRODUCTION

Tracheostomy refers to the creation of an opening in the tracheal cavity with a cutaneous incision to the anterior wall of the trachea. It is known to have been performed by the ancient Egyptians, but surgical tracheostomy was first described by Jackson in 1909 (1). Tracheostomy has been a longstanding and frequently performed intervention for critical intensive care unit (ICU) patients. Percutaneous dilatational tracheostomy (PDT) over a guidewire was described by Ciaglia in 1985. Surgical tracheostomy involves the dissection of the pretracheal tissues, followed by an incision to the trachea and visual insertion of a tracheostomy cannula. In contrast, PDT involves blunt dissection of pretracheal tissues, followed by tracheal expansion with a guidewire and insertion of the tracheostomy cannula using the Seldinger technique (2). PDT has a low complication rate, does not require an operating room setting, and can be easily performed at the point of care (3).

Prolonged endotracheal intubation can involve complications such as laryngeal damage, vocal cord paralysis, glottic and subglottic stenosis, infection, and tracheal damage (tracheomalacia, tracheal dilatation, and tracheal stenosis) (4). PDT is frequently performed in intensive care for the purpose of avoiding these complications, facilitating airway maintenance, achieving a safer airway in patient mobilization and transfer, and to facilitate oral nutrition (5). However, despite all these advantages PDT is an invasive procedure, with complications ranging from minor bleeding to mortality (6).

The purpose of this study was to review tracheostomy procedures performed in the ICU and the characteristics of the patients involved in a retrospective manner, and to report our results.

MATERIAL AND METHOD

Following receipt of İnönü University Health Sciences

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Non-Interventional Research Ethical Committee approval, patients aged over 18, admitted to the ICU between January 2018 and December 2022, and undergoing tracheostomies were screened. The patients' demographic data, diagnoses, comorbidities, lengths of hospitalization, APACHE II and SOFA scores, indications for tracheostomy opening, the time and day of tracheostomy opening, duration of mechanical ventilation, time spent tracheostomized, whether the patient was tracheostomized on departure from hospital, tracheostomy-related complications, anticoagulant use, and type of discharge from hospital were screened retrospectively and recorded. The patients were also compared by being divided into two groups, surviving and non-surviving. Surgically opened tracheostomies were excluded.

Candidates for tracheostomy in our unit are generally required to have routine activated thromboplastin and prothrombin times less than 1.5 times the control values, a platelet value above 50,000 (mm^3), and a normal tracheal and neck structure. Ultrasonographic assessment and airway examination are performed, and the intervention site is selected through ultrasonography (USG). Consultation for surgical tracheostomy is performed for cases that do not meet these criteria. Patients receive oxygenation with an FiO2 of 100% during tracheostomy. Anesthesia is administered with intravenous anesthetics such as midazolam and propofol. Analgesia is administered with an opioid analgesic, most commonly fentanyl. Local anesthesia is also used for analgesia. In addition, all patients are monitored throughout the procedure by means of electrocardiography, pulse oximetry, end-tidal carbon dioxide pressure, and invasive/non-invasive arterial pressure. Mechanical ventilation is performed in either volume- or pressure-controlled mode. The patient is first positioned, with a pillow beneath the head, after which PDT is performed using the Griggs technique. The endotracheal cuff is deflated, pulled beneath the vocal cord, and re-inflated. Tracheostomy is performed at an appropriate location determined by USG after the second tracheal ring. After the procedure, lung ventilation is assessed through auscultation, and chest X-rays are taken.

Biostatistical Data Analysis

The variables in the study were summarized as number (percentage) values. Normality of distribution of quantitative variables were evaluated using the Shapiro-Wilk test. Non-normally distributed quantitative data were summarized as median (minimum-maximum) values and normally distributed data as mean± standard deviation. The Mann-Whitney U test, independent sample t test, Pearson's chi-square test, the Yates corrected chi-square test, and Fisher's exact test were used as appropriate during statistical analyses. p values <0.05 were regarded as statistically significant. All analyses were performed on IBM SPSS Statistics version 26.0 for Windows software (NY, USA).

RESULTS

One hundred seven patients underwent percutaneous tracheostomy during the study period. Men represented 69 (64.5%) of the 107 patients, and the mean age of the entire patient group was 61.49±18.95 years. The patients' demographic and clinical characteristics are shown in Table 1.

Table 1. Patients' demographic and clinical data

	Mean±SD	Median (Min-Max)
Age (years)	61.49±18.95	63 (20-93)
Length of hospitalization (days)	71.15±59.9	57 (15-441)
APACHE	22.69±9.24	22 (2-59)
SOFA	4.93±2.61	4 (0-16)
Day of tracheostomy opening	21.79±9.58	21 (4-60)
Duration of MV (days)	67.57±55.69	54 (15-441)

The four most common diagnoses at the time of presentation were diseases of neurological origin (23.4%), return of spontaneous circulation after cardiac arrest (2.4%), infection (20.6%), and trauma (18.7%). Other conditions included cardiac conditions (3.7%), respiratory system diseases (4.7%), malignancy (1.9%), intoxications (2.8%), and post-surgical causes (1.9%). Comorbidity was present in 69 patients - respiratory diseases (17.8%), neurological disease (17.8%), hypertension (18.7%), coronary artery disease in (16.8%), diabetes mellitus (11.2%), chronic kidney failure (2.1%), and malignancy (0.9%). Late tracheostomy was performed in 89.7% patients, and re-intubation was observed in 19.6%. Anticoagulant use was present in 39.3% patients.

The most common indication for tracheostomy opening was prolonged intubation 78.5%, followed by prolonged mechanical ventilation requirements (16.8%) for reasons such as maxillofacial trauma. The most frequent complications were minor bleeding, observed in four (3.7%) patients, infection around the tube in 1.9%, subcutaneous emphysema in 0.9%, and trachea-esophageal fistula in 0.9%. The total complication rate was 7.4%. No procedure-related pneumothorax or mortality occurred in any patient. The patients' discharge status is summarized in Table 2.

Table 2. Patients' ICU discharge status

		n	%
Form of discharge	Transfer to the ward	2	1.9
	Transfer to intensive care	5	4.7
	Transfer to the palliative unit	4	3.7
	Discharge to home	22	20.6
	Exitus	74	69.2

Significant differences were determined between the surviving and non-surviving groups in terms of age (p=0.006), comorbidity (p=0.037), presence of tracheostomy at the time of discharge (p=0.001), and anticoagulant use (p=0.019), while the other parameters were similar between the groups (p<0.05) (Tables 3 and 4).

Table 3. A comparison of the surviving and non-surviving groups

	Group				
	Surviving		Non-surviving		
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	p
Age (years)	53.79±17.93	53 (20-82)	64.92±18.49	67 (22-93)	**0.006***
Length of hospitalization (days)	82.45±75.38	66 (21-441)	66.11±51.34	53 (15-365)	0.125*
APACHE	21.24±6.95	21 (5-35)	23.34±10.07	23.5 (2-59)	0.281**
SOFA	4.21±1.87	4 (0-11)	5.24±2.84	5 (0-16)	0.053*
Day of tracheostomy opening (days)	21.91±11.2	21 (5-60)	21.74±8.85	21 (4-45)	0.853*
Duration of MV (days)	75.45±75.83	60 (19-441)	64.05±44.06	51 (15-270)	0.513*

SD: standard deviation, *: Mann Whitney U test, **: Independent samples t-test MV: Mechanical ventilation

Table 4. Comparison between the surviving and non-surviving groups

Variables	Group		p
	Surviving (n, %)	Non-surviving (n, %)	
Sex			
Male	22 (66.67)	47 (63.51)	0.923**
Female	11 (33.33)	27 (36.49)	
Comorbidity	16 (48.48)	53 (71.62)	**0.037****
Tracheostomy timing			
Early	5 (15.15)	6 (8.11)	0.309***
Late	28 (84.85)	68 (91.89)	
Re-intubation	4 (12.12)	17 (22.97)	0.298**
Tracheostomy on discharge from intensive care	23 (69.70)	73 (98.65)	**<0.001****
Anticoagulation	7 (21.21)	35 (47.30)	**0.019****

* Pearson chi square, ** Yates's correction chi-square test, ***: Fisher's exact chi square, CPR: Cardiopulmonary resuscitation

DISCUSSION

PDT is widely used in mechanically ventilated patients in the ICU, increasingly replacing surgical tracheostomy. PDT is performed for several reasons, including facilitation of weaning, facilitation of tracheobronchial cleansing, reducing the risk of aspiration, reducing sedation requirements, for airway protection in patients due to be mobilized, in cases of prolonged endotracheal intubation, and for patients requiring long-term mechanical ventilation. Yeşiller et al. reported prolonged mechanical ventilation and prolonged coma as the most frequent indications for tracheostomy opening (1). Yeniaras et al. reported that prolonged endotracheal intubation was the reason for tracheostomy in 104 (89%) out of 114 patients undergoing the procedure in the ICU (7). Tracheostomy was also most frequently performed due to prolonged endotracheal intubation in the present study. The second most common reason in this study was long-term mechanical ventilation, another reason being maxillofacial trauma.

Early tracheostomy is reported to reduce lengths of hospital stay (8). In the SETPOINT2 study, Bosel et al. examined the effect on prognosis of early tracheostomy in patients with severe stroke, but reported no improvement in functional outcomes. Early tracheostomy was performed after a median four days in that study, compared to a median 11 days in the standard group (9). In their meta-analysis, Andriolo et al. reported that there was insufficient evidence for the effect of early and late tracheostomy opening on mortality, and that on the basis of the current evidence, early tracheostomy could go no further than being a recommendation in terms of reducing mortality (10). The length of hospital stay in the present study was approximately 71 days, and the mean time to tracheostomy opening was 21.8 days. We concluded that early or late tracheostomy had no significant effect on mortality.

Tracheostomy is generally performed in intensive care patients due to respiratory and neurological diseases (3). In a retrospective study of 38 patients who underwent tracheostomy using the Griggs technique, Öncül et al. described neurological problems as the most frequent reason for tracheostomy opening, followed by respiratory causes (11). Neurological pathologies were the most frequent cause in Yeşiller et al.'s study, followed by respiratory causes (1). Neurological problems were also the most frequent cause in the present study, followed by patients with spontaneous return of circulation following cardiac arrest.

The tracheostomy stoma generally heals within one week. Complications arising within the first week are considered early, while those occurring later are

termed late. Complications include bleeding, infection, posterior wall damage, obstruction of the tracheostomy tube, subcutaneous emphysema, tube displacement, pneumothorax, and tracheal stenosis (12). Complications in the current study included infection, posterior wall damage, subcutaneous emphysema and, most frequently, bleeding.

Mortality rates in tracheostomized patients in the literature range between 40% and 90% (3,13). The mortality rate in the present study was 69.2%. We also observed differences between the groups in terms of patients' ages, comorbidities, presence of tracheostomy on discharge, and anticoagulant use. This variation shows the presence of a more sever manifestation in the non-surviving patient group.

The main limitation of our study was that it was retrospective. In addition, the fact that it is a single center and the study data were obtained from patient files can be listed as other limitations.

CONCLUSION

PDT has finally assumed its proper place in intensive care and has become a frequently performed technique. Although a high risk of complications and mortality may be expected in the light of the patient group to which it is applied, we think that PDT can be safely applied to patients in the ICU due to its low complication and mortality rate and the fact it can be performed at the point of care, and because it increases airway control and permits easier removal of tracheal secretions.

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

Ethical approval: *Before the study, ethical approval was obtained from the İnönü University Health Sciences NonInterventional Clinical Research Ethics Committee (No: 2023/4664).*

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MEDICAL RECORDS-International Medical Journal

Research Article

The Effect of Circular Stapler Design Used in Hemorrhoidopexy on Medical Outcomes in terms of Medical Engineering

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Abstract

Aim: The aim of this study is to examine the advantages and disadvantages of the design of the circular stapler device by sharing our hemorrhoidectomy results with circular stapler, and to examine whether changes in the design of the circular stapler device have an effect on these complications.

Material and Methods: This study which was planned as retrospective archive search and approved by the Etlik Zubeyde Hanim Gynecology Training and Research Hospital Medical Specialization Education Board (TUEK) with decision number 17 dated November 15, 2019. Moreover, cases with missing or unclear data weren't included to the study. Descriptive patterns were revealed by examining the records of the patients in terms of length of hospital stay, pain level according to Visual Analogue Scale (VAS: 1-10), bleeding and other complications. In terms of descriptive analyses, percentage and frequency values were evaluated with mean and standard deviations in continuous variables.

Results: Between January 2014 and July 2018, a total number of 21 hemorrhoid cases were treated with circular stapler hemorrhoidopexy. All of the patients were in the young male age group (21-26), with a mean age of 23.5±1.2. The mean hospital stay was 1.95±0.75 (1-4) days. In the intraoperative period, anal bleeding was observed only in one patient. When the pain occurring in the first 24 hours after surgery was examined according to the VAS, there were 11 patients (52%) painless, 7 patients (33%) had mild pain, 2 patients (10%) were moderate pain and 1 patient (5%) was describing severe pain.

Conclusion: The circular stapler is a surgical instrument that stands out with its specific engineering features, and when used in the correct position and in the correct manner, it is an advantageous surgical tool with low complication rates for the surgeon in advanced hemorrhoids and mucosal sagging.

Keywords: Hemorrhoidopexy, circular stapler, anorectal surgery, surgery technique, medical engineering, hemorrhoid

INTRODUCTION

For hemorrhoids; the theory of slipping of the anal cushions put forward by Thomson is the most widely accepted theory (1). Constipation, chronic diarrhea, chronic straining, occupations requiring prolonged standing and pregnancy were the factors accused in the etiology of hemorrhoidal disease. Hemorrhoidal disease causes many clinical conditions such as bleeding, pain and itching that adversely affect people's social life. There are authors who divide hemorrhoidal disease into two groups: internal hemorrhoidal disease originating above the dentate line and external hemorrhoidal disease originating below the dentate line (2). Antonio Longo first described the stapled hemorrhoidopexy method in prolapsed symptomatic hemorrhoidal disease in 1998. In this method, approximately 3-4 cm of the dentate line is defined as suspending prolapsed hemorrhoid packs in their normal anatomical places as a result of disconnection of the superior rectal artery feeding both hemorrhoids and loosening of Thomson's Park's ligament by performing a mucosal-submucosal resection over the hemorrhoids (3). On the other hand, it is thought that the technique applied to the area where there is no pain sensation without touching the anoderm compared to conventional methods will theoretically result in less

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postoperative pain and therefore shorter hospitalization time (4,5). Considering that the pain can be very severe in some patients, especially after hemorrhoid surgeries, we can see that this method created a lot of excitement in the first days of its introduction (5). Because, in this method, the suture line stays on the dentate line, where sensation is less, and the postoperative pain will be equally less. As we mentioned above, in this technique, if the circular stapler (circular cutter and circular tissue stapler) device is incorrectly placed and this procedure is applied to the dentate line or lower region, a series of complications, especially pain, will occur. Although the footprints of the surgical stapler used today go back to the works of Humer Hültl in the early 1900s, today's instruments are spread over a much wider range thanks to the work of innovators such as Von Petz and Ravitch (6), and in this study, we will review the surgical experience of this surgical instrument. We shared our opinions and results on this subject by presenting the advantages and disadvantages of the state of the design of the device related to its use.

MATERIAL AND METHOD

This study, which was planned as retrospective research, was approved by the Etlik Zubeyde Hanim Gynecology Training and Research Hospital Medical Specialization Education Board (TUEK) with the approval date November 15, 2019 and decision number 17.

In this study, in which data between January 2014 and January 2018 were analyzed as a retrospective archive scan, descriptive patterns were revealed by examining the records of the patients in terms of length of hospital stay, pain level according to Visual Analogue Scale (VAS: 1-10), bleeding and other complications.

Surgical Approach

The cases were performed under general anesthesia in the lithotomy position. A slight dilatation was achieved by placing the anoscope of the circular stapler kit, together with its dilator, into the anal canal (Figure 1).

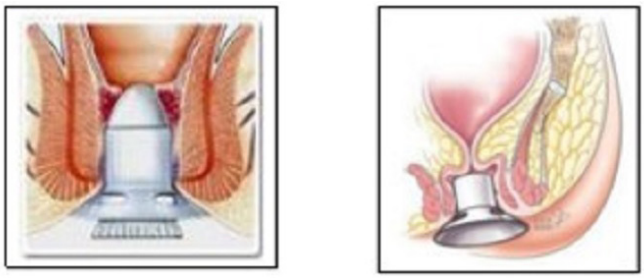

Figure 1. From Barlas (2008) (13)

After the dilator was removed, the 4 holes on the outside of the anascope were fixed to the perianal region with silk sutures. With the help of the kit's transparent anascope, a clear view of the endoscopic anatomy of the anal canal and rectum was provided. A circular purse-string suture was passed on the condition that it passes through the mucosa and submucosa with a 2-0 5/8 27 mm maxon approximately 4 cm above the dentate line (Figure 2).

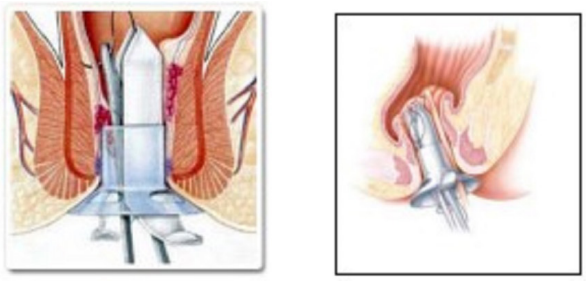

Figure 2. From Barlas (2008) (13)

The stapler was fully opened and a knot was tied by inserting the anvil into the purse-string suture. Both free ends of the suture were removed from the canals of the stapler with the help of a hook, and these ends were knotted and pulled outward slightly to provide traction on the stapler (Figure 3).

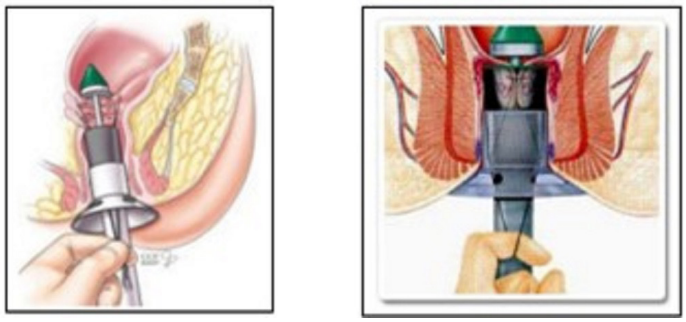

Figure 3. From Barlas (2008) (13)

At this time, the circular stapler was slowly pushed into the anal canal. When the dial on the device reached the level of 4 cm, the pushing process was terminated. The correct position of the stapler in the anal canal was reviewed one last time, the device was turned to the end point and fired. In order to provide hemostasis in the anastomosis line, it was waited for 20 seconds during the firing. Then, the device was slowly removed from the anal canal after one turn, and the operation was completed (Figure 4).

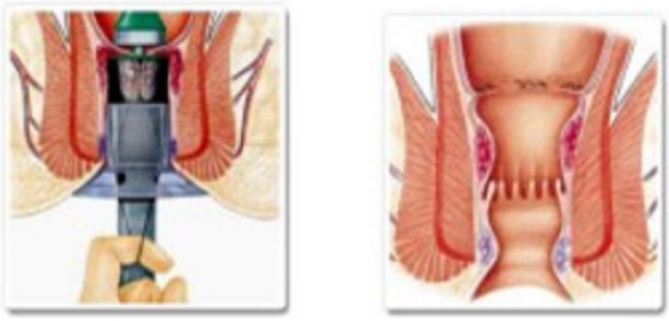

Figure 4. From Barlas (2008) (13)

RESULTS

A total of 21 hemorrhoid cases were treated with circular stapler hemorrhoidopexy between January 2014 and July 2018. All of the patients were in the young male age group (21-26). The mean age was 23.5±1.2 (21-26). Spinal anesthesia was applied to all of the patients. In the intraoperative period, anal bleeding was observed in

one patient. When the pain occurring in the first 24 hours after surgery was examined according to the visual analog scale, there were 11 (52%) painless patients, while 7 (33%) patients had mild pain, 2 (10%) patients were on moderate pain and only 1 (5%) patient described severe pain (Table1). We encountered hemorrhage in only one case (5%)(Table2). The mean operation time was 42.65±8.2 (30-55) minutes, and the mean hospital stay was 1.95±0.75 (1-4) days (Table 3).

Table 1. Pain occurring in the first 24 hours after surgery

	The VAS score distribution				
	Painless	Mild pain	Moderate pain	Severe pain	Very severe pain
n	11	7	2	1	0
Percentage (%)	52	33	10	5	0

VAS: Visual Analogue Scale

Table 2. Monitored complications

	n	Percentage (%)
Intraoperative bleeding	1	5
Severe pain	1	5

Table 3. The hospital length of stay

n	12	6	2	1	HLOS
Day	2	1	3	4	1.95±0.75 (1-4)

HLOS: hospital length of stay

DISCUSSION

The border between the normal skin containing hair follicles, sweat and sebaceous glands at the entrance of the anus and the anal canal skin deprived of these appendages is called the anal verge (verge) and is considered as the border in distance determinations (7,8). On the other hand, when we look at the term anal canal, its inner covering has changed mainly in two directions: It is the mucosa above and the skin below. The border between the two covers is called linea pectinea or linea dentata (pectinate line and dentate line, respectively). The surgical anal canal is defined to be the 3-4 cm section between the anal verge and the anorectal ring. The dentate line is located in the middle of the surgical anal canal (7,8).

The stages of the classification of disease stages in hemorrhoids suggested by Goligher et al. (1984), which is still in use, are the following:

Stage 1: Hemorrhoidal pads are located above the dentate line, they do not go down with straining.

Stage 2: Hemorrhoidal pads hang below the dentate line by straining, and return spontaneously after defecation.

Stage 3: Hemorrhoidal pads come out of the anal canal during straining, do not return spontaneously, but can be pushed in manually.

Stage 4: The hemorrhoidal pads are constantly hanging out of the anal canal and do not be pushed in manually (2).

Surgery is recommended for 3rd and 4th stage hemorrhoidal pouches. Standard surgical techniques consisting of ligation and removal of hemorrhoidal packs are still the most frequently used methods in the world and have been offered as the gold standard in the treatment of stage 3 and 4 hemorrhoids for a long time (9).

External hemorrhoids are located distal to the dentate line and covered with anoderm. In other words, they develop under the skin around the anus and because the innervation of the anoderm is rich, thrombosis of external hemorrhoids causes severe pain. On the other hand, internal hemorrhoids are located proximal to the dentate line and are covered by anorectal mucosa with numb morgagni columns. So they are inside the rectum. Internal hemorrhoids may prolapse, bleed, and do not cause pain. Severe pain develops in stage 4 internal hemorrhoids because of malnutrition. Finally, combined internal and external hemorrhoids are described as both internal and external hemorrhoids overlapping above and below the dentate line (10).

Circular stapler line placed under the dentate line may cause an operation that cannot reach its target, especially in the case of severe pain, because the mucosa is removed from an inappropriate place and an end-to-end anastomosis is performed. It may also lead to other serious complications such as abscess, gas, stool incontinence, and anal stenosis (11-13). Additionally, pain after hemorrhoidectomy has been one of the most important factors preventing patients from undergoing surgery. On the other hand, since there is no surgical incision or wound on the anus skin with the Longo method, there will be no need for post-operative dressing and sitting baths. Contrary to the expectation of most patients, when there is a need for defecation after surgery, there will not be excessive pain. Most of the patients are able to return to work within 3-5 days (13). There are two basic steps in the Longo method; The first step is to pass the perimeter seam. The second stage can only be passed after an ideal perimeter seam has been passed. In the second step, the punch is placed and fired, which is a mechanical process (14).

In the time period following the announcement of the Longo technique, more than 50,000 cases across Europe underwent surgery with this method (15). There are studies in the literature describing low pain scores and a shorter hospital stay in this period (16,17). While increasing the popularity of the technique, which began to be named after Longo, there were also studies published in the opposite view over time. Cheetham et al. (2000) observed pain in 31% of the patients they operated with stapled hemorrhoidopexy (18), while Ravo et al. (2002) reported severe pain in 5% of the patients at the end of the first week in their study, which included 1107 patients in 12 centers in Italy (19). These findings are similar to the study of Ougris et al. (2005), in which they examined the early and late complications of the technique and explained the severe pain rate as 2.3% (20). A clinical study by Thaha et al. (2005) compared multicenter randomized stapled hemorrhoidopexy with

closed hemorrhoidectomy, and observed post-defecation pain, which was defined as a complication specific to the technique using only staples, and which was not observed in any other study (21). Despite all these results, the number of publications reporting the superiority of stapled hemorrhoidopexy in postoperative pain control in studies comparing conventional methods is also quite high.

When our study was examined in terms of the pain occurring on the first postoperative day according to the Visual Analog Scale (22), 11 patients were painless (53%) 7 patients had mild pain (33%), and 2 patients were describing moderate pain (10%). Severe pain was detected with 1 patient (5%) and there was no patient with very severe pain. In all patients, the complaint of pain regressed within the first 72 hours. When the daily patient records are examined, it was observed that the anastomosis line was just at the border of the dentate line, as a result of the anoscope examination of the patient who complained of severe postoperative pain. It is thought that the reason for this severe pain is due to the stapler suture line being adjacent to the dentate line. The difference in pain sensitivity between the upper and lower dentate line is also due to the different nerve conduction of these two separate regions (4,5,13).

Abandoned bleeding is also a complication, which definitely needs to be stopped. It is usually due to the presence of the injury of the branches of the superior mesenteric artery, which provides 80% blood supply to the dentate line (23). In our study, a significant bleeding was detected in 1 case (5%) after completing the resection and anastomosis with circular stapler, and after removing the relevant instrument. In the later evaluation, the cause of the bleeding here was evaluated to be the possibility of opening the anvil and retraction of the circular stapler without waiting for at least 20 seconds of waiting time after the circular stapler was fired (for the suture line to settle and to provide hemostasis). At this point, the question of whether a double-row stapler mechanism can be made to reduce the risk of bleeding in the circular stapler in the field of surgical instrument engineering in the future still comes to mind. The use of three-row circular staplers independently reduced the risk of anastomotic leak and related morbidity after left-sided colorectal resection (24).

Although we did not encounter it in our study, and it is rarely reported in the literature, it is also important to determine the condition anorectal ring, 3-4 centimeter above from the anal verge, because this anorectal ring can be injured via circular stapler in the surgical treatment of abscess, fistula, grade 3-4 hemorrhoidal diseases, and rectal prolapses. Although the complete cutting of the anorectal ring in surgical interventions almost always results in incontinence, partial losses that may occur in the internal and external sphincter do not usually cause serious stool incontinence problems (11-13).

Anal stricture, on the other hand, is a rarely seen complication. A meta-analysis by Allen et al. (2008) reported that the risk of stricture in surgical igniters containing large-surface metal staples applied to the upper gastrointestinal tract is 2 times less than that of small surfaces surgical igniters (25). Peeters et al. (2016) observed in their study using three different types of circular staplers (Chex CPH32, Chex CPH 34 and Ethicon EndoSurgery PPH03) that anal stenosis occurred in 5 cases with Chex CPH32 circular stapler. They observed no stenosis developed in cases where they used PPH03 circular stapler (26). We did not observe in our study development of stenosis for the cases in which circular staplers were used. We attribute this to Allen et al. (2008), who stated that less stricture develops in the use of large-surface metal staples for the upper gastrointestinal tract (25). In addition, having a flat surface of the head part of the circular igniter, called anvil, removes the relevant mucosa all around, and contributes to the prevention of unnecessary tissue collection, as per the literature on the anvil surface (27). We further think that it may be important in preventing the development of anal stenosis.

Another complication is bleeding, which can be caused by the forced opening of the circular stapler sutures, as a result of the difficulty of the removal of the instrument from the staple (suture) line to separate it from the remaining intact tissue. The reason might be the instrument or the operator error; because, when the instrument is tried to be removed before the anvil is fully opened after ignition, it may damage the anastomosis by pulling the tissue behind it. However, less tension can be generated on the tissue, creating an anastomosis with less strain on the stapler suture line, thanks to the fixed anvil design feature of an important medical device used for this type of operations (28). As mentioned before, we encountered hemorrhage in only one case (5%) and we think that it may be related to the premature opening of the anvil.

CONCLUSION

We consider the large surface area of the staple metal suture material clips of the instrument used, as well as the flatness of the anvil surface, to be advantageous aspects. We think that operator errors will be reduced with the development of a circular stapler with a double row of seams in future works. Multicenter prospective studies will therefore yield much clearer results.

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MEDICAL RECORDS-International Medical Journal

Research Article

Assessment of Quality of Life Before and After Ileostomy Reversal After Low Anterior Resection for Rectal Cancer

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Abstract

Aim: Low Anterior Resection Syndrome negatively affects patients' quality of life after surgery for rectal cancer. Temporary loop leostomy is preferred to avoid anastomosis leakage related problems. Aim of this study is to evaluate Patients' quality of life before and after ileostomy reversal.

Material and Methods: Patients with laparoscopic low anterior resection with protective loop ileostomy were included in the study. Before and after the reversal of the ileostomy quality of life assessment was done by EORTC QLQ-C30 scale.

Results: Sixty two patients with temporary loop ileostomy after laparoscopic low anterior resection for rectal cancer were included in the study. Our study population showed better results in General health scale and social function scale after ileostomy reversal. However; there is no significant difference in general function and general symptom scale.

Conclusion: Temporary ileostomy can negatively affect patients' quality of life and patients have higher quality of life scores after ileostomy reversal.

Keywords: Rectal cancer, low anterior resection, quality of life, ileostomy

INTRODUCTION

Technological developments in surgical equipment changed the nature of rectal surgery. Sphincter sparing surgery became gold standard in low and very low rectal cancer. Although, staplers used for anastomosis enables surgeons to make ultra-low colo-anal anastomosis, leakage still remains a major problem (1). Thus, temporary loop ileostomy is very common to avoid leakage related complications.

Rectal surgery itself, seriously affect patients' quality of life. Frequency, urgency, incontinence and loose stools are common problems faced by the patients. Low Anterior Resection Syndrome is defined to demonstrate the undesired results of especially ultra-low rectal resections (2). Loop ileostomy prevents passage of loose stool though low anastomosis and low anterior resection related symptoms. After ileostomy reversal patients face those common problems.

There are many scales developed to assess the Quality of Life (QoL). QLQ-C30 scale is developed by The European Organisation for Research and Treatment of Cancer (EORTC) to evaluate patient reported, cancer related QoL (3).

The aim of this study is to evaluate patients' health related quality of life before and after ileostomy reversal.

MATERIAL AND METHOD

After approval from local ethical committee study was conducted in Uşak Training and Research Hospital. Patients undergone laparoscopic low anterior resection for rectal cancer between January 2018 and January 2022 were included in the study. Abdominoperineal resections, high anterior resections (above peritoneal reflection) were not included to make a standard evaluation. Patients with no temporary loop ileostomy were not included in the study.

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Patients completed EORTC QLQ-C30 questionnaire just before and 2 months after ileostomy reversal. All ileostomies were reversed six months after the primary surgery. QLQ-C30 scale consists of 28 four scale and two six scale questions divided in to three main domains as functional, symptom related and general health scores. All of the scales and single-item measures range in score from 0 to 100. A high scale score represents a higher response level. Thus a high score for a functional scale represents a high/healthy level of functioning, a high score for the global health status represents a high QoL, but a high score for a symptom scale represents a high level of symptomatology. General Health Scale (GHS), Social Function Scale (SFC), General Symptom Scale (GSS), Fatigue Scale (FAS) and General Function Scale (GFS) were calculated according to EORTC manual (4). The validation study of the Turkish version of QLQ-C30 scale was performed by Akduran F et al. (5).

There were 64 patients included in the study. Two cases excluded from study because of non-closure of loop ileostomy due to recurrent disease. Sixty-two patients were included in the final analysis.

IBM SPSS version 22.0 software was used for statistical analysis. Categorical data was presented as percentages and chi-square test was used for comparison. Quantitative data was presented as mean±standard deviation and Student T test was for comparison. A p value of 0.05 was accepted as statistical significance point.

RESULTS

The mean age of the patients was 67.4±11.8. There was 28 male and 34 female patients. Cancer stages were also evaluated and 62.9% of the cases had stage 2 rectal cancer. Sixteen (25.8%) patients had neoadjuvant radiotherapy. Sociodemographic data of the patients were presented in Table 1.

Table 1. Demographic data of the patients

Age (mean±sd)		67.4±11.8
Gender	**Male**	45.2% (n:28)
	Female	54.8% (n:34)
Tumor stage	**1**	16.1% (n:10)
	2	62.9% (n:39)
	3	19.4% (n:12)
	4	1.6% (n:1)
Radiotherapy	**Yes**	25.8% (n:16)
	No	75.2% (n:46)

General health scale significantly increased from 46.8±2.1 to 53.9±1.6 after ileostomy reversal (p=0.003). Mean social function scale was 55.4±2.6 before ileostomy reversal and increased to 72±1.5 after colostomy reversal (p<0.001). General symptom scale and general functional scale showed no significant difference before and after ileostomy reversal. Differences in QLQ subdomains are presented in Table 2.

Table 2. Mean scores obtained from QLQ-C30 questionnaire before and after ileostomy reversal

	Before ileostomy reversal	After ileostomy reversal	Difference	p
GHS	45.8±2.1	53.9±1.6	-8.02	0.003
GFS	49.2±1.9	50±1.8	-0.82	0.763
SFS	55.4±2.6	72±1.5	-16.61	0.001
GSS	46.1±2.06	47.4±1.2	1.24	0.584
FAS	59.8±2.4	76.3±1.1	-16.48	0.001

GHS: General Health Scale, GFS: General Functional Scale, SFS: Social Function Scale, GSS: General Symptom Scale, FAS: Fatigue Scale

In multivariate analysis age, gender, preoperative radiotherapy and tumor stage showed no difference in any domain of the QLQ questionnaire.

DISCUSSION

Nature of the rectal surgery dramatically affects patients' quality of life. Especially after the introduction of low and ultra-low anterior resections with colo-anal anastomosis negative aspect of the rectal surgery became more apparent. Increased stool frequency, loose stool, anal incontinence and incomplete emptying of the rectum are frequent symptoms related to Low Anterior Resection Syndrome (LARS). In most of the cases LARS is thought to be caused by absence of rectum and it's concentrating function. However, LARS can be seen even in right sided hemicolectomy. In right sided hemicolectomy, LARS development is thought to be dissection of nerves and removal of ileocecal valve (6,7). Even, Meurs et al suggested a new name to LARS as Colorectal resection syndrome.

In our study we found no significant difference in general functional scale and general symptom scale before and after ileostomy reversal. Studies evaluating QoL after rectal surgery with stoma commonly report bad QoL scores with stoma. Most of the studies report bad results in self-respect, depression, sexual problems, psychosocial adaptation and poor body image perception (8). Similarly our study demonstrates better QoL scores after ileostomy reversal.

In low anterior resection ileostomy is temporarily opened for protection against anastomosis leakage. Erarlu and late reversal of ileostomy is compared by Dulskas et al. (9). They found no difference in quality of life according to ileostomy reversal time. In our study ileostomy reversal time kept standard to prevent time caused differences in QoL. Even with standard closure time there were better results in general health scale and functional scale after ileostomy reversal.

Common aspect is poor results with stoma surgery. However, in patients with permanent stoma after surgery for inflammatory bowel disease; Deputy et al. reported high satisfaction and good quality of life results (10). According to their findings we can result that our findings of no difference in general functional scale and general symptom scale are similar with the literature. Loop

ileostomy does not have any effect on patient's perception of general functions.

Similarly Zevude et al. reported no difference in quality of life of patients with stoma (11). Main idea relying beyond this study is appearance of LARS after ileostomy reversal. However, after ileostomy reversal patients' QoL does adversely affected. They showed improvement in general health scale and social functional scale.

Main limitation of the current study is it does not involve all cases with rectal cancer and have a short follow up time. There is need for studies with longer follow up to better determine long term effects of LARS.

CONCLUSION

In conclusion, after low anterior resection for rectal cancer, patients' quality of life is positively affected after closure of temporary loop ileostomy.

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MEDICAL RECORDS-International Medical Journal

Research Article

Effects of Electromagnetic Field (1.8/0.9 GHz) Exposure on Spleen in Rats

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Abstract

Aim: To evaluate potential effects of whole-body 900 and 1800 MHz electromagnetic field (EMF) exposure on the rat spleen.
Material and Methods: The study was conducted on 9 Sprague–Dawley rats. Pregnant rats were assigned into 3 groups: 900 MHz EMF-exposure, 1800 MHz EMF-exposure and controls.
Results: Under light microscope, myeloid series cells, erythrocytes and megakaryocytes were observed in all groups. In the red pulp, dilated sinusoids were observed in both 900 and 1800 prenatal 24-hour groups with more prominent findings in the 1800 prenatal 24-hour group. Fused white pulps were apparent in 900 group while there was increase in the irregular white pulps (varying in size) with destruction in the1800 group. Biochemical evaluation showed that spleenmalondialdehyde level was higher while glutathione level waslower in the 900 MHz-exposure and 1800 MHz-exposuregroups compared to controls ($p<0.05$ for both).
Conclusion: Based on our results, it was concluded that EMF exposure at prenatal period led pathological changes in thespleenof pups. Again, it was revealed that it led oxidative stress through enhanced lipid oxidation and altered antioxidant defense systems. We also demonstrated that these effects were more evident as the level of EMF was increased from 900 MHz to 1800 MHz.

Keywords: Electromagnetic field, spleen, rat model, experimental animal models

INTRODUCTION

The electromagnetic (EM) wave is an area generated from a radiofrequency source which radiates in spacee. Electronic devices that we use frequenty and for a long period in our daily lives expose us to EMF. Today, the vast majority o mobile phones operates at 900 MHz and 1800 MHz in the GSM systems in Europe (1). The high- or low-power EM waves generated by electronic devices have deleterious effects on the human body. The high-frequency EM waves causemediated by heat while low-frequency EM waves createsharmful effects due to chemical changes in tissues (2). It is well-known that EMF exposure can produce biological and functional changes in cells through thermal and non-thermal effects on tissues (2-5). In the literature, it was shown thatEMF causes oxidative stress by increasing reactive oxygen species in tissues, causing DNA damage, protein folding defects and disruption Ca+2-dependent cell signaling due to increase in free radical and Ca+2 (2,6).

Immune system starts developing at the early embryogenesis and it proceeds with hematopoietic cell production, migration and differentiation at prenatal period. The early phases are sensitive to exogenous effects such as EMF. The spleen has a crucial role in the institution of immune responses against infection. Thus, exogenous factors which negatively affect thymus and spleen development can also influence on the immune system development. Although there is no definitive evidence that the electromagnetic field has harmful effects onin the researches about possible effects of EMF on the immune system, there are no comprehensive studies to rule out potential harm definitely (7). There is an increasing interest on potential deleterious effects of EMF on the immunee systems, which is promoted by reports on mobile phones used extensively and the carcinogenicity of EMF exposure (8).

The radiation-induced oxidative stress generates reactive oxygen species (ROS) which may result in injuries at

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tissue level (9). ROS plays a pivotal role in oxidative damage in lipids, DNA and proteins (10). They can trigger lipid peroxidation and cause alterations in reduced glutathione (GSH) and malondialdehyde (MDA) levels. MDA is produced during the premier chain reactions which promotepolyunsaturated fatty acids oxidation. Thus, it is a reliable marker of oxidative stress in tissues (11). On the other hand, GSH is a marker of antioxidant status (11).

The effects of EMF on several tissues were investigated; however, to best of our knowledge, there is no study on effects of EMF on the spleen, an important part of the immune system, in the literature. Thus, we aimed to evaluate the potential effects of whole-body 900- and 1800-MHz EMF on the spleenic tissues of rat in our study.

MATERIAL AND METHOD

Study Design

This study was conducted at Recep Tayyip Erdoğan University, Medicine School. The study was approved by Local Ethics Committee on Experimental Animals (approval: 2015/44-45). All procedures were performed in accordance to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

The study conducted on 9 female pregnant Sprague Dawley rats (weighing 280-300 grams) which were maintained on light-dark cycle of 12-hours at a steady environment of temperature (20-22°C) and humidity 50-55% relative humidity. The animals were fed ad libitum with free access to tap water. This experimental study was designed and conducted in accordance to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Pregnant rats were randomly assigned into 3 groups: sham-exposure (controls, n=3), 900 MHz EMF-exposure (n=3) and 1800 MHz EMF-exposure (n=3). No radiation was given to the control rats. Rats were subjected to continuous (24 hours) radiation for 20 days in 900 MHz EMF-exposure and 1800 MHz EMF-exposure groups.

Gestational Period

Female rats were housed in plastic cages (36 cm x 23 cm x 21 cm in size) as being 3 rats in each cage. In addition, a single male rat was put into all cages for one day in each cage for mating; the male rats were removed from cages thereafter. The vaginal secretions of the rats were collected by instilling 0.5 ml normal saline using a pipette. A vaginal smear was prepared from samples collected, which was examined under light microscope. The rat was considered to be conceived in the presence of sperm in microscopic examination (day 0 of gestation). The rats were regarded as be pregnant if vaginal membrane was observed during vaginal inspection and no microscopic evaluation was performed.

Following mating, radiation was applied to 6 pregnant rats (experimental group) using a digital signal generator. Remaining pregnant rats (control group) received no radiation.

Exposure System and SAR Calculation

In our study, rats in 900 MHz and 1800 MHz EMF-exposure groups were maintained separately in different experiment boxes. The control rats were isolated electromagnetically. In our study, 900 MHz EMF was generated from RF source identical to source used in the study by Koyu et al. (Everest GSM Simulator (Model: 900CW4, Türkiye). However, 1800 MHz EMF was generated by a distinct RF source (Everest GSM Simulator) (12). At exposure period, monopole antennas of exposure systems was mounted as close as possible (nearly 2 cm to the head) to the rat. In the experiment boxes (shielding effectiveness: appoximately100 dB at 1800 MHz), RF source radiation was measured by a spectrum analyzer (PROMAX, AE-566) using varying probes. In RF sources, there is a control switch in the front panel to adjust power output. In the exposure systems, the antenna power output values were maintained at a level mimicking effects of celullar and digital communication devices used in extensively in the society.

The newborn rats (n=8) without EMF were assigned into each group. The newborn rats were housed under above-mentioned conditions over two monts. On postnatal day 60, blood samples were drawn; in addition, liver was removed. Rats were sacrificied under anesthesia using intraperitoneal ketamine hydrochloride (50 mg/kg Ketalar®; Pfizer İlaçları Ltd. Şti., Istanbul, Türkiye) plus xylazine (10 mg/kg Rompun®; Bayer, USA). The spleens were also removed for histopathological examination.

Histopathological Analysis

Tissue samples were fixed in a 10% formaldehyde (Sigma – Aldrich Chemie, Steinheim, Germany) for 72 hours. Then, spleen tissue samples were gradually dehydrated using ethanol series of 50%, 70%, 80%, 90%, 96% and 100% (Merck GmbH, Darmstadt, Germany). The samples were placed intoxylol solution (Merck GmbH, Darmstadt, Germany) thereafter and subjected to mordanting process. Following mordanting process, the tisssue samples were embedded into paraffinblocks (Merck GmbH, Darmstadt, Germany). Then, sections (4-5 µm in thickness) were obtained and stained with Harris hematoxylin (Merck GmbH, Darmstadt, Germany) and eosin G (Merck GmbH, Darmstadt, Germany). The preparations were evaluated using a light microscope (Olympus CX51, Olympus Corporation, Tokyo, Japan) equipped with a digital camera (Olympus CX41, Olympus Corporation, Tokyo, Japan).

Biochemical Evaluation

The spleens removed were washed in cold phosphate buffered saline. In addition, liver samples obtained were weighed and underwent homogenization in phosphate buffered saline (pH 7.4) over 1 minute. Tissue weight: homogenization buffer ratio was 1:10. The homogenate obtained was centrifuged using following parameters: speed: 4500 rpm; duration: 20 min; temperature: 4°C.

MDA assays: The MDA level was quantified using the technique described by Draper and Hadley. Firstly, 10% trichloroacetic (0.5 mL) acid was added into liver tissue homogenate (0.1 mL), which then vortexed. After incubation at 94°C over 15 minutes, the mixture was cooled in cold water, and centrifuged using following parameters: speed: 3000 rpm; duration: 10 min. Then, 0.675% 2-thiobarbituric

acid (0.4 mL) was added to supernatatn (0.2 mL). After incubation at 94°C for 15 min, absorbance was measured at 532 nm by a spectrophotometer (13).

GSH assays: The GSH level was quantified by the Ellman method. The Na2HPO4 (0.5 mL) was added to liver tissue homogenate (0.125 mL); then, Ellman reagent was added to mixture. Absorbance was measured at 412 nm by a spectrophotometer (14).

Statistical Analysis

Statistical analyses were performed using the IBM SPSS version 21 (SPSS Inc., Chicago, IL, USA). Nonparametric data obtained as a result of semi-quantitative analyses were expressed as median, 25% and 75% interquartile ranges. After making the analyzes using non-parametric Kruskal Wallis test followed by a Tamhane T2 test using the differences between the groups, the numerical data of the groups were analyzed. All differences associated with a chance probability of 0.05 or less were considered statistically significant. Parametric data was calculated as mean standard deviation. Differences between the groups were tested using one-way analysis of variance (ANOVA) followed by a Tukey HSD test (p values<0.05 were regarded as significant).

RESULTS

Histopathological Analysis

Light microscopic evaluation of the spleen revealed myeloid series cells, erythrocytes and megakaryocytes in all groups. In the red pulp, it was seen that there wa dilated sinusoids in both 900 and 1800 prenatal 24-hour groups as being more severe in the 1800 prenatal 24-hour (Figure 1A, C). There was impairment on the central artery wall in both groups (Figure 1B, D). Fused white pulps were apparently seen in 900 prenatal 24-hour group and irregular white pulps (at varying siz) with destruction were increased in 1800 prenatal 24-hour group.

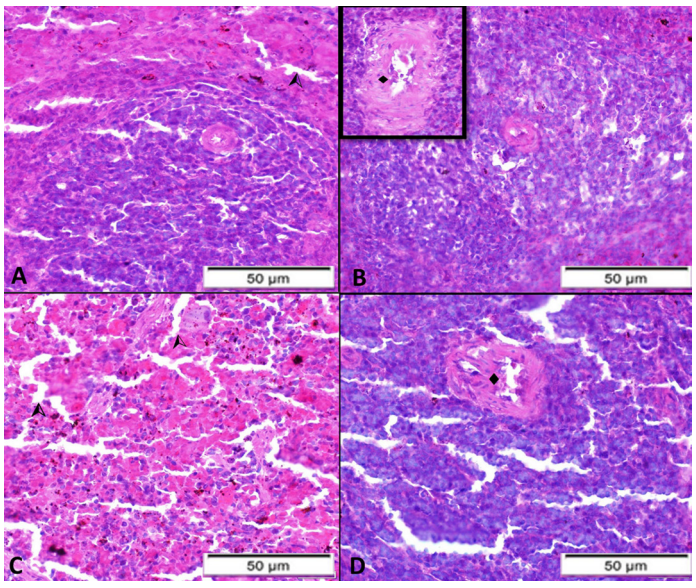

Figure 1. Light micrograph of the red and white pulps of the spleen sections in the 900 prenatal 24-hour group (1A, B), and in the 1800 prenatal 24 hour group (1C, D). Dilated sinusoids (×), are clearly seen. Inset (1B) demonstrate enlarged central artery with impaired arterial wall (®) (Hematoxylin and eosin, X50 µm)

Biochemical Results

In the biochemical evaluation, spleenic tissue MDA level wasfound to be higher in the 900 MHz EMF-exposure and 1800 MHz EMF-exposure groups than controls ($p<0.05$). Also, a significant difference was found in MDA levels in the 900MHz EMF-exposure group than those in 1800 MHz EMF-exposure group ($p<0.05$) (Table 1). The spleenic tissue GSH level was lower in the 900 MHz MF-exposure and 1800 MHz EMF-exposure groups than those in controls ($p<0.05$). Also, a significant difference was in GSH level in EMF900 group than EMF1800 group ($p<0.05$) (Table 1).

Table 1. Spleen tissue GSH and MDA levels in the control and EMF groups

	MDA (nmol/g protein)	GSH (nmol/g protein)
Control group	36.53 ± 0.68	8.60 ± 1.04
EMF900 group	41.04 ± 0.84	6.72 ± 0.43
EMF1800 group	48.54 ± 0.62	5.63 ± 0.64

EMF: electromagnetic field, MDA: malondialdehyde, GSH: glutathione
The results were given as mean±standard deviation

DISCUSSION

Although there are many studies about the prenatal effects of EMF on several tissues and organs of embryos and fetuses, our literature search revealed that there was no study on the splenic effects of EMF applied at 900-1800 MHz at prenatal period. Therefore, we aimed to evaluate whole-body 900- and 1800-MHz EMF exposure at prenatal period on the spleen inrats.

Specific absorption ratio-specific absorption rate (SAR) value has been determined and standards have been set in order to express the damages and heat effects of the radiation emitted by mobile phones and base stations in human tissues. Electromagnetic energy absorption, which increases the human body temperature by 1°C, is considered to be harmful to humans. This value is: 4 Watt/kg. One tenth of this value (0.4 W/kg) is considered as acceptable limit value for those exposed to electromagnetic fields because of their profession and 1/50 (0.08 W/kg) of this value is considered as acceptable limit value for general public exposure (15,16). In the literature studies, researchers report that biological functions change after 0.1 W/kg SAR value was delivered (17). It was reported that birth weights of pups were significantly decreased when pregnant Wistar albino rats were subjected to SAR value of 0.15 W/kg and emission of 890-915 MHz emission for 2 hours daily (18). In another developmental study, chicken embryos were exposed to mobile phone EMWs and mortality was calculated. It was reported that mortality rate was 70% in the EMW group vs. 16% in the controls (19). In our study, the rats were exposed to radio frequency up to 0.2 W/kg SAR value.

During prenatal period, exposure to environmental agents have significant effects on the thymus and spleen development and may threaten T cell-dependent responses in adults (20,21). Chagnaud and Veyret investigated

the effect of the GSM-tuned (900 MHz) microwave delivered 2 hours/day over tenconsecutive days, on the lymphocyte subgroups and normal mitogenic responses of Sprague-Dawley rats. Authors found no changes in the surface phenotypes or mitogenic activities of the spleen lymphocytes, suggesting no influence on immune system integrity (22). In the study on the effect of radiofrequency at GSM –tuned 900 MHz on mouse spleen cells, Gatta et al. found no change in total number of spleen cells, at B and T lymphocyte frequencies, and in CD69 and CD25 expression of T or B lymphocyte subgroups (23). Based on our histological results , dilated sinusoids in both 900 and 1800 prenatal 24-hour groups, being more severe in the 1800 prenatal 24-hour group were observed in the red pulp. Impairment on the central artery wall was evident in both groups. Fused white pulps were clearly observed in 900 group, and variable size of irregular white pulps with destruction were increased in 1800 group.

It is essential to keep oxidant-antioxidant systems in balance in the organism for maintaining health (24). Free radicals are endogeneous by products generated during the normal metabolic process. These radicals can interact with and cause injury in all types of cellular components (lipids, nucleic acidss and proteins in particular) as they reactive compounds. The increased free radical generation and/or failure of antioxidant defense system can result in xidative stress in living organism (25). MDA and GSH are well-known biomarkers for oxidative stress. MDA is produced during the premier chain reactions which promote polyunsaturated fatty acids oxidation. In their study, Esmekaya et al. reported elevation in MDA levels in heart, liver, testis and lung tissues resulting from 900 MHz EMF exposure (26). In the study by Aydin and Akar, effects of 900 MHz EMF on rat lymphoid organs, polymorphonuclear leukocytes and serum oxidative stress parameters were investigated. In agreement with our study, authors reported that MDA levels were significantly increased in spleen and thymus (27). Organisms have some antioxidant defense systems against oxidative injury resulting from formation of reactive oxygen species. By acting as a scavenger, GSH can remove ROS from organism and prevent oxidative damage resulting from ROS (28). In the present study, we found that spleen tissue MDA level was higher while GSH level was lower in EMF groups than in those in the controls. Also, there was significant differences were observed in MDA and GSH levels in the EMF900 group as compared with those in EMF1800 group.

There are major concerns about reliability of using animal models to investigate human toxicity; however, such concerns do not rely on the scientific grounds of translating results across species. Instead, they address soundpotentially correctable issues, such as technical competence in animal research, robustness of study design in animal experiments, and publication bias.

CONCLUSION

To best of our knowledge, this is the first study to investigate the effects of 900- and 1800-MHz EMF given at the prenatal period on the spleen. Based on the histological and biochemical results of the present study, it was concluded that EMF exposure at prenatal period led pathological changes in the spleenof pups. Again, it was revealed that it led oxidative stress through enhanced lipid oxidation and altered antioxidant defense systems. We also demonstrated that these effects become more evident as the level of EMF increases from 900 MHz to 1800-MHz.

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

Ethical approval: *This study was conducted at* Recep Tayyip Erdoğan *University, Medicine School. The study was approved by Local Ethics Committee on Experimental Animals (approval#: 2015/44-45). All procedures were performed in accordance to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.*

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MEDICAL RECORDS-International Medical Journal

Research Article

Anatomical Analysis of Foramen Magnum: A 3D Slicer CT Study

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Abstract

Aim: To analyze the anatomical structure of foramen magnum in healthy individuals according to age/gender.

Material and Methods: Cranial Computed Tomography (CT) images of 130 healthy individuals (60 female, 70 male) the 18-84 age were included in our study. Using the 3D Slicer software package on these images, the Anterior-Posterior Diameter (APD), Transverse Diameter (TD), perimeter, area, angle between the posterior edge of the foramen magnum and the clivus (FMC) value, and index were found. The shapes of the foramen magnum were analysed.

Results: There was no statistical significance between age, FMC, and foramen magnum index values in both genders ($p>0.05$). APD, TD, perimeter, and area values of males were statistically higher ($p<0.05$). The average foramen magnum index was 85.61±6.5 in the whole group, 84.94±7.04 in males, and 86.29±5.67 in females. There was no statistical relationship between age and measurement parameters in the whole group ($p>0.05$). Seven shapes of foramen magnum have been seen. The most common was the oval shape (27.7%) and the least common was the pentagon (1.5%).

Conclusion: Knowing the anatomical structure of the foramen magnum is important in terms of identity and ethnicity. We also think that our results may contribute to the surgical treatment of the foramen magnum and adjacent structures.

Keywords: Computed tomography, gender, foramen magnum, morphology, morphometry.

INTRODUCTION

Foramen magnum is a part of the occipital bone and is the largest opening in the lower part of the skull. Anatomically, it is located in the posterior cranial fossa and is surrounded by parts of the occipital bone (1). Although it is defined as oval-shaped, there is information in the literature that it has different shapes and sizes. It can be in different shapes such as ellipse, egg, round, hexagonal, pentagonal, tetragonal, and irregular (2-4). Foramen magnum is adjacent to structures at both ectocranial and endocranial borders (5). These structures are the spinal cord, meninges, accessory spinal nerve, dural sinuses, tectorial membrane, alar ligament, vertebral, and spinal arteries (1,6). For this reason, the foramen magnum is important for clinicians regarding hemodynamic, hydrodynamic, and locomotor function (5). In addition, the morphological and morphometric structure of human bones is used in identification in forensic medicine and archaeological studies (7). It is reported that there is an accuracy rate of around 90% in identification studies conducted on skull bones (8).

Foramen magnum may follow a course associated with brain development. It has been reported that diseases such as Chiari malformation and Syringomyelia show changes in bone structure (9,10).

Previous studies reported measurements from skull bones (2,6). In recent years, Computerised Tomography (CT) has been used for identification, especially in forensic cases (11).

Morphometric analysis of the area, circumference, anteroposterior diameter (APD), and transverse diameter (TD) of the foramen magnum was examined with different methods. In recent years, with the development of the field of computer science and bioinformatics, there are many new software tools. One of these software is 3D Slicer. 3D Slicer is free and open access and can make automatic calculations using medical image data of patients (12).

Our aim in this study is to examine the anatomical structure of the foramen magnum according to age/gender using the 3D Slicer software tool.

CITATION

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MATERIAL AND METHOD

Cranial CT images of 130 healthy individuals (60 female, 70 male) of the 18-84 age were included in our study between January 2023 and May 2023. Individuals who had not undergone cranial surgery had no traumatic pathology at the craniocervical junction, or had no congenital or traumatic craniofacial deformity were included in the study.

The approval of the Amasya University Non-Interventional Clinical Research Ethics Committee (Date: 13.06.2023, Approval Decision No: 2023/103) was obtained.

GE Healthcare Revolution EVO CT (multi-detector CT, 128 slices) device was used in the images. Cranial CT images with a section thickness of 0.625 mm were used. In our study, CT data were recorded in the DICOM format using the PACS system. Images were analysed with 3D Slicer version 5.2.1 (https://www.slicer.org/) (12).

In this study, the APD, TD, perimeter, area, and angle between the posterior edge of the foramen magnum and clivus (FMC) value were examined with the "Markups" tool in the 3D Slicer program. In addition, the index value was formulated and its anatomical shapes were examined (Figures 1,2 and 3). It was calculated as foramen magnum index (TD/APD)*100.

Classification of foramen magnum index values was made. Small: a–81.9, medium: 82.0–85.9, large: 86.0–a (13).

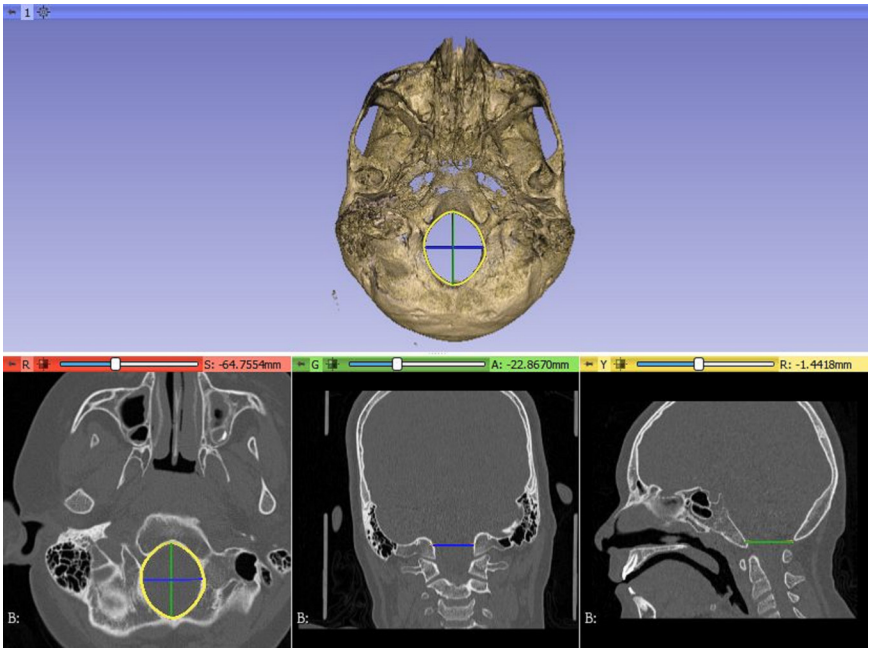

Figure 1. Axial, coronal and sagittal view of foramen magnum measurement with 3D Slicer (Green line----APD, blue line-----TD, yellow line----- Perimeter)

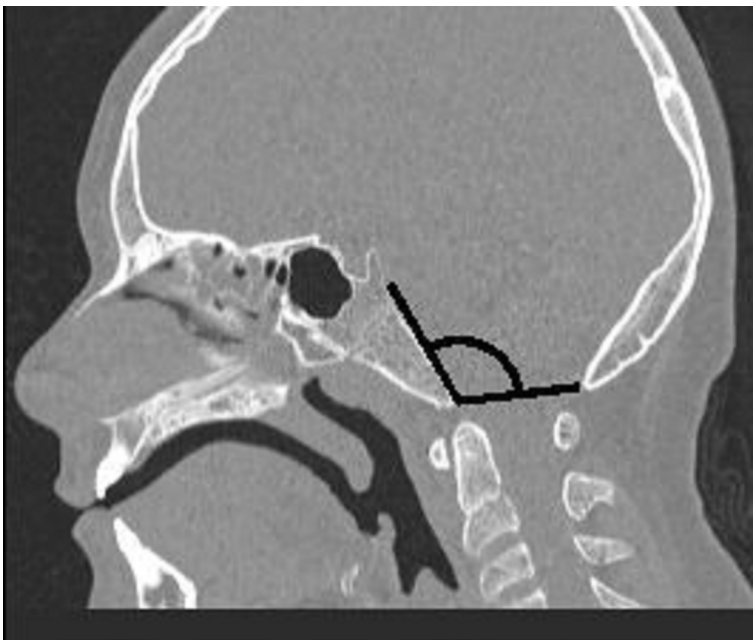

Figure 2. Angle between the posterior edge of the foramen magnum and the clivus (FMC)

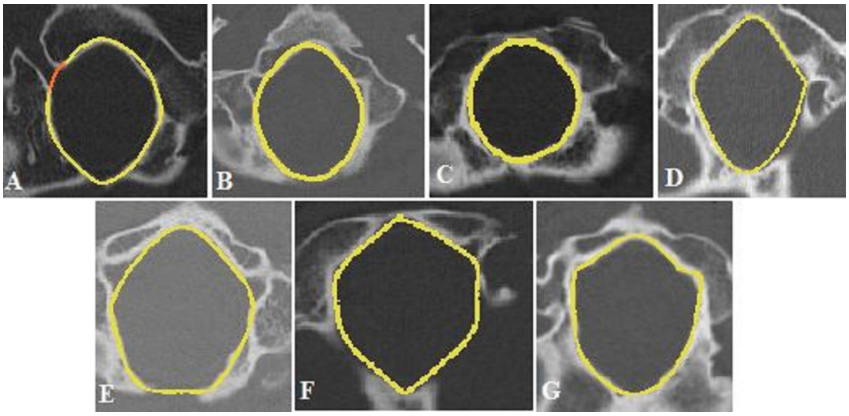

Figure 2. Angle between the posterior edge of the foramen magnum and the clivus (FMC)

Statistical Analysis

Data were analyzed in IBM SPSS V26 software. Descriptive statistics; It is expressed as several units (n), percentage (%), mean ± standard deviation, median and interquartile distance values. Data were evaluated with the Shapiro-Wilk normality test. The homogeneity of variance of the groups was analysed with the Levene test. Numerical variables by gender were performed by independent samples t-test or Mann-Whitney U test. The relationships between age and other numerical variables were evaluated with the Spearman correlation coefficient (14). The significance value was considered as statistical p<0.05.

There was no statistical difference between age, FMC and foramen magnum index values in both genders (p>0.05).

Table 1. Comparisons by gender

Variables	Sex		Test statistics	
	Male n=70	Female n=60	Test value	p value
Age	28.0 (27.0)	24.0 (31.5)	0.781	0.435‡
TD (mm)	31.77±2.30	29.84±2.08	4.977	<0.001†
APD (mm)	37.54±2.83	34.65±2.25	6.490	<0.001†
Perimeter (mm)	109.29±7.50	100.57±7.30	6.697	<0.001†
Area (cm²)	8.90±1.17	7.43±0.93	7.851	<0.001†
FMC	123.13±6.20	123.45±7.35	0.266	0.790†
Foramen Magnum Index	84.94±7.04	86.29±5.67	1.189	0.237†

Data are given as mean±standard deviation or median (interquartile) distance. †: Independent samples t test. ‡: Mann-Whitney U test.

Table 2: Correlations between age and other variables

Variables	Whole Group		Male		Female	
	rho	p	rho	p	rho	p
TD (mm)	-0.009	0.918	-0.057	0.637	-0.054	0.680
APD (mm)	-0.114	0.195	**-0.338**	**0.004**	-0.058	0.662
Perimeter (mm)	-0.082	0.352	**-0.274**	**0.022**	-0.042	0.750
Area (cm²)	-0.088	0.319	**-0.239**	**0.046**	-0.123	0.348
FMC	0.167	0.057	0.147	0.223	0.182	0.164
Foramen Magnum Index	0.164	0.062	**0.257**	**0.032**	0.055	0.676

TD: transvers diameter, APD: anterior posterior diameter, FMC: angle between the posterior edge of the foramen magnum and the clivus, rho: Spearman correlation coefficient

RESULTS

There was no statistical difference between age, FMC, and foramen magnum index values in both genders (p>0.05) (Table 1). The average foramen magnum index was found to be 85.61±6.5 (medium) in the whole group, 84.94±7.04 (medium) in males, and 86.29±5.67 (large) in females. The APD, TD, perimeter, and area values of males were higher (p<0.05). No significant relationship was found when age and other variables were compared throughout the group. Weak negative correlation between age and APD, perimeter and area values of males; there is a weak positive correlation with the foramen magnum index values. No relationship was found between age and measurement parameters in females (p>0.05) (Table 2).

DISCUSSION

Skull bones are important for forensic science and anthropologists in gender determination or ethnicity research. Because it has been reported that the skull bones show an accuracy of 90% in sex determination (15-17). Many studies have been conducted on the foramen magnum. Our aim in this study is to examine the anatomical structure and index value of the foramen magnum according to age/gender using the 3D Slicer software tool. The morphology and morphometry of skull bones have been examined in many studies (2,6). In addition, there are studies on CT images (18,19).

Chethan et al. (2) examined the morphometry of the foramen magnum in 53 dry bones. The average APD value was reported as 31±2.4 mm and the TD value as 25.2±2.4 mm. Another study analysed the foramen magnum on 77 dry bones of the Brazilian population aged 18 and over. The study reported the average APD value as 34.23 mm and TD as 28.62 mm. Additionally, the average index value was reported as 83.75 mm (20). The results differed from our study. We think that the reason for these differences may be due to sample size, measurement technique and the fact that the studies were conducted on dry bones.

Govsa et al. (6) study of 352 dry bones, the mean APD value was 37.2 mm, the TD value was 30.8 mm, and the foramen magnum index was 84.02 mm. Although our study was not conducted on dry bones, it is similar to the results obtained (APD: 36.2 mm; TD: 30.8 mm; foramen magnum index: 85.5). Vinutha et al. (18) study, the foramen magnum index value was reported as medium in both genders. Our study determined it as a medium in males and large in females. Differences in results may be related to ethnicity.

Meral et al. (21) reported in their study the APD, TD, area, and index values from the CT images of 600 people (300 males, 300 females) aged between 21 and 50. They examined the study according to age groups and genders, five years in each group. It has been reported that there is no difference between age groups and foramen magnum measurements. In addition, males were found to be higher than females in all measurements, and this difference was reported to be statistically significant. Aljarrah et al. (4) They examined 472 CT images (236 males, 236 females) in the Saudi Arabian population aged 18-72. It has been reported that there are significant differences when compared by gender. In their CT study, Tellioğlu et al. (22) analysed APD, TD, and perimeter and area measurements of 100 individuals (50 males, 50 females). Significant differences between genders were reported in all variables. It was also stated that males had a higher average. Meral et al. (21), Aljarrah et al. (4) and Tellioglu et al. (22) results are compatible with our study.

Botelho et al. (23) reported the FMC value of the control group as 126±9.4°, and Ferreira et al. (24) reported the same angle in the control group as 126.20±9.6° in their study. The findings are consistent with our results. Sun et al. (19) study, the average FMC value was reported as 153.46±9.1° in females and 149.93±8.6° in males. A significant difference was reported in both genders ($p<0.001$). It was reported to be significant according to the age variable. In our study, the FMC value was high in females. However, no difference was found between age and gender. The findings were higher than our results. Differences in results may be the sample size and the structural differences. We believe that the differences in the results may be important in terms of surgery.

It is known in the literature that the foramen magnum varies morphologically. This may cause differences in structures at the craniovertebral junction (25). In our study, oval-shaped foramen magnum was the most common type with 27.7%. Pires et al. (20) found oval-shaped foramen magnum in a dry bone study with a rate of 53.24%. Our results were lower than in this study (Table 3). Taib et al. (26) reported an oval-shaped foramen magnum at a rate of 14%, but this rate was lower than our results. Differences in results may be related to ethnic origin. Kum et al. (3) analysed 314 CT images in their study. They found the most common oval-shaped (39.09%) and the most rare egg-shaped (1.59%) foramen magnum.

Table 3. Frequency of different morphological shapes of foramen magnum

Types of shapes	Female		Male		Whole group	
	n	%	n	%	n	%
Oval	20	33.3	16	22.9	36	27.7
Egg	4	6.7	4	5.7	8	6.2
Round	2	3.3	11	15.7	13	10.0
Tetragonal	10	16.7	13	18.6	23	17.7
Pentagonal	1	1.7	1	1.4	2	1.5
Hexagonal	16	26.7	14	20.0	30	23.1
Ireegular	7	11.7	11	15.7	18	13.8
Total	60	100.0	70	100.0	130	100.0

In another CT study, the frequency of appearance of foramen magnum shapes was reported as follows: hexagonal (30.72%), irregular A (20.34%), oval (15.25%), irregular B (13.14%), round (7.42%), egg (5.72%), pentagonal (4.24%) and tetragonal (3.18%) (4). Chethan et al. (2) reported that round shape was the first and pentagonal shape was the last in terms of frequency. The results differ from our current study. Differences in results may be related to sample size.

Limitations of our study; this may be due to the fact that it is single-centered, retrospective and the number of samples is limited.

Foramen magnum is very important due to its location. While it may vary from person to person, differences in ethnicity may also affect bone structures. Therefore, we think that the anatomy of the foramen magnum will be important for clinicians and anthropologists.

CONCLUSION

In our study, the anatomical structure of the foramen magnum of a healthy individual was examined according

to age/gender variable, and the differences were recorded. Foramen magnum index was in the medium category in the entire group, medium in males, and large in females. Males' APD, TD, perimeter, and area values were statistically higher. It was determined that males showed a weak negative correlation between age and APD, perimeter, and area values in all parameters except the foramen magnum index. When age and other parameters were compared in females, the results were not significant.

We believe that measurements and analysis of the foramen magnum will contribute to clinical diagnosis and treatment.

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Research Article

Short-Term Amyloid Beta Application Decreased Glutamate Release, but Increased Glutamate Spillover in Hippocampal Neurons

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Abstract

Aim: Synaptic dysfunction is a characteristic linked with the early stages of Alzheimer's disease (AD), but the pathological mechanisms remain elusive. It was aimed to investigate how amyloid beta 42 (Abeta42) peptide affects miniature events mediated by glutamate release in hippocampal neurons.

Material and Methods: We performed all experiments in the primary cultured hippocampal neurons in control and Abeta42-treated neurons (24 h). Pharmacologically isolated miniature excitatory postsynaptic currents (mEPSCs) were obtained in whole-cell voltage-clamp configuration at – 70 mV. AMPAergic channel conductance and basic synaptic parameters were evaluated by performing peak-scaled variance analysis and cumulative event analysis and glutamate spillover is determined by application of DNQX.

Results: The oligomeric Abeta42 for 24h decreased the mEPSCs frequency (***p<0.001), while it has no any measurable effect on the amplitude of mEPSCs as well as unitary current and number of receptors. In addition, the incubation of neurons with oligomeric Abeta42 for 24h increased the glutamate spillover measured as baseline shift (***p<0.001).

Conclusion: The oligomeric form of the Abeta42 peptide has a significant effect on the presynaptic site of excitatory synapses in primary cultured hippocampal neurons. It lowers the release probability during short-term incubation, while it increases glutamate spillover.

Keywords: AMPAergic synapses, amyloid beta peptide, Alzheimer's disease, glutamate spillover

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that predominantly affects the elderly population and is characterized by a gradual decline in cognitive function, memory loss, and behavioural changes (1). One of the key pathological hallmarks of Alzheimer's disease is the accumulation of abnormal protein aggregates, including amyloid beta peptide and tau tangles, within the brain (2-4). While these proteinopathies have long been central to understanding the disease, emerging research has highlighted the critical role of the amyloid beta 42 (Abeta42) peptide on synaptic dysfunction and thus the development and progression of AD, since the amyloid beta accumulation precedes the hyperphosphorylated tau tangles (5,6).

Synapses, the specialized connections between neurons, play a fundamental role in transmitting and processing information in the brain. Proper synaptic function is essential for various cognitive processes, including learning, memory, and information processing. However, in AD, synaptic dysfunction emerges as a pivotal contributor to the cognitive decline observed in patients and animal models (7,8). The disruption of synaptic connections and neurotransmitter imbalances impair neural communication, leading to the cognitive impairments associated with the disease.

In this study, we aimed to determine the relationship between the early pathology of AD and excitatory synaptic dysfunction, exploring how the accumulation of Abeta42 peptides contribute to synaptic alterations, specifically excitatory synapses mediated by AMPA receptors. By understanding the cellular mechanisms underlying these possible synaptic alterations, we also aimed to

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uncover potential therapeutic targets that could mitigate cognitive decline and offer new avenues for managing this devastating neurological disorder.

MATERIAL AND METHOD

Cell Culture

To harvest tissues, animals were humanely euthanized by gradual exposure to increasing levels of CO_2 followed by swift cervical dislocation. The 18-day-old embryos of C57BL6 mice (Envigo, San Pietro al Natisone, Italy) were used to obtain hippocampal neurons. They were promptly retrieved through caesarean section and swiftly decapitated before extraction. Under sterile conditions, the rapidly dissected hippocampus was placed in cold high-glucose HBSS (4°C), and then subjected to digestion using papain (0.5 mg/mL) supplemented with DNAse (0.1 mg/mL) (9). Subsequently, the isolated neurons were plated at a density of 100 cells/mm² and cultivated in a medium composed of Neurobasal B-27 (at a 1:50 v/v ratio), 1% w/v glutamine, and 1% penicillin-streptomycin. A semi-weekly half-volume medium exchange was conducted over a span of 2-3 weeks.

Solutions and Drugs

To record miniature excitatory postsynaptic currents (mEPSCs), the extracellular solution employed was Tyrode's standard solution, comprising the following concentrations (in mM): 2 $CaCl_2$, 130 NaCl, 2 $MgCl_2$, 10 HEPES, 4 KCl, 10 glucose, with a pH of 7.4. The internal solution was composed of the following concentrations (in mM): 135 gluconic acid (potassium salt: K-gluconate), 5 NaCl, 2 $MgCl_2$, 10 HEPES, 0.5 EGTA, 2 ATP-Tris, and 0.4 Tris-GTP. To inhibit NMDA, GABAA, and GABAB receptors, respectively, d-(−)-2-Amino-5-phosphonopentanoic acid (d-AP5; 50 µM, Sigma-Aldrich), picrotoxin (100 µM, Sigma-Aldrich), and CGP58845 (5 µM, Tocris) were used. To prevent spontaneous action potential propagation, 300 nM tetrodotoxin was added into the external solution. 6,7-dinitroquinoxalone-2,3-dione (DNQX, 20 µM) was used to evaluate glutamate spillovers. Abeta42 obtained from Sigma-Aldrich was solubilized in a solution of dimethyl sulfoxide (DMSO) and then stored at a temperature of -20°C, a concentration of 1mM. As previously described (10), the hippocampal cultures were exposed to Abeta42 (1 µM) for 24 hours before the performing of the electrophysiological experiments.

Patch Clamp Experiments

Borosilicate glass (Hilgenberg, Mansfield, Germany) were used to obtain patch electrodes (3 to 5 MΩ). All measurements were executed in the whole-cell configuration. The mEPSCs were recorded from 22-day in vitro (22 DIV) primary cultured hippocampal neurons (22-24°C) at −70 mV (Vh). The mEPSCs were recorded at a sampling rate of 10 kHz (Bessel filter set at 1 kHz). The analysis of frequency and amplitude of mEPSCs was performed with Clampfit Software (Molecular devices). Mini Analysis program (Synaptosoft, Leonia, NJ, USA) was used to noise analysis.

Non-stationary noise analysis of mEPSCs was conducted, taking into account the parabolic relationship between the variance (σ^2) of current and the mEPSC amplitude (11). In order to separate current fluctuations attributed to the stochastic characteristics of channel gating from those resulting from variations in neurotransmitter release and the quantity of postsynaptic receptors, the average waveform derived from the mEPSCs was normalized to the peak of each individual mEPSC. The unitary current was estimated by fitting the relationship between peak-scaled variance $\sigma^2(t)$ and the mean amplitude I(t) with the following equation: $\sigma\ 2(t)=iI(t)-(I^2(t)/Nch)+\sigma B^2$, where i is the weighted mean unitary current and Nch is the number of channels activated by a signle quantum of vesicle release.

Glutamate spillover was estimated by application of AMPA receptor antagonist, 6,7-dinitroquinoxalone-2,3-dione (DNQX, 20 µM), and measured as baseline shift.

Statistical Analysis

The data are presented as mean±S.E.M. for a total of n cells. The normal distribution of data was evaluated using the D'Agostino Pearson's normality test. Unless otherwise indicated, the statistical significance has been assessed by considering two sample groups that are normally distributed, and the unpaired Student's t-test was applied. In instances where the two sample groups were not normally distributed, we employed the non-parametric Kolmogorov-Smirnov test. Data were considered statistically significant when $p<0.05$.

RESULTS

To investigate the presynaptic and postsynaptic effects induced by Abeta42 in AMPAergic synapses, we firstly focused on the miniature excitatory postsynaptic currents (mEPSCs) in primary cultured hippocampal neurons in in vitro day (DIV) of 22-23 in control and neurons of pretreated with the Abeta42 for 24 h (Figure 1a for WT and Abeta42, respectively).

With 24 h-incubation of neurons with oligomeric Abeta42, it was significantly rightward shifted the inter-event intervals (IEIs) of mEPSCs (Figure 1b) (***$p<0.0001$, Kolmogorov-Smirnov test), but has no any measurable effect on the amplitude of mEPSCs (Figure 1c). In fact, the mean of mEPSCs IEI (Figure 1b, inset bar graph) increased from 289.7±45.08 ms in ctrl to 478.0±48.96 ms in Abeta42-treated neurons for 24 h (n=9 cells for ctrl and Abeta42-treated neurons), whereas it has no any measurable effects on the mEPSCs amplitude (Figure 1c, inset bar graph) (15.86±0.64 pA in ctrl and 17.29±0.56 pA in Abeta42-treated neurons for 24 h (n=10 cells for ctrl and Abeta42-treated neurons) ($p>0.05$). We then evaluated how Abeta42 affects the number of AMPA receptors (AMPARs) and its single channel current through peak-scaled variance analysis (11). We found that the parabolic relationship between variance (σ^2) and mean mEPSCs amplitude were not affected by the Abeta42 (Figure 2a and b), observing unchanged steepness and width of parabolic fit. With

the result of the unchanged steepness and its width, it is possible say that the Abeta42 does not have any effect on the unitary current of AMPARs (1.14±0.09 pA in ctrl and 1.23±0.07 pA in Abeta42-treated neurons for 24 h) (n=10 cells for ctrl and Abeta42-treated neurons, p>0.05, Unpaired Student's t-test) (Figure 2c) and the number of AMPARs which is activated by one vesicle release (13.81±0.96 in ctrl to 15.36±1.25 in Abeta42-treated neurons) (n=10 cells for ctrl and Abeta42-treated neurons, p>0.05, Unpaired Student's t-test) (Figure 2d), respectively. These results imply that oligomeric Abeta42 has an initial impact only on the presynaptic site by reducing release probability, without affecting postsynaptic site of excitatory neurons in primary cultured hippocampal neurons.

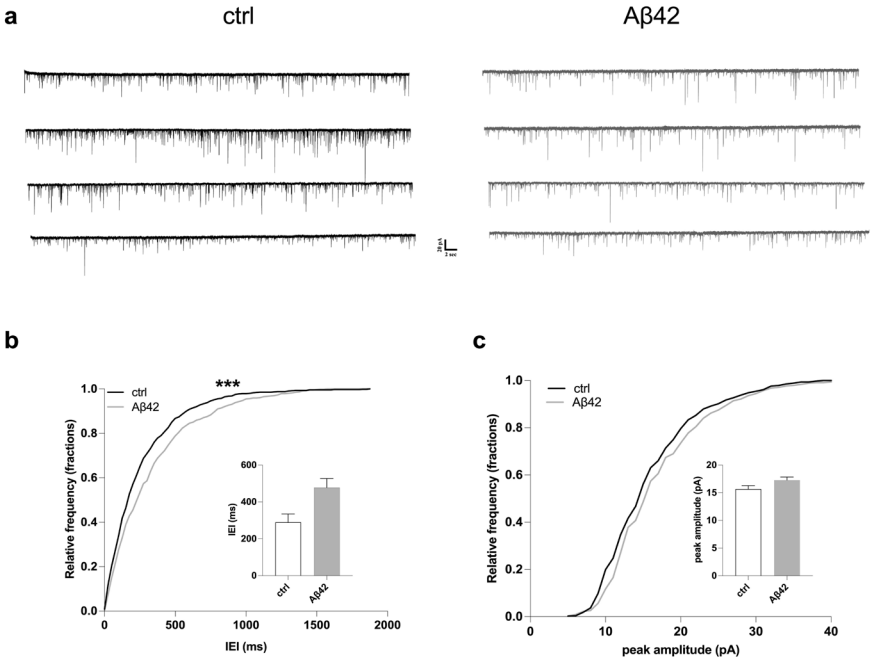

Figure 1. Abeta42 lowers the frequency of AMPAergic mEPSCs in the hippocampal neurons, **1a.** representative mEPSCs measured from four different cells in ctrl (left, black) and in Abeta42 treated neurons (right, grey), **1b.** and **1c.** cumulative distribution of mEPSCs inter-event interval (IEIs) and amplitude measured in ctrl (black) and Abeta42-treated neurons (grey) (***p<0.001). Inset bar graphs demonstrate the mean IEI and amplitude of mEPSCs in ctrl (black) and in the Abeta42-treated neurons (grey)

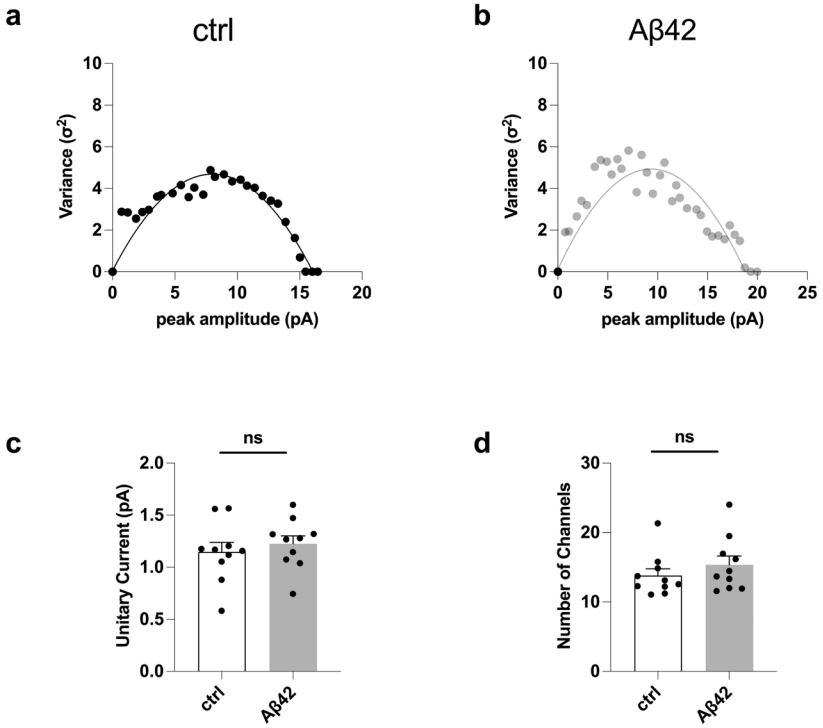

Figure 2. Abeta42 does not have any effect on the mean unitary current and number of receptors. a and b, parabolic fit of mEPSC variance (σ2) as a function of their amplitude in ctrl **2a.** and Abeta42-treated neurons **2b.** c and d, the parabolic fit provides information about unitary current **2c.** and number of AMPARs **2d.** (p>0.05)

Lastly, we investigated glutamate spillover by application of 20 μM DNQX (Figure 3a, ctrl and Abeta42-treated neurons, respectively). The results showed that oligomeric Abeta42 incubation for 24h induced a significant increase in the tonic excitation mediated by glutamate spillover (9.90±2.13 pA in ctrl vs. 24.29±4.66 pA in Abeta42-treated neurons) (n=10 cells for ctrl and Abeta42-treated neurons, P>0.05, Unpaired Student's t-test) (Figure 3b). This result indicated that oligomeric Abeta42 peptide has an early effect on the glutamate reuptake mechanism.

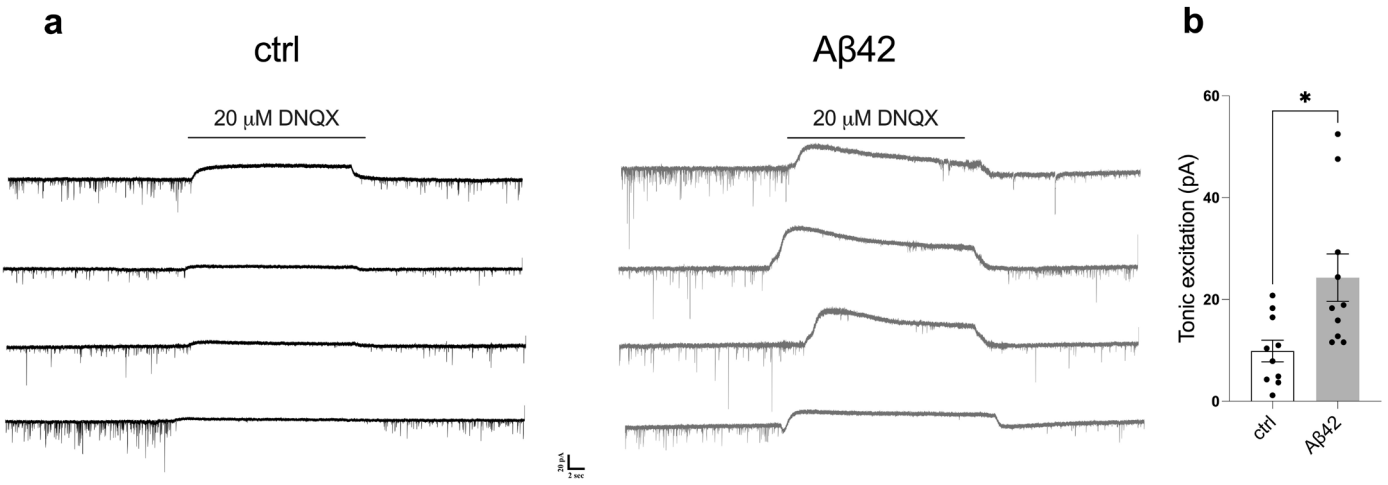

Figure 3. Abeta42 induces an increase in tonic excitation, indicating an augmentation in glutamate spillover, **3a.** representative mEPSCs recorded from four different cells in ctrl (left, black) and Abeta42-treated neurons (right, grey) with 20 μM DNQX, **3b.** bar graph summarizing the average tonic excitation measured by application of 20 μM DNQX (*p<0.05)

DISCUSSION

The hippocampus is a critical brain region located in the temporal lobe and composed of diverse array of neurons, including both excitatory and inhibitory neurons, which plays a pivotal role in the formation, consolidation and retrieval of memories as well as spatial navigation. In AD, the hippocampus is among the earliest and most severely affected brain regions and hippocampal overexcitability is observed during early stages of AD pathology. The underlying cause of this hyperexcitability is thought to be due to pathological changes in the glutamatergic system and reducing neuronal excitability might provide a potential therapeutic avenue for AD, but the exact mechanisms still remain unclear.

Previous studies suggest that accumulation of Abeta peptide can modulate the efficacy of excitatory neurotransmission by affecting the availability of synaptic vesicles, neurotransmitter release probability, and the function of postsynaptic receptors. These alterations may contribute to aberrant synaptic signaling and potentially contribute to cognitive impairments observed in AD. While the alterations in the excitatory system caused by the accumulation of Abeta are undoubtedly established, conflicting findings have emerged from various studies-likely attributed to differences in experimental models, time duration of incubation and oligomerization process of Abeta peptide. In hippocampal neurons, it has been observed that the release probability is significantly decreased following acute application of or incubation of neurons with Abeta42 peptide (<1μM) for 24 hours. For example, He. et al. (2019) have reported that both the frequency and the amplitude of mEPSCs are significantly decreased following application of 400 nM Abeta42 in the hippocampal neurons, suggesting reduced release probability. They also provided evidence that this type of reduction observed in release probability is mainly due to the presynaptically phosphatidylionositol-4-5-biphosphate (PIP2) depletion in axons (12). On the other hand, the hippocampal neurons, exposed for 24 h to various concentrations (from 5 nM to 10 μM) of Abeta, have exhibited different effects on the excitatory synaptic transmission in cultured hippocampal neurons. The high concentrations of Abeta, ranging from 50 nM to 1 μM, exhibited a reduction in the frequency of mEPSCs. However, this effect was absent when Abeta was present in low concentration (5 nM), indicating no influence on synaptic transmission. A separate investigation in this study noted that distinct variations of Abeta (monomers and fibrils) did not yield any significant effects on excitatory synaptic transmission (13). Our results are partly consistent with these studies except of the effects of Abeta42 on the amplitude of mEPSCs. In our study, we demonstrated a significant decrease in the frequency of mEPSCs following a 24-hour incubation of hippocampal neurons with oligomeric form of Abeta42 peptide, whereas no observable changes were detected in the amplitudes of these events. In addition, we did not observe any significant effects of oligomeric Abeta42 peptide on the single channel conductance and the number of AMPARs. These outcomes suggest that, under in vitro conditions, the primary influence of the oligomeric Abeta42 peptide pertains to the presynaptic site, without affecting the postsynaptic site.

Nevertheless, these findings appear incongruent with the increased neuronal activity reported in existing literature (14-16). One potential explanation for these contradictory results is that many studies reporting hyperexcitability involved prolonged Abeta exposure, disregarding its earliest effect, which entails a decline in glutamate release. The effects induced by the application of Abeta42 peptide for 48 hours were also comparable in excitatory synapses mediated by NMDA receptors and the authors have reported that Abeta42 activates synaptic responses mediated by NMDA through intracellular calcium release from internal stores through ryanodine receptors (RyRs) in the the early stages of AD (17). However, in this study, we observed that tonic excitation was increased by application of oligomeric form of Abeta42, suggesting that glutamate reuptake rate is decreased in early stages of amyloid pathology. This results with the overaccumulation of glutamate in synaptic cleft, inducing hyperexcitability in excitatory neurons. Considering all these findings, it is reasonable to conclude that the accumulation of Abeta42 peptide primarily affects presynaptic site by reducing glutamate release as well as reuptake rate in excitatory synapses. The limitation of this study is that glutamate reuptake mechanisms were not studied in detail. Therefore, in future studies, it is necessary to investigate the glutamate reuptake mechanisms and to what extent they are affected by Abeta pathology.

CONCLUSION

In conclusion, we here demonstrated that the oligomeric form of the Abeta42 peptide elicits presynaptic effects, leading to a reduction in glutamate release and it also disrupts glutamate reuptake mechanism on excitatory synapses in primary cultured hippocampal neurons. These findings highlight that a mechanism that can regulate the glutamate release and reuptake mechanism observed in the early stages could be an effective treatment method for AD pathology.

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

Ethical approval: *All experimental protocols received ethical approval from the University of Turin Animal Care and Use Committee in Turin, Italy and adhered to the National Guide for the Care and Use of Laboratory Animals as outlined by the Italian Ministry of Health (Authorization 695/2020-PR). The animals were provided with unrestricted access to water and food within their shelter. Every possible measure was taken to mitigate animal distress and limit the utilization of animals.*

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MEDICAL RECORDS-International Medical Journal

Research Article

The Consequences of Fasting During Pregnancy on the Thiole/ Disulfide Balance: An Observational Study

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Abstract

Aim: The objective of this research was to examine the impact of fasting on thiol-disulfide hemostasis in a population of healthy pregnant women.

Material and Methods: The study, conducted during Ramadan in 2021, included a control group of 53 pregnant women who were fasting and 57 pregnant women with similar demographics who were not fasting. The disulfide, native thiol, and total thiol concentrations in centrifuged blood plasma and venous blood plasma were all looked at. The ratios "disulphide/thiol 100," "disulphide/total thiol 100," and "thiol/total thiol 100" were all calculated.

Results: There were 32 pregnant women in the second trimester and 21 pregnant women in the third trimester among fasting participants, whereas there were 33 pregnant women in the second trimester and 24 pregnant women in the third trimester among non-fasting participants. Disulfide, disulfide/nativethiol*100, disulfide/totalthiol*100, and native/totalthiol*100 ratios were not significantly different between groups. A statistically significant difference was seen between the groups when comparing the mean values of native thiol and total thiol.

Conclusion: Our research is one of the first to examine homeostasis in pregnant women who were fasting, making it a remarkable advance in the field. The study demonstrated a discernible alteration in the thiol-disulfide balance, enhancing the process of oxidation. Fasting during pregnancy is associated with an increase in oxidative stress.

Keywords: Fasting, oxidative stress, pregnant women, ramadan, thiol-disulfide homeostasis

INTRODUCTION

Fasting is when you stop eating solid food, drinking drinks with calories, smoking, and using stimulants like coffee or tea on purpose for a certain amount of time. It typically involves some form of calorie restriction (1). During Ramadan, Muslims do a special kind of fasting, which means they can't eat, drink, smoke, or have sexual relations between dawn and dusk (1). The length of the fasting period is determined by the season in which Ramadan falls, and it lasts from about an hour before sunrise to sundown. For instance, fasting may last longer than 17 hours in several regions of the world during the summer (2,3).

Women are allowed to skip Ramadan fasts if they like, so long as they make up the days they miss after giving birth. However, most women would rather fast with their families throughout the month of Ramadan than make up the days later on their own. Hence, the practice of fasting throughout pregnancy occurs frequently among Muslim women (4,5).

The daytime rituals, lifestyle, and biorhythm of someone who is fasting change significantly throughout the month of Ramadan. The diurnal patterns of hormones, nutrient flow, and energy utilization may experience significant modifications during the period of fasting seen during Ramadan. These abnormalities might potentially lead to oxidative stress. "Oxidative stress" is described as a discrepancy between a cell's ability to produce reactive

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oxygen species (ROS) and its ability to do so while also protecting itself against oxidative damage (6).

There is a widely acknowledged consensus that pregnancy leads to an elevation in oxidative stress, which arises from a conventional systemic inflammatory response and heightened amounts of circulating (ROS). The placenta, which is the primary regulator of pregnancy, serves as the principal generator of ROS during this period (6-8). Accordingly, the elevated oxidative stress observed during pregnancy may result in possible tissue damage (6,8).

Numerous studies have looked at how Ramadan fasting affects inflammatory agents and oxidative damage using a variety of redox markers (9-16).

The cellular enzymatic and nonenzymatic processes that maintain redox equilibrium are essential for development and survival (17). However, conventional methods of measuring oxidative stress present a number of practical challenges. In the year 2014, Erel and Neselioglu introduced an innovative methodology that relies on evaluating the equilibrium between thiol and disulfide compounds in order to tackle this particular issue (18,19). Thiol sulfhydryl groups are essential for maintaining redox homeostasis, and thiols are responsible for the great majority of antioxidant capability (20). The oxidative balance is made up of the continuous and reversible exchange of thiols (the reduced state) and disulfides (the oxidized state). However, until recently, only the thiol side of the equilibrium could be identified, even using elaborate and costly technologies. But Erel and Neselioglu demonstrated that they could evaluate both factors rather well.

Thiol-disulfide hemostasis is not well studied in fasting pregnant women. There is only one study conducted by Ozturk et al., who studied oxidative stress in pregnant women during Ramadan (16). The number of participants in this research was small, and it was restricted to pregnant women in the 2nd trimester. The objective of this research was to investigate oxidative stress in fasting healthy pregnant. We used the serum concentrations of thiol and disulfide homeostasis species in the current investigation.

MATERIAL AND METHOD

This observational research was granted approval by the Trabzon Kanuni Training and Research Hospital's Ethics Committee for Clinical Research. Women who visited the Obstetrics and Gynecology Clinic at Trabzon Kanuni Training and Research Hospital between April 13 and May 12, 2021, were selected as the study's subjects (the month of Ramadan). Only pregnant women expecting a single child who were also fasting throughout Ramadan were considered eligible for this study. All pregnant individuals involved in the research provided informed consent.

The G*Power 3.1.9.4 statistical program's power analysis and the findings of previous studies were used to establish the necessary sample size. The research determined the critical values for Type I error (α) and Type II error (β) to be 0.05 and 0.95, respectively. In order to achieve a statistical power of 95% with a sample size of 96 participants, it was determined that a minimum of 52 patients were required in each group (16). A group of 60 participants was allocated to each experimental group, accounting for an anticipated attrition rate of 10% (Figure 1). Due to the increased oxygen pressure within the feto-maternal unit in the latter stages of the first trimester, this study did not include pregnant women.

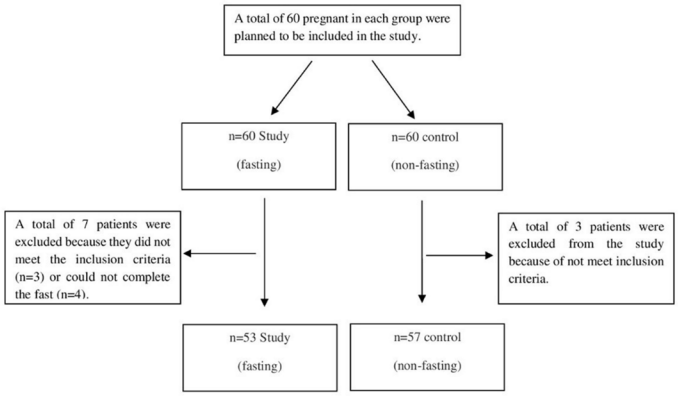

Figure 1. Participant flowchart

We excluded women who were in labor, had ruptured membranes, were carrying multiples, had fetal abnormalities, smoked or drank, or had significant medical disorders, including diabetes or inflammatory illnesses, and pregnant women who were COVID-19 positive during the study. All of the pregnant women in both the fasting and non-fasting groups took their prescribed medicines as usual, without any additional vitamin or mineral supplements. Oxidative stress values were examined in women who fasted for at least 15 days. Before and after at least 15 days of fasting, the native thiol and total thiol concentrations in the study were compared.

In the process, 10 cubic centimeters of blood from a vein are taken and put into tubes that have already been filled with ethylenediaminetetraacetic acid (EDTA). The cellular and plasma components were separated using centrifugation at a force of 1500 times the acceleration due to gravity for a duration of 10 minutes. The specimens were stored in a freezer maintained at a temperature of -80 degrees Celsius. Erel and Neselioglu used sodium borohydride (NaBH4) as a means to assess the levels of native thiol, total thiol, and disulphide in plasma (5). This was accomplished by converting the plasma's dynamic disulphide bonds into inert thiol groups. To inhibit further reduction of 5,50-dithiobis-2-nitrobenzoic acid (DTNB) and the newly formed disulfide bond resulting from the DTNB reaction, formaldehyde was used to eliminate the surplus NaBH4. Total thiol concentration was determined using a specialized Ellman's reagent. It was decided that mercaptoethanol solutions would be used for the calibrations. The concentration of disulfide was calculated as half of the difference between the total and the native

thiol amount. Lastly, we determined the "disulfide/thiol 100," "disulfide/total thiol 100," and "thiol/total thiol 100" ratios.

Statistical analysis was conducted using SPSS version 23.0 (SPSS, Chicago, IL, USA). The data were provided in the form of the mean plus the standard deviation (SD). Kolmogorov-Smirnov testing showed that the dataset was normally distributed. The difference between the study and control groups was determined using an independent t-test. Within-group comparisons were made using a student t test. The cutoff for significance was set at a p value of 0.05.

RESULTS

Of the 60 patients included in the study group, 2 were excluded from the study due to COVID-19, 4 due to inability to complete fasting, and 1 due to premature birth. Of the 60 patients included in the control group, 2 were excluded because of COVID-19 and 1 due to gestational diabetes. Table 1 provides an overview of the demographic characteristics of each subgroup. Table 2 presents the mean values for native thiol, total thiol, disulfide, and the ratios disulfide/native thiol*100, disulfide/total thiol*100, and native/total thiol*100 for pregnant women who fasted and those who did not. In the fasting group, there were 32 second trimester and 21 third trimester pregnancies. In the non-fasting group, there were 33 second- and 24 third-trimester pregnant women. Disulfide and disulfide/nativethiol*100, disulfide/totalthiol*100, and native/totalthiol*100 ratios did not differ significantly across groups.

Table 1. The table shows the demographic data of the groups

	Control (n=57)	Study (n=53)	p
Age (year)	28.40±8.81	27.8±6.3	0.258
BMI (kg/m2)	24.80±3.12	24.3±4.10	0.213
Parity	2.68±1.61	2.51±1.75	0.196
Gestational age (week)	26.34±8.50	27.26±9.60	0.120
Oral intake (hour)	6.42±1.21	6.28±1.27	0.089
Fasting time (hour)	16.14±1.18	16.25±1.23	0.143

Data is presented mean±std

Native thiol and total thiol levels were much lower in the study group than in the control group, but there were no major changes in the pre- and post-oxidative stress indicators in the control group (Table 2). A statistically significant difference was seen between the groups upon analyzing the mean values of native thiol (Figure 2) and total thiol (Figure 3).

Table 2. The levels of oxidative stress indicators in the study and the control groups

		Study (n=53)	Control (n=57)	p*
Native thiol	**Before**	430.15±56.3	431.23±64.4	0.246
	After	396.20±66.3	429.40±69.8	**0.012**
	p**	**0.022**	0.311	
Total thiol	**Before**	473.30±69.20	475.65±69.2	0.255
	After	433.40±72.10	471.80±73.1	**0.007**
	p**	**0.006**	0.295	
Disulfide	**Before**	19.80±8.77	20.22±8.87	0.121
	After	18.60±9.75	21.18±9.61	0.166
	p**	0.280	0.219	
Disulfide/Native thiol*100	**Before**	4.60±1.12	4.68±1.21	0.433
	After	4.75±2.53	5.04±2.38	0.527
	p**	0.512	0.499	
Disulfide/Total thiol*100	**Before**	4.18±1.67	4.25±1.83	0.457
	After	4.24±2.07	4.50±1.92	0.499
	p**	0.501	0.518	
Native thiol/Total thiol*100	**Before**	90.90±5.71	90.66±4.13	0.411
	After	91.52±4.15	91.01±3.83	0.496
	p**	0.388	0.405	

Data is presented mean±std. *Independent t test. **Paired t test

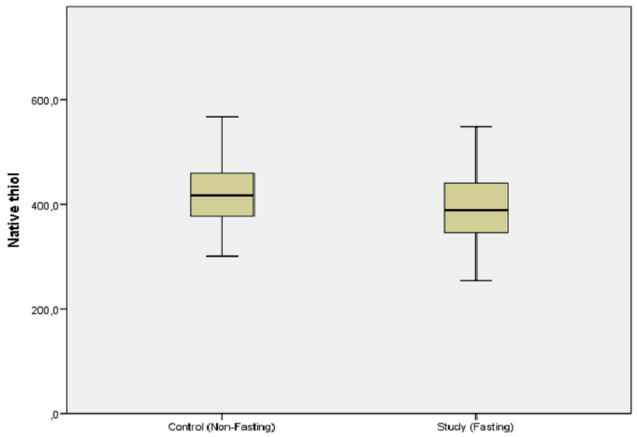

Figure 2. The mean values of native thiol in the study and control groups are shown in the box plot graph

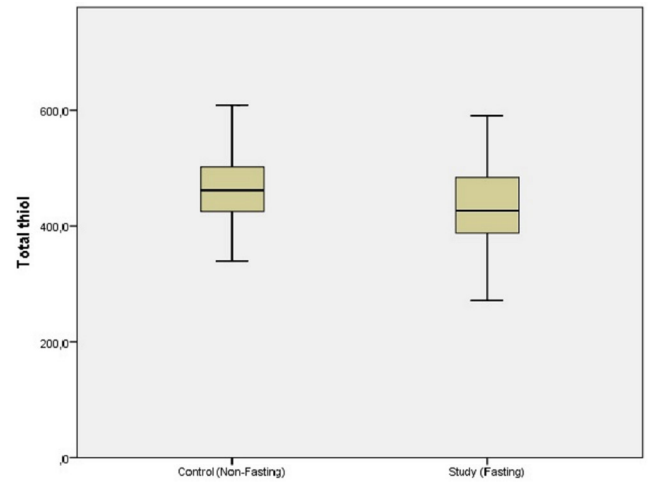

Figure 3. The mean values of total thiol in the study and control groups are shown in the box plot graph

DISCUSSION

The current investigation included an assessment of thiol disulfide hemostasis and, as a result, oxidative stress levels in pregnant women who fasted throughout the month of Ramadan. Based on the results obtained from this investigation, it was observed that the average concentrations of native thiol and total thiol in individuals who underwent fasting exhibited a statistically significant decrease compared to those who did not undergo fasting.

The prevailing consensus acknowledges that pregnancy is associated with an elevation in oxidative stress, which arises from a systemic inflammatory response and heightened levels of circulating (ROS). The placenta, which serves as the central regulator of pregnancy, is primarily responsible for generating (ROS) during this period (7). Accordingly, the elevated oxidative stress observed during pregnancy may result in possible tissue damage (8,21).

The biological significance of thiols and disulfides includes the static stability of the structures of proteins, the control of the activities of proteins and enzymes, receptors, transporters, Na-K channels, and transcription (19). The dynamic equilibrium state of thiol/disulfide plays crucial roles in several biological processes, including antioxidant defense, detoxification, apoptosis, control of enzyme activity, transcription, and cellular signal transmission systems (22). The plasma thiol pool is mostly composed of albumin (23).

To make disulfide bond structures that can be broken and put back together again, the thiol groups in cysteine residues, low molecular weight compounds, proteins, and other thiol groups are oxidized by interacting with oxidant molecules in the environment. The ability of disulfide bond structures to be broken down into thiol groups, which helps keep the thiol disulfide equilibrium, is important (24).

Since 1979, the measurement of just one aspect of this bilateral balance has been conducted. However, thanks to the invention of the novel method by Erel and Neselioglu, it is now possible to measure and evaluate both variable levels separately and collectively, allowing for a thorough evaluation (19). Thiol-Disulfide Homeostasis in fasting pregnant women has never been investigated until now, and our study is the first report in this area. In this study, we used an innovative methodology that allows the accurate quantification of cumulative thiol and disulfide levels. Additionally, we were able to quantify the specific amounts of native-total thiol (representing the reductive state) and disulfide (indicating the oxidative condition). The study revealed that the balance between thiol and disulfide was modified in response to variations in thiol levels among pregnant women who were fasting.

The research done by Ozturk et al. aimed to examine the impact of fasting during Ramadan on oxidative stress levels in pregnant women (16). The research revealed that there was no statistically significant disparity between the groups assigned to the study and the control conditions. Throughout their investigation, the researchers used total antioxidant status, total oxidant status, and the oxidative stress index as indicators. In the present investigation, a distinction was made from the previous research by including pregnancies in the third trimester. In the present work, an examination was conducted on the equilibrium of thiol-disulfide, a novel marker for oxidative stress. It was observed that there were substantial differences in the mean values of native and total thiols between the fasting and non-fasting groups. Furthermore, the individuals included in our research were subjected to a comparison both before and after a period of fasting. The study group exhibited a notable reduction in levels of native thiol and total thiol, while no discernible disparity was noted in the control group.

In the study conducted by Ibrahim et al., the impact of fasting during Ramadan on oxidative stress parameters and biochemical markers of cellular damage in healthy individuals was investigated. The findings revealed that, apart from a minor reduction in lipid peroxidative damage in erythrocytes, fasting during Ramadan did not result in any significant alterations in oxidative stress parameters or biochemical markers of cellular damage in the healthy subjects (14). In their study, Ibrahim et al. conducted an analysis of many biomarkers, including malondialdehyde

(MDA), glutathione, glutathione peroxidase, and catalase, in order to assess their potential as indicators of oxidative stress. In the present study, we conducted an assessment of thiol-disulfide levels, which diverged from the methodologies used in previous investigations. The cohort under scrutiny consisted only of pregnant females.

Numerous studies in the literature have documented a shift towards an oxidant direction in thiol-disulfide hemostasis in various obstetric complications, including preeclampsia, fetal distress, isolated oligohydramnios, gestational diabetes mellitus, and fetal growth restriction. These investigations specifically focused on examining thiol-disulfide dynamics in pregnant women. Based on the findings of previous research and the outcomes of our present investigation, it can be stated that the practice of fasting among pregnant women leads to a change in thiol-disulfide hemostasis towards an oxidant state (25-32). It can be said that pregnant women should not fast in order not to be exposed to oxidative stress during Ramadan and postpone it until after birth and even to the end of the breastfeeding period.

There is evidence indicating that, in pregnancies affected by COVID-19, there is a change in thiol-disulfide homeostasis towards an oxidant state. This observation suggests a potential involvement of ischemic processes in the etiopathogenesis of this emerging disease (33). It can be said that the COVID-19 pandemic period will also contribute to the oxidative stress caused by fasting.

The prospective design of this study was one of its strongest aspects. One of the limitations of our research is that we did not assess the incidence of obstetric problems such as preeclampsia, fetal distress, isolated oligohydramnios, prenatal diabetes mellitus, and fetal growth restriction. However, it was assumed that they would not provide statistically significant findings because of their low prevalence in otherwise healthy pregnancies. Obstetric complications can be evaluated in large scale studies. During the period of investigation, the dropout rate observed in the research exhibited fluctuations as a consequence of the global influence exerted by the COVID-19 pandemic.

CONCLUSION

In conclusion, this study is the first one in the scientific literature to look at the oxidative status of pregnant people who fasted during Ramadan, as well as the thiol-disulfide equilibrium of the mother's blood. The findings of the investigation indicated that there had been a change toward an oxidant direction in the thiol-disulfide balance. Antioxidant use may be recommended for fasting pregnant women.

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

Ethical approval: *Ethics committee approval was received for this study from the ethics committee of Trabzon Kanuni Training and Research Hospital. Decision No: 2021/52 Date: 04.04.2021.*

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Research Article

Evaluation of Demographic and Clinical Characteristics of the Patients with Syphilis Who Applied to the Dermatology Clinic of a Tertiary Referral Hospital Between the Years 2019-2023

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Abstract

Aim: The aim of this study is to evaluate the demographic and clinical features of syphilis patients followed up in a tertiary referral hospital, and thus to provide current contributions to epidemiological data related to syphilis.

Material and Methods: The demographic characteristics, clinical and laboratory findings, and treatments received of all patients diagnosed with syphilis and followed up in our clinic between January 2019 and June 2023 were evaluated retrospectively.

Results: We included a total of 118 patients, 24 women and 94 men. The average age of the patients was 36.56±5.1 years. Forty-one of the patients (34.7%) were married, 56 were single (47.4%) and the marital status of 21 (17.8%) was unknown. Sixty-two of the patients (52.5%) had primary syphilis , 29 (24.6%) had secondary syphilis and 27 (22.9%) had latent syphilis. When the patients' admission symptoms were evaluated, genital chancre was found in 58 (49.1%), roseola syphilitica in 24 (20.3%), mucous plaque in 11 (9.3%), condyloma lata in 7 (5.9%) and syphilis papulosa psoriasiformis in 3 (2.5%) patients. Twenty-five patients (21.1%) were asymptomatic. Transmission from spouse was in 14 (11.9%) patients while suspicious sexual contact was in 77 (65.3%) patients. The source of transmission was unknown in 27 (22.9%) patients. Response to treatment was obtained in all of the patients who were not lost to follow up. Twenty-two of the patients (18.64%) were HIV positive.

Conclusion: Rising syphilis incidence which is observed globally in the recent years indicates the need to raise the level of public awareness about the disease and its transmission routes. In addition, since it can mimic many diseases, it should always be considered in the differential diagnosis, especially in patients with a history of suspicious sexual contact. It is necessary to investigate other sexually transmitted diseases, especially HIV, in patients diagnosed with syphilis.

Keywords: Syphilis, sexually transmitted diseases, *Treponema pallidum*

INTRODUCTION

Syphilis is an infection caused by the spirochete group Treponema pallidum subspecies pallidum; it is transmitted sexually, through blood transfusions or transplacentally. It has different stages which can be symptomatic or asymptomatic and can affect all systems and organs if not treated with its chronic course (1). Being an endemic disease on the European continent since the 17th century, syphilis has caused numerous epidemics worldwide since the 1990s due to the HIV epidemic, polygamy, an increase in the number of substance addicts, and the prevalence of homosexual relationships. The first cases in Türkiye started to be seen after the 19th century, and the name "frengi" was used to describe the disease, indicating that it came to Türkiye from Europe (1,2).

The clinical symptoms of syphilis are not specific and can mimic many diseases. During the primary syphilis period, chancre; during the secondary syphilis period, skin rashes, mucocutaneous lesions, lymphadenopathy; and in tertiary syphilis, cardiac and neurological symptoms, and gummas can be observed (3). Moreover, since the pathogen cannot be cultured in vitro, serological methods are generally used in diagnosis (4). The most commonly used serological tests for screening the presence of infection are treponemal and non-treponemal tests. For screening purposes, the flocculation-based Veneral Disease Research Laboratory (VDRL) and agglutination-based Rapid Plasma Reagin (RPR) are most commonly used. Their false positivity rates are higher compared to treponemal tests. Treponemal tests display antibodies specific to treponemas and become positive from the

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second week of infection. The most frequently used are the Treponema Pallidum Hemagglutination Assay (TPHA) and the Fluorescent Treponema Antibody-Absorption Test (FTA-ABS). The false positivity rate in these specific tests is very low, hence they are used to confirm the diagnosis. Due to the lifelong positivity of treponemal tests, treatment response is monitored by titration tracking of non-treponemal tests (5).

In our study, we aimed to evaluate the demographic characteristics, clinical and laboratory findings, and treatments received by the patients diagnosed with syphilis and followed up in our clinic in the last 4.5 years. We believe that the obtained data will contribute to the improvement of current epidemiological data related to syphilis.

MATERIAL AND METHOD

Our study was conducted in a tertiary referral hospital. The demographic and clinical characteristics of all patients followed up with the diagnosis of syphilis in our clinic between January 2019 and June 2023 were evaluated retrospectively. Ethics committee approval with number E1-23-3654 was obtained from Ankara City Hospital before the study (07.06.2023). Our study was administered in accordance with the Helsinki Declaration. Patients with missing records in hospital records or with unconfirmed diagnosis of syphilis were not included in the study.

Statistical analysis calculations were performed with the SPSS 24.0 (IBM Corp., NY, USA) program. Continuous variables are expressed as mean and standard deviation, and categorical variables are expressed as number and percentage. Pearson's Chi-Square test statistic or Fisher's Exact tests were used as appropriate for comparing categorical measurements. The level of statistical significance was accepted as 0.05 in all tests.

RESULTS

A total of 118 patients, 24 women (20.3%), and 94 men (79.7%) were included in our study. The average age of the patients was 36.56±5.1 years. The average age of female patients was 30.3±3.2 years, while the average age of male patients was 38.16±4.2 years. Forty-one of the patients (34.7%) were married, 56 were single (47.4%), and the marital status of 21 (17.8%) was unknown. Five of the women were single, 19 of them were married. Twenty-two of the men were married, 55 were single, and the marital status of 21 was unknown. Table 1. shows demographic characteristics of our patients.

Table 1. Demographic characteristics of patients

	Female	Male	Total
Number of patients (N)	24	94	118
Mean age ±SD (years)	30.3±3.2	38.16±4.2	36.56±5.1
Marital Status N (%)			
Married	19	22	41 (34.7)
Single	5	55	56 (47.4)
Unknown	0	21	21 (17.8%)

Table 2. Clinical characteristics of the patients

Syphilis stage N (%)	Primary	62 (52.5)
	Secondary	29 (24.6)
	Latent	27 (22.9)
Presenting symptoms N (%)	Genital chancre	58 (49.1)
	Roseola syphilitica	24 (20.3)
	Mucous patch	11 (9.3)
	Condyloma lata	7 (5.9)
	Syphilis papulosa psoriasiformis	3 (2.5)
	Neurosyphilis	1 (0.8)
	Asymptomatic	25 (21.1%)
Laboratory results		
RPR N (%)	Positive	112 (95)
	Negative	6 (5)
TPHA N (%)	Positive	117 (99.2)
	Negative	1 (0.8)
Sources of infection N (%)	Spouse	14 (11.9)
	Suspicious sexual contact	77 (65.3)
	Unknown	27 (22.9)
Treatments received N (%)	Benzathine Penicillin G 2.4 MU 3 doses	52 (44)
	Benzathine Penicillin G 2.4 MU 2 doses	29 (24.6)
	Benzathine Penicillin G 2.4 MU single dose	31(26.2)
	Doxycycline 200 mg for 4 weeks	5 (4.2)
	Crystalline Penicillin for 10 days	1 (0.8)
Accompanying infectious diseases N (%)	HIV	22 (18.4)
	Hepatitis B	2 (1.7)
	Hepatitis C	(1.7)

RPR: Rapid Plasma Reagin, TPHA: Treponema Pallidum Hemagglutination Assay

Sixty-two of the patients (52.5%) had primary syphilis, 29 (24.6%) had secondary syphilis and 27 (22.9%) had latent syphilis. When the patients' admission symptoms were evaluated, genital chancre was found in 58 (49.1%), roseola syphilitica in 24 (20.3%), mucous plaque in 11 (9.3%), condyloma lata in 7 (5.9%) and syphilis papulosa psoriasiformis in 3 (2.5%). One patient had neurosyphilis. Twenty-five patients (21.1%) were asymptomatic. When the non-treponemal tests of the patients were evaluated, RPR was positive in 112 patients (95%), negative in 6 (5%) patients. TPHA was positive in 117 patients, and negative in 1 patient. When we examined the sources of transmission, there was transmission from husband/wife in 14 (11.9%) patients and due to suspicious sexual contact in 77 (65.3%) patients while the source was unknown in 27 (22.9%) patients. When we evaluated the treatments received, 52 patients (44%) received 3 doses of Benzathine penicillin G 2.4 million units with one week apart, 29 (24.6%) received 2 doses with one week apart and 31 (26.2%) received a single dose of penicillin treatment. Five patients (4.2%) with penicillin allergy received doxycycline 200 mg/day for 4 weeks. One patient

who was also HIV positive and in the latent stage had neurosyphilis. He received crystalline penicillin 6X4 million units for 10 days. A response to treatment was obtained in 110 patients, while the remaining 8 patients dropped out of the follow-up. Twenty two patients (18.64%) were HIV positive, 2 were hepatitis B positive, and 2 were hepatitis C positive. Table 2. shows clinical characteristics of our patients.

DISCUSSION

Syphilis represents a significant public health concern that affects a large proportion of the population (1,2). The number of syphilis cases reported between 2010 and 2019 (excluding the plateau between 2017 and 2018) has been on a steady increase (6,7). While a marked increase has been observed in men, a slow decrease has been noticed among women. This is thought to be due to the rise in men having sex with men (MSM) (7,8).

According to the 2019 data from the European Centre for Disease Prevention and Control (ECDC), the incidence of syphilis was calculated as 7.4/100,000. This surveillance study used data obtained from 29 European Union countries (7). According to the Centers for Disease Control and Prevention (CDC) 2019 data, the incidence of syphilis in the United States has increased five-fold compared to 2001, reaching 11.9/100,000 (9).

The number of studies and statistical data concerning the frequency of syphilis in our country is limited. According to the syphilis statistics from the Ministry of Health Public Health, the number of reported cases has increased from 502 in 2015 to 2801 in 2021 (10). The annual incidence of 3.35/100,000 is thought to be lower than the actual incidence, occasionally due to the treatment of syphilis cases under different diagnoses and limited reporting of the cases (11).

In our study, the male/female ratio was found to be 3.92. In the study by Oğrum et al. (1), this ratio was 3.5; in the study by Adışen et al. (2), it was 4.3. Our study shows similarity with these studies in terms of the male/female ratio. According to the CDC 2019 data, 83% of primary syphilis cases in the United States were male, and 57% of these were MSM (9). According to the ECDC data, the male/female ratio was found to be 8.6/1 (7). In the same study, it was found that the disease was most frequently seen in the 25-34 age range and that the frequency of occurrence in men was higher than in women across all age groups (7). The average age of our patients was 36.56±5.1 years. The average age of female patients was 30.3±3.2 years, and the average age of male patients was 38.16±4.2 years, which were found to be compatible with the ECDC data.

The majority of our patients, whose marital status was known, were observed to be single. Excluding the 21 patients whose marital status was unknown, 57.7% of the patients were single, and 42.3% were married. In studies conducted in our country, the percentage of married syphilis patients was observed to be 70% (1), 80.6% (2), and 78.7% (12). Our study differs from the results of other studies conducted in previous years in our country in that the rate of single patients was higher than the rate of married patients. On the contrary, we observed that the rate of married patients in our study was higher in the female patients' group (72.2%).

The most common stages of disease were found to be primary, secondary, and latent syphilis, respectively. The genital chancre, a primary syphilis lesion seen in approximately half of the patients, was the most common presenting symptom. Other presenting symptoms were roseola syphilitica, plaque mucosus, condylomata lata, and psoriasiform papular syphilis, in order, all indicative of the second stage. Our study did not have sufficient data on the frequency of generalized lymphadenopathy, a common symptom of the second stage. It is noteworthy that six patients with latent syphilis were serologically identified during screening tests when they applied to donate blood. This underscores the importance of raising awareness among potential donors about sexually transmitted diseases. In the study by Adışen et al., 75.5% of the patients had first-stage, 12.8% had second-stage, and 11.32% had third-stage syphilis. The most commonly observed symptom at the time of presentation in this study was also the genital chancre (2). Similarly, in the study by Karaosmanoğlu et al., patients were most commonly diagnosed in the primary stage, followed by the secondary stage (12). These findings are consistent with our study data. However, in the study by Oğrum et al., the most common reason for presentation was second-stage syphilis lesions. The authors attributed this to the first stage, where the only symptom is painless chancre, being overlooked, and the more noticeable course of the second stage, where dermatological and systemic symptoms are more prominent (1). According to the ECDC 2019 data, in contrast to the studies in our country, it was reported that latent syphilis was most commonly seen (34% primary, 25% secondary, 38% latent syphilis) (7).

When patients' serological tests were examined, the non-treponemal test RPR was positive in 112 patients (95%) and negative in 6 patients (5%). The prozone phenomenon was observed in 2 patients who tested negative, with initially negative values becoming positive with serum dilution. VDRL is not performed in our hospital. TPHA was negative in one patient, whose clinical presentation was compatible with syphilis and whose FTA-ABS test was found to be positive. Similar characteristics of syphilis serology have been observed in other studies conducted in our country (1,2).

When assessing the filiation status, 11.9% of patients had contracted the disease from their spouse, 65.3% due to a suspicious sexual relationship, whereas the source of infection was unknown for 22.9% (n=27) of the patients. When excluding the patients with an unknown source of infection, among the patients with known sources of infection, 81% of women (n=13) got infected by their spouses, while 98.6% of men got infected due to suspicious

sexual relationships. The content of the suspicious sexual relationships (sex worker or MSM) could not be clarified with the available data in the system. The striking difference in the source of transmission between men and women could be related to the sociocultural structure of society. Similarly to our study, in the study by Oğrum et al., the source of infection for 7 out of 8 married women was their husband, while for 26 out of 27 married men it was extramarital relationships (1). The study by Adışen et al. produced data parallel to our study (2). It was also noteworthy in our study that about a quarter of the cases did not describe any mode of transmission. This may be due to the lack of sufficient awareness and knowledge in society about the modes of transmission and course of the disease. In their study investigating the level of knowledge about sexually transmitted diseases among young adult males, Açıkel et al. showed that only 6.5% of this group had knowledge about syphilis (13). We believe that awareness about syphilis should be raised through health services and mass communication tools.

In the ECDC study, it was reported that, according to the available data from 16 countries, the source of transmission in 68% of cases was male-to-male relationships, 25% was heterosexual relationships, and in 7% the source of transmission was unknown (7). Factors associated with syphilis transmission have been reported as high-risk sexual behaviors (MSM, multiple partners), sex work, substance use (drugs or alcohol), poverty, homelessness, ethnic minority or migrant status (3).

The recommended treatment regimen for syphilis is a single dose of benzathine penicillin G 2.4 million units for primary, secondary, and early latent syphilis. In cases of HIV positivity, 3 doses with a week's interval is recommended. For late latent syphilis, Benzathine penicillin G is given as 3 doses at one-week intervals (4). Doxycycline can be used in cases of penicillin allergy (4,14). All of our patients, except for 5 with a penicillin allergy, had received penicillin treatment. The outcome of the 8 patients who were lost to follow-up is unknown, but all remaining patients had responded to treatment. No side effects or complications were reported. In the study by Karaosmanoğlu et al., similarly, 43 patients were successfully treated with penicillin, except for 3 patients with penicillin allergy (12). These findings demonstrate that penicillin remains as the first and most effective treatment option for syphilis.

Of our patients, 22 (18.64%) tested positive for HIV, 2 for each of hepatitis B and hepatitis C. The coexistence of syphilis and HIV has been increasing in recent years, and co-infections are frequently seen (15). Syphilis not only facilitates the transmission of HIV but also increases the frequency of complications and the progression of the disease. Therefore, all patients with syphilis should be screened for HIV (16). In a study published by Harman et al. in 2021, the frequency of HIV in patients with syphilis was investigated, and HIV positivity was detected in 45% of patients. A higher rate of HIV co-infection in patients with syphilis was found in that study than syphilis co-infection rates in patients with HIV, stated in previous studies. This was thought to be due to the perception of syphilis as less significant compared to HIV infection among sexually active individuals and the abandonment of protective measures (16). The ECDC 2019 study reported the coexistence of syphilis and HIV as 23% (7). While older studies in our country did not detect a coexistence of syphilis and HIV (1,12), the finding of HIV in nearly a fifth of our patients in our study may indicate a diversification of sexual preferences in our country, similar to European and American countries.

CONCLUSION

In conclusion, syphilis remains a significant public health problem in our country, as it is worldwide. The increase in the incidence of the disease compared to past years indicates the need to raise the level of awareness in society about the disease and its modes of transmission. In addition, due to its wide range of clinical manifestations, it can mimic many diseases, and therefore should always be considered in the differential diagnosis, especially in patients with a history of suspicious sexual contact. It is necessary to investigate other sexually transmitted diseases, especially HIV, in patients diagnosed with syphilis.

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Research Article

The Effect of Training Program Given to Multiple Pregnants by Motivational Interview Method on Fear of Birth and Delivery Style

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Abstract

Aim: Within the scope of our research, it was aimed to evaluate the effects of the training programs given with the motivational interview attitude given specifically to the mothers with multiple pregnancies on possible fears of childbirth and self-perception. In this direction, case and control studies were conducted with two groups. In summary, it focused on the effect of self-efficacy experienced during birth and self-efficacy experienced at the same time.

Material and Methods: Our study was carried out between August 2022 and November 2022 in Adana Seyhan State Hospital Obstetrics and Gynecology Polyclinic with 73 multiple pregnant women (intervention: 37, control: 36) who met the study. More than one pregnant mother in the sample was randomly selected according to the intervention and control groups. "Training Program Based on Motivational Interview Method for Fear of Birth" was given to 37 pregnant women in the case group, four sessions individually, once a week.

Results: In obtaining the relevant data within the scope of our study; Descriptive Personal Form, Wijma Birth Expectation/Experience Scale (W-DEQ) Versions A and B, Birth Self-Efficacy Scale (DSS) Short Version and Birth Evaluation Form were used. During the evaluation of the data, descriptive statistics such as the number of patients and the percentage of gender were recorded. Data are shown as mean±standard deviation, median (percentage). Chi-square test, Independent Groups t test, Mann Whitney U test, Wilcoxon test, repeated measures ANOVA test and Friedman test were used in statistical analysis.

Conclusion: In the training program conducted with the motivational interview approach with pregnant mothers, it was revealed that possible fears of birth were minimized, positive increases were observed in birth self-efficacy, and the effect on birth types was not much. Looking at the results of the research; In order to minimize the fear of birth in the prenatal period and to increase self-efficacy during birth, it is recommended to include it in nursing care in a training program based on the motivational interview approach for fear of birth.

Keywords: Birth fears, pregnancy, self-efficacy at birth, mode of delivery

INTRODUCTION

Every woman experiences different physical changes during the birth process. From the other point of view, the uncertainty caused by the unpredictability of the birth event, the emotional states of losing control during childbirth, and the problem of confidence in the pregnant mother's own responsibilities cause anxiety and stress for some women to reveal their fear of childbirth (1-3). Studies have shown that 6.3-75% of pregnant women experience fear of childbirth (4), and 6-10% experience fear of childbirth, which can affect their daily lives (1).

In case of birth fears; biological, psychological, social or secondary factors can play a significant role. In addition, it is stated that the gestational week and parity have an important effect on the emergence of the fear of childbirth (1,2).

As the gestational week progresses and the expectant mother approaches childbirth step by step, the fear of childbirth increases rapidly (1,2). Laursen et al. (5) stated that the number of pregnant women who had fear of childbirth in the 31st week of pregnancy was much higher than at the 16th gestational week.

CITATION

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Rouhe et al. also revealed that the fear of childbirth increased after the 20th gestational week (1). As the gestational week progresses, the reason for the fear of childbirth to increase; women pay attention to pregnancy in the first trimester, their babies in the second trimester, and birth in the third trimester (3).

Rouhe et al. state that many pregnant women experience a higher level of fear of childbirth than normal pregnant women, both in the early and late stages of pregnancy (1). The factors that cause this situation; Having thoughts such as hearing negative birth stories, not being able to cope with birth pain, not being able to give birth in a healthy way (2).

This research was carried out to observe the effects of motivational interview, self-efficacy and delivery method, and the training program given to pregnant women who have fear of childbirth.

Childbirth is an issue that women often worry about. Exaggerated birth stories and inconsistent narratives cause women to fear childbirth during and even before pregnancy. Bringing a new life to the world is, of course, a miraculous and challenging process. However, it should not be forgotten that pregnancy and childbirth are quite natural for the female body. Biologically, getting pregnant and giving birth are normal for the female body when the right conditions are met. Every woman has the strength to somehow handle childbirth. Whether it is a normal birth or a cesarean delivery, thinking about the pain to be experienced during childbirth can cause expectant mothers to experience tension during pregnancy (5).

Although fear of childbirth is a comprehensive concept, it does not have a precise definition. According to the transfer of Hofberg and Brockington (6); Fear of childbirth was first noticed by the French psychiatrist Louis Victor Marcé in 1858. Marce describes primitive anxiety arising from the expectation of unknown pain; The fear of multi-born people arising from their past memories and future expectations is defined as fear of birth. In 1981, the fear of childbirth was defined as a strong anxiety that negatively affects the daily life and well-being of pregnant women.

Saisto and Halmesmaki defined fear of childbirth as an anxiety disorder or a phobic fear that includes having nightmares, physical complaints, difficulty concentrating on daily activities, and often a cesarean delivery request (7). Ryding et al. (8) defined fear of birth as a negative expectation about birth, while Wijma et al. (9) defined it as a feeling of anxiety about birth that existed before, during or after birth.

Motivational interview method is first described by scientists William R. Miller and Stephen Rollnick. It is a clinical perspectives. These scientists defined motivational interviewing as “a directive and client-centered approach used to reveal behavior change that will help clients recognize and resolve conflicting emotions (ambivalence)” (10).

The main purpose of motivational interviewing; to stimulate the individual's intrinsic motivation for change and to ensure active participation in the change process. This approach is particularly useful for individuals who are unwilling to change and/or have conflicting emotions (11).

The motivational interview technique was first developed to make behavioral changes in individuals with problematic alcohol use (11). Today, it is used in the treatment of substance addiction (12), smoking cessation, diet management and weight control (13), compliance with exercise, continuation of breastfeeding (14), ensuring the use of contraceptives, preventing sexually transmitted diseases, reducing the rates of cesarean section (15), pregnant women. It is used to create behavioral change in individuals by encouraging participation in birth preparation classes.

MATERIAL AND METHOD

Purpose of the Research

Within the scope of this study, it was performed in order to evaluate the effects of birth fears, self-efficacy during childbirth and the way of giving birth in the training program given with the motivational interview approach to mothers who are in the process of multiple pregnancy. Ethical approval was taken from Clinical Researches Ethical Committee, Diyarbakır Gazi Yaşargil Training and Research Hospital (date: 25/11/2022 and no:314).

Research Hypotheses

H1: Birth fears differ significantly from mothers who have had multiple pregnancies compared to those who did not receive training in motivational interviewing approach.

H2: Among the mothers who had multiple pregnancies, there was a significant difference between the motivational interview approach and the education fields and their self-efficacy at birth compared to those who did not.

H3: There is a significant difference between the motivational interview approach and the way of giving birth among mothers who have had multiple pregnancies compared to those who did not receive education.

Data Collection Tools

Descriptive Personal Form, Wijma Birth Expectation/ Experience Scale (W-DEQ) Versions A and B, Birth Self-Efficacy Scale (DSS) Short Version and Birth Evaluation Form scales were applied.

Mothers with multiple pregnancies were included in our study. The mother candidates who applied the training programs given with the motivational interview attitude were included. Single pregnancies are not included.

Statistical Analysis

Statistical Package for the Social Sciences version 22.0 (SPSS Inc., Chicago, IL, USA) software was used for the statistical analysis of this study. While descriptive data and frequencies were calculated with the help of computer,

the normal distribution of the data was tested with the Shapiro-Wilk test. Continuous variables were expressed as mean±standard deviation and nominal variables as numbers (percentage). Normally distributed data were compared between the two groups using Student's t-test, while those that did not fit normal distribution were tested with the Mann-Whitney U test. For categorical variables, Pearson's chi-square or Fisher's exact test was used as appropriate. P value <0.05 was considered statistically significant.

RESULTS

Findings Related to Descriptive Characteristics of Pregnants

Table 1 shows the distribution of some sociodemographic characteristics of multiple pregnant mothers according to research groups. 67.6% of the pregnants in the intervention group and 65% of the pregnants in the control group were between the ages of 18-24, 77% of the pregnants in the intervention group were in high school and undergraduate or graduate school, and 33.3% of the pregnants in the control group were in primary or secondary school. determined to be a graduate.

It was determined that 77% of the pregnant women in the intervention group and 83.3% of the control group did not work in an income-generating job, and 88.9% of the pregnant women in the intervention group and 97.3% of the control group had social security.

Table 2 shows the distribution of some obstetric characteristics of the pregnant women participating in the study according to the study groups. It was determined that 100% of the pregnants in the intervention group and 83.3% of those in the control group had planned pregnancy, 100% of the pregnants in the intervention group and 77.8% of the control group did not receive prenatal education. There was a statistically significant difference between the case group and the control groups in terms of creating a pregnancy plan and educational approach before birth (p<0.05).

Intervention group (5.4% of the pregnant women) and control group (11.1% of the pregnant women) experienced abortion, 5.4% of the pregnant women in the intervention group and 8.3% of the control group had D/C experience. It was determined that 5.4% of them and 13.9% of those in the control group had health problems during pregnancy. Although not stated in the table, ıt was observed that there was bleeding at the beginning of pregnancy in two expectant mothers in the case group, two pregnant women in the control group had urinary tract infections, and three pregnant women had bleeding in the early stages of pregnancy.

It was revealed that 91.9% of the multiple pregnant mothers in the case group and 86.1% of the multiple pregnant mothers in the control group preferred vaginal delivery. It has been clarified that the starting time of the pregnant follow-up process in the case group who participated in the study was 5.95±1.93 weeks, while it was 5.81±1.28 weeks in the control group.

In Table 3, the distribution of the mean and median scores of the pregnant women on the Obstetrics Self-Efficacy Scale (WHO) in terms of research groups and follow-up periods can be seen. No significant changes were observed between the DSQ total scores, sub-dimension of DSQ outcome expectations and DSQ efficacy expectations sub-dimension score medians of expectant mothers in the case group and the median scores of the pregnant women in the control group (p>0.05).

Pre-training DSQ total score median of the pregnants in the intervention group was 273.00 (247.00-291.00), 310.00 (302.00-317.00) and 37-40 after the training. It was determined that it was 313.00 (309.50-317.50) at the gestational week. Post-training and 37-40th pregnant women in the intervention group. According to analysis results, the median of the total score of DSQ for the week of gestation was higher than the median of the total score of DSQ before the education, and this result was statistically significant (p<0.05).

Table 1. Distribution of some socio-demographic characteristics of pregnant women according to study groups

Some sociodemographic characteristics		Intervention group (n=37)		Control group (n=36)		Total		Statistical analysis	p
		S	%	S	%	S	%		
Age	18-24	25	67.6	20	65	45	63	0.123	0.739
	25-35	12	32.4	16	35	28	27		
Educational status	Middle school	7	23	12	33.3	19	28	0.773	0.674
	High school	15	38.5	12	33.3	27	36		
	University	15	38.5	12	33.3	27	36		
Working status	Works	10	23	6	16.7	16	21.2	0.337	0.580
	Not working	27	77	30	83.3	57	78.8		
Social security	Yes	32	88.9	35	97.3	67	93.2	-	0.199
	No	5	11.1	1	2.7	6	6.8		

Table 2. Distribution of some obstetric characteristics of pregnant women according to study groups

Some obstetric features		Intervention group (n=37)		Control group (n=36)		Total		Statistical analysis	p
		S	%	S	%	S	%		
Abortus	Yes	2	5.4	4	11.1	6	8.2	-	0.410
	No	35	94.6	32	88.9	67	91.8		
D/C	Yes	7	23	12	33.3	19	28	-	0.474
	No	15	38.5	12	33.3	27	36		
Planned pregnancy status	Planned	37	100	30	83.3	67	91.8	-	**0.013**
	Not planned	0	0	6	16.7	6	8.2		
Status of receiving prenatal education in pregnancy	Received	0	0	8	22.2	8	11	-	**0.010**
	Not received	37	100	28	77.8	65	89		
Preferred mode of birth	Vaginal birth	34	91.9	31	86.1	65	89.0	4.250	0.250
	Birth by cesarean	0	0	3	8.3	3	4.1		
	Indecisive	3	8.1	2	5.6	5	6.8		

Table 3. Distribution of some obstetric characteristics of pregnant women according to study groups

Birth Self-Efficacy Scale and its sub-dimensions		Intervention group (n=37) $\bar{X}$±SD Mean (25%p-75%p)	Control group (n=36) $\bar{X}$±SD Mean (25%p-75%p)	Statistical analysis	p
BSES total score	Pre-training	267.49±31.33 273.00 (247.00-291.00)	265.86±37.38 268.50 (246.50-287.00)	-0.353	0.724
	Post training	308.65±9.52 310.00 (302.00-317.00)	-	-	-
	37-40. Pregnancy week	311.49±9.28 313.00 (309.50-317.50)	256.28±22.63 256.50 (245.25-273.75)	-7.197	**<0.001**

In our study result, it is thought that it may be due to the anxiety of pregnant women about coping with childbirth as the delivery approaches. Based on the findings, the hypothesis "Among the mothers who had multiple pregnancies, there was a significant difference between the motivational interview approach and the education fields and their self-efficacy at birth compared to those who did not (H2)" was accepted.

It has been observed that women with cesarean section mostly are influenced by fear of childbirth and this fear can be used for prediction of fear16. Within the scope of our study, it was thought that it could be effective in promoting vaginal birth in the training program according to the motivational interview approach on fear of childbirth. In this context, it was also observed that 97.3% of the mothers in the intervention group and 94.4% of the pregnant women in the control group had vaginal delivery. There was no significant difference between the delivery approaches of the pregnant women in the intervention group and the delivery approaches of the pregnant women in the control group (p>0.05). Based on these findings, “There is a significant difference between the multiple pregnant women who received training with the motivational interview method and the multiple pregnant women who did not receive education in terms of delivery type. (H3)” hypothesis was rejected.

DISCUSSION

It was revealed that the mothers in the case group of our study were similar in age, education levels, a very short information about the job they had done, education levels of the spouses, working status of the spouses, and socially similar to the prospective mothers in the control group. At the same time, it was seen that among these similarities, the presence of insurance, family type, perceived income status and the place where they lived the longest (p>0.05).

There was a statistically significant difference between pregnancy planning and prenatal education in the case group and control groups (p>0.05). There was no significant difference observed between the case and control groups when considering the experience of abortion and D/C, health problems during pregnancy, preferred delivery methods, starting time of pregnancy follow-ups, numbers in pregnancy follow-ups and gestational week (p>0.05).

It was revealed that 91.9% of all expectant mothers in the case group and 86.1% of the mothers in the control group preferred vaginal delivery (p>0.05).

It was revealed that the rate of preferring vaginal deliveries in the future period of mothers who had just given birth in the case group was significantly higher than those in the control group (p<0.05). It was determined that the birth experiences of the mothers who had just given birth in the case group were much better than the control groups (p<0.05). It was determined that postpartum women in the intervention group had higher birth satisfaction than the control group (p<0.05). The negative impact of fear of childbirth on the quality of life of pregnant women (16,17) and the increase in cesarean section rates due to the needs of mothers have made the approach to fear of birth important (18).

In our age, there are very different approaches to the

management of the fears experienced by women about childbirth. One of the most used methods is birth preparation training. Childbirth preparation trainings appear as a type that helps women to overcome their less informed opinions and cope with pains of labor. However, it has been observed that this approach has a limited effect on minimizing the woman who are feared from childbirth and acquiring skills to cope with childbirth (19). In order to minimize this fear to obtained the behaviros of wanted in patients, there are some stargies requried such as cognitive and behavioral strategies as well as didactic expression in childbirth preparation education. In this direction, the effectiveness of the birth preparation education given by method of motivational interview used in revealing the behavior change in this study was evaluated (20,21).

Results of the intervention and control groups showed that feared women from childbirth was moderate in the pre-training evaluations of the pregnant women (p>0.05). Post-training (16.46±10.29) and 37-40. It was determined that the level of fear of birth at the week of gestation (15.51±7.35) lessened comparing to the period of the pre-training (49.22±21.15) (p<0.05). 37-40 of control pregnant women. Further analysis showed that fear developed due to childbirth at the gestational week (57.50±16.33) did not change any comparison to the pre-training period (56.63±16.03) (p>0.05).

CONCLUSION

Ultimately, though; According to the motivational interview approach on birth fears, the training program shows that they are effective in reducing birth fears. There are different results in the literature review showing the impacs of preparation for the birth training (19).

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Research Article

Role of SARS-CoV2 Virus in the Etiology of Acute Pancreatitis

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Abstract

Aim: To investigate whether severe acute respiratory syndrome coronavirus is involved in the etiology of acute pancreatitis.

Material and Methods: This study was conducted in Çiğli Educational Hospital, Bakırçay Univesity. The study included 2060 patients with AP admitted to hospital between March, 2020 and August, 2023. The patients were assigned into 2 groups based on presence of COVID-19 infection. Ethiological factors for AP were determined in all patients.

Results: Gallstone was the etiological factor in 614 patients (32.9%) who were COVID (-) but it was the etiological reason in only 19 patients (19%) in COVID (+) group. No etiology was identified in 217 (11.6%) of COVID (-) patients who were diagnosed as idiopathic pancreatitis. Idiopathic pancreatitis was diagnosed in 107 cases (54%) in COVID (+) group. There was significant difference presence of the diagnosis, which was made according to etiological factor, between groups.

Conclusion: There was no definitive etiological link between COVID-19 and AP; however, the fact that same team diagnosed such a different idiopathic AP in the same hospital with same diagnostic facilities implies an etiological role for SARS-CoV-2 virus in AP.

Keywords: COVID-19, etiology, acute pancreatitis

INTRODUCTION

The coronavirus disease-19 (COVID-19), manifested with world-embracing pandemics, is often linked to respiratory symptoms. When studies and case series on COVID-19 disease are reviewed in detail, it was seen that gastrointestinal (GIS) symptoms were prominent in clinical presentation in 25% of the COVID-19 patients. The gastrointestinal symptoms occurring during COVID-19 infection may be resulted from GIS multi-organ injury, including pancreas, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (1). Although the pathogenesis of organ injury in GIS system hasn't been clarified, it can be considered that, at least in part, angiotensin converting enzyme 2 (ACE2) receptor proteins present in pancreas, are the underlying cause for pancreas to be targeted (2). In the literature, there are clinical studies and case series favoring above-mentioned consideration. In their study, İnamdar et al. reported the rate of COVID-19 patients developed acute pancreatitis to all COVID-19 patients, risk factors and outcomes of acute pancreatitis (3). The findings support that acute pancreatitis can be regarded as gastrointestinal symptoms of COVID-19 infection but clinical course and outcomes of acute pancreatitis in COVID-19 patients haven't been clearly defined since AP is rare in COVID-19 (2).

In addition, it is not possible to draw definitive conclusions due to facts that clear definition is lacking for acute pancreatitis and that not all retrospective studies used Atlanta criteria (4).

In this study, it was aimed to determine acute pancreatitis prevalence and investigate whether COVID-19 leads acute pancreatitis.

MATERIAL AND METHOD

This is a retrospective, observational cohort study.

This retrospective study included 2901 patients who presented to emergency department of Çiğli Training and Research Hospital of Bakırçay University with diagnosis of acute pancreatitis (AP) between March 1, 2020 and August 1, 2023 during pandemics. This study was conducted after approval of Institutional Ethics Committee (1194/2023).

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Patients were diagnosed as AP if they fulfilled three of following criteria (Atlanta criteria) (5):

1. Serum lipase level at least three times of normal,
2. Computerized tomography or MRI) results indicative for pancreatitis,
3. Typical upper abdominal pain.

The exclusion criteria were:

- Diagnosis of chronic pancreatitis,
- Hereditary pancreatitis,
- Previous history of pancreatic or biliary surgery,
- Malignancy of pancreas or other organs,
- Presentation with sepsis, septic shock or multi-organ dysfunction.

Overall, 2586 patients with diagnosis of AP were recruited to the study after applying exclusion criteria. The patients were classified into two groups based on COVID-19. Only patients with positive PCR test for SARS-CoV-2 infection in nasal swabs were considered as positive for COVID-19 disease. The patients considered to have COVID-19 disease based on thoracic CT findings were excluded. The patients with negative PCR test result and thoracic CT findings were included in the COVID-19 negative group. Five hundred and twenty six patients without definitive diagnosis of COVID-19 (positive pulmonary findings but negative PCR test) were excluded. Final study population included 2060 patients with diagnosis of AP. COVID (+) group included 198 AP patients while COVID (-) group included 1862 AP patients.

The groups were compared regarding etiology of pancreatitis. The American College of Gastroenterology guidelines were used to differentiate etiologies in COVID-19 positive and negative AP patients.

Data were analyzed using Statistical Package for Social Sciences version 23.0 (SPSS, IBM, Chicago, IL, USA). Categorical data are presented as number and percent while continuous data are presented as mean± standard deviation. Fisher's exact test or Student's t test were employed to determine differences in clinical variables. A p value<0.05 was considered as significant.

RESULTS

The findings of the study are shown in Table 1.

The main characteristics of study population: Overall, 2060 patients were included in the study based on inclusion and exclusion criteria. COVID (+) group included 198 AP patients (9.6%) while COVID (-) group included 1862 AP patients (90.4%). Mean age was 52.2±17.3 in the COVID (-) group and 50.8±16.1 in the COVID (+) group, indicating no significant difference between groups (p>0.05). There were 1106 women and 756 men in the COVID (-) group while 122 women and 76 men in the COVID (+) group. Gender distribution was comparable between groups (p>0.05). When comorbid diseases were assessed in AP patients, it was found that there was diabetes mellitus in 558 patients (29.9%) in the COVID (-) group and 61 patients (30.8%) in the COVID (+) group. No significant difference between groups regarding diabetes mellitus (p>0.05). It was found that there was hypertension in 1171 patients (62.88%) in the COVID (-) group and 122 patients (61.61%) in the COVID (+) group. There was a significant difference in hypertension among groups (p<0.01). Congestive heart failure (CHF) was detected in 201 (10.8%) in the COVID (-) patients and 23 COVID (+) patients, indicating no significant difference (p=0.88).

When patients were classified according to AP etiology, alcohol was identified as etiological factor in 570 patients (30.6%) in the COVID (-) group while in only 8 patients (4%) in the COVID (+) group. Again, gallstone as demonstrated by sonography was found as underlying cause in 614 patients (32.9%) in the COVID (-) group and 39 patients (19%) in the COVID (+) group, indicating a significant difference between groups (p<0.01). It was found that hypertriglyceridemia (G>1000 mg/dL) was the etiological factor in 461 patients (4.7%) in the COVID (-) group and 44 patients (22.2%) in the COVID (+) group, indicating no significant difference (p>0.05). It was found that there was idiopathic AP in 217 patients (11.6%) in the COVID (-) group and 107 patients (54%) in the COVID (+) group, indicating a significant difference between groups (p<0.01).

Table 1. Demographic and ethiologic variables of patients

Variable	COVID (-)	COVID (+)	p value
N	1862	198	<0.05
Age	52.2±17.3	50.8±16.1	0.87
Female n (%)	1106 (59.80%)	122 (61.60%)	0.19
Diabetes mellitus n (%)	558 (29.90%)	61 (30.80%)	0.76
Hypertension n (%)	782 (41.99%)	122 (61.61%)	<0.01
Congestive heart failure n (%)	201(10.80%)	23 (11.50%)	0.88
Pancreatitis Etiology			
Alcohol n (%)	570 (30.60%)	8 (4.00%)	<0.01
Gallstone n (%)	614 (32.90%)	39 (19.00%)	<0.01
Hypertriglyceridemia n (%)	461 (24.70%)	44 (22.20%)	>0.05
Idiopathic n (%)	217 (11.60%)	107 (54.00%)	<0.01

Biochemistry results are summarized in Table 2. Among biochemistry parameters, mean ALP level was significantly higher in the COVID (-) than the COVID (+) group (233±32 IU/L vs. 128±22 IU/L; p<0.01). Mean GGT levels were significantly higher in the COVID (-) than COVID (+) group (224.21 IU/L vs. 120±11 IU/L; p<0.05). Mean blood glucose level was significantly higher in the COVID (+) than COVID (-) group (154±35 mg/dL vs. 192±23 mg/dL; p<0.05).

Table 2. Biochemical results of patients

Variable	COVID (-)	COVID (+)	p value
Amylase (U/L)	2765±541	2681±659	p>0.05
Lipase (IU/L)	1376±432	1288±541	p>0.05
ALT (IU/L)	110±16	99±13	p>0.05
AST (IU/L)	146±22	152±18	p>0.05
ALP (IU/L)	233±32	128±22	p<0.01
GGT (IU/L)	224±21	120±11	p<0.05
T. Bil (mg/dL)	1.26±0.35	1.1±0.22	p>0.05
D. Bil (mg/dL)	0.7±0.21	0.5±0.19	p>0.05
Glucose (mg/dL)	154±35	192±23	p<0.05
Triglyceride (mg/dL)	496±241	527±118	p>0.05
Creatinine (mg/dL)	0.6±0.13	0.7±0.22	p>0.05

DISCUSSION

It is, now, apparent that COVID-19 has diffuse effects in the body including gastrointestinal and pancreaticobiliary systems (1).

Although GI and pancreaticobiliary involvement has been described by multiple case reports, it is challenging to investigate pancreatitis due to low prevalence when compared to other GI symptoms (2,6-8).

Our study has several strengths including:

First of all the AP diagnosis was made according to Atlanta criteria. Each patient file was retrospectively reviewed to confirm diagnosis at presentation in all patients. So this made the diagnosis more clear. Alsa in our study the number of COVID-19 positive patients with AP (n=198) was remarkably higher when compared to previous studies.

It also has limitations originating from its retrospective nature. Although 3 criteria (fulfilling 2 of 3 criteria is sufficient for diagnosis of AP according to Atlanta criteria) were required to diagnose acute pancreatitis in our study and exclusion criteria limited number of patients included, our study had one of the largest sample sizes published in the literature.

The idiopathic pancreatitis rate was 11.6% COVID-19 negative patients (n=1862) who were diagnosed as AP while it was 54% among COVID-19 positive patients (n=198). The glucose levels were higher in COVID (+) group [COVID (-) patients 154±35 vs COVID (+) patients 192±23 p<0.05]. This may indicate that COVID 19 affects not only exocrine pancreas but also insulin secreting beta cells. This may cause a higher rate of a diagnosis of secondary diabetes mellitus after the COVID infection (9,10).

CONCLUSION

Acute pancreatitis is a condition where the pancreas becomes inflamed (swollen) over a short period of time. It also has long time complications that affects a patient's life. Although evidence existed for causal link between COVID-19 and acute pancreatitis, the substantial difference in rate of idiopathic AP diagnosed in the same hospital by the same team using same diagnostic tools suggests that SARS-CoV2 virus may have role in the etiology of AP.

Even though pandemics has relented today, the variants of SARS-CoV-2 virus are threatening healthcare system as a common infectious agent. As it has the potential risk for risks of chronic disease like secondary diabetes mellitus long time follow-up is needed in all COVID infected patients. Thus, there is need for larger studies to support our findings.

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