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European Journal of Therapeutics (Eur J Ther) is the double-blind peer-reviewed, open access, international publication organ of the Gaziantep University School of Medicine. The journal is a quarterly publication, published on March, June, September, and December. The journal publishes content in English.

European Journal of Therapeutics aims to contribute to the international literature by publishing original clinical and experimental research articles, short communication, review articles, technical notes, and letters to the editor in the fields of medical sciences. The journal's target audience includes researchers, physicians and healthcare professionals who are interested or working in in all medical disciplines.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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Short Communication: This type of manuscript present significant findings from tangential investigations that are offshoots from larger studies or from early results that will have to be confirmed through further study. An unstructured main text should be prepared for each short communication. Please check Table 1 for the limitations for Short Note.

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Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	30	6	7 or total of 15 images
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Short Communication	1500	200	20	5	1 or total of 5 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. Authors should avoid using references that are older than ten years. The limit for the old reference usage is 15% in the journal. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six authors should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal Article: Rankovic A, Rancic N, Jovanovic M, Ivanović M, Gajović O, Lazić Z, et al. Impact of imaging diagnostics on the budget – Are we spending too much? Vojnosanit Pregl 2013; 70: 709-11.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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ORIGINAL RESEARCH ARTICLES

REVIEWS

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Original Research

Investigation of the Distribution of Orthodontic Anomalies Among Patients in the Eastern Anatolia Region

Yasin Akbulut

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ABSTRACT

Objective: This study aimed to identify dental anomalies according to Angle's classification, skeletal anomalies according to Steiner's classification, and crowding regions in 2145 patients who presented for consultation at the Department of Orthodontics in the Faculty of Dentistry at Fırat University and to determine the distribution of orthodontic anomalies in the Eastern Anatolia Region.

Methods: In this study, anamnesis forms based on the radiographic and clinical examinations of 2145 patients with ages ranging from 6 to 29 years and who applied at the University were were evaluated their skeletal anomalies were classified according to Steiner's classification and dental anomalies were classified according to Angle's classification.

Results: Of the 2145 patients, 373 (64%) were women and 772 (36%) were men. For skeletal anomaly classification, anomalies in 1377 (64.2%) patients were classified as Class I, 569 (26.5%) as Class II, and 199 (9.3%) as Class III. For dental anomaly classification, anomalies in 957 (44.6%) patients were classified as Class I, 962 (44.8%) as Class II, and 226 (10.5%) as Class III. The distributions of these anomalies were also investigated in terms of age, sex, and crowding region.

Conclusion: In this sample of the Turkish population consisting of orthodontics patients in the Eastern Anatolia Region, statistically significant differences were observed in terms of age–skeletal and age–dental anomalies.

Keywords: Orthodontic anomaly, Angle's classification, crowding, prevalence, Turkish population

INTRODUCTION

Orthodontics is a field specialization in dentistry, which studies the relationship of teeth, tooth cavities, jaw and facial complexes with each other and soft tissues, and it attempts to correct abnormal ones by determining the ideal relationships between these or to prevent anomalies even before they are formed. Skeletal elements of the human face consist of numerous pieces and disproportions between these pieces result in orthodontic anomalies (1). Although many methods have been used in the classification of orthodontic anomalies to date, the most commonly used classification is Angle's classification (2). Edward H. Angle, the father of modern orthodontics, is recognized as the first person who limited his studies to orthodontics and the first orthodontics specialist in the world (3).

Several studies have been conducted on the distribution of orthodontic anomalies in different populations (4-19). Although the studies in the literature reported the frequencies of anomalies in different groups, few studies have been conducted within a certain population (20, 21). Therefore, this study aimed to identify orthodontic anomalies in patients who presented for consultation at the Department of Orthodontics in the Faculty of Dentistry at Fırat University, which serves a large area since the opening of the clinic, while investigating the frequencies of these anomalies in the Turkish population in the Eastern Anatolia Region, and their distribution in terms of age, sex, and crowding regions.

METHODS

Ethics Statement

This study was conducted in accordance to the ethical principles of medical research on human volunteers specified in the World Medical Association Declaration of Helsinki. Ethics Committee approval dated 17/10/2019 with meeting no. 15 and decree no. 07 was obtained from Fırat University for the research.

A total of 2145 patients from the ages of 6 to 29 years old, including 1373 females and 772 males, who presented for consultation at the Department of Orthodontics in the Faculty of Dentistry at Fırat University between 01/01/2016 and 31/08/2019 with orthodontic anomalies were evaluated in this study.

By investigating the anamnesis forms based on the radiographic and clinical examinations of the patients, the anomalies of patients were classified as Class I, Class II, and Class II for skele-

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Table 1. Distribution of Skeletal Anomalies According to Sex

	Female		Male		Total		
Skeletal Anomaly	**n**	**%**	**n**	**%**	**n**	**%**	**p***
Class I	901	65.6	476	61.7	1377	64.2	0.177
Class II	348	25.3	221	28.6	569	26.5	
Class III	124	9.0	75	9.7	199	9.3	
Total	1373	100.0	772	100.0	2145	100.0	

*p: Pearson Chi–Square

Table 2. Distribution of Dental Anomalies According to Sex

	Female		Male		Total		
Dental Anomaly	**n**	**%**	**n**	**%**	**n**	**%**	**p***
Class I	608	44.3	349	45.2	957	44.6	0.143
Class II Division 1	528	38.5	264	34.2	792	36.9	
Class II Division 2	56	4.1	34	4.4	90	4.2	
Class II Subdivision	48	3.5	32	4.1	80	3.7	
Total Class II	632	46.0	330	42.8	962	44.8	
Class III	133	9.7	93	12.0	226	10.6	
Total	1373	100.0	772	100.0	2145	100.0	

*p: Pearson Chi–Square

tal anomalies and as Class I, Class II Division 1, Class II Division 2, Class II Subdivision, and Class III for dental anomalies. Considering the fact that studies in the literature did not conduct dental classification in such detail, Class II Division 1, Class II Division 2, and Class II Subdivision anomalies were collected under a single Class II classification and examined once more to compare these findings with the findings of other studies. Additionally, classifications of the present crowding in all the patients were conducted according to their locations as incisor, premolar, and molar regions. The patients who previously went through treatment, who had insufficient material, and whose accuracies were doubted or hesitated about were excluded from the study.

Statistical Analysis

In the statistical analysis of the resulting data, IBM SPSS package software version: 24.0, (IBM SPSS Corp.; Armonk, NY, USA) was used to conduct the relevant analyses. The normality of the distribution of continuous variables was tested using the Shapiro Wilk test. In the descriptive analyses, categorical variables were presented as frequency and percentage [n (%)], and analyzed using Pearson's Chi-squared test. The results were interpreted at a 95% confidence interval while a p-value <0.05 was accepted as the statistical significance level.

Main Points:

- This study researched to identify orthodontic anomalies in patients who presented for consultation at the Department of Orthodontics in the Faculty of Dentistry at Fırat University.
- The aimed investigating the frequencies of orthodontic anomalies in the Turkish population in the Eastern Anatolia Region.

RESULTS

A total of 2145 patients were included in this study, including 1373 (64%) females and 772 (36%) males. The distributions of the skeletal anomalies, dental anomalies, and crowding in these patients according to sex are presented in tables 1, 2, 3, and 4. No statistically significant differences were found between sex and skeletal anomaly (p=0.177), dental anomaly (p=0.143), and crowding (p=0.915).

In the distribution of skeletal anomalies among the patients included in the study, it was observed that the Class I relationship was the most common in the 22–25 years group, Class II relationship was the most common in the 6–12 years group, and the Class III relationship was the most common in the 26–29 years old group (Table 5). Statistically significant differences were observed between age and skeletal anomaly (p<0.001).

In the distribution of the dental anomalies, it was observed that the Class I relationship was the most common in the 22–25 years

Table 3. Distribution of Dental Class II Anomalies According to Sex

	Female		Male		Total		
Class II Anomaly	**n**	**%**	**n**	**%**	**n**	**%**	**p***
Division 1	528	83.5	264	80.0	792	82.3	0.213
Division 2	56	8.9	34	10.3	90	9.4	
Subdivision	48	7.6	32	9.7	80	8.3	
Total	632	100.0	330	100.0	962	100.0	

*p: Pearson Chi–Square

Table 4. Distribution of Crowding Regions According to Sex

	Female		Male		Total		
Crowding	**n**	**%**	**n**	**%**	**n**	**%**	**p***
Incisors	1355	98.7	763	98.8	2118	98.7	0.915
Premolars	13	0.9	7	0.9	20	0.9	
Molars	5	0.4	2	0.3	7	0.3	
Total	1373	100.0	772	100.0	2145	100.0	

*p: Pearson Chi–Square

Table 5. Distribution of Skeletal Anomalies According to Age

	Class I		Class II		Class III		Total		
Age	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**p***
6–12	176	54.0	115	35.3	35	10.7	326	100.0	0.001
13–15	421	63.0	192	28.7	55	8.2	668	100.0	
16–18	501	67.5	187	25.2	54	7.3	742	100.0	
19–21	169	65.8	53	20.6	35	13.6	257	100.0	
22–25	71	77.2	12	13.0	9	9.8	92	100.0	
26–29	39	65.0	10	16.7	11	18.3	60	100.0	
Total	1377	64.2	569	26.5	199	9.3	2145	100.0	

*p: Pearson Chi–Square

group, Class II relationship was the most common in the 13–15 years group, and Class III relationship was the most common in the 26–29 years group (Table 6). Statistically significant differences were observed between age and dental anomaly ($p<0.005$).

In the patients included in this study, no statistically significant differences were observed between crowding region and age ($p=0.344$), sex ($p=0.915$), skeletal anomaly ($p=0.770$), and dental anomaly ($p=0.791$).

DISCUSSION

Due to the availability of various methods for the classification of orthodontic anomalies, it is challenging to establish a universal classification (6, 21, 22). Tang et al. (22) stated that Angle's classification neglects the ratio between the teeth and face, while Gravely and Johnson (23) stated that different people conducted different measurements in Angle's classification, which led to errors in the classification. Furthermore, Ackerman and Proffit (8) criticized certain aspects of Angle's classification of being weak. Despite all of these criticisms, Angle's classification remains the most commonly adopted and accepted classification to date, due to its reliability, simplicity, and practicality in clinical applications (2).

The anomalies of patients included in the study were classified into Class I, Class II, and Class III according to their skeletal anom-

Table 6. The Relationship between Dental Classification and Age Distribution

	Class I		Class II		Class III		Total		
Age Interval	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**p***
6–12	145	44.5	150	46.0	31	9.5	326	100.0	
13–15	279	41.8	325	48.7	64	9.6	668	100.0	
16–18	340	45.8	332	44.7	70	9.4	742	100.0	
19–21	119	46.3	104	40.5	34	13.2	257	100.0	0.005
22–25	49	53.3	30	32.6	13	14.1	92	100.0	
26–29	25	41.7	21	35.0	14	23.3	60	100.0	
Total	957	44.6	962	44.8	226	10.5	2145	100.0	

*p: Pearson Chi–Square

alies and into Class I, Class II Division 1, Class II Division 2, Class II Subdivision, and Class III according to their dental anomalies using Angle's classification. Given that previous studies in the literature did not conduct dental classification in such detail, Class II Division 1, Class II Division 2, and Class II Subdivision anomalies were regrouped under a single group, Class II, and evaluated once more. Another significant detail in the current study is that the studies in the literature conducted on the prevalence of anomalies in different populations were limited to investigating the distribution of dental anomalies. It is an authentic aspect of the current study to investigate the distribution of dental anomalies along with the distribution of skeletal anomalies. Furthermore, all the patients were classified according to the regions of crowding as incisor, premolar, and molar region, whereas crowding regions were investigated in terms of their relationship with the skeletal and dental anomalies, age, and sex.

In the classification of skeletal anomalies, it was observed that the number of Class I anomalies was higher than that of other anomalies. Of the 2145 patients investigated, 1377 (64.2%) had Class I anomalies, 596 (26.5%) had Class II anomalies, and 199 (9.3%) had Class III anomalies.

In the classification of dental anomalies, it was observed that the total number of Class II anomalies was higher than that of other anomalies. Of the 2145 patients investigated, 962 (44.8%) had Class I anomalies, 957 (44.6%) had Class II anomalies, and 226 (10.6%) had Class III anomalies. When Class II was evaluated in sections, it was determined that the 2154 patients included 957 (44.6%) patients with Class I anomalies, 792 (36.9%) patients with Class II Division 1 anomalies, 226 (10.6%) Class III anomalies, 90 (4.2%) patients with Class II Division 2, and 80 (3.7%) patients with Class II Subdivision anomalies. Compared with the findings of previous studies conducted on dental anomalies within the Turkish society, it was determined the number of dental Class II anomalies was increased (24, 25).

In this study, which investigated the Turkish population of patients in the Eastern Anatolia Region, it was observed that the number of Class I anomaly in the skeletal anomaly distribution and that of Class II anomaly in the dental anomaly were higher than the number of other anomalies. It was determined that there were statistically significant relationships between both skeletal and dental anomaly distributions and age groups.

The distribution of orthodontic anomalies in the Turkish population has not been reported in the literature in detail. In the study of Sarı et al. (24) conducted in the Turkish population, it was determined that 61.7%, 28.1%, and 10.2% of the patients were classified as Class I, Class II, and Class III, respectively. Although the rate of Class II anomalies was almost the same in this study, contrary to the current study, the rate of Class I anomalies was definitely higher in their study compared to the rate of Class II anomalies. This could be explained by the fact that this study evaluated patients who underwent treatments rather than all of the patients who consulted at their clinic. Additionally, the study conducted by Sari et al. (24) included only a dental classification and no skeletal classification.

In the study conducted by Yang (20) in Seoul, it was reported that 35.9% of the patients had Class I, 14.9% had Class II, and 49.1% had Class II anomalies. Here, it is observed that the frequency of Class III malocclusion is higher compared to those in the literature and the current study. This is believed to be due to ethnic differences.

In the current study, because only patients who wished to undergo orthodontic treatment were covered, it is not surprising to observe that the rate of Class I malocclusion is lower compared to the literature while the rates of Class II and Class III malocclusion are higher compared to the literature. This could be explained by the fact that the rate of anomalies in patients who presented to the orthodontics clinic with a desire to undergo treatment is higher compared to individuals who were investigated by prevalence studies conducted among individuals who did not have any desire to undergo treatment, such as students in schools, etc. Another reason could be the fact that the type of anomaly is a factor that directly affects patients' desire to undergo treatment. In the study conducted by Wilmont et al. (26), it was reported that the patients with severe Class II malocclusion had a greater

motivation to undergo orthodontic treatment compared to patients with Class III anomalies.

In the study conducted by Jones (19) with 132 patients in Saudi Arabia, the prevalence of orthodontic anomalies was investigated and it was reported that 53.8% of the patients had Class I anomalies, 33.3% had Class II anomalies, and 12.9% had Class III anomalies. Although the prevalence of the anomalies in this study was similar to those in the literature, it is believed that these results do not represent the prevalence of the population due to the rather small sample size. Furthermore, in this study, the prevalence of dental anomalies alone was investigated, while the prevalence of skeletal anomalies was not investigated.

CONCLUSION

When a sample of the Turkish population, which consisted of orthodontics patients in the Eastern Anatolia Region, was investigated, the following results were concluded:

1. Approximately two-thirds of the patients were females (64%). No statistically significant differences were observed in terms of sex-skeletal anomaly, sex-dental anomaly, and sex-crowding.
2. Statistically significant differences were observed between age–skeletal anomaly and age–dental anomaly.
3. It was determined that the most common skeletal anomaly was Class I anomalies while the least common anomaly was Class III anomalies.
4. It was determined that the most common dental anomaly was Class II anomalies while the least common anomaly was Class III anomalies.

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Original Research

Can Exercise Ameliorate Memory Impairment via PPAR Gamma Activation in Rats Fed A High–Fat Diet?

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ABSTRACT

Objective: This study aimed at determining the molecular effects of exercise on obesity treatment and cognitive impairment by examining the relationship between exercise (one of the non-pharmacological approaches) and PPAR-γ.

Methods: We classified 32 rats into four experimental groups: random control (C), obese (Ob), control exercise group (C+Ex), and obese exercise (Ob+Ex). The experimental groups were fed with a high-fat diet (45% fat) and standard rodent chow. The exercise program commenced after obesity diagnosis (30 min/day) and continued until the end of the study. At the end of the study, all rats underwent a learning–memory test in a Morris water tank, and the hippocampus of all rats were removed under anesthesia to study the PPAR gamma gene expression level.

Results: The escape latency was significantly different between the exercise groups and non-exercise groups ($p<0.05$). Molecular analysis revealed an increase in PPAR-γ gene expression levels in the exercise groups compared with that observed in the non-exercise groups, but no significant difference was found when comparing the gene expression levels between the groups ($p>0.05$).

Conclusion: PPAR-γ gene expression levels were upregulated in the exercise groups. In addition, the exercise groups performed better with regards to cognitive functions. This result provides a clue about the impact of exercise on the molecular pathway with respect to performance differences in cognitive function due to obesity.

Keywords: Experimental obesity model, exercise, learning–memory, PPAR gamma

INTRODUCTION

Obesity is mainly a preventable, complex, multifactorial disease that affects more than one third of the world's population (1). Based on World Health Organization's 2016 data, obesity has tripled worldwide since 1975 (2). While obesity is more common in developed countries, years, its incidence has also significantly increased in developing countries in recent years (3). If obesity continues to rise at the same rate, 2.16 billion people (38%) will reportedly be overweight and 1.12 billion people (20%) obese by 2030 (3).

Recent studies have shown that obesity affects the central nervous system spanning learning and memory centers in the brain (4). As mechanisms that cause cognitive impairment, obesity is responsible for systemic or chronic low-grade inflammation caused by increased levels of adiposity and cytokines (such as IL-6, TNF α, and CRP) (4).

Exercise reduces body weight, prevents obesity, reduces systemic inflammation and improves insulin resistance (5). In addition, physical exercise increases the levels of brain-derived neurotrophic factor (BDNF) and IGF-I in the hippocampus, leading to regeneration of neurons therein. This promotes differentiation of progenitor cells in the hippocampus and increase in BDNF gene expression. Both BDNF and IGF-1 (which provides stimulation of proliferation and differentiation of hippocampal progenitor neurons), are raised by astrocytes in hippocampus after exercise (6). Physical activity is an important factor that increases neuroplasticity (7).

PPAR-γ is a member of the nuclear receptor superfamily and a transcription factor (8). PPAR-γ plays a role in the differentiation of adipocytes, fatty acid storage and regulation of glucose metabolism (8). PPAR-γ is the target of antidiabetics, and its agonists (such as TZD; thiazolidinediones) improve insulin resistance against the effects of cytokines such as TNF-α (8). PPAR-γ agonists act as negative regulators of monocytes and macrophages and inhibit the production of proinflammatory cytokines (such as TNF-α, IL-β, IL-6), which cause neuroinflammation (9). Furthermore, PPAR-γ activation promotes BDNF expression level in the hippocampus, therefore improving cognitive deficit in diabetes patients (10).

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In this study, the experimental rats were deliberately fed a high-fat diet to become obese. The rats were then subjected to swimming exercise for treating obesity and improve their cognitive functions. This study intends to determine the molecular effects of exercise in the treatment of obesity and cognitive impairment by examining the relationship between exercise (a non-pharmacological approach) and PPAR-γ.

METHODS

Animals and Experimental Groups

Due to ease of care, resistance to diseases, and a universal acceptance in inducing experimental obesity model, Wistar albino rats (Rattusnovergicus var. albinus, Rodentia, Mammalia) were used in the study. Thirty- two male rats were randomly distributed into four experimental groups (eight rats in each group): Control (C), Control+Exercise (C+Ex), Obese (Ob) and Obese+Exercise (Ob+Ex). All rats were housed in standard plastic cages and maintained under 12 hourly light-dark cycle at constant temperature and humidity (23 ± 1°C and 40-50 %, respectively) in the Experimental Animals Research Center of Gaziantep University. The Local Ethics Committee of Animal Experiments for all surgical and experimental protocols approved our study through approval no:2017/16 and protocol no:32. All procedures were in compliance with NIH Guide for the Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals issued by the Local Ethics Committee of Animal Experiments of Gaziantep University. C and C+Ex groups were fed with standard laboratory feed while Ob and Ob+Ex groups were fed with a high-fat diet (45 % kcal fat) to induce obesity (Table 1). All groups were given *ad libitum* access to tap water throughout the study (for sixteen weeks) (ArdenIncCo., Ankara) (11). The body masses of all rats were measured weekly.

Exercise Protocol

Rats in the C+Ex and Ob+Ex groups were trained by swimming for 30 minutes per day, seven days a week (12). Morris water maze tank was used as a swimming pool. Each group swam separately. If a rat did not complete the 30 min-exercise, it was removed from the tank, after 30 min-exercise protocol, it swam for remaining time.

Morris Water Maze Test

The Morris water maze is a round pool of 180 cm in diameter and 70 cm in height. The reference memory of all animals was tested by applying spatial acquisition at the end of the 16th week (13). The tank was filled with tap water at 22 ± 2°C opacified with powder black food coloring (14). All rats performed four trials per day for four consecutive days. After the acquisition days, rats underwent a probe trial (15). Escape latency was recorded to assess memory by Etho Vision XT 11.5 video tracking system.

Main Points:

- We have found statistically significant difference between C – Ob and C+Ex -Ob+Ex.
- The escape latency was significantly different between the exercise groups and non-exercise groups.
- Molecular analysis revealed an increase in PPAR-γ gene expression levels in the exercise groups compared with that observed in the non-exercise groups, but no significant difference.

Hippocampus Isolation

The animals were anesthetized with ketamine and xylazine (80 mg/kg and 15 mg/kg, respectively), and hippocampus tissues were dissected from the whole brain.

Gene Expression Analysis

We extracted the total RNA from the hippocampus tissue using RN easy Mini kit (QIAGEN, No:74104) according to the manufacturer's protocols. RNA concentration was determined using Epoch Micro-Volume Spectrophotometer System (BioTech, Winooski, United States). The RNA samples were converted to cDNA using the Qiagen RT2 First Strand Kit (QIAGEN Cat No: 330404). Expression of the PPAR-γ gene was determined by using the Qiagen RT^{2}q PCR kit and primer assay for rat PPAR-γ gene (Cat No: PARN-149Z) GAPDH were used as housekeeping gene (Cat No: PPR06557B).

Gene expression data was obtained as ct values (ct = the cycle number at which logarithmic PCR plots cross a calculated threshold line). CT values were used to calculate ΔCT values (ΔCT =ct of the target gene - ct of the housekeeping gene). We obtained fold changes according to the transformation $2^{-\Delta CT} \times 10^{-4}$ and were expressed as arbitrary units.

Data Evaluation

To statistically analyze the weight values for the first eight weeks, the Student's t-test was applied using the Statistical Package for Social Sciences version 20.0.0 (IBM SPSS Corp.; Armonk, NY, USA)

Table 1. The Composition of High–fat diet

Component	% (Gr)
Casein	200.00
L–Cystin	3.00
Cornstarch	72.8
Maltodextrin	100.00
Sucrose	172.8
Soya oil	25.00
Cellulose	50.00
Lamb tail oil	177.5
Mineral Mix (S10026)	10.00
Dicalcium Phosphate	13.00
Calcium Carbonate	5.5
Potassium citrate (H2O)	16.00
Vitamin Mix (W10001)	10.00
Choline	2.0
Sum	858.1

Table 2. Average weight figures for the first eight weeks

Group	C	Ob	C+Ex	Ob+Ex
Mean±SD	307.00±6.00	385.00±44.00[δ]	315.00±11.00	377. 00±37.00[β]

[δ]The statistical significance between the control group (C) and the obese group (Ob) is illustrated in the table: $p<0.05$. [β]The statistical significance between the control exercise (C+ Ex) and the obese exercise group (Ob+Ex) is illustrated in the table: $p<0.05$.

Table 3. Morris Water Tank Learning/Memory parameters

	C	Ob	C+Ex	Ob+Ex
t(s)	51.25±12.34	74.38±14.14[a]	27.00±5.23[bc]	42.25±5.20[de]

[a]Statistically significant difference observed between the control (C) and obese groups (Ob): $p<0.05$.
[b]Statistically significant difference between the control (C) and control – exercise (C+Ex) groups: $p<0.05$.
[c]Statistically significant difference between the obese (Ob) and control – exercise (C+Ex) groups: $p<0.05$.
[d]Statistically significant difference between the obese (Ob) and obese – exercise (Ob+Ex) groups: $p<0.05$.
[e]Statistically significant difference between the control exercise (C+Ex) and obese – exercise (Ob+Ex) groups: $p<0.05$.

Table 4. Summary table for the elevation of the PPAR–γ gene expression levels

Control and Obese Group	Control and Control Exercise Group	Obese and Obese Exercise Group	Control Exercise and Obese Exercise Group
reduction by 2,914	elevation by 1,344	elevation by 3,127	reduction by 1,253
p: 0.524	p: 0.322	p: 0.6	p: 0.0045

and the weight data of the control, control exercise, obese, and obese exercise groups were described as mean ± standard deviation (mean ± SD) values.

We performed a one-way ANOVA Analysis with SPSS 20.0.0 for the "escape latency" data obtained from the results of the learning–memory experiment. Tukey's HSD was used post-hoc for the comparison of all groups, and the "escape times to the platform" data were provided in mean ± standard deviation values.

We applied the student's t-test to analyze the genetic data and compare two independent groups with the same version software. In the comparison of the groups, the expression level of the PPAR-γ gene was expressed in terms of the fold change values.

RESULTS

The Effect of a High-Fat Diet on the Weight of Rats

We observed a significant difference between the mean values of the control – obese group and the control exercise – obese exercise groups in the evaluation of the group weights for the first eight weeks ($p<0.05$) (Table 2).

Morris Water Tank Test Results

The test revealed a significant difference in the escape of latency between the C and Ob groups, C and C+Ex groups, Ob and Ob+Ex groups ($p<0.05$) (Table 3).

A video tracking system recorded their tracks of the rats asleep during the intervention period. The Ob group spent more time to find the platform, while the C+Ex group spent less time for the test purpose (Figure 1).

Gene Expression Results

On comparing PPAR-γ gene expression across the groups, we used the first group as reference and the second group as the target gene. In the obese group, the PPAR-γ gene expression level was 2,914 times lower than that in the control group (down-regulated), though no significant difference observed ($p>0.05$) (Table 4). Comparisons between other groups are summarized in Table 4 below and illustrated in Figure 2.

DISCUSSION

One of the approach to establish an experimental obesity model is to feed the experimental animals with a high-fat diet (16). In this study, obesity was induced in Wistar Albino rats using a high-fat diet. The rats were fed for eight weeks with 45% of the calories coming from fat. In the eighth week of the diagnosis of obesity, there was a difference between the average weight of the C and Ob groups, with a higher average weight in the obese group. A statistical evaluation of the average weights showed a significant difference between the C and Ob groups. It was observed that body weight increased with longer administration duration of the high-fat diet (11). Hundreds of hormones and adipokines are secreted from adipose tissue, and adipokines influence the peripheral and central nervous systems (17). Obesity leads to oxidative stress and the development of insulin resistance. The development of insulin resistance also reportedly causes cognitive impairment (18). It has been shown that when rats are fed a high-fat diet, oxidative stress occurs in the CA1 region of the hip-

Figure 1. a-d. Heat maps of acquisition days (group means). a, b, c, d represents C, Ob, C+Ex, Ob+Ex, respectively

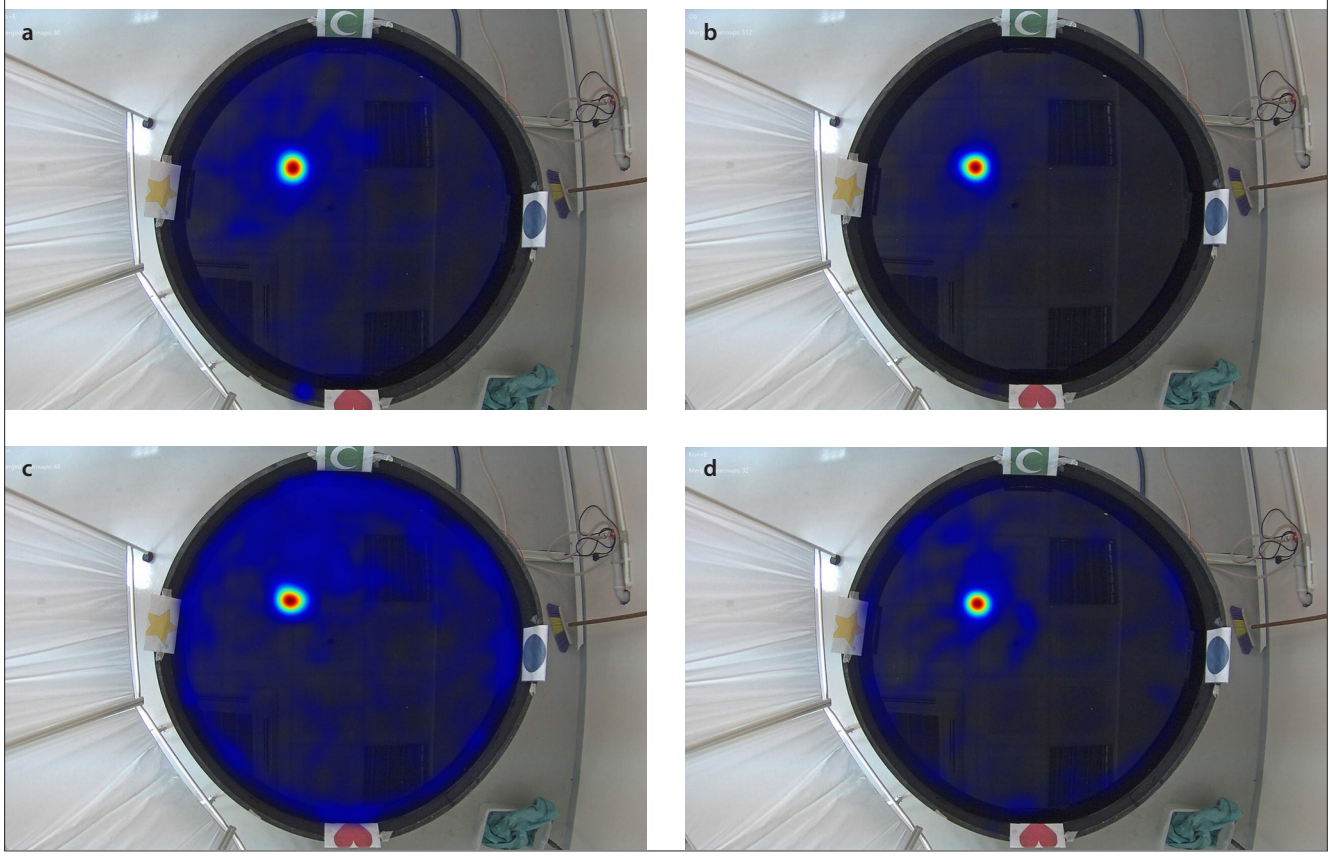
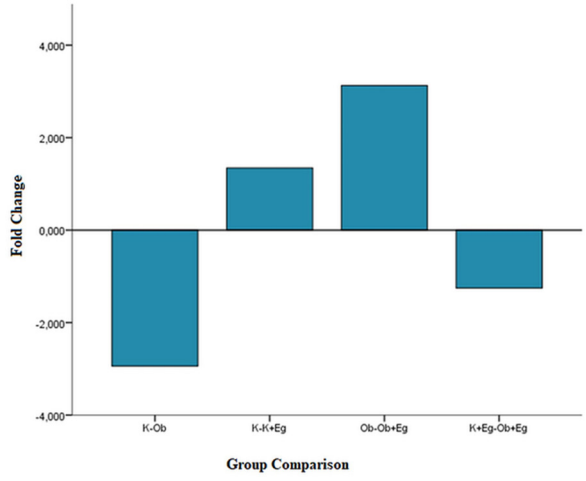


Figure 2. Chart showing the Fold changes of the PPAR γ gene expression level of the groups. C stands for control, Ob for obese, C+ Ex for control exercise and Ob + Ex for obese exercise groups



pocampus which is (important for learning and memory), and that the mitochondrial dysfunction of the brain worsens along with decreased synaptic plasticity and dendritic branching (19). In experimental epilepsy rat models, significantly elevated levels of proinflammatory cytokines such as L- 1β, IL-1Ra, IL-6, and TNF-α were observed in the hippocampus during epileptic episodes (20). Neuronal degeneration was observed in the dorsal and ventral CA1 and CA3 regions in the experimental animals of the same group (20).

Burning more calories is an effective strategy for treating obesity (21). With exercise, we accelerated glycogenolysis in the muscles and the liver, glycolysis in the muscles, citric acid cycle, and oxidative phosphorylation, lipolysis in the adipose tissue and the muscles, and fatty acid oxidation in the muscle (21). In a study in which an obesity model was developed, it was found that the adipocyte area was smaller in rats administered a swimming exercise compared with that observed in sedentary rats (22). We observed systemic low-grade inflammation as well as reduced neurodegeneration and cognitive impairment in rats that regularly exercised; furthermore, reduced levels of plasma IL-6, IL-8, CRP, and TNF-α in the central nervous system were also noted in them (23). Exercise alters the body's lipid profile, reduces obesity indicators, improves heart health, improves nutrient distribution and brain health by regulating cerebral blood flow, and also increases the volume of white and gray matter in the prefrontal, superior parietal, and temporal cortex (23). Physical activity improves hippocampal neurogenesis, increases synaptic plasticity, and significantly enhances hippocampal-based learning and memory (24). Many animal studies have demonstrated the functional benefits of exercise (25, 26). In addition, there are studies that show that treadmill and wheel running improve spatial learning and enhance hippocampal neurogenesis in experi-

mental animals. A study that examined the effect of exercise on synaptic plasticity and neurogenesis in rats found that exercise significantly increased BrdU-positive cells (bromodeoxyuridine) in the hippocampus of adult rats (25). Studies on humans have also shown that physical activity increases the volume of white and gray matter. One such study tested the association between a nine-year of physical activity and gray matter volume. We ultimately found that a greater frontal, occipital, entorhinal and hippocampal volume was associated with physical activity (26).

In the last week of this current study, the Morris Water Tank Spatial Memory Test revealed a significant difference between exercise and non-exercise groups in terms of the escape latency. Similarly, another experimental study applying a Morris water tank test to rats fed with a high-fat diet for eight weeks found that the escape latency was longer compared to the control group (27). Rats in which obesity was induced following a 13-week long high-fat diet performed a treadmill exercise. The memory test showed that the escape latency of the sedentary obese group fed with a high-fat diet was significantly longer than that of the sedentary group fed with a normal diet and the obese group fed a high-fat diet on an exercise regimen (28). In line with similar studies in the literature, obesity has been shown in this study to lead to changes in cognitive performance and that physical activity improves cognitive performance.

In this study, we isolated the hippocampus of animals from all experimental groups to better demonstrate the effect of this change in cognitive performance due to obesity and exercise. In this way, the change in the molecular level of the PPAR-γ gene expression for learning performance was studied for all groups. The activation of PPAR-γ has an important therapeutic potential in brain diseases playing a vital role in regulating proliferation, metabolism, differentiation, development, and the inflammatory response of the central nervous system. The genetic analysis in this current study showed that the PPAR-γ gene expression level in the hippocampal tissue reduced in the obese group compared to the control group. Alongside the problem of memory impairment due to obesity caused by a high-fat diet, the spatial reference memory test revealed a significant difference between the control and obese groups in terms of the escape times to the platform. In exercise groups, PPAR-γ gene expression levels increased in the control exercise group compared to the control group and in the obese exercise group compared to the obese group. Natural agonists for PPAR-γ activation include oleic, linoleic and polyunsaturated fatty acids (29). A study conducted using herring oil containing n-3 polyunsaturated fatty acids (PUFA) for PPAR-γ activation investigated the spatial learning performances and the hippocampal PPAR-γ gene expression of the groups (30). It was found that the PPAR-γ gene expression was significantly higher in rats administered with high doses of herring oil compared to rats administered with low or no doses at all. Having analyzed the escape times to the platform, it was found that it was significantly lower for the group that received high-dose herring oil than the group that received a low-dose and none at all (30). Another study investigated that activation of PPAR gamma by rosiglitazone (a synthetic ligand of PPAR-γ used in improvement of the cognitive impairment), improve spatial cognitive deficits by repairing expression of AMPA receptors in seipin knock out mice. Spatial memory deficits are caused by knocking out seipin gene in mice. The study has shown that seipin deficiency in neurons reduced the PPAR gamma level in the hippocampus compared with the wild type or control group but rosiglitazone repaired the spatial cognitive impairment caused by knocking out seipin and it has been shown in the Morris water maze by determining the escape latency compared to wild type. The escape latency was reduced in seipin knock out group treated with Rosiglitazone with respect to non-treated group and this decrease has shown a statistically significant difference between these two groups (31). The study by Gao et al. investigated the effect of pioglitazone, another synthetic ligand of PPAR gamma, on learning, and memory. They induced experimental type 2 diabetes mellitus and used pioglitazone to treat the animals. The results have shown that, pioglitazone treated group had lower Fasting Blood Glucose compared to non-treated diabetic and control group and the groups shown a statistically significant difference. In Morris water maze test, pioglitazone treated group revealed a significantly lower escape latency than non-treated diabetic group but higher than control group (32).

The synaptic function is based on special neural extensions called dendritic spine (33). These are the neurotrophins where BDNF is among the mediators that increase dendrite density and synaptic plasticity (30, 33). PPAR-γ agonists (such as rosiglitazone) increase synaptic plasticity, prevent loss of dendritic spine and improve synaptic function in the hippocampus (33). Exercises trigger expression of BDNF in the hippocampus (34). This way, BDNF regulates neuronal survival, neurogenesis, synaptogenesis and synaptic plasticity (34). In this study, we analyzed PPAR-γ gene expression levels from tissue samples obtained from the hippocampal tissues of the experimental groups. As a result, we observed an increase in gene expression in the exercise groups, though not significant (Table 4). Further studies are recommended to carry out extensive molecular investigations and to increase the number of samples to assess learning performance.

CONCLUSION

To conclude, our study revealed that impairment in cognitive functions (such as memory), occurs in obesity caused by a high-fat diet. According to the learning–memory test data from the experimental groups, exercise has a beneficial effect on cognitive functions. Hence, we observed in increase in the PPAR-γ gene expression level as well as better outcomes from the learning–memory test in the exercise groups.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University (2017/16, 07.06.2017).

Informed Consent: All participants signed informed consent forms before study inclusion.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.A., C.D., S.O.B., İ.Y.; Design -E.A., C.D., S.O.B.; Supervision – E.A.; Resources - E.A., C.D.; Materials - İ.Y., C.D., S.O.B.; Data Collection and/or Processing - İ.Y., C.D., S.O.B.; Analysis and/or Interpretation - İ.Y., C.D.; Literature Search - İ.Y., C.D., S.O.B.; Writing Manuscript - S.O.B., C.D., E.A., İ.Y.; Critical Review - E.A.; Other – E.A., C.D., S.O.B., İ.Y.

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Original Research

Effect of Past and Current Dental Treatment on Children's Dental Anxiety Status and Oxidative Stress Biomarkers: A Pilot Study

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ABSTRACT

Objective: This study aimed to detect dental anxiety caused by past and current dental treatment in children using saliva biomarkers and to investigate the correlation between these markers and psychometric analysis.

Methods: A total of 43 children aged 6–13 years were recruited for this study. The participants were divided into two groups: those with dental treatment experience (G1) and those who presented to the dentist for the first time (G2). Anxiety scale data and saliva samples were obtained. Oxidative stress (OS) markers, namely, total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI), were analyzed from the saliva samples. After current treatments such as fluoride application or restorative procedures, anxiety scale data, and saliva samples were obtained.

Results: TAC and OSI values were higher in G1 than in G2 at baseline ($p<0.05$), and anxiety scale values were similar in both groups ($p>0.05$). After the current treatment, a significant decrease in TAC and OSI values was found in G1 ($p<0.05$), but no significant difference was observed in G2. Anxiety scale values were significantly decreased after treatment in G2 ($p<0.05$). Although a strong correlation was found between baseline TAC, TOS, OSI, and post-treatment TAC and OSI values ($p<0.001$), no correlation was noted between OS biomarkers and anxiety scale values ($p>0.05$).

Conclusion: These results suggested a potential relationship between anxiety and OS biomarkers, but additional studies are needed to understand the relationship between dental anxiety and pathophysiological changes in OS biomarkers.

Keywords: Dental anxiety, oxidative stress, dental treatment, anxiety scale

INTRODUCTION

Dental anxiety is a state of constant and extreme fear of dental stimulants and procedures. If a patient has anxiety, the frequency of dental caries may increase due to the decrease in the frequency of visit to the dentist (1). Although its etiology is unclear, three mechanisms were proposed for the development of dental anxiety: indirect development via vicarious learning/modeling, direct development through direct conditioning, or a person's inherent personality traits (2). In the literature, the term state anxiety, which means "anxiety at the present moment," has also been defined (3). While dental anxiety involves the individual emotional state for dental treatment shaped by experiences or modeling, state anxiety can reflect a situation-specific emotional experience for a particular dental procedure, and studies have shown that it can fluctuate at different treatment stages (4). Thus, treatment planning should be directed in a flexible and patient-based manner in stages, and treatment should be started with the least fearful, painless, and nontraumatic techniques, especially in children with anxiety and no dental treatment experience (5).

Four different measurement techniques are recommended for the assessment of dental anxiety in children. These include projective tests, psychometric techniques, various scales used to examine and evaluate a child's behavior during a dental visit, and physiological measurements (6). It is difficult to talk and agree with younger patients. Thus, studies have recommended the use of simple and visual projective methods that do not require verbal communication and are applicable in this patient group (7, 8). In psychometric techniques that include self-report measurements, a dental anxiety score is obtained by asking children directly about their concerns with the help of a scoring scale. This method is usually performed through a survey or an interview (9). One of the most widely used psychometric techniques in children is the modified child dental anxiety scale (MCDAS) (10). This scale has been modified for easier application in younger

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children. The MCDAS faces version ($MCDAS_f$), which was created by adding a cartoon faces rating scale to the original numerical form, is a versatile scale that can be applied to children aged 5–12 and with limited cognitive function (11).

In addition to psychological changes in dental anxiety observed in children, dental anxiety may lead to the secretion of some mediators, that is, biochemical changes, as a result of stress or mood disorders affecting the adrenal system. Studies have suggested that continuous stimulation of the hypothalamus–pituitary–adrenal axis due to prolonged stress causes oxidative damage (12, 13). Oxidative stress (OS) results from increased production of reactive oxygen species and reduced concentration/activity of antioxidants responsible for their neutralization (14). Imbalances in the levels of free radicals and antioxidant-reactive oxygen species can play an important role in the onset and development of various inflammatory oral pathologies (15). Total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) are biochemical markers that can be used to measure OS. TAC is the total capacity of both enzymatic and non-enzymatic antioxidants, TOS provides an assessment of the content of all oxidants in a sample, and OSI shows the relationship between antioxidant mechanisms and oxidant concentrations (16). Evaluation data of these OS biomarkers in the saliva have also been used to identify or describe anxiety-causing events such as dental treatment in children (17).

To the best of our knowledge, no study has evaluated the relationship between dental experience-based anxiety and OS biomarkers. In the light of this, this pilot study aimed to detect dental anxiety caused by past and current dental treatment in children by using saliva biomarkers and to investigate the correlation between these markers and psychometric analysis. The study hypotheses were as follows:

- Increased OS biomarker values would be obtained in children with dental treatment experience compared with children without any dental treatment experience.
- There would be a significant decrease in OS biomarkers in both groups after the procedure.
- The anxiety scale data obtained at baseline and after treatment would be correlated with OS biomarkers.

METHODS

Participants

In this study, ethics committee approval was obtained from the Gaziantep University/Turkey Clinical Research Ethics Committee (2019/153). The study included 43 patients aged 6–13 years.

Main Points:

- High OS biomarker values decreased significantly in patients who had undergone surgical treatment.
- Decreased anxiety scale values were observed in each group, especially in G2, after the treatment.
- A strong correlation was found between OS biomarkers at baseline and after treatment.

Patients with systemic and congenital disorders, receiving chemotherapy and radiotherapy, taking medications that affect the saliva such as antidepressants and corticosteroids, and giving insufficient saliva samples were excluded from the study. Before the procedure, children and parents were informed about the study and consent forms were obtained. The participants were divided into two groups: those with dental treatment experience including surgical procedures (G1, n=18) and those who visited a dentist for the first time (G2, n=25). A questionnaire including demographic data and $MCDAS_f$ data was administered to the participants.

Anxiety Scale

The $MCDAS_f$ questionnaire contains eight questions about dental procedures that can trigger stress such as local anesthesia, sedation, general anesthesia, and tooth extraction, and five faces indicating a visual emotional state for each question. Scores range from 8 to 40: the happiest and unhappiest faces were rated 1 and 5, respectively. The Turkish version of this scale was filled out by the dentist in an interview, in line with the answers given by the patient.

Collection of Saliva Samples and TAC, TOS, and OSI Analyses

The unstimulated saliva samples of the participants were collected by a researcher between 9 and 12 o'clock in the morning, which was 1 hour after the participants brushed their teeth. During sample collection, the children were seated with their heads slightly down and their saliva was allowed to accumulate in the mouth for 2 min. Later, they were asked to spit the collected saliva into the pet cups. In addition, they were asked not to move their mouth, buccinator muscles, tongue, and lip during the procedure to increase the amount of saliva, and not to swallow them. The collected saliva was transferred to Eppendorf microtubes through volume samples and centrifuged (NF 200 centrifuge machine) at 5000 rpm for 10 min. All samples were stored at −20°C after centrifugation, and TAC, TOS, OSI levels were analyzed using an enzyme-linked immunosorbent assay kit (DRG Salivary Cortisol ELISA; DRG International, Inc., USA) according to manufacturer's instructions.

After baseline data were obtained, fluoride application or restorative procedures were performed, and then anxiety scale data and saliva samples were obtained.

Statistical Analysis

The compatibility of all numerical data to normal distribution was tested by the Shapiro–Wilk test. Mann–Whitney U test was used to compare non-normally distributed variables between groups. Analysis of variance and Tukey comparison tests were used to compare normally distributed numerical data, and Kruskal–Wallis and allpairwise tests were used to compare non-normally distributed data. Relationships between numerical variables were tested with Spearman rank correlation coefficient. The average statistics are presented as mean ± standard deviation for introductory statistics. SPSS Statistics v22.0 (IBM SPSS Corp.; Armonk, NY, USA) for Mac was used in the analysis. $p<0.05$ was considered significant.

Table 1. Mean Oxidative stress marker values and anxiety scale data at baseline and after treatment

	TAC		TOS		OSI		Scale	
	B	AT	B	AT	B	AT	B	AT
G1 (n=18)	7.37±2.63*	5.44±2.39*$^{\delta}$	4.54±2.64	5.07±1.91	2.16±1.48*	1.18±0.57$^{\delta}$	19.32±5.53	18.17±5.3
G2 (n=25)	3.08±1.89	3.68±2.19	5.51±2.44	5.24±2.03	0.76±0.77	0.89±0.7	20.17±5.26	17.09±4.9$^{\delta}$

*Statistical significance between parameters between groups
$^{\delta}$Statistical significance relative to baseline.
TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index; B, baseline; AT, after treatment

Table 2. Correlation coefficient values of the parameters

	TAC_b	TOS_b	OSI_b	TAC_at	TOS_at	OSI_at	Scale_b	Scale_at
TAC_b	1.000	−.211	.793**	.515**	−.136	.447**	.081	.112
TOS_b	−.211	1.000	−.702**	.056	−.124	.049	−.005	.034
OSI_b	.793**	−.702**	1.000	.280	−.046	.250	−.035	−.021
TAC_at	.515**	.056	.280	1.000	−.133	.766**	.086	.063
TOS_at	−.136	−.124	−.046	−.133	1.000	−.688**	−.101	−.276
OSI_at	.447**	.049	.250	.766**	−.688**	1.000	.130	.264
Scale_b	.081	−.005	−.035	.086	−.101	.130	1.000	.584**
Scale_at	.112	.034	−.021	.063	−.276	.264	.584**	1.000

** p<0.001 (strong correlation); * p<0.05 (correlation)
TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index
b, baseline; at, after treatment

RESULTS

No statistically significant difference in baseline anxiety scale values was found between G1 and G2 (p>0.05). Anxiety values were decreased after treatment in both groups. While the anxiety scores obtained after dental procedure in G2 showed a statistically significant decrease compared with the baseline values (p<0.05), the decrease in the anxiety scoresin G1 was not statistically significant (p>0.05) (Table 1). In the analysis of baseline OS biomarkers, TAC and OSI values were higher in G1 than in G2, and this increase was statistically significant (p<0.05). After the current treatment, a significant decrease in TAC and OSI values was obtained in G1 (p<0.05). No significant difference in the values of OS biomarkers at baseline and after treatment was noted in G2 (p>0.05) (Table 1).

In the correlation analysis, a strong correlation was found between baseline TAC, TOS, and OSI values and post-treatment TAC and OSI values (p<0.001). Although no correlation exist between OS biomarkers and anxiety scale values (p>0.05), a strong correlation was found in baseline and post-treatment anxiety scale data (p<0.001) (Table 2).

DISCUSSION

Studies have shown that OS biomarkers increase in body fluids such as serum and saliva during or after stress. A study has also suggested that OS biomarkers are potential markers of stress (18). OS is considered an important component of various diseases. From the oxidation of deoxyribonucleic acid to proteins, lipids, and free amino acids, numerous methods have been developed and used in nearly all diseases to measure the extent and nature of OS (19). While OS measurements in the spinal cord and tissues are limited to certain diseases, measurements in venous blood and urine samples are the most common methods in clinical practice (20). As researchers show that saliva contains oxidation biomarkers similar to serum, saliva is increasingly used to measure OS markers (21). In addition, saliva analysis has been proposed as a noninvasive and low-cost method for screening OS (22). In pediatric patients, this method can be preferred to prevent potential stress that may occur during sample collection. Moreover, saliva is considered the first line of defense against OS (23). Therefore, in this study, saliva samples were used to evaluate the relationship between OS biomarkers and anxiety caused by dental treatment in children.

Many studies have shown the relationship between OS and periodontal diseases (23, 24), malignant oral disorders (25),various systemic diseases (14, 26), and dental caries (27, 28). However, to the best of our knowledge, no study has evaluated the relationship between dental experience-based anxiety and OS biomarkers. For this reason, this pilot study was conducted. In this study, children without dental treatment experience will have lower OS biomarker values than those with dental treatment

experience, as shown in the statistically significant increase in baseline TAC and OSI values in G1. TAC describes the combined ability of a group of enzymes, including saliva antioxidants, saliva peroxides, saliva uric acid, and several small enzymes (29). Saliva peroxidase catalyzes the peroxidation of thiocyanate ion to produce oxidation products. This prevents the growth and metabolism of many microorganisms, thereby inhibiting caries or at least slowing the progression of caries (27). In this study, increased TAC and OSI values in G1 may be due to the patient's past dental treatment experience being a surgical procedure. Statistically significant decrease in post-treatment TAC and OSI values in G1 also shows that dental anxiety affects OS biomarkers. Increased OS biomarker levels in a child who had undergone surgery decreased with more easily tolerated processes, such as preventive or restorative applications. In G2, no significant difference was found in baseline and post-treatment values of OS biomarkers. In this case, the hypothesis that there would be a significant decrease in OS biomarkers in both groups after the procedure could be rejected. However, a statistically significant decrease in anxiety scale value was found. Perhaps, saliva samples collected immediately after the procedure does not reflect any changes in OS biomarker levels. Similar to the present study, Zarbanand et al. (30) reported no significant change in TAC values before and after treatment. However, Al Anaziand et al. (28) reported a decrease in TAC values in saliva measurements at 1 week and 3 months after the treatment.

The third hypothesis that initial and post-procedure anxiety levels would correlate with OS biomarkers can also be rejected because no correlation was noted between these two parameters. However, considering other results of this study, this correlation could be achieved by increasing the number of patients. More comparable results could be obtained by increasing the number of patients, differentiating the dental treatment experience, and repeating saliva measurements at regular intervals after treatment.

This study has several limitations. First, this was conducted as a pilot study. Second, although dental anxiety scores were recorded, general anxiety scores and psychiatric stress were not evaluated. Third, even if the analysis was performed in a pediatric population, variation between age groups may affect the secretion of saliva. This could result in differences in salivary biomarkers.

CONCLUSION

Within the limitations of this study, increased OS biomarker values in patients who has undergone surgery decreased significantly after treatment. In patients without dental treatment experience, baseline and post-treatment anxiety scale data decreased, but no significant change in salivary parameters was observed. Although this finding is unclear, there may be a relationship between anxiety and OS biomarkers. Additional studies are needed to understand the relationship between dental anxiety and pathophysiological changes in OS biomarkers due to dental treatment experience.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of Gaziantep University (2019/153).

Informed Consent: All participants were informed and consent forms were obtained.

Peer-review: Externally peer-reviewed.

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Original Research

The Predictive Role of Heat Shock Proteins 27 and 60 in Pediatric Patients with Ataxia Telangiectasia

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ABSTRACT

Objective: This study aimed to measure the levels of heat shock proteins (HSP) 27 and 60 as mediators for hypoxia and tissue injury in pediatric patients with ataxia telangiectasia (AT). Another aim was to determine the prognostic role of HSP 27 and 60 in AT.

Methods: This study analyzed the data of AT patients (n=15) aged 4-16 years and of age-matched healthy controls (n=20). HSP 27 and 60 levels were measured from serum samples of AT patients using an enzyme-linked immunosorbent assay and compared with those of healthy controls.

Results: Serum levels of HSP 27 and 60 were higher in patients with AT than in age-matched healthy controls (p=0.010 and p=0.000, respectively).

Conclusion: In AT patients, levels of HSP 27 and 60 are increased to prevent tissue injury; therefore, treatment targeting HSPs may prevent disease progression and development of secondary malignancy in this patient population.

Keywords: Ataxia telangiectasia, heat shock protein 27, heat shock protein 60, chaperone, children

INTRODUCTION

Ataxia telangiectasia (AT) or Louis–Bar syndrome (OMIM 208900) is a pediatric autosomal recessive disorder characterized by progressive neuromotor dysfunction, variable immunodeficiency, genomic instability, hypersensitivity to ionizing radiation, and predisposition to malignancies (1, 2).

Most AT patients die of recurrent pulmonary infection due to severe immune deficiency or cancer development following chromosome rearrangements. An early symptom of AT is ataxia, which is the lack of movement coordination and the inability to control body posture. Ataxia is caused by neurodegeneration and, in particular, by death of Purkinje cells (3).

The ataxia telangiectasia mutated (ATM) gene, also referred to as ATM serine/threonine kinase that is located on the long arm (q) of chromosome 11 between positions 22 and 23, controls several aspects of cell cycle and promotes repair of double-strand breaks. As a result, clinical manifestations lead to the absence of ATM. Previous studies have linked this defect to cancer, sterility, radiosensitivity, and neurodegeneration (1). Identification of the molecular mechanism of ATM gene function in neural tissues provides insight into the mechanisms of neurodegeneration.

Heat shock protein 27 (HSP 27) is a molecular chaperone expressed in cells under various stress conditions and offers cytoprotection from various deleterious molecular events (4). HSP 60 influences the development of autoimmune diseases, including rheumatoid arthritis in humans, systemic sclerosis, Parkinson's disease, psoriasis, Kawasaki disease, Behcet's disease, and early-onset atherosclerosis (5). The important role of HSP 60 in neuron maintenance has been supported by the finding that homozygosity for a missense mutation in the HSPD1 gene (p.Asp29Gly) is associated with a fatal hypomyelinating leukodystrophy (Mit-CHAP60 disease) (6).

In this study, we aimed to measure the levels of HSP 27 and 60 as mediators of hypoxia and tissue injury in pediatric patients with AT and determine their value as prognostic indicators of AT.

METHODS

This study included 15 patients (7 boys and 8 girls) aged 4–16 years who were diagnosed with AT (AT group) and treated and followed up at Gaziantep University Faculty of Medicine Pediatric Neurology Department between March 2014 and April 2015. Twenty (9 boys and 11 girls) healthy age-matched subjects served as controls (control group). Patients who had symptoms or diseases other than AT syndrome (e.g., eating disorders, endocrine, metabolic, hepatic, renal diseases, etc.) were excluded from the study. The diagnosis of AT syndrome was based on history, neurologic examination, and magnetic resonance imaging data. Upon physical examination, ataxic gait and telangiectasia

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were determined. To rule out ataxia associated with oculomotor apraxia, patients were examined for oculomotor apraxia, and the absence of sensory motor neuropathy findings in electromyography and the presence of normal deep tendon reflexes were documented. Levels of creatinine kinase were within normal ranges. Genetic mapping of the ATM gene was not performed due to technical impossibilities. The International Cooperative Ataxia Scale (ICARS) (7) was used for evaluating ataxic score, and the results associated with ICARS and HSP 27 and 60 were evaluated by statistical analyses.

Blood samples were collected from the patients confirmed to have no fever and/or signs of infection. To measure HSP levels in the AT group and control group, 5 mL of blood was collected in gel separator tubes. After centrifugation (4,000 rpm for 10 min), serum was separated and stored at – 80°C. HSP 27 and 60 (AssayPro, USA) levels in the serum samples were analyzed using an enzyme-linked immunosorbent assay (ELISA) method.

To measure HSP 27 and 60 levels, 50 µL of samples from each specimen was added to the wells, followed by 2-h incubation. After incubation, 50 µL of biotinylated HSP 27 and 60 primary antibodies were added to the wells and incubated for 2 h. Thereafter, the wells were washed, and 50 µL of streptavidin-peroxidase conjugate was added, followed by the addition of a stop solution. The mixture was analyzed with an ELISA reader (Biotech Instruments, USA) at 450 nm. The relationship between the levels of HSP 27 and 60 with alpha fetoprotein, ataxia score, age, and sex in the AT group was examined.

Gaziantep University's ethical committee approved the study, and informed consent was given by all patients and families of the healthy controls.

Statistical Analysis

All analyses were performed using SPSS 13.0 (SPSS Inc.; Chicago, IL, USA) statistical software package. Descriptive results were expressed as mean±standard deviation. The significance of the differences between repeated measurements in each patient was evaluated using the Wilcoxon matched-pairs test. The significance of the differences in terms of seizure type and sex was compared using the Mann–Whitney U test. Correlations between parameters were evaluated using the Spearman correlation test. For all tests, a p-value less than 0.05 indicated a statistically significant difference. The diagnostic validity analysis included receiver operating characteristics (ROC) curve analysis, with specific values for the area under the curve, the significance of the area, and confidence intervals. Sensitivity (SS), specificity (SP), positive and negative predictive values, and positive and negative likelihood ratios are also expressed in the analysis. The calculation of the overall accuracy (ACC) of the prediction was based on previous parameters. The cut-off point for measurement the calculation of which is based on the coordinates of the ROC curve is also presented in the analysis. The positive likelihood ratio is calculated as sensitivity/1 - specificity. The negative likelihood ratio is calculated as 1- specificity/sensitivity. The overall accuracy is calculated as the sum of the positive and negative predictive values.

Main Points:

- HSPs provide protection for normal cellular functions, including stabilizing cellular cycle and inhibiting apoptosis due to unknown mechanisms.
- HSP 27 and 60 levels were found to be significantly higher in patients with ataxia telangiectasia compared to controls.
- Development of therapies targeting HSPs could prevent disease progression and the development of secondary malignancies in patients with AT.

RESULTS

The mean age of the AT group and control group was 10.2±3.48 (4–16) years and 10.1±3.56 years, respectively. No statistically significant difference was found in the mean age (p=0.987) or sex (p>0.05).

The mean serum HSP 27 levels in the AT group and control group were 3.238±3.566 ng/mL and 0.537±0.253 ng/mL, respectively. This result indicates that the HSP 27 levels in the AT group were statistically different from those in the control group (p=0.010) (Table 1).

The mean serum HSP 60 levels in the AT group and control group were 2.142±1.514 ng/mL and 0.357±0.284 ng/mL, respectively. A statistically significant difference was found between the groups (p=0.000) (Table 1).

In the AT group, serum HSP 27 and 60 levels was not statistically significantly associated with alpha fetoprotein levels and ataxia score (ICARS) (7) (p>0.05). In addition, serum HSP 27 and 60 levels were not associated with patient age and sex (p>0.05).

Receiver operating characteristic (ROC) curve analysis of serum HSP 27 and 60 levels:

As depicted in Figure 1, the ROC curve analysis revealed an area under the curve (AUC) of 0.758 and 0.953 for HSP 27 and 60, re-

Figure 1. ROC curve for serum HSP 27 compared HSP 60. HSP 60 shows higher AUC than HSP 27

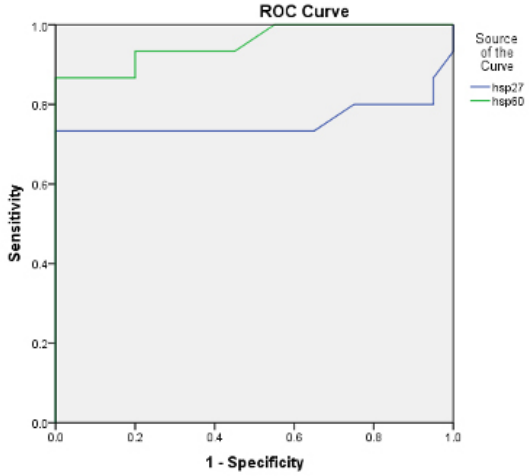

Table 1. Mean±SD values of the AT group and control group

	AT group	Control group	p
HSP 27	3.238±3.566 ng/mL (min–max/0.04–12.2)	0.537±0.253 ng/mL (min–max/0.08–0.93)	p=0.010
HSP 60	2.142±1.514 ng/mL (min–max/0.35–6.68)	0.357±0.284 ng/mL (min–max/0.03–0.91)	p=0.000

spectively. Serum HSP 60 showed higher AUC than HSP 27 as well as higher sensitivity to stressful conditions implicated in the pathogenesis of AT, indicating the potential of HSP 60 serum levels to serve as an early diagnostic marker.

DISCUSSION

HSPs were first identified as increased levels of polypeptides in response to excessive heat in *Drosophila melanogaster*. HSPs provide protection for normal cellular functions, including stabilizing cellular cycle and inhibiting apoptosis due to unknown mechanisms. HSP may be divided into the following groups according to their molecular sizes: high-molecular mass HSPs (110, 90, 70–72, and 55–60 kDa) and small HSPs (HSP 27, ubiquitin, αB-crystallin) (4). HSPs are expressed at low levels in most eukaryotic cells but are induced by cellular stress such as increased temperature, radiation, chemical exposure, oxidative stress, and physiological and pathological stimuli (8, 9).

HSP 27 is expressed in various tissues such as in cancer and in the brain, and it is responsible for resistance to cell damage and development of tumors. Increased HSP 27 levels can prevent apoptotic mechanisms. Recent studies have shown that this protein has protective effects against neurodegenerative diseases, including Huntington disease, amyotrophic lateral sclerosis, and Alzheimer's disease (5, 10). The protective effects and expression of HSP 27 and 60 are potential targetable proteins for therapy in AT pathology.

The HSP family can provide cellular protection for maturation under normal conditions and may enhance the progression of tumor cells through the cell's life cycle. In addition, new drugs for each HSP may affect the mechanisms of the cell's life cycle. Therefore, HSPs may be a promising and important target for the development of new therapies, especially in cancer. A recent study (11) revealed that HSPs were upregulated in the hippocampus, inferior parietal lobe, and cerebellum in patients with mild cognitive impairment. The authors suggested that an alteration in the chaperone protein function contributed to the pathogenesis and progression of Alzheimer's disease. Targeting HSPs could be a therapeutic approach to delay disease progression.

Hyperthermia enhances X-ray killing in cells derived from both normal and AT individuals. However, normal cells that were allowed to recover at 37°C between heat and X-ray treatments do not exhibit heightened radiosensitivity, whereas AT cells remain sensitive (12). Thus, heat, or HSPs induced by heating, may modulate ATM protein function and affect cell survival. Heat and inducible HSPs can change ATM gene functions and tend to be radiosensitive.

Akbar et al. (13) reported that HSP 27 expression particularly decreased neuronal cell death in the hippocampal CA3 region, resulting in significant reductions in kainate-induced seizure severity and mortality rate. In HSP 27 transgenic animals, modulation of caspase-3 induction and apoptotic features were responsible for the reduction of seizure severity in HSP 27. These studies indicate that HSP 27 playsa major neuroprotective role in the central nervous system.

HSP 60 is a molecular chaperone that ensures correct folding of mitochondrial proteins in mitochondria. It is a predominant mitochondrial protein with important homeostatic functions. Induction of HSP 60 has been demonstrated in cerebral ischemia models, possibly reflecting mitochondrial stress. Similarly, increased HSP 60 levels were found in the cerebrospinal fluid (CSF) of children with traumatic brain injury (TBI), and this increase was independent of the severity of trauma (14). Increased HSP 60 levels in the CSF were suggested to reflect the severity of mitochondrial stress or damage after TBI. A study involving patients who had acute cerebral infarction and transient ischemic attack showed that serum HSP 60 levels were higher in these patients than in controls and that these levels increased as a response to neuronal injury (15).

According to Xi et al. (16), HSP 27 may have protective role against the intractable epilepsy. HSP BAP1 (heat shock 27-kDa-associated protein 1), a protein inhibiting the function of HSP 27, is abnormally expressed in the neocortex of patients with intractable epilepsy and may have inhibit the protective role of HSP 27 in epilepsy. A similar study established that overexpression of HSP 27 was used as a marker, because seizures increased in the cortical regions due to stress. They reported that HSP 27 levels in intractable epileptic patients undergoing temporal lobe surgery were higher than HSP 27 levels in autopsied brain tissue from the control group without known neurologic disease (5). In addition, Chen et al. (17) found massive expression of HSP 27 in the frontal cortex of AT patients compared with controls in postmortem autopsy. They did not find a difference in the levels of other stress proteins (HSP 70, αB-crystallin, and prohibitin) in cortical and cerebellar tissues. Because of an increase in HSP levels in the frontal cortex of AT patients, they claimed that HSP 27 prevents increased oxidative stress.

As one limitation of this study, patients could not be tested for the ATM gene mutation. However, our patients were diagnosed with AT clinically based on examination and radiological findings, and they were followed up by our department. We could not find a significant correlation between HSP levels and ataxia scores. However, HSP 27 and 60 levels were analyzed in a small number of patients. None of the enrolled patients had secondary malignancy. Thus, these proteins should be investigated in a larger group of AT patients with or without malignancy.

CONCLUSION

Despite the limited number of patients, levels of HSP 27 and 60 were significantly higher in pediatric patients with AT than in healthy controls. The increased levels and their preventive role against tissue damage or as predictors of cancer risk should be investigated in future studies. This would help in the determination of follow-up and treatment options. As there is growing knowledge on extracellular chaperones, chaperone networks, and therapeutic use of chaperones (i.e., chaperonotherapy), awareness of chaperonology will most likely be widespread in the near future for AT patients. Since the analyzed patients did not have malignancy, we thought that increased HSP levels may prevent tissue injury. We believe that the development of treatments and target HSPs may prevent disease progression and development of secondary malignancies in AT patients. HSPs have promising potential as diagnostic markers and as prognostic indicators due to their ability to improve cell performance during stress. For this reason, HSPs may be a plausible target in AT.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University of School of Medicine (2015/92).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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

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Original Research

The Importance of Thiol/Disulfide Homeostasis- and Ischemia-Modified Albumin Levels in Acute Coronary Syndrome and Their Relationship with Angiographic Scoring Systems

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ABSTRACT

Objective: The objective of this study was to investigate the differences in thiol/disulfide homeostasis- and ischemia-modified albumin (IMA) levels that are known to be associated with oxidative damage in patients with acute coronary syndrome between ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction/unstable angina pectoris (NSTEMI/USAP) groups and their relationships with angiographic scoring systems.

Methods: A total of 142 patients were included in this study, with 49 in group 1 (STEMI) and 93 in group 2 (NSTEMI/USAP). Thiol/disulfide homeostasis was determined using a recently developed novel method. We investigated whether thiol/disulfide homeostasis and parameters such as IMA, troponin I, and creatine kinase MB fraction levels were associated with Gensini, and Syntax I and II scores, which are angiographic scoring systems.

Results: The native and total thiol levels were found to be statistically significantly lower in the STEMI group than in the NSTEMI/USAP group (both, $p < 0.05$). The serum IMA levels were statistically significantly higher in group 1 (0.59±0.12 vs 0.46±0.23 absorbance units, $p<0.05$). A significant positive correlation was found between the IMA and peak troponin I levels.

Conclusion: Thiol/disulfide homeostasis was shifted in favor of disulfide in the STEMI group, indicating a significant correlation between increased myocardial damage and disulfide. Similarly, the significantly higher IMA levels and positive correlation between IMA and peak troponin I in the STEMI group indicate its vulnerability in the infarcted myocardium area in addition to its vulnerability known in ischemia.

Keywords: Thiol/disulfide homeostasis, ischemia-modified albumin, acute coronary syndrome

INTRODUCTION

Irreversible endothelial cellular damage occurs with insufficient enzymatic and nonenzymatic antioxidant mechanisms against the effects of oxidative damage occurring with reactive oxygen species (ROS) (1). This is the most important cause of atherosclerosis (2). Increased oxidative stress increases both the incidence of coronary artery disease (CAD) and the vulnerability of an existing stable plaque, increasing the risk of the acute coronary syndrome (ACS) (3-5). Thiol groups in cysteine, homocysteine, glutathione, albumin, and other proteins, which are constituents of the antioxidant defense mechanism, are oxidized by ROS, giving rise to reversible disulfide bonds (6). The disulfide bonds can be reversibly reduced to thiol groups by several antioxidants; in this way, the thiol/disulfide homeostasis is maintained (7, 8). Dynamic thiol/disulfide homeostasis has a crucial role in redox reactions such as antioxidant defense, oxidation of proteins, and apoptotic activity of enzymes. Abnormal thiol/disulfide homeostasis levels are involved in the pathogenesis of many diseases. Many studies are investigating the existence and prevalence of CAD and disulfide and thiol levels and homeostasis in patients with ACS (9-11). The N-terminal amino end of the albumin molecule is the binding site of metal ions such as Co^{+2}, Ni^{+2}, and Cu^{+2}. In oxidative stress, in cases of exposure to acidosis, hypoxia, free iron, and copper, this N-terminal

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end is modified, and its ion-binding feature decreases (12). This modified form of albumin is named ischemia-modified albumin (IMA) (13). The serum IMA level is known to increase in ACS (14). The IMA level can be used as a cardiac biomarker in clinical practice both by increasing in the early period of ischemia and by its high levels up to 6–12 hours in myocardial infarction (MI) (15-18). Studies have been conducted in patients with CAD and ACS (19-21). Thiol/disulfide homeostasis has been measured since 1979 only in one direction, but the novel method recently developed by Erel and Neselioglu (8) allows the levels of both variables to be measured separately and jointly.

In this study, we investigated the differences between the two groups as ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction/unstable angina pectoris (NSTEMI/USAP) in terms of both dynamic thiol/disulfide homeostasis parameters with a method developed by our researchers and serum IMA levels associated with increased myocardial damage in ACS patients. In addition, we investigated their correlations with Gensini, and Syntax I and II scores, which are angiographic scoring systems showing the prevalence and severity of CAD.

METHODS

The study was designed as a prospective single-center study. Group 1 consisted of 49 patients with ST-STEMI who were immediately taken to the catheter laboratory, and group 2 included 93 patients admitted in the coronary intensive care unit with NSTEMI and unstable angina pectoris (USAP) who then underwent coronary angiography. Both groups were informed of the procedure before the study, and written consent was obtained. The ethics committee approved the study protocol. The study complied with the Declaration of Helsinki. The patients' baseline characteristics, age, sex, and cardiovascular risk factors (hypertension [HT], diabetes mellitus [DM], hyperlipidemia [HL], obesity, smoking, family history, etc.), and previous history of percutaneous or surgical revascularization were recorded. In addition to the Gensini and Syntax anatomical scores, the Syntax II (SS II) score was calculated after coronary angiography. The Gensini score was computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its geographic importance (22). Each lesion was 1.5 mm in diameter, and 50% stenosis was scored using the online SS Calculator version 2.1 (www.syntaxscore.com). Fluoroscopic visualizations were evaluated by two experienced cardiologists who were not aware of the clinical specifications of the patients. In case of disagreement, the opinion of a third observer was received, and the final decision was made by consensus. To calculate the SS II score, ejection fraction (EF) was studied using the Simpson method with echocardiography, and GFR values calculated with Cockcroft Gault formula were recorded. In addition, patients were evaluated by the thoracic clinic for chronic obstructive pulmonary disease (COPD), and those with COPD and peripheral vascular disease (PVD) were recorded (23-25).

Main Points:

- Both thiol and IMA levels are markers that demonstrate dynamic variability as oxidative stress increases.
- In this study, we investigated the differences of thiol, disulfide, or index values and the angiographic scoring systems and serum IMA levels between the two groups as ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI)/unstable angina pectoris patients.
- We found statistically significantly lower thiol levels and higher IMA levels in the STEMI group in which the affected myocardial area is more and therefore oxidative stress is higher.

Patients with severe systolic heart failure (EF < 40%), those with end-stage renal failure or nephrotic syndrome, severe liver disease, active infection, known rheumatological or hematological disease, and histories of cerebrovascular stroke and cancer were excluded from the study.

Laboratory Analysis

Venous blood samples were collected on admission or within the first 24 hours from patients with ACS into both EDTA-containing and serum-separating tubes. The samples were then centrifuged at 1500 rpm for 10 minutes to separate the plasma and serum. After centrifugation, the separated plasma and serum samples were frozen at −80°C until analysis. Samples with significant absorptions in the red region of the visible spectrum, which equates to extracellular hemoglobin levels of >0.3 g/l, were excluded from the study analyses.

Thiol/disulfide homeostasis parameters were determined with a novel method as described previously [8] by a spectrophotometric method using an automatic clinical chemical analyzer (Roche, Cobas 501, Mannheim, Germany). Serum IMA values were measured using the colorimetric method described by Bar-Or et al. (26) and IMA results were reported as absorbance units (ABSU). The albumin level was measured using the bromocresol green method. Creatinine (Cr) level and creatine kinase MB fraction (CK-MB) were measured using an enzymatic colorimetric method. Other laboratory parameters were measured with the Abbott Chemistry Analyzer. Cardiac troponin I (cTnI) level was measured with the Abbott Architect.

Statistical Analyses

A sample size of n=48 per group was required to provide 80% power to detect a difference in mean level with a significance of 0.001 (two-sided α). The normal distribution of the data was tested using a one-sample Kolmogorov-Smirnov test. Continuous variables are presented as mean±SD. Categorical variables are presented as counts. The statistical comparisons were performed using the two-sided Student t test. Categorical variables were compared using the Chi-square test or Fisher exact test for small samples. The Pearson correlation analysis was used for the numerical data. A multivariate logistic regression model was used to determine the effect of thiol/disulfide homeostasis and IMA levels on the angiographic scores. P values < 0.05 were considered statistically significant. The statistical analyses were performed using the the Statistical Package for Social Sciences version 20.0 software for Windows (IBM SPSS Corp.; Armonk, NY, USA).

Table 1. Demographic characteristics and laboratory findings of the study groups

	STEMI (n=49)	NSTEMI/USAP (n=93)	p
Age, years	59±12.9	61.2±12.6	0.324
Sex (% female)	10 (20.4)	28 (30.1)	0.238
Hypertension (%)	26 (53.1)	51 (54.8)	0.861
Diabetes mellitus (%)	17 (34.7)	37 (39.8)	0.590
Hyperlipidemia (%)	16 (32.7)	34 (36.6)	0.714
COPD (%)	6 (12.2)	7 (7.5)	0.372
PVD (%)	9 (18.4)	14 (15.1)	0.637
Smoking (%)	29 (59.2)	43 (46.2)	0.161
LMCA (%)	3 (6.1)	10 (10.8)	0.543
Number of diseased vessels			
1 (%)	10 (20.4)	26 (28)	0.418
2 (%)	18 (36.7)	33 (35.5)	0.883
3 (%)	21 (42.9)	34 (36.6)	0.475
PCI (%)	49	90 (96.8)	0,758
Glucose (mg/dL)	145±78	136±63	0.437
Creatinine (mg/dL)	0.9±0.3	1±0.4	0.137
eGFR	77±15	73±18	0.135
T. Cholesterol (mg/dL)	193±37	199±50	0.464
HDL-C (mg/dL)	43±10	46±25	0.302
LDL-C (mg/dL)	134±36	134±47	0.957
Triglyceride (mg/dL)	140±77	178±131	0.068
CRP (mg/dL)	14.7±29.8	16.9±31.1	0.685
Leukocyte count (K/uL)	11.6±3.7	11.4±6.4	0.906
Hemoglobin (g/dL)	14.1±1.6	13.6±1.9	0.154
Platelet count (K/µL)	245±60	244±65	0.896
Albumin (g/dL)	3.9±0.5	4±0.4	0.550
C-reactive protein/ albumin ratio	3.7 ± 7.4	4.4 ± 8.6	0.605
Peak CK-MB (U/L)	168±170	38±47	<0.001
Peak troponin I (pg/mL)	33157±19539	7163±11788	<0.001
LVEF (%)	48.4±7.1	53.2±8.6	0.001
Gensini	56±25	43±32	0.010
Syntax score	17±6	16±10	0.431
Syntax II score- CABG	22±12	23±12	0.529
Syntax II score- PCI	29±11	28±13	0,144

COPD: chronic obstructive pulmonary disease; PVD: peripheral vascular disease; T. Cholesterol: total cholesterol; HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, CRP: C-reactive protein, LVEF: left ventricular ejection fraction; CABG: coronary artery bypass grafting; PCI: percutaneous coronary intervention; LMCA: left main coronary artery

Table 2. Thiol/disulfide homeostasis and IMA levels of the study groups

	STEMI (n=49)	NSTEMI/USAP (n=93)	p
Native thiol (µmol/L)	331±64	366±51	0.001
Total thiol (µmol/L)	372±67	404±53	0.003
Disulfide (µmol/L)	20.4±6.5	22.8±8.3	0.112
Index I (disulfide/total thiol) (%)	6.4±2.4	5.8±2.5	0.184
Index II (disulfide/native thiol) (%)	5.6±1.8	5.1±1.9	0.148
Index III (native thiol/total thiol) (%)	88.8±3.6	89.7±3.8	0.143
IMA (absorbance units)	0.59±0.12	0.46±0.23	<0.001

IMA: ischemia-modified albumin.

RESULTS

The demographic features of both groups (group 1: STEMI; group 2: NSTEMI/USAP) are presented in Table 1. Age was similar between the groups (59±12.9 vs 61.2±12.6, p=0.32). The numbers of patients with HT, DM, and HL were higher in group 2, and the numbers of patients with peripheral artery disease, left main coronary artery, and smokers were higher in group 1. CK-MB and troponin I levels were higher in the STEMI group as expected. EF was significantly lower in the STEMI group. When the angiographic scoring systems were examined, only the Gensini score differed between the groups. The mean Gensini score was 56±25 in group 1 and 43±32 in group 2 (p<0.05). No significant difference was found between the groups in terms of Syntax and SS II scores calculated by adding clinical factors. In the STEMI group, PCI was performed in all the patients. In the STEMI/USAP group, two patients underwent CABG and one patient received an intense medical follow-up. Except for three patients, all the patients successfully underwent PCI.

Disulfide levels were similar between the two groups. Native and total thiol levels were statistically significantly lower in group 1 (Table 2, Figures 1, 2). In groups 1 and 2, the mean native thiol levels were 331.62 and 366.57 µmol/L (p<0.05) and the mean total thiol levels were 372.47 and 404.61 µmol/L, respectively (p<0.05). Among the index values defined as index 1 (disulfide/thiol), index 2 (disulfide/native thiol), and index 3 (native thiol/total thiol), the index 1 and 2 values were higher in group 1, although the differences did not reach statistical significance. The IMA level was significantly higher in group 1 (Table 2, Figure 3). The mean IMA level was 0.59±0.12 ABSU in group 1 and 0.46±0.23 ABSU in group 2 (p<0.05). The results of the correlation analysis are presented in Table 3. The IMA level weakly positively correlated with peak troponin I (r=0.185, p<0.05). The native thiol level negatively correlated with peak troponin I (r=–0.247, p<0.05). The total thiol level negatively correlated with peak CK-MB (r= –0.175, p<0.05) and peak troponin I (r= –0.232, p<0.05). The disulfide level positively correlated with LVEF (r=0.200, p<0.05).

Table 3. Correlations of the study parameters with IMA and thiol/disulfide homeostasis

	CAR	IMA	Native Thiol	Total Thiol	Disulfide
	r	r	r	r	r
IMA	-0.085				
Native thiol	-0.152	0.025			
Total thiol	-0.134	-0.067	0.966**		
Disulfide	-0.043	-0.056	0.058	0.299**	
Index I	0.019	-0.028	-0.507**	-0.281**	0.789**
Index II	0.026	-0.032	-0.529**	-0.308**	0.770**
Index III	-0.026	0.032	0.529**	0.308**	-0.770**
Gensini	-0.032	0.085	-0.062	-0.019	-0.134
Syntax I score	-0.006	0.034	0.083	0.116	-0.159
Syntax II score -PCI	0.184*	-0.001	-0.034	0.025	-0.010
LVEF	0.056	-0.098	0.004	-0.037	0.200*
Peak CK-MB	0.203*	0.094	-0.164	-0.175*	-0.145
Peak troponin I	0.018	0.185*	-0.247*	-0.232*	-0.192
Leukocyte	0.022	0.054	-0.071	-0.137	0.043
CRP (mg/dL)	0.994**	-0.098	-0.163	-0.142	-0.038
Creatinine	0.300**	-0.177*	-0.019	-0.024	-0.147
Age	0.214*	-0.058	0.039	0.077	-0.047

PCI: percutaneous coronary intervention; LVEF: left ventricular ejection fraction; CRP: C-reactive protein; CAR: C-reactive protein-to-albumin ratio.
*p<0.05, **p<0.001

Figure 1. Box-and-whisker plot of the native thiol levels (μmol/L) in the ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) groups. The middle lines, and upper and lower margins of the boxes represent median values

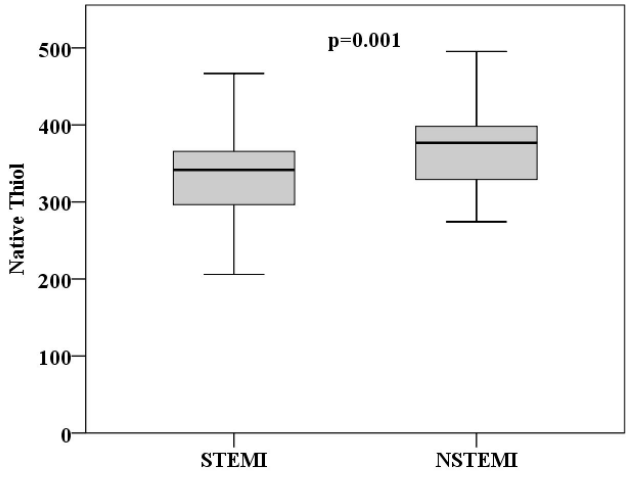


Figure 2. Box-and-whisker plot of the total thiol levels (μmol/L) in the ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) groups. The middle lines, and upper and lower margins of the boxes represent median values

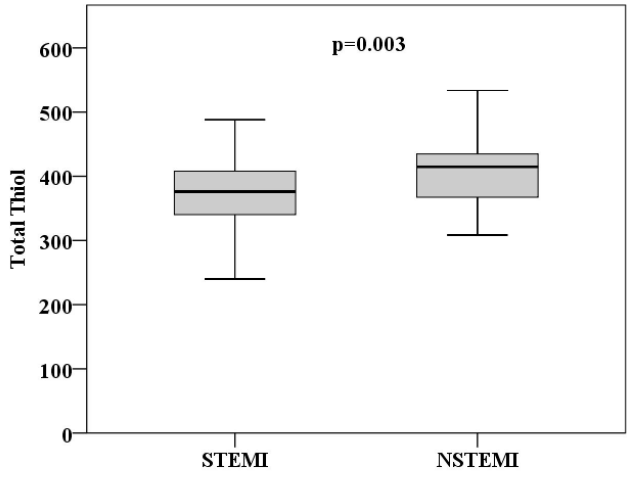
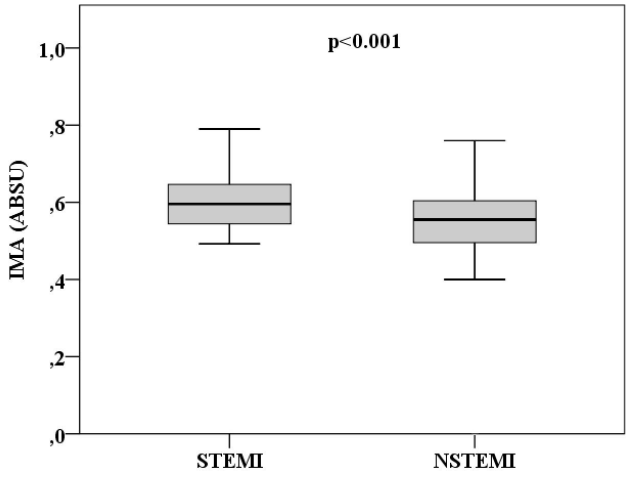


Table 4. Results of the logistic regression analysis between the Gensini score and thiol/disulfide homeostasis and IMA levels of the study groups

				Confidence Interval	
	Beta	t	p	Lower Bound	Upper Bound
Native thiol (μmol/L)	–0.076	–0.732	0.466	–0.143	0.066
Total thiol (μmol/L)	–0.075	–0.701	0.485	–0.139	0.066
Disulfide (μmol/L)	–0.104	–0.996	0.322	–1.119	0.372
IMA (absorbance units)	0.271	2.598	0.011	8.976	67.585

IMA: ischemia–modified albumin

Figure 3. Box-and-whisker plot of the IMA levels (absorbance units) in the ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) groups. The middle lines, and upper and lower margins of the boxes represent median values



In addition, the C-reactive protein/albumin ratio (CAR) positively correlated with Syntax II score (r=0.184, p<0.05), peak CK-MB level (r=0.203, p<0.05), creatinine level (r=0.300, p<0.001), and age (r=0.214, p<0.05; Table 3). We found no relationship between the Syntax scores and the IMA and thiol/disulfide levels. Only the Gensini score was related to the IMA level (p=0.011, 95% confidence interval=38.280 [8.976–67.585]) in the study group. The results of multivariate logistic regression analysis between the Gensini score and thiol/disulfide and IMA levels in the study groups are shown in Table 4.

DISCUSSION

In our study, disulfide levels were similar between the STEMI and NSTEMI/USAP groups. However, the native and total thiol levels were lower in the STEMI group; thus, thiol/disulfide homeostasis shifted in favor of disulfide, indicating the correlation between increased myocardial damage and disulfide, which is the oxidative form of thiol. In addition, the significantly higher IMA levels in the STEMI group and its significant positive correlation with peak troponin I show that the serum level increases as the infarcted myocardial area increases.

As an indicator of chronic inflammation, low thiol level is interpreted as a marker of atherosclerosis and CAD as in many systemic diseases (27). In a study by Altıparmak et al. (9), patients were divided into those with and those without CAD. Even CAD patients were classified into those with noncritical and those with critical stenosis according to stenosis severity. The lowest levels of native thiol, total thiol, and disulfide were found in the CAD group with critical stenosis. Disulfide levels were similar between the noncritical and critical stenosis groups, with significantly lower native and total thiol levels in the critical stenosis group. A negative correlation was found between the Gensini score, which is a marker of the prevalence of CAD and an anatomical score, and thiol level, and native thiol was found to be an independent predictor of the Gensini score. In the first of the two separate studies by Kunti et al. in patients with ACS, patients with MI were compared with the control groups. They found that the thiol levels were significantly lower and thiol/disulfide homeostasis shifted in favor of disulfide in the MI group (10). In a study that included 290 patients (11), this time, only patients with NSTEMI were included and divided into two groups, those with low (<23) and those with high (≥23) Syntax scores. The thiol/disulfide ratio was significantly lower in the high Syntax group, and a significant negative correlation was found between the thiol/disulfide and Syntax scores. These results indicate that the disulfide level was linearly increased with the prevalence and CAD severity. In our study, a lack of correlation between the angiographic scoring systems and the thiol and IMA levels might have resulted from the small number of patients. We included only patients with ACS in our study. In conclusion, we observed that thiol levels were significantly decreased with the increase in myocardial area. However, we could not find a correlation between the thiol, disulfide, or index values and the angiographic scoring systems. We thought that the presence of clinical factors such as age, EF, COPD, or PVD, which are used to calculate the SS II score, may have affected the thiol levels, but the SS II values were similar between the two groups. In a 6-month follow-up study, Akkuş et al. investigated the association of thiol levels with major cardiovascular events (MACEs) in patients with STEMI (28) by comparing 241 patents with STEMI patients with controls

with normal coronary arteries. The authors found that the native and total thiol levels correlated with N-terminal brain natriuretic peptide (NT-proBNP) level, Cr level, and contrast nephropathy, and the disulfide levels correlated with the NT-proBNP level. At a 6-month follow-up, they found that besides EF, low-density lipoprotein cholesterol, and serum albumin levels, the native and total thiol levels were also involved among the factors contributing to the development of MACEs and long-term morbidity. Therefore, according to the results of the study, thiol levels can provide information to clinicians about the prognosis of patients with MI.

Normally, the IMA level increases with the modification of albumin level as a result of ischemia and hypoxia occurring when coronary blood flow decreases, even before the levels of the cardiac markers increase with elevated blood levels (CK, CK-MB, lactate dehydrogenase, cardiac troponin I, and cardiac troponin T [cTnT], and myoglobin) in myocardial necrosis (14, 29). IMA is an efficient and inexpensive test with high sensitivity for the early diagnosis of ACS, which has been approved by the Food and Drug Administration in 2003. The IMA level increases within minutes immediately after the onset of ischemia, remaining high for 6 to 12 hours and returning to normal within 24 hours (16). In a diagnostic study by Sinha et al. for ACS, the sensitivity of IMA level was shown to increase to 95% when the marker was used together with electrocardiography and cTnT (17). In addition, in a study by Kazanis et al, IMA levels after exercise were demonstrated to be useful to detect ischemia not only in ACS but also in stable CAD. IMA levels were higher and total antioxidant status was lower in patients with stable CAD than in healthy controls (30). Again, in a study by Manneewong et al. about ischemia and infarction, serum IMA levels were significantly higher in patients with NSTEMI (31). It can be said that ROS leading to protein oxidation is less severe in association with minor myocardial damage rather than major myocardial necrosis in patients with NSTEMI. On the basis of this opinion, we tested the correlation between the affected infarction area and IMA with a categorization of the patients into STEMI and NSTEMI/USAP groups. We observed that EF was significantly lower and peak troponin I levels were significantly higher; thus, the infarction area was larger, and IMA levels were higher in the STEMI group. Moreover, we found a significant positive correlation between the IMA and peak troponin I levels. The results suggest that like thiol or serum albumin level, IMA level may be a predictive factor of MACEs and prognosis in patients with MI. However, we believe that further long-term follow-up studies are needed to confirm these results.

Another remarkable result of the study is the positive correlation of CAR with SS II score. CRP level is known to indicate endothelial dysfunction, and hypoalbuminemia is associated with new MI development and increased mortality in patients with ACS (32). On the basis of the same rationale, Çınar et al. recently published a study in which patients with STEMI were examined for CAR values. They showed that CAR is an independent predictor of all-cause mortality and associated with increased myocardial damage. They observed that increased CAR was significantly associated with advanced heart failure during in-hospital stay and long-term follow-up, and myocardial reinfarction (33). In our study, we included all patients with ACS (STEMI and NSTEMI/USAP) and observed no significant difference in CAR between the two groups. We found no significant relationship between CAR with IMA, thiol/disulfide homeostasis, and LVEF. Only a positive correlation was found between CAR and SS II score. SS II is known to be superior to the Syntax score in revascularization decision because it is calculated together with the clinical features and coronary anatomical score of patients. This relationship seems important in terms of providing a preliminary idea to the interventional cardiologist simply by laboratory examination before scoring. However, these results should be supported by larger studies.

CONCLUSION

Both thiol and IMA levels are markers that show dynamic variability as oxidative stress increases. The statistically significantly lower thiol levels and higher IMA levels in the STEMI group than in the NSTEMI group show that oxidative stress was increased and the antioxidative stress capacity was decreased with the reduced thiol levels in the STEMI group. Both blood tests are studied with a spectrophotometric method, which is a relatively inexpensive and rapid technique. As these tests are likely to guide clinicians, the present study will contribute to the literature.

Study Limitations

The lack of a correlation between the angiographic scores and the thiol, disulfide, and IMA levels may be attributed to the small number of patients included in the study. One of the other limitations of this study is the lack of a long-term follow-up of the patients because this study was aimed at investigating the association between blood test results and the affected myocardium. We believe that further long-term follow-up studies are needed to investigate the associations between clinical course and prognosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Biruni University.

Informed Consent: The patients were informed before the study and written consent was obtained.

Peer-review: Externally peer-reviewed.

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

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Original Research

In Vitro Effects of Natural Garlic Juice on Some Fungal Strains

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ABSTRACT

Objective: Garlic (Allium sativum) is a commonly available plant that is presumed to have antimicrobial properties. This study aimed to investigate the antifungal effects of natural garlic juice on different fungal isolates.

Methods: Garlic bulbs obtained from the market were aseptically cut cross-sectionally into slices, and the juice was collected via a sterilized electric mincer. First, a susceptibility screening test was performed using the slices and juice for *Candida, Epicoccum, Fusarium, Epidermophyton, Trichophyton, Aspergillus,* and *Penicillium* genera. Sabouraud dextrose agar (SDA) medium was inoculated with fungal isolates. Circular wells were drilled in the center of the SDA, and 270 µl of garlic juice were added to these wells. After incubation, the inhibition zones were evaluated, and a dilution test was performed for the most susceptible isolate. Increasing dilutions of garlic juice from 1/2 to 1/1024 were added to the wells that were drilled on Mueller Hinton agar, and the inhibition zone diameters were measured after incubation.

Results: All isolates were inhibited by the garlic juice. In the dilution test for Candida albicans, an inhibition zone larger than 25 mm was observed in up to the 1/128 dilution. After the 1/256 dilution, the inhibition zone gradually got smaller and no inhibition was observed at the 1/1024 dilution.

Conclusion: Natural garlic juice was effective in all the isolates. The most significant antifungal effect was observed against the Candida albicans isolate. In underdeveloped areas where access to healthcare and medicine is difficult, garlic juice may be an inexpensive and easily accessible alternative to classical antifungal drugs.

Keywords: Garlic, antifungal, fungi, Candida

INTRODUCTION

Garlic (*Allium sativum*) is a species in the onion-like genus—*Allium* (the genus name *Allium* is Latin name for garlic); the genus includes many other plants that are consumed by humans like onion, scallion, shallot, leek, and chives (1). Garlic is originally native to Central and South Asia, but China is still the largest producer of garlic with approximately 80% of the total global production (2). However, garlic has been widely consumed from ancient times all over the world for culinary and medicinal purposes (3-5). The historically appealing culinary and medicinal uses of garlic are well attributed to the fact that its cloves have a pungent smell and taste, caused by the numerous sulfur compounds present in garlic (6). Several organosulfur compounds have been identified in garlic, many of which had been studied for their potential medical benefits. The following are examples of such sulfur-containing compounds: three γ-glutamyl peptides, including γ-L-glutamyl-S-(2-propenyl)-L-cysteine (GSAC), γ-L-glutamyl-S-(trans-1-propenyl)-L-cysteine (GSPC), and γ-L-glutamyl-S-methyl-L-cysteine (GSMC); their three corresponding sulfoxide derivatives, including (+)-S-(2- propenyl)-L-cysteine sulfoxide (alliin), (+)-S-(trans-1-propenyl)- L-cysteine sulfoxide (isoalliin), and (+)-S-methyl-L-cysteine sulfoxide (methiin), respectively; and a seventh compound, (1S,3R,5S)-5-methyl-1,4- thiazane-3-carboxylic acid 1-oxide (cycloalliin) had also been investigated (7). The latter sulfoxides of garlic have been reported to have some medicinal properties; for example, alliin has anticancer effects (8) and also lipid-lowering effects (9, 10). On the other hand, the former γ-glutamyl peptides have been reported to lower blood pressure (11) and to have cholesterol-lowering and antioxidant effects (12). However, when garlic bulbs are crushed, these organosulfur compounds are transformed into other compounds such as allicin, ajoene, dithiins, and diallylpolysulfides (13).

This is mainly due to the release of enzymes such as alliinase, which act on the already present organosulfur compounds in garlic tissues, thereby transforming them into novel forms as mentioned above (13, 14). One of these compounds of interest is allicin. Upon garlic crushing or chopping, the enzyme alliin lyase (alliinase) catalyzes the cleavage of alliin into allicin, which reacts further to form ajoene (14). Allicin (allylthiosulfinate, diallyl disulfide-S-monoxide) is a molecule with strong aroma and is probably involved in the defense mechanism by the garlic plant against pests (15).

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Allicin material was first isolated and studied in the laboratory by Chester J. Cavallito and John Hays Bailey in 1944 (16), and in that early study, it was shown that allicin has antibiotic activity in the form of an antibacterial effect. Thereafter, the antibiotic activity of allicin and its derivative, ajoene, against various microorganisms, including bacteria, fungi, viruses, and even parasites, was well studied (17-20). Regarding studies that specifically target the antifungal activity of garlic products, the available scientific literature suggests that the antifungal activity of garlic was first established in 1936 by Schmidt and Marquardt whilst working with epidermophyte cultures (21). Since then, several studies have tested different components of garlic against different species of medically important fungi (22, 23). To this end, the present study aimed to investigate the antifungal effects of natural garlic juice on several fungal genera and *Candida albicans* species.

METHODS

Garlic bulbs were obtained from over-the-shelf grocery markets, and was first peeled and aseptically cut cross-sectionally into slices to be placed directly into solid agars plates for the first step. For the second step, the garlic juice was obtained by mincing the peeled garlic bulbs in a sterilized electric mincer. The bulb solid remnants were discarded and only the homogenous watery part of the juice was collected and kept in sterile bottles at +4°C for one week. One week later, the clear supernatant fluid was taken and used for the study, while the sediment was discarded.

The Screening Step

A total of seven isolates from different fungal genera, including *Candida, Epicoccum, Fusarium, Epidermophyton, Trichophyton, Aspergillus,* and *Penicillium,* were investigated. The isolates were obtained from the samples of patients in Gaziantep University Hospital. The isolates were previously identified via the routine work flow in the medical microbiology laboratory of Gaziantep University Hospital at the genus level (except *Candida albicans* species) and selected randomly for the study. The isolates were stored at –80°C before the study. In the screening step, we used a freshly cut slice from a garlic bulb to evaluate the antifungal activity of fresh garlic as a whole plant on several fungi. Then, we used the undiluted fresh garlic juice to screen and detect the most susceptible fungal genus to the garlic juice as described below.

First, pure colonies of the fungal strains were transferred into normal saline solutions (0.85% NaCl) until a fungal suspension of 0.5 McFarland turbidity is obtained. Sabouraud dextrose agar (SDA) plates were inoculated with 100 microlitres of 0.5 McFarland fungal suspensions and kept at room temperature for 5 minutes. Two millimeters thick slices of garlic were placed on the surface of each SDA just at the center to determine the inhibitory effect of the whole plant. For the other set, a single circular well was drilled in the center of the SDA, which was already inoculated with fungal suspensions, and 270 µl of fresh garlic juice was added to the well on the SDA without diluting. This procedure was repeated for all the seven isolates to obtain more homogenous and clearer inhibition zones.

Finally, the 14 plates were incubated at 37°C for 24 hours for *Candida* and 4 weeks for the other fungi. At the end of the incubation, the inhibition zones were noted for each isolate, and the isolate with the most significant inhibition zone was tested with natural garlic juice in the second dilution step.

The Dilution Step

For the dilution test using the natural garlic juice, serial saline dilutions of the garlic juice were prepared at 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, and 1/1024 concentrations. A single circular well was opened in the center of each Mueller Hinton agar (MHA), which was already inoculated with 100 µl of 0.5 McFarland suspension of the selected fungal isolate (*Candida albicans*), and 270 µl of fluid at increasing garlic juice concentrations were added to the wells on the MHA plates. A total of 10 plates with increasing concentrations of the garlic juice were incubated at 37°C for 24 hours. As mentioned above, the inhibition zone diameters of each media were measured using a compass and noted after the incubation.

Main Points:

- Natural garlic juice has antifungal effect on various fungal genera.
- Natural garlic juice inhibited growing of *Candida albicans* even at 1/128 dilution.
- Natural garlic juice has a potential to be used as antifungal agent.

RESULTS

In the screening step, with the cross-sectional slices of the garlic bulb, we could not observe a regularly shaped inhibition zone even if there was a visible inhibition.

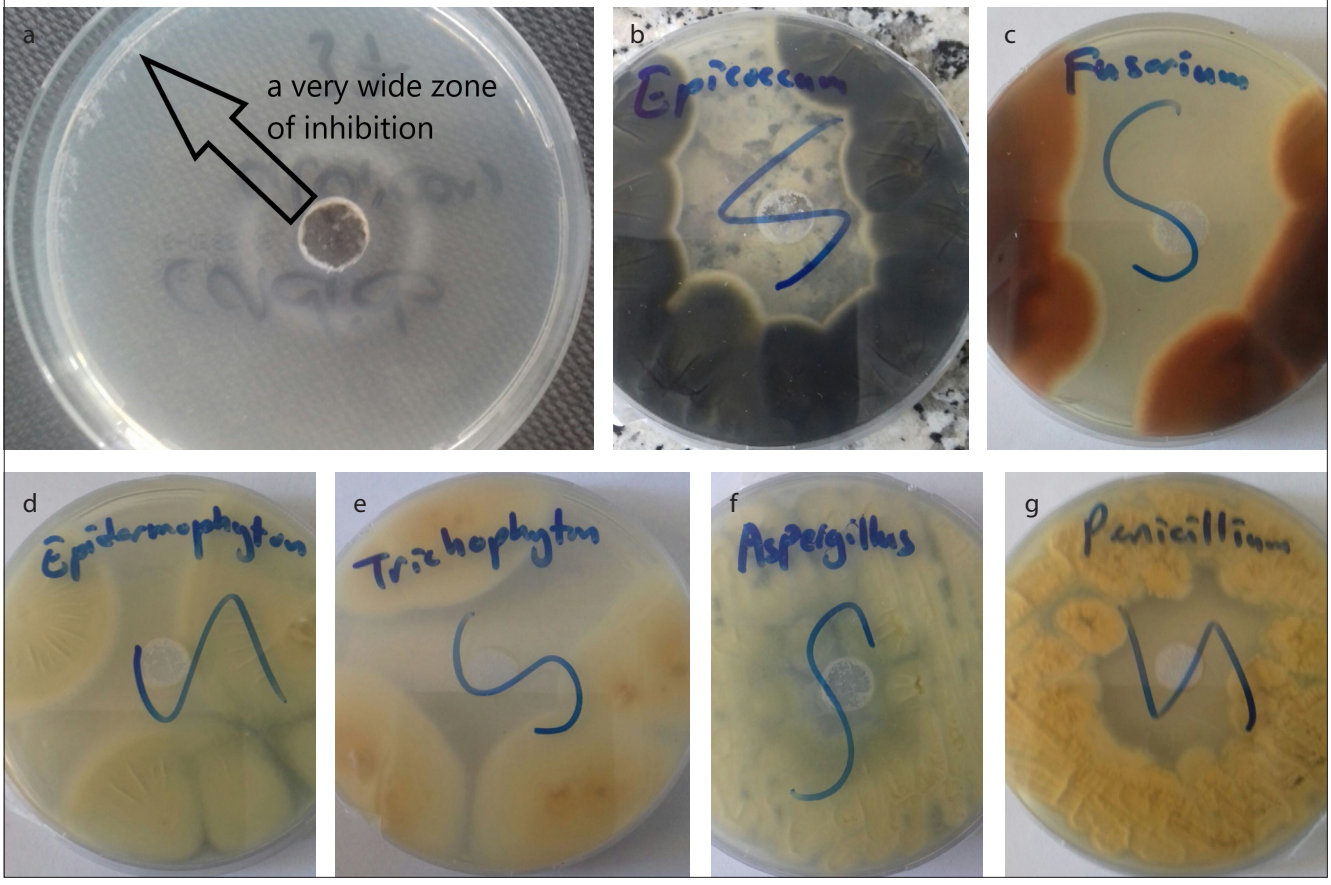
In the screening step, using the fresh garlic juice, the inhibition zones were clearer, especially for the *Candida albicans* isolate (Figure 1a). For all the isolates, including *Aspergillus, Epicoccum, Fusarium, Epidermophyton, Trichophyton,* and *Penicillium*, full concentration of the garlic juice showed an inhibitory effect but with a variable and an irregular extent (Figure 1. b-g). In addition, the inhibition zone was very small for the *Aspergillus* genus (Figure 1F). The largest and the clearest inhibition zone was detected in plate of the *Candida albicans* isolate (Figure 1a); therefore, this isolate was selected for the dilution test step.

In the dilution step, the *Candida albicans* isolate was subjected to a full serial dilution susceptibility test using different concentrations of garlic juice. In this step, we observed the largest inhibition zone at the 1/2 dilution of the garlic juice (almost all plates) (Figure 2A). At up to the 1/128 dilution, we observed significant inhibition zones greater than 25 mm in diameter (Figure 2. b-e). A small inhibition zone diameter was observed at the 1/256 dilution and then, the widths of the inhibition zones gradually decreased with higher dilutions (Figure 2. f, g). The inhibition zone did not appear at the 1/1024 dilution (Figure 2h).

DISCUSSION

The mechanism by which allicin and its derivatives function as an antimicrobial agent may involve several biological effects, in-

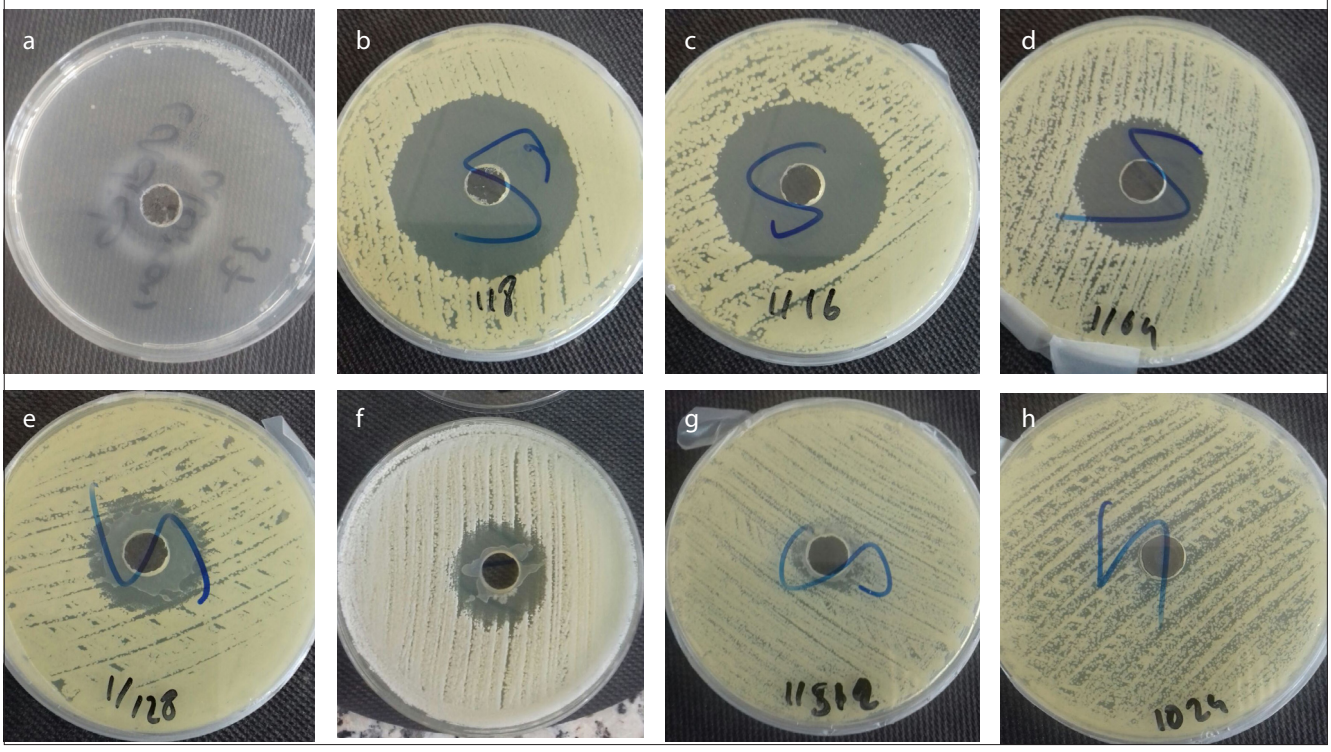
Figure 1. a-g. a) Effect of full concentration of garlic juice on *Candida albicans* b) Effect of the full concentration of garlic juice on *Epicoccum* c) Effect of the full concentration of garlic juice on *Fusarium* d) Effect of the full concentration of garlic juice on Epidermophyton e) Effect of the full concentration of garlic juice on Trichophyton f) Effect of the full concentration of garlic juice on *Aspergillus* g) Effect of the full concentration of garlic juice on *Penicillium*



cluding its permeability through cell membranes and its reactivity with various biomolecules within the target cells, regulation of enzymatic activity, protein synthesis, and nucleic acid synthesis (15, 24). Most previously cited studies used methods involving steam distillation or solvent extraction (such as those using alcoholic compounds) to obtain the essential oils from garlic plants that contain allicin and ajoene prior to testing these compounds or even used synthetic preparations of such compounds (16-20). However, we used natural garlic juice instead of chemically or physically processed garlic in our study. We opted to test the *in vitro* effect of garlic on clinically relevant fungal species, especially the *Candida* isolate (*Candida albicans*), from patients using freshly cut garlic bulb slices or mechanically obtained garlic juice from locally sold culinary garlic that is available at grocery markets, as this provides the easiest and cheapest means of obtaining garlic's active molecules (allicin and ajoene). In our study, garlic juice was found to be more effective than garlic slices, most probably due to the formation of garlic's active molecules. When the results were analyzed, garlic juice was found to be extremely effective on *Candida albicans*; moreover, garlic juice was effective on all fungal genera except *Aspergillus*. Fungal infections are a major public health concern, with an increasing demand for treatment, especially for topical infections caused by various fungi including dermatophytes and *Candida* species (25). *Candida albicans* is one of the most widespread causes of superficial cutaneous fungal infection, and when the immune system is weakened, it can spread to deeper tissues and even into the blood, where it causes systemic candidiasis (26). Therefore, natural garlic juice might be an alternative for the treatment of fungal infections caused by *Candida albicans.*

The limitation of our study is that it was only an *in vitro* study, which may not reflect the *in vivo* effect of garlic juice against human infections. Secondly, we only tested a limited number of fungal agents, which may not present all the members of the genus. However, in most of the fungi tested, natural garlic juice was shown to have significant inhibitory effects. Another limitation is that we performed the diffusion tests using different dilutions instead of the broth microdilution test, which can determine a minimum inhibitory concentration (MIC). Since our study was about the potential effect of natural garlic juice for topical use in fungal infections and there is no standard MIC for garlic juice, we preferred using the diffusion method, which was more practical. In a similar study that tested the antibacterial effect of fresh garlic juice using a similar well diffusion method, Yadav et al. (27) also found an efficiency of fresh garlic juice against the tested strains of bacteria. In a study designed to investigate the antifungal activity of garlic against *Candida albicans* at molecular levels,

Figure 2. a-h. a) Effect of the 1/2 dilution of garlic juice on *Candida albicans* b) Effect of the 1/8 dilution of garlic juice on *Candida albicans* c) Effect of the 1/16 dilution of garlic juice on *Candida albicans* d) Effect of the 1/64 dilution of garlic juice on *Candida albicans* e) Effect of the 1/128 dilution of garlic juice on *Candida albicans* f) Effect of the 1/256 dilution of garlic juice on *Candida albicans* g) Effect of the 1/512 dilution of garlic juice on *Candida albicans* h) Effect of the 1/1024 dilution of garlic juice on *Candida albicans*



Li et al. (28) found that garlic products have a more significant efficiency against Candida species than for other fungi; similar to our findings that garlic juice has a potential fungotoxic activity against Candida albicans specifically. We suggest that new *in vivo* and *in vitro* studies evaluating the effects of natural garlic juice should be performed with much more strains and various other fungal species in the future.

CONCLUSION

In our study, natural garlic juice was found to be effective against *Candida albicans* even at very low concentrations such as 1/128 dilution. Since garlic juice was evaluated as an effective antifungal agent in our study and it is a naturally occurring, easily accessible, and cheaply available product, natural garlic juice might be recommended as an alternative agent for the treatment of several fungal infections, especially for the *Candida albicans*, in the countries with a poor healthcare system and where accessing the classical antifungal drugs is difficult. However, more *in vivo* and *in vitro* studies are necessary to confirm the antifungal effects of natural garlic juice.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University (protocol no: 2018/171, 04.07.2018).

Informed Consent: N/A

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Original Research

Comparison of CRP, Full Blood Count Parameters and Transaminases across Different Age Groups of Children with Mycoplasma Pneumonia

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ABSTRACT

Objective: Mycoplasma pneumonia (MP) is a common cause of community-acquired pneumonia in about 10%–30% of children. The combination of polymerase chain reaction (PCR) with serology tests appears to provide better accuracy in the diagnosis of MP; however, these tests are not widely available. Therefore, its diagnosis could be challenging in younger children and would still require other conventional approaches with clinical examination, chest X-ray, and basic laboratory tests such as full blood count and c-reactive protein (CRP) across the most of the clinical settings. In this regard, the objective of this study is to investigate how CRP, full blood count parameters, and transaminases differ among older and younger children.

Methods: We reviewed the data of 2,246 patients aged between 0 and 18 years who were diagnosed with pneumonia between January 2011 and December 2018, and finally included 49 patients who had positive MP IgM in this study. Thereafter, we divided the patients into two groups as children aged ≤5 years and children aged >5 years, and compared the values of hemoglobin, white blood cell (WBC), neutrophils, lymphocytes, eosinophils, monocytes, platelet, CRP, and transaminases in the groups.

Results: The analyses showed that WBC (p=0.001), lymphocytes (p=0.001) and monocyte counts (p=0.004), and CRP (p=0.013) were significantly higher in the children younger than five years. On the contrary, Hb was significantly lower in the children younger than five years (p=0.001).

Conclusion: Children younger than five years who have been diagnosed with MP might exhibit more inflammatory response, which is represented with higher CRP levels. Although monocyte and lymphocyte counts were also higher in the younger group (less than five years), this might be an age-related finding.

Keywords: Mycoplasma pneumonia, CRP, children, full blood count

INTRODUCTION

Mycoplasma pneumonia (MP) is a common cause of community-acquired pneumonia in about 10%–30% of children (1, 2). Although it is common in school-aged children, MP can be observed at any age. The diagnosis of MP remains challenging. Basically, the diagnosis can be made via serological tests, culture tests, and molecular detection of pathogen-specific antigens. Additionally, polymerase chain reaction (PCR) might be used as an alternative in practice, but can pose various challenges because of its limited worldwide availability. However, none of these methods are accurate enough to establish diagnosis of MP via a single method alone (3-5). The combination of PCR with serology tests appears to provide a better accuracy (6). Furthermore, diagnostic testing is recommended based on the availability of the test and its effect on the management of the disease (6). Hence, the diagnosis of MP still requires other conventional approaches such as clinical exam, chest X-ray, and basic laboratory tests such as full blood count and c-reactive protein (CRP) across the most of the clinical settings. In this regard, the objective of this study is to investigate how CRP, full blood count parameters, and alanine amino transferase (ALT) differ among older and younger children.

METHODS

We reviewed the data of 2,246 patients aged 0–18 years who were diagnosed with pneumonia between January 2011 and December 2018 at our hospital from the patient records. Thereafter, we enrolled the patients who were tested positive for serum-specific MP IgM in this study. We checked MP IgM by EUROIMMUN immunofluorescence kits. MP IgM had been studied as a part of pneumonia panel. The patients who met the criteria of pneumonia provided below were involved in the initial review of records. The definition of pneumonia was based on the clinical manifestations (fever, cough, or wheezing), physical examination findings, and/or chest imaging scans. Patients who met at least one of the aforementioned criteria were included in the cohort, and finally 49 qualified patients were enrolled in this study.

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Table 1. Comparison of laboratory parameters in children aged between ≤5 and >5 years with MP infection±

	≤60 months	>60 months	p
WBC (10^3/μL)	11.84±3.61	8.52±2.91	0.001
Neut (10^3/μL)	6.29±2.9	4.87±2.86	0.098
Lymph (10^3/μL)	4.26±1.87	2.58±0.95	0.001
Eu (10^3/μL)	0.19±0.26	0.19±0.17	0.959
Mono (10^3/μL)	0.94±0.48	0.61±0.14	0.004
Hb (g/dL)	11.10±1.48	12.65±1.65	0.001
PLT (10^3/μL)	402.52±134.30	354.12±110.45	0.182
CRP (mg/mL)	18.71±22.37	5.46±5.51	0.013
ALT (U/L)	20.96±19.15	21.60±18.314	0.906
AST (U/L)	31.65±9.71	28.36±10.73	0.271

WBC: White blood cell count, Neut: Neutrophil, Lymp: Lymphocyte; Eu: Eosinophil, Mono: Monocyte, Hb: Hemoglobin, PLT: Platelets, CRP: C-reactive protein, ALT: alanine amino transferase, AST: aspartate amino transferase.

We classified the patients into two groups: children aged 5 years and children aged >5 years. Next, we compared the values of hemoglobin (Hb), white blood cells (WBC), neutrophils, lymphocytes, eosinophils, monocytes, platelets, CRP, aspartate amino transferase, and ALT between the groups.

Statistical Analysis

We used the Statistical Package for Social Sciences version 20 (IBM SPSS Corp.; Armonk, NY, USA) statistical software for this study's analyses. Importantly, we used frequency analysis and Student's t-test for analyzing the parametric variables. P-value <0.05 were accepted as statistically significant for this study. We obtained ethical permission from the local ethics committee.

RESULTS

The study cohort comprised 49 out of 2,249 patients (2.1%) who had pneumonia with positive IgM specific to MP. The age of the patients ranged between 3 and 150 months, and the average age of patients was 75.9±54.9 months. The male population in this study cohort was 53.1%. The analyses showed that WBC (p=0.001), lymphocyte (p=0.001) and monocyte counts (p=0.004), and CRP (p=0.013) were significantly higher in the group of children younger than five years. On the contrary, Hb was significantly lower in the group of children younger than five years (p=0.001) (Table 1).

Main Points:

- CRP levels might be elevated in children younger than five years during mycoplasma pneumonia infection.
- Pneumonia due to mycoplasma pneumonia can be observed throughout the year but have a slightly higher prevalence in winter seasons.
- Increased transaminases with mycoplasma pneumonia could be observed in one of the ten patients.

We also performed receiver operating characteristic (ROC) curve analysis between the age groups. The results of ROC are as follows: WBC: AUC=0.753, cut-off=12.25 (10^3/μL), sensitivity/specificity=40.9%/95.7% (p=0.004); lymphocyte count: AUC=0.786, cut-off=3.63 (10^3/μL), sensitivity/specificity=50%/95.7% (p=0.001); monocyte count: AUC=0.709, cut-off=0.775 (10^3/μL), sensitivity/specificity=63.6%/87% (p=0.016); and CRP (mg/L) values: AUC=0.771, cut-off=19.25, sensitivity/ specificity=31.8%/95.7% (p=0.002).

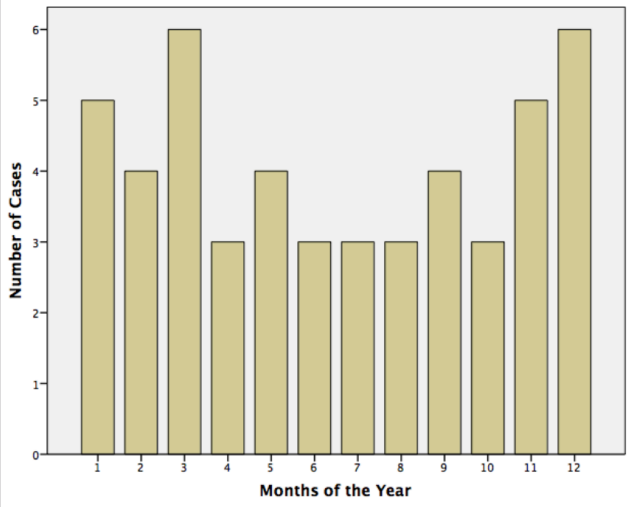
MP infection is observed throughout the year; however, the highest number of cases was recorded in the 3rd and 12th months (Figure 1).

DISCUSSION

MP infection accounts for an important proportion of community-acquired pneumonia cases among children (7). No single test is reliable in terms of detecting MP infection; however, IgM detection was found to be one the most accurate tests in children (8). On the contrary, although PCR has been found to be superior than serological methods, it may underestimate or overestimate the diagnosis in the cases of only the carrier state because of technical issues. Therefore, a combination of these two methods is advised in terms of diagnosis; however, PCR may not be available in most of the clinical settings worldwide and may not be cost effective as well (9). In this context, the differences in basic blood tests such as full blood count and CRP across different age groups might be essential and convenient.

Our study showed significantly increased WBC count and CRP in the children younger than five years. Yun-Ju Ma et al. similarly reported higher WBC and CRP levels in this patient group (10). In contrast, another study showed normal CRP levels in the younger age groups but they reported significantly increased lymphocyte count, which was consistent with our findings (11). Similarly, Defilippi et al. demonstrated higher lymphocyte and monocyte counts in the group of younger children; both of these values

Figure 1. Distribution of the cases over the years



were in agreement with our findings (12). Furthermore, the ROC analysis demonstrated that children younger than five years who are suspected to have MP might have WBC counts greater than 12.5 (10^3/μL) with sensitivity and specificity of 40.9% and 95.7%, respectively. Similarly, while considering lymphocyte counts, values greater than 3.63 (10^3/μL) can be observed in younger children with a possible diagnosis of MP infection with sensitivity and specificity of 50% and 95.7%, respectively. Moreover, the monocyte counts showed a similar pattern, and the values greater than 0.775 (10^3/μL) might be related to a younger age group with sensitivity and specificity of 63.6% and 87%, respectively, during a suspected MP case. The ROC analysis of CRP, which was another significantly higher parameter in the younger age group, showed that values greater than 19.25 (mg/L) can be expected with sensitivity and specificity of 31.8% and 95.7%, respectively, in the context of suspected MP. As it could be observed from the analyses, although the specificities were in the acceptable levels, the sensitivity values were far from the desired levels. However, we need to mention that the given cut-off values along with sensitivity and specificity percentages for WBC, lymphocytes, monocytes, and CRP should not be misinterpreted as a surrogate marker of MP. We analyzed these figures to differentiate the age groups so that these numbers might be helpful to clinicians at limited clinical practice settings for correctly diagnosing children who are suspected to have MP based on the conventional diagnostic methods such as physical examination, history, X-rays, and vital signs.

Additionally, our analysis demonstrated lower Hb values in younger children with MP infection. However, both lymphocyte and Hb counts physiologically differ in the patients who are older and younger than five years (13, 14). Therefore, our findings may not be related to MP infection. We could not prove this statement in either way as we did not had a control group.

We could not find any significant difference in the neutrophil counts across the groups; importantly, neither of the abovementioned studies also could not do the same. Some studies have reported significantly higher platelet counts in younger children. In our study, the platelet counts were also higher in the younger patients; however, this difference was not statistically significant (11, 12).

No specific cumulation of cases with respect to the months of the year was observed; however, the highest number of cases were in the 3rd and 12th months of the year, which are relatively colder months. MP has reported a greater incidence in higher temperatures, whereas some other studies have demonstrated a higher incidence of MP in colder seasons, which was consistent with our findings (10, 15). Generally, MP can be observed throughout the year.

Furthermore, hepatitis, which is one of the extrapulmonary manifestations of MP, was observed in four patients (8.16%) of this study based on their ALT values. Although the data regarding the percentage of hepatitis among patients infected with MP is scarce, our values were in agreement with one of the prospective studies in which the percentage was determined as 7.7% (16).

There are some limitations of this study. One of these limitations is the relatively lower number of patients. Second, as it is known, the retrospective collection of data might lead to data inaccuracy and a lack of information as compared to prospectively acquired data. The reason for high CRP in younger children might be related to co-infections, which we were not able to report as they were not tested but they have been reported in other studies (17, 18).

CONCLUSION

The children younger than five years who were diagnosed with MP infection were found to exhibit more inflammatory responses that are basically represented by increased CRP levels. Furthermore, monocyte and lymphocyte counts were also higher; however, this increase might be related to the age difference rather than MP infection. Overall, while making the diagnosis of MP infection in children younger than five years at limited clinical settings, the findings of higher CRP levels in younger children and lower CRP levels in older children should definitely be interpreted as the support of diagnosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (2020/136 / 02.04.2020).

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Original Research

Comparison of Incisional Hernias with Another Type of Abdominal Hernias in Terms of Predisposing Factors

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ABSTRACT

Objective: Incisional hernia (IH) is a common late complication of abdominal surgery. Factors such as wound infection, type of incision, wound closure technique, and suture material used and patient-related factors such as age, gender, body mass index (BMI), diabetes mellitus (DM), and smoking are also involved in the development of IH and other types of abdominal hernias (OTAH). In this article, we compared the predisposing factors for IH and OTAH in light of the literature.

Methods: Among 130 patients operated for abdominal hernia between January 2015 and December 2018 at the Department of General Surgery of GÜLHANE Training and Research Hospital, we analyzed the predisposing factors for IH and OTAH.

Results: The female-to-male ratio was 28:102, mean age was 58.6 years, and mean BMI was 29.3 kg. The prevalence of DM and smoking was also evaluated. The rate of drain application and hospitalization duration was 56.2% to 4.1% and 8.6–5.3 days in IH and OTAH groups, respectively.

Conclusion: We determined that male gender is a dominant risk factor for OTAH and high BMI for IH. Age, DM, and smoking are equivalent risk factors for both. Drain application for IH is highly statistically significant, which results in prolonged hospitalization. These results suggest an important complication of DM and obesity and conclude that obesity is a major risk factor for IH.

Keywords: Abdominal hernia, diabetes mellitus, herniorrhaphy, incisional hernia, obesity

INTRODUCTION

Incisional hernia (IH) is a type of abdominal hernia that occurs at the previous surgical incision site. The incidence of IH at midline incisions is higher than the incisions in other regions. Despite advances in abdominal wall closure techniques, the rate of development of IH after laparotomy ranges from 15% to 20% (1). More than 50% of IHs originating from abdominal incisions occur within the first year after surgery and 80% within the 3 years (2, 3). Other types of abdominal hernias (OTAH) are the hernias that do not originate from previous abdominal incisions but from the anatomically weak sites of the abdominal wall. The abdominal hernia of a patient independent from being IH or OTAH has the risk of strangulation and may cause other life-threatening complications if it is huge in size and irreducible and consists of abdominal luminal organs. Particularly in such cases, surgical treatments for hernias are strongly recommended.

Factors such as wound infection, location or type of incision, wound closure technique, and suture material and patient-related factors such as age, body mass index (BMI), presence of diabetes mellitus (DM), and smoking are considered other important risk factors for developing IH. In addition, poor nutritional status, chronic lung disease, renal failure, malignancies, and steroid therapies are also considered facilitating factors for developing IH (1, 4).

In this study, we examined the data of 130 patients with abdominal hernia operated between January 2015 and December 2018 at the Department of General Surgery of Gülhane Training and Research Hospital, University of Medical Sciences. Among 130 patients, 32 patients had IH and 98 patients had OTAH. The data of the 2 groups were also compared statistically. This study aimed to determine the factors for developing abdominal hernias and whether the factors have the same impact on both IH and OTAH. In addition, we compared the herniorrhaphy techniques, drain application, and hospitalization durations of the patients.

METHODS

The data of 130 abdominal hernia patients operated in General Surgery Department of Gülhane Training and Research Hospital, University of Medical Sciences, between January 2015 and December 2018 were retrospectively analyzed. The analyzed data of the patients were age, gender, BMI, DM smoking, hernia repair technique, hospitalization duration, and drain applications. Notably, 32 operated 32 had IH and 98 had OTAH. Because this study was a retrospective analysis of patient medical data without collecting any personal data, no informed consent was obtained from the patients. As authors of the manuscript, we declare that all procedures followed in this study were in accordance with the ethical standards of the responsible committee on human exper-

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Table 1. Sociodemographic and clinical features of patients with incisional hernia and other types of abdominal hernias

	IH (n = 32)		OTAH (n = 98)		Total (n = 130)		
Gender	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**P**
	19 (41%)	13 (59%)	83 (84%)	15 (16%)	102 (78%)	28 (22%)	0.02
Age distribution (mean)	24–75 (56.8±5.7) years		21–86 (59.2±8.1) years		21–86 (58.6±7.5) years		0.26
BMI distribution (mean)	18.8–39.8 (29.3±3.4)		19.5–40.1 (27.2±3.6)		18.8–40.1 (27.8±4.1)		0.03
DM	Yes	No	Yes	No	Yes	No	
	6 (18.7%)	26 (83.3%)	14 (14.3%)	84 (85.7%)	20	110	0.57
Smoking	Yes	No	Yes	No	Yes	No	
	14 (44.6%)	18 (55.4%)	47 (47.9%)	51 (52.1%)	61	69	0.67
Drain application	Yes	No	Yes	No	Yes	No	
	18 (56.2%)	14 (43.8%)	4 (4.1%)	94 (95.9%)	22	108	<0.00
Duration of hospital stay (mean)	2–19 (8.6±3.2) days		1–18 (5.3±4.1) days		1–19 (7.2±4.0) days		<0.00

IH: incisional hernia, OTAH: Other types of abdominal hernia, BMI: Body mass index, DM: Diabetes Mellitus

imentation (institutional and national) and with the Declaration of Helsinki of 1964 and its later amendments.

Statistical Analysis

Gender, age, BMI, DM, smoking, postoperative drain application, and hospitalization duration of the 2 groups were evaluated using SPSS version 16.0 (SPSS Inc.; Chicago, IL, USA) for statistically significant differences. $p<0.05$ was considered statistically significant. Nonparametric test chi-square was used for gender, DM, smoking, and postoperative drain application, whereas Mann–Whitney U test was used for age, BMI, and hospitalization duration. Age, BMI, and hospitalization duration parameters were also evaluated for descriptive statistics. Gender, DM, smoking, and postoperative drain application parameters were evaluated for percentage distribution.

A statistically significant difference of 3 operation techniques used for the 2 groups were evaluated using SPSS version 16.0. Percentage distribution for 3 operation techniques was evaluated, and nonparametric chi-square test was used for statistical analysis. $p<0.05$ was considered statistically significant.

RESULTS

The majority of all patients with abdominal hernia were male. The female-to-male ratio was 13:19 in the IH group and 15:83 in the OTAH group with a total of 102 male and 28 female patients (Table 1). Males are found to be highly statistically significant in the OTAH group. The ages of the patients ranged from 24 to 86 years (58.6 ± 7.5) (Table 1). No statistically significant difference was found in mean age between the groups.

Patients were evaluated for BMI, DM, and smoking. The mean BMI was 29.3 ± 3.4 kg/m^2 in patients with IH and 27.2 ± 3.6 kg/m^2 in patients with OTAH (Table 1). Statistically significant difference was found between the 2 groups. The presence of DM in patients between the 2 groups was compared; 6 (18.3%) of the patients in the IH group and 14 (14.3%) patients in the OTAH group had DM, which showed no statistically significant difference between the 2 groups (Table 1). One patient in each group was treated with insulin and the others were with oral antidiabetic agents. The prevalence of smoking was found to be 44.8% in the IH group and 47% in the OTAH group (Table 1) with no statistically significant difference between the groups.

Regardless of the type of repair technique, the rate of drain application was 56.2% in the IH group and 4.1% in the OTAH group (Table 1), which showed a statistically significant difference between the 2 groups.

The duration of hospital stay ranged from 1 to 19 days, with a mean duration of 8.6 ± 3.2 days in the IH group and 5.3 ± 4.1 days in the OTAH group, showing that hospitalization of IH patients is statistically longer than OTAH patients (Table 1).

Patients were evaluated in 3 subgroups in terms of hernia repair technique: classical herniorrhaphy using primary suturing, herniorrhaphy using mesh repair, and fence-darning technique (Table 2). The rate of classical herniorrhaphy using primary suturing, herniorrhaphy using mesh repair, and fence-darning technique in the IH and OTAH groups were 12.5%, 28.1%, and 59.4% and 13.2%, 61.2%, and 25.5% respectively (Table 2).

Main Points:

- The male gender is the dominant risk factor for OTAH and obesity is dominant for IH.
- Drain application for IH is highly statistically significant for delayed hospitalization.
- Newly defined complication of DM and obesity is abdominal hernia in which obesity has more effect on developing IH.

Table 2. Distribution of hernia repair types

	IH (n = 32)	OTAH (n = 98)	Total (n = 130)	p
Primary repair	4 (12.5%)	13 (13.2%)	17 (13.1%)	<0.01
Mesh herniorrhaphy	9 (28.1%)	60 (61.2%)	69 (53.1%)	
Fence darning	19 (59.4%)	25 (25.5%)	44 (33.8%)	

IH: Incisional hernia, OTAH: Other types of abdominal hernia

DISCUSSION

Age and gender are both believed to be the risk factors for abdominal hernias. Older age affects wound-healing impairment as DM, which will be discussed later on, and causes IH. In addition, as patients get older, the strength of connective tissues decreases, which causes weakening of the abdominal anterior wall and OTAH. Therefore, there is no doubt that age is a predisposing factor for abdominal hernia. However, in our study, no statistical difference was found in age between the 2 groups. We conclude that age is an equivalent risk factor for both IH and OTAH.

Gender is also known as a risk factor for abdominal hernia; however, its effect on OTAH and IH is unknown. We compared its predisposing effect in each group separately. We found that male gender is more of a risk factor for OTAH than IH. This may be a result of males dealing with more muscle works and more increased intraabdominal pressure. As they get operated, they decrease their muscle works owing to the advice of the care provider; hence, male gender is not considered a dominant predisposing factor for IH.

Obesity is an important risk factor for developing IHs after abdominal surgery and may cause problems with wound healing in a significant proportion of patients with a BMI of >30 kg/m^2 in the early or late postoperative period (2, 5, 6). Wound complications, such as wound infections and wound separation, are frequently associated with obesity because of poor vascularization of increased adipose tissue and proliferation of proinflammatory tissue factors. Obesity increases the rate of IH by impairing wound healing or causing infections. In addition, the increase in the risk of developing IH should be noted because of the increased intraabdominal pressure in obesity. In many animal experiments, physical and pathological events that increase intraabdominal pressure cause herniation of the abdominal wall in weak areas or sutured incision sites. The weak areas of the abdominal wall are not only the incisional scars but also the physiological and anatomical locations such as umbilicus and the inguinal region. Therefore, obesity is also considered a risk factor for OTAH (7). However, our study resulted that obesity is a more dominant risk factor for IH.

It is very difficult to conduct human experimental studies on intraabdominal pressure and tissue resistance. Because of their different anatomical structures, the results of animal studies are not fully scientifically valid for humans. Therefore, Kroese et al. created a simulator called AbdoMAN and conducted different studies on this model, which has very similar features to the muscles and fascia of the human abdominal wall. Furthermore, they clearly demonstrated the importance of increased intraabdominal pressure for developing IH (8, 9). These studies have reported that factors that increase intraabdominal pressure, such as coughing, straining, vomiting, obesity, and heavy physical exercise, may increase the risk of IH independent of other factors. Therefore, patients should be investigated in the postoperative period for constipation, pulmonary infection, or difficulty in urination, and if necessary, medical treatment should be initiated for the diseases causing these symptoms.

In our study, the IH and OTAH groups were evaluated for obesity by measuring the BMI of the patients. We found out that the BMIs of the 2 groups were statistically different, which supports the data in the literature that obesity can cause both the IH and OTAH and is a more dominant risk factor for IH. From this result, it can also be stated that the incisional scars are weaker than the anatomical weak areas of the abdominal wall. In conclusion, we believe that obesity is a risk factor for both IH and OTAH, and patients with high BMI should be advised to lose weight to reduce the risk of IH.

DM is one of the known risk factors for developing IH and is responsible for many possible local and systemic complications (10, 11). DM causes these adverse effects by disrupting the vascular structures, resulting in tissue ischemia, or by further compromising the general condition of the patients owing to previously developed systemic complications related to cardiac or nephrological system. DM, which causes delay in wound healing and increases wound complications, also demonstrates these effects by impairing collagen synthesis. Similar to DM, smoking also has a negative impact on wound healing through the mechanism of collagen synthesis and is therefore seen as a risk factor for developing IH. Studies have indicated that approximately 8% of patients with abdominal hernias had DM and 43% had smoking. Our results showed that the frequency of DM was slightly higher, but the remaining results were consistent with data from the literature (9, 12-15). However, we could not find statistically significant difference between the IH and OTAH groups for DM and smoking. Because DM and smoking are associated with wound-healing impairment and weakening of normal tissues, we determined that they have an equal effect on both IH and OTAH. This may be a result of not only affecting the wound healing but also weakening the abdominal wall by the pathophysiology described earlier.

Studies on the role of obesity, DM, smoking, and related collagen synthesis disorders in the etiology of IH have yielded conflicting results. The connective tissue consists of 3 groups of extracellular proteins, namely, proteoglycans, glycoproteins, and collagens. Proteoglycans regulate the structure and permeability of tissues, whereas glycoproteins are proteins that are effective in cell-to-cell interactions. Connective tissue dysfunctions are associated with collagen synthesis disorders because collagen is responsible for matrix structure and connective tissue support (16). In fact, some experimental studies have found that the most intense changes in collagen metabolism occur directly in the anterior sheath of the abdominal rectus muscle. However, despite

these proven functional properties of collagen structures, many clinical observational studies reported no statistically significant difference between the hernia types in terms of age, DM, and smoking (9, 17). A published systematic analysis analyzed 55 original articles evaluating connective tissue changes in patients with abdominal hernias and reported no statistically significant difference in collagen changes between IH and OTAHs (18). These studies support the results of our study that there is no statistically significant difference between IH and OTAH in terms of DM and smoking.

Apart from DM and smoking, wound infection, location, type of incision, wound closure material, and wound closure technique are other important factors involved in the etiology of IH. The development of wound infection releases many mediators in the surgical area, disrupts the general resistance of the patient, delays the formation of granulation tissue in the wound area, and prevents wound healing by disrupting the collagen synthesis. It has been shown that collagen synthesis has been reduced especially after contaminated surgical procedures and in patients with infected wounds, and this result is defined as a risk factor for developing IH. One study reported that IH developed in the first year after surgery in 21% of patients who underwent colorectal surgery (6). The published series reported that half of patients with IH had a history of wound infection in the postoperative period and reported that the risk of developing IH in the first postoperative year was 5-fold higher in patients with wound infection than in those without wound infection (4, 8). In our study, we had no wound infections after surgery and cannot conclude for the effect of would infection on IH and OTAH. In addition, we did not evaluate our patients for the location of the incision, type of incision, and wound closure material. However, we found a statistically significant difference among the wound closure techniques used for the repair of IH and OTAH.

The closure technique and materials for abdominal incisions are thought to affect the development of IH (19, 20). Although there are still discussions on the wound closure technique and the materials used in surgery, it is considered sufficient to use any nonallergic material that does not increase the risk of infection and can provide adequate tissue resistance. Abdominal closure is underestimated by most surgeons and is generally considered an educational activity for inexperienced residents. The closure of laparotomy should be taken as seriously as all previous operative procedures and handled with appropriate techniques and materials (9). One of the key points in preventing the development of IH is the use of fascia sutures, which can last for a long time and can resist tissue resistance. In patients undergoing laparotomy, only 70% of the tensile strength of fascia can be recovered 1 year after fascia repair. Therefore, suture materials that are absorbed and lose their strength in a short time are not suitable for fascia repair. Although studies have indicated that wound-stretching force is higher in the single suture technique than the continuous suture technique for laparotomy closure, single or continuous or monofilament/polypropylene sutures could not be found to have a significant effect on wound healing and the development of IH. Some authors argue that the use of continuous suture technique increases the risk of IH based on studies indicating that this suture technique causes insufficient wound tensile strength (21, 22).

The literature data given earlier obviously state that the closure technique is a factor for abdominal hernias, and we compared the wound closure techniques in both groups. Even though we did not evaluate the relapse rates after the wound closure techniques, we found that the primary saturation technique is applied with the same ratio for the IH and OTAH. However, the fence-darning technique is used more in IH and mesh herniorrhaphy in OTAH. This may be a result of habits for inguinal herniorrhaphy, which is the Lichtenstein technique. Because the Lichtenstein technique is a gold standard for inguinal hernia repair, which is evaluated in the OTAH group, the rate of mesh herniorrhaphies is higher in the OTAH group than the IH group. However, fence darning is safer because a prosthetic material is not applied, resulting in a lower risk of wound infection. This may result in surgeons choosing a fence-darning technique in reoperated wounds as IH.

In patients undergoing mesh repair, some precautions should be taken, such as the use of drains and antibiotic agents to avoid the risk of seroma, hematoma, and mesh reactions. Thus, we compared the drain application in both groups. We found out that there is statistically significant difference between the IH and OTAH groups, in which drain application is higher in the IH group. However, mesh herniorrhaphy is higher in the OTAH group, which is just the reverse of drain application indication for the presence of mesh. This may be because the only indication for drain application is not the presence of mesh. It is also applied for the risk of hemorrhage. Reoperated IHs has more risk of bleeding. This may cause surgeons to apply the drain to incisional IH.

Hospitalization duration is related to the expected postoperative complications and patient postoperative recovery. In our study, we found that hospitalization of IH patients is longer than of OTAH patients, which may be owing to IH being more complicated than OTAH. In addition, we found that the rate of drain application is higher in the IH group than the OTAH group, which may also cause the longer hospitalization of the patients for drain monitoring. However, because no bleeding and wound infection are found in both groups, the unnecessary application of drain owing to the surgeon's obsessions should be accepted (23).

CONCLUSION

Many factors described earlier predispose patients to develop abdominal hernias, but the significance of these factors for IH and OTAH are not much debated. In this study, we specified the significance of the predisposing factors according to the type of abdominal hernia classified as IH and OTAH. We determined that the male gender is the dominant risk factor for OTAH and obesity is dominant for IH, whereas age, DM, and smoking are the equivalent risk factors for both IH and OTAH. We also found out that drain application for IH is highly statistically significant in IH, which results in delayed hospitalization. All these results state that age, DM, and smoking are risk factors not only for IH but also for OTAH. In addition, these results show another complication

of DM and obesity, which is abdominal hernia. Therefore, physicians have one more reason to cure obesity and DM aggressively. In addition, we found out that obesity has more effect on developing IH; hence, we believe that surgeons should recommend patients to lose weight after surgery if the patient has a high BMI to prevent IHs.

Ethics Committee Approval: N/A

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Original Research

Knowledge of Dentistry Students on Local Anesthetic Systemic Toxicity and Intravenous Lipid Rescue Therapy: A Cross-Sectional Questionnaire-Based Study

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ABSTRACT

Objective: This study aimed to evaluate the level of knowledge of dentistry students on local anesthetic systemic toxicity (LAST), which will provide important information that will help in scheduling the educational content of the future syllabus before graduation, in order to prepare the students for possible challenges in the future.

Methods: This study included 234 dentistry students who were in the 3rd, 4th, and 5th years of education, at 2018–2019 educational year of Gaziantep University, Faculty of Dentistry. It was conducted in a cross-sectional questionnaire-based manner. The revised questionnaire form included questions regarding the frequency of encountered LAST cases,
signs of LAST they had observed, and treatments for LAST that they had used, particularly lipid treatment.

Results: The questionnaire was sent to 234 dentistry students who were in the 3rd, 4th, and 5th degree in the Gaziantep University Faculty of Dentistry and 215 of them responded to the study (91,88%). The majority of the participants (93%, n = 200) declared that they had received training about LAs. Among the participants, 38,60% (n = 83) of them preferred only one LA agent, whereas the remaining participants preferred multiple agents. In addition, most of the participants 79,5% (n = 171) declared that they had not observed LAST, while only 15 of the students stated that they have encountered LAST (7%) and had used an alternative therapy rather than the intravenous lipid rescue therapy. None of the students personally administered the lipid rescue therapy.

Conclusion: The results of this current study demonstrate the evident need for additional educational effort to create awareness regarding LA use and the effective management of LAST among dentistry students.

Keywords: Local anesthetics, dentistry, toxicity, local anesthetic systemic toxicity, lipid emulsion

INTRODUCTION

Local anesthetics (LAs) are frequently used in routine clinical practice and may sometimes be associated with systemic toxicity. Studies in the literature regarding the awareness of local anesthetic systemic toxicity (LAST) among different medical specialties are lacking due to the misdiagnosis or underreporting of similar events (1-3).

Therefore, we conducted a cross-sectional questionnaire-based study to assess the level of knowledge on LA use and the effective management of LAST among dentistry students in Gaziantep University Faculty of Dentistry. This study will provide important information that will help in scheduling the educational content of the future syllabus before graduation, in order to prepare the students for possible challenges in the future.

METHODS

This study included 234 dentistry students who were in the 3rd, 4th, and 5th degree at 2018–2019 educational year, second semester of Gaziantep University Faculty of Dentistry, after obtaining approval from the ethical committee (2019/318). Oral informed consent was obtained and the participants filled a questionnaire form. The study was conducted in a cross-sectional questionnaire-based manner, which was adapted from a previous study by Öksüz et al. (4). Students are supposed to have one semester of a lecture entitled 'Local anesthesia in dentistry' in the third year and one semester of another lecture entitled 'General anesthesia in dentistry', including a lecture on LAs, in the fourth year in Gaziantep University Faculty of Dentistry. All 4th and 5th degree students use local anesthesia during their clinical practice on behalf of their preceptors during various rotations

The revised questionnaire form includes questions regarding the frequency of LAST cases encountered, signs of LAST they had seen, and treatments for LAST, particularly lipid treatment, they had used. The questionnaire contains multiple-choice questions that are shown at Figure 1.

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Figure 1. Study Questionnaire (revised from Öksüz et al [4])

Thank you for participating our questionnaire about local anesthetic systemic toxicity (LAST) and treatment.

1. Age:
2. Degree of class:
3. Did you have training about local anesthesia (LA)?

   Yes ( ) No( ) Don't remember ( )
4. Choose the local anesthetics that you most frequently use.
   Articaine ( ) Bupivacaine ( ) Lidocaine ( ) Prilocaine ( ) Mepivacaine ( )
   Articaine with vasoconstrictor ( ) Lidocaine with vasoconstrictor ( ) Prilocaine with vasoconstrictor ( )
   Mepivacaine with vasoconstrictor ( )

Evaluation of degree of knowledge about local anesthetics.

5. LA dose: No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
6. LA contraindications: No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
7. LA complications: No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
8. LA maximum dose: No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
9. LA side effects: No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
10. Treatment of LA side effects: No idea ( ) Not sure ( ) Know well( ) Know Very Well ( )
11. Recognize signs and symptoms:

    Tachycardia ( ) Syncope ( ) Irritability ( ) Tinnitus ( ) Metallic taste in the mouth ( ) Allergic reactions ( ) Hypotension ( )
    Hypertension ( ) Stupor ( ) Convulsion ( )
12. Have you ever seen LAST?

    Yes ( ) No ( ) Unaware ( ) Don't remember ( )
13. Do you know intravenous lipid treatment in LAST?

    Had no idea about intravenous lipid rescue therapy ( )

    Had heard but did not have enough knowledge about it ( )

    Had read an article about lipid rescue therapy ( )

    Know how to use lipid rescue therapy ( )
14. Have you ever use intravenous lipid treatment in LAST?

    Had never seen local anesthetic toxicity ( )

    Had seen it but used treatments other than lipid rescue therapy ( )

    Had seen it and used intravenous lipid therapy ( )

Main Points:

- Studies in the literature concerning awareness of local anesthetic systemic toxicity (LAST) among different medical specialties is lacking due to misdiagnosis or underreporting of similar events.
- The level of consciousness of LAST among dentistry student that will provide helpful information for scheduling the educational content of the future syllabus before graduation to prepare the students for possible challenges in the future.
- There is evident need for additional educational effort to create awareness regarding LA use and effective management of LAST among dentistry students.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences for Windows version 11.5® (SPSS Inc.; Chicago, IL, USA) and the results are shown in tables and presented as descriptive statistics.

RESULTS

The questionnaire was sent to 234 dentistry students who were in the 3rd, 4th, and 5th degree in Gaziantep University Faculty of Dentistry and 215 of them responded to the study (91,88%). Mean age of the participants was 22,52±1,41 years (Min. 20 – max. 27). Most of the participants (93%, n=200) declared that they had received training about LAs. The most preferred LAs are shown in Table 1.

Among the participants, 38,60% (n=83) of them preferred only one LA agent, whereas the remaining participants preferred multiple agents. The degrees of knowledge of the participants regarding the LAs they had used are shown in Table 2.

In addition, most of the participants 79,5% (n = 171) declared that they had not observe any LAST before, but only 15 of the students stated that they had encountered LAST (7%), although they used an alternative therapy rather than the intravenous lipid rescue therapy. None of the students personally administered the lipid rescue therapy.

We showed that 42,8% (n = 92) of the participants had knowledge about lipid rescue therapy in LAST, although they could not remember the management of this clinical situation. Twelve of the students (5,6%) declared that they knew how to administer the lipid rescue therapy with intravenous lipids. Approximately 10,7% (n = 23) participants had read articles about the therapy, whereas 40,9% (n = 88) of the participants had received no information concerning this therapy.

Most of the common LA adverse effects observed in clinical practice are shown in Table 3.

DISCUSSION

The side effects frequently observed with the use of LA are often minor and/or transient. The symptoms of the side effects are on a broad spectrum, ranging from mild symptoms to life threatening severe ones, such as cardiac arrest and involvement of the central nervous system.

Individual patient risk factors, concurrent medications, location, and technique of the block, specific LA compound, total LA dose, timing of detection, and adequacy of treatment are risk factors that determine the severity of LAST. History of LAST articles in the literature goes back to 1884, with the introduction of cocaine to clinical practice in 1884, bupivacaine in the 1970s, and ropivacaine and levobupivacaine in the late 1980s (5, 6). Research studies are aimed at clarifying the pathophysiology of LAST and novel treatment modalities like lipid emulsion. The first guideline regarding the role of lipid emulsion to manage LAST was published by the Association of Anesthetists of Great Britain and Ireland in 2007 (7). The American Society of Regional Anesthesia and Pain Medicine (ASRA) reported practice guidelines regarding the prevention and treatment of LAST in 2010 (8). According to these guidelines, the treatment of refractory LAST requires conventional therapies (airway management with 100% O_2, convulsion therapy, cardiopulmonary resuscitation if cardiac arrest occurs), including lipid emulsions 20% intravenous lipid solutions at the dose of 1,5 mg/kg intravenously followed by 15 ml/kg/h infusion for maintenance. In case of persistent symptoms, a bolus dose can be administered twice without exceeding the limit of 10 ml/kg.

LAST rarely occurs in dentistry. However, they can become a serious problem if the clinical symptoms and signs are underestimated and appropriate steps are not taken. Unfortunately, to our best of knowledge, epidemiologic studies regarding the frequency of LAST in dentistry are not available (9).

The performance of inferior alveolar nerve blockade is relatively common in dentistry (15.3%). Therefore, the expected risk of LAST may be higher while performing the aforementioned nerve block.

The frequency of use of ester-type local anesthetic agents is low. Among amide-type LAs, lidocaine is the most commonly used local anesthetic agent, which has a low potency (10).

According to our results, the most commonly used local anesthetic was lidocaine (74.41%). Bupivacaine is a long-acting local anesthetic agent, which has a severe cardiotoxic potential. Car-

Table 1. Most commonly preferred local anesthetic agents among participants

Agent Preferred	Number of Participants	Percentage (%)
Lidocaine	160	74,41
Lidocaine + vasoconstrictor	4	1,86
Articaine + vasoconstrictor	2	0,93
Articaine	88	40,93
Bupivacaine	18	8,37
Prilocaine	9	4,18
Mepivacaine	7	3,25

Table 2. The degree of knowledge of the participants regarding the LAs used

	Know very well % (n)	Know well % (n)	Not sure % (n)	No idea % (n)
LA doses	10,7 (23)	33,5 (72)	46 (99)	9,8 (21)
LA contraindications	8,4 (18)	23,3 (50)	56,3 (121)	12,1 (26)
LA complications	8,4 (18)	25,1 (54)	52,6 (113)	14 (30)
LA maximum doses	4,2 (9)	34,9 (75)	45,6 (98)	15,3 (33)
Adverse effects of LA	8,4 (18)	21,4 (46)	56,7 (122)	13,5 (29)
Management of adverse events	11,2 (24)	40 (86)	37,7 (81)	11,2 (24)

Table 3. Most common LA adverse effects observed in clinical practice

Signs and symptoms	Percentage (%)	Number
Tachycardia–palpitation	52,09	112
Syncope	27,44	59
Irritability	24,18	52
Tinnitus	5,58	12
Metallic taste in the mouth	6,04	13
Allergic reactions	25,11	54
Hypotension	21,86	47
Hypertension	9,30	20
Stupor	4,18	9
Convulsion	0,93	2
None	0,93	2

diac arrest cases due to bupivacaine-induced LAST are known to be resuscitation-resistant cases (11). Among the amide-type LAs, percentage of choice of bupivacaine was relatively low (8.37%). We need to remember that our study population consisted of dentistry students. The more they become experienced, the more they will treat complicated cases that may need long-acting nerve blockade with bupivacaine.

Even if the practitioner's choice is an amide-type local anesthetic, the risk of LAST is still present. Furthermore, if a patient is allergic to this drug, ester-type LAs, which have high potency, have to be chosen (9).

Unfortunately, most of the clinics that administer LAs do not readily have anesthesiologists in charge at their clinic. All non-anesthesiologist practitioners including dentists have to be conscious of LAST symptoms and signs and also the therapeutic modalities. In a study by Öksüz et al. (4), of 600 dentists, 404 (67.3%) of the respondents declared that they had no idea about lipid treatment, 128 (21.3%) had heard about lipid treatment but said they did not have enough knowledge of it, 59 (9.8%) had read an article about lipid treatment, but only 9 (1.5%) knew how to administer the lipid treatment. Another study among 124 dentists demonstrated that they were aware of some side effects of LAs with vasoconstrictors; however, they had inadequate knowledge about the signs and symptoms of LAs overdose (12).

Published case reports regarding LAST (13-15) in the literature most commonly depend on the experience of non-anesthesiologists. Interestingly, a Danish survey study among anesthesiologists in 2011 concluded that there was limited knowledge of lipid rescue therapy in LAST (3). It can be speculated that the guidelines about lipid emulsion therapy were relatively new at the time period of the study. A study performed at a similar time period among dermatologists found similar results. According to this study, the awareness of intravenous lipid rescue therapy was lower than expected (22%) (2).

Nurses who work in preoperative and postoperative care units, outpatient services, labor and delivery units, and even operation room circulating nurses usually do not receive formal education or training about the diagnosis and treatment of LAST events (16).

Ophthalmologists are another group of specialists who frequently use LAs. A questionnaire study performed among 104 ophthalmologists reported that 76% of the participants declared that they use LAs every day or more than twice a week; however, 56.7% of them had no specific training about this clinical situation (17).

CONCLUSION

Dentistry practitioners who perform various nerve blocks multiple times a day also have to be aware of LAST. A dentist who encounters a LAST case should accurately know about its rapid recognition. In addition, treatment with lipid emulsion therapy has to be kept in mind. We have to incorporate education on LA safety and the treatment of LAST in mandatory training sessions. The introduction of national guidelines on lipid rescue therapy would probably accelerate this process.

In this context, academic trainers have a very important mission to prepare their students. The content of the local anesthesia lectures have to be reviewed and arranged regarding these concerns. The results of this current study demonstrate the evident need for additional educational efforts to create awareness regarding LA use and the effective management of LAST among dentistry students.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University Clinical Investigations Ethical Committee (2019/318).

Informed Consent: Oral informed consent was obtained from patients who participated in this study.

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Original Research

Comparative Evaluation of Micropercutaneous Nephrolithotomy and Retrograde Intrarenal Surgery in the Management of Renal Stones 10–20 mm in Size

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ABSTRACT

Objective: To assess the effects of micropercutaneous nephrolithotomy (microperc) and retrograde intrarenal surgery (RIRS) on treating 10–20-mm kidney stones.

Methods: Twenty-eight patients who underwent microperc (group 1) and 30 patients who underwent RIRS (group 2) between February 2015 and April 2017 were examined. This study included patients with 10–20-mm kidney stones located at a single location. Stone characteristics, fluoroscopy and operation times, stone-free rates (SFRs), and postoperative complications were compared between the two groups.

Results: Age, gender, size, laterality, and stone location,and operation times were similar between the two groups. Moreover, the two groups had similar SFRs (92.9% vs. 90%, p=1.00, respectively). The mean fluoroscopy time was higher in group 1 than in group 2 (p=0.001). The two groups were similar in terms of SFRs (92.9% vs.90%, p=1.00, respectively). The decrease in hemoglobin levels was signifcantly more in group 1 than in group 2 (p=0.001).In terms of postoperative complications, no statistically significant difference was observed between the groups (p=0.277). The mean hospitalization time was 50.21±9.62 and 27.46±7.23 hours in groups 1 and 2, respectively (p=0.001).

Conclusion: Both techniques are successful in treating renal stones and have low complication rates. Microperc is an effective method for managing medium-sized renal stones. However, longer fluoroscopy time and longer hospital stay are the main disadvantages of this technique.

Keywords: Micropercutaneous nephrolithotomy, retrograde intrarenal surgery, renal stone

INTRODUCTION

Urinary system stone disease is a common disease in urology practice. Shock wave lithotripsy (SWL), percutaneous nephrolithotomy (PCNL), and, less frequently, open or laparoscopic pyelolithotomy are standard approaches for treating renal stones (1). The appropriate method is chosen based on the following factors: the location of the kidney stone and its size and the urinary system anatomy. With technological advances, the efficacy of treating kidney stones has improved significantly (1, 2). Recently, retrograde intrarenal surgery (RIRS) has become a more common, effective, and safe treatment option for stones smaller than 20 mm due to the development of the optical system and the introduction of the Holmium: YAG (Ho: YAG) laser (2, 3). RIRS has potential advantages as follows: lower morbidity than percutaneous procedures and a higher success rate in the first session than SWL (4, 5). In 1976, Fernström and Johansson performed the first successful percutaneous removal of renal stones (6). Since then, PCNL has been increasingly used by urologists. PCNL is a successful method for treating kidney stones; however, it has a high risk of complications (7). Technical improvements such as miniaturization of the instruments and the development of smaller sheaths (mini, ultramini, etc.) reduced the procedure-associated morbidity, thereby decreasing the rate of complications without negatively affecting its therapeutic efficacy (8). On the other hand, micropercutaneous nephrolithotomy (microperc) is a single-step procedure performed using an optical puncture system. According to a microperc series by Desai (9) in 2011, high success rates were reported in both adult and pediatric populations (10), indicating that this technique has a high SFR, provides renal access, and reduces the time to initiate lithotripsy (9, 10).

Therefore, in this study, we determined the success and complication rates of microperc and RIRS techniques in patients with medium-sized (10–20 mm) kidney stones retrospectively.

METHODS

We retrospectively analyzed the data of 58 patients who underwent surgery for 10–20-mm kidney stones at a single location

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from February 2015 to April 2017. Twenty-eight patients were treated with microperc (group 1) and 30 patients with RIRS (group 2). A single surgeon conducted all the procedures. Patients with a single renal stone up to 20 mm in size without contraindication to microperc and RIRS were included in the study. Exclusion criteria were as follows: patients who underwent different surgical treatments such as ureterorenoscopy (URS), those who had multiple stones at different locations, and those with active urinary tract infection or hypersensitivity to anesthetic drugs. All patients were informed in detail about the success rates and complications of both treatment methods. The patients' requests and malfunctioning of the devices were factors taken into account when choosing the suitable surgical technique.

Stone size and location, intraoperative findings, and postoperative complications were recorded for all patients. Noncontrast computed tomography (CT) was used to measure maximum stone length defined as stone size. Residual stones were detected with CT 3 months postoperatively; stones of 4 mm or smaller were considered insignificant residues. We evaluated the urine culture results of all patients preoperatively. The antibiotic treatment protocol was similar in both groups. Stone size and location, operation and fluoroscopy times, postoperative hospital stay, analgesic requirements, complications (using the Clavien grading system), hemoglobin levels, and SFRs were compared between the two groups. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki Declaration and its later amendments.

Microperc Technique

First, a 6-French (Fr) ureteral catheter was inserted into the ureter using a cystoscope in the lithotomy position and fixed to the Foley catheter. Then, the patients were placed in the prone position. A 16-gauge 4.8 Fr all-seeing needle (PolyDiagnost, Pfaffenhofen, Germany) under fluoroscopy was employed to access the appropriate calyx of the kidney. After the stone was visualized, the inner part of the instrument was removed and a three-way connector was attached to the outer part. A connector side port was used for irrigation and the telescope was inserted through the other side. An optical fiber was inserted into the central connector side port, whereas the other port was used for the laser probe (Ho: YAG laser device, Quanta System, S.p.A., Italy). Generally, 272 µm laser probes were employed and the energy settings of the device were set to dust conditions of 0.8 J energy and 8 Hz frequency instead of fragmentation. The foot pedal was used for irrigation. The ureteral catheter was usually removed on the first postoperative day. A double-J (JJ) stent was inserted if there was a significant stone burden or residual stones. The operative time was calculated from the time from accessing the collecting duct system to the removal of the microperc system from the kidney.

Main Points:

- We observed that the success and complication rates of the microperc and RIRS techniques were similar in patients with medium-sized renal stones.
- However, microperc was associated with longer fluoroscopy time, greater decrease in hemoglobin levels, and longer hospital stay.
- Where there is a narrow infundibulopelvic angle, microperc is more useful and favored than a flexible ureteroscope because it provides direct access to low calyx stones.

RIRS Technique

Under fluoroscopy guidance, a guidewire (Sensor ™, Boston Scientific, USA) was inserted into the ureter in all patients under general anesthesia. The collecting duct system was reached using a flexible ureteroscope (7.5 Fr FlexX™ 2, Karl Storz, Tuttlingen, Germany) with a 12 F ureteral access sheath (Flexor®, Cook Medical, USA). Ho: YAG laser was applied with a 200 µ laser probe and the stones were broken in situ. For lithotripsy, the laser was used at a frequency of 0.8–1.5 joules, 10–25 pulses. If necessary, a grasper and 2.4 F Zero Tip Nitinol Basket were employed to manipulate small stone fragments after fragmentation. Fragments of about 2 mm or smaller were not broken down any further. After the procedure, a fluoroscopy-guided placement of a 4.8 F JJ stent or a 5 F ureter catheter was conducted. The ureter catheters were used in the following cases: patients who were declared stone-free postoperatively or when there were no postoperative complications; they were removed on the first postoperative day. On the other hand, the JJ stents were placed in patients with prolonged operation time or minor ureteral wall damage. The operative time was calculated from the beginning of the cystoscopy procedure to the insertion of the ureter catheter or JJ stent into the ureter.

Patients who had no additional complications or complaints in both groups were discharged on the first postoperative day.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM SPSS Corp.; Armonk, NY, USA). Mann–Whitney U test was utilized for comparing the nonparametric values. Pearson's chi-square test was used to compare proportions in different groups. In the power analysis using the G-power computer program for lower calyx stone subgroups, the large effect size (d:0.8) was detected with a power of 80% and an alpha of 0.05. A p value of <0.05 was considered statistically significant.

RESULTS

The patients' demographic characteristics are listed in Table 1. Age, gender, operation times, laterality, and size and location of stones were similar in both groups. The mean fluoroscopy times were 91.00±15.99 seconds (sec) and 48.33±11.78 sec in groups 1 and 2 (p=0.001), respectively. The SFRs were similar in the two groups (92.9% vs. 90%, p=1.00, respectively). In the microperc group, noncontrast urinary CT detected residual stones in two patients had on the third postoperative month; subsequently, they underwent re-microperc and became stone-free. On the other hand, in the RIRS group, residual fragments were observed in 3 patients who became stone-free after the re-RIRS. In group 2, JJ stents were inserted in two patients with minimal ureteral wall injury (Clavien II) and two patients due to the risk of steinstrasse formation, whereas in group 1, JJ stent was placed in one patient

due to the migration of residual stone to the ureter (p=0.354). Hemoglobin levels decreased in both groups; however, group 2 had significantly low levels compared to group 1 (1.47±0.61 g/dL vs. 0.76±0.31 g/dL, p=0.001, respectively). Postoperative fever was observed in four and three patients in the RIRS group and the microperc group, respectively (p=1.00), who were treated with antipyretics (Clavien I). Two patients in group 1 and one patient in group 2 required narcotic analgesics postoperatively (p=0.60). The mean hospitalization time was significantly longer in the microperc group (50.21±9.62 hours vs. 27.46±7.23 hours, p=0.001, respectively). Table 2 summarises the intraoperative and postoperative parameters of the study groups.

Table 1. Patient demographics and stone characteristics for each group

	Microperc group	RIRS group	p
Patients, n	28	30	
Mean (SD) age, years	48.75±8.26	48.03±12.20	0.796
Gender, male:female	19:9	20:10	0.923
Mean (SD) stone size, mm	15.07±2.37	15.43±2.50	0.589
Laterality, right:left	15:13	16:14	1.000
Stone location, n			0.865
Pelvis	11 (39.3%)	14 (46.7%)	
Upper calyx	3 (10.7%)	2 (6.7%)	
Middle calyx	3 (10.7%)	2 (6.7%)	
Lower calyx	11 (39.3%)	12 (52.2%)	

p<0.05 values are statistically significant. SD: standard deviation; n: number; microperc: micropercutaneous nephrolithotomy; RIRS: retrograde intrarenal surgery.

DISCUSSION

SWL, standard PCNL/miniperc, and RIRS are some treatment options for small kidney stones. RIRS and different PCNL sizes (mini, ultramini, and microperc) have similar success and complication rates. In this study, we compared the outcomes of applying microperc and RIRS techniques for treating patients with medium-sized kidney stones and observed that their success rates were similar. However, microperc was associated with longer fluoroscopy time, greater decrease in hemoglobin levels, and longer hospital stay.

SWL is a popular method due to its noninvasive nature; however, it has several drawbacks such as requiring many sessions and additional procedures to achieve renal stone clearance (11). A Cochrane review has reported that PCNL is more effective than SWL in treating renal stones (12). PCNL and RIRS techniques are favored techniques with high success rates, but PCNL is more invasive and has a higher complication rate (13). Blood loss is one of the PCNL complications and might be associated with tract size (14). The use of miniaturized instruments (mini, ultramini, micro, etc.) is starting to be popularized to reduce complication rates. Accordingly, microperc, which involves a single-step stone fragmentation is applied using an optical puncture system for small stones, is speculated to be associated with SFRs and the lowest incidence of morbidity in selected cases (9).

Table 2. Comparison of intraoperative and postoperative variables in the two study groups

Variables	Microperc group	RIRS group	p
Operation time, minutes (mean±SD)	57.60±6.31	56.43±6.08	0.477
Fluoroscopy time, seconds (mean±SD)	91.00±15.99	48.33±11.78	0.001
Intraoperative double-J stenting, n (%)	1 (3.6%)	4 (13.3%)	0.354
Intraoperative complications, n (%) Minor ureteral wall injury (Clavien II)	0 (0.0%)	2 (6.7%)	0.492
Postoperative complications, n (%)	8 (28.5%)	5 (16.6%)	0.277
Hematuria (Clavien I)	3 (10.7%)	0 (0.0%)	0.242
Fever (Clavien I)	3 (10.7%)	4 (13.3%)	0.617
Colic pain (Clavien I)	2 (7.1%)	1 (3.3%)	0.605
Hemoglobin drop g/dl (mean±SD)	1.47±0.61	0.76±0.31	0.001
Postoperative narcotic analgesic requirements, n (%)	2 (7.1%)	1 (3.%)	0.605
Hospital stay, hours (mean±SD)	50.21±9.62	27.46±7.23	0.001
Stone-free rates, n (%)	26 (92.9%)	27 (90%)	1.000

p<0.05 values are statistically significant. SD: standard deviation; n: number; microperc: micropercutaneous nephrolithotomy; RIRS: retrograde intrarenal surgery.

In 2010, Desai et al. (9) first described the microperc technique. Since then, several case series were conducted on the treatment of 10–20-mm renal stones in both children and adults, with approximately 90% SFR and up to 10% complication rates (15).

RIRS is an effective and safe surgical management option for treating medium-sized kidney stones (<20 mm), which has high SFRs and a low risk of major complications (16). Severe bleeding or infection after RIRS is rare. Sabnis et al. reported that SFRs after RIRS ranged from 84% to 97% and that the risk of complications for small renal stones was low (17). In Tepeler et al.'s study, using microperc in 21 patients with lower pole stones resulted in 85.7% SFR (18). In the current study, SFRs were similar in both the groups (92.9% v.s 90%, respectively). Many studies have demonstrated that there were no significant differences between the two techniques in terms of complications (19-21). Cepeda et al. compared the microperc and RIRS procedures and found no significant differences for mild and severe complications (20). In this study, the intraoperative and postoperative complications were statistically insignificant between the two groups, which is in line with Cepeda et al.'s results. None of the patients complained of organ injury or sepsis. Kandemir et al. stated that there was no significant difference in hemoglobin decrease between the microperc and RIRS groups (22). Alternatively, in this study, we observed a significant decrease in hemoglobin levels in the microperc group, which is in agreement with Sabnis et al.'s results (23).

Moreover, in this study, the fluoroscopy time was longer in the microperc group than in the RIRS group, which was similar to the results of Armağan et al. and Kandemir et al. (19, 22). All-seeing needle access was applied to the renal unit under fluoroscopic guidance, leading to prolonged fluoroscopy time, which is a disadvantage of the microperc procedure.

Furthermore, the mean operative time was similar in both the groups, agreeing with the findings of Kandemir et al. and Sabnis et al. (22, 23).

In the RIRS group, the JJ stent was placed in two patients due to the risk of steinstrasse and in two patients for minimal ureteral wall injury, whereas in the microperc group, it was inserted in one patient due to the migration of residual stone to the ureter. However, no significant differences were observed between the two groups. These findings were in line with the findings of Cepeda et al. and Kandemir et al. (20, 22).

Two patients in the microperc group and one patient in the RIRS group required narcotic analgesics postoperatively. Additionally, Sabnis et al. reported that the mean requirement for postoperative narcotic analgesia was higher in the microperc group (23).

In our study, the mean hospitalization time was longer in the microperc group than that in the RIRS group that is similar to Armağan et al. and Kandemir et al. studies (19, 22). Since the incidence of complications associated with bleeding was higher in the microperc group, the length of hospital stay was longer. However, in terms of hospital stay, these findings were not similar to those in Cepeda et al. and Bağcıoğlu et al. studies (20, 21). The number of patients with lower calyx stones was low in both groups; as a result, subgroup analyses for lower calyx stones were conducted. Where there is a narrow infundibulopelvic angle, microperc is more useful and favored than a flexible ureteroscope because it provides direct access to low calyx stones; besides, it is preferred over RIRS in the case of the ureteral strictures or narrow ureters.

The retrospective nature and small sample size were the main limitations of this study. To obtain more accurate results, multicentre studies with large numbers of patients are warranted.

CONCLUSION

Both techniques had high success rates and low complication rates The microperc technique is an effective and reliable management option for renal stones smaller than 2 cm. However, this method has major disadvantages, such as prolonged hospital stay, longer fluoroscopy times, and more hemoglobin drops. Therefore, prospective controlled studies with large sample sizes are required to confirm these results.

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Original Research

The Top 100 Cited Articles on Ocular Trauma: A Bibliometric Analysis

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ABSTRACT

Objective: Eye injuries are one of the leading causes of disabling ocular morbidity. The objective of this bibliometric study was to evaluate the top 100 cited articles on ocular trauma published between 1975 and 2018 via multidimensional citation analysis.

Methods: We analyzed the top 100 cited articles among 3,768 ocular trauma articles published between 1975 and 2018; these articles were obtained from the databases in Web of Science and PubMed based on their citation rates per article, publication years, countries of origin, institutions or organizations, the most common subjects, funding status, article types, and levels of evidence. The data obtained were analyzed with the SPSS® 20.0 software package program.

Results: In the top 100 cited articles on ocular trauma, the total number of authors was 420 and the average authorship was 4.20±2.23 (range: 1–14). In our study, 70 of the top 100 cited articles were published in journals with an impact factor (IF) of ≥2.00 (range: 2.016–8.806), and Q index or quartile score of these journals was mostly Q1. Although the most preferred journal was Ophthalmology according to the total number of citations and articles (n=2,183 and n=23, respectively), Eye was the most preferred journal according to the mean number of citations per article. Besides, the three most common topics among the top 100 cited articles were mechanical eyeball injury (40 articles), epidemiology of ocular trauma (19 articles), and traumatic eye infection (17 articles). The average level of evidence was found to be 3.14±0.66 (range: 1–4), and the mean number of citations per article was the highest level at 2. Moreover, we also found that the most commonly preferred article type by authors was clinical research (92 articles), and most of them were in the B level of evidence group (70 articles).

Conclusion: Analysis of the top 100 most cited articles on ocular trauma as an update study can provide us scientific contributions and vital current data in clinical implementations.

Keywords: Ocular trauma, top cited article, bibliometric analysis

INTRODUCTION

Ocular trauma is a common, treatable, and preventable health problem (1). It is the most common cause of monocular blindness and visual impairment worldwide (2). Ocular trauma is directly responsible for bilateral blindness in about 5% of population (3). The World Health Organization Program for the Prevention of Blindness had presented the following findings: some 55 million eye injuries that restrict activities for more than one day occur each year, in which 750,000 cases, including some 200,000 open globe injuries, will require hospitalization each year; approximately 1.6 million people are blinded from eye injuries; and an additional 2.3 million people with bilateral low vision and almost 19 million with unilateral blindness or low vision are blinded from eye injuries (4). Many studies report that one out of five adults have a history of ocular trauma (5). The rate of ocular trauma history in the childhood is around 12%–38%, and it is one of the most important preventable causes of blindness in the childhood (6). Poor vision or visual impairment due to ocular trauma causes a severe financial load and workforce loss on both individuals and the national economy. This burden occurs because trauma-inflicted eye injuries are imminent eye diseases that require long-term hospitalization, special patient visits, long-term treatment and patient follow-up, and visual rehabilitation. In addition to the prevention of ocular traumas, several scientific studies have been conducted worldwide on ocular traumas to determine and develop their quick and effective medical and surgical treatments. Furthermore, we can categorize these studies mainly as epidemiologic, diagnostic, therapeutic, and prognostic. To the best of our knowledge, we have not found any update study that comprised all these fields in the literature.

A bibliometric study is the quantitative analysis of the publications in the literature via statistical and mathematical methods (7, 8). These studies are trending topics or updates studies, which can significantly help in determining the research tendencies of scientific studies conducted over a certain time period. Nowa-

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days, bibliometric studies are more increasingly involved in the current scientific literature for determining and evaluating the influential literature (9, 10). Furthermore, bibliometric analysis studies are also considerably helpful in determining the impact and number of citations of a scientific article after it is published (11). Garfield E. was the first researcher to present the "citation classics" concept in 1987 for the most cited articles published in *JAMA* (12). The trend of bibliometric analysis of current studies started with Garfield at *JAMA* spread with other studies comprising bibliometric article analyses conducted by many researchers in different medical fields in the following years (13). However, the review of current literature revealed that there are few bibliometric studies in the realm of ophthalmology, and most of them are focused on ophthalmic epidemiology as well as the evaluation of ophthalmology journals, cataract surgery, and dry eye (7, 11, 14, 15). However, we did not found any bibliometric analysis studies on ocular trauma in the current literature.

The objective of this study is to systematically analyze the most cited articles "key papers or classic papers" according to the data obtained from PubMed and Web of Science (WoS) in the field of ocular trauma. We have determined the number of citations with ranking; publications by years and journals; mean number of citations per article by journals, type, and subtype of articles; institutions and countries of origin; the most common topic of frequently cited articles; level of evidence with the number of mean citations per article; and authorship status of the classic papers in this bibliometric research.

METHODS

Study Design

Study type: Retrospective clinical research

Level of evidence: 3 or Group B (according to Scottish Intercollegiate Guidelines Network; SIGN) (16).

Data Collection and Inclusion Criteria

The data used in this bibliometric citation analysis were obtained from Thomson Reuters' WoS Core Collection database (Philadelphia, Pennsylvania, USA) and PubMed (US National Library of Medicine – National Institutes of Health). We accessed the WoS database (accessed date: December 2, 2018) using the keyword "ocular trauma" for the period between 1975 and 2018. Consequently, we obtained 3,768 articles and conducted analysis of the top 100 cited articles among these results. We accessed the remaining data pertaining to the analyzed articles via PubMed. Three of the authors (X, Y, and Z) independently evaluated the top 100 cited articles with consensus. Being a first author or co-author was accepted as the authorship criterion in this study. To shorten the same obtained table, we limited the quantitative values to "2 or more and 3 or more." The level of evidence of the top 100 cited articles was detected in accordance with the SIGN criteria (16). According to these criteria, Group A evidence (levels 1a and 1b) comprised randomized clinical trials (RCTs) or meta-analysis of RCTs. Group B evidence (levels 2a, 2b, and 3) comprised cohort studies, case–control studies, and comparison of groups, where the data were collected retrospectively, as well as semi-experimental studies. Group C evidence (level 4) comprised case reports and series as well as expert opinions or expert committee reports (excluding levels 1 and 3 of evidence).

Main Points:

- This study was performed a bibliometric analysis of popular ocular trauma articles via the use qualitative and quantitative methods.
- Trending topics or updates ophthalmologic studies, which can significantly help in determining the research tendencies of scientific studies conducted over a certain time period.
- Ocular traumas are important and have revealed the need for a new and more comprehensive classification on ocular traumas.

Statistical Analysis

We used descriptive statistical methods in this study. The mean (±SD) was calculated for continuous variables while categorical variables were presented as frequencies. The normal distribution of the data was tested using the one-sample Kolmogorov–Smirnov test. The statistical analyses were performed using the Statistical Package for Social Sciences 20.0 software (IBM SPSS Corp.; Armonk, NY, USA) for Windows.

Ethical Statement

All authors declare that the research was conducted according to the principles of the World Medical Association Declara-tion of Helsinki "Ethical Principles for Medical Research Involving Human Subjects." This study did not need to be approved by an ethics committee because it performed a bibliometric analysis or citation analysis of existing published classical papers studies.

RESULTS

Contributions, Attributions, and Research Groups

We found that the average citation of the top 100 cited articles on ocular trauma was 88.07±41.97 (range: 50–269), the sum of times the article were cited was 8,807 and the number of self-citations was 239 (according to Thomson Reuters' WoS Core Collection). The publishing language was English in all the articles. We determined that the most cited article (times cited: 269) on ocular trauma was the perspective article by Pieramici DJ et al. about the classification of mechanical ocular trauma with the following topic: "A system for classifying mechanical injuries of the eye (globe). *American Journal of Ophthalmology*; 1997: 123(6): 820–831." The last three articles in the rankings that had the same number of citations (50). Their subjects were fungal keratitis (Wang L et al.), traumatic macular hole (Yamada H et al.), and blindness after facial trauma (Zachariades N et al.), respectively (Appendix 1). In this study, there was no clearly defined study group but we found that the most cited article had an ocular trauma classification group.

The analyses of the publications (between 1982 and 2013) and citation rates in each year (between 1982 and 2018) of the "key papers" on ocular trauma revealed that the highest number of publications was observed in 1996 (10 publications) and the highest number of citations was observed in 2012 (661 citations)

(Fig. 1 and Fig. 2). The total number of self-citations for top 100 cited articles was 239. Besides, we have added the current citation information to this study for determining the articles' influence on this bibliometric study (Appendix 1).

Authorship

We found that the total number of authors was 420, and the mean number of authorships was 4.20±2.23 (1-14) in these influential papers (according to Thomson Reuters' WoS Core Collection and PubMed). We analyzed the distribution of 25 authors who were included in two and more articles among the classic papers and found that the first three ranks were shared by Kuhn F, Tielsch JM, and Witherspoon CD, respectively, with five articles and more. Additionally, we observed that the most frequent first author of key papers included in our research was Kuhn F with five articles, and Tielsch JM, Witherspoon CD, and Morris R were found to be the most frequent co-authors with four articles and more (Table 1).

Origins of Countries and Institutions or Organizations

The three most commonly listed countries with two and more publications in our study were the USA (53%), the UK (7%), and Germany (5%), respectively. In total, 23 countries were listed 117 times in the top 100 cited articles (range: 1–62) (Table 2). This study showed that the most commonly listed institution or organization was the Johns Hopkins University (USA), which was listed 13 times in the top 100 cited articles. Moreover, the number of institutions or organizations that published two and more publications were found to be 19/25 (76%), and majority of these institutions were in the USA (Table 3).

Funding Status

In addition to this finding, we also found that 22 of the top 100 cited articles on ocular trauma, which are considered "topic trends," received grants from 9 different funding agencies, and

Figure 1. The top 100 cited articles published in each year (1982–2013)

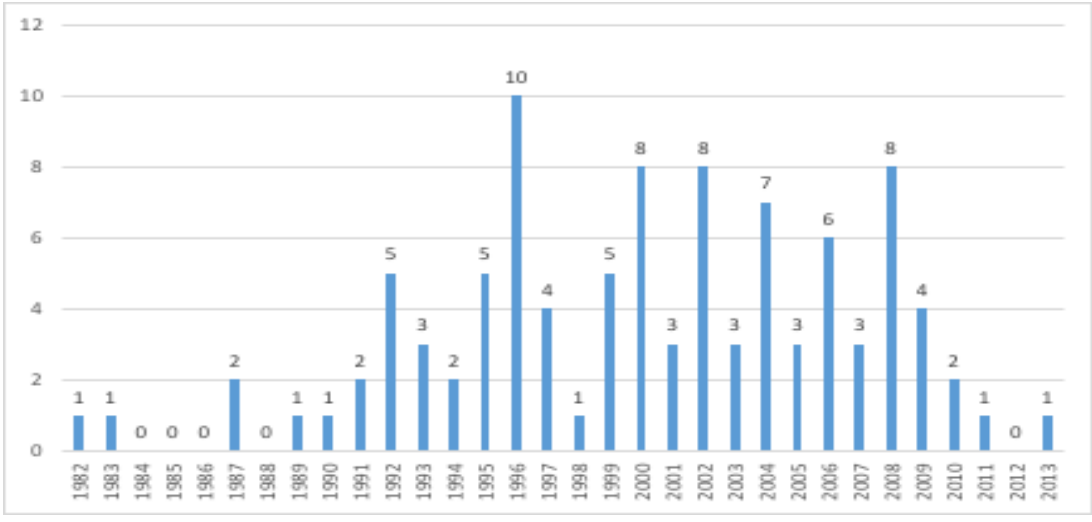


Figure 2. Citations in each year (last 36 years, source: Web of Science database)

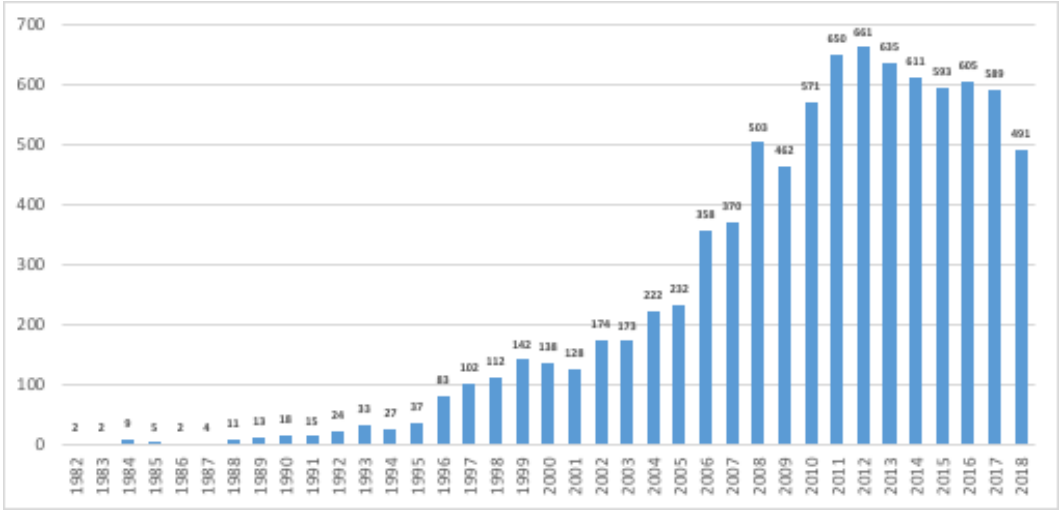


Table 1. The most common first and total authors with presence in two or more articles in the top 100 cited articles on ocular trauma

Author	Affiliation	First	Total
Kuhn F	University of Alabama at Birmingham (USA)	5	6
Tielsch JM	Wilmer Eye Institute, John Hopkins University, Baltimore (USA)	1	5
Witherspoon CD	University of Alabama at Birmingham/Eye Foundation Hospital, Birmingham (USA)	0	5
Morris R	University of Alabama at Birmingham/Eye Foundation Hospital, Birmingham (USA)	0	4
Bower KS	Johns Hopkins Medicine, Baltimore (USA)	0	3
Colyer MH	Walter Reed Army Medical Center, Washington (USA)	2	3
Desai P	London School of Hygiene and Tropical Medicine, London (UK)	2	3
Gilliland MGF	East Carolina University School of Medicine, Greenville (USA)	2	3
MacEwena CJ	University of Southampton, Southampton General Hospital, Southampton (USA)	1	3
Ryan SJ	University of Southern California School of Medicine, and the Doheny Eye Institute, Los Angeles (USA)	1	3
Weichel ED	Walter Reed Army Medical Center, Washington, District of Columbia (USA)	1	3
Wong TY	Singapore National Eye Center and Singapore Eye Research Institute (Singapore)	3	3
Aaberg TM	Emory University School of Medicine, Atlanta (USA)	0	2
Baines P	London School of Hygiene and Tropical Medicine, London (UK)	0	2
Brechner RJ	The Johns Hopkins University School of Hygiene and Public Health, Baltimore (USA)	0	2
Bucolo C	Ocular Pharmacology, University of Catania, Catania (Italy)	0	2
Cardillo JA	University of Southern California School of Medicine, Los Angeles (USA)	1	2
Danis RP	University of Wisconsin School of Medicine and Public Health, Madison (USA)	0	2
Dannenberg AL	The Johns Hopkins University School of Hygiene and Public Health, Baltimore (USA)	1	2
De Juan E	Wilmer Ophthalmological Institute, Johns Hopkins Hospital, Baltimore (USA)	0	2
Dick JS	Kaiser Permanente Health System, San Diego (USA)	0	2
Dutton GN	Tennent Institute of Ophthalmology, Western Infirmary Glasgow (UK)	0	2
Flynn HW	Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami (USA)	0	2
Folberg R	University of Iowa College of Medicine, Iowa (USA)	0	2

Table 2. The most commonly listed countries with presence in two and more articles in the top 100 cited articles

Country	Number
The United States of America (USA)	62
The United Kingdom	9
Germany	6
India	5
Scotland	5
Australia	4
Italy	3
Japan	3
Singapore	3
Israel	2
Nepal	2
People's Republic of China	2

most of them were NEI NIH HHS (12 studies), NCRR NIH HHS (2 studies), and PHS HHS (2 studies) (according to Thomson Reuters's WoS Core Collection).

Journals and Proceedings Papers

Our study showed that 70 of the top 100 cited articles were published in the journals with an IF of ≥2.00 (range: 2.016–8.806) (according to Clarivate Analytics, 2018), and Q categories of these journals according to the Scimago Journal & Country Rank, 2017 were Q1, except for *Journal of Inflammation*, which was Q2. Although the most preferred journal was *Ophthalmology* according to the total number of citations and articles (n=2,183 and n=23, respectively), *Eye* was the most preferred journal according to the mean number of citations per article (Table 4). Besides, we observed that the number of proceedings papers among the top 100 articles in 1 national and international scientific activities was 6, and the most presented proceedings paper was presented at the "56th Annual Meeting of the AAST" (date: November 11–14, 2006; Las Vegas, USA)" (according to Thomson Reuters' Core Collection).

Table 3. Institutions of origin with two or more appearances in the top 100 cited articles

Rank	Institutions	Number*
1	Johns Hopkins University (USA)	13
2	The United States Army (USA)	7
3	The United States Department of State (USA)	7
4	University of Alabama at Birmingham (USA)	5
5	University of Alabama System (USA)	5
6	Walter Reed National Military Medical Center (USA)	5
7	Wilmer Eye Institute at Johns Hopkins (USA)	5
8	Baylor College of Medicine (USA)	4
9	Uniformed Services University of the Health Sciences (USA)	4
10	University College London (United Kingdom)	4
11	University of London (United Kingdom)	4
12	University of Southern California (USA)	4
13	Doheny Eye Institute (USA)	3
14	East Carolina University (USA)	3
15	Madigan Army Medical Center (USA)	3
16	Medical College of Wisconsin (USA)	3
17	Singapore National Eye Centre (Singapore)	3
18	Thomas Jefferson University (USA)	3
19	University of Cologne (Germany)	3
20	University of Dundee (United Kingdom)	3
21	University of Melbourne (Australia)	3
22	University of Miami (USA)	3
23	University of North Carolina (USA)	3
24	Alaska Native Medical Center (USA)	2
25	American Society of Ocular Trauma (USA)	2

*Number of times listed out of total 25 institutions in the top 100 cited articles.

Main Topics

The three most common topics among the top 100 cited articles were mechanical eyeball injury including open and closed globe injury (40 articles), the epidemiology of ocular trauma (19 articles), and traumatic eye infection (17 articles), respectively (Table 5). Mechanical eye trauma was more common than non-mechanical ones among the top 100 cited ocular trauma articles (Fig. 3).

Study Types and Levels of Evidence with Mean Number of Citation per Article

Additionally, the most preferred study type and subtype among researchers in the top 100 cited articles on ocular trauma were clinical studies (92 articles) and retrospective–descriptive study studies (38 articles), respectively. Our study found that the mean level of evidence was 3.14±0.66 (range: 1–4), and the mean number of citations per article was the highest level 2, which was experimental study according to the levels of evidence. Furthermore, the evidence group of 70 articles was group B (prospective/retrospective comparative studies, retrospective cohort studies, case–control studies, descriptive studies, cross-sectional studies, and validation studies), and the evidence group of 28 articles was group C (reviews, expert committee reports, and expert opinions). Finally, the level of evidence of two articles were group A (meta-analysis of RCT, systematic review, RCT, and prospective cohort study) (Table 6).

We determined that the most commonly preferred article type and subtype by authors were clinical research (92 articles) and retrospective–descriptive study studies (38 articles), respectively.

DISCUSSION

The eye is an important sense organ, to such an extent that it was mentioned in the Codes of Hammurabi, the Babylonian King (17). Eye injuries are one of the main causes of disabling ocular morbidity; hence, they require an immediate and comprehensive care. For ocular trauma to be better interpreted and understood in the literature, many studies on ocular trauma's classification and epidemiology have been conducted in the past. However, because there is no mutual consensus on this subject, we believe that there will be more studies in the future.

Although it is often criticized for its limited analysis and methodology, bibliometric research is quite important in providing scientific and topic trends for a specific period of time. For these reasons, we conducted a bibliometric study in the realm of ocular trauma. In particular, this kind of studies can present important clues about the current best-cited or landmark articles to researchers because it reflects scientific improvements in the respective field along with landmark papers and topic trends in chronological and systematic perspectives (12, 13, 18). In the last 30 years, many bibliometric analyses of articles have been conducted in the different fields of clinical medicine (19, 20). Scientometric analysis, which includes altmetric scores at different publication levels or values, has also been added to these studies in the recent years (21, 22).

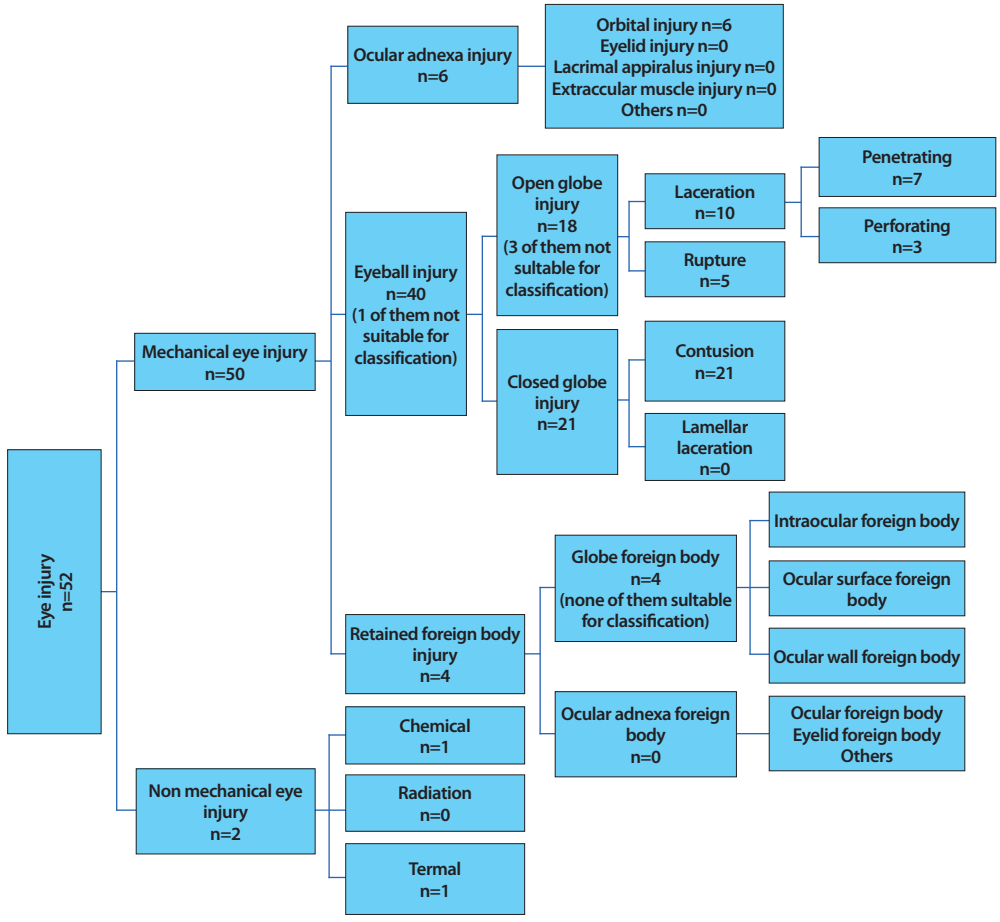
We used the contemporary medical databases, primarily the WoS database and PubMed, in our bibliometric analysis. Although many international medical databases (such as PubMed, ScienceDirect, Medline, Scopus, Embase, EBSCO Host, and so on) have been used in citation analysis studies, the most frequently used source is Thomson Reuters' WoS Core Collection. The WoS database includes important information about the number of citations and researching other relevant academic effects (23).

Table 4. List of journals that have two and more published articles on ocular trauma

Rank	Journals	Number of articles	Impact Factor (2017)*	Total number of citations	Mean number of citation per article±SD	Q categories (2017)**
1	Ophthalmology	23	2.016	2183	94.91±43.66	Q1
2	JAMA Ophthalmology (formerly known as the Archives of Ophthalmology)	10	6.669	800	77.03±22.76	Q1
3	American Journal of Ophthalmology	7	4.795	708	101.14±78.37	Q1
4	British Journal of Ophthalmology	7	3.384	580	81.12±18.30	Q1
5	Survey of Ophthalmology	5	3.764	314	62.80±10.18	Q1
6	Cornea	4	2.464	387	96.75±81.74	Q1
7	Eye	3	2.478	368	122.66±70.60	Q1
8	Graefe's Archive for Clinical and Experimental Ophthalmology	3	2.249	250	83.33±29.67	Q1
9	Biomaterials	2	8.806	229	101.75±74.24	Q1
10	Drugs	2	4.690	152	76.00±29.69	Q1
11	Inflammation	2	2.884	179	89.50±0.70	Q2
12	Clinical and Experimental Ophthalmology (formerly known as the Australian and New Zealand Journal of Ophthalmology)	2	3.217	155	72.45±38.89	Q1

*2017 Journal Citation Reports® (Clarivate Analytics, 2018) **2017 Scimago Journal & Country Rank (SJR)

Figure 3. New perspective of ocular trauma classification



Various research methods are employed along with bibliometric studies to determine the effect size of a study; for example, the altmetric score studies have becoming increasingly popular in recent years among these methods.

Table 5. The most common topics among the top 100 cited articles

Main subject	Number
Mechanical eyeball injury (Open and closed glob injury)	40
Epidemiology of ocular trauma	19
Traumatic eye infection	17
Ocular adnexa injury	6
Medical therapeutic approaches for ocular trauma	6
Classification of mechanical ocular trauma	4
Retained foreign body–induced eye injury	4
Diagnostic method for ocular trauma	2
Thermal ocular injury	1
Chemical ocular injury	1

In our study, we found that the total number of citations of the top 100 cited articles between 1982 and 2018 is 8,568, except self-citations (range: 50–269). The average number of citations was 88.07, and the values of the mode and median were 52 and 78, respectively. Two of the three top cited articles were on ocular trauma classification, whereas one was a review related to ocular infection epidemiology caused by ocular trauma. The highest cited article in our study was written by Pieramici et al. (24), whose research included the ocular classification group. They presented the first classification concept on ocular trauma in their article, which was included in the perspective category. In their study, they not only classified mechanical eye injury but also standardized the terminology that will be used on ocular trauma cases.

Factors such as the number of citations being in favor of old journals and publications, scientific articles less cited within one or two years after their publication, and post-publication citation peak being between three and ten years, and articles losing their importance after that period are the main limitations in determining the value of articles in citation classics studies (13, 25). We found that the quick increase in the number of citations in the last ten years was related to the previous articles being published in the previous years.

Table 6. Study design and levels of evidence by SIGN* of the top 100 cited articles on ocular trauma

Study type and subtype	Level of evidence	Group	Number	Mean number of citation per article±SD
Clinical research				
Systematic review	1	A	1	94.50±44.54
Randomized controlled trial (RCT)	1	A	1	
Retrospective comparative study	2	B	2	89.00±32.52
Retrospective cohort study	3	B	1	81.50±32.38
Case–control study	3	B	1	
Case series	3	B	9	
Case report	3	B	1	
Prospective–descriptive study	3	B	9	
Retrospective–descriptive study	3	B	38	
Cross–sectional–descriptive study	3	B	1	
Review	4	C	24	97.00±55.89
Expert committee report	4	C	3	
Expert opinion (editorial, letter, and note)	4	C	1	
Experimental human cadaveric study				104.25±50.88
Prospective comparative study	2	B	1	
Experimental animal study				
Prospective comparative study	2	B	7	

*SIGN: Scottish Intercollegiate Guidelines Network

Garfield stated that the review type studies are mainly the most cited articles (12). However, in our study, the most cited study was expert opinion or expert committee report with review being the second most cited article. However, it is noteworthy that the review type studies have received a large number of citations.

Our research data show that among the 100 most classical articles in ocular trauma, USA has an impressive stronghold both in terms of the number of authors who were included in more than one article and the number of presented proceedings papers and scientific activities (23).

The official language of the country of origin of publications is an important factor for the selection of language for articles. The publication language of the top 100 cited articles was English in our study. Although different languages were used, the common ones in the recent medical literature were published in English (25).

Our bibliometric study showed that the institutions or organizations located in the USA are more prominent both in terms of the number of institutions producing publications and in the number of publications per institution. The USA has the highest production of scientific publications, including in medicine and other health science fields. Moreover, the USA has the highest number of scientific publications in many fields (26, 27).

The result of our bibliometric study demonstrates that 22% of the classic papers are being supported by funding agencies in the USA. Although the amount of allowance has been reduced in recent years, educational and scientific studies are generally supported with funding in developed countries to a greater extent relative to developing countries (28, 29).

High IF is an important scientometric criterion in determining the journal quality (30). However, in the recent years, Q categories have gained more prominence in the determination of the scientific value of a journal (31). IF of the most of the journals in our "The Top 100 Cited Articles on Ocular Trauma" study was ≥2.0. This observation means that the key articles on ocular trauma are preferred by journals with a high IF. When we evaluated the top 3 journals in our study based on the most number of publications and citations on ocular trauma, *Ophthalmology* is the leading journal (23 articles and 2,183 citations). The second position was held by *JAMA Ophthalmology*, formerly known as the *Archives of Ophthalmology* (10 articles and 800 citations). The third rank belonged the *American Journal of Ophthalmology* (7 articles and 708 citations). Although the first three journals based on total number of citations are listed above, it is significant that the leader based on mean citation per article is *Eye*. Besides, we also realized that the name of *Australian and New Zealand Journal of Ophthalmology* was changed to *Clinical and Experimental Ophthalmology* during the literature review. Lin et al. (7) and Liu et al. (14) conducted bibliometric studies in different fields of ophthalmology. They stated that the most published and cited journal was *Ophthalmology* in their studies. The joint evaluation of their results with ours indicates that the *Ophthalmology* is an influential and preferred journal in this field.

Although Garfield reported that the most cited articles were review type (level of evidence: 4), we determined the retrospective–descriptive type studies (level of evidence: 3) as the most cited article (12). Moreover, most articles in the list were clinical outcome studies with an evidence level of 3, indicating that case series or prospective and retrospective–descriptive studies can still attract the interest of researchers and readers in the ocular trauma. Clinical type studies are the more preferred ones because of their conduct and easily accessible data (15).

For the first time, Kuhn et al. (32) standardized the ocular trauma terminology and classified it under the title of "classification of eye injuries" to provide a more understandable communication between the physicians attending to clinical ophthalmology. Later on, other studies suggested the extended classifications comprising all the eye injuries (33, 34). By evaluating the articles that are included in our study, we preferred to classify the eye injuries as mechanical and non-mechanical instead of only mechanical. This way, all the eye injuries could be gathered under a single algorithm. We used the classification of mechanical eye injuries suggested by Xiao et al. (33) in 2014, which they called the mechanical eye trauma and included the eyeball and ocular adnexa all together. For non-mechanical eye injuries, we based our study on the classification suggested by Shukla et al (34). Additionally, we used these classifications and grouped the eye injuries detailed in the top 100 ocular trauma articles in a new classification chart (Fig. 3). This update study can provide a new point of view for ocular trauma classification that includes all the eye injuries.

The most common topic in our study is mechanical eyeball injury; these injuries are divided into two groups—open and closed globe injuries. In this study, we determined that closed globe injuries were more common than open globe injuries. When the articles detailing the injuries are evaluated among these ocular trauma articles, it was observed that the most commonly reported type of closed globe injuries was contusion type injury, wherein the zone 3 area is largely affected. Additionally, penetrating eye injuries, wherein zone 3 area is mostly affected, are more common types of open globe injuries. In their study, in which they retrospectively reviewed the epidemiology of eye injuries, Oum et al. (35) stated that the prevalence of closed globe injuries was six times higher than that of open globe injuries. Moreover, Karaman (36) and Syal et al. (37) reported that contusion is the most common type of closed globe injuries, whereas penetration is the most common type of open globe injuries. We also found that eight (38%) of the closed globe traumas were associated with head traumas in children. Retinal bleedings and retinal folds suggested the presence of diagnostic findings for severe head injuries in childhood head trauma in some studies (38, 39).

The second most common topic in our study is ocular trauma epidemiology. The importance of this topic comes from ocular trauma being a preventable major cause of monocular blindness and visual impairment (2). It is important to reduce the cost of treatment and rehabilitation that will result from ocular morbidity and eye injuries. Therefore, it is important to adopt preventive

measures for eye injuries as well as identify agents and risk factors causing eye injuries to decrease the related economic costs. Hence, this might be the other reason for researchers to focus on articles on ocular traumas. Many of the studies in this field have stated that the victims who underwent these eye injuries are mostly men and that the incident locations are mostly home or workplace (40, 41).

The third most common topic in our study are traumatic eye infections comprising keratitis and endophthalmitis. Endophthalmitis is mostly observed in open globe injuries and is a devastating complication with undesirable consequences on visual prognosis. Andreoli et al. (42) evaluated endophthalmitis rate in a large series of open globe traumas, and the comparison of their results with other studies revealed that the rate of endophthalmitis cases was lower in their study. They attributed the lower rate of endophthalmitis in their study on the usage of prophylactic broad-spectrum antibiotics and early surgical applications. Fungal keratitis is more commonly observed in the closed globe injuries, wherein the cornea is affected. This is because cornea traumas are mostly observed in agricultural workers (43). Many studies have observed *Fusarium* species as the most commonly observed traumatic fungal keratitis pathogen. Although bacterial keratitis is rare in these traumas, *Staphylococcus species* is the most commonly observed bacterial pathogen (44, 45).

Strengths

Beyond a bibliometric analysis, the strength of this study is that we have determined the average number of citations per journals and the average number of citations by the level of evidence together with Q categorization of journals. Furthermore, this study offers a new perspective on the ocular trauma classifications.

Future Directions

This bibliometric analysis study suggested that ocular traumas are important and have revealed the need for a new and more comprehensive classification on ocular traumas. Additionally, we realized that preventive medicine is important on ocular traumas as in all the preventable traumas.

CONCLUSION

Bibliometric studies are the analysis of publications in the literature via the use qualitative and quantitative methods. If their analysis can be conducted very well and produce a deducible interpretation of results, then bibliometric studies will not only provide valuable information for future literature but can also gain new scientific approaches to ocular trauma. This study's results suggest the importance of mechanical eyeball injuries as the most serious eye injuries that can still threaten vision, emphasize the determination of preventable causes in eye injuries, and present the standardization of ocular trauma terminology with the classification of ocular trauma including all the eye injuries necessary for the ophthalmologist to communicate more comprehensively with each other.

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Original Research

miR-96-5p regulates autophagy through targeting ATG9A in lung cancer

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ABSTRACT

Objective: Lung cancer is the leading cause of cancer-related deaths among all types of cancer worldwide. Autophagy is a cellular process involving lysosomal degradation of damaged organelles and long-lived proteins. It provides nutrients and energy to cancer cells by breaking down damaged proteins and organelles and contributes to tumor-cell survival by enhancing stress tolerance and supplying nutrients to meet the metabolic demands of tumors. Therefore, the combination of cytotoxic chemotherapy and autophagy inhibition strategies has been proposed. This study aimed to investigate how miR-96-5p regulates autophagy in A549 and HTB-54 lung cancer cell lines.

Methods: MiR-96-5p expression levels in lung cancer cell lines and normal bronchial epithelium were measured by qPCR. The functional role of miR-96-5p on autophagy and its modulatory effects were investigated in vitro by overexpression studies.

Results: mirR-96-5p was found to be overexpressed in A549 and HTB-54 lung cancer cell lines compared to the normal Beas2B cell line. Overexpression of miR-96-5p resulted in the attenuation of starvation-induced autophagy. It was shown that miR-96-5p suppressed autophagy by targeting ATG9A. Both mRNA and protein cellular levels of ATG9A were decreased in cells upon miR-96-5p overexpression.

Conclusion: This study demonstrated that miR-96-5p might be a candidate for autophagy inhibition in lung cancer. The effects of autophagy inhibition by miR-96-5p and cytotoxic chemotherapy should be further examined.

Keywords: Autophagy, microRNA, miR-96-5p, lung cancer

INTRODUCTION

Lung cancer is the most common type of cancer worldwide, contributing to 11,6% of all new cancer cases according to the Globocan 2018 (1). Surgery, radiotherapy, and chemotherapy are the main therapeutic strategies. However, despite treatment, the survival rate is approximately 5 years, which is still at a very low level (2). Treatments can reduce tumor growth but usually relapse occurs because genetic heterogeneity and tumor plasticity contribute to drug resistance and metastasis, which are responsible for mortality (3).

The term autophagy was first used by Christian de Duve in 1963, shortly after lysosome discovery. It is derived from ancient Greek and literally means "self-eating" (4). Autophagy is an evolutionarily conserved mechanism from yeast to mammals and it is a stress response regulating cellular metabolism and homeostasis by eliminating toxic components (5). Three different types of autophagy were defined mechanistically and morphologically: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy (herein referred to as autophagy) involves lysosomal degradation of intracellular damaged proteins and organelles by sequestering them into autophagosomal double membrane vesicles first, fusing with lysosomes and then the re-introduction of the building blocks to the cell. Microautophagy uptakes cargo directly into the lysosome to be degraded by invagination of the lysosomal membrane. Chaperone-mediated autophagy relies on the transportation of unfolded proteins to the lysosomal membrane (6).

When cells are exposed to stress conditions such as growth factor deficiency, oxygen deficiency, and starvation, autophagy is stimulated for the destruction of organelles and proteins, and the resulting building blocks are involved in cell homeostasis by providing nutrients and energy to cells (7). Recent studies have shown that autophagy contributes to physiological processes such as cell differentiation, regulation of cell metabolism, and destruction of intracellular pathogens. Furthermore, it has been shown that defects in the autophagy mechanism are associated with pathophysiological conditions such as cancer, neurodegenerative, and infectious diseases (8).

Autophagy has both oncogenic and tumor-suppressor roles in cancer. Its role in tumorigenesis is context dependent and is affected by cellular conditions, type, and stage of the tumor. It

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functions as a tumor suppressor by eliminating damaged proteins and organelles at early stages and prevents cancer formation by assisting in reducing genomic instability. However, autophagy supports cancer cells against cellular stresses such as oxygen and nutrient deficiency, endoplasmic reticulum and mitochondrial stress, and chemotherapy and radiotherapy at later stages. It contributes to chemotherapy resistance by supplying energy to the cancer cells through recycling of damaged proteins and organelles (9). The currently used chemotherapeutic agents for treatment of lung cancer promote cell death. However, relapse and drug resistance are common.. Therefore, autophagy inhibition strategies have been proposed together with cytotoxic chemotherapy in order to increase the effectiveness of treatment. However, the only autophagy inhibitor approved by the United States Food and Drug Administration (FDA) is hydroxychloroquine, a chloroquine derivative (10). Because hydroxychloroquine is a chemical compound and causes various adverse effects, researchers have focused on finding miRNAs targeting autophagic genes.

MicroRNAs (miRNAs) are single-stranded noncoding RNAs of 17–25 nucleotides in length. They exert their functions by degrading their target mRNAs or suppressing their translation into protein through base-paring with targets. Therefore, miRNAs can be defined as post-transcriptional negative regulators of genes. miRNAs have been shown to be associated with several diseases, including cancer (11). As noted in all cancers, there are miRNAs correlated with lung cancer that promote cancer or suppress invasiveness (12). Further, several miRNAs have already been identified, patented as biomarkers, phase I and phase II studies have been conducted on them and can be found in Chakraborty's review for further reading (13).

This study aimed at reavealing whether miR-96-5p has a role in suppressing autophagy pathway in non-small-cell lung carcinoma model A549 and HTB-54 cell lines.

In order to produce mechanistic data explaining the mechanism by which gene autophagy is regulated, ATG9A, one of the autophagy genes, was selected as a target gene of miR-96-5p by using bioinformatic data tools. The miR-96-5p mimic transfection was performed on lung cancer cell lines and its effect on autophagy was investigated. It has been shown to suppress autophagy by regulating ATG9A.

METHODS

Cell lines and Cell Culture

For cancer cell lines, A549 (CCL-185) and HTB-54 (Calu-1) were used and Beas2B (CRL-9609) was used as a normal bronchial epithelium. They were purchased from ATCC (American Type Culture Collection, VA, USA). Beas2B was cultured in Roswell Park Memorial Institute; A549 and HTB-54 were cultured in Dulbecco's modified eagle's medium, both supplemented with 10% fetal bovine serum and 50 U/ml penicillin/streptomycin and cultured in a 5% CO_2-humidified incubator at 37°C. Research procedures were in accordance with the ethical standards of the Declaration of Helsinki of 2018.

Main Points:

- miR-96-5p is a modulator of autophagy.
- miR-96-5p is repressor of ATG9A.
- miR-96-5p might be used to sensitize cancer cells to chemotherapeutic agents.

Selection of ATG9A as a Target of miR-96-5p

The miRNAs interact with their target mRNAs through base-pairing. miRBase, TargetScan, and miRDB bioinformatic databases were used to select miR-96-5p target based on base-pairing strength and the presence of conserved 7-mer and 8-mer sites that match the seed region of the miR-96-5p. ATG9A was computationally selected as a strong predicted target which plays a role in autophagosome formation.

Transfection

Cells were transfected with miR96-5p mimic (Cat no. MSY0000095 Qiagen, Germany) using HiPerFect Transfection Reagent (Qiagen, Germany) following the manufacturer's instructions. All-Stars Negative Control siRNA (Cat:1027281 Qiagen, Germany) was used as negative control for fluorescence microscopy analysis of autophagy pMRX-IP-GFP-LC3-RFP-LC3-ΔG plasmid cotransfected with miR-96-5p mimic using Lipofectamine 3000 (Thermo Fisher Sci. US).

RNA Isolation and RT-PCR Analysis

Total RNA was isolated using miScript miRNeasy Mini Kit (Qiagen, Germany), cDNA was synthesized using miScript II RT Kit (Qiagen, Germany) following the manufacturer's instructions. The microRNA and mRNA gene expression analysis were performed using miScript SYBR Green PCR kit (Qiagen, Germany). For the analysis of relative gene expression data and calculation of fold changes, $2^{-\Delta\Delta CT}$ method was used. To normalize data in terms of RNA added to the reverse transcription reactions, internal control genes were used. Whereas RNU-6 (Cat: 218300 Qiagen, Germany) was used as an internal control for miR-96-5p (MS00003360, Qiagen, Germany) expression analyses, GAPGH (Cat:QT00079247 Qiagen, Germany) was used for ATG9A (QT01025262 Qiagen, Germany). In order to normalize data and to calculate ΔCt, difference between Ct values of the gene of interest and the housekeeping gene was calculated. Then, to calculate ΔΔCt; difference of ΔCt values between the test and control samples was calculated. The fold change was then equal to $2^{-\Delta\Delta Ct}$.

Western Blot

Cells were washed with cold 1X PBS, and then total protein was extracted using RIPA buffer containing protease inhibitors cocktail. Total protein concentration was determined using Pierce BCA Protein Assay Kit (Thermo Fisher Sci. US). An SDS-PAGE gel was loaded with 20 µg total protein and transferred using wet transfer electroblotting (Bio-Rad, America) under a constant current overnight at +4 ^{0}C. After membranes were incubated in 5% (w/v) skimmed milk, incubated with primary antibodies overnight at 4°C. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature. Chemiluminescent imaging system was used to obtain protein bands. (Vilmer Lourmat, Germany).

GFP-LC3 Analysis

Cells were seeded on cover slides. After 48 hours of cotransfection of mimic and the plasmid, cells were fixed with 3.7% formaldehyde for 20 min. Images were obtained with Leica DMIL Led DFC450 microscope (Leica, Germany). After normalization with a threshold, the GFP dots were counted and compared between groups.

Statistical Analysis

Statistical analyses and comparisons between groups were performed by Student's t-test and Mann–Whitney U test using SPSS Statistics V22.0 (IBM SPSS Corp.; Armonk, NY, USA) programme. A $p<0.05$ was considered statistically significant.

RESULTS

miR-96-5p was Overexpressed in Lung Cancer Cell Lines

Expression levels of miR-96-5p were determined by RT-PCR. Normal lung cell line and lung cancer cell lines were compared and values were normalized with RNA-U6. The miR-96-5p was found 2.5 fold increased in A549 and 2.3 fold increased in HTB-54 cell line compared to Beas2B normal cell line (Figure 1) ($p = 0.0023$ and $p = 0.0053$, respectively).

miR-96-5p Blocked Starvation-Induced Autophagy

Protein levels of LC3, marker of the autophagy, were detected by western blot, and the cells were cotransfected with GFP-LC3-containing plasmid and the mimic in order to show whether miR-96-5p regulates autophagy. The cells were incubated with HBSS for 4 hours before fixation to induce autophagy. Change in autophagosome numbers were evaulated. It was shown that overexpression of miR-96-5p repressed starvation-induced GFP-LC3 dot accumulation (Figure 2) which represents autophagic vesicles. These results showed that miR-96-5p is an autophagy regulating microRNA. This was also confirmed by western-blot analysis. When autophagy is induced, Arginine amino acid at the C-terminus of LC3 is cleaved by an enzyme called Atg4 (cysteine protease) and LC3-I type is formed. Binding LC3-I to phosphatidylethanolamine (PE) leads to the formation of LC3-II. As shown in Figure 3, lipid conjugation of LC3-I was attenuated after miR-96-5p transfection.

miR-96-5p Repressed Autophagy-Related Target ATG9A

Bioinformatics tools were used to identify a predicted target of miR-96-5p, and autophagy-related gene—ATG9A—the only transmembrane autophagy protein required for autophagosome formation, was selected as a target to demonstrate the mechanism by which autophagy is regulated. Cells were transfected with miR-96-5p and the effects on ATG9A was evaluated. MiR-96-5p has been shown to suppress autophagy by targeting ATG9A. Both ATG9A mRNA (Figure 4) and protein levels (Figure 5) were decreased after transfection in both cell lines.

Figure 1. Expressions of miR-96-5p in lung cancer cell lines

Expression levels in A549 and HTB-54 were given as fold changes which were calculated from $2^{-\Delta\Delta Ct}$ compared to normal lung cell line Beas2B. *$p<0.05$; **$p<0.01$; ***$p<0.001$, ****$p<0.0001$

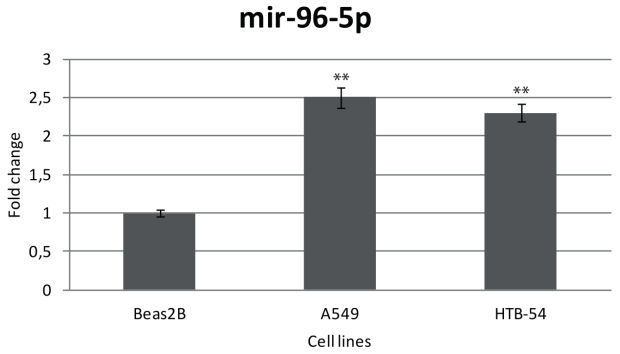


Figure 2. a, b. The effect of miR-96-5p overexpression on starvation induced autophagy

(A) Immunofluorescence imaging of autophagosomes. For immunofluorescence analysis, cells were transfected with miR-96-5p mimic or negative control siRNA (miR-cnt) simultaneously with the plasmid containing GFP-LC3. Cells were starved for 4 hours prior to fixation to induce autophagy. Decreased numbers of GFP-LC3 puncta is evidence of decreased autophagosomes. (B) miR-96-5p supressed autophagy in lung cancer compared to negative control siRNA (miR-cnt). Since LC3-II is degraded by autophagy mechanism, it is used as a marker of autophagy and band density of LC3-I, and LC3-II was evaluated. When autophagy is stimulated, arginine amino acid at the C-terminus of LC3 is cleaved by an enzyme ATG4 (cysteine protease) and LC3-I type is formed. LC3-I is bound to phosphatidylethanolamine (PE) to form LC3-II. The increase in LC3-II band density indicates that autophagy is stimulated if there is no problem with lysosomal fusion and degradation, whereas the decrease of LC3 is interpreted vice versa

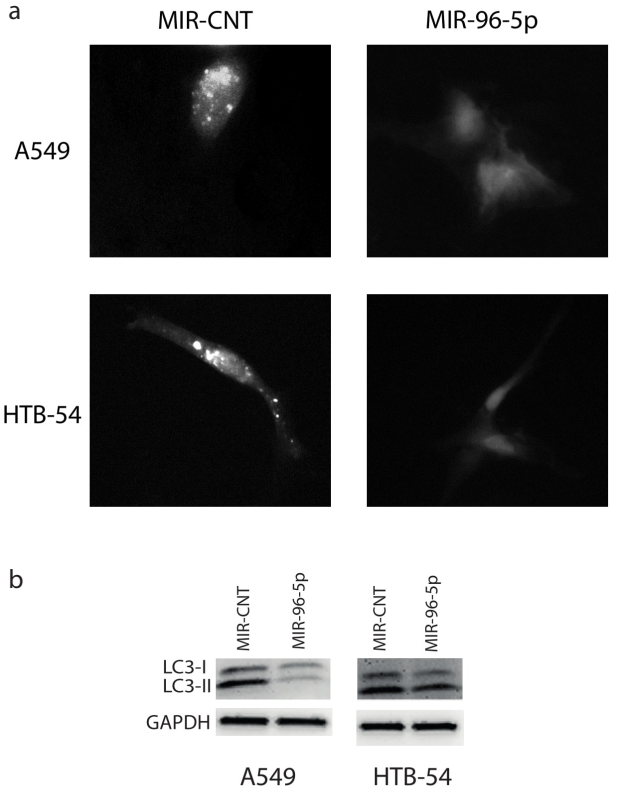
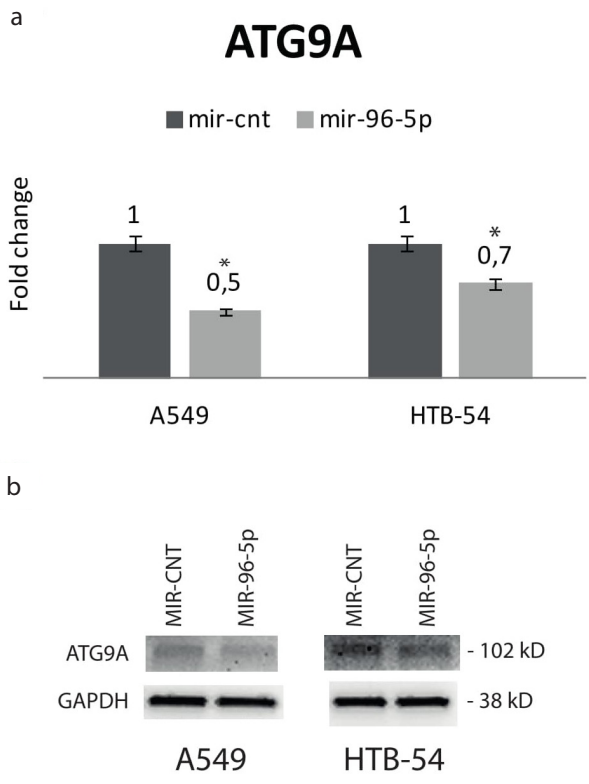


Figure 3. a, b. The effect of miR-96-5p overexpression on ATG9A

(A) miR-96-5p supressed ATG9A at mRNA level in lung cancer compared to negative control siRNA (miR-cnt). ATG9A was computationally found as a strong estimated target of miR-96-5p and it was shown to be downregulated at mRNA level after miR-96-5p mimic transfection. *p<0.05; **p<0.01; ***p<0.001, ****p<0.0001. (B) Overexpression of miR-96-5p resulted in decreased ATG9A protein level compared to negative control siRNA (miR-cnt). Bioinformatically estimated target ATG9A was shown to be affected by miR-96-5p overexpression



DISCUSSION

MicroRNAs are small noncoding RNAs that negatively regulate gene expression at the transcriptional or post-transcriptional level. They have been found to regulate many cellular processes, including autophagy, and have been associated with many diseases, including cancer (14, 15). The role of miRNA-mediated autophagy in cancer treatment is controversial. Whether autophagy needs to be suppressed or induced should be decided based on tumor type, stage, and tumor microenvironment.

Many cancers have been associated with miR-96-5p in previous studies (16-19). miR-96-5p was found to be overexpressed in breast, prostate, bladder, and colorectal cancers compared with that observed in normal tissue and is defined as an oncogenic miRNA (16-19). In addition, overexpression of miR-96-5p has been shown to increase cell proliferation in prostate, lung, and breast cancer and also induce cell migration in lung and breast cancer (17, 20, 21). Conversely, miR-96-5p suppresses cell migration in osteosarcoma and renal cancer and has been reported as a tumor suppressor (22, 23). The expression level of miR-96-5p was found higher in non-small cell lung cancer and non-small cell epidermoid cancer cell lines compared to the normal lung cell line in this study. Furthermore, miR-96-5p has been revealed to inhibit autophagy by suppressing Atg9A when it is overexpresssed by mimic transfection. ATG9A supression was shown at both mRNA and protein levels. It is important to show protein expression levels when studying miRNAs because at the molecular level, miRNAs mainly exert their function by affecting target mRNA stability and/or by down regulating their translation.

In humans, ATG9 has two different isoforms; ATG9A and ATG9B. ATG9A is found in all tissues, while ATG9B is concentrated in the placenta and pituitary, and is stimulated by hypoxic conditions (24). ATG9 is the only transmembrane autophagy protein required for autophagosome formation. It plays an important role in organization of nucleation site for formation of precursor autophagosomal structures and in phagophore elongation (25). Previous studies have shown that there are numerous membrane sources that contribute to autophagosome formation, such as endoplasmic reticulum, golgi apparatus, and plasma membrane (26). ATG9 is thought to play an important role in autophagosome formation by directing membrane from these donor organelles (27). Therefore it is important to understand ATG9-related regulatory mechanisms to better understand autophagic machinery. There have been studies in which autophagy was associated with both cell survival and cell death. Therefore, the contribution of autophagy to cancer cell death is controversial and context-dependent. Many anti-cancer treatments including chemotherapy, radiotherapy, and targeted therapies have been shown to induce autophagy (28, 29). In response to these anti-cancer treatments, autophagy is activated to protect cells from stress-related damages and it appears as a mechanism that contributes drug resistancy in cancer cells as in the study of Chen *et.al* (30). Therefore, targeting of autophagy with combination of chemotherapy has been proposed as a new strategy and clinical studies based on this approach are being conducted (10). Since hydroxychloroquine is the only FDA approved chemical compound that inhibits autophagy and causes various adverse effects, researchers have been focused on finding miRNAs targeting autophagic genes. As miRNAs are already present physiologically in the human body, they are thought to lead fewer adverse effects. Our study showed that miR-96-5p has a regulatory role on autophagy by downregulating ATG9A. Structural analysis of microRNA-target interaction is needed in order to show whether ATG9A is a direct target of miR-96-5p. A mutation should be introduced in miRNA target recognition site by site directed mutagenesis and then direct targeting could be finally confirmed. We also bioinformatically checked for regulatory roles of miR-96-5p on other autophagy genes and we found that ATG7 might be a predicted target gene of miR-96-5p. Ubiquitin-like modifier-activating enzyme ATG7 is essential for autophagy and required for cytoplasm-to-vacuole targeting (Cvt). Interaction between ATG7 and miR-96-5p should be further investigated to obtain deeper mechanistic view of autophagy regulation by miR-96-5p.

CONCLUSION

After suppression of autophagy, cancer cells became more sensitive to chemotherapy and radiotherapy. Previous studies showed that a combination of chemotherapy and autophagy inhibition by miRNAs can be used as an effective approach for treatment.

Although miR-96-5p has been shown to induce proliferation and cell migration in lung cancer, this is a dose-dependent effect and its effect on autophagy and cell survival with chemotherapeutic agents should be investigated.

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Informed Consent: N/A

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Review

Pregnancy During the Covid–19 Pandemic: What an Obstetrician Needs to Know

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ABSTRACT

In February 2020, the World Health Organization identified the coronavirus disease 2019 (COVID-19). The virus that causes COVID-19 is known as severe acute respiratory syndrome coronavirus 2. Nations worldwide have reported high death tolls and are adopting measures, particularly in Turkey wherein efforts are made to ensure that pregnant women are minimally affected by this epidemic that has such terrible effects. To date, a total of 206,844 cases have been confirmed in Turkey in approximately 115 days. However, the exact number of pregnant COVID-19 patients in Turkey or worldwide is not yet known. There are no conclusive data that confirm whether pregnant women are at a higher risk of acquiring this infection compared to the rest of the population. It is also not yet known if or how fetuses will be affected by this infection. However, the body of research evidence concerning COVID-19 is rapidly growing, and multiple organizations are constantly updating and expanding resources for interim guidance. In this review, we aimed to summarize the latest available research on COVID-19 virology and epidemiology as well as the status of pregnant healthcare workers together with the reported data on pregnant women and other recent findings and to discuss COVID-19 during pregnancy based on the available data.

Keywords: COVID-19, SARS-CoV-2, pregnancy, pandemic

INTRODUCTION

Coronaviruses are important pathogens for humans and animals. In late 2019, a new coronavirus was found to be the cause of a subset of pneumonia cases in the Wuhan province of China. Since then, it has spread worldwide, resulting in a pandemic. The World Health Organization (WHO) coined the term coronavirus disease 2019 (COVID-19) (1). The virus that causes COVID-19 was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This severe acute respiratory syndrome seems to spread from one person to another mainly through the transmission of SARS-CoV-2 by respiratory droplets, Similar to the case of influenza (2). Authorities have recommended several infection control measures in order to reduce the transmission of COVID-19. These include controlling the major source of the infection (e.g., using physical barriers to cover the nose and mouth in order to contain respiratory secretions), the early identification and isolation of patients suspected of being infected, the use of adequate personal protective equipment while providing care to COVID-19 patients, and the disinfection of the environment. Limiting the transmission of SARS-CoV-2 is an important component in the management of patients with suspected or identified COVID-19. An early report of COVID-19 from China consisting of 138 patients estimated that 43% of them had contracted the infection in a hospital setting (3).

Most studies describing the clinical characteristics of COVID-19 were conducted among hospitalized patients. A report of COVID-19 pneumonia from Wuhan consisting of 138 patients, determined that the most frequent clinical findings at the onset of the disease were fever (99%), fatigue (70%), dry cough (59%), anorexia (40%), myalgia (35%), dyspnea (31%), and thick mucus from coughs (27%) (3).

Individuals of all ages can be infected with SARS-CoV-2, although middle-aged and especially elderly people with serious diseases are the most frequently affected. A report from the Chinese Center for Disease Control and Prevention (CDC) stated that of 44,500 confirmed COVID-19 patients, 87% were aged between 30 and 79 years (4).

Common laboratory findings of hospitalized COVID-19 patients include lymphopenia, elevated amino transaminase and lactate dehydrogenase levels, and increased inflammatory markers (e.g., ferritin, C-reactive protein, and erythrocyte sedimentation rate) (3).

Disease severity can range from mild common cold symptoms to pneumonia and even death. Currently, the virus has been detected in every continent except Antarctica, and there are serious outbreaks especially in China, Italy, and New York. Current findings

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show that the average incubation period is 5 days, but can vary from 2 to 14 days (5). The morbidity and mortality of COVID-19 have been largely associated with acute viral pneumonitis that evolves into acute respiratory distress syndrome (ARDS). Common complications of ARDS associated with COVID-19 include acute renal failure (ARF), elevated liver enzymes, and cardiac injury involving cardiomyopathy, pericarditis, pericardial effusion, arrhythmia, and sudden cardiac death. For example, a single-center retrospective cohort study from China assessed 52 COVID-19 patients who were in a critical condition and reported the following complications: ARF (29%), liver dysfunction (29%), and heart damage (23%) (6).

In this review, we will summarize the latest available research on COVID-19 virology and epidemiology, the status of pregnant healthcare workers together with the reported data on pregnant women and other recent findings, and we will discuss COVID-19 during pregnancy together with the available data.

Clinical and Research Consequences

Virology and Epidemiology

Full genome sequencing and phylogenetic analysis have revealed that the virus associated with COVID-19 is a betacoronavirus from the same subgenus as the severe acute respiratory syndrome (SARS) coronavirus (and also a few bat coronaviruses), but belongs to a different clade. The structure of the receptor-binding gene region of COVID-19 is quite similar to that of the SARS coronavirus, and both viruses use the same receptor (angiotensin-converting enzyme 2 for cellular entry (7). The International Committee on Taxonomy of Viruses Coronavirus Study Group recommended naming the virus as SARS-CoV-2 (8). A phylogenetic analysis of 103 SARS-CoV-2 strains from China defined two different strains of SARS-CoV-2 called type L (accounting for 70% of the strains) and Type S (accounting for 30%) (9).

More than three million confirmed COVID-19 cases have been reported worldwide. Since the first case was reported from the city of Wuhan in the Hubei province of China at the end of 2019, more than 80,000 cases of COVID-19 have been reported in China, most of which were from Hubei and the surrounding provinces. A joint WHO-China fact-finding mission estimated that the Chinese outbreak peaked between the end of January 2020 and the beginning of February 2020 (10), and the rate of new cases declined significantly in early March. In the United States (US), cases of COVID-19 have been reported in all the 50 states, Washington DC, and at least four territories (11).

Pregnancy and COVID-19

Guidelines suggest that pregnant women avoid exposure by following the same precautions recommended to non-pregnant individuals such as social distancing, hand hygiene, and disinfection of surfaces. Pregnant women with a history of epidemiological contact should be monitored.

Main Points:

- The exact number of pregnant women affected by COVID-19 is not known.
- Vertical transmission and the fetal and maternal effects of COVID-19 are still not clearly understood.
- COVID-19 does not affect the mode of delivery.

Pregnant Healthcare Professionals

All pregnant women are considered to be at a high risk for COVID-19 because of their high sensitivity to changes in immune responses. These women should adopt comprehensive preventive measures, including practicing hand hygiene, disinfecting surfaces with >60% ethanol, and strictly following social distancing measures when interacting with other people (12). Accordingly, pregnant healthcare professionals have additional concerns, and there is no standard occupational guidance for them. Some human resources departments recommend that pregnant women in the third trimester, especially those at ≥36 weeks of gestation, should suspend face-to-face patient consultations in order to help reduce their risk of acquiring the infection and its consequences. The International Society for Infectious Diseases in Obstetrics and Gynecology suggests that pregnant women at ≥24 weeks of gestation who are working in settings with an increased risk of exposure (e.g., labor and delivery, operating rooms, intensive care, or high dependency units) should switch to positions where they would be subjected to a lower risk of exposure (12).

Clinical Characteristics

All pregnant women should be monitored for the development of COVID-19 signs and symptoms, especially if they were in close contact with a suspected or confirmed case. A systematic review of 33 studies that included 356 pregnant COVID-19 patients reported that the most common symptoms were fever (67%), cough (66%), dyspnea (7%), sore throat (7%), fatigue (7%), and myalgia (6%) (13). Other reported symptoms included rhinorrhea, nasal congestion, loss of appetite, nausea and vomiting, headache, and possibly abnormalities in the sense of smell and/or taste. The most common laboratory findings included lymphopenia (14%), moderate liver enzyme elevation (5%), and thrombocytopenia (1%) (13).

Classification

The US National Institute of Health has provided the following system for the classification of COVID-19 according to severity (14):

- Asymptomatic or presymptomatic infection: Tested positive for SARS-CoV-2 but without symptoms.
- Mild disease: Any signs or symptoms (fever, cough, sore throat, fatigue, headache, myalgia) without shortness of breath or abnormal chest radiography.
- Moderate disease: Evidence of lower respiratory tract disease in the clinical evaluation or imaging and oxygen saturation (SaO_2) of >93% when breathing room air at sea level.
- Severe disease: Respiratory rate of >30/min and SaO_2 of ≤93% when breathing room air at sea level, ratio of arterial oxygen over the fraction of inspired oxygen ($PaO2/FiO_2$) of <300.
- Critical disease: Respiratory failure, septic shock, and/or multiple organ dysfunction.

Pregnancy Complications and Vertical Transmission

Early data show similar intensive care unit (ICU) admissions for pregnant and non-pregnant individuals who develop COVID-19 pneumonia but an increased risk of preterm and cesarean deliveries (15).

A preliminary report from the US showed that 4 (2.8%) out of 143 pregnant COVID-19 patients were admitted to the ICU; however, it was indicated that the data were incomplete (16). The first US experience from New York that included 43 pregnant patients with confirmed COVID-19 reported that the course of the disease was mild in 37 patients (86%), severe in 4 patients (9.3%), and critical in 2 patients (4.7%) (17). A more extensive cohort study of 147 pregnant patients included in the WHO-China Joint Mission Report and a different report of 118 pregnant patients in Wuhan indicated that 8% of all subjects had severe disease and 1% were in a critical condition (18). Only one maternal death was reported in the medical literature, caused by multiorgan failure (19), but there are several anecdotal reports (20).

An early review of 51 well-documented pregnant COVID-19 patients have reported that 39% delivered before 37 weeks of gestation and 96% delivered by cesarean section (21); however, a larger systematic review including 252 pregnant COVID-19 patients indicated that 15% delivered before 37 weeks of gestation and 70% delivered by cesarean section (13). When we reviewed the limited available studies in the literature, we found that pregnancy was not an additional risk factor for severe COVID-19 prognosis. Therefore, follow-up without treatment should be considered first for pregnant women with uncomplicated COVID-19 infections. Testing positive for COVID-19 should not be considered as an indication for delivery. Delivery needs to be decided according to the patient's clinical condition and gestational age. In other words, premature birth should not be considered if the patient is asymptomatic or stable. The decision for the type of delivery should be based on the indications of pregnancy and not be influenced by COVID-19 positivity. There are currently no data on whether pregnancy delays recovery from the viral disease or causes treatment resistance.

The frequency of spontaneous abortions may not increase, but there are limited data regarding infections in the first trimester (13). As reported previously, fetal deaths were noted in two patients in critical conditions—one of whom died, whereas the other required extracorporeal membrane oxygenation (ECMO) (19).

More than 95% of newborns reportedly had a good overall condition at birth, and neonatal complications were thought to be largely associated with premature birth (13). The criteria for the definitive diagnosis of vertical SARS-CoV-2 transmission are unclear. Researchers have suggested various methods for this purpose, such as a SARS-CoV-2-positive neonatal nasopharyngeal swab within 1–2 hours of birth and before contact with an infected individual and an elevated SARS-CoV-2 IgM level in cord blood. To date, SARS-CoV-2 has not been detected in cord blood or amniotic fluid (22). However, there is at least one case where a confirmed COVID-19 patient had a second trimester miscarriage, and examinations revealed that the placental cotyledon and submembrane were positive for SARS-CoV-2, whereas all fetal, amniotic fluid, cord blood, maternal blood, and vaginal samples were negative (23). A review that included 51 pregnant women with COVID-19 did not report any cases of intrauterine transmission (21).

Diagnostic Method

A positive reverse transcription-polymerase chain reaction usually confirms the diagnosis of COVID-19, although it is possible to have false-positive results. False-negative results were common in the first tests (approximately one in four) (12) and were also reported in pregnant women (24). If the initial nasopharyngeal test is negative but COVID-19 is still suspected and the determination of the infection is important for management or infection control, the test should be repeated within 24 hours to several days. Infection control measures for COVID-19 should continue during reassessments. Two more consecutive negative results usually exclude infection (12). If COVID-19 infection is strongly suspected and the diagnosis is required for treatment, lower respiratory tract samples (sputum, bronchoalveolar lavage, etc.) can be tested due to their higher sensitivity (25). In most hospitalized COVID-19 patients, lung radiography is sufficient for the initial assessment of lung complications. The fetal radiation dose for a single lung radiograph is very low at 0.0005–0.01 mGy. Computed tomography (CT) should be performed if indicated because the fetal radiation dose is not associated with increased risk of fetal anomalies or miscarriage for a routine chest CT. Some authors advocate for pulmonary ultrasound in the rapid diagnosis of pneumonia in pregnant women and argue that this would be the fastest approach to detect COVID-19 infection in highly suspicious pregnant women (26).

Pregnancy Monitoring

There are many ways of reducing the duration of pregnancy follow-up consultations, especially for patients with high-risk pregnancies (27). For example, a clinician may utilize the 2-hour 75-gram glucose tolerance test (GTT) or the 3-hour 100-gram GTT (in patients with positive results); the cell-free DNA scanning can be used for Down syndrome (≥10 weeks). In patients without symptoms, ultrasonography should be performed within the first trimester and detailed ultrasonography should be performed between 18 and 22 weeks. Any further ultrasonographic examinations should not be done unless necessary.

The psychological impact of COVID-19 should also be recognized and addressed. One study reported that approximately one-third of all subjects indicated moderate-to-severe anxiety (28). Another longitudinal study found that there was no major change in anxiety, depression, and stress symptoms at baseline and four weeks into the COVID-19 pandemic (29). Psychological interventions include online cognitive behavior therapy and mindfulness-based therapies via smartphone applications will help to alleviate anxiety and depression in pregnant women during the COVID-19 pandemic (30). Most pregnant patients with known or suspected COVID-19 have mild disease that does not require hospital care if there are no associated obstetric problems. Pregnant COVID-19 patients in the third trimester should be mindful of the number of fetal movements and report to their physicians if they are reduced.

Other than this, the home care and other relevant instructions are similar to those for non-pregnant individuals (12). The US Food and Drug Administration has expanded its approval for the use of non-invasive fetal and maternal monitoring devices at home among patients requiring fetal and/or maternal monitoring for non-COVID-19-related cases (31). This could help reduce patient and healthcare provider contact and potential COVID-19 exposure during the pandemic.

Pregnant women with mild disease and comorbidities and those with moderate to critical illness are hospitalized. Hospitalized pregnant patients with severe disease, ventilation requirements, and comorbidities or those who are in critical condition should be monitored by a multidisciplinary team (12). These patients should be monitored with fetal monitoring and for preterm labor while paying close attention to the maternal oxygen levels. There are limited data regarding the association between COVID-19 and the risk of thromboembolism, but the available data suggest an increased risk. The American Society of Hematology, the Society of Critical Care Medicine, and the International Society of Thrombosis and Hemostasis recommend routine pharmacological venous thromboembolism (VTE) prophylaxis for pregnant patients with COVID-19 if there are no contraindications (32).

Antiviral Therapy

Remdesivir is a novel nucleotide analogue that is effective against SARS-CoV-2 *in vitro* (33) and against other related coronaviruses *in vitro* and in animal studies (34). It has been used in pregnant women with Ebola and Marburg viral diseases without causing fetal toxicity (35) and has been used to treat pregnant patients with severe COVID-19. Pregnant and breastfeeding women were not included in randomized trials of the drug during the COVID-19 outbreak.

Both hydroxychloroquine and chloroquine have been reported to inhibit SARS-CoV-2 *in vitro*, but data from early randomized studies have shown that they were not generally beneficial. Hydroxychloroquine crosses the placenta. Animal studies have reported its accumulation in fetal ocular tissues; however, given that the drug is commonly used by pregnant women to treat systemic lupus erythematosus or to prevent malaria, it is not associated with fetal ocular toxicity in humans. Adverse maternal effects include abnormal heart rhythms (36, 37).

Studies have investigated various other drugs such as lopinavir/ritonavir, which is used primarily for the treatment of HIV infection, including during pregnancy. It crosses the placenta, can increase the risk of premature birth, and has not been observed with an increased risk of teratogenic effects in humans (38).

Tocolysis

In patients with known or suspected COVID-19, the recommended tocolytic agent is nifedipine. It is a suitable alterative to indomethacin and beta sympathomimetics (39).

Corticosteroid Therapy

For the general population, the CDC recommends avoiding glucocorticoids in COVID-19 positive individuals. This is because it has been associated with increased risk of mortality in influenza patients and delayed viral clearance in patients with Middle East Respiratory Syndrome- Coronavirus infection. However, the CDC has not evaluated the use of antenatal glucocorticoids to reduce neonatal morbidity and mortality resulting from premature birth in pregnant COVID-19-positive patients. Due to the clear benefits of antenatal betamethasone administration between 24 + 0 and 33 + 6 weeks in patients at a high risk of early preterm birth within seven days, the American College of Obstetricians and Gynecologists (ACOG) continues to recommend its use for standard indications in suspected or confirmed COVID-19 patients (40). However, for pregnant patients with suspected or confirmed COVID-19 at 34 + 0 to 36 + 6 weeks of gestation and at risk of preterm birth within seven days, the benefits to the newborn are less clear, and the ACOG has advised that betamethasone should not be administered to these patients.

Low-dose Aspirin Therapy

For non-COVID-19-positive pregnant women, the ACOG has stated that low-dose aspirin should continue to be prescribed as medically indicated (e.g., the prevention of preeclampsia) (41). For pregnant patients with suspected or confirmed COVID-19 for whom low-dose aspirin is indicated, the decision to use the drug should be made at an individual level.

Timing of Birth

Delivery is not indicated for most women with preterm mild-to-moderate COVID-19 who do not have any medical or obstetric indications for an emergency delivery. Ideally, delivery is performed after a negative test result is obtained or isolation is no longer required. This aims to minimize the risk of transmission of the virus to the newborn after birth (42).

The timing of birth is difficult for pregnant women with COVID-19 who are intubated or in a critical condition. Some authors argue that delivery is indicated after 32 to 34 weeks of gestation if the patient is stable; however, this approach can worsen the mother's condition. Fetal monitoring and maternal support are recommended for patients between the viability limit and <32 weeks of gestation as long as the mother's condition remains stable or improves. In some cases, maternal ECMO may be required (43).

Type of Delivery

COVID-19 is not an indication for changing the mode of delivery, and birth by cesarean section should be performed only for standard obstetric indications (44).

Postpartum Period

• Venous thromboembolism prophylaxis

VTE prophylaxis should be decided on a case-by-case basis after risk assessment for all postpartum women with COVID-19. The optimal duration of anticoagulant therapy after delivery is unclear. Some authors recommend the discontinuation of prophylaxis after discharge, and others recommend continued prophylaxis for up to 10 to 14 days (12, 45).

• Postpartum analgesia

For the treatment of postpartum pain in patients with COVID-19

in whom pain cannot be control with acetaminophen, non steroidal anti-inflammatory drugs can be used as the alternative of opioids (46).

• Newborn assessment

Babies born to mothers with COVID-19 are considered suspicious for COVID-19 and should be tested. They need to be isolated from other healthy infants and cared for according to infection control measures for confirmed or suspected COVID-19 patients (47).

It is recommended that newborns be temporarily separated from suspected or confirmed COVID-19-positive mothers to reduce the risk of mother-to-baby transmission, but this application may also have adverse consequences (48). The WHO recommends that mothers who are suspected, likely, or certain to have the COVID-19 infection should stay together with their babies and have skin-to-skin contact (44).

The CDC recommends that the decision on whether to separate the baby from their confirmed or suspected COVID-19-positive mother should be taken on a case-by-case basis through a decision process involving both the mother and the healthcare team (47).

The infectiousness of the virus through breast milk is unknown. Breast milk samples from 26 infected women tested negative for SARS-CoV-2 (13).

Discharge

To limit the risk associated with hospital stay, early post-delivery discharge is recommended: one day after vaginal delivery and no more than two days after cesarean delivery (49).

The Current State in Turkey

In Turkey, the respiratory tract samples of patients who meet the criteria of suspected COVID-19 are evaluated with polymerase chain reaction diagnostic tests for COVID-19 in the Microbiology Reference Laboratories of the Public Health General Directorate (50), and COVID-19 rapid diagnostic kits have been available in Turkey since March 23, 2020. Our review of the literature indicated that there are currently no studies that have reviewed COVID-19 in pregnancy cases in Turkey, although there are individual case reports.

CONCLUSION

With a rapid increase in the number of cases, deaths, and affected countries, the COVID-19 outbreak is spreading rapidly. However, the full effects of the virus are not clearly understood due to limited data. Particularly, vertical transmission and the fetal and maternal effects of COVID-19 are still not clearly understood. It is especially important to put in place measures for the protection of pregnant healthcare workers who are under increased risk of infection. In addition, considering that the outbreak may have a psychological impact on pregnant patients and pregnancy-related side effects, pregnant women should be educated, provided with psychological support, and informed about the subject. During the outbreak, data should be recorded and evaluated reliably and the effects of the disease on the mother and fetus should be investigated.

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Review

Gut Microbiota: Formation, Lifelong Development and Relation to Cytochrome P450 System, Diseases, Drug Bioavailability and Drug Interactions

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ABSTRACT

The human microbiota is an essential and individual part of the human body composed of microorganisms that live in symbiosis with the human body. It is formed prenatally and changes throughout a person's life due to endogenous and exogenous factors such as birth, geography, gender, sex hormones, genetics, mode of birth, age, lifestyle, nutrition, use of antibiotics, and disease-related changes. Studies on the microbiota have mostly focused on its relation to diseases, but the gut microbiota is also very sensitive to xenobiotics, which directy or indirectly alter the pharmacokinetics of drugs used by the host. Although the gut microbiota recovers in a few weeks, some changes may be permanent. It is important to consider changes in drug bioavailability in patients who were recently or currently treated with antibiotics for the safe and efficient pharmacotherapy of patients. With advanced knowledge about the microbiota and advances in the field of microbiome genetics, it may be possible that in the future, a person's microbiota map is used in personalizing drug treatment. In this review, we aim to emphasize the importance of microbiota and drug interactions.

Keywords: Microbiota, bioavailability, pharmacokinetics, personalized medicine, CYP P450s

INTRODUCTION

The human body is composed of trillions of cells. While 10% of these are human cells, 90% are microbial cells located in this macroscopic host (1). All microorganisms found in humans are called "microbiota" and the genome of microorganisms is called "microbiome" (2). The term microbiota is very old; however, the knowledge of its importance and relationship to diseases dates back only to a decade or two. The skin, mouth, vagina, gastrointestinal, urinary system, or even the placenta contain various microorganisms (3) and therefore form the different types of microbiota like the gut microbiota, oral microbiota, skin microbiota, vaginal microbiota, and urinary microbiota.

The microbiota mediates several functions including the production of essential vitamins, toxin detoxification, regulation of cholesterol metabolism, bile deconjugation, inhibition of pathogenic microorganism colonization, and regulation of gene expression of microbial metabolites such as short-chain fatty acids production for epithelial cells. Therefore, the microbiota is a basic part of a healthy life (4).

The human microbiota has a multifactorial interaction and changes throughout a person's life due to endogenous and exogenous factors like geography, genetics, mode of birth, age, lifestyle, nutrition, use of antibiotics and disease-related changes (5).

Mode of delivery and maternal microbiome transmitted by birth, nutrition during infancy, diet, habitat, social interaction, exposure to xenobiotics, pathogen, and parasitic organisms have been shown to be environmental factors affecting the formation of the microbiome content (6). Microbiota begins to form in the human body during the intrauterine life. The first stage of a baby's microbiota forms when the newborn comes into contact with microorganisms in the vaginal canal of the mother. With normal birth, the maternal microbiota passes predominantly to the child. In general, permanent microbiota develops at the end of the first month after birth (7, 8). Considering all these, it is thought that each person has an unique microbiota. The Human Genome Project (Human Genome Project-1990–2003) and the Human Microbiome Project (Human Microbiome Project-2008–2012) are two important projects that have as a common goal to analyze the characterization of the human microbiota and its role in human health and disease (9). Another important project on this topic is the ELDERMET Project, which was conducted between 2007–2013, in which the relationship between the diet, microbiota, and functional outcomes was investigated and associated with diseases in 500

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elderly Irish persons >65 years. It was outlined that changing the gut microbiota by altering the diet may promote healthy aging, which is associated with microbial diversity (10, 11). Although there are many studies examining the state of the human microbiome in disease and health status, these projects mostly focused on the relationship between microbiome and diseases (12).

Our microbiota contains a certain proportion of beneficial bacteria and opportunistic pathogens (13). When this ratio of beneficial/opportunistic pathogens decreases, a pathological process called "microbial dysbiosis" begins. This has been found to be associated with many diseases including cancer, non-alcoholic fatty liver disease, obesity, diabetes, coronary heart disease, kidney dysfunction, and neurodegenerative disorders (2-4, 7, 14, 15). When using drugs, this important factor should also be taken into consideration as conditions such as drug side effects, decreased drug efficacy, or drug toxicity may be related to the patient's microbiota (6, 7, 15). This review summarizes the formation and development of gut microbiota and the relationship between gut microbiota and diseases, drug bioavailability, and the cytochrome P450 (CYP) System.

Formation and Development of Gut Microbiota

Formation of the microbiota in the human body starts in the prenatal period. It is mainly shaped in the first three years of life, and feeding style plays an important role in the development of a healthy microbiota (16, 17). Infancy microbiota contributes to the development and maturation of the gastrointestinal mucosa. Breast milk is thought to contain especially Bifido bacteria. The gut microbiota starts to take shape during birth. After birth, it changes with factors such as the use of antibiotics, diseases, infections, hormonal changes, circadian rhythm, and nutrition. Subsequently, certain changes occur in the microbiota with aging (18). Studies have examined the importance of being exposed to microorganisms and microbiota development has been found to be an important determinant of the child's future health status (17). In humans, the digestive system microbiota begins to take shape immediately after birth. During delivery, the newborn encounters many microorganisms in the vaginal canal of the mother leading to the formation of the microbiota of the digestive tract. Studies in newborns have shown that the mode of delivery is directly related to the baby's digestive system microbiota. In babies born through vaginal delivery, their gut microbiota is composed of microorganisms of the mothers' genitourinary system, while for those born by cesarean section, their gut microbiota is similar to that of the maternal skin microbiota (8, 19). Other important factors affecting the gut microbiota of infants include the diet, gestational age, hospitalization, and frequent antibiotic use during the infantile period (17-20). Studies in geriatric populations have shown that there is significant reduction both in bacterial density and diversity of the microbiota with aging (17, 21).

Main Points:

- The development of gut microbiota which is essential for a healthy life, starts to take shape during birth in the vaginal canal and changes throughout life due to many factors.
- Gut microbiota is metabolically active and contains their own CYP enzyme systems which can alter the pharmacokinetics and thus the therapeutic results of an administered drug.
- The ratio of beneficial/opportunistic pathogens in the gut is important and changes in gut microbiota composition is associated with many diseases including cancer, non-alcoholic fatty liver disease, obesity, diabetes, coronary heart disease, severe asthma, food allergies, autism, major depressive disorders, kidney dysfunction and neurodegenerative disorders.
- The effects of antibiotics on microbiota composition, metabolism and host interaction are dramatic and may change the efficacy of a drug drastically.
- Knowledge of interaction of microbiota and drugs is essential for safe and efficient pharmacotherapy of patients and individual differences in gut microbiota are an important obstacle in choosing drug treatment.

Relationship Between Gut Microbiota and Diseases

There is increasing evidence that gut microbiota is associated with many diseases (5, 15, 21, 22). Infection is one of the most common diseases that occur as a result of microbiota dysbiosis. Most importantly, infectious diseases and their treatment have a great impact on the human microbiota, which determines the outcome of infectious diseases in the human host. Pathogenic microorganisms colonize the intestinal mucosa, thereby resulting in the induction of a strong inflammatory response, followed by translocation of the intestinal bacteria. Chronic inflammation associated with *Helicobacter pylori* is thought to be the strongest risk factor for gastric cancer (23-26).

An increasing number of *in vivo* and human studies have shown that the interaction between the gut microbiota and host genotype or dietary changes may be important contributing factors to obesity and related metabolic disorders (3, 7, 25, 26). Studies have shown that the gut microbiota is an important modulator. It is an established fact that the gut microbiome changes according to dietary intake (4, 6). Gut microbiome is regulated by circadian rhythms via intrinsic circadian clocks as well as via the host organism. Furthermore, recent studies have shown that the gut microbiota affects the circadian rhythm and is itself exposed to circadian oscillations (27, 28). It has been shown that bacterial rhythms in female mice are more robust than those observed in male mice (29). Gut microbiota differs according to sex (30) *Lactobacillaceae* are more prevalent in women, while *Ruminococcaceae* and *Rikenellaceae* are more common in men. Furthermore, *Bacteroides* and *Prevotella* bacteria were found to be more prevalent in males than in females in females in a cohort (31). Testosterone, which is responsible for the microbial difference between the sexes has an impact on the gut microbiome. The gut microbiome is affected and regulated by estrogen and testosterone levels (32, 33). Testesterone and non-ovarian estrogen levels can also be affected by alterations in the gut microbiome. In a cohort investigating the contribution of microbiome to extra-ovarian estrogen levels, a correlation was found between men and postmenopausal women and estrogen levels.

There is increasing evidence that changes in the microbiota are involved in the pathogenesis of other diseases such as severe asthma, food allergies, autism, and major depressive disorders (15, 34-36). Human studies of the gut microbiota in food-allergic individuals have yielded variable findings because food allergy is a complex and heterogeneous disease. More studies are needed on gut microbiota and food allergies (36). There is evidence that allergic asthma susceptibility increases with antibiotic-induced changes in the gut microbiota (35, 37).

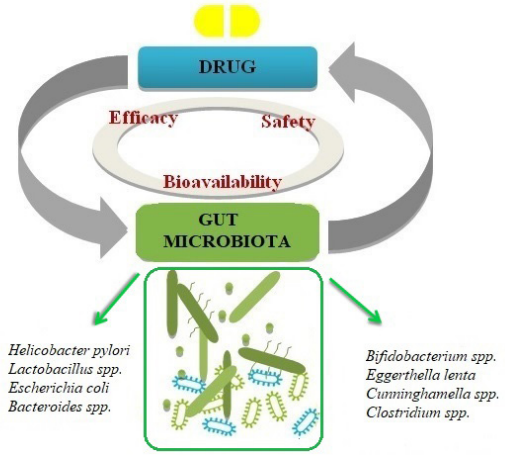
Microbiota differs between genders, both in animal models and in humans. Immunity is affected by sex hormones. The "microgenderome" is the term used for the sexually dimorphic microbiome and defines the interaction between microbiota, sex hormones, and the immune system. Sex differences in systemic immunity and susceptibility to a multitude of infections and chronic diseases are influenced by the gut microbiota. The gut microbiota drives multiple interactions locally with immune cells that regulate the homeostatic environment. The course and treatment of diseases in males and females including psychological disorders such as anxiety and depression and psychosomatic disorders such as irritable bowel disease are affected differently due to the microgenderome. Treatment of these disorders by changing the microbiota with pre-, pro-, syn- and post-biotics, is sometimes a therapeutic option that will respond differently in males and females (38-41).

Relationship Between Gut Microbiota and Drug Bioavailability

Many studies have suggested that the gut microbiota has metabolic potentials equivalent to that of the liver. It is also known that gut microbiota influences drug pharmacokinetics by the direct metabolism of xenobiotics or the indirect interaction with the host enzyme system. Human and animal studies have shown that the gut microbiota has a variety of metabolic activities and can alter the pharmacokinetics and thus the therapeutic results of an administered drug. Studies have suggested that the suitability of the microbiota should be considered when determining the pharmacokinetics of a drug (42).

In addition, a newly developed drug may have more microbial interactions if it has a low solubility or permeability and a long passage time in the gastrointestinal tract, leading to a marked change in the effect of the drug. An example for this is amiodarone, a class III antiarrhythmic drug, which shows marked increase in its bioavailability due to the increased formation of active metabolites, when exposed to the *Escherichia coli* strain *Nissle 1917 (EcN)* in the gut (43). *Helicobacter pylori (Hp)* reduces the absorption of levodopa, a drug commonly prescribed in Parkinson's disease (44). It has been shown that the efficacy of simvastatin, a prodrug used in the treatment of primary hypercholesterolemia, varies in the presence of *Lactobacillus bacteria* (45). Changes in the bioavailability of nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, indomethacin, or ketoprofen have been related to an increase in microorganisms containing β-glucuronidase like *Bacteroides, Clostridium, and Bifidobacterium spp* (46). Irinotecan, an antineoplastic drug for the intravenous treatment of colorectal cancer, may lead to drug toxicity due to an increase of the active metabolite of the drug, SN-38 (7-ethyl-10-hydroxycamptothecin), when the microbiota contains excess amounts of *Escherichia coli, Bacteroides vulgatus, Clostridium ramosum,* or β-glucuronidase (47). *Eggerthella lenta* can reduce drug bioavailability by causing the inactivation of digoxin and hence may cause problems in patients taking the drug (Figure 1) (48).

Figure 1. The human body contains many different microorganisms. Depending on the type and amount of these microorganisms, the efficacy, safety and bioavability of drugs which are taken changes. Gut microbiota may effect the bioavailability of drugs by microbial enzyme activity, drug pharmacokinetics by the direct metabolism of xenobiotics or interaction with host enzyme systems



When talking about bioavailability, the relationship between antibiotics, a widely used drug group, and the microbiota should not be ignored. The effects of antibiotics on microbiota composition, metabolism, and host interaction are dramatic. It may take several weeks for the altered microbiota to recover after antibiotic treatment. However, there are also studies indicating that the population of some subtypes of bacteria in the microbiota can be affected permanently. Therefore, when using antibiotics, it should be taken into consideration that the bioavailability of other drugs used by the patient may be affected.

Relationship Between Gut Microbiota and the Cytochrome P450 System

Cytochrome P450s (CYPs) are a group of enzymes metabolizing xenobiotics. The activity of CYP P450 is altered by various factors such as gender, the environment, disease, alcohol, and drugs. Diet is another important influential factor. Cytochromes involve individual, interindividual, and interracial genetic polymorphisms. The metabolic activity of the microbiota is related to CYP enzymes catalyzing phase I and II reactions in drug metabolism (49, 50).

There are a lot of CYP isoforms in humans. It is known that CYP enzymes are involved in drug metabolism in humans. Cytochromes are found in many different tissues of the human body including the intestine and liver. The majority of drug oxidation appears to be related to the main enzymes: CYP 2D6, 2C9, 1A2, 2C19, 2E1, and 3A4. The most important CYP enzymes are CYP3A4 and CYP2D6. CYP3A4 is expressed in both liver and intestinal tissue. It is

also involved in extrahepatic metabolism. The human intestinal tissue has the ability to metabolize drugs. CYP enzymes are associated with a majority of phase I drug metabolism reactions (50, 51). There is an association between CYP cytochrome and different diseases and nutritional and environmental toxic effects. Cytochromes demonstrate interindividual genetic variation. Gut microbiota regulates liver and metabolic functions, regulates the secretion of bile acids, and participates in the pathogenesis of alcoholic and non-alcoholic fatty liver disease (14). Cytochrome P450 enzymes oxidize substances and are also able to metabolize. CYP enzymes are involved in reactions including O-dealkylation, S-oxidation, epoxidation, and hydroxylation. The activity of cytochrome P450 is influenced by a variety of parameters, such as genus, environment, diseases, alcohol use, smoking, diet, and drugs. Diet plays an essential role and has effects on the metabolism of xenobiotics. Cytochromes have different interindividual polymorphisms (49, 51). The gut microbiota possesses a metabolic capacity because of phase I (oxidative) and phase II (conjugative) drug metabolism. Bacteria have been shown to express CYP genes. Human cytochrome P450s play important roles in the bioactivation and detoxification of numerous therapeutic drugs. The first-pass metabolism of orally administered drugs is related to drug-metabolizing enzymes, P450s, and the gut microbiota. Genetic polymorphisms in P450 genes may affect the pharmacokinetics of their drugs (52). The molecular mechanisms of the interaction between gut bacteria and the metabolism of drugs by the host warrant further research. *Cunninghamella spp. elegans, C. Blakesleeana,* and *C. echinulata* have been employed in the transformation of drugs and xenobiotics via phase I and phase II reactions. The transformation of drugs and xenobiotics in *Cunninghamella spp* involves a number of reactions catalyzed by different CYP isoforms. For example, flurbiprofen is transformed to 40-hydroxyflurbiprofen by *C. elegans* and in humans, this transformation is catalyzed exclusively by CYP2C9 (50). Phase II metabolism of drugs by *Cunninghamella spp.* has been reported to be less frequent. *Bacillus megaterium* is found in the human ileum and can express CYP102, which can affect the cell cycle in epithelial cells. CYP102 in *B. megaterium* can be induced by barbiturates like phenobarbital indirectly, peroxisome proliferators, and nonsteroidal anti-inflammatory drugs. Taken regularly, ibuprofen and other drugs create an environment that is favorable to the pathogens in the gut microbiome (53).

In addition to bacterial P450s, fungal P450s present a new area for xenobiotic biotransformation. Fungal P450s can metabolize antibiotics, β-blockers, and anti-inflammatory drugs (54). The function of microbiome P450s is affected by drugs. It is important to understand how microbial P450s may influence host health and drug efficacy.

Regulating microbial P450s may be important for personalized medicine and drug bioavailability. Due to the fact that irinotekan is changed to its active metabolyte by β-glucuronidase, the inhibition of β-glucuronidases by *Escherichia coli* prevents the toxicity of the drug. Thus, inhibiting or inducing microbial P450s may be a new method of altering the pharmacokinetics of drugs (46, 47, 50, 53).

When the urinary metabolite ratio of acetaminophen metabolism was investigated with regard to the microbiome, patients who had high urinary levels of p-cresol sulfate before exposure to acetaminophen had a low acetaminophen to acetaminophen glucuronide ratio. In another study, probiotic exposure of *Lactobacillus reuteri* KCTC3679 increased the metabolism of acetaminophen, decreased the AUC (Area Under the Curve) of oral acetaminophen, and increased the bacterial load of *L. reuteri* as well as that of cyanobacteria (55).

Cytochromes show interindividual and interethnic genetic polymorphisms. Changes in the pharmacokinetic profile of drugs are associated with toxicity, reduced metabolism, and adverse drug interaction. The high metabolic capacity of the gut microbiota is due to enzymes, which catalyze reactions in phase I and phase II drug metabolism. However, drug discovery studies lack focus on the gut microbiota. Understanding the importance of gut microbiota may be essential to providing personalized medical healthcare. In the future, human gut microbiota may play an important role in the metabolism and oral bioavailability of drugs.

CONCLUSION

The importance of the human microbiota in health and disease is receiving increasing attention. The gut microbiota is essential for a healthy life. Knowledge of the interaction between the microbiota and drugs is essential for the safe and efficient pharmacotherapy of patients. Individual differences in the gut microbiota are an important obstacle in choosing a drug treatment. With advanced knowledge about microbiota and advances in the field of microbiome genetics, it may be possible that in the future, a persons microbiota map is used in personalizing drug treatment. Gut microbiota-drug interaction is explained by the changing toxicity, metabolism, absorption and efficacy parameters of the drug and gut homeostasis. Gut microbiota is another metabolically active compartment together with the classical drug metabolism in the body, including the liver that affects the bioavailability, safety and efficacy of drugs. In addition, the gut microbiota may be a good target for regulating drug pharmacological or toxicological effects. Since the gut microbiota plays a major role, these factors should be investigated in the future.

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Review

Social Capital in the Emergency Department

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ABSTRACT

The concept of social capital is a comprehensive social phenomenon consisting of social support, social integration, values, and norms. In social and economic transactions and economic and physical capital, non-monetary human, cultural, and social capital types have been accepted as neoclassical capital theories. The increase in information communication technologies, especially in economic relations, has now caused individuals to connect with weaker bonds compared with that in the past. Social capital parameters have gained importance to achieve this interaction. This article reveals that the issue of providing corporate loyalty to emergency department employees with a good team spirit is considered as a strong social capital parameter.

Keywords: Emergency service employees, social capital, relations, responsibility, individual

INTRODUCTION

Social capital is a type of capital that cannot be immediately transformed into economic capital, and it takes time to establish and maintain it (1). The importance and effectiveness of the concept of social capital have been sociologically adopted by philosophers such as Smith, Marx, Durkheim, and Weber in solving economic and social problems (2). Bourdieu, Coleman, Burt, Putnam, and Lin are considered as early social capital thinkers (3). This concept was born in response to the excessive centralization of individuality by traditional social science (4). It is based on the idea that relationships are important and social communication networks are valuable (5). It can be used to serve different purposes such as friendship, social support, financial assistance, and the fulfillment of psychological needs. It emphasise that power and influence should be seen as an important resource rather than being a monetary asset in social capital (6).

Social Capital Returns

It is believed that social capital has two important returns: individual and collective. This concept has begun to be seen as a complement to human capital, the source being the civil society. According to a report published by the Organization for Economic Co-operation and Development (OECD), the Wellbeing of the Nations reports a positive interaction between human and social capital. Both concepts are powerful supplements that feed each other (7). As a qualification, it is seen as an investment of one's self and the surplus value of an organization's collective goals. Relationships are inherent. Unlike human and economic capital, actors cannot control the type of social capital (8). The collective aspect of this concept demonstrates that individuals' social capital may be for the benefit of the organization (9) and that it reduces transaction costs in the economic sphere (10). Some authors have argued that the main problem in the development of developing countries is the lack of physical or human capital and also of social capital (11-13). For this reason, in the 1990s, some Western organizations supported non-governmental organizations in developing countries intending to structure social capital (14). The community and organization benefit directly from the collective returns; individuals are indirect and secondary beneficiaries. Different authors have indicated that social capital has three dimensions: structural, relational, and cognitive (15).

One of the distinguishing features of social capital from other types of capital is that it is a type of investment wherein its return in the future cannot be ensured. It can be converted to a lesser degree of economic capital compared to physical and human capital. Serious time and energy must be spent on relationships to ensure continuity. Unlike other types of capital, they wear out unless they are used (16). It does not take action on its own, and it is created by entrepreneurial individuals. In addition, it is based on the internalization of virtues such as loyalty, honesty, and reliability by the society (17). Moreover, it is not at the disposal of a single individual (18). Given that individuals are a byproduct of their activities, the formation and disappearance of social capital are not under the control of individuals (19). Due to its collective returns, it is seen as a public good in a way and it provides less returns than the investment made by the person.

Criticisms of Social Capital

Portes (20) has critically approached the concept of social capital. He saw this concept as a micro concept that could be analyzed at a much more individual level. He stated that the concept can be attributed to the society but requires to be theoretically careful. Portes believes that this concept is the property of individuals,

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therefore, it would be detrimental to reflect it on larger communities. He stated that if it is to be reflected in societies, theoretical improvement should be made through conceptual stretching.

In addition, Portes finds it wrong to consider social capital as a recipe for solving systematic social problems because he thinks that it may be incorrect to evaluate social capital benefits while all other factors are constant (21). Portes stressed that if independent variables are not well established, there is a risk that social capital will become an ambiguous concept (20, 22).

Basic Perspectives in Social Capital

Bourdieu, Coleman, Burt, Putnam, and recently Lin are thinkers who contributed to the development of the concept of social capital through pioneering research (3). Initially, the work of social capital thinkers was dispersed and contradictory. However, Lin attempted to combine them under a single roof with different approaches (17). Another feature is that it is symbolic and can be converted into symbolic capital (23). Reciprocity is an investment with risks because expectations are unlikely to occur. This is because the size of the responsibilities and the return of the earnings are unclear (24). In other words, social capital is an indirect gain. Further, individuals do not relate to social capital (19). However, the byproduct of their relationship constitutes social capital. Coleman states that there are three types of social capital—responsibility and expectations, information channels, and social norms. These are the returns of social relations that serve the interests of individuals. Responsibilities and expectations mean that the good will comes back in the future and that the other party feels responsible. This expectation depends on the reliability of the social environment and the extent of responsibility. Access to information is always costly. Individuals generate returns by using the information obtained through social capital for their purposes. Conversely, norms create a control mechanism through relationships and ensure the implementation of sanctions. Norms require some degree of consensus and provide social order (25).

Institutionalization Efforts & Lin

With his work, Lin has gathered the different perspectives of Bourdieu, Coleman, and Putnam for social capital under one roof (3). Lin contributed to the institutionalization of social capital. According to Lin, social capital is the investment that is obtained from embedded sources in networks of relationships between actors, which can be accessed and mobilized for a specific purpose. The definition emphasizes three elements of social capital— the resources embedded in social structures, the access of individuals to these resources, and the mobilization of these resources for the purpose. Here, acquisition of new resources and the protection of existing ones are two important parameters (26). Individuals who can mobilize buried resources in a better way achieve better results (27). One reason of social networks is claimed to be for social capital. Therefore, social networks establish a conceptual link between actions that connect individuals and social structures and structural constraints. Simultaneously, a social network enables fusion of micro and macro levels and connects relational and collective processes (28). Unlike some other authors, Lin believes that social capital maintains relationships without waiting to benefit individually or as a group. Lin divided the gains from social capital into four classes: facilitating individuals' information flow, increasing the influence of the individual on other actors, increasing the consultative power of the individual, and increasing the individual's identity and recognition. He stated that this classification is the characteristic that differentiates social capital from other types of capital (29). According to Lin, three sources constitute social capital—the strength of the structural position, the network position (weak or strong bonds), and the purpose of the actor's actions and behavior. In structural position, the actor has a place in the social hierarchy and communication with other strata. Lin emphasized that social capital preserves its physical existence and mental health and provides satisfaction with life. He defined this as a social benefit (reputation gain). He stated that these returns may also be purposes (30).

Main Points:

- Man is the most beautiful fruit of this tree of life, the most valuable asset of this universe.
- If the abilities of this fruit are opened, it will be a small world on its own and it will do great things.
- Beautiful and logical communications become an elixir and can take people from coal grade to diamond grade.
- No matter how far a person goes in his own profession, he will definitely need friends he can trust. Good friends are earned and continued through good relationships.
- In a safe and beautiful environment, hard work gets easier, big troubles get smaller, and misfortunes become light.

Solidarity and Intermediary Social Capital

The authors divided social capital into solidarity and intermediary social capital in terms of returns. The basis of this distinction lies in the collective and individual return differences of social capital. Coleman (24) is known as solidarity social capital and Burt (30-32) is known as intermediary social capital. Solidarity social capital comprises strong bonds and protection of resources. It is thought that social networks of this nature are necessary for social capital and those in these networks are rich in social capital. It focuses on relationships within the group.

Intermediate social capital focuses on external relationships and individual returns; it is provided by weak links between actors from different social and cultural backgrounds and is more decisive in accessing new resources. The element of trust in the intermediary social capital is not very much processed. In contrast to tight and strong relationships, extended networks with weak and familiar ties are encouraged (33). Both authors continued their claims by giving examples in their work. Coleman (25) stated that good relationships between parents and their children's teachers positively affects the success and bad behavior of children. However, Burt (31) and Borgatti (34) emphasized that companies restrict each other due to intense relationships and that companies can obtain innovative and creative ideas only from outside the group. Brut states that individuals with high intermediary social capital become more advantageous (30). However, some writers do not agree with Brut on this subject (35, 36).

Risks of Social Capital

Although social capital has many benefits, many studies have also reported its risks. Intermediary social capital is often unsustainable, the legitimacy of bridging actors is undermined, and the risk of exclusion from the group increases; they can even be punished by the subgroups (30, 31).

In particular, solidarity social capital can develop hostile attitudes toward non-group actors while ensuring compliance and belonging to intra-group rules (16, 10). The high level of intra-group solidarity is associated with it the risk of restricting the free will of individuals. Individuals may choose to restrict their freedom based on the threat of isolation from the group and sacrifice their freedom with the motive of protection from the dangerous outside world. They can evaluate these behaviors as survival strategies. The negative effects of social capital are also observed in cases such as terrorist groups and mafia relations (36). In intermediary social capital, actors can use their bridge role and knowledge for bad purposes. They can steal information in legal or unethical ways. Social capital and relations give rise to hate groups and thus form their bureaucracy. However, these negative results do not eliminate the fact that it is a type of capital (37) because when physical and human capital is abused, it can cause serious harm to individuals and societies. It is therefore unjustifiable to argue that social capital has fewer benefits than other types of capital (14).

Social Capital in Health

In this regard, the studies focused on access to and benefits from health services of the society or individual, human behaviors of diseases, and the quality of life of health. The results of different theories are included in these studies (38-41), which are built based on social support, trust, and interdependence. It emphasizes that social capital shapes the behaviors of individuals toward their health status, increases their quality of life, and decreases the risk of developing certain diseases or is protective against certain diseases. However, studies that are directly related to the interest of health service providers are limited.

To the best of our knowledge, there is no study in the literature on social capital and emergency services. Therefore, the relationship between social capital and emergency services is emphasized in this study. However, this process has been handled differently from what has been done so far. In this study, unlike the other studies, the theme of looking at the relationships between emergency service workers from the social capital window was discussed and possible gains were pointed out. When viewed from this perspective, it will be seen to give a new perspective. The social capital window of the relationships between emergency service staff and patient and patient relatives was excluded from this study to make the subject of another. Since there is no significant information in the literature about the following section, it is written entirely from clinical experience. The topic will begin with an emphasis on the job descriptions of employees working in the emergency department and how they have reached the current position.

Personnel Working in the Emergency Department

- **Those who come with the in-house (reluctant) assignment:** This group can include all other health personnel, including doctors, and often consider this assignment as a kind of exile. In the subconscious codes, the emergency department has long been registered as a place of exile. They do not digest and accept the event of exile. This group is the most difficult to adapt to the emergency department. They do not feel like they belong to the emergency room (ER). They do their tasks very unintentionally. They do not satisfy the patient they care for and the other employees they work with, nor do they enjoy themselves. They knock on every door to go back to where they came from. From time to time, they confront hospital administration and emergency room managers. They come late to work, give unnecessary excuses to leave, and receive reports from other institutions without notification and send them to the emergency department responsible. They usually get very sick. According to the experience so far, almost all of those who were exiled to the emergency department with the change of hospital administration have either returned to their previous wards or have been reassigned in another field of study. Therefore, their reluctant marriages with the emergency department result in divorce.
- **Those who have their wishes to be assigned to the emergency department:** These are usually
  - Young and single nurses who have just started work and love adrenaline,
  - Some senior staff who wants to go to a different place and have a discussion in other parts of the hospital,
  - And some senior physicians who want to work in emergency services due to financial concerns. They certainly add joy to the emergency room and bring mobility. Working with them, keeping watch, and looking after patients becomes easier and even enjoyable. They are satisfied with the patients and the patients are satisfied with them. They see the emergency room as a big part of their families. They rejoice in those who come, and they feel sorry for those who go. Very few of these people continue to work in the emergency department with their initial willingness. Most of them leave the emergency department after their marriages, having children, or thinking that they are very worn out. However, they never forget the emergency room; they do not neglect to stop by as soon as they find the opportunity.
- **Employees with an assignment:** These are Emergency Medical Physicians (EMP), paramedics, Emergency Medical Technicians (EMT), and more recently, established emergency room nurses. This group is not a tenant in an emergency; they are the permanent staff of the emergency service. Therefore, they are the hosts. They can only go to another emergency room with the assignment. Some of them love their profession and certainly add strength to the institution. In particular, EMPs have made revolutionary positive changes in all emergency services they have worked to date. They have made amazing contributions in every field related to emergency services in the country, changed the face of these institutions, and brought them to world standards.

The inclusion of ATUs in the health system has significantly reduced the workload of other branches, mortality, and morbidity of patients in the acute phase. They deserve great appreciation for all these and for similar reasons. Apart from these, some of the personnel who come by appointment continue to think that they are obliged and sentenced to the emergency room. Those who are unhappy can make their working life unbearable both for themselves and for the friends with whom they work in the service.

- **Employees who come with an exam result:** This group comprises research assistants. The assistants come to make an informed decision by considering that their working life will continue in the emergency services. Although the range of work in the training process (four years) has already been determined, they also work with the knowledge that they are the hosts. They learn and teach. They are the strawberries of the ER. It is possible to see their signatures at every step of the emergency services.
- **Faculty or training staff:** They see themselves as the staff of the emergency services, and they are seen and accepted as such. Their most important tasks are to conduct research, provide training, and direct and manage emergency services. However, these three important tasks slow down after a certain academic title. Those who are enthusiastic and have great goals make an incredible contribution to Emergency Medicine. I have to emphasize an important point here. As in every group, there may be conflicts from time to time in this group and their reflections in the service can be seen. However, a prolonged conflict at the upper level of the service leads to different and harmful perceptions throughout the service. Other staff working in the service sometimes cannot decide who to listen to and may have to play a different personality role. Certain conflicts cannot be prevented despite all goodwill and solidarity. Sometimes even if we are right, we should be able to correct our behavior that can make many people uneasy and learn to waive some of our rights. We do not and should not have the right to make many people uneasy for a piece of righteousness and for our self, which we misunderstand a lot of times. The most important step in preventing this situation that is harmful and unfair and which affects the service in every cell and disrupts the motivation of the employees is to take the decisions considered for the service together. This does not only provide justice but also facilitates the implementation of decisions and helps to achieve faster results. There may be different behaviors in the details and these should be accepted. Besides basic principles, everyone should act as one body.
- **Non-fixed employees:** These are patient transport, cleaning and security guards and interns. The duration of the education of the students in the emergency department is predetermined and is a little out of this article. Therefore, it will not be mentioned in detail. For other employees, the time of arrival and departure to the emergency department is often not known in advance. Their working order is completely at the disposal of the hospital administration. Some of them work in the emergency department for a long time, while others come and go without being recognized. The employees in this group should be constantly educated about sterile procedures, about dealing with colleagues-patients-relatives, and about time to job, drinking, eating, and resting. Although they are not in the decision-making position and do not have the decision-making authority, they have the potential to significantly affect the quality of the service and the service they produce. For example, if your patient arrives, and examination, diagnosis, and treatment is excellent but the toilets smell, floors, stretchers, and chairs are dirty, it will not be easy to talk about quality service. Therefore, the controls, follow-up, and necessary training of the employees in this group have to be daily. However, these employees who are lower in economic terms should not be miserly in terms of personal rights. Their happiness and motivation can only be achieved through this

Gaining Corporate Belonging

First of all, the communication between the existing employees must be sound. A full consensus should be reached in the management of the institution. It is useful to make a definition so that the full agreement is not misunderstood.

- **Full reconciliation in the emergency department**
  - Improving quality at every stage of patient care in the emergency department,
  - Ensuring an adequate level of physical space,
  - Provision of adequate equipment according to the level of emergency services,
  - To act fairly in the protection of the personal rights of the personnel,
  - Arranging annual permits,
  - Adjusting and maintaining milk or breastfeeding permits,
  - Preparation of disease reports,
  - Distribution of shifts,
  - Ensuring that legal and excuse permits are granted for weddings, engagements, and deaths
  - Ensuring the allocation of out-of-work wages,
  - Preventing mobbing on any subject,
- Providing the necessary training to each group of personnel for the development of Emergency Medicine and ensuring the continuation of them,
- Protection of the rights of emergency personnel against other services
- Protecting the revenues of the institution and service and ensuring that all services provided for patient rights are fully recorded. In cases where full reconciliation is impossible, at least there should not be serious differences of opinion and power that affect each other negatively and cause negative forces instead of a synergy.

First Welcome and First Meeting in the Emergency Department

For both newcomers and active workers, the first impressions at the first meeting are like the first teachings of young children that cannot be erased. These moments are very precious; they are never forgotten with their pangs and dessert. If you see the first meeting big, it grows bigger and if you see it small, it becomes smaller; if you give importance, it develops and if you do

not give importance, it goes out. People are both satisfied and happy at the first meeting or are anxious and uneasy. Both parties agree that the first encounter in the emergency room and the interview can be very impressive. There is the first communication step before the first encounter. Before they arrive, including those who think they have been exiled, they do research about their destination on social media or call the service and ask for information about everything. I would like to make an evaluation based on our experience so far for useful communication and meeting.

- The information that can be given in the first interview
  - The number of staffs in each group (doctor, nurse, paramedic, Emergency Medical Technician)
  - Number of patients per day,
  - Distribution, number, and duration of seizures,
  - Patient population,
  - Patient transfer,
  - Invasive procedures,
  - Security status,
  - Keeping records,
  - Inter-shift
  - Annual and other permits,
  - How nutrition, dressing, and other daily needs are met,
  - Clothes to be worn during the service,
  - Patient transfer and delivery between departments,
  - The location of the hospital in the city,
  - House prices and rents by region,
  - Monthly fees
- Describe the other supervisors in the department and their areas of interest (areas of responsibility of the professors); the newcomer should be contacted by those in charge. People must not provide confusing information about the department. Possibly, different information and ideas can cause frustration and insecurity in newcomers. It might make them think they are in a confrontational environment. To prevent this from happening, the service must be in full agreement, as described above. When these encounters do not occur, communication accidents occur. Many people know from their own experience that suddenly in the service, we may come across someone in uniform who is interested in the patient or another job of the service. Asking him who you are or what are you doing here, may offend both parties. Answers like these from our nurse friends who started four months ago make the whole thing ugly. Sometimes, it may not be appropriate for everyone to meet at the same time. In this case, communication and promotion can be provided by social media networks such as WhatsApp or other communication devices established within the service. Taking a photo of the person with his/her consent and his/her name and surname can be shared in the group together with the study area. Those who are certain to start can be included in the groups at the moment and it can be called a welcome, good luck friend
- When the person is a new faculty member, it is important to introduce him to the hospital chief, dean, and head of the internal/surgical departments. When the new person is a nurse, paramedic, or ATT, care must be taken to introduce him to the nurse and hospital director or assistant principals in charge of the department. These interviews are extremely important in terms of corporate identity.
- After the interviews, a meal with the new person, if possible, within the hospital, reinforces the sense of belonging.
- Beginners may not have arranged accommodation in the first place. In this case, it is a courtesy to show them hospitality, arrange an accommodation where they can be comfortable. This may be temporary in our own homes or in suitable guesthouses. Against such ownership, the new officer who has moved to full settlement will never forget the fidelity of his hospitality.
- It is a good communication tool that should not be skipped when the first interviews are over, providing information to the newcomers about the city. The person in charge can inform the new person about the city›s population, cosmopolitanism, shopping centers, parks and gardens, the kitchen, and the means of transportation.
- New staff should also be assisted in renting, buying or moving into a house. After all, we know the city and the environment we work in, better than newcomers. It is not right to leave these people who will be our colleagues alone at the mercy of unknown people like real estate agents in the city. Without our help, the problems that may arise as a result of renting a house in an unnecessary place will be reflected in the work environment; we will all be negatively affected.
- The introduction of the materials and working systems in the service to the newcomers in the early period is one of the aids that may be useful in opening up the whole service. This promotion is particularly important, as a large number of new staff are appointed or dispatched to the emergency room without any experience. Sometimes, a staff member who does not know how the blood pressure cuff works, how to turn the monitors on and off, how to work sterile in the service, and even how to open the vascular access, can come. Releasing such a person to learn for himself or herself over a long period will cause harm to all employees and patients. The sooner one gets the job, the safer we all are. Angry questions such as where you graduated, how you graduated, and why you do not even know the simple tasks, do not help the young people who do not know the job at this time. Since we cannot send the incoming staff back, because the new staff will not be replaced even when the staff goes back, training the incoming staff is the least harmful and most profitable way. However, this teaching work should be done with compassion and good faith, not by offending the person. Then, the new person will know you as a lifetime master.
- One of the secrets of gaining corporate belonging to the newcomers and being a good team with them is to visit their homes as a department and to welcome them and share their joy and grief with them in cases of birth, marriage, death or illness. These visits are social capital parameters and are very useful. The department can visit all of their staff by making these visits routine throughout the year.
- As for those who believe that they have been sent to the emergency room as exile, we can talk with them about a common working environment and discuss the errors or

negativity about the emergency room in their minds. They may indeed have been sent to the emergency department as a punishment for friction with the administration. Some of them are people who do not hold stitches anywhere and eventually coming to the emergency room. Perhaps they are experienced and usable elements. The boundaries with them should be drawn. We can tell them that: they are not sent to the emergency room as a result of our mistakes, we are not in favor of punishment, we have no intention of tormenting them in the emergency room, we can do good work together within the legal framework, and most of the reservations about the emergency service may be wrong and incomplete. This type of treatment causes them to separate us from the hospital managers who sent them to the emergency room. It prevents them from looking at us as a common criminal, and they are convinced that they can work with us.

- One of the many components of social capital in the emergency department is the positive behavior we have against the employees of the institution who come to the emergency department for any discomfort or special job. When in-house employees come to the emergency room for their discomfort, we need to make them feel that they are an employee of the institution. A significant number of patients who come to the emergency department wait for such behavior and enjoy it. As an emergency room manager or responsible nurse, we can convey our wishes to our sick employees. We can stand next to them and share their troubles. If necessary, we can invite them to our room and offer tea and coffee. If we are not present in the emergency room, we can call our sick colleagues and tell them that we are aware that they are coming to the emergency room as a patient and that our other colleagues will do their best for them. We can guarantee that they can reach us in case of any need. This favoritism, which is shown as VIP treatment, can be viewed as a right of the employees of the institution rather than a compliment. Such behaviors should not be viewed from a window of interest, such as «now someone from a different service came to us; we should treat them well so that they can treat us well when we go to that service." As the emergency department, this kind of positive behavior toward our sick friends is more sympathetic compared with just giving them a nice gift; it increases their trust and confidence in us and the emergency department. It is also social capital to inform our colleagues who are actively working in the emergency department on these issues. When one of our colleagues approaches our as a patient, we must ensure that the service officers are notified. Thus, a protocol of the emergency department should be created for the internal employees.

Those Who Leave the Emergency Service

We believe that this issue has been partially neglected in emergency services. Regardless of the long or short term, everyone working in the emergency department has more or less labor, which should not be ignored. From time to time, if we had problems with some people and even if we were right, these people looked after us in the same place, treated patients, experienced stress, gave their days and nights, remained sleepless, exposed the violence of the patients and their relatives with us, and shared our problems; they stood side by side with us many times and had dinner and tea; they may have borrowed or lent money to us and added a useful new vision to the service. For all these reasons, people should leave satisfied when they have to leave us. They should leave without saying, "Oh, I'm leaving you, I'm getting rid of you." We should not be doing any injustice that makes them say that. Combining the experiences of a large number of people, there are good points for those who will leave the emergency room for any reason. These include:

- Departure/leaving must be announced to all service employees.
- Gifts should be given within the facilities. This can be a meal or something like the clothes that one likes.
- Before leaving, it will be an honor to give the person a certificate of appreciation signed by the person in charge of the department or the hospital administrator for his contribution to the emergency room.
- It is very valuable to accompany people to the bus station or airport.
- It would be detrimental to remove people from communication groups immediately they leave. These people often leave the groups themselves. If the time is prolonged or people forget, they may be reminded later by implication.
- Calling on days like holidays and festivals, and asking them to remember their memories shows that they are not forgotten, which will make them happy.
- When there are situations like birth, marriage, death, or illness, visiting them if they are nearby, searching for distant ones, and sharing their joy and grief is an example of omnipotence.
- It is a humanitarian and conscientious task not to leave them alone when they or their relatives come to the emergency room due to illness; the required sensitivity must be shown. This approach makes our old colleagues extremely happy. You should not spare this much.
- Those who will leave are expected to submit a report on the service (without intent, a frankly negative report) to the department responsible for problems and solutions. These reports should always be evaluated at the point of service benefit.

Why These?

Emergency services are constantly changing. Due to shifting principles, we may not meet face-to-face for a long time. Such rapid changes and stressful environments can also result in changes in people's material and spiritual worlds and work ethics. Changes that occur in the spirit world of individuals cannot be noticed. We may be faced with a serious problem with a colleague at an unexpected moment. Sometimes, we scold our friends for not informing us of what is going on. Moreover, the work of the emergency department requires a very serious team than other branches. The success of the team being united around a purpose in the same event. A secret to our success is to know each other well. In other words, it should be essential for the team to work as a sino-atrial node. Thus, water and elec-

tricity go to every focus of the service, distant and near points are fed and do not develop into ischemia. Otherwise, with such intense work volume and rapid variability, everyone works as atrial fibrillation to their strength but cannot prevent the service from entering into ischemia. Ensuring an ideal environment is also possible through good communication. It is useful to evaluate the activities described in this section such as acquaintance, solidarity, and ownership as social capital. This capital is free of charge, may not be easily won but not lost easily, does not deteriorate after separation, daily currency changes do not affect it, and it does not even end in death. They should not be seen as unnecessary, insignificant, and dysfunctional and should not be considered useless.

CONCLUSION

Social capital does not have a unique definition. Social issues play an important role in the background of problems and difficulties in all aspects of life. The challenges encountered in economic life and in managing organizations are not only associated with the lack of material and physical resources. Therefore, the concept of social capital considers an individual as an important parameter of productivity and efficiency without ignoring the social aspect of the individual. He considered the social deficiencies of individuals as a problem that needs to be solved and considered it as a manageable resource. In this respect, at the end of the 20th century, a new perspective and conceptual framework were created by examining the social relationships of individuals. Globalization and the development of information communication technologies have also seriously affected interactions between individuals. To meet complex economic transactions and non-monetary needs, relationships with minimal confidence were needed. Especially in the 1990s, different capital concepts such as social capital, which belong to many disciplines, were added alongside classical capital concepts. The mentioned reasons have increased the importance of social relationships and provided an atmosphere suitable for the development of social capital in an academic sense. However, it is a panacea and a mistake to look through a prescription. Some authors represent the individuality of social capital, whereas others represent the collective aspect of social capital, focusing on social returns. In the literature, majority of the have investigated the positive aspects of social capital and especially social solidarity.

This paper more closely conforms to the definitions of social capital in the literature by Coleman and Lin. Although there are many reasons for the success and failure of institutions, the responsibility of the employees in that institution has the most important factor. The advantages such as the love and ownership of the employees add value to the organization; negativity such as hating the institution and downgrading it everywhere can degrade the value of the institution. The emotions and feelings that people experience during their start-up and departure have an important place in owning the institution or trying to escape from it at every opportunity. These events are very effective in gaining corporate identity or eroding an identity. Emergency services have some disadvantages. Due to the balance of supply and demand, the type and number of patients are constantly changing and the number of staff and the staff itself is always changing. This rapid variability can make it difficult for those who come to have a sense of belonging to their institution. This disadvantage can be advantageous with good corporate organization and management.

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