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The Medical Bulletin of Haseki is the official scientific journal of the University of Health Sciences Haseki Training and Research Hospital. It covers subjects on general medicine, published both in Turkish and English, and is independent, peer-reviewed, international periodical and is published quarterly (January, March, June, September and November).

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Case Report

Original Article

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Effects of Fibrin Glue, 2-Octyl Cyanoacrylate, and N-Butyl Cyanoacrylate on Cartilage Healing: An Experimental Animal Study

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Abstract

Aim: Following septoplasty surgery, nasal packing is performed to stabilize the healing of septum and prevent septal hematoma. The purpose of this study was to investigate the efficacy of fibrin glue, 2-octyl cyanoacrylate, and n-butyl cyanoacrylate on the healing of the septum cartilage following septoplasty.

Methods: Since rabbit ear cartilages resemble septal cartilage, 10 New Zealand rabbits were used in our study which was performed between March and May 2016. A total of 20 ears of 10 rabbits were used, with 4 separate incisions in each ear. Perichondrial flaps were raised and the following chemical adhesives were applied separately on the cartilage: Fibrin glue, 2-octyl cyanoacrylate, n-butyl cyanoacrylate. As the control group, no material was applied to the remaining incision. The right ears were examined at 15 days and the left ears at 30 days; samples were removed with solid tissue around the edges, treated with formol and histopathologically examined.

Results: Epithelial integrity and re-epithelialization were adequate in all groups. As the epithelium and perichondrium thicknesses were compared in the 15-day samples, epithelium and perichondrium were thicker in the n-butyl cyanoacrylate and 2-octyl cyanoacrylate groups than the other groups.

Conclusion: N-butyl cyanoacrylate, fibrin glue and 2-octyl cyanoacrylate are potential alternatives for cartilage and mucosal support.

Keywords: Cartilage, fibrin tissue adhesive, octyl 2-cyanoacrylate, cyanoacrylates

Introduction

Septoplasty is among the most common operations in otolaryngology. Primarily the mucoperichondrial and mucoperiosteal flaps are elevated and the deviated parts of the cartilage and/or bony septum are repositioned or resected. Following septal surgery, internal nasal packing is applied to stabilize the septum and to prevent septal hematoma, as well as to support septal flap and remove the dead space between the subperichondrial flaps and cartilage (1). If the internal packing of the nose is not adequately provided, bleeding, septal hematoma, perforation, septal instability, septal synechia, infection and a septal abscess may develop (2).

As a foreign body, nasal packing has a number of disadvantages such as prophylactic antibiotic use, nasal pain and discomfort, edema, insufficient nasal breathing that leads to mouth breathing and dry mouth, increased lacrimation, difficulty in sleeping and cardiopulmonary complications (2-4). Various absorbable materials, tissue adhesives or suture techniques have been used to overcome the disadvantages of removable nasal packing (1,5,6).

Fibrin glue (FIB), n-butyl cyanoacrylate (NBC) and 2-octyl cyanoacrylate (2OC) are commonly used tissue adhesives. These adhesives are not only hemostatic, but also possess bacteriostatic properties (4,7,8), so they may eliminate the need for nasal packs. The ear cartilages of

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rabbits are very much alike the septal cartilage, therefore, we found it appropriate to carry out this study in a rabbit model.

In this study, we aimed to investigate the efficacy of FIB, 2OC, and NBC on the healing processes of cartilages in a rabbit model. Those three tissue adhesives are also compared to each other both clinically and histopathologically.

Methods

All experiments were performed at the Animal Research Laboratory of İstanbul Bagcilar Training and Research Hospital between March and May 2016 with the approval of the Animal Experiments Ethics Committee of İstanbul Bagcilar Training and Research Hospital (date: 23.03.2015, no: 2015/30). Experiments were conducted in accordance with the principles of the Helsinki Declaration on laboratory animals. Due to the ethical regulations of the animal ethical committee, a routine reduction in the number of experimental animals was enforced, and a total number of 10 New Zealand rabbits were provided.

Study Design

Four different groups were formed; one was the control group and the other three groups were designed as the study groups for study defined as, FIB, 2OC, and NBC groups.

Experimental Animal Model

Both ears of all rabbits were used, and for each ear, four separate incisions were made. Four flaps in the subperichondrial plane were raised delicately for each ear (Figure 1). In the control group, perichondrial flap was laid in its place without using any material between the cartilage and perichondrium. In the FIB group, FIB was placed between the cartilage and perichondrium. In the 2OC group, 2OC was placed between the cartilage and perichondrium. In the NBC group, NBC was placed between the cartilage and perichondrium. All flaps in the FIB, NBC and 2OC groups were retained to their original positions and the incision sites were pressed against the ear for 20 seconds. All incisions were then sutured.

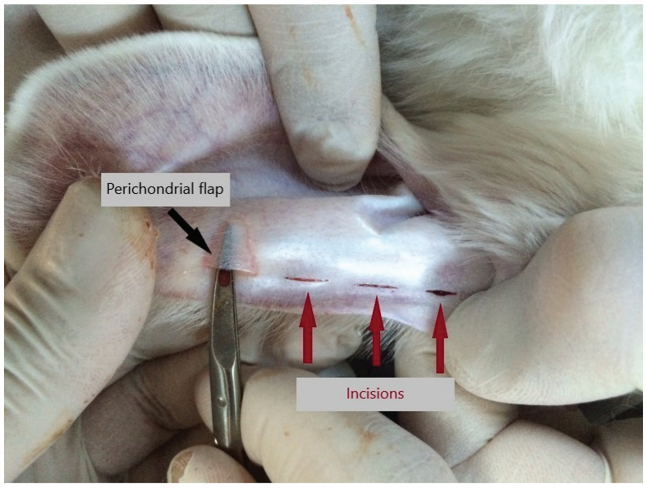

Figure 1. Separate incisions were performed and four flaps were raised in the subperichondrial plane for each ear

The right ears were examined on the 15th day and the left ears on the 30th day; samples were removed with solid tissue around the edges, treated with formol, and prepared for histological examination.

Histopathological Examination

The relevant areas of rabbit ears were isolated and incubated for 72 hours at room temperature in 10% buffered neutral formaldehyde. Histological examinations were performed on a tissue monitoring device (Leica TP 1020). After routine tissue monitoring, tissue blocks were implanted in paraffin. Sections of 5 µm thickness were stained with haematoxylin eosin. A light microscope (Leica 6000B) and a LASX program attached to a digital camera (Leica DC490, Wetzlar-Germany) were used for histological examinations. Each sample was scored and semi-quantitatively assessed for epithelial integrity and renaturation, edema, vascularity, and inflammation (0=no, 1=mild, 2=moderate, 3=severe, and 4=very severe). Epithelium and perichondrium thickness (µm) were measured with a 10-magnification image analysis program (Leica Application Suite software, Leica) and the mean values for the groups were determined.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows version 10.0. The Mann-Whitney U and Kruskal-Wallis tests were used to compare quantitative data. The chi-square and Fisher's Exact tests were used to compare qualitative data. Data were expressed as the mean ± standard deviation of the mean. A value of $p<0.05$ was considered as statistically significant.

Results

In all groups, a multi-layered flat epithelium was observed. Epithelial integrity was preserved in all groups with re-epithelization. Slight edema was observed in connective tissue under the epithelium in all groups on 15th and 30th days (Table 1).

The epithelium and perichondrium thicknesses were compared on the 15th day and were found thicker in NBC and 2OC. 2OC epithelial thickness was nearly two-fold compared to the control. Thirty-day samples exhibited decreased thickening of the epithelium in NBC and 2OC whereas the thickness of the perichondrium was increased. In FIB, epithelium thickness and perichondrial thickness did not change after 30 days (Table 2).

Table 1. Histopathological scores of the study groups

		Re-epithelialization	Edema	Inflammation	Vascularisation
15th day	Control	**3**	**1**	**2**	1
	FIB	4	1	2	2
	2OC	3	1	2	2
	NBC	3	1	1	1
		Re-epithelialization	Oedema	Inflammation	Vascularisation
30th day	Control	4	1	2	2
	FIB	4	1	2	2
	2OC	4	1	2	2
	NBC	3	1	1	1

Each sample belonging to each group has epithelial integrity and regeneration, edema, vascularization, inflammation in the range of 0-4; 0=none, 1=mild, 2=moderate, 3=severe and 4=very severe. The data reflect the mean scores of the groups. FIB: Fibrin glue, 2OC: 2-Octyl cyanoacrylate, NBC: N-Butyl cyanoacrylate

Table 2. Comparison of epithelium and perichondrium thickness in the 15th and 30th days

	15th Day		30th Day	
Groups	**Epithelium thickness**	**Perichondrium thickness**	**Epithelium thickness**	**Perichondrium thickness**
Control	368.106±6.407	55.943±1.111	489.133±6.487	49.090±0.886
FIB	327.113 ±3.138	68.593±1.377	524.514±6.677	68.600±1.758
2OC	384.893±5.455	82.776±1.941	426.680±5.399	73.413±1.573
NBC	576.073±11.794	118.553±2.069	340.093±5.450	138.706±2.036

Epithelium and perichondrium thickness measurements were made in µm in three sections of each sample and given as mean + SEM for the groups. FIB: Fibrin glue; 2OC: 2-Octyl cyanoacrylate; NBC: N-Butyl cyanoacrylate

Moderate vascularity was observed in 15-day samples from the groups treated with 2OC and FIB. Vein occlusion was observed in 15-day samples in the control and NBC groups. Moderate inflammation was observed in all 15-day samples. In NBC, thicker collagen fibrils were observed in connective tissue.

Mild inflammation was observed in all test groups except the NBC on the 30th day, but NBC had moderate inflammation. Inflammation in the control group was similar to FIB and 2OC but less in NBC.

On the 15th day, samples from 2OC and FIB exhibited a larger number of chondroblasts. Extracellular matrix synthesis was apparent in 2OC and the basophilic area around the cells was similar to the extracellular matrix in the hyaline cartilage.

On the 30th day, a round-cytoplasmic chondrocyte embedded in lacunae, a basophilic extracellular matrix in the periphery, and a cartilaginous tissue composed of flattened outer chondroblasts were observed in the control group. On the 15th day, immature cartilaginous tissue was observed in FIB; chondrocytes forming isogen groups and more chondroblasts were observed in NBC; and chondrocytes, mitochondrial chondrocytes, and chondroblast cells were observed in the group treated with 2OC.

Discussion

Nasal packing after septoplasty is still used worldwide. Although the need for nasal packing remains a matter of debate, many surgeons prefer to use it. It's used to reattach mucoperichondrial flaps onto septal cartilage, to prevent epistaxis and septal hematoma. However, nasal packing has a number of disadvantages such as nasal pain, edema, prophylactic antibiotic use, mouth breathing, dry mouth and these effects decrease the quality of life (2,4). Various absorbable materials have been used to overcome the pain during the removal of the packing. Absorbable materials do not require subsequent removal, providing the patient with increased comfort while achieving positive effects on haemostasis.

A lack of packing may have advantages such as increased comfort, decreased chance of postoperative complications associated with packing, lowered costs. A few alternative techniques to nasal packing have been described in the literature. The most commonly used technique is suturing the septum (9,10). Korkut et al. (11) compared trans-septal suturing and intranasal packing after septoplasty. Postoperative symptoms were less in the transseptal suturing group, patients were more comfortable. However, the mean duration of surgery was higher in the suture group. Gunaydin et al. (12) compared transseptal suturing and nasal packing. They suggested

that trans-septal suturing is more comfortable and cost-effective, but also they reported that postoperative discomfort due to foreign body sensation was seen in transseptal cases. Tami et al. (13) used bioresorbable septal stapler in 24 subjects, complete coaptation of the mucoperichondrial flaps was achieved in all of the patients and septal hematoma did not occur in any patient.

Habesoglu et al. (2) used FIB to fix the mucoperichondrial flap to the septal cartilage and compared outcomes with non-absorbable nasal packing. They found that pain scores in the FIB group were significantly lower than in the intranasal packing group. The postoperative bleeding was also lower in the FIB group. Erkan et al. (14) investigated the changes in rabbit nasal septal tissues after using FIB in septoplasty. They found that the use of FIB caused mucosal inflammation, increased mucosal thickness, decreased perichondrial and cartilaginous thickness and created mucosal damage. Coey et al. (15) compared the use of fibrin tissue adhesive and nasal packing in endoscopic nasal surgery in their meta-analysis and their results showed that fibrin tissue adhesive had minor advantages against nasal packing.

NBC and 2OC are bacteriostatic, hemostatic, biodegradable and tissue-compatible powerful tissue adhesives (8,16). Cyanoacrylate fixatives were approved by the Food and Drug Administration (FDA) to facilitate skin closure, but in recent years they have been used in different areas of medicine. Üstün et al. (17) repaired the incisions made on the tongues of rats with 2OC or Vicryl sutures. In their short and long-term results, they claimed that 2OC is a good alternative to primary suturing in mucosa lacerations. Alkan et al. (16) fixed the nasal septum to the anterior nasal spine in rabbits using NBC and they did not observe any kind of foreign body reaction, histotoxicity, cartilage necrosis or inflammation. Dabb et al. (18) used 2OC for the fixation of nasal cartilage grafts and reported that using 2OC was an effective method for prefabrication and fixation of nasal cartilage grafts. Brown et al. (19) reported in their short-term animal study that the stabilization of cartilage to bone with 2OC was better than the suturing technique.

In our study, we found that the epithelium and perichondrium were thicker in NBC and 2OC. In FIB, epithelium and perichondrium thicknesses were the same after 30 days. Moderate vascularity was observed in all groups, pointing out to stable cartilage viability. The number of chondroblasts were increased in all groups, suggesting that these three materials are compatible for use with cartilage as a packing material.

The ear cartilage of the rabbits is very much alike the human septal cartilage. In need of search for different materials to be used as nasal packing after septoplasty, we aimed to compare previously mentioned three chemical adhesives to a control group and examined and compared the effects and potential biohazards of them to tissues histopathologically.

Study Limitations

Our study had one limitation: due to legal and ethical regulations, the number of rabbits provided according to the ethical committee directives were less in number to conclude a concrete result, nevertheless the data we collected deemed a success in the favour of chemical adhesives.

Conclusion

Application of NBC, FIB, and 2OC between septal cartilage and perichondrium are potential alternatives to nasal packing after septoplasty. These materials have an acceptable histopathological profile in animals, based on post-operative inflammation levels, vascularisation, and the cellular characteristics of bone and cartilage regrowth. Studies can be conducted in which these adhesives are used in septoplasty surgeries in humans.

Authorship Contributions

Concept: D.H., H.A., O.U., D.Z.,B.G., Y.U., Design: D.H., H.A., O.U., D.Z.,B.G., Y.U., Data Collection or Processing: D.H., H.A., O.U., D.Z.,B.G., Y.U., Analysis or Interpretation: D.H., H.A., O.U., D.Z.,B.G., Y.U., Literature Search: D.H., H.A., O.U., D.Z.,B.G., Y.U., Writing: D.H., H.A., O.U., D.Z.,B.G., Y.U.,

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References

1. Genç E, Ergin NT, Bilezikçi B. Comparison of suture and nasal packing in rabbit noses. Laryngoscope 2004;114:639-45.
2. Habesoglu TE, Kulekci S, Habesoglu M et al. Comparative outcomes of using fibrin glue in septoplasty and its effect on mucociliary activity. Otolaryngol Head Neck Surg 2010;142:394-9.
3. Muluk NB, Apan A, Ozcakir S, Arikan OK, Koç C. Risk of respiratory distress in the patients who were applied nasal packing at the end of nasal surgery. Auris Nasus Larynx 2008;35:521-6.
4. Aksoy F, Yilmaz F, Yildirim YS, Gideroglu K, Tatar Z. Use of N-butyl cyanoacrylate in nasal septoplasty: histopathological evaluation using rabbit nasal septum model. J Laryngol Otol 2010;124:753-8.
5. Lemmens W, Lemkens P. Septal suturing following nasal septoplasty, a valid alternative for nasal packing? Acta Otorhinolaryngol Belg 2001;55:215-21.

6. Reiter D, Alford E, Jabourian Z. Alternatives to packing in septorhinoplasty. Arch Otolaryngol Head Neck Surg 1989;115:1203-5.
7. Vaiman M, Eviatar E, Segal S. The use of fibrin glue as hemostatic in endonasal operations: a prospective, randomized study. Rhinology 2002;40:185-8.
8. Min JY, Jang YJ. Use of 2-octylcyanoacrylate (Dermabond) tissue adhesive for tip graft fixation in open rhinoplasty. Otolaryngol Head Neck Surg 2011;145:737-41.
9. Bajaj Y, Kanatas AN, Carr S, Sethi N, Kelly G. Is nasal packing really required after septoplasty? Int J Clin Pract 2009;63:757-9.
10. Wang D, Liu T, Liao C, Tang G, Tian T, Tian L. Is nasal septal suturing an alternative technique to nasal packing?: A protocol for systematic review and meta-analysis. Medicine (Baltimore) 2020;99:23535.
11. Korkut AY, Teker AM, Eren SB, Gedikli O, Askiner O. A randomised prospective trial of trans-septal suturing using a novel device versus nasal packing for septoplasty. Rhinology 2010;48:179-82.
12. Günaydın RÖ, Aygenc E, Karakullukcu S, Fidan F, Celikkanat S. Nasal packing and transseptal suturing techniques: surgical and anaesthetic perspectives. Eur Arch Otorhinolaryngol 2011;268:1151-6.
13. Tami TA, Kuppersmith RB, Atkins J. A clinical evaluation of bioresorbable staples for mucoperichondrial flap coaptation in septoplasty. Am J Rhinol Allergy 2010;24:137-9.
14. Erkan AN, Cakmak O, Kocer NE, Yilmaz I. Effects of fibrin glue on nasal septal tissues. Laryngoscope 200;117:491-6.
15. Coey JG, Whittaker PJ, Williams G, Ikram UH, Page OJR. Fibrin tissue adhesive versus nasal packing in endoscopic nasal surgery: a systematic review and meta-analysis. Rhinology 2019;57:21-31.
16. Alkan S, Dadaş B, Celik D, Coskun BU, Yilmaz F, Başak T. The efficacy of N-2-butyl cyanoacrylate in the fixation of nasal septum to the anterior nasal spine in rabbits: experimental study. Eur Arch Otorhinolaryngol 2007;264:1425-30.
17. Üstün O, Kumral TL, Atar Y, et al. Histopathological Comparison of 2-Octyl Cyanoacrylate and Primary Suturing for Tongue Lacerations. J Craniofac Surg 2020;31:e334-e7.
18. Dabb RW, Gaffield JW, Camp LA. Use of cyanoacrylate (super glue) for the fixation and prefabrication of nasal cartilage grafts. Aesthet Surg J 2001;21:328-33.
19. Brown PN, McGuff HS, Noorily AD. Comparison of N-octyl-cyanoacrylate vs suture in the stabilization of cartilage grafts. Arch Otolaryngol Head Neck Surg 1996;122:873-7.

Original Article

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Development of a New and Simple Postoperative Pain Fear Scale for Elective Surgeries in Adult Patients

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Abstract

Aim: There is a need to assess the pain fear levels of surgical patients simply and appropriately before the surgery. This study aimed to develop and test the psychometrics of an instrument to evaluate the fear of postoperative pain.

Methods: This methodological study was conducted at four surgical clinics including general surgery, orthopedics and traumatology, neurosurgery, heart and vessel surgery of a university hospital between 28 March and 19 October 2018. Totally, 150 patients who were scheduled for elective surgery and at their preoperative day were included. This scale contained 10 items related to the postoperative pain sources and aimed to identify the pain fear of patients preoperatively. To measure the sampling adequacy, Kaiser-Meyer-Olkin index and Bartlett's test of sphericity was used. Explanatory and confirmatory factor analyses were conducted to evaluate the construct validity. The internal consistency of the scale was evaluated by Cronbach's alpha calculation. All hypotheses were tested in two directions.

Results: The total variance explained 55.5% of the variance for one factor structure consisting of 10 items. The model fit index values through the confirmatory factor analysis were found to support this structure.

Conclusion: The scale is appropriate to be used in clinical settings to quickly evaluate the elective surgical patients' fear level of postoperative pain preoperatively.

Keywords: Postoperative pain fear scale, postoperative pain, scale development, surgical patient, Turkish

Introduction

Fear is defined as an emotional response to traumatic events such as surgical interventions (1,2). Patients waiting for an upcoming surgery may describe the fear and this may be related to many conditions (3,4). In the previous studies, fear of anesthesia, surgical procedure and unknown were reported as the factors of surgical fear (5,6). Many studies have found that surgical fear is related to fear of postoperative pain and reported that preoperative predictors of postoperative pain, fear were associated with postoperative pain (4,7).

Pain-related fear is identified to be an important target of pain treatment and with reducing pain fear, improvement in the pain management outcomes is evidenced to be possible (8). Fear-avoidance beliefs are evidenced to play a significant role in pain experience, and psychological well-being, so that assessing the fear beliefs with standardized instruments is recommended for the first step of patients who present with a pain condition (9,10). Clinical nurses and physicians are the primary professionals in taking care of patients and evaluating their pain levels. Screening patients preoperatively to identify their pain fear helps to reduce unwanted pain expectations and increase the pain coping abilities of patients (11,12).

Fear of pain is possible to be measured with some assessment tools including the Fear of Pain Questionnaire III (13), Parent Fear of Pain Questionnaire (14) and Pain Catastrophizing scale (15). However, they are not specific for surgical pain fear, complex and not practical to be used at the busy surgical clinics for preoperative evaluation. Therefore, there is a need for a simple, timesaving and appropriate tool to assess pain fear levels at the clinic preoperatively or before transferring to the operating room.

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Preoperative investigation of postoperative pain fear among surgical patients for planning an effective pain management regimen is essential. Knowing the predictors of postoperative pain may be helpful for making decisions about optimal pain management techniques. Unfortunately, there is not any tool to evaluate surgical pain fear. The development of this will fill the gap in the literature and help health professionals to evaluate the fear of postoperative pain before surgical interventions of the patients. With the evaluation of the pain fear levels of the surgical patients, it is intended to decrease the postoperative pain and anxiety by providing interventions on patient care and medical treatments. The purpose of this study was to develop and test the psychometrics of an instrument to evaluate the fear of postoperative pain.

Methods

Ethical Considerations

This study was approved by the ethical review board of the University Ethics Committee of the Trakya University Medical Faculty (date: 19.02.2018 decision number: 03/24) and permission to conduct the study was obtained from the hospital directory. Before the interviews start, the aim and context of the study was explained to the patients and they were asked to sign an informed consent form if they are volunteered.

Research Design and Sample

The study was designed as a methodological study and included the development and validation of the postoperative pain fear scale. The study took place at the four common surgical clinics (general surgery, orthopedics and traumatology, neurosurgery and heart and vessel surgery) at a university hospital in Turkey between 28.03.2018-19.10.2018 with 150 patients. While the reliability and validity studies require a sample size at least 5-10 times of the total number of scale items, the sample size is calculated as 150, fifteen times the 10 items of the present study (16). Inclusion criteria were being voluntary, scheduled for elective surgery, being at the preoperative day, able to communicate in Turkish, had no mental problem.

Data Collection

For data collection, a data collection form with third parts is used. In the first part, questions about age, gender, clinic and previous surgery history were included; in the second part, the Postoperative Pain Fear scale (POPFS), was included to develop within this research; in the third part, the Surgical Fear Questionnaire (SFQ) and Fear of Pain Questionnaire-III (FPQ-III) were included to evaluate criterion validity.

The SFQ was developed by Theunissen et al. (4) in 2014 and the Turkish adaptation was conducted by Bagdigen and Ozlu (17) in 2018. It consists of 8 items based on an 11-point Likert type scored 0 "I have no fear at all" to 10 "I have a very great fear". It has two sub-scales. Items 1 to 4 assess the source of fear caused by short-term results of the surgery and items 5 to 8 assess long-term results. The higher scale score indicates a higher level of surgical fear. Cronbach's alpha internal consistency coefficient was found as 0.95 in the Turkish version (17).

The FPQ-III was developed by McNeil and Rainwater (13) in 1998 and the Turkish adaptation was conducted by Unver and Turan (18) in 2018. It consists of 30 items based on a 5-point Likert type scored 1 "not at all" to 5 "extreme". It has three sub-scales. Items 1,3,5,6,9,10,13,18,25,27 assess the severe pain fear, items 2,4,7,12,19,22,23,24,28,30 assess the minor pain fear and items 8,11,14,15,16,17,20,21,26,29 assess the medical pain fear. The higher scale score indicates the higher fear. Cronbach's alpha internal consistency coefficient was found as 0.92 in the Turkish version (18). In this study, the cross-validation of this scale was also completed, and the results supported that this scale is valid, reliable and can be used in surgical patients.

The data collection form was administered to the patients in their rooms at the clinic before their surgery via face-to-face interviews by the same researcher. It took approximately 10 to 15 minutes to complete.

Scale Development

The scale development was completed following three steps. The first step included item generation. In this step, a pool of items was prepared by a literature review to shape the draft of the scale. Within the context, the studies conducted national and international were examined in terms of postoperative pain and fear, and the expressions that could be used in the scale were determined (3,4,17,19). It was noted that these expressions were related to the fear experienced by the patients who were planned to undergo surgery. It was also aimed that each of the items will be directed towards the fear of pain that are easy to be understood by patients. Totally 13 items were identified which specifically focus on the postoperative pain sources (such as wound-related pain, suture related pain, moving related pain, dressing changing related pain, deep breathing-related pain, coughing related pain, getting out of bed-related pain, feeding-related pain, urinary drain related pain, nasogastric drain related pain, intra-abdominal gas-related pain, medical treatment-related pain).

The second step included the expert consultation. In this step, draft scale items were sent to the experts via e-mail to evaluate their opinions. Ten experts in the field of

anesthesiology (1 medical doctor), psychology nursing (1 academician), surgery (4 academicians - working at general surgery service), surgeon (2 medical doctors), surgical nursing (2 academicians). They were asked to control if the scale items related to the fear of pain after surgery, to express their opinions about each item, to check the items according to grammar and word suitability, cultural compatibility, clear to understand and a need for another item to add.

The third step included the psychometric tests. In this step, psychometric tests of the 10 items were conducted to evaluate the validation of POPFS.

Statistical Analysis

In the analysis of data, the package program IBM Corp. Released 2010 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used. To evaluate the data distribution, Shapiro-Wilk test was used. Arithmetic mean ± standard deviation, median (minimum-maximum) values were given as descriptive statistics. Correlation between nonparametric variables was determined by the correlation coefficient of Spearman rho.

To measure the sampling adequacy, Kaiser-Meyer-Olkin (KMO) index and Bartlett's test of sphericity was used. Principal component analysis (PCA) was assessed for the construct validity and confirmatory factor analysis (CFA) was used to confirm the factor structure. For confirmatory factor analysis, LISREL 9.30 for Windows (Scientific Software International, Inc.) was used. In calculating the scope validity ratio, the content validity ratio (CVR) formula that was developed by Lawshe (20) to calculate how much each item was required was used. The internal consistency (reliability) of the scale was evaluated by Cronbach's alpha calculation. $p<0.05$ was considered statistically significant and all hypotheses were tested in two directions.

Results

Sample Characteristics

The mean age of the patients was 54.13±16.36 years and 51.3% of them were male. The percentage of the patients that will have a surgery at general surgery clinic was 45.3% and 72% of them had previous surgery history. The demographic characteristics are shown in Table 1.

Cross-Validation of FPQ-III

The cross-validation of this scale was completed with the 150 patients waiting for surgery analyzed using the Cronbach's alpha reliability coefficient, KMO measure of sampling adequacy and Bartlett's test of sphericity. As a result of the analysis, the total variance was found as 58.21% for three-factor structure. According to the explanatory factor analysis (EFA) test, the KMO sampling adequacy was found as 0.918 and Bartlett's test score was found as χ^2=3210,226, $p<0.001$. The factor loads of the scale ranged between 0.352-0.876. The Cronbach's alpha coefficient for the total scale was 0.955, and 0.938 for the severe pain fear subscale, 0.895 for the minor pain fear subscale, 0.889 for the medical pain fear subscale.

Table 1. Demographic characteristics of the patients (N=150)

Demographic Characteristics	Value
Age (years) (mean ± SD)	54.13±16.36
Gender (n)	
Female Male	73 (48.7%) 77 (51.3%)
Clinic (n)	
General surgery Orthopedics and traumatology Neurosurgery Heart and vessel surgery	68 (45.3%) 25 (16.7%) 32 (21.3%) 25 (16.7%)
Previous surgery history (n)	
Yes No	108 (72%) 42 (28%)
SD: Standard deviation	

Table 2. Responses of the experts

POPFS Items	Appropriate	Quite appropriate	Inappropriate	Total expert number
POPFS Item 1	10	0	0	10
POPFS Item 2	10	0	0	10
POPFS Item 3	10	0	0	10
POPFS Item 4	10	0	0	10
POPFS Item 5	10	0	0	10
POPFS Item 6	9	1	0	10
POPFS Item 7	10	0	0	10
POPFS Item 8	8	2	0	10
POPFS Item 9	8	2	0	10
POPFS Item 10	10	0	0	10

The responses of the experts according to the suitability of the items. POPFS: Postoperative pain fear scale

Scale Development

Draft scale form with 13 items was formed by gathering the determined items according to the expressions. Selected items were about fear of pain-related to wound, sutures, walking, breathing, coughing, feeding, intestinal gas, the tubes and drains. Numeric rating was used from 0-2 (0=no fear, 1=a little fear, 2=so much fear) for each item. According to the experts' comments and opinions, two items about pain while walking and getting out of bed were picked up in one item; two items about pain-related with tubes in the stomach and urinary were picked up in one item; two items about pain-related sutures and

wound were picked up in one item and the numeric rating was changed from 0-2 to 0-10 and all items were scored ranging from 0 (not at all afraid) to 10 (very afraid) on a Numeric Rating scale. After completing these changes, opinions of experts were derived from to the same experts for the second time to conduct the content validity. The responses of the experts are shown in Table 2.

Before the content validity, the relationship between the scale items and the total scale was evaluated. The correlation coefficients between the POPFS total score and all the items that make up the scale ranged from 0.691 to 0.869 and were strongly and statistically significant (Table 3).

After the responses of the experts, content validity ratios (CVR) were calculated for each item and as a result, the items of the scale were 10. The content validity index (CVI) value was calculated as well and as a result, for each item it was found as +1.00.

Validity

To evaluate the construct validity of the scale, the KMO test was used to analyze the sample size sufficiency for the EFA. The calculated KMO for 150 samples was found as 0.88. Bartlett's test was applied for the suitability of the data set and it was found significant (χ^2= 807.616, p<0.000). A PCA was used and as a result, the significant factor loadings of each item were found 0.45 or greater. Table 4 showed the communalities from the single factor analysis.

Table 3. Scale total score and correlation between scale items

	Total scale score	
POPFS Items	**r**	**p**
POPFS Item 1. I am afraid that I will have pain due to wounds and sutures in the operation area.	0.790	0.000
POPFS Item 2. I am afraid that I will have pain during dressing change.	0.725	0.000
POPFS Item 3. I am afraid that I will have pain when getting out of bed and walking.	0.691	0.000
POPFS Item 4. I am afraid that I will have pain while breathing deeply.	0.755	0.000
POPFS Item 5. I am afraid that I will have pain when coughing.	0.869	0.000
POPFS Item 6. I am afraid that I will have pain while eating meal.	0.722	0.000
POPFS Item 7. I am afraid that I will have pain due to a stomach/urinary catheter.	0.755	0.000
POPFS Item 8. I am afraid that I will have pain due to drains/inserted tubes.	0.809	0.000
POPFS Item 9. I am afraid that I will have pain due to gas in my intestines.	0.694	0.000
POPFS Item 10. I am afraid that I will have pain during medication (intramuscular, intravenous).	0.700	0.000

The Spearman rho test. The correlation coefficients between the total score and all the items were found to be strongly and statistically significant. POPFS: Postoperative pain fear scale

Table 4. Communalities from the single factor analysis

Communalities		
POPFS Items	**Initial**	**Extraction**
POPFS Item 1	1.000	0.608
POPFS Item 2	1.000	0.555
POPFS Item 3	1.000	0.471
POPFS Item 4	1.000	0.582
POPFS Item 5	1.000	0.730
POPFS Item 6	1.000	0.522
POPFS Item 7	1.000	0.508
POPFS Item 8	1.000	0.626
POPFS Item 9	1.000	0.450
POPFS Item 10	1.000	0.477

Extraction Method: Principal Component Analysis. The significant factor loadings of each item were found 0.45 or greater. POPFS: Postoperative pain fear scale

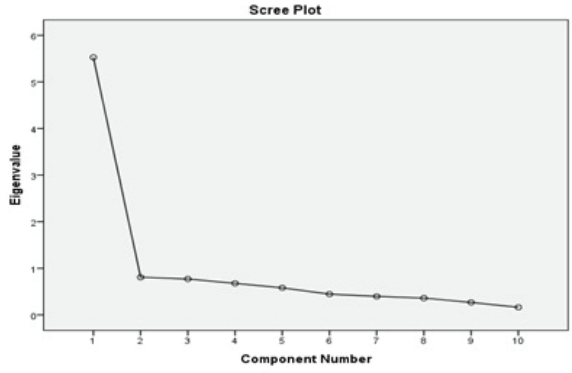

Figure 1. The scree plot (exploratory factor analysis)

After evaluating the sample size sufficiency, EFA was conducted to evaluate the construct validity. The total variance explained 55.5% of the variance for one factor. The one-factor structure was also confirmed by the scree plot (Figure 1).

After the EFA was performed, CFA was used to check if the model defined by EFA was working in a new sample. According to the model fit indexes, the chi-square/df ratio (3.15), and goodness of fit indexes [Root Mean-Square Error of Approximation (RMSEA)=0.120, Standardized Root Mean-Square Residual=0.050, Comparative Fit index (CFI)=0.907] showed that the model of the scale was fit with the data and POPFS was able to be used (Figure 2 and Table 5).

The structural elements (standardized factor loads, t-values, and R2) of the model were examined to fit the indexes. The R2 values ranged between 0.40% and 0.73% indicating that the variability in the scale model was mostly explained by POPFS item 5, while the rarely explained POPFS item 10 (Table 6). The mean score of the

Table 5. The goodness of fit statistics of the model*

Statistics	Value	Recommended value	Fitness
X^2/df	3.15	2≤ X^2/df ≤3	Marginal fit
RMSEA	0.120	0.05≤ RMSEA ≤0.08	Marginal fit
CI	0.09-0.14	close to RMSEA	Marginal fit
SRMR	0.050	0.05≤ SRMR ≤0.10	Good fit
NFI	0.871	0.90≤ NFI ≤0.95	Marginal fit
NNFI	0.881	0.95≤ NNFI ≤0.97	Marginal fit
CFI	0.907	0.95≤ CFI ≤0.97	Marginal fit
GFI	0.884	0.90≤ GFI ≤0.95	Marginal fit
AGFI	0.817	0.85≤ AGFI ≤0.90	Marginal fit

*Measures of goodness of fit (Schermelleh-Engel, Moosbrugger and Müller, 2003), RMSEA: root mean-square error of approximation, CI: Confidence interval, SRMR: Standardized root mean-square residual, NFI: Normed fit index, NNFI: Non-normed fit index, CFI: Comparative fit index, GFI: Goodness of-fit index, AGFI: Adjusted goodness-of-fit index Confirmatory, Factor Analysis showed the model was fit and the scale was able to be used.

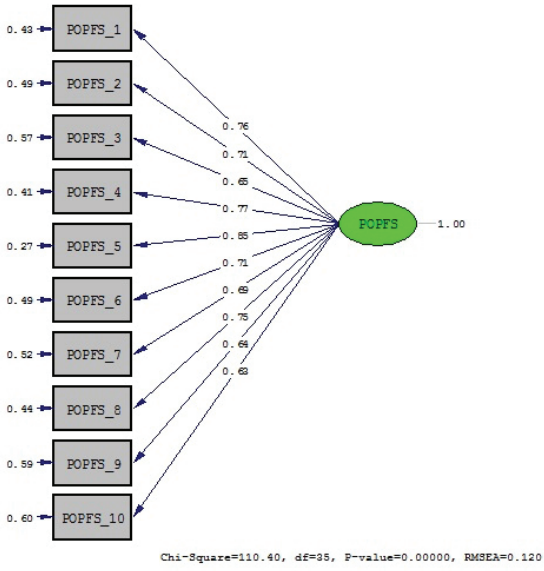

Figure 2. Path diagram of the postoperative pain fear scale
POPFS: Postoperative Pain Fear scale, RMSEA: Root Mean-Square Error of Approximation

items was 3.24±2.37 for total scale items (Table 7). To measure the reliability, internal consistency was concerned and Cronbach's alpha for the 10-item postoperative pain scale was found as 0.91.

Discussion

Surgical pain is unavoidable in surgery patients and fear is an expected response to pain. Healthcare professionals should be aware of patients' pain fear to reduce such fear and implement supportive strategies to improve the patient surgical care quality. In the literature, numerous studies reported that patients experience significant levels of anxiety and fear before undergoing surgical procedures

Table 6. Statistics on observed variables

POPFS Items	Standardized factor loads	t	R2
POPFS Item 1	0.76	10.63	0.58
POPFS Item 2	0.71	9.78	0.51
POPFS Item 3	0.65	8.73	0.43
POPFS Item 4	0.77	10.83	0.59
POPFS Item 5	0.85	12.73	0.73
POPFS Item 6	0.71	9.83	0.51
POPFS Item 7	0.69	9.43	0.48
POPFS Item 8	0.75	10.51	0.56
POPFS Item 9	0.64	8.54	0.41
POPFS Item 10	0.63	8.40	0.40

R2= squared multiple correlations. Standardized factor loads, t-values, and R2 values of the final model items obtained from confirmatory factor analysis. POPFS: Postoperative pain fear scale

Table 7. Descriptive statistics of POPFS

POPFS Items	Mean ± SD*	Median (Min-Max)
POPFS Item 1	3.82±3.15	4.00 (0.00-10.00)
POPFS Item 2	2.87±3.11	2.00 (0.00-10.00)
POPFS Item 3	2.95±2.84	2.50 (0.00-10.00)
POPFS Item 4	2.98±2.94	2.00 (0.00-10.00)
POPFS Item 5	4.00±3.57	3.50 (0.00-10.00)
POPFS Item 6	2.43±2.98	1.00 (0.00-10.00)
POPFS Item 7	3.58±3.51	3.00 (0.00-10.00)
POPFS Item 8	4.11±3.48	4.00 (0.00-10.00)
POPFS Item 9	3.03±3.14	2.00 (0.00-10.00)
POPFS Item 10	2.64±3.22	1.00 (0.00-10.00)
Total scale	3.24±2.37	3.30 (0.00-9.90)

*SD: Standart deviation descriptive statistics (mean ± SD, median, min, max), POPFS: Postoperative pain fear scale

(6,21,22). It was also reported that preoperative fear was associated with acute and long-term postoperative pain (4,23). Expecting fear was reported as one of the strong psychological factors that influence the postoperative pain (7,24). Although preoperative pain fear is so important and affects patients' postoperative pain levels, there is not a valid scale to evaluate the fear of postoperative pain.

According to the cross-validation results of the FPQ-III scale, the three factors accounted for 58.21% of the total variance and it was between the expected values of 40-60% (25). The KMO (expected to be >0.5) and Bartlett's test scores (expected to be p<0.05) showed that the sampling was adequate for the factor analysis (26). In the literature, the Cronbach's alpha coefficient value was reported to be >0.70 for the internal reliability of a scale (27,28), and in this scale, the total and subscale Cronbach's alpha values were over 0.80.

Additionally, the present study met the goal of developing the POPFS. In this scale, the correlation

coefficients between the POPFS total score and all the items were strongly and statistically significant (p<0.001). The existence of a relationship between the items of the scale was related to the validity of the scale. The scale items were to be understood and appropriate to the sample to be applied. These results showed the content validity of the scale.

For the analysis of sampling adequacy, the KMO index for 150 samples was found as 0.88 to be close to perfect for factor analysis. In the literature, it was reported to be >0.50 (26-29). Also for the suitability of the data set the Barlett's test of sphericity showed the sample was coming from a multivariate normal scatter and was found significant with values of (χ^2=807.616, p=0.000). In the literature, it was reported that Bartlett's sphericity test should be significant (p<0.05) (26). It is also important to test the range sufficiency and partial correlations whether they are small or large. These parameters showed the sampling was appropriate to conduct factor analysis (25,26,29).

Construct validity was proven using EFA and CFA. There are many studies that both factor analyses were conducted on the same data (30,31). In this study, according to the total variance explained, EFA was analyzed for one factor and examination of the screen plot also suggested the factor structure with one factor. The obtained model fit index values through the CFA, including RMSEA, Normed Fit index, Non-normed Fit index, CF, Goodness of-Fit index and Adjusted Goodness-of-Fit index, were close to the acceptable values. In the literature, it is reported that the model fit cutoff indexes are arbitrary and they are affected by many reasons such as estimation methods, sampling fluctuations, small sample size, etc (32,33). For this reason, although one or more fit index values are not highly acceptable, this model may fit the data. In this study, the sample included patients from four different surgical clinics and this may have affected the model fit index values.

The Cronbach's alpha coefficient value of the scale was 0.91 and it met the recommended value for the development of an instrument (27,28). In the literature, the Cronbach's alpha values of the developed Experience of Cognitive Intrusion of Pain scale was 0.94 and of the developed Daily Pain Catastrophizing scale was 0.892 for the total score (34,35). The Cronbach's alpha coefficient values of these pain scales resembled the present study.

With this sufficiently validated scale POPFS, evaluation of the fear of postoperative pain will be possible preoperatively. This instrument will provide health professionals particularly surgery nurses and guidance in knowing the predictors of postoperative pain to make decisions about optimal pain management techniques. With the evaluation of the pain fear levels of the surgical patients, it is intended to decrease the postoperative pain and anxiety by providing interventions on patient care and medical treatments.

Study Limitations

One of the limitations of the study is that the study data was collected from four different surgical clinics and this may differ the pain fear levels of the patients. So that, this may affect the model fit index values of this scale. The second limitation is that the scale was about the fear of postoperative pain and it is not possible to test-retest the fear preoperatively. The third limitation is that patients who had previous surgery were also included in the study. However, pain fear levels of patients may be affected by whether they are having surgery for the first time or have had a previous surgery history. Despite these limitations, this study gains a valid and reliable POPFS to the literature. With this scale, it will be able to evaluate the surgical patients' postoperative pain fear during the preoperative period.

Conclusion

The POPFS is a reliable and valid instrument to measure the fear level of postoperative pain. The scale is appropriate to be used in clinical settings to quickly evaluate the surgical patients' fear level of postoperative pain preoperatively. For future studies, we recommend researchers to use this scale for larger samples and specific surgical patient groups. Further external validation studies are needed to fully appreciate the generalizability of this suggested scale.

Authorship Contributions

Concept: S.U., F.N.T., Design: S.U., F.N.T., Data Collection or Processing: S.U., Analysis or Interpretation: F.N.T., Literature Search: S.U., F.N.T., Writing: S.U., F.N.T.

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References

1. Strøm J, Bjerrum MB, Nielsen CV, et al. Anxiety and depression in spine surgery-a systematic integrative review. Spine J 2018;18:1272-85.
2. Alodaibi FA, Fritz JM, Thackeray A, Koppenhaver SL, Hebert JJ. The Fear Avoidance Model predicts short-term pain and disability following lumbar disc surgery. PLoS One 2018;13:e0193566.
3. Londhe SB, Shah RV, Patwardhan M, Doshi AP, Londhe SS, Subhedar K. Understanding the apprehension and concern haunting patients before a total knee arthroplasty. Arthroplasty 2021;3:1-5.

4. Theunissen M, Peters ML, Schouten EGW, et al. Validation of the surgical fear questionnaire in adult patients waiting for elective surgery. PLoS One 2016;11:e0162737.
5. Burton D, King A, Bartley J, Petrie KJ, Broadbent E. The surgical anxiety questionnaire (SAQ): development and validation. Psychol Health 2019;34:129-46.
6. Cheng JYJ, Wong BWZ, Chin YH, et al. Preoperative concerns of patients undergoing general surgery. Patient Educ Couns 2021;104:1467-73.
7. Wang Y, Liu Z, Chen S, et al. Pre-surgery beliefs about pain and surgery as predictors of acute and chronic post-surgical pain: A prospective cohort study. Int J Surg 2018;52:50-5.
8. Angelini E, Wijk H, Brisby H, Baranto A. Patients' Experiences of Pain Have an Impact on Their Pain Management Attitudes and Strategies. Pain Manag Nurs 2018;19:464-73.
9. Turk DC, Wilson HD. Fear of pain as a prognostic factor in chronic pain: conceptual models, assessment, and treatment implications. Curr Pain Headache Rep 2010;14:88-95.
10. Yüksel A, Çetinkaya F, Karakoyun A. The effect of mindfulness-based therapy on psychiatric symptoms, psychological well-being, and pain beliefs in patients with lumbar disk herniation. Perspect Psychiatr Care 2021;57:335-42.
11. Lewis GN, Rice DA, McNair PJ, Kluger M. Predictors of persistent pain after total knee arthroplasty: a systematic review and meta-analysis. Br J Anaesth 2015;114:551-61.
12. Rajput K, Vadivelu N. Acute Pain Management of Chronic Pain Patients in Ambulatory Surgery Centers. Curr Pain Headache Rep 2021;25:1.
13. McNeil DW, Rainwater AJ 3rd. Development of the Fear of Pain Questionnaire–III. J Behav Med 1998;21:389-410.
14. Simons LE, Sieberg CB, Carpino E, Logan D, Berde C. The Fear of Pain Questionnaire (FOPQ): assessment of pain-related fear among children and adolescents with chronic pain. J Pain 2011;12:677-86.
15. Sullivan MJL, Bishop SR, Pivik J. The pain catastrophizing scale: development and validation. Psychol Assess 1995;7:524-32.
16. Esin N. Data collection methods and tools and reliability and validity of data collection tools. In. Erdoğan S, Nahcivan N, Esin N, editors. Nursing research process, application and critic. 3th ed. İstanbul: Nobel Tıp Kitabevleri; 2015. p. 193-232.
17. Bağdigen M, Karaman Özlü Z. Validation of the Turkish Version of the Surgical Fear Questionnaire. J Perianesth Nurs 2018;33:708-14.
18. Ünver S, Turan FN. Ağrı korkusu ölçeği-III'ün Türkçe geçerlilik ve güvenilirlik çalışması [Turkish validity and reliability study of fear of pain questionnaire-III]. Agri 2018;30:18-27.
19. Altun ÖŞ, Özlü ZK, Kaya M, Olcun M. Does the fear of surgery prevent patients from sleeping? J Anatol Nurs Health Sci 2017;20:260-6.
20. Lawshe CH. A quantitative approach to content validity. Pers Psychol 1975;28:563-75.
21. Masjedi M, Ghorbani M, Managheb I et al. Evaluation of anxiety and fear about anesthesia in adults undergoing surgery under general anesthesia. Acta Anaesthesiol Belg 2017;68:25-9.
22. Mulugeta H, Ayana M, Sintayehu M, Dessie G, Zewdu T. Preoperative anxiety and associated factors among adult surgical patients in Debre Markos and Felege Hiwot referral hospitals, Northwest Ethiopia. BMC Anesthesiol 2018;18:155.
23. Lin D, Huang X, Wang J, Hao Y, Geng M, Wei C. Effect of preoperative anxiety, depression, and insomnia on acute postoperative pain after non-cardiac surgery. Res Sq April 20 2021. Inpress. https://doi.org/10.21203/rs.3.rs-429163/v1
24. Jayawardane M, Gankanda W, Gunathilake M. Prevalence of pre-operative anxiety and associated factors among a group of women undergoing gynaecological surgeries at a single unit in a tertiary care hospital in Sri Lanka. F1000Res 2021;10:74.
25. Cokluk O, Sekercioğlu G, Büyüköztürk Ş. Exploratory factor analysis. In: Multivariate statistics for Social Sciences SPSS and Lisrel applications. 5th ed. Ankara: Pegem Yayınevi; 2018. p. 190-240.
26. Field A. Exploratory factor analysis. In: Discovering statistics using SPSS. 5th ed. London: Sage Publications Ltd; 2018. p. 1922-2065.
27. Almanasreh E, Moles R, Chen TF. Evaluation of methods used for estimating content validity. Res Social Adm Pharm 2019;15:214-21.
28. Howard MC. A review of exploratory factor analysis decisions and overview of current practices: What we are doing and how can we improve? Int J Hum-Comput Int 2016;32:51-62.
29. Ekşi H, Kardaş S. Spiritual well-being: Scale development and validation. Spirit Psychol Couns 2017;2:73-88.
30. Saray Kilic H, Tastan S. Development of post hip replacement comfort scale. Appl Nurs Res 2017;38:169-174.
31. Ozturk M, Ersanli E. Development of philanthropy scale. Sci Educ 2018;1:22-33.
32. Hu L, Bentler PM. Fit indices in covariance structure analysis: Sensitivity to underparameterized model misspecification. Psychol Methods 1998;3:424.
33. Schermelleh-Engel K, Moosbrugger H, Müller H. Evaluating the fit of structural equation models: Tests of significance and descriptive goodness-of-fit measures. Methods Psychol Res Online 2003;8:23-74.
34. Attridge N, Crombez G, Van Ryckeghem D, Keogh E, Eccleston C. The Experience of Cognitive Intrusion of Pain: scale development and validation. Pain 2015;156:1978-90.
35. Darnall BD, Sturgeon JA, Cook KF, et al. Development and Validation of a Daily Pain Catastrophizing Scale. J Pain 2017;18:1139-49.

Original Article

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Evaluation of Fibrosis and Histopathological Changes in the Psoas Muscle with E- Cadherin, Claudin-5 Expression and Demographic Data: An Autopsy Study

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Abstract

Aim: It is known that muscle mass decreases with age. Sometimes it may be possible to adversely affect this reduction. It was aimed to investigate the contribution of claudin-5 and E-cadherin to this process.

Methods: Samples were taken from the psoas muscle of 55 cases autopsied for different reasons between 2018-2019. Age, gender, weight, height, chronic disease, and addiction were recorded. Histopathological degeneration parameters were evaluated. In addition, samples were prepared for immunohistochemical study. Evaluation for E-cadherin and claudin-5; no staining, weak staining, moderate staining and severe staining. Fibrosis was evaluated with Masson trichrome.

Results: There was a very strong and statistically significant inverse relationship between acidophilic sarcoplasma and staining with claudin-5 ($p<0.01$). There was also a strong positive correlation between cellular fatty cell degeneration status and staining with claudin-5 ($p=0.04$). No correlation could be established between sociodemographic characteristics of the cases and staining with claudin-5 and E-cadherin, nor could there be a correlation between staining with E-cadherin and staining with claudin-5 ($p>0.05$).

Conclusion: Claudin-5 can be a target protein that can be used in the detection and prophylaxis of degeneration and atrophy in striated muscles.

Keywords: Claudin-5, cadherins, socioeconomic factors, muscle, striated

Introduction

Muscle mass in humans is observed at maximum values at the ages of 25-30 and decreases by about 25-30% by age 65. This decrease in muscle mass is accompanied by an increase in non-contractile structures such as adipose tissue and connective tissue. In addition to the causes of age-related decrease in muscle mass, nutrition, sedentary lifestyle and chronic diseases can also affect this situation. The opposite situation may also have a protective effect (1).

The intersection complex consists of two main components [tigh junctions (TJ) and adherent junctions]. TJs are important in the epithelium in terms of barrier function and polarity. They are also involved in tissue differentiation and homeostasis (2). There is evidence that these functions are compromised, especially in older organisms, but these mechanisms have not been fully elucidated (3).

Claudins are the main transmembrane proteins of TJ. So far, 24 members of different species belonging to the claudin family have been identified (2,4,5). Claudin proteins appear to be important for TJ formation and play an important role in controlling the paracellular permeability of ions. Indeed, Claudin gene expression is sufficient to induce the formation of TJ fibrils in fibroblast cells (6).

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Cadherins constitute the most basic component of adhesive junctions. Cadherins are associated with the intercellular connection and the cytoskeletal system with the help of the catenin family (7). It has been observed that different expression patterns and dynamic changes of cadherins during development affect the function of cadherin in various morphogenetic events (8).

In this study, it was aimed to reveal the expression differences of claudin-5 and E-cadherin which are components of the junction complex, in the psoas muscle and the relation of these differences with the histopathological changes in the muscle with aging, fibrosis, adipose tissue as well as demographic data.

Methods

Study Design

This study was ethically approved by the Ordu University clinical research Ethics Committee (217/2020). In addition, the material in the study was autopsy samples and necessary legal and official permissions were obtained.

In this study, samples were taken from the psoas muscle of 55 cases autopsied for different reasons between 2018 and 2019. Simultaneous excel record files were created by selecting appropriate autopsies between 2018-2019. Age, gender, weight, height, chronic disease, and addiction were recorded. Histopathological fatty cell degeneration, fibrosis, acidophilic sarcoplasma, streaking, vacuolization, disruption in fibers, fragmentation, cellular infiltration, lipofuscin pigment accumulation indicating tissue degeneration and injury were evaluated (8-10).

Paraffin blocks were prepared using the manual microarray method from existing samples. Five µm thick sections were taken from these muscle samples. Sections were stained with H&E stain. The preparations were evaluated under light microscopy (Nikon eclipse Ni-U Tokyo/Japan) at different magnifications. Histopathological evaluation was graded as 1/0 for each parameter [Figure 1. (A-E)] (8-10).

In addition, the scores of these parameters were summed up and the degeneration score was obtained. The average score was obtained according to age groups. The score belonging to the degeneration was evaluated as 1/0. The score for claudin-5 and E-cadherin were evaluated as semiquantitatively (11,12).

Immunohistochemical Study

Samples were taken from the tissues and then sections with a thickness of 5 µ were taken on the poly- laminated slide. Prepared for immunohistochemical (IHC) study. A Leica Bond-Max IHC staining device (Vision Biosystems, Melbourne, Australia) was used for the IHC study. It was stained with claudin-5 (Epitomics (AC-0212A), 0.1 mL (1: 100). Slides were evaluated with a light microscope. Cytoplasmic membrane staining was considered positive.

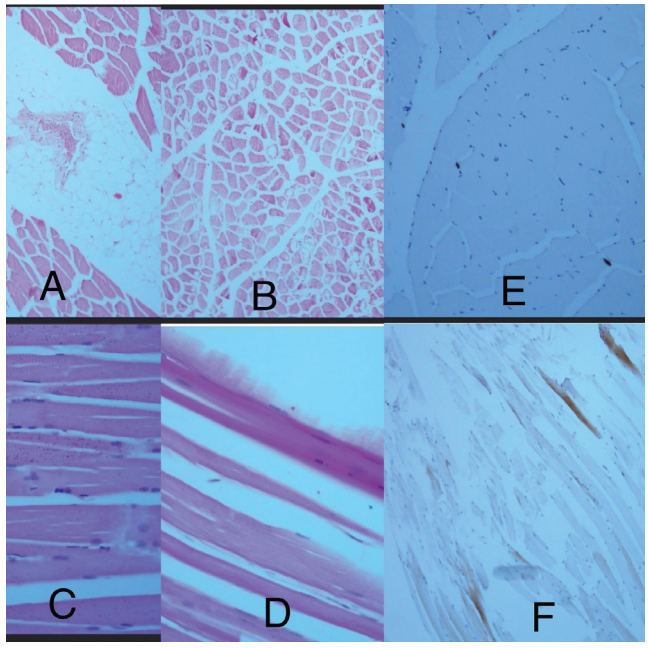

Figure 1. (A-E): A- Fat cell accumulation in muscle tissue (fatty degeneration) (H&EX100), B- Vacuolization and disruption, fragmentation in muscle tissue (H&EX40), C- Deposition of lipofuscin pigment in muscle tissue (H&EX400), D- Acidophilic-like changes in muscle tissue (H&EX400), E-claudin-5 expression in muscle tissue (grade 1) (x100), F-claudin-5 expression in muscle tissue (grade 2) (x100)

Four grades were evaluated. 0 staining (no staining), 1+ (weak) (1% to 10%) staining, 2+ (moderate) staining (11% to 50%), 3+ (severe) staining (over 50%) were evaluated (11,12).

E-cadherin [mouse monoclonal antihuman antibody, Biogenex (10 microliters diluted per 1 mL)] was applied. The stained slides were examined with a Nikon Eclipse Niu microscope and photographs were taken. Grading of IHC slides was done semiquantitatively.

Evaluation for E-cadherin; 0 grading (no staining), 1+ (weak) (1% to 10% staining), 2+ (moderate) (11% to 50% staining), 3+ (severe) (over 50% staining) were evaluated (11,12).

Histochemical Study

Masson's Trichrome Stain kit was used to evaluate fibrosis. The presence of fibrosis was evaluated.

Statistical Analysis

The data were collected and analyzed using Statistical Package for software. In addition to descriptive statistics, the chi-square test was used in classifying categorical data. Spearman Correlation analysis was performed to investigate the relationship between staining and sociodemographic data and histopathology findings. In addition, the quality of the relationship was revealed by performing multiple regression analyses. In all types of analyzes, a 5.0% significance level was used.

Table 1. Distribution of sociodemographic data according to BMI index					
	BMI <18.5	**18.6 < BMI <24.9**	**25.0 < BMI <29.9**	**30.0 < BMI <39.9**	**p**
	n (%)	**n (%)**	**n (%)**	**n (%)**	
Gender					p=0.46
Female	-	1 (%1.81)	3 (5.45)	2 (3.63)	-
Male	4 (7.27)	19 (34.54)	19 (34.54)	7 (12.72)	-
Age					p=0.86
35-65	2 (3.63)	12 (21.81)	16 (29.09)	7 (12.72)	-
66-85	2 (3.63)	7 (12.72)	5 (9.09)	2 (3.63)	-
85 and above	-	1 (1.81%)	1 (1.81%)	-	-
Alcohol					p=0.62
Using	3 (5.45)	3 (5.45)	4 (7.27)	2 (3.63)	-
Not using	1 (1.81)	17 (30.90)	18 (32.72)	7 (12.72)	-
Cigarette					p=0.16
Using	4 (7.27)	15 (27.27)	13 (23.63)	4 (7.27)	-
Not using	-	5 (9.09)	9 (16.36)	5 (9.09)	-
Disease state					p=0.67
No known disease	1 (1.81)	7 (12.72)	8 (14.54)	2 (3.63)	-
CAD	3 (5.45)	10 (18.18)	10 (18.18)	6 (10.90)	
CAD and COPD	-	1 (1.81)	1 (1.81)	-	-
Psychiatric disease	-	-	3 (5.45)	-	-

CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, BMI: Body mass index

Table 2. Results of multiple regression analysis of factors associated with claudin staining						
	β	**S.E**	**p**	**OR**	**95% CI**	
					Lower	**Upper**
Y	-1.35	0.66	0.04	0.25	0.07	0.94
A	2.04	0.72	p<0.01	7.73	1.87	31.87

Y: Fatty cell degeneration, A: Acidophilic sarcoplasma, OR: Odds ratio, CI: confidence interval. An inverse relationship was observed between claudin-5 and adipose tissue that increased with age

Results

89.1% (n=49) of 55 patients were male and 10.9% (n=6) were female. The mean age of the cases was 60.67±13.38 and the youngest case was 35 and the oldest case was 90 years old. The sociodemographic data obtained from the cases according to their body mass index (BMI) are presented in Table 1.

As a result of staining the muscle samples of the cases with E-cadherin, 92.7% (n=51) of the samples were not stained, but only 7.3% (n=4) were stained. Staining the psoas muscle samples of 55 cases with claudin-5, 29.1% (n=16) were not stained, and 70.9% (n=39) were stained. Five (9.1%) of the stained samples were strongly stained with claudin-5.

No correlation could be established between sociodemographic characteristics of the cases and staining with claudin-5 and E-cadherin, nor could there be a correlation between staining with E-cadherin and staining with claudin-5 (p>0.05). In addition, there was no correlation between E-cadherin staining and histopathological features (p>0.05).

In the correlation analysis made to evaluate the relationship between histopathological findings (Graph 1) and staining (Graph 2); It was observed that there was a very strong and statistically significant inverse relationship between acidophilic sarcoplasma and staining with claudin-5 (r=-0.41, p<0.01). There was also a strong positive correlation between cellular fatty cell degeneration status and staining with claudin-5 (r=0.28, p<0.05) (Graph 2); Staining with claudin-5 in non-fatty cells is statistically significant 0.25 times higher. In addition, staining with claudin-5 in cells with acidophilic sarcoplasma is statistically significantly higher than 7.73 times. The relationship between histopathological findings and age groups was demonstrated by Graph 1, and when age groups were grouped as 35-45, 46-55, 56-65, 66 and above, it was observed that degeneration increased as age progressed. The same relationship with claudin-5 is shown in Table 2,3.

Discussion

There are many publications stating that myofibrillary decline starts around the age of 25 and the rates of this

Table 3. Relationship between age groups and degeneration score, claudin score, chronic disease							
Age		**35-45**	**46-55**	**56-65**	**66-90**	**65 age below**	**66 age above**
Degeneration score	mean	3.5	3.57	3.52	4	3.56	4
Claudin score		0.8	1	0.75	0.77	0.81	0.77
Chronic disease		0.2	0.57	0.85	0.94	0.62	0.94
When the youngest age and the oldest age are compared, an inverse relationship is observed between claudin-5 and degeneration							

decline increase with age. In some of these publications, it has been noted that the decrease between the ages of 30-50 is approximately 15% and after this age it is 30% every ten years. In addition, there are determining factors such as changes in the nervous system with age, changes in the structure and function of the neuromuscular junction, fat infiltration, cellular and molecular modifications of the muscle. Again, lipofuscin (age-related pigment) and fat deposit into the muscle and promote muscle tissue loss. This ensures less tone and ability to contract (9,10). Although adult skeletal muscle is fully differentiated, fibers retain the ability to regenerate and replace it in response to an injury. However, in pathological conditions or during the aging process, functional muscle damage may result in the formation of fibrotic tissue regeneration (9,10).

One of the parameters evaluated in this study was the association of psoas muscle fibrosis with age and chronic diseases. In this evaluation, age was evaluated individually as well as in age groups. They had chronic cardiovascular disease, allergy and psychiatric diseases in terms of chronic diseases. The other two diseases were excluded from the group and their relationship with the more common chronic cardiovascular disease was examined. It was observed that this disease increased with age, and fibrosis increased with age but it was not statistically significant. This is probably because the sample is small.

Atrophy and degeneration often occur for different reasons. They may occur due to mechanical reasons or immobility. Degeneration can also occur by many factors such as inflammation, abnormal mechanical strength, and altered vascularization (13).

In animal models of muscle injuries, atrophy characterized by fat accumulation has been found to be effective in early and middle-stage diseases. It has also been shown that high levels of inflammation exhibit an active cycle of degeneration and regeneration. In this study by Gibbons et al. (10) muscle loss was so severe that in the vast majority of samples, muscle tissue was reported to be replaced by an irregular, vascular connective tissue network with high macrophage density. It has been stated that in clinical imaging of such tissues, this appearance resembles a muscle, which may prevent the detection of true loss (10).

Although there is no muscle injury known in this study, parameters such as BMI as well as age and chronic diseases are associated with degeneration and atrophy.

In this study, it was observed that fatty degeneration, histopathological signs of degeneration and chronic diseases tended to increase with age, and this trend was accompanied by loss of claudin-5 (Table 3).

Inflammation is prominent in an increasing number of disease states where changes in TJ have been observed. Changes in TJs has been associated with inflammatory diseases associated with many autoimmune mechanisms such as collagenous colitis, psoriasis, multiple sclerosis/encephalomyelitis, Crohn's disease, ulcerative colitis and arthritis. In addition, proinflammatory cytokines have also been shown to induce apoptosis at the cell level and affect permeability by affecting the structure of TJs (13).

In a study on changes in claudin expression profiles, it was reported that it contributed to epithelial lung dysfunction during infection and inflammation (14).

It has been reported in the literature that the change in claudin levels contributes to the disruption of the blood barrier (15,16). In several diseases related to barrier dysfunction, various pathologies of the nervous system characterized by a pronounced neuroinflammatory component such as Alzheimer's disease, multiple sclerosis, diabetic retinopathy and retinopathy of prematurity, the dysfunction of the brain and retinal barriers contributes to the pathogenesis of these diseases and even to the pathogenesis of these diseases (17).

Recent reports have also shown that claudin-5 is reduced in retinal endothelial cells due to endoplasmic reticulum stress, which also plays a role in vascular dysfunction in diabetic retinopathy (17).

In this study, a statistical relationship was not found when evaluated in terms of chronic diseases. When it is divided into age groups and evaluated, it has been observed that it tends to decrease (muscle mass). Since the youngest adult case was 35 years old, no comparison was made with muscle tissue under 35 years old. An evaluation was made with claudin-5 in the form of muscle expression, and the blood level was not measured. However, when the degeneration score was compared with the claudin score, an inverse relationship was observed. No relationship was found between E-cadherin and claudin-5. The reason for this is that E-cadherin is mostly in epithelial localization and claudin-5 is in endothelial localization, and therefore, it can be difficult to show this relationship in the striated muscle. This situation constitutes the limitation of our study.

In previous references, the increase in fat tissue and fibrosis in muscle tissue with age is indirectly indicative of atrophy. It can also indicate degeneration (13,14,17). In this study, an inverse relationship was observed between the increase in adipose tissue and claudin-5, and it was observed that the increase in adipose tissue increased with age. Based on this result, we can say that atrophy and degeneration occur with age, and as a result, claudin expression decreases. Although a positive correlation was observed between acidophilic sarcoplasma and claudin-5, it was seen that this parameter showed a homogeneous distribution when the age distribution was examined. When the degrees of degeneration including acidophilic sarcoplasma were scored, we observed that claudin expression decreased with age and the score of the degeneration increased.

In a study by Ozawa (18), it was shown that expression of the DsRed-labeled E cytoplasmic domain in C2C12 myoblasts inhibits myoblast fusion as well as the transport of endogenous cadherins, including N-cadherin and M-cadherin (18).

Hollnagel et al. (19) in their study, analyzed the known components of adhesive plaques on Western blot of proteins from regenerated muscle to investigate alternative molecules that could mediate cell adhesion in the absence of M-cadherin. Interestingly, heterozygous and homozygous animals reported that they contained levels of N- and E-cadherin, both of which were regulated by a significant increase during degeneration (19).

In this study, since the expression of E-cadherin was very low, no relationship was found with age groups when evaluated in terms of the trend in expression of E-cadherin. Likewise, no relationship was found with histomorphological findings. As stated above, the very low expression level could possibly be the cause.

Study Limitations

Causes of death such as drowning or gas poisoning were not included in this study. Again, those with a color change on the corpse were not included in the study. The sampled muscles were chosen from areas that could be hidden in order not to disturb the integrity of the body. These reasons are the limitations of the study.

Conclusion

As a result, claudin-5 tends to decrease in striated muscle tissue in degeneration and atrophy. Therefore, it can be a target protein that can be used in the detection and prophylaxis of degeneration and atrophy in striated muscles. No relationship was found between E-cadherin and striated muscle degeneration.

Authorship Contributions

Concept: H.E., Design: H.E., H.Y.T., Y.S., Data Collection or Processing: H.E., H.Y.T., Analysis or Interpretation: H.E., H.Y.T., Y.S., Literature Search: H.E., Y.S., Writing: H.E., H.Y.T.

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References

1. Thompson LV. Skeletal muscle adaptations with age, inactivity, and therapeutic exercise. J Orthop Sports Phys Ther 2002;32:44-57.
2. González-Mariscal L, Betanzos A, Nava P, Jaramillo BE. Tight junction proteins. Prog Biophys Mol Biol 2003;81:1-44.
3. D'Souza T, Sherman-Baust CA, Poosala S, Mullin JM, Morin PJ. Age-related changes of claudin expression in mouse liver, kidney, and pancreas. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences 2009:64:1146-53.
4. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2001;2:285-93.
5. Förster C. Tight junctions and the modulation of barrier function in disease. Histochem Cell Biol 2008;130:55-70.
6. Furuse M, Sasaki H, Fujimoto K, Tsukita S. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occluding in fibroblasts. J Cell Biol 1998;143:391-401.
7. Redfield A, Nieman MT, Knudsen KA. Cadherins promote skeletal muscle differentiation in three-dimensional cultures. J Cell Biol 1997;138:1323-31.
8. Hafez MS, Makhlouf NA. Histological changes in sarcopenia and the possible protective role of angiotensin-converting enzyme inhibitors in male albino rats. The Egyptian Journal of Histology 2011;34:762-77.
9. Ríos IDP. Loss of Muscle Mass Induced by Aging. Rev Cienc Salud Bogotá Colombia 2019;17:223-4.
10. Gibbons MC, Singh A, Anakwenze O, et al. Histological Evidence of Muscle Degeneration in Advanced Human Rotator Cuff Disease. J Bone Joint Surg Am 2017;99:190-9.
11. Erdem H, Canakcı E, Karatas A, Akcay Celik M, Kilinc A. Evaluation of Distant Organ Effect of Renal Ischemia and Reperfusion with Claudin-5. J Crit Intensive Care 2020;11:3-7.
12. Sahin A, Erdem H, Akçay Çelik M, Cankaya S, Aslan Ali. Correlation of E-cadherin/Beta-Catenin Expression with Localization in Squamous Epithelial Cell Carcinoma and Basal Cell Carcinoma. Online Turkish Journal of Health Sciences 2020;5:464-73.
13. Skrovanek S, Valenzano MC, Mullin JM. Restriction of sulfur-containing amino acids alters claudin composition and improves tight junction barrier function. Am J Physiol Regul Integr Comp Physiol 2007;293:R1046-55.
14. Van Itallie CM, Anderson JM. The role of claudins in determining paracellular charge selectivity. Proc Am Thorac Soc 2004;1:38-41.

15. Huang M, Chen SP, Dai YY, et al. [Cathepsin L mediates glomerular endothelial cell injury by cleavaging complement C3 in trichloroethylene-sensitized mice]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2021;39:5-11.

16. Zhang S, Wang X, Cheng F, et al. Network Pharmacology-Based Approach to Revealing Biological Mechanisms of Qingkailing Injection against IschemicStroke: Focusing on Blood-Brain Barrier. Evid Based Complement Alternat Med 2020;2020:2914579.

17. Gonçalves A, Ambrósio AF, Fernandes R. Regulation of claudins in blood-tissue barriers under physiological and pathological states. Tissue Barriers 2013;1:e24782.

18. Ozawa M. E-cadherin cytoplasmic domain inhibits cell surface localization of endogenous cadherins and fusion of C2C12 myoblasts. Biol Open 2015;4:1427-35.

19. Hollnagel A, Grund C, Franke WW, Arnold HH. The cell adhesion molecule M-cadherin is not essential for muscle development and regeneration. Mol Cell Biol 2002;22:4760-70.

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Emergency Physicians' Knowledge and Attitudes Towards Childhood Traumatic Tooth Avulsion in Turkey: A Multicenter Questionnaire-Based Cross-Sectional Study

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Abstract

Aim: In children presenting to the emergency room with traumatic tooth avulsion (TTA), the immediate replantation of the avulsed permanent tooth is required. We study aimed to investigate the knowledge and attitude of emergency physicians (ER) regarding emergency management of avulsed teeth

Methods: This multi-center cross-sectional survey study was conducted from October to December 2020. A self-administered questionnaire that had been designed in the Internet environment was sent to directors of ER facilities in hospitals regarding physicians at the emergency room in the hospitals. A total of 381 physicians participated by filling out the questionnaire.

Results: Data revealed that 92.1 % of the participants did not find prior knowledge sufficient about avulsion and only 8.9% would replant the tooth by themselves. 56.2% of the physicians did not know the importance of tetanus prophylaxis. Only 48% of the participants selected the best transport media for an avulsed tooth. Experienced physicians and the emergency specialist had significantly better knowledge about the management of avulsed teeth ($p=0.005$ and $p<0.001$, respectively).

Conclusion: The knowledge of avulsed teeth among emergency physicians in Turkey ranges from low to moderate, which highlights the need to improve the knowledge of the management of traumatic dental injuries among emergency physicians.

Keywords: Surveys and questionnaires, tetanus, emergency service, tooth avulsion, specialization, general practitioners

Introduction

The prevalence of childhood traumatic dental injuries (TDIs) has been increasing in recent years, and studies in this field in literature estimate that TDIs will in time come to exceed tooth decay and periodontal disease in terms of prevalence (1). According to epidemiological studies, oral region traumas account for 5% of all body traumas across all age groups, and this rate increases to 18% in preschool children (2). Avulsion, as one form of TDI, is defined as the complete displacement of a tooth from its socket due to trauma (3). Avulsion injuries are more common in primary teeth than in permanent teeth, with previous studies reporting the prevalence of avulsion injuries to be 0.5-16% in permanent teeth and 7-21% in primary teeth (4). It has further been reported that 75% of avulsion traumas to primary tooth also cause damage to the developing permanent tooth (3). Avulsions occur more frequently during permanent dentition, and especially in those age 7-9 years, at the time when the anterior teeth are still erupting, due to the weakness of the periodontal tissue surrounding the teeth. While fighting and sports injuries are frequently involved in the etiology of avulsion injuries in permanent teeth, avulsions involving the primary teeth mainly result from falls on hard surfaces (5). In general, only one tooth is affected in avulsion cases, and the most commonly avulsed teeth

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among both the primary and permanent teeth are the maxillary central incisors (6).

Today, even though considerable steps have been taken as a result of the investments made by the Ministry of Health into Oral and Dental Health, it may be difficult to directly access dental centers, due especially to socioeconomic reasons (7). In rural areas in particular, where the full integration of dental services with the social security institution is lacking, physicians inevitably perform emergency dental interventions in cases presenting to the emergency rooms (ER) of hospitals and primary healthcare centers with such injuries. A study conducted in Chile found the emergency room of hospitals to be the most common first stop for the treatment of TDIs, since dental services may not always be convenient or accessible (8). Although the literature contains publications examining the knowledge and attitudes toward such topics as the early detection of caries, fluoride applications and nutritional problems, due to their effects on oral and dental health in children, there is a lack of publications examining the knowledge of, and attitudes toward, tooth avulsion injuries that may be caused by TDIs among emergency physicians (9,10).

The present study evaluates the attitudes and knowledge levels of emergency physicians in Turkey related to traumatic tooth avulsion (TTA).

Methods

Study Design

Approval for the study was obtained from the Istanbul Medipol University Non-invasive Clinical Research Ethics Committee (decision no: 10840098-604.01.01-E65339, date: 18 December 2019). This multicenter cross-sectional questionnaire-based study was conducted between October and December 2020. A link to the questionnaire that had been designed in the Internet environment. was sent via a mailing database list to emergency physicians employed at the different hospitals (public, university or private hospitals) in Turkey. Emergency medicine specialists emergency medicine assistant doctors, and general practitioners working in hospitals that did not have a dental emergency service room were included in the study. All participants gave written informed consent. After filling out the questionnaire, the respondent sent it back to the database, and it was checked for any missing parts before being included in the evaluation. Incomplete questionnaires were returned to the relevant respondent with a request that they complete the missing parts.

Questionnaire Evaluation

The questionnaires included questions that had been used previously and tested for validity and reliability in similar studies published in the literature (11-13). The section part of the three-part questionnaire garnered data on the respondents' age, gender, specialization (if any), years of professional experience and the type of healthcare institution. The second section included questions about physicians' previous experience with TTA; prior knowledge on this subject; the source of prior knowledge, if any; and attitudes in the event of coming across such cases. The third and final section was aimed at establishing the knowledge level of emergency physicians as regards to TTA, for which questions were asked about the definition of an avulsed tooth, its prognosis, the time from avulsion of the tooth to admission to the healthcare institution for intervention, and the required storage conditions of the avulsed tooth during this period. Additionally, the knowledge of the respondents related to such issues as the importance of the tooth type (permanent or primary tooth) for replantation; the appropriate part of the tooth for handling; and the method of cleaning the tooth if contaminated, were examined. The knowledge level of the participants was based on their scores from the third section of the questionnaire, with 1 point given for each correct answer.

Statistical Analysis

All statistical analyses were made using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY). Descriptive analyses were presented using mean, standard deviation, median, frequency and percentage values, where appropriate. Shapiro-Wilk test was used to control whether the variables were normally distributed. Chi-square and Fisher's Exact test were used for categorical data. The student's t-test was used for the comparison of normally distributed parametric variables. Intergroup comparisons were performed using Kruskal-Wallis test and One-Way ANOVA. Bonferroni-Dunn test was used as a post-hoc test for significant cases while with post-hoc Tukey HSD test was used for parametric variables. The statistical significance level was taken as 0.05 in all tests

Results

The questionnaire was completed by a total of 381 emergency physicians. The distribution of physicians participating in the study by gender, age, institution status, professional experience and position in the hospital, garnered from the first section of the questionnaire, is presented in Table 1.

The responses of the physicians to the second part of the questionnaire, examining their attitudes toward traumatic tooth avulsion, are presented in Table 2. A vast majority (92.1%) of the respondents stated that they did not think they had adequate knowledge of TTA and the number of those stating that they had received knowledge

Table 1. Demographic data and knowledge level of the participants

Demographic data			Knowledge level		
		N(%)	Mean (SD)	Median (range)	p
Gender	Female	135 (35.6%)	4.49 (2.0)	5 (0-8)	0.280*
	Male	244 (64.4%)	4.26 (1.98)	4 (0-8)	-
Age	20-30	166 (43.6%)	4 (2.11)	4 (0-8)[a]	**0.004**†
	31-40	171 (44.9%)	4.44 (1.91)	5 (0-8)[ab]	-
	41-50	39 (10.2%)	5.33 (1.42)	5 (2-8)[b]	-
	> 50	5 (1.3%)	4.4 (1.82)	5 (2-6)[ab]	-
The status of the health institution	Public university hospital	40 (10.5%)	5.15 (1.53)	5 (1-8)	0.067
	Training and research hospital	230 (60.4%)	4.8 (2.49)	5 (1-7)	-
	Public hospital	97 (25.5%)	4.3 (2.01)	5 (0-8)	-
	Private hospital	9 (2.3%)	4.14 (2.07)	4 (0-8)	-
	Private university hospital	5 (1.3%)	3.67 (1.12)	4 (2-5)	-
Specialty	General practitioner	97 (25.5%)	3.84 (1.98)[a]	4 (0-8)	**<0.001**‡
	Emergency medicine assistant doctor	126 (33.1%)	4.18 (2.06)[a]	4 (0-8)	-
	Emergency medicine specialist	158 (41.4%)	4.91 (1.73)[b]	5 (1-8)	-
Professional experience	0-5	175 (45.9%)	3.98 (2.07)[a]	4 (0-8)	**0.005**†
	6-10	109 (28.6%)	4.41 (1.87)[a b]	4 (1-8)	-
	11-15	68 (17.8%)	4.82 (1.97)[b]	5 (0-8)	-
	16-20	19 (5%)	5.11 (1.49)[b]	5 (2-8)	-
	> 20	10 (2.6%)	5.2 (1.23)[b]	5.5 (3-7)	-

Student's t-test, † Kruskal-Wallis test, ‡ANOVA. Different lowercase letters in a column indicate the statistically significant difference between group

on TTA during vocational courses was quite low (n=14). Of the physicians, 91.1% stated that they needed more training in the management of tooth avulsion following trauma.

In a question on the medical treatment to be administered to patients presenting with TTA, the majority (67.7%) of respondents marked the correct answer, which is the use of antibiotics, anti-inflammatory and analgesic agents and 43.8% of the respondents stated that they would administer tetanus prophylaxis after TTA. The ratio of physicians who stated that they would refer the patient to another center for the necessary treatment was high (84.2%), while those who stated that they could do the intervention themselves accounted for 8.9% of the total. Regarding the referral center, 32.5% of the respondents stated that they would refer pediatric patients to a pediatric dentist.

The responses of the participants to the third section of the questionnaire, measuring the level of the knowledge related to TTA, are presented in Table 3. Although the majority (56.4%) of physicians correctly understood the definition of an avulsed tooth, a considerable number did not. Of the physicians who participated in the study, only 36.5% chose the response "If the avulsed tooth is a permanent tooth, it appropriate for replanting" regarding the effect of the type of avulsed tooth on treatment. The ratio of physicians who chose the correct responses to questions of how to clean an avulsed tooth after contamination, which is rinsing under running water, and the appropriate part of the tooth for handling during this procedure, which is holding from the crown, were 51.4% and 52%, respectively. Of the respondents, 82.7% expressed the importance of the elapsed time for the success of the treatment of the avulsed tooth, whereas the rate of those who knew the best time for replantation was 18.1%. Regarding the storage of an avulsed tooth when replantation is not immediately possible, 48.0% of the respondents gave the optimal answer, while 24.9% stated that the tooth could be carried in dry cotton or gauze and 4% in alcohol.

A comparison of the knowledge scores inquired in the third section of the questionnaire according to the demographic characteristics of the participants revealed no difference between genders (p=0.280) or institution types (p=0.067), while the knowledge scores were lower among those aged 20-30 in terms of the age factor (p=0.004) (Figure 1). Regarding professional experience, the participants with 0-5 years of professional experience

Table 2. Previous experience and attitudes of participants toward dental avulsion		
Questions	**Options**	**N (%)**
What is your medical approach to a patient presenting with avulse dental trauma?	If the patient has no special condition, I do not prescribe antibiotics, I prescribe anti-inflammatory and analgesic.	111 (29.1%)
	I prescribe antibiotics, anti-inflammatory, and analgesic	258 (67.7%)
	I add topical antibiotics	14 (3.7%)
	Tetanus prophylaxis should be administered	167 (43.8%)
Have you ever come across a patient with an avulsed tooth?	Never	164 (43%)
	1-5	164 (43%)
	6-10	26 (6.8%)
	> 10	27 (7.1%)
Do you have any prior knowledge about the management of avulsed teeth?	No	253 (66.4%)
	Yes	128 (33.6%)
If your answer is yes, what was your source of information?	In-service course	14 (10.9%)
	National-international congress	3 (2.3%)
	Medical books	60 (46.9%)
	Other	51 (39.8%)
Do you find your knowledge about teeth avulsed after trauma sufficient?	No	349 (92.1%)
	Yes	32 (7.9%)
In your opinion, learning about traumatic dental injuries is	Not important	12 (3.1%)
	Somewhat important	126 (33.1%)
	Important	186 (48.8%)
	Very important	57 (15%)
Would you like to receive more information to properly manage traumatic dental injuries?	No	34 (8.9%)
	Yes	347 (91.1%)
What do you do for the replantation of the avulsed tooth?	I do it myself	34 (8.9%)
	I refer to any dentist	197 (51.7%)
	I refer to the pediatric dentist	124 (32.5%)
	No idea	26 (6.8%)

had lower knowledge scores than the other groups (p=0.005) (Figure 2), while the total scores of general practitioners and emergency medicine residents were lower than those of the emergency medicine specialists (p<0.001) (Figure 3).

Table 4 presents the results of a statistical comparison of emergency medicine specialists, emergency medicine residents and general practitioners based on their responses to the third section of the questionnaire. The rate of correct responses to the question "What is the definition of tooth avulsion after trauma?" was higher among the participants who were working as emergency medicine specialists than those working as general practitioners and emergency medicine residents, and the difference was significant (p=0.003). To the question "What is your opinion of the prognosis of an avulsed tooth?", the highest rate of correct responses was among the emergency medicine specialists, while the lowest rate was among the general practitioners (p<0.001). The rate of correct responses to the question of cleaning a contaminated tooth after trauma was higher among the emergency medicine specialists than the general practitioners (p=0.001). To the question "Does the time from trauma to intervention effect the success of intervention to the avulsed tooth?", the rate of correct responses were given by the emergency medicine specialists was higher than that of the emergency medicine residents (p=0.025). The rate of correct responses to the question "How much time do you think should elapse for increased the success of replantation?" was lower among emergency medicine residents than emergency medicine specialists and general practitioners (p<0.001) (Table 4).

Discussion

Traumatic tooth avulsions account for approximately 16% of all dental injuries, and the loss of an anterior tooth can lead to esthetic, social and psychological problems (14,15). The majority of maxillofacial traumas in particular lead to tooth avulsion in children (16). In cases

Table 3. Participants knowledge about traumatic dental avulsion		
Questions	**Options**	**N (%)**
What is the definition of a tooth avulsion?	No idea	33 (8.7%)
	Total dislodgement of intact tooth out of its socket due to any trauma†	215 (56.4%)
	Dislodgement of fractured segment of the tooth due to any trauma	99 (26.0%)
	Tooth fracture due to any trauma	34 (8.9%)
What is your opinion about the prognosis of an avulsion tooth?	Recoverable †	260 (68.2%)
	Unrecoverable	24 (6.3%)
	No idea	97 (25.5%)
What are the possible options after an avulsed tooth is replanted?	It will not help; the tooth will fall off again	24 (6.3%)
	There is a risk of infection spreading throughout the body	85 (22.3%)
	Implanted teeth may be rejected as foreign bodies †	14 (3.7%)
	Replantation can damage adjacent teeth	57 (15.0%)
	No idea	201 (52.8%)
Which of the following is true about the replantation of a tooth with avulsion?	It must be guaranteed to be the primary tooth. Permanent teeth should not be placed	15 (3.9%)
	It must be guaranteed to be a permanent tooth. Primary teeth should not be placed †	139 (36.5%)
	There is no need to define the tooth type. Need to relocate both	120 (31.5%)
	No idea	107 (28.1%)
If the tooth you are replanting has fallen to the ground and is dirty, what would you do?	I used to gently brush the tooth with a toothbrush	41 (10.8%)
	I gently wipe the dirt stuck on the tooth with my hand	13 (3.4%)
	I used to rinse the tooth under running water †	196 (51.4%)
	I clean the tooth with disinfectant	35 (9.2%)
	No idea	96 (25.2%)
How to hold an avulsed tooth?	The crown part of the tooth (outside the alveolus) †	198 (52.0%)
	From the root part of the tooth (the part remaining in the alveoli)	26 (6.8%)
	No crown or root	11 (2.9%)
	No idea	146 (38.3%)
Does extra-oral time affect the prognosis of an avulsed tooth?	No	10 (2.6%)
	Yes †	315 (82.7%)
	No idea	56 (14.7%)
What is the best time for an avulsed tooth replacement?	First 15 minutes †	69 (18.1%)
	First 1 hour	121 (31.8%)
	first 12 hours	64 (16.8%)
	First 24 hours	46 (12.1%)
	No idea	81 (21.3%)
In pediatric cases, If you did not replant the tooth, which one is the most appropriate media to carry the tooth to the dentist?(24)	Childs' sublingual	94 (25.2%)
	Saliva	133 (35.7%)
	Dry cotton or gauze	93 (24.9%)
	Ice water	73 (19.6%)
	Saline †	179 (48%)
	Alcohol	15 (4%)
† Correct answers		

of TTA, there are several critical points that should be taken into account, such as which tooth requires the intervention, who should perform the intervention, the condition and storage of the avulsed tooth before the intervention, and the time from the occurrence of the trauma to the moment of intervention (6). It is believed that increasing the knowledge of emergency physicians in this area will contribute positively to the success of treatment, as healthcare professionals who inevitably come across dental injuries resulting from trauma in ER

that provide emergency medical services 24 hours a day (17-20).

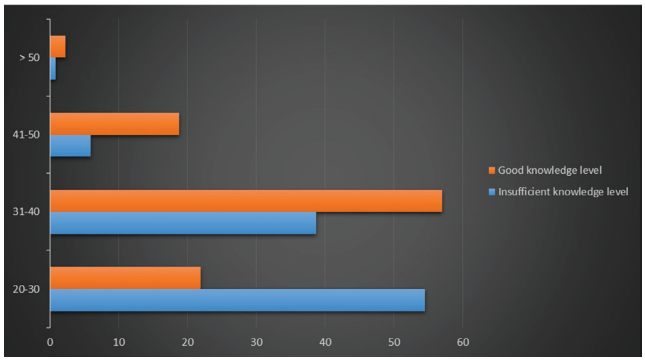

Figure 1. Percentage of knowledge level according to age

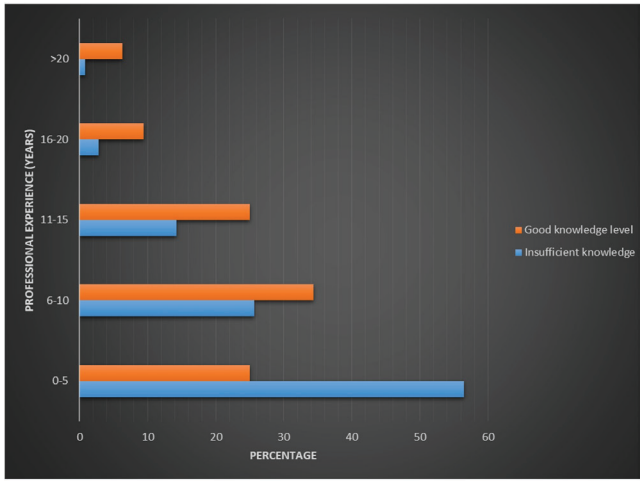

Figure 2. Percentage of knowledge level according to professional experience

In children presenting to the emergency room with TTA, the immediate replantation of the avulsed permanent tooth is required, while replantation is considered inappropriate in avulsions of primary teeth. Previous studies have revealed that the number of physicians who believe that permanent teeth should be replanted is not as high as expected, with a rate of 15.9% noted in a study by Ulusoy et al. (13) and 10.32% in another study by Aren et al. (19). In the present study, 36.5% of the physicians believed that the tooth should be replanted if it is a permanent tooth. These rates reveal that the majority of emergency physicians lack sufficient knowledge of the replantation of permanent teeth.

In two studies conducted in Turkey involving emergency physicians, it was established that the rate of exposure to TTA at least once during the professional lives of the respondent physicians was 55.56% and 68.1% (13,19). These two studies further reported that the number of respondents who gave the answer "a dentist should perform the procedure" to the question "who should perform the intervention" was 73.9% and

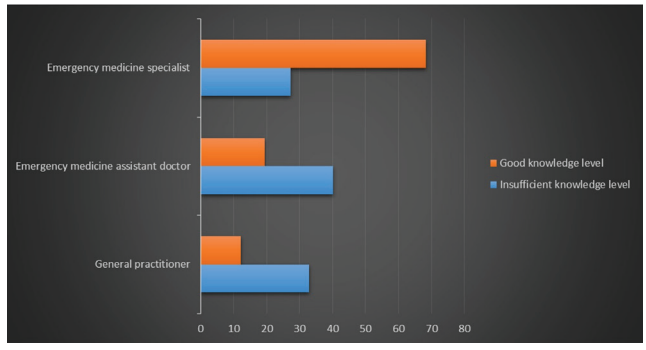

Figure 3. Percentage of knowledge level according to specialty

Table 4. Emergency physicians' right response about traumatic dental avulsion according to their specialties

Questions	General practitioner (n=91) N(%)	Emergency medicine assistant doctor (n=117) N(%)	Emergency medicine specialist (n=147) N(%)	p
What is the definition of a tooth avulsion?	43 (47.3%)[a]	61 (52.1%)[a]	100 (68%)[b]	**0.003***
What is your opinion about the prognosis of an avulsion tooth?	43 (47.3%)[a]	80 (68.4%)[b]	122 (83%)[c]	**<0.001†**
What are the possible options after an avulsed tooth is replanted?	4 (4.4%)	3 (2.6%)	5 (3.4%)	0.763
Which of the following is true about the replantation of teeth with avulsion?	35 (38.5%)	47 (40.2%)	51 (34.7%)	0.642
If the tooth you are replanting has fallen to the ground and is dirty, what would you do	35 (38.5%)[a]	57 (48.7%)[a b]	93 (63.3%)[b]	**0.001***
How to hold an avulsed tooth?	39 (42.9%)	64 (54.7%)	85 (57.8%)	0.072
Does extra-oral time affect the prognosis of avulsed tooth?	73 (80.2%)[a b]	90 (76.9%)[a]	131 (89.1%)[b]	**0.025***
What is the best time for an avulsed tooth replacement?	24 (26.4%)[a]	8 (6.8%)[b]	34 (23.1%)[a]	**<0.001***
In pediatric cases, if you did not replant the tooth, how would you carry the tooth to the dentist?	53 (58.2%)	79 (67.5%)	101 (68.7%)	0.223

Data are presented as n (%). *Chi-square test, †Fisher's Exact test. Different lowercase letters in a row indicate the statistically significant difference between groups.

7.94%, respectively. The studies also reported a low rate of recommended referral to a pediatric dentist (18.8% and 1.59%, respectively). Studies of emergency physicians by Holan and Shmueli, and Subhashraj identified a tendency to refer the patient to a dentist of 96% and 94.5%, respectively, suggesting that emergency physicians abroad consider the treatment of TTA to be a technical issue (21,22).

In the present study, 56.9% of physicians reported encountering to avulsed dental injury at least once during their professional life and 7.9% of physicians reported having sufficient knowledge to undertake an intervention, while in the study by Ulusoy et al. (13) 14.5% of physicians believed they had sufficient knowledge to treat this type of trauma. Some 51.7% stated that they would turn to dentists for professional support in this area, while 32.5% stated a preference for pediatric dentists in this regard (13). While the findings of the present study concur with Ulusoy et al. (13) they differ considerably from those established by Aren et al. (19). The findings of the present study suggest that the respondents do not consider themselves trained in TTA, leading them to avoid intervention, due mainly to the fact that there are physicians who are specialists or residents in this field.

The questions included in the third section of the questionnaire aimed to measure the level of the knowledge level of emergency physicians regarding TTA management. For the medical treatment of children presenting to the emergency department with an avulsed tooth after trauma, 67.7% of the respondent emergency physicians in the present study stated that they would prescribe an antibiotic, anti-inflammatory and analgesic combination, which is the correct answer, while in the study by Aren et al. this rate was 80.96% (19). The present study, unlike other studies on this subject in literature, also established the level of the knowledge of the physicians regarding post-traumatic tetanus prophylaxis, with 43.8% of the respondents stating that they would administer tetanus prophylaxis (14). While these rates determined in the study reveal that emergency physicians have sufficient knowledge of the potential for infection and inflammation following TTA, the rates indicate that they do not have the same level of the knowledge about tetanus prophylaxis.

The studies also differ in the level of knowledge among the respondents of the correct handling of an avulsed tooth and the appropriate method of cleaning if contamination is present. In the study by Aren et al. (19) 48.41% of the physicians knew the correct handling approach, and 50.79% correctly answered what to do in the event of the avulsed tooth being contaminated. These rates were 34.3% and 33.3%, respectively, in the study by Ulusoy et al. (13) and 51.6% and 48.4%, respectively, in the study by Bahammam (23). In the present study, 51.4% knew the correct approach to cleaning a contaminated tooth, which is washing gently with water, while those who knew the tooth should be held by the crown amounted to 52%.

The question about the critical time for intervention in TTA was correctly answered by 26.19% of the participants in the study by Aren et al. (19) while this rate was 10.1% in the study by Ulusoy et al. (13) falling short of the desired rate. In the studies by Subhashraj (22) and Bahammam (23) this rate was reported as 52% and 54%, respectively. The present study found that the rate of physicians who expressed the time from the occurrence of the trauma to the required intervention as the first 15 minutes was 18.1%, indicating that the majority of the respondents lacked sufficient knowledge of timing.

In previous studies in the literature, correct answers to the question of the most appropriate method of transportation vary between 31.1% and 31.9% among physicians, although Aren et al. (19) recently reported a rate of 94.3% in a study conducted again with emergency physicians. In the present study, 48% of the participants gave the correct answer to the question to emergency physicians about the appropriate media for storage (24). It is believed that the vast difference between the findings of Aren et al.'s (19) study and those of other studies may result from the greater number of appropriate storage media provided to the participants.

Previous studies have examined the effects of demographic factors on knowledge of TTA, comparing the effects of age, specialty and professional experience on knowledge levels. The study by Bahammam (23) found that demographic factors had an effect on knowledge levels, while both Aren et al. (19) and Ulusoy et al. (13) found that the knowledge scores from the section of their questionnaires measuring the level of the knowledge did not statistically significantly differ based on demographic factors. That said, the study by Ulusoy et al. (13) found physicians working in the ER of public hospitals to be better informed about the appropriate emergency management of an avulsed tooth, and that getting professional help would be more appropriate compared to the physicians working in the same departments of universities ($p<0.05$). In the present study, an examination of the relationship between demographic factors and knowledge scores revealed no significant difference between genders and the type of institution in which the respondent was employed, while the level of the knowledge was found to increase with increasing years of professional experience and with increasing age, which concurs with the findings of Bahamman's (23) study ($p<0.001$ for both). Furthermore, an analysis of the position of the respondents in the institution revealed specialist emergency physicians to

have more accurate knowledge of TTA than emergency residents and general practitioners ($p<0.001$).

Study Limitations

Since the involvement of participants in the survey studies such as this is on a voluntary basis, it is believed that emergency physicians with an interest in dental traumas may increase the rate of correct responses to the posed questions. That said, the present study has some prominent characteristics, such as being one of the few studies conducted into TTA in Turkey involving emergency physicians, and including those working in the peripheral regions outside big cities, by conducting the questionnaire online throughout Turkey. The study also benefits from having the highest number of participants among those conducted to date into TTA.

Conclusion

It can be concluded from the present study that emergency physicians lack sufficient knowledge and experience of TTA, which can be attributed to shortfalls in their education and post-specialty training programs. It is therefore believed that it would be appropriate to include dental trauma training in specialist training programs due to the potential for frequent exposure to this condition during and after medical education.

Authorship Contributions

Concept: B.K.E., Design: B.K.E., B.G.Y., Data Collection or Processing: B.G.Y., Analysis or Interpretation: B.K.E., Literature Search: B.K.E., Writing: B.K.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Petti S, Glendor U, Andersson L. World traumatic dental injury prevalence and incidence, a meta-analysis-One billion living people have had traumatic dental injuries. Dent Traumatol 2018;34:71-86.
2. Glendor U. Epidemiology of traumatic dental injuries–a 12 year review of the literature. Dent Traumatol 2008;24:603-11.
3. Andersson L, Andreasen JO, Day P, et al. Guidelines for the Management of Traumatic Dental Injuries: 2. Avulsion of Permanent Teeth. Pediatr Dent 2017;39:412-9.
4. Mazur M, Jedliński M, Janiszewska-Olszowska J, et al. Knowledge of Emergency Management of Avulsed Teeth among Italian Dentists-Questionnaire Study and Next Future Perspectives. Int J Environ Res Public Health 2021;18:706.
5. Wu TT, Li JY, Yang KY, et al. A cross-sectional evaluation of knowledge among Chinese dentists regarding the treatment of traumatic injuries in primary teeth. Dent Traumatol 2021;37:188-95.
6. Yang X, Sun W, Wang Z, Ji AP, Bai J. [Clinical analysis of children and adolescents emergency dental trauma cases]. Beijing Da Xue Xue Bao Yi Xue Ban 2021;53:384-9.
7. Özyavaş S. Türkiye'de Ağız Diş Sağlığı Politikası: Mevcut Durum Analizi. Hacettepe Sağlık İdaresi Dergisi 2018;21:789-805.
8. Onetto JE, Flores MT, Garbarino ML. Dental trauma in children and adolescents in Valparaiso, Chile. Endod Dent Traumatol 1994;10:223-7.
9. Bottenberg P, Van Melckebeke L, Louckx F, Vandenplas Y. Knowledge of Flemish paediatricians about children's oral health-results of a survey. Acta Paediatr 2008;97:959-63.
10. Salama F, Alwohaibi A, Alabdullatif A, Alnasser A, Hafiz Z. Knowledge, behaviours and beliefs of parents regarding the oral health of their children. Eur J Paediatr Dent 2020;21:103-9.
11. Hadjipanayis A, Grossman Z, Del Torso S, Michailidou K, Van Esso D, Cauwels R. Oral health training, knowledge, attitudes and practices of primary care paediatricians: a European survey. Eur J Pediatr 2018;177:675-81.
12. Sezer RG, Paketci C, Bozaykut A. Paediatricians' awareness of children's oral health: Knowledge, training, attitudes and practices among Turkish paediatricians. Paediatr Child Health 2013;18:e15-9.
13. Ulusoy AT, Onder H, Cetin B, Kaya S. Knowledge of medical hospital emergency physicians about the first-aid management of traumatic tooth avulsion. Int J Paediatr Dent 2012;22:211-6.
14. Fouad AF, Abbott PV, Tsilingaridis G, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent Traumatol 2020;36:331-42.
15. Antipovienė A, Narbutaitė J, Virtanen JI. Traumatic Dental Injuries, Treatment and Complications in Children and Adolescents: A Register-Based Study. Eur J Dent 2021 Feb 3.
16. Lam R. Epidemiology and outcomes of traumatic dental injuries: a review of the literature. Aust Dent J 2016;61:4-20.
17. Ng L, Malandris M, Cheung W, Rossi-Fedele G. Traumatic dental injuries presenting to a paediatric emergency department in a tertiary children's hospital, Adelaide, Australia. Dent Traumatol 2020;36:360-70.
18. Li J. Emergency department management of dental trauma: recommendations for improved outcomes in pediatric patients. Pediatr Emerg Med Pract 2018;15:1-24.
19. Aren A, Erdem AP, Aren G, et al. Importance of knowledge of the management of traumatic dental injuries in emergency departments. Ulus Travma Acil Cerrahi Derg 2018;24:136-44.
20. Mitchell J, Sheller B, Velan E, Caglar D, Scott J. Managing pediatric dental trauma in a hospital emergency department. Pediatr Dent 2014;36:205-10.

21. Holan G, Shmueli Y. Knowledge of physicians in hospital emergency rooms in Israel on their role in cases of avulsion of permanent incisors. Int J Paediatr Dent 2003;13:13-9.

22. Subhashraj K. Awareness of management of dental trauma among medical professionals in Pondicherry, India. Dent Traumatol 2009;25:92-4.

23. Bahammam LA. Knowledge and attitude of emergency physician about the emergency management of tooth avulsion. BMC Oral Health 2018;18:57.

24. Ballıkaya E, Öztürk Ş, Çehreli ZC. Avulse Dişleri Taşıma Ortamları: Bir Güncelleme. Turkiye Klinikleri Dis Hekimligi Bilimleri Dergisi 2020;26:487-95.

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Plasma Native Thiol and Lipid Hydroperoxide Levels in the Patients with A Non-Aura Migraine Attack Admitted to the Emergency Department: A Case-Control Study

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Abstract

Aim: We emphasize the importance of serum native thiol (SH), lipid peroxidation (LOOH), and ceruloplasmin (CP) levels in the management of patients with a migraine attack and clarify the possible relationships among these parameters in the emergency department (ED)

Methods: The study was designed as a case-control study which is a type of observational study by including the patients who applied to the University of Health Sciences Turkey, University Diyarbakır Gazi Yasargil Training and Research Hospital between December 2018 and July 2019. A total of 88 participants (study group, n=46 and healthy control group, n=42) consecutively admitted to the ED. with similar demographic characteristics were included in the study. SH, LOOH, and CP levels in both groups were recorded and studied.

Results: The serum SH level was significantly lower in the patient than in the control group (p=0.002). However, the LOOH and CP levels were significantly higher in the patient group than in the control group (p<0.001). A significant correlation was observed between LOOH and CP (r=0.332, p=0.002). The receiver operating characteristic analysis indicated that a serum LOOH level of 11.9 µmol/L predicted an acute migraine attack with 89% sensitivity and 71% specificity.

Conclusions: Our results suggest that the oxidative status is activated in patients with a non-aura migraine attack, and this may lead to other oxidative and inflammatory processes, with an increase in the serum LOOH level and a decrease in the native thiol level.

Keywords: Non-aura migraine, native thiol, lipid peroxidation, emergency department

Introduction

Migraine is a neurovascular disease characterized by brain and brain vascular inflammation with episodic attacks. Migraines with and without aura have been described by the International Society of Headaches. There are generally two types of migraine, migraine with aura accompanied by transient focal neurological symptoms that often start with pain, sometimes before headache, and migraine without aura, which is associated with certain symptoms (ICHD) (1). Although many theories on the pathogenesis of migraine have been proposed, it is not yet fully understood. Activation of neuropeptides and mechanisms associated with inflammation are believed to be responsible for the pathogenesis of migraine (2-4).

Thiols are compounds that scavenge free oxygen radicals. Thiols are important antioxidant agents in humans, and thiols containing the sulfur analog in alcohol are in a free or oxidized form in the plasma. Thiol groups are oxidized by disulfide bonds and reduced by reactive oxygen species (5).

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Oxidative stress is responsible for migraine, and the thiol-disulfide balance is likely to deteriorate during a migraine attack. In fact, there are a limited number of studies on migraine patients with a thiol-disulfide balance (2,5). It is clear that these studies are inadequate to explain the clinical and physiopathological status of migraine.

Hydroperoxides (LOOH), which are more stable than free radicals, in serum, may be markers of oxidative stress in tissue and their levels increase in the presence of stress (5). We hypothesized that acute migraine attacks may be associated with enhanced oxidative status and that determination of the plasma levels of LOOH and native thiol (NT), which are antioxidants, may be beneficial in the management of patients with migraine in the emergency department (ED). In this study, we emphasize the importance of serum NT and LOOH levels in the management of patients with a migraine attack and clarify the possible relationships among these parameters. We also investigated the relationships of these parameters with the migraine disability assessment score (MIDAS) and determined Whether NT and LOOH were independent risk factors in patients with migraine in the ED.

Methods

Study Design

The local ethics committee approved the study and all participants gave informed written consent. The study was designed as a case-control study which is a type of observational study by including the patients who applied to the University of Health Sciences Turkey, University Diyarbakır Gazi Yasargil Training and Research Hospital between December 2018 and July 2019 (date: 2018, number: 169). All procedures were carried out in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. A total of 46 patients with non-aura migraine consecutively admitted to the ED (Patients group: 8 males and 38 females) and 42 healthy controls (12 males and 30 females) with similar demographic characteristics were included in this study.

Inclusion criteria: 1. Admitted to the ED with headache and diagnosed with migraine; 2≥18 years of age; 3. any other known chronic or acute disease; and 4. nonsmoker and no substance dependence.

Exclusion criteria: Patients who were diagnosed with any secondary cause of headache; known febrile illness or other cardiovascular disorder; history of alcohol consumption or alcohol intake; active somatic disease in the past month; active somatic or psychiatric disease; drug use for any reason within 7 days; taking drugs such as alcohol or ecstasy, or taking drugs with suicidal intent; likely to become pregnant or suspected pregnancy; chronic disease; exposed to trauma in the last week; and 12. <18 years of age (n=13).

Clinical Evaluation

The patients were initially treated in the ED. Vital signs such as blood pressure, pulse, and body temperature were evaluated. After a migraine episode was detected, 5 cc of blood were obtained and treatment with non-steroidal anti-inflammatory drugs and/or selective 5HT1B/1D agonists (Triptan) was started. The patients were taken to quiet, low-light observation rooms with appropriate conditions. All of the patients were discharged from the ED after their symptoms were resolved. The age, sex, migraine type, duration of migraine (years), monthly frequency of migraine attacks, duration of migraine attacks (hours), and headache severity [visual analog scale (VAS)] of the patients were recorded. It was not taken into consideration whether the patient received treatment for a migraine attack. Headache severity was evaluated using a VAS (score 1-10). The diagnosis of migraine was made according to the latest diagnostic criteria classification of ICHD (1). Diagnostic criteria of the ICHD for migraine indicate that a patient should have at least five migraine attacks (untreated or unsuccessfully treated) lasting 4-72 hours with at least two of the following characteristics: 1) unilateral placement, 2) a vibrating quality, 3) moderate or severe pain intensity, and 4) aggravation that causes avoidance of daily physical activity. After arriving at the ED, the patients were evaluated by the same healthcare professional who used MIDAS to evaluate headache-related disability. The procedure involved a questionnaire to determine the effect of migraine headaches on the patient's performance routine activity. The patients were divided into three groups according to their MIDAS scores (6).

Laboratory Assessments

NT and LOOH levels in both groups were recorded as primary independent variables and demographic and clinical characteristics of the patients and other biochemical and hematological data were recorded as secondary independent variables.

A 5 mL blood sample was taken within the first hour from all patients who suffered from migraine without aura and from controls. All blood samples were placed in flat tubes and centrifuged at 3,500 rpm for 15 min to obtain the plasma or serum. All samples were stored in aliquots at -80 °C until use.

Hemogram counts were measured using an automated analyzer in K2EDTA samples (Sysmex K-1000; Block Scientific, Bellport, NY USA). Routine biochemical variables such as serum urea, creatinine, sodium, and potassium were measured using commercial kits (Abbott; Block Scientific). Serum CP was measured by the Erel (7) method, an automatic calorimetric method based on enzymatic oxidation of ferrous ion to ferric ion. Details of this method are available in Erel (7).

Ferrous ion oxidation of xylenol orange was used to assess serum LOOH levels (8). In the test, the ferric ion concentration was measured using xylenol orange. LOOH is reduced by triphenylphosphine (TPP), a specific lipid reductant. The presence or absence of TPP during pretreatment determines the difference in LOOH level. LOOH values are expressed in μmol/L (8).

NT concentrations were measured using a new and fully automated analytical method (9). In this method, the reducible disulfide bonds were reduced to form free functional thiol groups. Reductive sodium borohydride was used and extracted with formaldehyde, and the native and reduced thiol groups were determined after reacting with 5.5-dithiobis-(2-nitrobenzoic) acids. Then, the natural thiol levels were measured and calculated. The measurements were performed using a Cobasc 501 instrument (Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

The demographic and clinical data of the patients were written into excel files at the time of patient admission. Serum biochemical data of the participants were also transferred to this excel file. Study calculations were performed using SPSS version 20.0 software package (Chicago, IL, USA). In the power analysis for SH, differences in the mean levels of patient and control group variables were compared. The power of the test to detect a difference between the two groups was 80% for NT. The Shapiro-Wilks test was performed to determine the normality of the distribution. Student's t-test was used for comparisons between groups of numerical variables showing a normal distribution, and an analysis of variance (ANOVA)-Tukey test for multiple comparisons within groups. Categorical variables were analyzed using the chi-squared or Fisher's Exact tests. A receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory ability of NT and LOOH. Binary logistic regression analyzes were used for NT and LOOH in patients with migraine attacks. P-values of <0.05 were considered indicative of significance.

Results

Of the 46 migraine patients included in the study, 8 (17.4%) were males, and 38 (82.6%) were females; 12 (28.6%) of the 42 control individuals were males, and 30 (71.4%) were females. The mean age of the migraine patients was 36.04±8.06 years, and that of the control group was 37.32±12.55 years. The sex and age distributions were similar between the groups (p=0.211, p=0.509, respectively). While the baseline clinical characteristics of the patients with acute migraine attacks are shown in Table 1, the demographic and laboratory data of the patient and control groups are in Table 2.

The serum NT level was meaningfully lower in the patient than in the control group (0.49±0.15 vs. 0.60±0.19 μmol/L, p=0.002, Table 2). However, the LOOH and CP levels were meaningfully higher in the patient group than in the control group (17.81±5.19 and 407.88±119.23 in the patient group; 10.86±3.05 and 343.24±104.83 in the control group, respectively, p<0.001, p=0.009, respectively; Table 2).

In Table 3, an ANOVA-Tukey test was performed according to the MIDAS classification to determine the

Table 1. Baseline clinical characteristics of patients with acute migraine attack

Pain localization	Number (N)	Proportion (%)
Frontal	19	(41.3%)
Occipital	7	(15.2%)
Temporal	14	(30.4%)
Verteks	6	(13.1%)
Symptomsα	Number (N)	Proportion (%)
Nausea-vomiting	39	(84.8%)
Yawning	7	(15.2%)
Irritability	15	(32.6%)
Sensitivity to light, sound or motion	25	(54.3%)
Dark circles under the eyes	4	(8.7%)
History	Mean ± SD	Minimum-maximum
Duration of disease/year	6.08 ± 2.42	1.00-12.00
Attack time/hour	19.28±10.43	6.00-48.00
Frequency of attacks/month	2.73±1.52	1.00-7.00
Pain severity (VAS)	7.65±1.40	5.00-10.00

α: Each patient had one or more symptoms, VAS: Visual analog scale, SD: Standard deviation

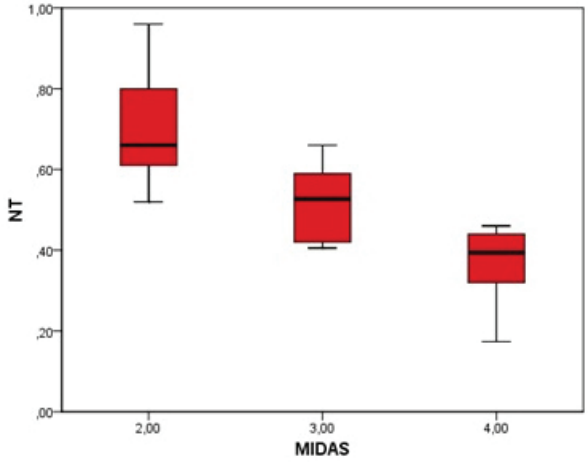

Figure 1. NT level comparison between MIDAS classification in patients with migraine attack
MIDAS: Migraine disability assessment score, NT: Native thiol

Table 2. Age, gender and baseline laboratory characteristics of patients with acute migraine attack and controls			
Parameters	**Migraine patients (52.2%, n=46) (n, % or mean ± SD)**	**Controls (47.8%, n=42) (n,% or mean ± SD)**	**p***
Gender			
(Male/female)	8/38	12/30	0.211
Age (years)	36.04±8.06	37.32±12.55	0.509
LOOH (µmol/l)	17.81±5.19	10.86±3.05	<0.001
NT (mmol/L)	0.49±0.15	0.60±0.19	0.002
CP (mg/dL)	407.88±119.23	343.24±104.83	0.009
LDL cholesterol (mg/dL)	109.52±34.81	114.23±31.06	0.506
Ca (mg/dL)	9.79±1.66	10.16±0.92	0.200
Glucose (mg/dL)	96.10±24.50	100.98±23.35	0.313
Urea (mg/dL)	23.64±7.50	29.79±7.85	<0.001
Creatinin (mg/dL)	0.60±0.16	0.81±0.19	<0.001
AST (U/L)	24.82±9.70	21.21±8.25	0.067
ALT (U/L)	26.89±17.52	17.52±7.34	0.063
T. Chol (mg/dL)	182.18±51.14	196.04±38.15	0.156
HDL (mg/dL)	38.28±12.61	40.40±11.99	0.426
K (mEq/L)	4.22±0.78	4.21±0.47	0.894
LDH (U/L)	286.75±101.35	257.18±94.64	0.162
Hemoglobin (g/L)	13.93±4.07	14.20±1.81	0.715
MPV (fL)	7.44±1.27	7.39±1.08	0.894
PLT (x109/L)	258.05±84.76	282.16±92.47	0.205
APTT (Sec)	34.14±4.07	31.95±7.85	0.258
PT-INR	1.31±1.28	1.10±0.21	0.295
Hs-CRP	0.542±0.210	0.580±0.170	0.453

*Student's t-test and chi-square tests. ALT: Alanine aminotransferase, APTT: Activated partial thromboplastin time, AST: Aspartate aminotransferase, Ca: Calcium, HDL: High-density lipoprotein, Hs-CRP: High-sensitivity-C-reactive protein, K: Potassium, CP: Ceruloplasmin, NT: Native thiol, LOOH: Lipid peroxidation, LDL: Low-density lipoprotein, LDH: Lactate dehydrogenase, MPV: Mean platelet volume, PLT: Platelet, PT-INR: Prothrombin time- International normalized ratio

clinical severity of patients presenting to the ED with a migraine attack. The mean NT level of the group with the most severe clinical presentation was lowest (for comparisons among three groups, p<0.001; Table 3 and Figure 1, respectively). In contrast, the mean plasma LOOH level increased in proportion to the clinical severity of migraine (p=0.002, Table 3, Figure 2).

A significant, positive correlation was observed between MIDAS score and LOOH (r=0.449, p=0.002). Conversely, the MIDAS score was significantly negatively correlated with NT level (r=-0.777, p<0.001). As seen in Figure 3, the ROC analysis indicated that a cut-off serum LOOH level of 11.9 µmol/L predicted acute a migraine attack with 89% sensitivity and 71% specificity (area under the curve=0.896; 95% confidence interval (CI): 0.832-0.960).

In binary logistic regression analyses, NT and LOOH were independent markers of a migraine attack in the ED (Table 4); the odds ratio (OR) was significantly lower for NT (OR <0.001; 95% CI, 0.00-1.04; p=0.002) and significantly higher for LOOH (OR: 1.89; 95% CI, 1.42-2.52; p<0.001)

Discussion

This is one of the few studies in which serum NT was studied in patients who presented to the ED with a migraine attack without aura. No previous study has evaluated NT and LOOH levels in patients admitted to the ED with a migraine attack without aura. Our results show that the NT level was meaningfully lower and the LOOH level was higher in patients admitted to the ED with a migraine attack without aura compared to those of healthy participants. Moreover, the NT level decreased and the LOOH level increased in proportion to the severity of the migraine attack. Also, the MIDAS score was significantly positively correlated with the LOOH level and significantly negatively correlated with that of NT. In addition, binary logistic regression analyses showed that the NT and LOOH levels were independent markers of a migraine attack in the ED.

Migraine patients are susceptible to neurovascular reactions caused by different factors. The Impaired balance between inhibition and stimulation of the nervous system leads to some clinical variations, including headache

Table 3. Serum NT, CP and LOOH levels comparison between MIDAS classification in patients with migraine attack					
MIDAS	**N**	**Mean ± SD**	**Minimum-maximum**	**ANOVA**	**Tukey HSD**
Clas				**Px**	**Pα**
NT II	8	0.70±0.14	0.52-0.96	<0.001	II & III → <0.001
III	20	0.51±0.08	0.41-0.66	-	II & IV → <0.001
IV	18	0.36±0.09	0.17-0.46	-	III & IV → <0.001
LOOH II	8	13.75±2.55	10.78-17.30	0.002	II & III → 0.112
III	20	17.58±3.37	10.63-24.10	-	II & IV → 0.005
IV	18	20.04±5.91	12.10-32.52	-	III & IV → 0.218
CP II	8	456.09±110.25	297.86-647.50	0.426	II & III → 0.436
III	20	393.92±119.33	244.20-641.84	-	II & IV → 0.541
IV	18	401.95±123.89	268.26-678.03	-	III & IV → 0.977
Pα : Tukey test for Multiple Comparisons (the mean difference is significant at the 0.05 level), Px: One-Way ANOVA test. MIDAS: Migraine disability assessment score, LB: Lower bound, UB: Upper bound, CP: Ceruloplasmin, NT: Native thiol, LOOH: Lipid peroxidation					

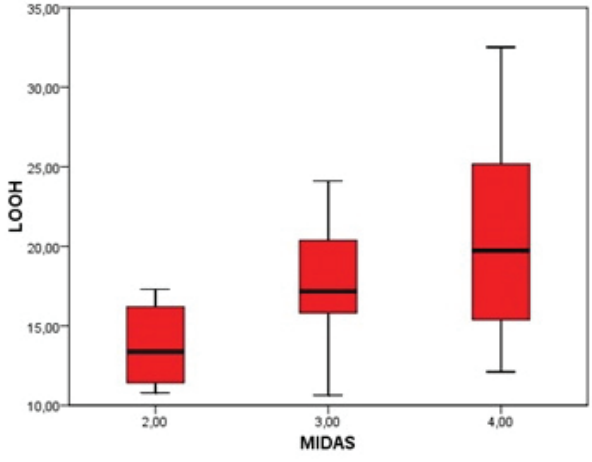

Figure 2. LOOH level comparison between MIDAS classification in patients with migraine attack
LOOH: Lipid peroxidation, MIDAS: Migraine disability assessment score

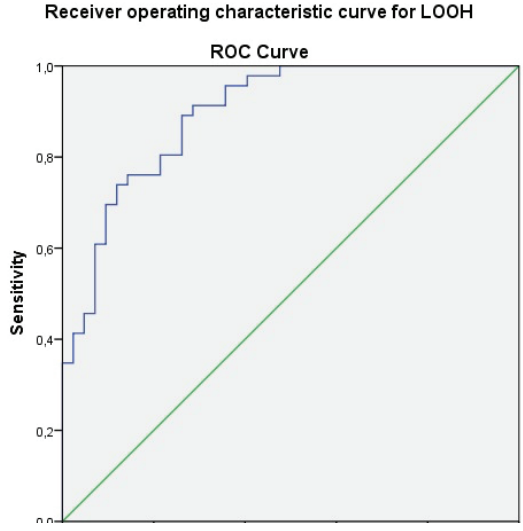

Figure 3. Receiver operating characteristic analysis for NT
NT: Native thiol, LOOH: Lipid peroxidation, LOOH: Lipid peroxidation ROC: Receiver operating characteristic

(10,11). Clinical studies have indicated that oxidative stress increases and antioxidant defense decreases in patients with migraine (12,13). The role of the acute inflammatory response caused by oxidative stress has been reported in some clinical studies in patients with a headache due to a migraine (13,14). LOOH is a primary product of lipid peroxidation, and it may be useful in predicting oxidative stress in the blood (15). Few studies have shown levels of lipid peroxidation in migraine patients or the levels of these antioxidant enzymes (12,16).

Lipid peroxidation levels can be assessed based on serum total oxidative stress measures using a recently developed method (16,17). Native thiol may be useful in determining antioxidant activity. Gumusyayla reported that NT levels were higher in migraine patients than in healthy controls (5). In the same study, NT level was reported to be similar in migraine patients with and without aura. In another study, the relationships of changes in dynamic thiol-disulfide homeostasis with pain severity and duration of disease were investigated in children with migraine (14). In that study, a negative correlation was found between the NT level and headache severity in patients with both

Table 4. Binary logistic regression analyses of study variables					
Variables	**OR 95%**	**CI**	**p**	**Sensitivity**	**Specificity**
NT	<0.001	0.00-1.04	0.002		
LOOH	1.89	1.42-2.52	<0.001	84.3	85.7
CP	1.00	0.99-1.01	0.317		
Hs-CRP	0.48	0.18-1.29	0.149		
NT: Native thiol, LOOH: Lipid peroxidation, CP: Ceruloplasmin, Hs-CRP: High-sensitivity-C-reactive protein, OR: Odds ratio, CI: Confidence interval					

migraine and tension-type headaches. NT levels have been reported to decrease as headache severity increases. In our study, serum NT levels were meaningfully lower in patients who presented to the ED with a migraine attack without aura than in the control group. We also classified the patients with migraine according to their MIDAS score; the mean NT level was significantly lower in patients with the most severe migraine attacks. Moreover, there was a negative correlation between the MIDAS score and NT level.

In a study of patients with coronary slow flow, the LOOH levels were higher than those in the normal population (18). Similarly, Yigit et al. (13) reported that the levels of oxidative parameters were high in patients presenting to the ED with migraine attacks. Similarly, levels of CP and LOOH, which are inflammatory markers closely associated with oxidative stress, were meaningfully higher in migraine patients than in controls in the present study. Furthermore, the level of LOOH, the main product of lipid peroxidation and a marker of the level of oxidative stress, increased meaningfully. In addition, the mean plasma level of LOOH increased in proportion to the severity of the migraine attack according to the MIDAS classification; patients with the highest MIDAS score had the highest level of LOOH. So, there was a significant, positive correlation between the MIDAS score and the LOOH level. However, the patients in the MIDAS-II, -III, and -IV groups had similar CP levels. Moreover, the NT and LOOH levels were independent markers of a migraine attack in the ED. The high serum LOOH levels suggest that oxidative stress plays a role in migraine. The oxidant state is dominant in cases of an acute attack. The increased serum levels of LOOH during migraine attack suggest that an acute inflammation event is triggered in the dominant state of oxidation. However, whether oxidative stress is a cause of migraine or the result of metabolic processes associated with this condition is unclear.

Study Limitations

The limitations of our study should be mentioned. The migraine diagnosis was based on subjective criteria. This was a single-center study with a relatively small number of participants. We used a pain severity scale for migraine patients, and we could not evaluate the effects of previously used drugs that may have affected the levels of SH, LOOH, or CP. This study was based on a single blood sample to determine NT, LOOH, and CP levels in each patient. A comparison could not be made regarding the pain location and symptoms of the patients, because the primary parameters in our study are based on the analysis of laboratory data. The fact that such a comparison was not made in the study is an important limiting factor. In our study, we can say that dehydration may affect oxidative stress. In this respect, perhaps this is an important limitation of our study.

Conclusion

The levels of the antioxidant NT were meaningfully lower in patients with migraine without aura than in controls. Moreover, as the MIDAS score increased, the level of NT decreased and the severity of disability increased. In contrast, the LOOH level, which reflects the oxidative state, was meaningfully higher in migraine patients than in the controls. Moreover, we observed a positive correlation between MIDAS score and LOOH level. In addition, NT and LOOH levels were independent risk factors for migraine attack in the ED. Our results suggest that the oxidative status is activated in patients with a migraine, and this may lead to other oxidative and inflammatory processes, with an increase in the serum LOOH level and a decrease in the NT level.

Authorship Contributions

Concept: M.T.G., Design: O.S., Data Collection or Processing: C.G., E.D., Analysis or Interpretation: O.S., M.T.G., Literature Search: H.E., Writing: O.S., M.T.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Göbel CH, Karstedt SC, Münte TF, et al. ICHD-3 is significantly more specific than ICHD-3 beta for diagnosis of migraine with aura and with typical aura. J Headache Pain 2020;21:2.
2. Albay VB, Tütüncu M. Evaluation of the Relationship Between Thyroid Dysfunction and Menstrual Migraine in Adult Females. Med Bull Haseki 2020;58:110-4.
3. Poyrazoğlu HG, Öztürk AB. Predictive value of laboratory parameters in childhood migraine. Acta Neurol Belg 2020;120:907-14.
4. Gross EC, Putananickal N, Orsini AL, et al. Mitochondrial function and oxidative stress markers in higher-frequency episodic migraine. Sci Rep 2021;11:4543.
5. Gumusyayla S, Vural G, Bektas H, Neselioglu S, Deniz O, Erel O. A novel oxidative stress marker in migraine patients: dynamic thiol-disulphide homeostasis. Neurol Sci 2016;37:1311-7.
6. Dönmez H ,Yoldaş TK, Solak EB, Çolpak AI. Demographic/ Clinical Characteristics and Midas Scores of 103 Consecutive Migraine Patients. Turk J Neurol 2008;14:388-93.
7. Erel O. Automated measurement of serum ferroxidase activity. Clin Chem 1998;44:2313-9.
8. Nourooz-Zadeh J. Ferrous ion oxidation in presence of xylenol orange for detection of lipid hydroperoxides in plasma. Methods Enzymol 1999;300:58-62.

9. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32.
10. Yu ES, Priyadharsini S S Y, Venkatesan T. Migraine, Cyclic Vomiting Syndrome, and Other Gastrointestinal Disorders. Curr Treat Options Gastroenterol 2018;16:511-27.
11. Söğüt Ö, Kaya H, Gökdemir MT, et al. Early Oxidative Status in Adult Patients With İsolated Traumatic Brain Injury. Turk J Med Sci 2012;42:1010-9.
12. Kim S, Bae DW, Park SG, Park JW. The impact of Pain-related emotions on migraine. Sci Rep 2021;11:577.
13. Yigit M, Sogut O, Tataroglu Ö, et al. Oxidative/antioxidative status, lymphocyte DNA damage, and urotensin-2 receptor level in patients with migraine attacks. Neuropsychiatr Dis Treat 2018;14:367-74.
14. Eren Y, Dirik E, Neşelioğlu S, Erel Ö. Oxidative stress and decreased thiol level in patients with migraine: cross-sectional study. Acta Neurol Belg 2015;115:643-9.
15. Argüelles S, Gómez A, Machado A, Ayala A. A preliminary analysis of within-subject variation in human serum oxidative stress parameters as a function of time. Rejuvenation Res 2007;10:621-36.
16. Bolayir E, Celik K, Kugu N, Yilmaz A, Topaktas S, Bakir S. Intraerythrocyte antioxidant enzyme activities in migraine and tension-type headaches. J Chin Med Assoc 2004;67:263-7.
17. Papurica M, Rogobete AF, Sandesc D, et al. Redox Changes Induced by General Anesthesia in Critically Ill Patients with Multiple Traumas. Mol Biol Int 2015;2015:238586.
18. Baysal SS, Koc S. Oxidant-Antioxidant balance in patients with coronary slow flow. Pak J Med Sci 2019;35:786-792.

Original Article

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The Impact of Body Mass Index on Intraoperative Blood Loss, Blood Transfusion and Fluid Management in Patients Undergoing Liver Transplantation: A Retrospective Analysis from a Tertiary Referral Center

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Abstract

Aim: Some parameters affecting intraoperative bleeding have been investigated, but there is not enough data on body mass index (BMI) and bleeding in liver transplantation (LT). We aimed to evaluate the relationship between the pretransplant BMI of recipients and blood loss, blood product transfusion, and fluid replacement during LT.

Methods: In this retrospective cross-sectional study, patients aged ≥18 years who underwent LT between April 2014 and June 2020 were analyzed. Patients <18 years of age, with incomplete data, and those using cell salvage were excluded from the study. The BMI of the patients was grouped according to the definition of the World Health Organization (BMI <18.5 in Group 1, BMI 18.5-24.9 in Group 2, BMI 25-29.9 in Group 3, and BMI >30 in Group 4). Groups were compared according to operative hemodynamics, blood loss, blood transfusion, and fluid management-related parameters.

Results: Two hundred and sixteen patients were included in the study. The mean blood loss was higher in obese patients than in others, but it was not statistically significant. The mean red blood cell, fresh frozen plasma, platelet, and cryoprecipitate transfusions were similar for the groups. The mean IV crystalloid fluid amount gradually shortened from underweight to obese patients but there was no significant difference. Preoperative mean international normalized ratio and prothrombin time were significantly higher in underweight patients than others (p=0.025).

Conclusion: LT can be performed safely in patients with different BMI with similar blood loss and transfusion rates.

Keywords: Anesthesia, blood transfusion, body mass index, hemorrhage, liver transplantation, obesity

Introduction

Liver transplantation (LT) is a life-saving procedure for patients with end-stage liver disease (ESLD). It is a complex and technically difficult surgery with multiple vascular transactions and anastomoses that increase the risk of blood loss. Although IV fluids are used in the first step of blood loss management, sometimes massive blood transfusion may be required. To make proper patient preparation before surgery, it is very important to be able to identify patients with a high probability of bleeding during LT and anticipate their transfusion needs. In this way, a prediction can be formed for both the preparation of blood products and the timing of the transplantation (1).

The increase in the overweight population is an important health problem worldwide. Obesity is known to be a risk factor for the development of diabetes mellitus, hypertension, and coronary artery disease. It is also thought to increase the perioperative morbidity and mortality resulting from surgery (2). The effects of body mass index (BMI) on clinical outcomes, complications, and perioperative parameters in surgery have been investigated before (3). It has also been reported that obesity is associated with higher infectious complications and cancer events leading to death in liver transplant recipients (4).

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Although some parameters affecting intraoperative bleeding have been investigated, there is not enough data about BMI and bleeding in LT. Therefore, in this study, we evaluated the relationship between the pretransplant BMI of recipients and blood loss, blood product transfusion, and fluid replacement during LT.

Methods

Study design

After obtaining approval from This study was approved by the Ethics Committee of the Istanbul Medipol University (approval number: 2021/126) the medical records of patients aged 18 years and over who underwent LT in our hospital between April 2014 and June 2020 were retrospectively analyzed. Data were collected using patients' charts and hospital medical records. Demographic data of the patients, parameters related to ESLD, anesthesia and surgery-related data, and laboratory test results were recorded. The exclusion criteria were being under the age of 18, incomplete data, and cell salvage (autologous blood transfusion system) usage during surgery. Verbal consent was obtained by informing the participating patients or their legal guardians.

BMI was calculated by dividing weight (kg) by square of height (m) and the World Health Organization (WHO) definition was used in its classification. Patients were divided into 4 groups according to the body mass index, as the BMI <18.5 (underweight) in Group 1, BMI 18.5-24.9 (normal weight) in Group 2, BMI 25-29.9 (overweight) in Group 3, and the BMI >30 (obese) in Group 4. Groups were compared according to preoperative demographic characteristics, operative hemodynamics, blood loss, blood transfusion, and fluid management-related parameters.

Anesthesia management

General anesthesia was employed for all LT surgeries. After preoxygenation, general anesthesia was induced with IV propofol, fentanyl, and vecuronium. Sevoflurane (2-3%) was used as an inhalation agent to maintain anesthesia. A central venous catheter was placed for fluid infusion, central venous pressure (CVP), and mixed venous oxygen saturation monitoring. A radial arterial line was used for blood pressure measurement. Intraoperative monitoring includes serial arterial blood gases, temperature, thromboelastogram (TEG), pulse index contour continuous cardiac output, and standard laboratory tests. We applied the restrictive fluid management technique before the anhepatic phase to reduce blood loss.

The transfusion strategy was recommended according to the guideline by the American Association of Blood Banks for transfusion. If the hemoglobin was below 7 g/dL, allogeneic red blood cell (RBC) transfusion was performed. For hemoglobin levels between 8 and 10 mg/L, the decision to transfuse was made based on symptoms and signs of anemia. If bleeding continued, allogeneic fresh frozen plasma (FFP), platelet, and cryoprecipitate transfusion were used according to TEG results. All patients were transferred to the intensive care unit (ICU) for close follow-up after surgery.

Statistical Analysis

The normality distribution of the variables was checked by the Shapiro-Wilk test and Q-Q plots. One-Way ANOVA test was used for comparison of variables showing normal distribution between groups, and the Kruskal-Wallis test was used for data that did not show normal distribution. Quantitative data are showed as mean ± standard deviation. Categorical variables were grouped and compared using the χ^2 test or Fisher's Exact test. The data were analyzed at a 95% confidence level and a p-value of less than 0.05 was accepted as statistically significant.

Results

Two hundred and sixteen patients [Group 1=6 (2.8%), Group 2=56 (25.9%), Group 3=82 (38%) and Group 4=72 (33.3%) patients] were included in the study (Figure 1). In the study cohort, the highest number of patients, with a rate of 38%, was in the overweight group. The mean ages were 47.5±12.8, 47.9±14.3, 54.4±10.0, and 56.1±11.2 years for Group 1, Group 2, Group 3, and Group 4, respectively (p=0.001). Gender, diabetes mellitus, hypertension, coronary artery disease and model for end-stage liver disease (MELD) score were comparable between the groups (p=0.078, p=0.347, p=0.863, p=0.486 and p=0.119, respectively) (Table 1). The most common cause of ESLD was the Hepatitis B virus (47 patients) and the second most common cause was cryptogenic cirrhosis (45 patients).

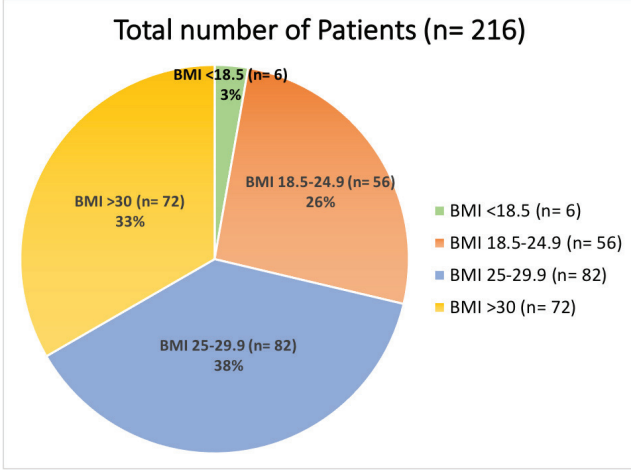

Figure 1. Number of patients according to groups
BMI: Body mass index (kg/m^2)

Table 1. Demographic and clinical characteristics of the patients who underwent liver transplantation					
	BMI <18.5 (n=6)	**BMI 18.5-24.9 (n=56)**	**BMI 25-29.9 (n=82)**	**BMI >30 (n=72)**	**p**
Age, years	47.5±12.8	47.9±14.3	54.4±10.0	56.1±11.2	0.001*
Gender (Male/Female)	2/4	40/16	62/20	45/27	0.078
Diabetes mellitus	2 (33.3%)	11 (19.6%)	26 (31.8%)	25 (34.7%)	0.347
Hypertension	0 (0%)	6 (10.7%)	6 (7.3%)	7 (9.7%)	0.863
Coronary artery disease	0 (0%)	11 (19.7%)	13 (15.9%)	8 (11.1%)	0.486
MELD score	22.5±8.1	16.4±6.1	16.8±6.4	15.4±5.6	0.119
BMI: Body mass index (kg/m^2), MELD: Model for end-stage liver disease, *Kruskal-Wallis test					

Table 2. Comparison of anesthesia and surgery related parameters according to the body mass index of the patients					
	BMI <18.5 (n=6)	**BMI 18.5-24.9 (n=56)**	**BMI 25-29.9 (n=82)**	**BMI >30 (n=72)**	**p**
Donor type (Living/deceased)	6/0	47/9	74/8	56/16	0.152
Cold ischemia time, minutes	38.5±20.9	114.0±151.8	77.7±92.5	105.5±115.7	0.320
Warm ischemia time, minutes	33.0±21.9	39.4±22.8	42.6±22.6	44.6±25.4	0.515
Urine output, mL	3108.3±1437.5	2454.1±1568.7	2392.9±1399.9	2264.3±1425.0	0.460
CVP (cm H_2O)					
At the induction	8.7±4.5	11.3±3.6	10.9±4.3	12.1±3.4	0.320
At the extubation	5.0±1.7	9.7±3.7	9.0±2.7	9.2±2.8	0.104
Blood loss, mL	2449.7±1470.5	2862.4±3329.6	2412.9±2186.3	3094.4±3454.6	0.636
Intraoperative transfusions, unit					
Red blood cell	3.5±3.2	3.9±4.0	3.0±2.9	3.7±3.4	0.415
Fresh frozen plasma	7.1±5.3	7.7±9.0	6.8±4.9	8.6±6.9	0.615
Platelet	0.2±0.5	0.9±1.5	0.6±1.0	1.0±1.6	0.704
Cryoprecipitate	0	1.0±2.3	1.1±1.9	0.9±1.6	0.372
Crystalloid fluid amount, mL	8000.0±4147.3	6631.2±3022.4	6227.3±2400.3	6212.1±3490.3	0.467
Colloid fluid amount, mL	400.0±223.6	448.6±253.5	475.4±293.6	560.4±354.8	0.549
Anesthesia duration, hours	8.0±1.7	7.9±1.5	7.9±1.7	7.9±1.9	0.939
Length of stay in ICU, days	2.8±1.3	2.8±1.9	2.3±1.7	3.5±4.3	0.081
Length of stay in hospital, days	19.3±8.9	16.4±9.7	17.7±12.1	16.2±12.8	0.669
BMI: Body mass index (kg/m^2), CVP: Central venous pressure, ICU: Intensive care unit					

Donor type, cold ischemia time, warm ischemia time, urine output, CVP at induction and at extubation, anesthesia duration, length of stay in ICU and length of stay in hospital were similar between the groups (p=0.152, p=0.320, p=0.515, p=0.460, p=0.320, p=0.104, p=0.939, p=0.081 and p=0.669, respectively). The mean blood loss was higher in Group 4 (3094.4 mL) than in other groups, but it was not statistically significant between the groups (p=0.636). The mean RBC, FFP, platelet and cryoprecipitate transfusions were also similar for the groups (p=0.415, p=0.615, p=0.704 and p=0.372, respectively). The mean IV crystalloid fluid amount gradually shortened from group 1 to group 4 but there was no significant difference between the groups (8000, 6631.2, 6227.3, and 6212.1 ml, respectively, p=0.467). Similarly, groups were comparable in terms of colloid fluid replacement (p=0.549) (Table 2).

The comparison of laboratory parameters is shown in Table 3. The mean platelet count, hemoglobin, activated partial thromboplastin time and fibrinogen were all similar between the groups. All these parameters did not differ between the groups in the preoperative and postoperative periods. The mean preoperative international normalized ratio (INR) was 2, 1.5, 1.8, and 1.5 for Group 1, Group 2, Group 3, and Group 4, respectively (p=0.033). Also, preoperative mean prothrombin time (PT) was significantly higher in Group 1 than in other groups (p=0.025). Both postoperative INR and PT were comparable between the groups (p=0.896 and p=0.711, respectively).

Discussion

Obesity is an important public health problem with an increasing worldwide prevalence. Globally, obesity has nearly tripled in the last 40 years. According to WHO, 39% of adults were overweight and 13% were obese in

Table 3. Comparison of laboratory parameters according to the body mass index of the patients					
	BMI <18.5 (n=6)	BMI 18.5-24.9 (n=56)	BMI 25-29.9 (n=82)	BMI >30 (n=72)	p
Platelet count (1000/mm³)					
Preoperative	148.2±84.9	123.4±103.0	87.3±47.3	90.6±54.9	0.136
Postoperative	10.8.0±59.4	74.8±52.8	62.9±36.8	58.4±28.3	0.360
Hemoglobin (g/dL)					
Preoperative	9.9±1.4	10.2±1.9	10.6±2.2	10.9±2.0	0.375
Postoperative	8.1±0.1	8.4±1.4	8.5±1.3	8.9±1.7	0.610
INR					
Preoperative	2.0±1.4	1.5±0.4	1.8±0.9	1.5±0.4	0.033*
Postoperative	2.2±0.3	2.2±0.9	2.2±0.7	2.0±0.5	0.896
Prothrombin time (seconds)					
Preoperative	24.0±10.4	17.5±3.9	21.2±7.7	18.8±5.3	0.025*
Postoperative	25.9±5.7	23.0±8.5	23.3±6.1	22.6±5.4	0.711
Activated partial thromboplastin time (seconds)					
Preoperative	28.3±3.3	37.6±6.0	37.9±2.6	38.9±6.1	0.086
Postoperative	38.5±2.1	54.1±40.4	39.4±4.9	48.2±22.5	0.833
Fibrinogen (mg/dL)					
Preoperative	239.8±141.5	273.4±97.0	216.5±85.4	244.8±111.4	0.103
Postoperative	118.0±32.5	145.6±44.2	111.9±34.5	114.7±37.1	0.116
BMI: Body mass index (kg/m²), INR: International normalized ratio, *Kruskal-Wallis test					

2016 (5). The rate of obese patients in LT recipients has also increased from 20% to 33% over the years (6). Non-alcoholic steatohepatitis, a consequence of obesity, is the fourth leading indication for LT and it is estimated to pass hepatitis C as the main indication in the next years (7). In this study, the rates of overweight and obese patients were 38% and 33.3%, respectively. Our rate of overweight patients was similar to population-based studies, and our obese patients' rate was higher than the overall prevalence as in other LT studies (6).

High BMI increases the risk of diseases such as cardiovascular and musculoskeletal diseases, and several cancers. It is also a predictor of decompensation of ESLD and is known to worsen portal hypertension (8). Regarding LT complications, it has been demonstrated that infectious and biliary complications are higher in obese patients (9). It can be thought that all these parameters may increase the duration of surgery and anesthesia. Obesity has been shown to increase the duration of surgery in some, but there are also studies reporting that it does not change the duration of surgery (10,11). In a study conducted on 813 liver recipients, it was reported that the mean operation time in morbidly obese patients (8.2 hours) was significantly longer compared to those with normal weight and underweight patients (7.2 hours) (9). However, Conzen et al. (7) concluded that there was no difference between the BMI groups in terms of operation time. In our study, anesthesia durations were similar between the groups, and obesity was not a factor affecting the duration of anesthesia.

Transplantation is surgery with a high risk of bleeding due to portal hypertension and coagulopathy in ESLD patients. The amount of bleeding can vary from hospital to hospital depending on many variables such as the recipient's preoperative clinical status, the condition of the graft, and the surgical technique. In our study, the mean blood loss of the groups is 2412-3094 ml and is not affected by the BMI category. These results are contrary to some studies showing that the amount of bleeding increases as BMI increases, but they are consistent with some other studies (7,9,12). We think that excessive fluid overload during LT is also one of the determining factors of bleeding. Therefore, we apply the restrictive fluid resuscitation strategy before the anhepatic phase in anesthesia management. Previously, preoperative blood products or agents have been used to correct an existing coagulopathy and fibrinolysis to reduce bleeding during LT (9). However, nowadays, we prefer to use these managements not preoperatively but during surgery, if needed.

Different results have been reported between obesity and blood transfusion in the literature. Although there are studies reporting that blood transfusion increases as BMI increases, there are also studies reporting that there is no difference (6,13). In studies conducted in 2001 and 2005, they found RBC transfusion as 13-16 units for the obese group and 11-16 units for the non-obese patients. In these studies, there was no difference between the groups in terms of both RBC, platelet, and FFP transfusion

(12,14). Correspondingly, in our study, the number of intraoperative transfusions was similar between the groups. Over the years, blood transfusion rates have decreased due to the increase in anesthesia and surgical experience and improvement of medical technology. In all groups, we performed LT with an average of 3-3.9 units of RBC transfusion intraoperatively. One of the factors that increase the blood product transfusion is the prolonged donor ischemia time (15). Our study groups were similar in terms of both cold ischemia time and warm ischemia time. We think that this is also effective in the similarity of blood transfusion rates.

Various strategies have been adopted to limit the amount of blood transfusion, including the use of autotransfusion devices, avoiding excessive fluid administration, and maintaining relative hypovolemia. During liver resections, a low CVP through volume restriction has been shown to reduce surgical blood loss and transfusion rate (16). However, it is not clear what an optimal CVP value is to prevent bleeding during LT. Accordingly, we apply restrictive fluid therapy before hepatectomy in anesthesia management to prevent volume overload. Although in the current study there was no difference in the mean intraoperative fluid replacement amount between the groups, the crystalloid amount was higher in Group 1. We argue that this may be due to the higher MELD scores of the patients in Group 1. Feltracco et al. (16) reported that individualized proper intraoperative fluids and prevention and treatment of coagulation abnormalities had a positive effect on the amount of bleeding and transfusion requirements. We found that, with the same approach as Feltracco et al. (16) similar fluid management can be performed in patients with different body mass indexes.

Significant changes are observed in the biochemical variables of the hemostatic system in patients with ESLD. These changes include thrombocytopenia, platelet functional defects, low coagulation factors and inhibitor levels, and low fibrinolytic protein levels. Similarly, the preoperative platelet count, INR and PT values of the patients in our study were abnormal. Preoperative INR and PT values of the patients in Group 1 were significantly higher than the other groups. We allege that these differences might be due to the higher MELD scores and severity of ESLD of the patients in Group 1. In those with only hemostatic disorders without liver disease, these abnormalities usually indicate a bleeding tendency. But the situation is different in patients with ESLD and the approach to hemostasis is changing recently. It is thought that the abnormal coagulation tests seen in patients with liver disease do not definitely specify an increased bleeding tendency (17). In a study of 206 liver transplants, it was found that the most important factors regarding the number of RBC transfusions were preoperative platelet count and duration of surgery (18). In accordance with this study, we found that our groups were similar in terms of these two parameters and blood product transfusions were also similar. In addition to routine coagulation tests, TEG quickly assesses functional coagulation status. TEG has been shown to improve the detection of intraoperative coagulation profiles and reduce blood transfusion (19). For the stated benefits, we use TEG in blood transfusion management during LT.

Long ICU and hospital stay are indicators of increased morbidity and factors that increase costs. Recipient age, severity of ESLD, preoperative nutritional status, donor age and weight, and complications are risk factors predicting a longer hospital stay (20). Previous reports have shown inconsistent results regarding ICU and hospital stay after LT in obese patients (7,21,22). This inconsistency can be explained by the differences in BMI categories in the groups, the attitudes of the institutes about the duration of discharge, and the number of patients in the studies. In our study, obesity is not a factor that affects on length of ICU and hospital stay. Our length of ICU stay was in concordance with a study by Conzen et al. (7) reporting 2-6 days, but our length of hospital stay was higher than their result which was 7-12 days.

Study Limitations

This study has its own limitations. First, the number of patients in the groups was not similar. Secondly, subgroup analysis was not performed (e.g., obese-nonobese, morbid obese-normal weight). Lastly, early postoperative blood transfusion data and graft-patient survival data were not included. These limitations should be considered in further studies.

Conclusion

Clear guidelines on the management of blood transfusion in obese patients during LT are lacking. In the present study, we found the same blood loss, blood transfusion, and fluid replacement between groups. We concluded that LT can be performed safely in patients with different BMI with similar blood loss and transfusion rates. Our results should be supported by prospective multicenter studies from different populations.

Authorship Contributions

Concept: T.U.Y., P.K., Design: T.U.Y., P.K., Data Collection or Processing: T.U.Y., Analysis or Interpretation: T.U.Y., P.K., Literature Search: T.U.Y., Writing: T.U.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Pandey CK, Singh A, Kajal K, et al. Intraoperative blood loss in orthotopic liver transplantation: The predictive factors. World J Gastrointest Surg 2015;7:86-93.
2. Wang M, Chen M, Ao H, Chen S, Wang Z. The Effects of Different BMI on Blood Loss and Transfusions in Chinese Patients Undergoing Coronary Artery Bypass Grafting. Ann Thorac Cardiovasc Surg 2017;23:83-90.
3. Villavicencio A, Lee Nelson E, Rajpal S, Vivek N, Burneikiene S. The impact of BMI on operating room time, blood loss, and hospital stay in patients undergoing spinal fusion. Clin Neurol Neurosurg 2019;179:19-22.
4. Higuera-de-la-Tijera F, Córdova-Gallardo J, Buganza-Torio E, et al. Hepamet Fibrosis Score in Nonalcoholic Fatty Liver Disease Patients in Mexico: Lower than Expected Positive Predictive Value. Dig Dis Sci 2021;11.
5. World Health Organization. Obesity and overweight. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed March 2021).
6. Spengler EK, O'Leary JG, Te HS, et al. Liver Transplantation in the Obese Cirrhotic Patient. Transplantation 2017;101:2288-96.
7. Conzen KD, Vachharajani N, Collins KM, et al. Morbid obesity in liver transplant recipients adversely affects longterm graft and patient survival in a single-institution analysis. HPB (Oxford) 2015;17:251-7.
8. Nishikawa H, Enomoto H, Nishiguchi S, Iijima H. Sarcopenic Obesity in Liver Cirrhosis: Possible Mechanism and Clinical Impact. Int J Mol Sci 2021;22:1917.
9. LaMattina JC, Foley DP, Fernandez LA, et al. Complications associated with liver transplantation in the obese recipient. Clin Transplant 2012;26:910-8.
10. Hotouras A, Ribas Y, Zakeri SA, et al. The influence of obesity and body mass index on the outcome of laparoscopic colorectal surgery: a systematic literature review. Colorectal Dis 2016;18:O337-O66.
11. Muelleman T, Shew M, Muelleman RJ, et al. Obesity Does Not Increase Operative Time in Otologic Surgery: An Analysis of 5125 Cases. Otol Neurotol 2018;39:e103-e7.
12. Hillingsø JG, Wettergren A, Hyoudo M, Kirkegaard P. Obesity increases mortality in liver transplantation–the Danish experience. Transpl Int 2005;18:1231-5.
13. Sawyer RG, Pelletier SJ, Pruett TL. Increased early morbidity and mortality with acceptable long-term function in severely obese patients undergoing liver transplantation. Clin Transplant 1999;13:126-30.
14. Nair S, Cohen DB, Cohen MP, Tan H, Maley W, Thuluvath PJ. Postoperative morbidity, mortality, costs, and long-term survival in severely obese patients undergoing orthotopic liver transplantation. Am J Gastroenterol 2001;96:842-5.
15. Braunfeld MY, Chan S, Pregler J, et al. Liver transplantation in the morbidly obese. J Clin Anesth 1996;8:585-90.
16. Feltracco P, Brezzi M, Barbieri S, et al. Blood loss, predictors of bleeding, transfusion practice and strategies of blood cell salvaging during liver transplantation. World J Hepatol 2013;5:1-15.
17. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med 2011;365:147-56.
18. Massicotte L, Sassine MP, Lenis S, Roy A. Transfusion predictors in liver transplant. Anesth Analg 2004;98:1245-51.
19. Hannaman MJ, Hevesi ZG. Anesthesia care for liver transplantation. Transplant Rev (Orlando) 2011;25:36-43.
20. Tovikkai C, Charman SC, Praseedom RK, Gimson AE, van der Meulen J. Time spent in hospital after liver transplantation: Effects of primary liver disease and comorbidity. World J Transplant 2016;6:743-50.
21. Amiri M, Toosi MN, Moazzami B, et al. Factors Associated With Length of Hospital Stay Following Liver Transplant Surgery. Exp Clin Transplant 2020;18:313-9.
22. Leonard J, Heimbach JK, Malinchoc M, Watt K, Charlton M. The impact of obesity on long-term outcomes in liver transplant recipients-results of the NIDDK liver transplant database. Am J Transplant 2008;8:667-72.

Original Article

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Endocan and Asymmetric Dimethylarginine as an Etiological Indicator in the Maternal and Umbilical Cord Serum in Pre-Eclampsia

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Abstract

Aim: Preeclampsia is a pregnancy-specific disease of unknown etiology. This study was planned to determine the place of asymmetric dimethylarginine (N, N-dimethylarginine, ADMA) and endothelial cell specific molecule-1 (ESM1, endocan) levels in etiology. The aim of this study was to determine ADMA and endocan levels in maternal and fetal umbilical cord serum of patients with preeclampsia and to evaluate them with clinical data.

Methods: This case-control study was conducted between June and December 2020. The clinical and demographic characteristics of the participants were evaluated in the department of obstetrics and gynecology. Thirty-tree women with preeclampsia and 55 healthy women in the same age group were included in our study. Serum ADMA and endocan values were determined by the ELISA method.

Results: Maternal and umbilical cord ADMA levels in the preeclampsia group were statistically significantly higher than the control group (p=0.001, p=0.001, respectively). Likewise, the levels of the umbilical cord and maternal serum endocan were statistically significantly higher in the preeclampsia group compared to the control group (p=0.001, p=0.037, respectively).

Conclusion: We found that ADMA and endocan molecules associated with endothelial dysfunction in the pathogenesis of preeclampsia significantly increased in maternal and umbilical cord serum.

Key words: N, N-dimethylarginine, preeclampsia, pregnancy, proteoglycans, umbilical cord

Introduction

Preeclampsia is the accompanying of proteinuria to new-onset hypertension in pregnancy. In some preeclampsia, proteinuria may not evidently develop. Therefore, preeclampsia is the presence of one of the findings of proteinuria accompanying gestational hypertension or gestational hypertension accompanied by at least one of thrombocytopenia (platelet <100,000/mm³), renal failure (creatinine doubled from baseline or >1.1 mg/dL), liver findings [Alanine aminotransferase (ALT) or aspartate transaminase (AST) doubling the normal], cerebral findings (headache, seizure, visual disturbances) or pulmonary edema findings (1,2).

Asymmetric dimethylarginine (ADMA) is an amino acid naturally occurring in plasma and is an endogenous nitric oxide synthase (NOS) inhibitor (3). Nitric oxide (NO) is a free radical, synthesized by the NOS from L-arginine. NO regulates endothelium-dependent vasodilation, proliferation of smooth muscle cells in the vascular wall, aggregation by platelet adhesion and monocyte adhesion inhibition. It also plays a role in maintaining vascular balance and blood supply to the organs (4). Since NO is a major endothelial vasoactive mediator, ADMA is thought to play a key role in endothelial dysfunction. The endocan molecule, also known as endothelial cell-specific molecule-1 (ESM-1), is a proteoglycan synthesized from endothelial cells and detectable in serum. It is found especially in lung, kidney

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cells, adipocyte and endothelium (5). ADMA is associated with many conditions such as intracellular signaling, differentiation, migration, proliferation and adhesion of different cell types. Increased release of ADMA in the tissue or its level in the blood reflects endothelial activation and neovascularization, which indicates inflammation and tumor progression (6).

The main problem in preeclampsia is endothelial cell damage due to increased inflammatory response. Endothelial cell damage and subsequent endothelial dysfunction make sense of clinical findings detected in preeclampsia disease. The cause of the symptoms that play a role in preeclampsia clinic is diffuse endothelial dysfunction (7). It is thought that the molecule NO has a key role in the regulation of endothelial function. The aim of this study was to evaluate the maternal and fetal umbilical cord serum levels of ADMA and endocan molecules, which are associated with endothelial dysfunction and have different results in the literature, and to investigate the relationship between preeclampsia clinical effects and these parameters.

Methods

Study Population

This case-control study was conducted between June and December 2020. The clinical and demographic characteristics of the participants were evaluated in the department of obstetrics and gynecology. This study was approved by the Ethics Review Board of Yozgat Bozok University Faculty of Medicine (document no: 2017-KAEK-189_2020.06.23_08). The study was carried out in accordance with the principles of medical research provided by the Helsinki Declaration. Written informed consent was obtained from each participant. Preeclampsia diagnosis after the 20th week of gestation was made by blood pressure measurements taken at least four hours intervals and determined by systolic pressure above 140 mmHg and diastolic pressure above 90 mm Hg and also by measuring proteinuria in 24-hour urine ≥0.3 g; proteinuria/creatinine ratio ≥0.3 or ≥+1 proteinuria in spot urine sample (8). Diabetes mellitus, thyroid diseases, cardiovascular diseases, chronic renal failure, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia and 21-hydroxylase deficiency were accepted as exclusion criteria for the preeclampsia group. The control group was composed of normotensive 18-40 years old 370/7-406/7 w healthy singleton pregnant women. Body mass index (BMI) was calculated by dividing body weight by the square of height (kg/m²). Patients' age, gravidity, parity, gestational weeks, systolic/diastolic blood pressure levels, protein levels in spot urine protein, hemoglobine, platelet counts, creatinine, urea, liver function markers (AST, ALT, lactate dehydrogenase), angiogenic and antiangiogenic factors sEng, sFlt and Pgf levels were recorded. Gestational weeks were determined according to the last menstrual period confirmed by ultrasonography.

Determination of Serum ADMA and Endocan Levels

Venous blood samples were taken from the participants before giving birth and taken from the umbilical cord at birth. Venous blood samples were collected in a 5 mL serum-separating vacuum tube. Blood samples were collected and centrifuged for 10 min at 2.000 g. The supernatant was quickly removed and kept frozen at -80 °C until the assays were performed. Serum ADMA (cat. no REA201/96, BioVendor, Czech Republic) and Endocan (cat. no E3160Hu, Bioassay Technology Laboratory, China) levels were measured with commercially available enzyme-linked immune sorbent assay (ELISA) kits, with a minimum detectable concentration of 0.4 µmol/L and 5 ng/L, respectively, according to the manufacturer's instructions. Optical density values for samples and standard samples were detected on Thermo Scientific (USA) Multiscan Go Microplate Reader ELISA reader at 450 nm. The results are presented as µmol/L and ng/mL.

Statistical Analysis

Statistical analysis was performed using SPSS (version 20, SPSS Inc., Chicago, IL, USA). For each continuous variable, normality was checked by Kolmogorov-Smirnov and Shapiro-Wilk tests. The categorical variables between the groups were analyzed by using the chi-square test or Fisher's Exact test. Comparisons between groups were applied using Student's t-test (normally distributed data) and Mann-Whitney U test (not normally distributed data). A multivariate logistic regression analysis was performed

Table 1. Comparison of demographic data between preeclampsia and control groups

	Control (n=55)	Preeclampsia (n=33)	p
Age (year)	29.8±4.8	29.3±6.3	0.650**
BMI (kg/m²)	29.5±4.1	29.1±3.3	0.761*
Gravida	2.8±1.8	3.3±2.2	0.423*
Parity	1.8±1.5	1.8±1.9	0.557*
Gestational week	37.5±1.1	33.3±4.3	**0.001***
Gender Male (n) (%) Female (n) (%)	 23 (44.2%) 29 (55.8%)	 20 (51.3%) 19 (48.7%)	0.505*
Neonatal weight (g)	3203±553.9	2252.8±1017.6	**0.001***
Systolic pressure (mmHg)	109.8±14.7	135.1±24.5	**0.001***
Diastolic pressure (mmHg)	70.4±9.7	86.3±14.3	**0.001***

Values are presented as mean ± standard deviation. *Mann-Whitney U test, **Independent samples t-test. BMI: Body mass index

Table 2. Comparison of data between preeclampsia and control groups

	Control (n=55)	Preeclampsia (n=33)	p
AST (U/L)	17.3±8.4	24.9±20.5	0.019*
ALT (U/L)	15.4±14.2	15.2±12.6	0.533*
BUN (mg/dL)	7.5±2.5	19.4±10.6	**0.001***
CRE (U/L)	0.5±0.1	0.6±0.2	**0.001***
LDH (U/L)	198.7±26.6	304.3±167.4	**0.001***
Spot urine protein (mg/dL)	0±0	1.5±2	**0.001***
24-h urine (mg/day)	-	1500.9±1378.6	-
Hemoglobin (g/dL)	11.6±1.4	11.8±1.8	0.488**
Hematocrite (%)	34.1±3.9	35.2±4.5	0.291**
Platelets (10^3/mm^3)	234.1±41.2	205.9±64.2	**0.015****
Maternal ADMA (µmol/L)	0.8±0.2	1.2±1.1	**0.001***
Umbilical cord ADMA (µmol/L)	1.2±2.2	1.5±0.5	**0.001***
Maternal Endocan (ng/mL)	2.7±1.1	3.1±0.5	**0.037***
Umbilical cord Endocan (ng/mL)	3.7±1.9	4.9±0.8	**0.001***

Values are presented as mean ± standard deviation. *Mann-Whitney U test, **Independent samples t-test. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, CRE: Creatinine, LDH: Lactate dehydrogenase, 24-h urine: 24 hours urine

to determine independent risk factors. A p-value of less than 0.05 was considered significant.

Results

The demographic characteristics of the groups in our study were presented in Table 1. When the groups were compared statistically, there was no significant difference in age and BMI averages, but systolic pressure and diastolic pressure were significantly higher in the preeclampsia group (p<0.001).

Biochemical parameters and ELISA results of the study groups were presented in Table 2. Maternal serum and umbilical cord serum ADMA values were significantly higher in the preeclampsia group compared to the control group (p<0.001). Likewise, the umbilical cord and maternal serum endocan levels were found to be significantly higher in the preeclampsia group (p<0.05) (Table 2).

In the multivariate logistic regression analysis, the most important independent risk factor predicting preeclampsia was maternal ADMA [Odds ratio (OR): 6.8, 95% confidence interval (CI): 1.7-8.9], and the other independent risk factor was determined as cord endocan (OR: 3.7, 95% CI: 2.1-5.5) (Table 3). This result indicated that the rising of maternal ADMA increases the risk of preeclampsia 6.8 times, and the rising of cord endocan increases the risk of preeclampsia 3.7 times.

Table 3. Multivariate logistic regression analysis results of ADMA and endocan values

	B	SE	p	OR	95% CI	
					Lower	Upper
Maternal ADMA (µmol/L)	4.6	3.7	**0.001**	6.8	1.7	8.9
Umbilical cord ADMA (µmol/L)	3.6	0.2	0.076	1.5	0.8	2.5
Umbilical cord endocan (ng/mL)	1.4	0.5	**0.011**	3.7	2.1	5.5
Maternal endocan (ng/mL)	0.4	0.6	0.200	1.2	0.8	6.8

ADMA: Asymmetric dimethylarginine, B: Regression coefficient, CI: Confidence interval, OR: Odds ratio, SE: Standart error

Discussion

Endothelial dysfunction is accepted to be the basis of the pathogenesis of preeclampsia (9). It is a disease with vasospasm due to endothelial damage, activation of the coagulation system, edematous ischemic and thrombotic sequelae that progresses negatively in humoral and local control affecting blood pressure and fluid volume (10). Maternoplacental ischemic environment occurring after defective placentation causes placental factors to be released and pass into the maternal circulation. This condition initiates maternal endothelial cell damage and dysfunction. The vasodilator effect is disrupted by the anticoagulant effect of the intact endothelium, the balance of prostaglandin production and the release of NO (9,11,12). In this study, the maternal and umbilical cord serum levels of NOS inhibitor ADMA and a prostaglandin endocan were evaluated in preeclampsia patients. ADMA and endocan levels were found to be high in the maternal and umbilical cord in the preeclampsia group. Serum ADMA and endocan levels can be considered as independent risk factors for preeclampsia.

ADMA levels have been associated with many diseases such as renal diseases, Alzheimer's, liver failure, cirrhosis, cardiovascular diseases, diabetes and preeclampsia. The inhibition of NO synthesis by ADMA causes endothelium-dependent vasodilation (3). Increased levels of ADMA have been associated with endothelial dysfunction, thus preeclampsia (13). In the study conducted by Fickling et al. (14) ADMA was associated with preeclampsia and the ADMA level was found to be significantly higher in the preeclampsia group compared to healthy pregnant women. Data supporting the same result were presented and various authors emphasized that increasing ADMA levels may play an important role in the pathogenesis of preeclampsia. These data indicated that high circulating ADMA concentration in pregnant women could be defined as a potential biomarker of preeclampsia (15-17). It has also been reported that the ADMA level decreases during normal pregnancy, decreases to a minimum at

the end of the first trimester and then increases with the gestational age (18). On the other hand, it was determined that serum ADMA levels of pregnant women who developed preeclampsia were high in the first trimester and in the second trimester (19), and serum ADMA levels of pregnant women who did not develop preeclampsia and had Small for Gestational Age were normal (20). It was demonstrated that the increase in ADMA levels developed before the clinical findings of preeclampsia at 23 weeks of gestation (21). In preeclampsia studies performed in the umbilical cord serum, there are different data in the literature as in maternal serum ADMA results. Albayrak et al. (22) brought out no change in maternal serum ADMA level of the preeclampsia group, while they found the umbilical cord serum ADMA level higher than controls. In another study, both maternal serum and umbilical cord serum levels were detected to be higher in the preeclampsia group compared to the control (23). Even in the preeclampsia group, serum ADMA levels of the umbilical cord were noticed to be significantly higher in the severe preeclampsia group compared to the mild preeclampsia group (13).

Endocan is a proteoglycan secreted from vascular endothelia cells, indicative of endothelial function. It has been propounded that serum concentrations are elevated in conditions associated with endothelial activation or dysfunction. Studies have suggested that endocan can be a marker in many diseases such as tissue damage, angiogenesis, oncogenesis, inflammation, and sepsis (24). When we looked at the studies conducted with preeclampsia pregnant women in the literature, there were studies reporting that serum endocan levels were statistically higher than healthy pregnant women (5,10,11). In a meta-analysis study involving 451 preeclampsia pregnant women, it was determined that the level of endocan was higher in pregnant women with preeclampsia compared to normal healthy pregnant women (25). However, in other studies, no difference was found between serum endocan levels in the preeclampsia and the control groups (26,27). Even though the endocan protein and its expression in the maternal placenta were studied out to be higher than the control, no significant difference was found in the maternal serum level (27). A factor affecting the serum endocan level is the gestational week and it has been reported that as the gestational week increases, the serum endocan level decreases (26). A similar result was determined in patients with early onset preeclampsia, and no significant result was found (28). Moreover, no significant difference was observed between the groups with early and late preeclampsia and normotensive pregnant women (29). Schuitemaker et al. (30) highlighted that the endocan molecule has an angiogenic function in the first and second trimesters; therefore, the endocan level may be low in early-onset preeclampsia because angiogenesis is disrupted. A study on the endocan level in the fetal umbilical cord in preeclampsia patients is not included in the literature. In one of the existing studies, the umbilical cord endocan level in pregnancies complicated by intrauterine growth restriction (31) and in the other one, the umbilical cord endocan level was evaluated according to the delivery type (32). In this study, ADMA and endocan levels were found to be high in the maternal and umbilical cord in the preeclampsia group. Differences in the results of the studies may be based on the fact that they were performed without subgrouping, different samples (plasma, serum or placenta) were studied, and the number of different pregnant women.

Study Limitations

Our study has a few limitations. First, the sample size is relatively small. Second, there may be different ELISA kits used. However, evaluating both umbilical cord and maternal serum levels together is a factor that strengthens our study. At the same time, evaluating these two molecules, which are important in etiology, makes the study valuable.

Conclusion

In conclusion, the most important reasons for the different results between studies on ADMA and endocan include the difference in gestational week, the difference in tissue studied and the sample number. In future studies, expanding the sample size, measurements of each trimester of pregnancy, expression, protein and serum levels of maternal, placenta and umbilical cord samples and dividing preeclampsia into different subgroups according to its severity will provide more confirmed results.

Authorship Contributions

Concept: N.I., E.B., Design: N.I., E.B., Data Collection or Processing: N.I., E.B., D.A.K., Analysis or Interpretation: N.I., Literature Search: N.I., E.B., D.A.K., Writing: N.I., D.A.K.

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References

1. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol 2013;122:1122-31.
2. Nirupama R, Divyashree S, Janhavi P, Muthukumar SP, Ravindra PV. Preeclampsia: Pathophysiology and management. J Gynecol Obstet Hum Reprod 2021;50:101975.

3. Böger RH, Vallance P, Cooke JP. Asymmetric dimethylarginine (ADMA): a key regulator of nitric oxide synthase. Atheroscler Suppl 2003;4:1-3.
4. Guerby P, Tasta O, Swiader A, et al. Role of oxidative stress in the dysfunction of the placental endothelial nitric oxide synthase in preeclampsia. Redox Biol 2021;40:101861.
5. Hentschke MR, Lucas LS, Mistry HD, Pinheiro da Costa BE, Poli-de-Figueiredo CE. Endocan-1 concentrations in maternal and fetal plasma and placentae in pre-eclampsia in the third trimester of pregnancy. Cytokine 2015;74:152-6.
6. Leite AR, Borges-Canha M, Cardoso R, Neves JS, Castro-Ferreira R, Leite-Moreira A. Novel Biomarkers for Evaluation of Endothelial Dysfunction. Angiology 2020;71:397-410.
7. Feng X, Liu Y, Zhang Y, et al. New views on endothelial dysfunction in gestational hypertension and potential therapy targets. Drug Discov Today 2021;26:1420-36.
8. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010;376:631-44.
9. Mauro AK, Khurshid N, Berdahl DM, et al. Cytokine concentrations direct endothelial function in pregnancy and preeclampsia. J Endocrinol 2021;248:107-17.
10. Adekola H, Romero R, Chaemsaithong P, et al. Endocan, a putative endothelial cell marker, is elevated in preeclampsia, decreased in acute pyelonephritis, and unchanged in other obstetrical syndromes. J Matern Fetal Neonatal Med 2015;28:1621-32.
11. Cakmak M, Yilmaz H, Bağlar E, et al. Serum levels of endocan correlate with the presence and severity of pre-eclampsia. Clin Exp Hypertens 2016;38:137-42.
12. Cunningham FG. Hypertensive disorders. In: F. Gary Cunningham, Kenneth J. Leveno, Steven L. Bloom, et al. editors. Williams Obstetrics. 24th ed. New York: Mc Graw Hill; 2014. p. 728-79.
13. Pettersson A, Hedner T, Milsom I. Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. Acta Obstet Gynecol Scand 1998;77:808-13.
14. Fickling SA, Williams D, Vallance P, Nussey SS, Whitley GS. Plasma concentrations of endogenous inhibitor of nitric oxide synthesis in normal pregnancy and pre-eclampsia. Lancet 1993;342:242-3.
15. Böger RH, Diemert A, Schwedhelm E, Lüneburg N, Maas R, Hecher K. The role of nitric oxide synthase inhibition by asymmetric dimethylarginine in the pathophysiology of preeclampsia. Gynecol Obstet Invest 2010;69:1-13.
16. Yuan J, Wang X, Xie Y, et al. Circulating asymmetric dimethylarginine and the risk of preeclampsia: a meta-analysis based on 1338 participants. Oncotarget 2017;8:43944-52.
17. Meng W, Zhanashunbayaer, E L, Li R. Association between asymmetric dimethylarginine level and preeclampsia: a meta-analysis. Int J Clin Exp Med 2017;10:8720-7.
18. Holden DP, Fickling SA, Whitley GS, Nussey SS. Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. Am J Obstet Gynecol 1998;178:551-6.
19. Bian Z, Shixia C, Duan T. First-Trimester Maternal Serum Levels of sFLT1, PGF and ADMA Predict Preeclampsia. PLoS One 2015;10:0124684.
20. Speer PD, Powers RW, Frank MP, Harger G, Markovic N, Roberts JM. Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational-age infants. Am J Obstet Gynecol 2008;198:112.e1-7.
21. Savvidou MD, Hingorani AD, Tsikas D, Frölich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. Lancet 2003;361:1511-7.
22. Albayrak M, Öncül M, Uludağ S, et al. Comparison of maternal serum and umbilical cord concentrations of nitric oxide and asymmetric-dimethyl-arginine in pre-eclamptic and uncomplicated pregnancies. J Exp Clin Med 2013;30:125-8.
23. Gumus E, Atalay MA, Cetinkaya Demir B, Sahin Gunes E. Possible role of asymmetric dimethylarginine (ADMA) in prediction of perinatal outcome in preeclampsia and fetal growth retardation related to preeclampsia. J Matern Fetal Neonatal Med 2016;29:3806-11.
24. Yilmaz MI, Siriopol D, Saglam M, et al. Plasma endocan levels associate with inflammation, vascular abnormalities, cardiovascular events, and survival in chronic kidney disease. Kidney Int 2014;86:1213-20.
25. Lan X, Liu Z. Circulating endocan and preeclampsia: a meta-analysis. Biosci Rep 2020;40:BSR20193219.
26. Yuksel MA, Tuten A, Oncul M, et al. Serum endocan concentration in women with pre-eclampsia. Arch Gynecol Obstet 2015;292:69-73.
27. Chang X, Bian Y, Wu Y, Huang Y, Wang K, Duan T. Endocan of the maternal placenta tissue is increased in pre-eclampsia. Int J Clin Exp Pathol 2015;8:14733-40.
28. Ovayolu A, Karaman E, Turgut A, Guler S, Bostancieri N. Maternal serum endothelial cell-specific molecule-1 level and its correlation with severity of early-onset preeclampsia. J Obstet Gynaecol 2020:1-6.
29. Lopes ACDS, Martins SR, Dusse LMS, Pinheiro MB, Alpoim PN. Are Endocan Plasma Levels Altered in Preeclampsia? Rev Bras Ginecol Obstet 2021;43:232-4.
30. Schuitemaker J, Woudenberg J, Wijbenga G, Scherjon S, van Pampus M, Faas M. PP077. New prognostic marker for the risk to develop early-onset preeclampsia. Pregnancy Hypertens 2013;3:95-6.
31. Szpera-Gozdziewicz A, Kosicka K, Gozdziewicz T, et al. Maternal Serum Endocan Concentration in Pregnancies Complicated by Intrauterine Growth Restriction. Reprod Sci 2019;26:370-6.
32. Aksoy M, Aksoy AN, Laloglu E, Dostbil A, Celik MG. Umbilical cord blood endocan levels according to the delivery mode. Clin Exp Obstet Gynecol 2015;42:776-80.

Original Article

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Risk Factors and Seroprevalence of Syphilis Among Naive HIV Patients: A Cross-Sectional Study From a Tertiary Center

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Abstract

Aim: Syphilis coinfection is common among human immune deficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) diagnosed patients. We aimed to investigate the rates of syphilis coinfection, social-demographical features, viral and immunological properties among naive HIV/AIDS patients.

Methods: Socio-demographical data, CD4+ T lymphocyte counts, HIV viral load, syphilis antibody results and physical examinations at the time of diagnosis of naive HIV infected patients who were admitted to our center between January 2017 and January 2021, were evaluated retrospectively from the medical records.

Results: Three hundred-two naive HIV patients were enrolled in the study. Positive syphilis antibody rate was detected as 21.5% and 86.2% of those patients were male and also found 31.4% ($p<0.01$) in patients in which route of transmission was men sex with men (MSM). Positive syphilis antibody rate was found to be statistically higher in patients with substance abuse ($p<0.01$), CD4+ T lymphocyte counts were under 200 cell/mm^3 ($p=0.018$) and viral load above 100.000 copies/mL ($p<0.01$).

Conclusion: Among HIV-infected patients, those with MSM and substance abuse constitute the group at highest risk of syphilis. Therefore, syphilis must be screened and treated, especially at HIV diagnosis and high-risk behaviors.

Keywords: AIDS, coinfection, HIV infections, syphilis

Introduction

Syphilis is a systemic infectious disease caused by the spirochete bacterium *Treponema pallidum* (1). Human immune deficiency virus (HIV) and syphilis are sexually transmitted infections with similar transmission routes, such as unprotected sexual contact, blood transfusion, and vertical transmission from mother to baby (2). For early-stage HIV-positive individuals, the already existing high risk of transmission might be further increased with the incidence of syphilis due to genital/oral ulcers, decreased CD4+ T lymphocyte count and an increase of viral load. Besides, the disease course of syphilis progresses differently in HIV patients with diminished host defense due to impaired immune response and results in shortening of incubation time and increasing in number and infectiousness of syphilis lesions (3,4).

Since the first reported HIV case in 1985, the total number of HIV cases in Turkey has reached 25,809, according to the Turkish Ministry of Health surveillance between 1985 and 2020 (5). On the other hand, in studies regarding the seroprevalence of syphilis in potential risk groups in Turkey, rates between 0.11% and 19.3% were revealed. The highest rates were detected among men sex with men (MSM), followed by sex workers and

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blood donors (6-8). Syphilis coinfection is common in HIV-infected individuals due to sharing similar transmission routes (9). Although there are several studies and reports on the seroprevalence of HIV and syphilis separately, there is a lack of data regarding the seroprevalence of syphilis in HIV patients. Because of these close relationships, current guidelines recommend routine serological testing for syphilis during HIV infection screening and/or in patients diagnosed with HIV infection (10,11).

In order to prevent the spread of HIV and syphilis, determining the seroprevalence and associated risk factors of syphilis among HIV-infected patients will provide important information. The present study which contributes to the literature intended to investigate the rates of syphilis coinfection, social-demographical features, viral and immunological properties among naive HIV/AIDS patients.

Methods

Study Design

The study was approved by the University of Health Sciences Turkey, Izmir Bozyaka Training and Research Hospital's Local Ethics Committee with the approval number (2020-172). Informed consent was obtained from all participants included in the study. This study was conducted at the University of Health Sciences Turkey, Izmir Bozyaka Training and Research Hospital, Clinical Microbiology and Infectious Diseases outpatient clinic, between January 2017 and January 2021. Syphilis test results at the time of diagnosis of naive HIV patients those admitted to our outpatient clinic were investigated retrospectively and evaluated. Besides, social-demographical data regarding age, gender, marital status, sexual orientation, education, smoking, alcohol usage, substance abuse, HIV transmission route and physical examination findings were obtained from medical records. CD4+ T lymphocyte count, HIV viral load and syphilis antibody status at the time of diagnosis were evaluated.

Laboratory Assessment

CD4+ T lymphocyte counts were determined by using flow cytometry BD FACSCanto II (BD Diagnostic Systems, Sparks, MD, USA). According to the Centers for Disease Control and Prevention (CDC) staging system for classification of HIV-infected persons, CD4+ T lymphocyte counts <200 cell/mm³ were defined as stage-3; CD4+ T lymphocyte counts 200-499 cell/mm³ were defined as stage-2 and CD4+ T lymphocyte counts >499 cell/mm³ were defined as stage-1 (12). HIV-RNA levels were measured by polymerase chain reaction method (Artus HI Virus-1 QS-RGQ Kit, QIAGEN, Germany). According to the viral load, HIV-RNA >100.000 copy/mL was defined as high viral load whereas, HIV-RNA <100.000 copy/mL was defined as low viral load. Since January 2017, a reverse algorithm has been used in terms of serological diagnosis of syphilis (13). A chemiluminescent microparticle immune assay (CMIA) based test (Architect Syphilis TP, Abbott, USA) was used in terms of the initial screening test. According to the algorithm, an additional test was not performed for patients with CMIA negative, whereas patients with positive CMIA were tested with rapid plasma reagin (Omega Diagnostics, England). Positive results in both tests were considered to be syphilis-positive. In case of incompatible results, positive results with CMIA were verified with Treponema pallidum hemagglutination (Omega Diagnostics, England) test.

Statistical Analysis

All statistical analyses were performed using SPSS 18.0 (Chicago, IL USA). A p-value less than 0.05 was considered statistically significant. Comparisons between the two groups were assessed by chi-square and Fisher's exact test analysis. Comparison of numerical variables between two groups was made using Student's t-test when the normal distribution condition was met. On the other hand, when the normal distribution condition was not met, numerical variables were made between groups using the Mann-Whitney U test. All of the numerical variables with normal distribution were expressed as the means ± SD. All of the numerical variables without normal distribution were expressed as median interquartile range values. Pearson analysis was used for univariate correlations between variables.

Results

A sum of 302 naive HIV patients were enrolled in the study. Sixty-five patients (22%) were syphilis-positive, and 237 patients (78%) were syphilis-negative. Male gender dominancy existed in both syphilis-positive and negative patients, but there was no significant difference between groups (p=0.11). There was no statistically significant difference between syphilis-positive and syphilis-negative patients in terms of age (p=0.20).

In terms of sexual orientation, 159 patients (53%) were homosexual, 128 patients (42%) were heterosexual, and 15 patients (5%) were transsexual. Syphilis-positivity rates were most common among homosexual patients (31%) p<0.01.

There was no significant difference between syphilis-positive and syphilis-negative patients in terms of smoking and alcohol usage. On the other hand, a considerable difference between syphilis-positive and syphilis-negative patients was found in substance abuse (p<0.01).

Patients with high viral load, defined as HIV-RNA levels above 100.000 copies/mL, were detected in 69% of syphilis-

positive patients, whereas it was 50% in Syphilis- negative patients. There was a significant difference between groups in terms of high viral load existence (p<0.01).

According to the CDC staging system of HIV-infected patients, the rate of patients in stage-3 was higher in syphilis-positive patients (28%) compared to syphilis-negative patients (15%). In terms of classifying patients according to CD4 counts (stage-1, stage-2 and stage-3), there was a statistically significant difference between syphilis-positive and syphilis-negative patients (p=0.01), respectively.

According to the physical examination findings and symptoms of 65 syphilis-positive patients, 9 patients (13.8%) were considered primary syphilis, and 25 patients (38.5%) were considered secondary syphilis. Two of the patients with secondary syphilis were with syphilitic uveitis. The remaining 31 patients (47.7%) without either symptom or physical examination findings were considered latent Syphilis.

The demographical features and laboratory results of patients with positive and negative syphilis are presented in Table 1.

Discussion

Syphilis and HIV have mutual adverse effects on disease courses. Syphilis results in diminishing CD4 T lymphocyte levels and increasing HIV viral load during the HIV disease course. On the other hand, HIV results in increasing neurological complications and decreasing response to therapy during the syphilis disease course (14). Therefore, screening of HIV-infected patients in terms of other sexually transmitted diseases, including syphilis, is recommended at the time of diagnosis (15). Considering the high prevalence of syphilis and the increasing prevalence of HIV in many countries, early diagnosis of syphilis and HIV coinfection is very important. Although the relationship between HIV and syphilis has not been clarified yet, it was shown in the studies that syphilis enhances the contamination risk of HIV (16). The seroprevalence of syphilis among newly diagnosed HIV patients was reported as 25% in Mexico, 21% in Spain, 19.8% in China and 15.7% in the USA (17-20). The differences between countries regarding social, cultural, and economic differences seem to affect the incidence of sexually transmitted diseases. In Turkey, the seroprevalence of syphilis among HIV patients was reported between the range of 7.6% and 25%, and it was presented in Table 2 (21-26). In our study, the seroprevalence of syphilis among HIV patients was found to be 22%. This might be due to the differences between regions in terms of social, cultural, and economic reasons, which was shown in the studies in the literature (24).

Table 1. Demographical features and laboratory results of syphilis (-) and syphilis (+) patients

Total n=302	Syphilis (-) (n=237) n (%)	Syphilis (+) (n=65) n (%)	p
Gender Female Male Transgender Total (302)	29 (12.2) 196 (82.7) 12 (5.1) 237 (78.5)	3 (4.6) 56 (86.2) 6 (9.2) 65 (21.5)	0.11*
Age years	35±11	37±11	0.20**
Sexual orientation Heterosexual Homosexual Transsexual	 116 (48.9) 109 (46.0) 12 (5.1)	 12 (18.5) 50 (76.9) 3 (4.6)	**<0.01***
Marital status Single Married	 169 (71.3) 68 (28.7)	 54 (83.1) 11 (16.9)	0.05*
Education illiterate Primary school Secondary High school Universite	7(3) 49 (20.7) 35 (14.8) 73 (30.8) 73 (30.8)	1 (1.5) 14 (21.5) 9 (13.8) 18 (27.7) 23 (35.4)	0.73*
Reason for HIV testing another illness screening	89 (37.6) 148 (62.4)	32 (49.5) 33 (50.8)	0.08*
Smoking No Yes	 95 (40.1) 142 (59.9)	 23 (35.4) 42 (64.6)	0.49*
Alcohol No Yes	 107 (45.1) 130 (54.9)	 23 (35.4) 42 (64.9)	0.15*
Substance abuse No Yes Used to	 178 (75.1) 33 (13.9) 26 (11.0)	 40 (18.3) 20 (37.7) 5 (7.7)	**<0.01***
CD4 + T lymphocyte count (/mm³) ≥500 (stage-1) 200-499 (stage-2) <200 (stage-3)	 87 (36.9) 114 (47.9) 36 (15.3)	 27 (41.5) 20 (30.8) 18 (27.7)	**0.01***
HIV-RNA level(copymL) ≤100.000 >100.000	 119 (50.2) 118 (49.8)	 20 (30.8) 45 (69.2)	**<0.01***

*Chi-square test p<0.05 **Student's t-test

Table 2. The studies about HIV-syphilis coinfection in Turkey

Researcher	Publication year	HIV (+) patients (n)	Syphilis patients n (%)
Kaptan et al. (21)	2009	92	9 (9.8)
Sayan et al. (22)	2012	117	9 (7.6)
Aydin et al. (23)	2013	308	40 (12.9)
Sarigül et al. (24)	2018	3641	291 (8)
Sarigül et al. (25)	2018	384	97 (25)
Korkusuz et al. (26)	2020	1057	194 (18.3)

HIV: Human immunodeficiency viruses

The dominancy of male gender among patients with HIV-syphilis coinfection, which was found in our study and

similar studies in the literature, is a remarkable finding. In the report of CDC, which was published in 2018, the rise of the incidence of HIV-syphilis coinfection was attributed to the rapid increase in the number of HIV-infected patients who were MSM and also, it was reported that, syphilis prevalence is increased among bisexual and homosexual men (27). In Turkey, while the contamination rate of HIV/AIDS among heterosexual patients was declined to 35.4% from 50%, the contamination rate among patients who were MSM, increased from 8% to 13.4% (24). In a recently published study, the contamination rate of HIV-syphilis coinfection among patients who were MSM was found to be increased from 23% to 43% between 2012 and 2015 (28). This rate was found as 52.6% in our study. In a meta-analysis in West Europe, the seroprevalence of syphilis was found between the range of 14% and 59% among HIV-infected patients who were MSM (29). In a study conducted in China this rate was found as 22% (30). In two recently published studies in Turkey, the rate of syphilis among HIV-infected patients who were MSM was between 22% and 55.9% (25,26). In our cohort, syphilis seroprevalence was found 31.4%, 9.4%, and 20% among MSM, heterosexual, and transexual patients, respectively. In addition, the syphilis-positivity rate was found statistically significant ($p<0.01$) among patients who were MSM, which was following the other studies.

The higher incidence among these patient groups was mostly attributed to various reasons such as multiple sexual partners among patients who were MSM, inappropriate condom usage, the increase in the tendency of easy partner finding at web/smartphone applications, commonly usage of psychostimulant agents and sexual performance-enhancing drugs, the common belief about the idea that oral sex is a safer way for sexual contact (31). In a study conducted in Turkey, the syphilis-positivity rate was found 16.8% among newly diagnosed HIV patients and the incidence was found to be increased at 18.3% during the follow-up period. The rise in the incidence rate was attributed to sexual contact with multiple partners and the low rates of condom usage (26).

In terms of marital status, 73.8% of our patients were single and the rate of single patients among syphilis-positive patients was 83%. Although it was not found to be statistically higher ($p=0.056$), the high rates were remarkable. Studies revealed that single patients have tendency in multiple sex partners compared to the patients who are married. As a consequence, the seroprevalence of syphilis and HIV was found to be increased in parallel to multiple sexual partners. On the other hand, men display more risky behavior in terms of sexual contact (30).

In a recent study conducted in Istanbul, 50.7% of HIV patients were found to be primary school graduates and syphilis seropositivity rates were found to be associated with low education levels (23). However, in our study, 31.8% of HIV patients were college degree educated and was seen no association between education level and syphilis seroprevalence. This might be due to the regional differences in terms of education since Istanbul is the biggest and most cosmopolitan city in Turkey. The education level in the general population is relatively better in Izmir compared to Istanbul.

The most common reason for HIV testing was found to be due to application for another illness (40.1%) and 26.4% of these patients were found to be syphilis-positive. The second common reason for HIV testing was own request for sexual transmitted disease testing (25.5%). The syphilis seropositivity rate was found 23.4% in these patients who were recorded to declare risky sexual contact.

Coinfection of HIV-syphilis was shown to affect disease course of HIV via increase in viral load and/or decrease in CD4+ T lymphocyte count (32,33). The coexistence of these two highly contagious diseases was investigated in several studies in public health literature (34). In our study, 17.9% of our patients were stage-3 patients (CD4+ T lymphocyte counts ≤200) and the syphilis-positivity rate was 33.3% which was statistically significant compared to patients at stage-1 and stage-2 ($p=0.018$). Classification of patients in terms of viral load revealed that 54% of patients had a high viral load (HIV-RNA >100,000 copy/mL). The syphilis-positivity rate was found 27.6% in this situation, in which contamination risk is highest, and is found statistically significant ($p<0.01$).

The existence of uveitis in patients with secondary syphilis and the dominancy of patients in latent period were in accordance with the findings in the literature which might be due to the variable clinical features in HIV-syphilis coinfection.

Study Limitations

The only limitations of this study are that it is a single-centre study. Not all patients were evaluated in terms of other sexually transmitted diseases at the time of diagnosis. Despite the limitations, the study will contribute to the literature by demonstrating the rise of syphilis seroprevalence in newly diagnosed HIV-positive patients in recent years.

Conclusion

According to the HIV/AIDS patient profile in Turkey, sexual contact seems to be the main transmission route. The possibility of HIV-syphilis coinfection should be kept in mind because of sharing the same transmission route. Therefore, screening tests for syphilis should be considered in the routine follow-up procedures of patients with suspicious clinical findings and risky behavior and the evaluation of newly diagnosed naive HIV patients.

Authorship Contributions

Concept: H.O.O., Design: H.O.O., I.B., S.T., Data Collection or Processing: I.B., Analysis or Interpretation: H.O.O., I.B., Literature Search: H.O.O., S.T., I.B., Writing: H.O.O., I.B.,

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References:

1. Tudor ME, Al Aboud AM, Gossman WG. Syphilis. Last Updated March 22, 2021. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534780/. Accessed May 6, 2021.
2. Maan I, Lawrence DS, Tlhako N, et al. Using a dual antibody point-of-care test with visual and digital reads to diagnose syphilis among people living with HIV in Botswana. Int J STD AIDS 2021;32:453-61.
3. Kotsafti O, Paparizos V, Kourkounti S, Chatziioannou A, Nicolaidou E, Kapsimali V, Antoniou C. Early syphilis affects markers of HIV infection. Int J STD AIDS 2016;27:739-45.
4. Frunza-Stefan S, Acharya G, Kazlouskaya V, Vukasinov P, Chiou Y, Thet Z. Immune reconstitution inflammatory syndrome associated with secondary syphilis. Int J STD AIDS 2017;28:302-5.
5. TC.Sağlık Bakanlığı HIV-AIDS İstatistik Available from: https://hsgm.saglik.gov.tr/tr/bulasici-hastaliklar/hiv-aids/hiv-aids-liste/hiv-aids-istatislik.html. Accessed May 6, 2021.
6. Oncul O, Emekdas G, Cavuslu S, Artuk C, Aksoy A. The sixteen-year trend of syphilis in Turkey: data from blood donors. Trop Doct 2008;38:181-2.
7. Akın L. Epidemiology of sexually transmitted infection: review. Turkiye Klinikleri J Med Sci 2006;26:655-65.
8. Köksal MO, Beka H, Evlice O, et al. Syphilis seroprevalence among HIV-infected males in Istanbul, Turkey. Rev Argent Microbiol 2020;52:266-71.
9. Karumudi UR, Augenbraun M. Syphilis and HIV: a dangerous duo. Expert Rev Anti Infect Ther 2005;3:825-31.
10. European AIDS Clinical Society (EACS) Guidelines. Version 10.1 - October 2020. Available from: https://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.htmlAccessed May 6, 2021.
11. HIV infection: detection, counseling, and referral. Sexually transmitted diseases treatment guidelines CDC; 2021 Available from: https://www.cdc.gov/std/tg2015/hiv.htm. Accessed May 6, 2021.
12. Centers for Disease Control and Prevention (CDC). Revised surveillance case definition for HIV infection–United States, 2014. MMWR Recomm Rep 2014;63(RR-03):1-10.
13. French P, Gomberg M, Janier M, et al. IUSTI: 2008 European Guidelines on the Management of Syphilis. Int J STD AIDS 2009;20:300-9.
14. Salado-Rasmussen K. Syphilis and HIV co-infection. Epidemiology, treatment and molecular typing of Treponema pallidum. Dan Med J 2015;62:B5176.
15. TC. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü. HIV/AIDS Tanı Tedavi Rehberi 2019. Available from: https://hsgm.saglik.gov.tr/depo/birimler/Bulasici-hastaliklar-db/hastaliklar/HIV-ADS/Tani-Tedavi_Rehberi/HIV_AIDS_Tani_Tedavi_Rehberi_Yeni.pdf.Accessed May 6, 2021.
16. Staneková D, Kramárová P, Wimmerová S, Hábeková M, Takáčová M, Mojzesová M. HIV and risk behaviour among men who have sex with men in Slovakia (2008-2009). Cent Eur J Public Health 2014;22:239-44.
17. Mata-Marín JA, Sandoval-Sánchez JJ, Huerta-García G, et al. Prevalence of antibodies against Treponema pallidum among HIV-positive patients in a tertiary care hospital in Mexico. Int J STD AIDS 2015;26:81-5.
18. Pérez C C, Cerón A I, Fuentes L G, et al. Coinfecciones por virus hepatitis B, virus hepatitis C, Treponema pallidum y Toxoplasma gondii en la cohorte de pacientes VIH positivos en control en la Pontificia Universidad Católica de Chile [Hepatitis B, C, Treponema pallidum and Toxoplasma gondii co-infections in HIV infected patients]. Rev Med Chil 2009;137:641-8.
19. Hu QH, Xu JJ, Zou HC, et al. Risk factors associated with prevalent and incident syphilis among an HIV-infected cohort in Northeast China. BMC Infect Dis 2014;14:658.
20. Bernstein KT, Stephens SC, Strona FV, Kohn RP, Philip SS. Epidemiologic characteristics of an ongoing syphilis epidemic among men who have sex with men, San Francisco. Sex Transm Dis 2013;40:11-7.
21. Kaptan F, Örmen B, Türker F, et al. İnsan immun yetmezlik virüsü ile enfekte 128 olgunun retrospektif olarak değerlendirilmesi. Türkiye Klinikleri J Med Sci 2011;31:525-33.
22. Sayan M, Willke A, Ozgunes N, Sargın F. HIV-1 subtypes and primary antiretroviral resistance mutations in antiretroviral therapy naive HIV-1 infected individuals in Turkey. Jpn J Infect Dis 2013;66:306-11.
23. Aydin ÖA, Karaosmanoğlu HK, Sayan M, İnce ER, Nazlıcan Ö. Seroprevalence and risk factors of syphilis among HIV/AIDS patients in Istanbul, Turkey. Cent Eur J Public Health 2015;23:65-8.

24. Sarigül F, Sayan M, İnan D, et al. Current status of HIV/AIDS-syphilis co-infections: a retrospective multicentre study. Cent Eur J Public Health 2019;27:223-8.

25. Sarıgül F, Üser Ü, Öztoprak N. HIV/AIDS hastalarında sifilis koinfeksiyonu seroprevalansı ve risk faktörleri. Klimik Dergisi 2019;32:161-4.

26. Korkusuz R, Şenoğlu S. Syphilis Seroprevalence and Associated Risk Factors in HIV-infected Individuals. Mediterr J Infect Microb Antimicrob 2020;9:13.

27. United States Centers for Disease Control and Prevention. Sexually TransmittedDiseaseSurveillance2018. Available from: https://www.cdc.gov/std/stats18/default.htm Accessed May 6, 2021.

28. Sayan M. Turkey's HIV dynamics and the patient universe. In: HIV epi¬demiological status and prevention strategies in Turkey: KLIMIK HIV Study Group Meeting 2017 Oct 14; Istanbul, Turkey Available from: https://www.klimik.org.tr.

29. Pines HA, Wertheim JO, Liu L, Garfein RS, Little SJ, Karris MY. Concurrency and HIV transmission network characteristics among MSM with recent HIV infection. AIDS 2016;3:2875-83.

30. Tong ML, Lin LR, Liu LL, et al. Analysis of 3 algorithms for syphilis serodiagnosis and implications for clinical management. Clin Infect Dis 2014;58:1116-24.

31. Forsyth AD, Valdiserri RO. A State-Level Analysis of Social and Structural Factors and HIV Outcomes Among Men Who Have Sex With Men in the United States. AIDS Educ Prev 2015;27:493-504.

32. Jarzebowski W, Caumes E, Dupin N, et al. Effect of early syphilis infection on plasma viral load and CD4 cell count in human immunodeficiency virus-infected men: results from the FHDH-ANRS CO4 cohort. Arch Intern Med 2012;172:1237-43.

33. Farhi D, Dupin N. Management of syphilis in the HIV-infected patient: facts and controversies. Clin Dermatol 2010;28:539-45.

34. Rompalo AM, Joesoef MR, O'Donnell JA, et al. Clinical manifestations of early syphilis by HIV status and gender: results of the syphilis and HIV study. Sex Transm Dis 2001;28:158-65.

Original Article

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The Effect of Prone Position on the Integrated Pulmonary Index (IPI) Score in Lumbar Disc Surgeries Performed with Spinal Anesthesia

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Abstract

Aim: It is a known fact that the prone position has curative effects on respiratory parameters. To evaluate the effect of prone position on respiratory status in lumbar discectomy operations with spinal anesthesia using integrated pulmonary index (IPI), which is a novel tool that incorporates different respiratory parameters.

Methods: A total of 40 patients were enrolled in this prospective, observational study between December 2020 and February 2021. The IPI parameters included end-tidal carbon dioxide, respiratory rate, pulse rate and oxygen saturation recorded at the time of admission to the operating room, at ten minutes after spinal anesthesia administration, at ten minutes following prone positioning and ten minutes after the end of the operation.

Results: The mean end-tidal carbon dioxide value significantly increased after prone positioning and at the end of the operation. The mean oxygen saturation similarly increased at the end of the operation. There was a moderately significant correlation between the mean IPI scores after prone positioning and ten minutes after the administration of spinal anesthesia.

Conclusion: Prone position did not show any negative effect on respiratory mechanics as obtained from IPI, while it increased oxygenation. IPI may be a valuable tool in clinical practice to monitor respiratory mechanics in the prone position in patients undergoing lumbar disc surgeries.

Keywords: Prone position, integrated pulmonary index, respiratory mechanics, anesthesia, spinal, diskectomy

Introduction

Lumbar disc herniation (LDH) is a localized displacement of intervertebral disc tissue beyond its physical margins, causing lower back pain, radicular pain, numbness and motor weakness. The incidence of LDH is between 5 and 20 per 1,000 adults with a male/female ratio of 2:1 (1). Lumbar discectomy, one of the most common treatment methods of LDH, is the most frequently performed spinal surgery operation by neurosurgeons (2).

Spinal anesthesia provides a safe and highly satisfying alternative to general anesthesia, especially or patients undergoing limited lumbar surgeries (3). The advantages of spinal anesthesia over general anesthesia in lumbar disc surgery include decreased use of antiemetics and analgesics, lower rates of hemodynamic and respiratory complications, shorter length of stay in hospital and reduced costs (4,5). In addition, there are studies showing shorter operational times with spinal anesthesia (6).

The prone position is used during anesthesia to provide operative access in a wide variety of surgical operations, especially spinal surgeries. However, intraoperative complications such as accidental extubation and postoperative complications such as airway edema have been associated with prone position (7). In addition, since the abdomen and chest are restricted during the prone position, respiratory compliance decreases (8). Thus, placing an anesthetized patient into the prone position during spinal surgeries increases the risk of improper ventilation, making continuous monitoring of the respiratory status mandatory (9). Nevertheless, currently very little is known about the effects of prone position on respiratory mechanisms.

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The ıntegrated pulmonary ındex (IPI) incorporates four real-time respiratory measurements [end-tidal CO_2, respiratory rate, pulse rate and peripheral oxygen saturation (SpO_2)] into a single value between 1 and 10, which represents the respiratory status of the patients (10). There is no study in the literature investigating the effect of prone position on respiratory status using the IPI. Therefore in this study, we aimed to evaluate the effect of prone position on respiratory status in diskectomy operations with spinal anesthesia using the IPI.

Methods

Before the beginning of the study, ethics approval was received from the Recep Tayyip Erdogan University, Non-interventional Clinical Research Ethics Committee with the 2020/214 numbered decision. All patients were informed about the objectives of the study and gave written consent. The study was conducted in accordance with the Declaration of Helsinki.

This prospective, observational, cross-sectional study included patients aged between 18-65 years, classified as American Society of Anesthesiologists (ASA) 1-2 according to the ASA, and scheduled for L_4-$_5$, L_5-S_1 lumbar discectomy operation under spinal anesthesia between December 2020 and February 2021.

A total of 45 patients were included in the study. Two patients were excluded from the study, because spinal anesthesia was contraindicated, one patient because an adequate blockage could not be achieved and two patients due to missing data. Finally, the study was completed with 40 patients.

Patients with a basal mean arterial pressure (MAP) <65 mmHg, a room air SPO_2<92, and a heart rate (HR) out of the range of 45-120 bpm, those with a body mass index (BMI) >30 m^2/kg, patients with previously defined respiratory problems, chest and spinal deformity, patients with insufficient blockage and those who needed additional analgesics and sedatives during the procedure were excluded from the study.

After taken to the operating room, all patients were followed-up with routine monitorization including electrocardiogram, noninvasive arterial blood pressure and IPI (Capnostream 20 Oridion Medical 1987 Ltd., Jerusalem, Israel). This software provides a single index between 1-10 based on 4 physiological parameters including end-tidal carbon dioxide pressure ($EtCO_2$), respiratory rate (RR), SpO_2 and HR (Table 1).

Ringer lactate solution was initiated at mL/kg/h in patients with intravenous (i.v.) access provided. The patients were sedated with i.v. midazolam (0.02 mg/kg) received O_2 support at 2L/min with IPI monitor nasal cannula throughout the operational procedure. For spinal anesthesia, 3 mL 0.5% hyperbaric bupivacaine was administered with a 25 G spinal needle from the L_{3-4} or L_{4-5} intervertebral space. The patients were kept waiting for ten minutes and given a prone position after the adequate block was evaluated with the pin-prick test and Bromage score. 10 mg i.v. ephedrine was administered in the patients with a MAP that dropped more than 30% of the baseline value, and 0.5 mg i.v. atropine in those with an HR ≤45 bpm after spinal anesthesia.

Table 1. Integrated pulmonary ındex scoring

Integrated pulmonary ındex	Patient status
10	Normal
8-9	Within normal range
7	Close to normal range - requires attention
5-6	Requires attention and may require intervention
3-4	Requires intervention
1-2	Requires immediate intervention

Patients' demographic (age, gender, BMI, ASA) and operative data were recorded and analyzed. Monitored MAP, $EtCO_2$, RR, SpO_2, HR and IPI values were recorded at the time of admission to the operating room (T*0*), at ten minutes after spinal anesthesia administration (T*SPI*), at ten minutes following prone positioning (T*PR*) and ten minutes after the end of the operation in the supine position (T*SPII*).

Statistical Analysis

For the statistical analysis of the data obtained in this study, NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) software was utilized. Descriptive statistical methods (mean, standard deviation, frequency, percentage, minimum, maximum) were used in the expression of the variables and the distribution was analyzed with the Shapiro-Wilk test. The comparison of non-normally distributed variables between two groups was made using the Mann-Whitney U test. Whereas, Friedman test was used for comparisons of quantitative data for three or more non-normally distributed periods and the Wilcoxon test for comparison of the quantitative data between non-normally distributed two periods. The correlations within the variables were analyzed with Spearman's correlation analysis. P<0.05 values were considered statistically significant.

Results

A total of 40 patients, including 22 (55.0%) male and 18 (45.0%) female, were included in this prospective observational study. The mean age of the patients was found as 48.03±12.9 (range: 21-65) years. Physical status of the patients at the time of admission was found as ASA1 in 17 (42.5%) patients and ASA2 in 23 (57.5%) patients.

Table 2. Demographic features of the patients		
Age (Years), Mean ± SD	-	48.02±12.9
BMI (Kg/m²), Mean ± SD	-	26.02±2.73
Gender, n (%)		
Male	22 (55)	-
Female	18 (45)	-
ASA Status, n (%)		
ASA I	14 (42.5)	-
ASA II	23 (57.5)	-
SD: Standard deviation, ASA: American Society of Anesthesiologists, BMI: Body mass index		

The mean BMI was calculated as 26.02±2.73 (range: 21.04-29.94) kg/m^2. Demographic features of the patients are given in Table 2. The mean sensory level was as 8.95±1.01 (range: 8-10) and the mean Bromage value was 2.73±0.45 (2-3). The mean operational time was measured as 55.50±13.89 (38-90) minutes.

When monitoring values of the patients were evaluated; $ETCO_2$ and SpO_2 values showed statistical significance according to the time points. Accordingly, the mean $ETCO_2$ was significantly lower at T*0* compared to T*PR* and T*SPII* (POSTOP) values (p=0.001, p<0.01; respectively). Similarly, the mean $ETCO_2$ value was statistically significantly lower at T*SPI* compared to T*PR* and T*SPII* (POSTOP) values (p=0.001, p<0.01; respectively). The mean SpO_2 value was statistically significantly lower at T*0* and T*SPI* compared to T*SPII* (POSTOP) (p=0.001, p<0.01; respectively). No statistically significant difference was found between HR, MAP, RR and IPI values according to the time points (p>0.05) (Table 3).

Operative data included in the study were further compared between genders based on the time points. Accordingly, there was no statistically significant difference between the male and female patients in terms of the mean HR, MAP, $ETCO_2$, SpO_2 and IPI values at all time points (for all p>0.05). The mean RR value was significantly higher at T*SPI* in the female patients compared to the male patients (p=0.02).

The correlations between the variables at different time points were evaluated with the Spearman's correlation analysis. Accordingly, there was a statistically significant difference between the mean HR value at T*PR* and the mean HR values at T*0*, T*SPI* and T*SPII* time points (for all p<0.001). Similarly, significant correlations were found between the mean MAP and $ETCO_2$ values at T*PR* and the mean $ETCO_2$ values measured at other time points. A positive correlation was found between the mean RR values at T*PR* and the mean RR values measured at T*0* and T*SPI* time points. The mean IPI value measured at T*PR* was positively correlated with the mean values measured at T*SPI* time point. Table 4 shows the correlation between the monitored values measured at ten minutes after prone positioning and the other time points.

Discussion

In the present study, we investigated the effects of the prone position on respiratory mechanics using the IPI scoring. Risks and benefits of the prone position during anesthesia in several surgeries have been controversial in the literature. Some studies have emphasized the increased oxygenation with prone position, while the others have underlined compromised pulmonary compliance (11,12).

In a study by Palmon et al. (13) it was reported that the prone position increases the risk of improper ventilation during spine surgery. On the contrary, Miller reported that the lungs expanded better and oxygenation improved in patients under anesthesia in the prone position (14). The improvement of oxygenation with the prone position has been attributed to a reduction in intrapulmonary shunt that results in better ventilation (15). Furthermore, in this position, movement of the chest wall significantly increases, contributing to improved oxygenation. On the other hand, other beneficial hemodynamic effects with the prone position have been reported including increased exhalation volume, better blood circulation in the lungs and transformation of the effective area of the lungs (16). In a study by Black and Hawks, (17) SPO_2 was significantly increased in 75% of the patients 30 minutes after prone positioning. Similarly, in a study by Yazdannik et al. (18) SpO_2 value was significantly increased 30 minutes after prone positioning of the patients. In the present study, the mean SPO_2 value was significantly higher at the end of the operation compared to the baseline value, showing a gradual improvement in oxygenation.

In an animal study comparing hemodynamic parameters between various positions during surgery, no difference was found between the positions in terms of the MAP (19). Again in a study by Cheng et al. (20) no significant difference was found between the MAP values during and at the end of the operation in the prone position. In our study, we could not find a significant difference between the mean MAP values at different time points.

In a study by Yadav et al. (21) with patients undergoing cervical spine surgery in supine and prone positions, no significant change was found in $ETCO_2$ values with the prone position. In the present study, the mean $ETCO_2$ value significantly increased after prone positioning compared to the baseline value. In a study by Ariagno et al. (22) less variability was reported in HR with the prone position. Studies in the literature reported no significant differences in RR with the prone position (23,24). Similarly in our study, among the IPI parameters HR and RR values did not show significant variability with the prone position.

However, all the above-mentioned studies have been conducted in different patient populations undergoing different surgical operations in the prone position. Since

Table 3. Monitoring values according to the time points						
		T0	TSPI	TPR	TSPII (POSTOP)	p
HR	Mean ± SD	76.23±9.04	73.8±10.13	74.55±10.31	76.9±10.22	0.057
MAP	Mean ± SD	98.83±12.61	94.75±11.82	95.05±11.87	98.63±11.15	0.355
RR	Mean ± SD	16.88±3.86	18.18±4.55	16.65±3.1	16.85±3.01	0.165
$ETCO_2$	Mean ± SD	35.18±4.33	34.53±4.62	36.75±3.39	36.73±3.8	**0.001****
SpO_2	Mean ± SD	97.2±2.37	97.6±2.12	97.28±6.39	98.6±1.46	**0.001****
IPI	Mean ± SD	9.65±0.7	9.33±1.12	9.78±0.42	9.78±0.58	0.11

Friedman test **p<0.01: Statistical significance
HR: Heart rate, MAP: Mean arterial pressure, RR: Respiratory rate, $ETCO_2$: End-tidal carbon dioxide, SpO_2: Oxygen saturation, SD: Standart deviation

Table 4. Correlations between the variables measured at prone position and the other time points						
TPR	T0		TSPI		TSPII (POSTOP)	
	r	p	r	p	r	p
HR	0.608	<0.001	0.701	<0.001	0.65	<0.001
MAP	0.544	<0.001	0.708	<0.001	0.532	<0.001
RR	0.531	<0.001	0.317	<0.001	-	-
$ETCO_2$	0.619	<0.001	0.672	<0.001	0.534	<0.001
SpO_2	-	-	0.433	<0.001	0.673	<0.001
IPI	-	-	0.467	<0.001	-	-

Spearman correlation analysis; p<0.05: Statistical significance
HR: Heart rate, MAP: Mean arterial pressure, RR: Respiratory rate, $ETCO^2$: End-tidal carbon dioxide, SpO_2: Oxygen saturation

there is no study in the literature to evaluate the effects of the prone position on respiratory mechanics, we could not exactly compare our results with the other studies.

IPI is a decision-making algorithm that incorporates four respiratory parameters including $ETCO_2$, RR, pulse rate and SpO_2 into a single value between 1 and 10 to indicate the respiratory status of patients (25). It combines ventilation monitoring and oxygenation monitoring and is measured by capnography. IPI is used by clinicians to evaluate the need for additional intervention. IPI score has been investigated in limited studies in the literature to evaluate respiratory mechanics. In a study by Mermer et al. (26) with geriatric patients undergoing spinal anesthesia, IPI scores were found to be in the normal range both in unilateral and bilateral spinal anesthesia. The authors reported that the IPI score may be valuable in early identification of respiratory failure. However, there was no study in the literature to investigate the effects of prone position on the IPI. In our study, there were no statistically significant changes in the mean IPI scores at all time points in the patients undergoing lumbar discectomy. On the other hand, there was a moderately significant correlation between the mean IPI scores after prone positioning and ten minutes after the administration of spinal anesthesia.

IPI score may be a useful monitoring tool to use in the operating room in the evaluation of respiratory status and to take necessary action timely. But more evidence is needed to routinely introduce this tool in anesthesia practice.

Study Limitations

This study has several limitations. First, the study included a relatively small number of patients treated in a single center. Second, IPI parameters could be compared with another group of patients in various variants of the prone position or with standard monitoring. However, the prospective design of the study is our strength. In addition, this study is the first in the literature to investigate the effects of the prone position on the IPI. Studies on IPI monitoring during anesthesia are very limited. Further studies are needed on this issue to confirm utility of the IPI index in monitoring during lumbar disc surgeries.

Conclusions

The results of this study indicate that the prone position did not show any negative effect on respiratory mechanics as obtained from IPI, while it increased oxygenation. IPI may be a valuable tool in clinical practice to monitor respiratory mechanics in the prone position in patients undergoing lumbar disc surgeries. It is an easy to handle tool showing many respiratory parameters together to timely alert anesthetists when necessary.

Authorship Contributions

Concept: S.B., Design: S.B., O.E.B., Data Collection or Processing: O.E.B., Analysis or Interpretation: S.B., Literature Search: S.B., O.E.B., Writing: S.B., O.E.B.,

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Al Qaraghli MI, De Jesus O. Lumbar Disc Herniation. In: StatPearls. Treasure Island (FL): StatPearls Publishing; April 2, 2021.
2. Hareni N, Strömqvist F, Strömqvist B, Sigmundsson FG, Rosengren BE, Karlsson MK. Back pain is also improved by lumbar disc herniation surgery. Acta Orthop 2021;92:4-8.
3. Sung TY, Jee YS, You HJ, Cho CK. Comparison of the effect of general and spinal anesthesia for elective cesarean section on maternal and fetal outcomes: a retrospective cohort study. Anesth Pain Med (Seoul) 2021;16:49-55.
4. Walcott BP, Khanna A, Yanamadala V, Coumans JV, Peterfreund RA. Cost analysis of spinal and general anesthesia for the surgical treatment of lumbar spondylosis. J Clin Neurosci 2015;22:539-43.
5. De Cassai A, Geraldini F, Boscolo A, et al. General Anesthesia Compared to Spinal Anesthesia for Patients Undergoing Lumbar Vertebral Surgery: A Meta-Analysis of Randomized Controlled Trials. J Clin Med 2020;10:102.
6. Pierce JT, Kositratna G, Attiah MA, et al. Efficiency of spinal anesthesia versus general anesthesia for lumbar spinal surgery: a retrospective analysis of 544 patients. Local Reg Anesth 2017;10:91-8.
7. Chui J, Craen RA. An update on the prone position: Continuing Professional Development. Can J Anaesth 2016;63:737-67.
8. Stone JG, Khambatta HJ. Pulmonary shunts in the prone position. Anaesthesia 1978;33:512-7.
9. Manna EM, Ibraheim OA, Samarkandi AH, Alotaibi WM, Elwatidy SM. The effect of prone position on respiratory mechanics during spinal surgery. Middle East J Anaesthesiol 2005;18:623-30.
10. Turan G, Kuplay Y, Karip C, Koksal C, Akın C, Akgun N. Integrated Pulmonary Index: A New Strategy for Respiratory Patients Evaluation. Int J Anesthetic Anesthesiol 2016;3:042.
11. Taccone P, Pesenti A, Latini R, et al. Prone positioning in patients with moderate and severe acute respiratory distress syndrome: a randomized controlled trial. JAMA 2009;302:1977-84.
12. Pelosi P, Croci M, Calappi E, et al. Prone positioning improves pulmonary function in obese patients during general anesthesia. Anesth Analg 1996;83:578-83.
13. Palmon SC, Kirsch JR, Depper JA, Toung TJ. The effect of the prone position on pulmonary mechanics is frame-dependent. Anesth Analg 1998;87:1175-80.
14. Gropper M, Eriksson L, Fleisher L, Wiener-Kronish JP, Cohen N, Leslie K. Elsevier Health Sciences; 2014. Miller's Anesthesia.
15. Tawhai MH, Nash MP, Lin CL, Hoffman EA. Supine and prone differences in regional lung density and pleural pressure gradients in the human lung with constant shape. J Appl Physiol (1985) 2009;107:912-20.
16. Jozwiak M, Teboul JL, Anguel N, et al. Beneficial hemodynamic effects of prone positioning in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 2013;188:1428-33.
17. Black JM, Hawks JH. Saunders/Elsevier; 2009. Medical-surgical nursing: Clinical Management for Positive Outcomes, 8e without CD A st edition.
18. Yazdannik A, Gholami F, Zerraatkari K, Saghaei M. Effect of prone position on cardiac and pulmonary indices in patients under mechanical ventilation. IJNR 2006;1:15-21.
19. Hwang JH, Kwon YS, Kang EH, et al. Prone positioning improves oxygenation without adverse hemodynamic effects during partial liquid ventilation in a canine model of acute lung injury. Korean J Intern Med 2004;19:237-42.
20. Cheng MA, Todorov A, Tempelhoff R, McHugh T, Crowder CM, Lauryssen C. The effect of prone positioning on intraocular pressure in anesthetized patients. Anesthesiology 2001;95:1351-5.
21. Yadav M, Reddy EP, Sharma A, Kulkarni DK, Gopinath R. The Effect of Position on PaCO2 and PETCO2 in Patients Undergoing Cervical Spine Surgery in Supine and Prone Position. J Neurosurg Anesthesiol 2017;29:298-303.
22. Ariagno RL, van Liempt S, Mirmiran M. Fewer spontaneous arousals during prone sleep in preterm infants at 1 and 3 months corrected age. J Perinatol 2006;26:306-12.
23. Antunes LC, Rugolo LM, Crocci AJ. Efeito da posição do prematuro no desmame da ventilação mecânica [Effect of preterm infant position on weaning from mechanical ventilation]. J Pediatr (Rio J) 2003;79:239-44.
24. Levy J, Habib RH, Liptsen E, et al. Prone versus supine positioning in the well preterm infant: effects on work of breathing and breathing patterns. Pediatr Pulmonol 2006;41:754-8.
25. Kumar V, Taft A, Johnson S, Herrington R, Castresana M. Integrated Pulmonary Index for Successful Weaning Obese Patients After Cardiac Bypass Surgery. Crit Care Med 2013;41pA90.
26. Mermer D, Turan G, Koksal C, Tas B, Karadogan F, Akgun N. Correlation of integrated pulmonary index with clinical observation in unilateral and bilateral spinal anaesthesia in geriatric patients. Int J Res Med Sci 2016;4:4438-43.

Original Article

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Thiol Disulfide Homeostasis of Pediatric Oncology Patients After the Positron Emission Tomography/ Computerized Tomography Imaging: A Cross-Sectional Study

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Abstract

Aim: Positron emission tomography/computerized tomography (PET/CT) is used for oncologic imaging as hybrid imaging. Pediatric patients are exposed to ionizing radiation when they undergo hybrid medical imaging examinations. It is especially important to minimize the radiation exposure when children and infants are imagined, as immature tissues are more susceptible to damage induced by radiation. This study aims to reveal the oxidant and antioxidant status in oncology patients before and after PET/CT imaging.

Methods: Between February 2019 and July 2019, pediatric oncology patients who underwent PET imaging at the time of diagnosis in our center were included in the study. Eighteen newly diagnosed cancer pediatric patients participated in this prospective cross-sectional study. Data were saved to synchronously kept excel log files and used in the study. Disulphide amounts, total and native thiols, native thiol/total thiol percent ratios, disulphide/native thiol percent ratios, disulphide/total thiol percent ratios, and Ischemia-Modified Albumin were calculated before and after the PET/CT imaging.

Results: Disulphide levels, disulphide/total thiol and disulphide/native thiol ratios of patients' serum samples were significantly higher and the ratio of native thiol/total thiol was reduced after the PET/CT.

Conclusion: There was disturbed thiol-disulfide homeostasis and the balance changed in the direction of oxidant damage. Our results indicate the possible oxidative stress condition due to radiation exposure with PET/CT in pediatric oncology patients.

Keywords: Thiol disulfide homeostasis, ionizing radiation, PET/CT

Introduction

Positron emission tomography (PET) is a noninvasive imaging procedure and it is useful for clinical diagnosis, staging and treatment (1). According to the recent reports, there are significantly higher specificity, sensitivity, and accuracy in the hybrid PET/computerized tomography (CT) imaging than those in the conventional imaging for evaluation of the pediatric malignancies (2,3). A baseline PET/CT scan is also the best tool available for subsequent assessment of response. PET/CT enables the characterization and detection of disease in the early stages and gives worthy information for diagnosis not easily obtained based on the traditional techniques for imaging (4-6).

Stochastic effects such as cancer are due to translocations in cells and radiation-induced DNA mutation. These effects follow a hidden period between 2 and 30 years (7). Due to longer life periods of children for the deleterious stochastic effects after irradiation, they are more exposed toin coordin ionizing radiation than adults. Therefore, radiation exposure during PET/

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CT imaging is important in pediatric patients in terms of oxidant damage. Although the oxidant damage of ionizing radiation is known, the effect after PET/CT imaging is not fully known.

The antioxidant defense system inhibits the oxidative effects of free radicals. There is a sulfhydryl group in thiols with an essential role in the coordination of the antioxidant defense system. Oxidation reaction can occur in the thiols throughout oxidant, forming disulphide bonds. When there is oxidative stress, sulfhydryl groups are changed into disulphide bridges subsequently converted into thiols again. There is a balanced continuation of the cycle. Thus, thiol disulfide homeostasis (TDH) is kept. TDH is essential in apoptosis, oxidative stress, protection against antioxidants, detoxification, enzymatic activity, and cellular signal transmission (8).

In the present study, thiol levels were evaluated in cancer patients to show oxidant-antioxidant balance due to radiation exposure before and after PET/CT imaging. To our knowledge this is the first study, presenting data that cancer patients oxidative stress status with thiol/disulfide homeostasis.

Methods

Study Design

Approval from the University of Health Sciences, Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital Ethics Review Committee obtained (no: 2019-019). Between February 2019 and July 2019, pediatric oncology patients who underwent PET imaging at the time of diagnosis in Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital were included in the study. Eighteen newly diagnosed cancer pediatric patients participated in this prospective study. Data were saved to synchronously kept excel log files and used in the study. Written informed consent was obtained from all patients before participation in the study.

Inclusion criteria were: newly diagnosed cancers; Non-Hodgkin lymphoma, Hodgkin lymphoma, neuroblastoma, Langerhans cell histiocytosis, primitive neuro-ectodermal tumor, rhabdomyosarcoma, Wilms tumor and exclusion criteria were: scans required or recommended at primary site such as magnetic resonance imaging (MRI), Ultrasound, ECHO, CT other soft tissue sarcomas, scans for patients who relapsed. Patients with clinical findings such as infection, fever and vomiting that may cause oxidant damage were excluded from the study too.

Each patient was required to be admitted as at least 6 hours of fasting. Blood glucose levels were measured in each child before radiopharmaceutical injection. Two hundred mg/dL of blood glucose level was used as criteria for postponing the imaging procedure. F-18 Fluorodeoxyglucose (F-18 FDG) calculated as 3-5 MBq/kg body weight was injected intravenously to the child. They were advised to rest i low light and quiet room for approximately 45-60 min after injection. CT, PET and PET/CT fusion images were taken through vertex to mid-thigh scanning on 3D PET/CT device (Philips Gemini TF 16 w/TOF Performance PET/CT, Philips Medical Systems Nederland B.V.). Images were interpreted by a nuclear medicine physician experienced in pediatric studies. For the staging of newly diagnosed patients, 2 mL of blood was taken from the patients half an hour before and 2 hours after PET imaging and thiol metabolite measurements were made.

Measurement of Thiol/Disulphide Homeostasis

An automated spectrophotometric method which was described by Erel and Neselioglu (9) was used to measure the Thiol/Disulphide Homeostasis tests. For short, free functional thiol groups were formed with sodium borohydride by reducing the disulphide bonds. The reduction of 5.5'-dithiobis-(2-nitrobenzoic) acid (DTNB) was prevented by consuming and removing the reductant sodium borohydride which has not been used with formaldehyde, and all of the thiol groups, including native and reduced thiol groups, were specified after reacting with DTNB. The dynamic disulphide amount is provided by half of the difference between the native thiols and total thiols. After the native and total thiols were specified, disulphide/native thiol percent ratios (SS/SH), disulphide/total thiol percent ratios (SS/SH+SS), disulphide amounts, and native thiol/total thiol percent ratios (SH/SH+SS) were measured (9).

Ischemia-Modified Albumin Measurement

Venous blood samples were used during admittance within one hour to obtain the measurement of Ischemia-Modified Albumin (IMA) levels. Specimens were kept at room temperature for 30 minutes and then centrifuged for five minutes at 3,500 rpm. Later samples were taken to Eppendorf tubes and kept at -80 ºC for analysis. The Albumin Cobalt Binding test was performed to detect the presence of IMA. Fifty mL 0.1% cobalt (II) chloride ($CoCl_2$, $6H_2O$) (Steinheim, Sigma-Aldrich Chemie GmbH Riedstrasse 2, Germany) was added to the patient serum to perform this test. After mixing, 50 mL 1.5 mg/mL dithiothreitol was added before 10 minutes of incubation for albumin cobalt binding. After mixing, the binding capacity was reduced by adding 1.0 mL of a 0.9% sodium chloride solution before incubation for two minutes. Instead of dithiothreitol, the blank was prepared like the distilled water. A spectrophotometer was used to measure the sample's absorbance at 470 nm. The results were given as absorbance units (ABSU) (10).

Statistical Analysis

SPSS 20.0 package program was used for statistical analysis was done with. The frequencies and percentages are given in the evaluation of qualitative data. During the analysis of quantitative data, the Shapiro-Wilk test was applied and as a result of this analysis, it was observed that some parameters were normally distributed and some parameters were not normally distributed.

For data distributed normally, paired samples test was used and for data not normally distributed, The Wilcoxon-Signed rank test was used. Receiver operating characteristics (ROC) analysis was performed for the parameters that were found to be significant in these two tests. All statistical calculations were evaluated at a significance level of $p<0.05$ at a 95% confidence interval.

Results

Oncology patients participating in the study, 44.4% (8) were girls and 55.6% (10) were boys. The children's average age in this study was 11.3 years from 6.0 to 16 years. Patients diagnosis were; non-Hodgkin lymphoma (n=6, 33.3%), hodgkin lymphoma (n=5, 27.8%), neuroblastoma (n=2, 11.1%), Langerhans cell histiocytosis (n=2, 11.1%), primitive neuro-ectodermal tumor (n=1, 5.6%), rhabdomyosarcoma (n=1, 5.6%), Wilms tumor (n=1, 5.6%).

The radiation dose exposed by CT imaging in PET/CT was 6.37 mSV, the radiation dose exposed after PET imaging was 2.53 mSV, and the total radiation dose was 8.9 mSv.

No statistically significant difference was found in Total thiol (µmol/L), Native thiol (µmol/L), IMA (ABSU) and albumin (gr/dL) values before and after PET/CT ($p>0.05$).

Before PET/CT disulfide level (µmol/L) was median (min-max): 8.12 (1.25-13.30), after PET/CT disulfide level (µmol/L) was median (min-max): 14.15 (1.35-23.55) (Table 1).

Disulfide-native thiol ratio, disulfide level, and disulfide-total thiol ratio after PET/CT were found to be higher than before PET/CT ($p=0.001$, $p=0.002$, $p=0.002$) (Figure 1,2,3). Native thiol/ total thiol ratio before PET/CT was higher than after PET/CT ($p=0.02$).

When the cut-off value was accepted as 93.45 caused by the native thiol/ total thiol values of the patients in the study, the sensitivity was calculated as 72.20% and specificity 33.30%. The ROC curve of the disulfide and thiol values is given in Figure 4.

Discussion

In the present study, the aim was to evaluate homeostasis of thiol disulfide in pediatric oncology patients who underwent PET/CT imaging in a pediatric hematology oncology clinic before and after extraction. When free oxygen radicals are excessively produced, there will be

Table 1. Average and median values of values measured before and after PET/CT in oncology patients participating in the study

	Before PET/CT (n=18)	After PET/CT (n=18)	p
	Median (min, max)	Median (min, max)	
Native thiol (µmol/L)	332.20 (152.6-407.5)	287.70 (148.20-433.50)	0.824
Disulfide (µmol/L)	8.12 (1.25-13.30)	14.15 (1.35-23.55)	0.001
IMA (ABSU)	0.62 (0.38-1.08)	0.62 (0.34-1.25)	0.691

IMA: Ischemia-Modified Albumin, Paired Sample's t-test has been applied: Disulfide level after PET/CT was found to be higher than before PET/CT. PET/CT: Positron emission tomography/computerized tomography, min, max: Minimum, maximum, ABSU: Absorbance units

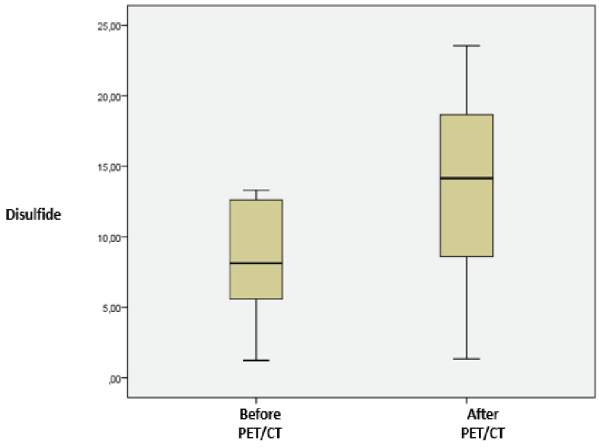

Figure 1. Distribution of disulfide values before and after PET/CT
PET/CT: Positron emission tomography/computerized tomography

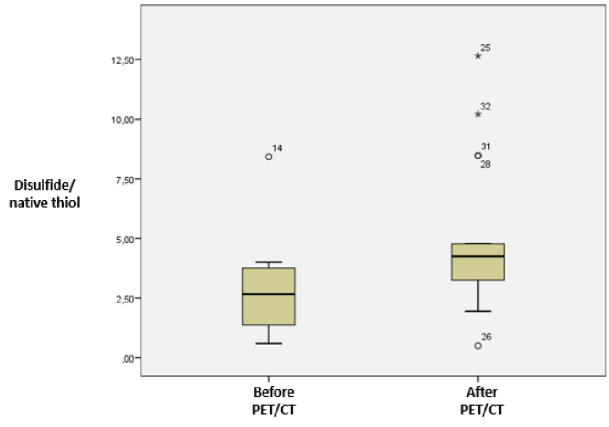

Figure 2. Distribution of disulfite/ nativethiol values before and after PET/CT
PET/CT: Positron emission tomography/computerized tomography

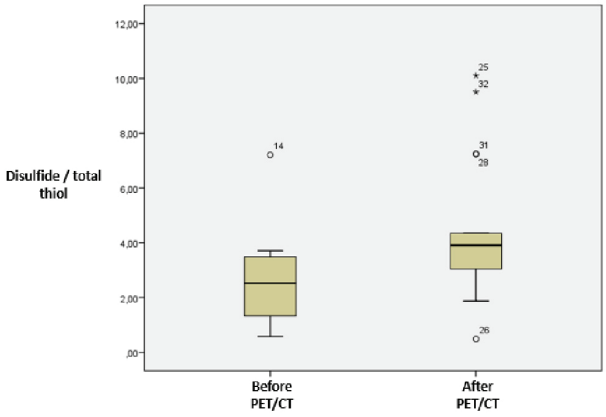

Figure 3. Distribution of disulfide /total thiol values before and after PET/CT
PET/CT: Positron emission tomography/computerized tomography

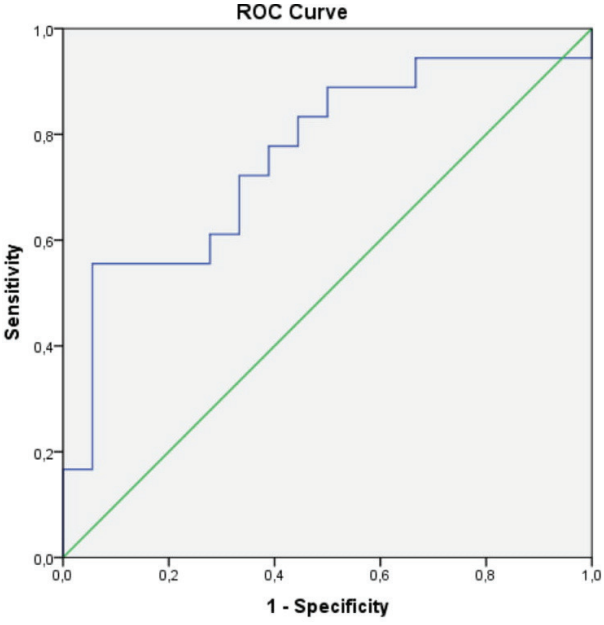

Figure 4. Native thiol/ total thiol values ROC curve
ROC: Receiver operating characteristics
PET/CT: Positron emission tomography/computerized tomography

systemic damage after exposure. It causes changes the prooxidant/antioxidant balance of the tissues, ultimately causes the oxidation of the basic structures of the cell. It is important to evaluate TDH to radiation exposure on PET/CT. According to our results, imaging methods that may have lower radiation exposure such as PET/MR may be preferred in pediatric patients. As far as we know, this study is the first one in the literature.

PET/CT studies show considerable exposure to radiation, particularly for the children who undergo follow-up exams regularly (11,12). There is a significant variation radiation dose depending on the number of the performed PET/CT studies (13). According to the results, the CT part of the examination causes the largest radiation dose. The pediatric malignancies treatment has different and considerable long-term effects. These include avascular necrosis of the hip, the development of cardiomyopathy, early onset of heart disease, cognitive delay, higher risk of secondary malignancies, and pulmonary fibrosis. Children receiving radiation and alkylating chemotherapeutic agents as their treatment regimens are exposed to the development of secondary malignancies. The significant issue of exposure to radiation in children increases with enhancing use of PET/CT in managing the pediatric malignancies, particularly when children are reported to have a higher risk of developing secondary malignancies caused by exposure to radiation compared with adults (14,15). In the present study, the radiation dose exposure by CT imaging in PET/CT was 6.37 mSV, the radiation dose exposed after PET imaging was 2.53 mSV, and the total radiation dose was 8.9 mSv. Therefore, to evaluate whether the radiation dose exposed in patients changes the oxidant/antioxidant balance, we evaluated oxidant/antioxidant status of pediatric new diagnostic oncology patients with the measurement of the thiol levels before and after the PET/CT imaging.

Mercaptans or thiols are a group of organic compounds containing a sulfhydryl group containing hydrogen and sulfur atoms bound to a carbon atom. There are albumin thiols in the plasma pool, and other protein thiols contributed by thiols with low molecular weight such as cysteine, cysteinyl glycine, glutathione, homocysteine, and γ-glutamylcysteine (8).

Cellular and tissue injury due to reactive oxygen species is prevented through the reaction between organic thiol compounds and free radicals. When there is oxidative stress, sulfhydryl groups are changed into disulphide bridges subsequently converted into thiols again. There is a balanced continuation of the cycle. Thus, TDH is kept. TDH is essential in apoptosis, oxidative stress, protection against antioxidants, detoxification, enzymatic activity, and cellular signal transmission (8). This study found no statistically significant difference before and after the PET/CT imaging in native and total thiol values but disulphide levels, disulphide/native thiol, and disulphide/total thiol ratios increased significantly after the PET/CT imaging (p=0.001, p=0.002, p=0.002). Native thiol/total thiol ratio was reduced after the PET/CT imaging (p=0.02) and native thiol/total thiol values of the patients in the study, the sensitivity was calculated as 72.20% and specificity 33.30% with ROC curve. The reason is the high levels of oxidant radicals in patients exposed radiation after the PET/CT imaging. Arıcan et al. (16) were investigated the effect of low dose ionizing radiation exposure on thiol/disulfide homeostasis and ischemia modified albumin levels.

They were found no statistically significant difference in terms of IMA levels in radiation exposed group like the present study.

Children are widely known to be more exposed to the radiation effects than adults. Not only are children's soft tissues and organs have more radiosensitivity than the adults since the cell is divided rapidly, but children have a longer life expectancy after exposure to show adverse effects of radiation after radiation exposure. It is hardly surprising that the risk of causing fatal cancer per exposure unit at 10 years is estimated to be about 5-15 times as much as that at 70 years, and this risk is seven times as much as that for younger children (17).

In some studies, there is an increase in the activity of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase, and GPx to be protected against the higher free radicals. In other studies, long-term exposure to chronic oxidative stress and low ionizing radiation is shown to reduce antioxidant defense among workers (18,19). As far as we know, disulfide level, disulfide/total thiol and native thiol/total thiol, and disulfide/native thiol ratios in pediatric oncology patients were first determined before and after the PET/CT imaging. It was found that in this study, thiol-disulfide homeostasis was disturbed and the balance changed in the direction of oxidant damage, in radiation exposure.

The importance of increasing exposure to radiation as shown in the serial PET/CT scans for children receiving high-dose radiation therapy as part of their treatment is not clear. Whole-body PET/CT remains as an important non-invasive staging/diagnostic modality for malignancies (20). PET/MRI scanners can be used to reduce the exposure to radiation from PET/CT imaging studies in cancerous children (21).

The diagnostic performance of F-18 FDG PET/MRI was compared with that of F-18 FDG PET/CT by Sher et al. (22) in a cohort of pediatric patients suffering from lymphoma. They showed that the two modalities had no statistical difference in lesion detection. Classification accuracy of lymph node groups was not different as negative or positive for lymphoma to us involvement. According to our results, PET/CT imaging in pediatric patients with cancer can cause oxidative stress. Considering other studies in the literature, PET/MR may be preferred.

Study Limitations

Our study has some limitation; sample groups were small. Oxidative stress could not be evaluated with different parameters such as reduced glutathione content, glutathione S-transferase activity, and malondialdehyde levels and not compared with thiol groups. The technical variations were limited using the PET scanner technology for PET/MR examination. Therefore, we could not compare the thiol groups between PET/CT and PET/MR. Despite these limitations, the study will contribute to the literature with oxidative damage by evaluating the thiol groups before and after PET/CT imaging.

Conclusion

The exposure to radiation from studies of serial PET/CT in pediatric malignancies may be regarded as significant. Our results indicate the possible oxidative stress condition due to radiation exposure with PET/CT in pediatric oncology patients. PET/CT remains an important modality of surveillance, noninvasive diagnostic, and staging for specific pediatric malignancies. The decision should be made to use PET/CT in children individually who particularly know the exposure to cumulative radiation and the total benefit of the scan. It is concluded that pediatric patients with cancer can be evaluated using the PET/MR due to its equivalent information at a considerable radiation dose savings compared with PET/CT.

Authorship Contributions

Concept: N.E., Design: N.E., G.S., N.M.C.G., Data Collection or Processing: S.Y., A.F., B.B.K., E.C., Analysis or Interpretation: O.E., Literature Search: N.E., Writing: N.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Gambhir SS. Molecular imaging of cancer with positron emission tomography. Nat Rev Cancer 2002;2:683-93.
2. Barrington SF, Mikhaeel NG, Kostakoglu L, et al. Role of imaging in the staging and response assessment of lymphoma: consensus of the International Conference on Malignant Lymphomas Imaging Working Group. J Clin Oncol 2014;32:3048-58.
3. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol 2014;32:3059-68.
4. Tatsumi M, Miller JH, Wahl RL. 18F-FDG PET/CT in evaluating non-CNS pediatric malignancies. J Nucl Med 2007;48:1923-31.
5. Treves ST, Baker A, Fahey FH, et al. Nuclear medicine in the first year of life. J Nucl Med 2011;52:905-25.
6. Kleis M, Daldrup-Link H, Matthay K, et al. Diagnostic value of PET/CT for the staging and restaging of pediatric tumors. Eur J Nucl Med Mol Imaging 2009;36:23-36.
7. Mothersill C, Seymour C. Implications for human and environmental health of low doses of ionising radiation. J Environ Radioact 2014;133:5-9.

8. Eroglu H, Turgal M, Senat A, Karakoc G, Neselioglu S, Yucel A. Maternal and fetal thiol/disulfide homeostasis in fetal growth restriction. J Matern Fetal Neonatal Med 2021;34:1658-65.
9. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32.
10. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000;19:311-5.
11. Prasad KN, Cole WC, Hasse GM. Health risks of low dose ionizing radiation in humans: a review. Exp Biol Med (Maywood) 2004;229:378-82.
12. Steinert M, Weiss M, Gottlöber P, et al. Delayed effects of accidental cutaneous radiation exposure: fifteen years of follow-up after the Chernobyl accident. J Am Acad Dermatol 2003;49:417-23.
13. Alkhorayef M. Effective radiation doses in pediatric PET/CT examinations: Pilot study. Appl Radiat Isot 2021;168:109412.
14. Chawla SC, Federman N, Zhang D, et al. Estimated cumulative radiation dose from PET/CT in children with malignancies: a 5-year retrospective review. Pediatr Radiol 2010;40:681-6.
15. Picardi M, Cavaliere C, Della Pepa R, et al. PET/MRI for staging patients with Hodgkin lymphoma: equivalent results with PET/CT in a prospective trial. Ann Hematol 2021;100:1525-35.
16. Arıcan S, Dertli R, Baktik S, et al. Efeito da exposição à radiação ionizante de baixa dose na homeostase dinâmica de tiol-dissulfeto e níveis de albumina modificada por isquemia: estudo observacional [The effect of low dose ionizing radiation exposure on dynamic thiol-disulfide homeostasis and ischemia modified albumin levels: an observational study]. Braz J Anesthesiol 2020;70:233-9.
17. Brenner D, Elliston C, Hall E, Berdon W. Estimated risks of radiation-induced fatal cancer from pediatric CT. AJR Am J Roentgenol 2001;176:289-96.
18. Durović B, Spasić-Jokić V, Durović B. Influence of occupational exposure to low-dose ionizing radiation on the plasma activity of superoxide dismutase and glutathione level. Vojnosanit Pregl 2008;65:613-8.
19. Kłucinski P, Wójcik A, Grabowska-Bochenek R, et al. Erythrocyte antioxidant parameters in workers occupationally exposed to low levels of ionizing radiation. Ann Agric Environ Med 2008;15:9-12.
20. Shapira N, Scheuermann J, Perkins AE, et al. Quantitative positron emission tomography imaging in the presence of iodinated contrast media using electron density quantifications from dual-energy computed tomography. Med Phys 2021;48:273-86.
21. Song H, Guja KE, Iagaru A. 18F-FDG PET/CT for Evaluation of Post-Transplant Lymphoproliferative Disorder (PTLD). Semin Nucl Med 2021;51:392-403.
22. Sher AC, Seghers V, Paldino MJ, et al. Assessment of Sequential PET/MRI in Comparison With PET/CT of Pediatric Lymphoma: A Prospective Study. AJR Am J Roentgenol 2016;206:623-31.

Original Article

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Determination of the Normal Anal Location in Neonates: A Prospective Cross-Sectional Study

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Abstract

Aim: Anterior displacement of the anus might be a form of anorectal malformations. The study aims to measure the anal position index (API) in neonates with a modified method.

Methods: The study was conducted between March 1, and December 31, 2019, in the neonatal unit at Tekirdag Namık Kemal University Hospital. The neonates between 35 and 42 gestational weeks and those without congenital malformation were included in the study. Four hundred five neonates were evaluated prospectively. API was determined by using distances between posterior fourchette - anus and posterior fourchette - coccyx in girls; scrotum - anus, and scrotum - coccyx in boys.

Results: Of 405 participants, there were 230 males. API was found 0.52±0.05 and 0.39±0.04 in boys and girls, respectively. For the diagnosis of the anterior location of the anus, the lower limit was 0.33 cm in girls and 0.43 cm in boys. According to these results, nine boys (3.9%) and seven girls (4%) had an anteriorly located anus.

Conclusion: API could be measured more accurately by the modified method and this resulted in a lower incidence of anterior location when compared with the previously defined techniques.

Keywords: Newborn, anorectal malformation, constipation

Introduction

Constipation is a common problem in infancy and childhood. The main reason for it in the pediatric population is dietary irregularities. Toilet training also has an important effect in preventing constipation. Besides functional constipation, the anatomic abnormalities of the anal region can also lead to difficulty in defecation. Anorectal malformations are one of those common congenital anomalies in pediatric surgery. The etiology is unknown and the incidence is reported to be 1/2000-1/5000 (1). It is especially together with other system anomalies (2-4). Anorectal malformations (ARM) lead to a spectrum of symptoms ranging from fecal incontinence to severe constipation (5-6). The higher type ARM, in which some serial operations are indicated, generally results in fecal incontinence. The lower type anomalies, on the other hand, may present with constipation as the normal propagation of stool is not allowed (7). On the other hand, some studies confirmed no relation with constipation. The anomaly, especially the higher type, might be detected prenatally in 16% of the cases (3). But generally, the diagnosis of ARM is made with the initial examination of the neonate just after the delivery.

The normal location of the anal opening is defined to be the midpoint of the distance between the coccyx-vaginal fourchette or coccyx-distal scrotum. This is an anatomic definition but the most important aspect of this location is its relation with the sphincter complex (8). Anteriorly located anus may be diagnosed with inspection but there might have difficulties if the standard measures are not used, especially in minor forms (9-10). Reisner et al. (8) described a quantitative measuring method in 1984. They suggested the lower limit of 0.34 and 0.46 in girls and boys, respectively for the diagnosis of the anterior location (8). The limits for an anterior displacement of the anus were reported to be <0.44 in girls and <0.53

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in boys in the study of Davari and Hosseinpour (11). The normal ranges of anal position index (API) were different in different studies and differences between ethnic groups were detected (11,12).

The study aims to evaluate the anal location using API measurement in otherwise healthy neonates and to make the comparison with the prior studies.

Methods

Study Design

The study was planned prospectively and approval was obtained from the local Ethics Committee (Protocol No: 2019.24.02.08). The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and written informed consent was obtained from the legal guardians of all the patients before enrollment.

The babies who were born between March 1-December 31, 2019, and examined in the Tekirdag Namık Kemal University neonatal unit were included in the study. The exclusion criteria were the existence of chromosomal abnormalities (n=1), anencephaly (n=1), congenital heart diseases (n=3), congenital malformations (n=2), ambiguous genitalia (n=1), congenital spine deformities (n=1), extremity deformities such as achondroplasia (n=1).

The baby born before $37^{0/7}$ gestational weeks was accepted to be preterm; born between $37^{0/7}$-$38^{6/7}$ and at/after $39^{0/7}$ gestational weeks were early term and late-term, respectively. The age of the mother, type of delivery, birth height/weight/head circumference in girls and boys, the age at pregnancy, body mass index (BMI) (weight/height^2), APGAR scores in the first and fifth minute, and the time of meconium passage were evaluated. All these data of the patients were obtained from the hospital records.

Anal Position Index Measurement

The neonate was in a supine position with both proximal lower extremities were in extension abduction. The longitudinal axis in the midline of the perineum together with the anus was covered with transparent adhesive tape. The anal circle and the lower/upper limits were marked. All the measurements were made by the same neonatologist. In girls, API was detected with the proportion between distances of the posterior fourchette to the anus and posterior fourchette to the coccyx. It was calculated using the distances between the scrotal base to the anus and the scrotal base to the coccyx in boys (Figure 1, drawn by the authors).

Statistical Analysis

The compatibility of the variables to normal distribution was evaluated with histogram graphics and the Kolmogorov-Smirnov test using Statistical Package for the Social Sciences version 17.0 program. The mean, standard deviation, and median values were used while presenting descriptive analyzes. Categorical variables were compared with the Pearson chi-square test. Variables that are not normally distributed (nonparametric) were evaluated in two and more than two groups by using Mann-Whitney U test and the Kruskal-Wallis test, respectively. Spearman correlation test was used in the analysis of measurement data. $P<0.05$ was considered statistically significant.

Results

The demographics and clinical features are presented in Table 1. There were 230 (57%) boys and 175 (43%) girls. The 52 (13%) of them were preterm, while 202 (50%) and 151 (37%) were early term and late term, respectively. Normal vaginal delivery was detected in 96 (24%) of them. The mean gestational age was 38.4±1.3 weeks. Birth weight ranged between 3130.0±475.8 grams. The height and head circumference at birth were found to be 49.3±2.5 and 34.5±1.8 cm. The mean maternal age was 29.2±5.8 years.

Table 2 documents the percentile scale of API values in the presented series. The values detected below the fifth percentile were used in the diagnosis of the anterior location of the anus after measuring API in all the neonates. These values were <0.33cm and <0.43 cm in

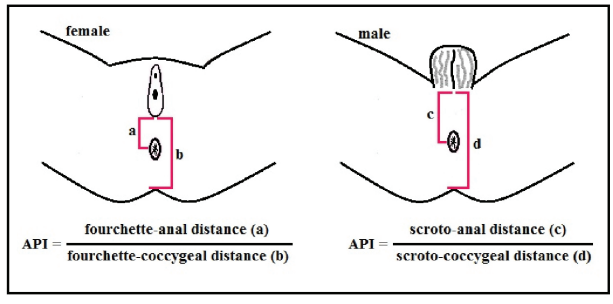

Figure 1. The method of measuring anal position index in girls and boys
API: Anal position index

Table 1. The demographic and clinical features of the patients

Features	mean ± SD
Gestational age (weeks)	38.4±1.3
Birth weight (gram)	3130±475.8
Birth height (cm)	49.3±2.5
Birth head circumference (cm)	34.5±1.8
Body mass index	1.28±0.13
Maternal age	29.2±5.8
First day of admittance (day)	4.3±6.8
The time of meconium passage (hour)	10.5±8.4
Apgar score 1. minute	8 (median)
Apgar score 5. minute	9 (median)

SD: Standard deviation

Table 2. Anal position index percentile in males and females		
API[1]	males	females
Percentile 05	0.43	0.33
Percentile 25	0.49	0.36
Percentile 50	0.52	0.38
Percentile 75	0.55	0.42
Percentile 95	0.60	0.46
Percentile 99	0.67	0.51
[1]API: Anal position index		

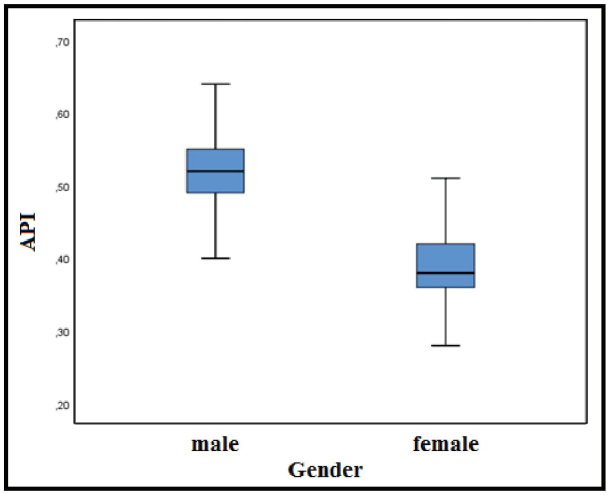

Figure 2. The anal position index values in different genders
API: Anal position index

Table 3. Measurement results of the patients					
Features	Mean	Standard deviation	Median	95% CI* Lower limit	95% CI Upper limit
Scrotum-Anus (cm)	2.87	±0.49	2.90	2.81	2.94
Scrotum-Coccyx (cm)	5.55	±0.72	5.55	5.46	5.64
API in boys	0.52	±0.05	0.52	0.51	0.52
Fourschette-Anus (cm)	1.44	±0.20	1.50	1.41	1.47
Fourschette-Coccyx (cm)	3.73	±0.49	3.70	3.65	3.80
API in girls	0.39	±0.04	0.38	0.38	0.39
*CI: Confidence interval, API: Anal position index					

girls and boys, respectively (Figure 2). The anus was found to be anteriorly located in nine (4%) boys and seven (4%) girls.

Table 3 presents the API measurement of the patients. The distance between the scrotal base to anus was 2.87±0.49 cm and the scrotal base to coccyx was 5.55±0.72 cm in boys. The posterior fourchette to anus was 1.44±0.20 cm and the posterior fourchette to coccyx was 3.73±0.49 cm in girls. The mean API was detected to be 0.52±0.05 cm in males and 0.39±0.04 cm in females. There was a direct correlation between the distance of scrotal base to the coccyx in boys and posterior fourchette to the anus in girls with increasing BMI and increasing gestational age (For gestational age boys r=0.165, p=0.012; girls r=0.186, p= 0.014), (For BMI boys r=0.241, p<0.01; girls r=0.343, p<0.01).

The rate of the anterior location did not differ between genders. The API values were higher in males (median; 0.52) than the girls (median; 0.38) (p<0.01). No difference in API was detected when gestational age was considered. The API value in total had a direct correlation with the birth weight, height, and head circumference. There was no statistically significant relationship between the existence of anteriorly located anus and the time of first meconium passage in our study. The mean time for the first meconium passage was detected to be 12.4 hours and 10.8 hours in the anteriorly displaced anüs and normally located anus, respectively (p>0.05).

The API did not have any statistically significant relation with gestational age-adjusted birth weight, type of delivery, presence of consanguinity (p>0.05 in each variable).

Discussion

Hendren (13) and Leap (5) reported in 1978 that they detected a relation between idiopathic constipation and anteriorly located anus in some cases. But they did not mention the exact anal location. When making a comparison between the normal or anterior located anus, the API should be used as an objective parameter. For this reason, API has to be evaluated in neonates and infants, especially when the physical examination reveals lead to a spectrum location. Its anterior placement solely does not mean that the child has an ARM. The diagnosis of a lower type ARM should be made together with its relation to the sphincteric complex. This anterior displacement might create a tendency to constipation (14). Bornman et al. (7) defined that the anal position was affected in male neonates if there was an exposure to dichloro diphenyl dichloroethylene in earlier stages of pregnancy. This was also documented with experimental studies (7). Reisner et al. (8) defined the normal anal location and API with a simple measurement in 1984. They reported that API for girls and boys should be 0.44±0.05 and 0.58±0.06, respectively in the neonatal period (8). While a study from Egypt mentioned an API value of 0.48 for boys and 0.34 for girls, they were defined to be 0.53 and 0.38 for Indian boys and girls, respectively (14,15). In the literature, the mean API values range widely in different populations (0.43-0.58 in boys, 0.34-0.46 in girls). The mean values in the presented series are suitable with these ranges

previously reported (16-18). Besides measuring the API of the cases, we created a percentile scale different than the other studies.

The diagnosis of the anteriorly located anus was made when the API was <0.34 in girls and <0.46 in boys according to Reisner et al. (8). On the other hand, Davari and Hosseinpour (11) accepted the limit as <0.44 and <0.53 for girls and boys, respectively. In the presented study the fifth percentile is accepted as the base for defining anterior location. Although the mean values are appropriate with the literature data, the limits of the fifth percentile are a bit lower than the previous studies (7,10,11,14). If the Reisner et al. (8) values were used in this series there would be 2.45 and 2.1 fold overdiagnoses in boys and girls, respectively as the lower limits of API in the Reisner et al. (8) study fall into the 5-25 percentile of ours and this was prevented by the modification presented.

The meconium passage was not affected in any patient presented in this study as all the neonates passed stool on the first day after birth regardless of their API. We detected that the API was higher in boys in all gestational age-adjusted weight groups and this finding is similar to the literature (11-12,17). Sharma et al. (19) also reported in their meta-analysis that API was higher in males.

The effect of the anteriorly located anus on constipation is evaluated in different studies (18,20). Leape and Upadhyaya supported that it increased the incidence of constipation (5,21). But Herek et al. (22) found that the incidence of constipation did not differ in two groups with the normal and anterior displaced anus. They included infants older than one month in their study and concluded that the anterior displacement, especially in girls, was a common variant (22).

We evaluated the anal position and created a percentile scale of API in a relatively high number of patients when compared with the published data. Using this scale diminished the number of cases that would be diagnosed to have an anteriorly displaced anus. This also aids in choosing the appropriate patients for referral to Pediatric Surgery to start the further examination. The API, by itself, does not reveal the existence of an ARM but will select the candidates for anal sphincter stimulation.

Study Limitations

The strength of the study is that it includes the highest number of patients in the literature. All the measurements in 405 cases were made by a single person and standardized. The limitation of this study is that it does not include long-term follow-up of the patients.

Conclusion

The measurement of API in neonates can be easily performed. This index is a noninvasive and useful modality to determine the abnormalities in the anal location. This study presents a percentile scale that more accurately differs the cases with an anomaly that should be further evaluated.

Authorship Contributions

Concept: S.T., E.Y., Design: S.T., E.Y., Data Collection or Processing: S.T., Analysis or Interpretation: S.T., E.Y., Literature Search: S.T., E.Y., Writing: S.T., E.Y.

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References

1. Gangopadhyay AN, Pandey V. Anorectal malformations. J Indian Assoc Pediatr Surg 2015;20:10-5.
2. Oh C, Youn JK, Han JW, Yang HB, Kim HY, Jung SE. Analysis of Associated Anomalies in Anorectal Malformation: Major and Minor Anomalies. J Korean Med Sci 2020;35:e98.
3. Rohrer L, Vial Y, Gengler C, Tenisch E, Alamo L. Prenatal imaging of anorectal malformations - 10-year experience at a tertiary center in Switzerland. Pediatr Radiol 2020;50:57-67.
4. Nakamura H, Puri P. Concurrent Hirschsprung's disease and anorectal malformation: a systematic review. Pediatr Surg Int 2020;36:21-4.
5. Leape LL, Ramenofsky ML. Anterior ectopic anus: a common cause of constipation in children. J Pediatr Surg 1978;13:627-30.
6. Weinberg G, Boley SJ. Anorectal continence and management of constipation. In: Ashcraft KW, Holder TM. Pediatric Surgery. Philadelphia: Saunders Company: 2000. p. 502-10.
7. Bornman MS, Chevrier J, Rauch S, et al. Dichlorodiphenyltrichloroethane exposure and anogenital distance in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) birth cohort study, South Africa. Andrology 2016;4:608-15.
8. Reisner SH, Sivan Y, Nitzan M, Merlob P. Determination of anterior displacement of the anus in newborn infants and children. Pediatrics 1984;73:216-7.
9. Kauvar DR, Boley SJ. Anorektal continence and management of constipation. In: Ascgraft KW, Holder TM, editors. Pediatric Surgery. 2nd ed. Philadelphia: Saunders; 1993. p. 402-9.
10. Pena A, Hong AR. Anorektal malformations. In: Mattei P, editör. Surgical directives pediatric surgery. Philadelphia: Lippincott Williams&Wilkins; 2003. p. 413-20.
11. Davari HA, Hosseinpour M. The anal position index: a simple method to define the normal position of the anus in neonate. Acta Paediatr 2006 Jul;95:877-80
12. Genç A, Taneli C, Tansuğ N, et al. Evaluation of the location of the anus by a modified technique in the neonate. J Pediatr Surg 2002;37:80-2.

13. Hendren WH. Constipation caused by anterior location of the anus and its surgical correction. J Pediatr Surg 1978;13:505-12.

14. Patel JN, Kumar A, Yadav PS, et al. The position of the anal dimple in newborns and infants with anorectal malformations and its correlation with the normal anal position. J Pediatr Surg 2018;53:1560-5.

15. Shahin M, Abdelsalam M. The anal position ındex: a simple method to define the normal position of the anus in neonate its significance ın diagnosis constipation. AAMJ 2011;9:374-84.

16. Mohta A, Goel MR. Determination of anal position index. Indian Pediatr 2004;41:91-2.

17. Rerksuppaphol S, Rerksuppaphol L. Normal anal position index in Thai newborns. J Med Assoc Thai 2008;91:1839-44.

18. Bar-Maor JA, Eitan A. Determination of the normal position of the anus (with reference to idiopathic constipation). J Pediatr Gastroenterol Nutr 1987;6:559-61.

19. Sharma S, Perumal V, Sharma K, Gupta DK. Varied parameters and utility of the anal position index: a systematic review and meta-analysis. Pediatr Surg Int 2021;37:469-77.

20. Hendren WH. Constipation caused by anterior location of the anus and its surgical correction. J Pediatr Surg 1978;13:505-12.

21. Upadhyaya P. Mid-anal sphincteric malformation, cause of constipation in anterior perineal anus. J Pediatr Surg 1984;19:183-6.

22. Herek O, Polat A. Incidence of anterior displacement of the anus and its relationship to constipation in children. Surg Today 2004;34:190-2.

Original Article

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Are Preoperative Systemic Immune Index and Neutrophil-to-Lymphocyte Ratio Sufficient to Predict Lymph Node Positivity and Overall Survival in Muscle-Invasive Bladder Cancer Cases?

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Abstract

Aim: The neutrophil-lymphocyte ratio (NLR) is a parameter that has been shown to be effective as a prognostic marker in many solid tumors. It is aimed to investigate NLR and the systemic immune-inflammation index (SII) in predicting the overall survival and lymph node positivity in bladder cancer (BC).

Methods: The retrospective study included patients that underwent radical cystoprostatectomy/radical cystectomy (RC) due to muscle-invasive bladder cancer (MIBC), high-grade T1 BC, or carcinoma *in situ* in our clinic between January 2010 and March 2020. All the patients had no history of preoperative metastasis, lymph nodes, chemotherapy, hematological malignancies, and preoperative urinary tract infection. Data on neutrophil, lymphocyte, platelet, and hemoglobin levels and total white blood cell counts were retrieved from clinical records and data on disease stage and lymph node positivity were retrieved from pathology reports. Follow-up times and survival times were recorded.

Results: The 213 patients comprised 196 (92%) men and 17 (8%) women with a mean age of 63.17±11.25 years. Lymph node positivity was detected in 49 (23%) patients. The mean overall survival time was 75.04, 63.77, and 84.4 months in all patients, patients with lymph node positivity, and patients with lymph node negativity, respectively. No significant difference was found between patients with lymph node positivity and negativity with regard to NLR and SII values (p=0.975 and p=0.745, respectively). In the receiver operating characteristics (ROC) analysis NLR and SII had no significant effect in predicting lymph node positivity [Area under the ROC curve (AUC) 0.499 (95% confidence interval (CI): 0.403-0.594) and AUC 0.485 (95% CI: 0.394-0.575), respectively] and in predicting overall survival [AUC 0.423 (95% CI: 0.346-0.501, p=0.056) and AUC 0.435 (95% CI: 0.357-0.514, p=0.107), respectively].

Conclusion: The results indicated that SII and NLR are not sufficient to predict lymph node positivity and survival in patients with organ-confined BC.

Keywords: Bladder cancer, neutrophils, lymphocytes, lymph nodes, systemic immune-inflammation index

Introduction

Bladder cancer (BC) is among the most frequently seen cancers in the world, ranking the 7th most common cancer in men and 11th in women (1). In Turkey, BC is the 4th most common cancer in men and 13th in women (2). At the time of diagnosis, 70-75% of the cases are non-muscle invasive bladder cancer (NMIBC). In the first five years after diagnosis, 50-70% of NMIBC cases will recur. On the other hand, 10-15% of NMIBC cases progress to muscle-invasive bladder cancer (MIBC) and approximately 25% of BC cases are MIBC at the time of diagnosis. The five-year survival rate in MIBC is only 50% (3,4). Radical cystectomy (RC) is the most effective treatment in MIBC. Regardless, some patients may recur. Therefore, neoadjuvant chemotherapy

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is recommended particularly in patients with T2-T4a, N0M0 tumors in order to increase overall survival and disease-free survival. Nevertheless, this therapy has been shown to provide positive contributions only to overall survival (5). To our knowledge, there are no reliable indicators showing patient suitability for neoadjuvant chemotherapy and/or patient prognosis after cystectomy.

Accumulating evidence suggests that immune response cells (monocytes, lymphocytes, and neutrophils) and platelets along with their associated signaling pathways are an important component of the tumor microenvironment and that these factors can play a significant role in tumor progression and metastasis (6). In particular, the effectiveness of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and C-reactive protein-to-albumin (CRP/ALB) ratio in predicting recurrence and life expectancy has been investigated (7-9). These studies have shown that the systemic immune-inflammation index (SII), which is calculated by multiplying the NLR ratio by the number of platelets, has a prognostic value in solid tumors such as gastric and colorectal esophageal tumors and also in BC. The role of NLR and SII in predicting the prognosis of prognosis BC is already known (10-14). Also, higher SII was shown to be related to prostate cancer with a high Gleason score (15). Moreover, lymph node positivity is known as an indicator of poor prognosis in BC (3).

In this study, we aimed to determine whether NLR and SII values are effective in predicting the overall survival and lymph node positivity in BC.

Methods

This retrospective study was approved by Erciyes University Medical School Clinical Research Ethics Committee (date: June 10, 2020; no: 220/273). Patients with complete follow-up data and who underwent radical cystoprostatectomy/RC due to MIBC, high-grade T1 BC, or carcinoma in situ in our clinic, between January 2010 and March 2020 were evaluated retrospectively. Patients whose age was between 18-85 years old, who had no solid organ and lymph node metastasis on preoperative imaging [computed tomography (CT), magnetic resonance imaging (MRI), or positron emission CT (PET-CT)], without history of bladder sparing protocol and without the indication of salvage cystectomy due to conditions such as hematuria and postrenal acute renal failure, without a known history of hematological malignancies, neoadjuvant chemotherapy within the last one month and preoperative diagnosis of cystitis/pyelonephritis were included. Having the known history of hematological malignancies and neoadjuvant chemotherapy, having indication for salvage cystectomy due to persistent symptoms of BC despite chemotherapy performed according to the bladder-sparing protocol, being below 18 years old and above 85 years old, having preoperative diagnosis of cystitis and/or pyelonephritis were settled as exclusion criteria.

In all patients included in the study, data on neutrophil, lymphocyte, platelet, and hemoglobin levels and total white blood cell counts were retrieved from clinical records and data on disease stage and lymph node positivity were retrieved from pathology reports. NLR and SII values were calculated as previously described in the literature (14). Follow-up durations and overall survival times were calculated from follow-up data. Overall survival was defined as the time from surgery to death. The 2017 TNM classification was used for tumor staging (16). Informed consent was obtained from each patient.

Statistical Analysis

Data were analyzed using SPSS for Windows version 22 (Armonk, NY: IBM Corp.). The normal distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Continuous variables with normal distribution were expressed as mean ± standard deviation median and continuous variables with nonnormal distribution were expressed as median (25^{th}-75^{th} percentile). Categorical variables were expressed as frequencies (%). Continuous variables with nonnormal distribution were compared using the Mann-Whitney U test. The prognostic values of NLR and SII in predicting lymph node positivity were calculated using the area under the receiver operating characteristics (ROC) curve (AUC).

Results

Of the 475 patients evaluated, 213 patients that met the inclusion criteria were included in the study. These patients comprised 196 (92%) men and 17 (8%) women with a mean age of 63.17±11.25 years. Lymph node positivity was detected in 49 (23%) patients, while no surgical margin positivity was present in any patient (Table 1).

No significant difference was found between patients with lymph node positivity and negativity with regard to NLR and SII values (p=0.975 and p=0.745, respectively). In the ROC analysis that was performed to evaluate the effectiveness of NLR and SII in predicting lymph node positivity, the AUC value for NLR and SII was 0.499 [95% confidence interval (CI): 0.403-0.594 and 0.485 95% CI: 0.394-0.575], respectively. These values indicated that NLR and SII had no effect in predicting lymph node positivity (Table 2, Figure 1).

The mean overall survival time was 75.04, 63.77, and 84.4 months in all patients, patients with lymph node positivity, and patients with lymph node negativity, respectively. No significant difference was found between patients with lymph node positivity and negativity with regard to overall survival (p=0.227). In contrast, the

Table 1. Characteristics of patients	
Variables	**(n=213)**
Age (years)	63.17±11.25
Sex	
Male Female	17 (8%) 196 (82%)
Pathological stage (n, %)	
T0 Tis Ta T1 T2a T2b T3a T3b T4a T4b	12 (5.6%) 5 (2.3%) 7 (7.3%) 22 (10.3%) 8 (3.8%) 68 (31.9%) 38 (17.8%) 36 (36%) 14 (6.6%) 2 (0.9%)
Type of the urinary diversion applied	
Cutaneous incontinent urinary diversion Orthotopic bladder	146 (69%) 67 (31%)
Follow up in month	29.9 (12.7-78.4)*
5 year survival (n, %)	74/213 (34.7%)
Overall survival with 95% CI	75.04 (63.22-86.85)
*Median (25^{th}-75^{th} percentile) CI: Confidence interval	

Table 2. Comparison of SII, NLR and survivals according to lymph node status			
	Lymph node positive (n=49)	**Lymph node negative (n=164)**	**p**
NLR	2.57 (1.87-3.69)*	2.67(2.04-3.52)	0.975**
SII	700 (474.9-876.5)	678.5(502.1-1025.1)	0.745**
5 year survival rate	10/49 (20.4%)	64/164 (39.0%)	0.016***
Overall survival	63.77 (29.32-98.21)	84.40 (72.13-96.66)	0.227***
*Median (25^{th}-75^{th} percentile) **The Mann-Whitney U test ***Chi-square, SII: Systemic immune-inflammation index, NLR: Neutrophil-to-lymphocyte ratio,			

mean five-year survival rate was 34.7%, 20.4%, and 39%, respectively, and a significant difference was found between patients with lymph node positivity and negativity (p=0.016). In the ROC analysis performed to evaluate the effectiveness of NLR and SII in predicting survival, the AUC value was 0.423 (95% CI: 0.346-0.501, p=0.056) and 0.435 (95% CI: 0.357-0.514, *p*=0.107), respectively. Accordingly, NLR and SII were found to have no significant effect in predicting survival (Figure 2,3).

Discussion

The present study investigated the effect of NLR and SII in predicting lymph node positivity, which is inflammatory indicators calculated from preoperative blood parameters, in patients who underwent RC due to clinical T1/T2 N0M0

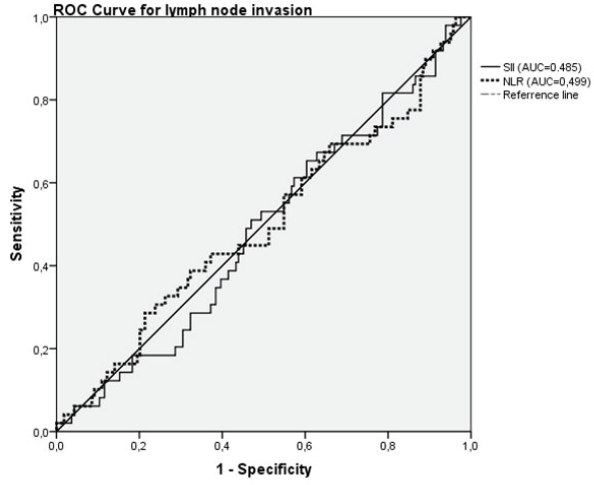

Figure 1. ROC curve for lymph node invasion
ROC: Receiver operating characteristics, SII: Systemic immune-inflammation index, NLR: Neutrophil-to-lymphocyte ratio

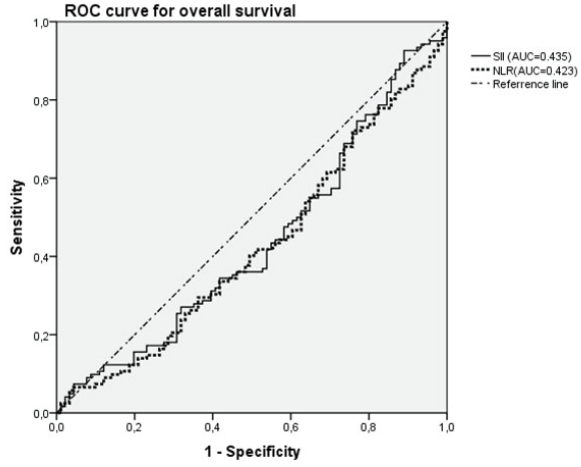

Figure 2. ROC curve for overall survival
ROC: Receiver operating characteristics, SII: Systemic immune-inflammation index, NLR: Neutrophil-to-lymphocyte ratio

MIBC. The median follow-up period was 29.9 months. Although no significant difference was found between patients with lymph node positivity and negativity with regard to overall survival time (63.77 and 84.4 months, respectively) (p=0.227), the five-year survival rate was higher in patients with lymph node negativity (p<0.05). No significant difference was found between the two groups with regard to NLR and SII values (p=0.975 and p=0.745, respectively). Moreover, ROC analysis indicated that NLR and SII had no effect in predicting lymph node positivity and overall survival. To our knowledge, our study is the first of its kind to show that SII and NLR have no significant effect in predicting lymph node positivity in patients with MIBC.

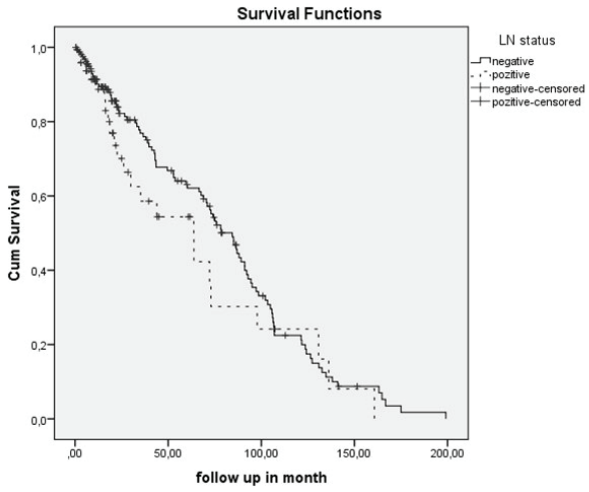

Figure 3. Overall survival according to lymph node (LN) status-Kaplan-Meier curve

Gorgel et al. (13) evaluated a cohort of 191 patients who underwent RC and reported that SII values greater than 843 were cancer-specific and indicated a poor overall survival. However, the authors did not state whether they only included organ-confined MIBC. Additionally, the authors did not perform an analysis regarding the prediction of lymph node positivity (13). Similarly, Zhang et al. (14) evaluated a patient series of 209 patients that were randomized into primary (n=139) and validation (n=70) cohorts and reported that SII is an independent factor in predicting overall survival and is more effective than NLR. However, the researchers examined the effect of SII and NLR only in predicting overall survival. Similarly, the authors did not state whether they only included organ-confined MIBC (14). Bi et al. (17) found that peripheral nutritional index (PNI) and SII are independent factors in predicting overall survival in high-risk pT1 patients. Nevertheless, the group analyzed in that study consisted of 387 patients who received intracavitary Bacillus Calmette-Guérin (BCG) treatment due to high-risk pT1, and only 42 of the patients underwent RC. Accordingly, this patient group had a better prognosis when compared to the patients evaluated in the present study (17). In a retrospective study, Peng et al. (18) evaluated 516 patients who underwent RC and reported that PNI and NLR were independent factors in predicting overall survival and progression-free survival. In that study as well, the authors did not state whether they only included patients with organ-confined MIBC, and they performed no evaluation regarding the role of the investigated indicators in predicting lymph node positivity (18).

Study Limitations

The main limitations of this study were its retrospective manner and including highly selected small group of patients. The present study only included patients with organ confined MIBC and aimed to seek an answer to the following question: "If SII and NLR are effective parameters in predicting lymph node positivity, can they be used to determine candidate patients for neoadjuvant chemotherapy?". Nonetheless, the results indicated that SII and NLR had no significant effect in predicting lymph node positivity and overall survival in this patient group. We consider that this finding could be attributed to the inclusion of a highly selected patient group in our study. However, to our knowledge, it is the first study in the literature to investigate the role of SII and NLR in predicting lymph node positivity. Also with respect to the prognosis of BC, the data about cancer specific survival could not be given in this study due to incomplete data entry about reason of death.

Conclusion

From the results obtained from this study it can be concluded that SII and NLR are not sufficient to predict lymph node positivity and survival in patients with organ-confined BC. However, these results should be confirmed in prospective studies with larger cohort.

Authorship Contributions

Concept: S.T.T., A.D., Design: S.T.T., A.D., Data Collection or Processing: S.T.T., G.S., Analysis or Interpretation: S.T.T., G.S., T.D., Literature Search: S.T.T., G.S., T.D., Writing: S.T.T., A.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Kara F, Keskinkılınç B. Türkiye Kanser İstatistikleri 2016, Halk Sağlığı Genel Müdürlüğü, Sağlık Bakanlığı. Ankara,2019. (www.hsgm.saglik.gov.tr/depo/birimler/kanser-db/istatistik/Turkiye_Kanser_statistikleri_2016.pdf) Accessed at 20 July 2020
3. Cumberbatch MGK, Jubber I, Black PC, et al. Epidemiology of bladder cancer: a systematic review and contemporary update of risk factors in 2018. Eur Urol 2018;74:784-95.
4. Marcos-Gragera R, Mallone S, et al. Urinary tract cancer survival in Europe 1999-2007: Results of the population-based study EUROCARE-5. Eur J Cancer 2015;51:2217-30.

5. Witjes JA, Bruins M, Cathomas R, et al. EAU Guidelines on Muscle-Invasive and Metastatic Bladder Cancer. Edn. presented at the EAU Annual Congress Milan 2021. p 40-56. ISBN 978-94-92671-07-3. Publisher: EAU Guidelines Office. Place published: Arnhem, The Netherlands.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
7. Marchioni M, Primiceri G, Ingrosso M, et al. The Clinical Use of the Neutrophil to Lymphocyte Ratio (NLR) in Urothelial Cancer: A Systematic Review. Clin Genitourin Cancer 2016;14:473-84.
8. Kaynar M, Yıldırım ME, Badem H, et al. Bladder cancer invasion predictability based on preoperative neutrophil-lymphocyte ratio. Tumour Biol 2014;35:6601-5.
9. Guo Y, Cai K, Mao S, et al. Preoperative C-reactive protein/albumin ratio is a significant predictor of survival in bladder cancer patients after radical cystectomy: a retrospective study. Cancer Manag Res 2018;10:4789-804.
10. Shi H, Jiang Y, Cao H, et al. Nomogram Based on Systemic Immune-Inflammation Index to Predict Overall Survival in Gastric Cancer Patients. Dis Markers 2018;2018:1787424.
11. Xie QK, Chen P, et al. The systemic immune-inflammation index is an independent predictor of survival for metastatic colorectal cancer and its association with the lymphocytic response to the tumor. J Transl Med 2018;16:273.
12. Geng Y, Shao Y, Zhu D, et al. Systemic Immune-Inflammation Index Predicts Prognosis of Patients with Esophageal Squamous Cell Carcinoma: A Propensity Score-matched Analysis. Sci Rep 2016;6:39482.
13. Gorgel SN, Akin Y, Koc EM, Kose O, Ozcan S, Yilmaz Y. Retrospective study of systemic immune-inflammation index in muscle invasive bladder cancer: initial results of single centre. Int Urol Nephrol 2020;52:469-73.
14. Zhang W, Wang R, Ma W, et al. Systemic immune-inflammation index predicts prognosis of bladder cancer patients after radical cystectomy. Ann Transl Med 2019;7:431.
15. Sonmez G, Demirtas T, Tombul ST, Akgun H, Demirtas A. Diagnostic efficiency of systemic immune-inflammation index in fusion prostate biopsy. Actas Urol Esp (Engl Ed) 2021;45:359-65.
16. TNM classification of malignant tumors. UICC International Union Against Cancer. 8th edn., G.M. Brierley JD, Wittekind C, Editor. 2017, Wiley-Blackwell and UICC: New York, USA. Available from: https://www.uicc.org/sites/main/files/private/TNM_Classification_of_Malignant_Tumours_Website_15%20MAy2011.pdf
17. Bi H, Shang Z, Jia C, et al. Predictive Values of Preoperative Prognostic Nutritional Index and Systemic Immune-Inflammation Index for Long-Term Survival in High-Risk Non-Muscle-Invasive Bladder Cancer Patients: A Single-Centre Retrospective Study. Cancer Manag Res 2020;12:9471-83.
18. Peng D, Gong YQ, Hao H, et al. Preoperative Prognostic Nutritional Index is a Significant Predictor of Survival with Bladder Cancer after Radical Cystectomy: a retrospective study. BMC Cancer 2017;17:391.

Original Article

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Low Serum Magnesium Level Can be a Risk Factor for Retinopathy in Diabetic Patients: A Cross-Sectional Controlled Study

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Abstract

Aim: Uncontrolled diabetes can lead to complications which are related to high blood sugar and insulin resistance (IR). A decrease in serum magnesium (Mg) levels can cause an increase in IR and a worsening of glycaemic control. In this study, we aimed to investigate the relationship between diabetic retinopathy (DR) and serum Mg levels.

Methods: The study was designed as a cross-sectional study in the internal medicine department of a tertiary referral center (Adana Numune Training and Research Hospital) in Turkey. A total of 554 subjects, including 176 patients with DR patients, 209 patients without DR, and 169 healthy individuals were included in this study. Serum fasting glucose levels, insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR), HbA1c percentages, and Mg levels were measured for all subjects.

Results: Serum Mg level was lower in patients with DR ($p<0.001$). Furthermore; HOMA-IR, HbA1c and fasting glucose levels were higher in patients with DR ($p<0.001$, respectively). Incidence of DR was associated with serum Mg levels (odds ratio: 2.1, confidence interval: 95% 1.2-3.6, $p=0.005$).

Conclusion: Low Mg level can lead to retinopathy by impairing glucose homeostasis. In patients with diabetes, Mg levels should be checked since Mg may be a supporting treatment.

Keywords: Diabetic retinopathy, hyperglycemia, insulin resistance, magnesium, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) has become an important disease for the reason that increasing prevalence and complications. Approximately, 347 million people have diabetes worldwide and a significant portion of them have an increased risk of cerebrovascular diseases, kidney failure, heart disease, non-traumatic lower-limb amputations, blindness, and premature death (1).

Diabetic retinopathy (DR) is a common microvascular complication of T2DM and a recent report on the prevalence of DR showed that it is one of the major causes of visual impairment in the adult population in industrialized countries (2,3). The pathogenesis of DR is still unknown. The decrease in retinal perfusion due to the endothelial dysfunction in diabetes leads to many biochemical and metabolic alterations. The retina is also disturbed by non-enzymatic glycosylation due to chronic high blood glucose. Ischemia, inflammation, and vitreoretinal traction are relationship with the evolution of DR (4,5).

Magnesium (Mg) has a role in many enzymatic reactions and affects glucose metabolism and insulin homeostasis (6). The Mg and T2DM relationship has been shown previously (7,8). In our study, we aimed to investigate the effects of low serum Mg levels on the development of retinopathy in patients with T2DM.

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Methods

This cross-sectional study was performed in the internal medicine department of a tertiary, training and research hospital in Turkey, from 11 July 2013, to 20 March 2015. The study was approved by the local ethics committee and informed consent was provided, and then received from all participants. Approval was obtained from Adana Numune Training and Research Hospital's non-invasive clinical research ethics committee (IRB approval number: ANEAH.EK.2015/126). The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice standards and ethical standards of the Human Experiments Responsible Committee.

Study Population

A total of 554 subjects, including 176 patients with DR patients, 209 patients without DR, and 169 healthy participants were included in this study. The study group (patients with retinopathy) was divided into two subgroups according to the retinopathy type (proliferative and non-proliferative). The subgroups included 81 and 95 patients with proliferative retinopathy (PR) or non-proliferative retinopathy (NPR), respectively.

Patients with a history of smoking or alcohol intake, renal failure, hypertension, malignancies, acute or chronic diarrhea, sepsis, malabsorption, and who were lactating or pregnant were excluded from this study. Eighteen patients were excluded due to the incomplete data.

Body mass indexes (BMI) of all the subjects were calculated (weight in kg/height in m^2). Sphygmomanometers (Erka, Germany) were used to measure the blood pressures of the subjects. When the systolic blood pressure ≥140 mmHg, or a diastolic blood pressure ≥90 mmHg was diagnosed as hypertension. Patients with hypertension were excluded from our study.

The diagnosis of DR was done by mydriatic fundus examination and fluorescence angiography. An experienced ophthalmologist classified the retinopathy status according to the internationally accepted classification (9).

Biochemical Parameters

Serum Mg level, fasting glucose, HbA1c percentage, insulin, creatinine, systolic and diastolic blood pressures, BMIs and duration of diabetes were measured. The calorimetric method was used to analyze the Mg levels with Roche C-501 (Japan) device (reference range: 1.8-2.6 mg/dL). Creatinine and serum fasting glucose levels were analyzed by commercially available kits with Beckman Coulter Synchron LX 20 (Massachusetts, USA). A high-performance liquid chromatography technique was used to analyze HbA1c percentages. Insulin levels were measured by Abbott Architect I 2000 SR analyzer system (Illinois, USA). IR [homeostasis model assessment of insulin resistance (HOMA-IR)] was calculated according to the fasting insulin x fasting glucose/405 formula.

Statistical Analysis

Statistical analyses were performed with the MedCalc software program (version 15.6.1; MedCalc, Belgium). The distribution of normality of numerical variables was evaluated using the Kolmogorov-Smirnov test. To compare the categorical measurements between the groups the chi-square test was used. The independent groups Mann-Whitney U tests or t-test were used for the comparison of the numerical variables between two groups, and the ANOVA (post-hoc: Scheffé's test) or Kruskal-Wallis tests (post-hoc: Dunn's test) were used between the groups more than two. Pearson and Spearman correlation analysis was used to evaluate the relationship between numerical variables. Odds ratio (OR) was used to evaluate the association between serum Mg levels and DR. A multiple linear regression model was used to identify independent predictors of HbA1c. A p-value of <0.05 was considered significant in all tests.

Table 1. Clinical and demographical data of the groups

	T2DM with retinopathy	T2DM without retinopathy	Healthy group	p
	N=176	N=209	N=169	
Age (years)	53.7±7.5	54.1±10.9	51.9±10.3	0.693
Female (N) (%)	100 (56.9%)	130 (62.3%)	95 (56.3%)	0.798
Magnesium (mg/dL)	1.88±0.25	1.94±0.19	2.10±0.26	<0.001[a]
HbA1c (%)	9.2±2.1	8.1±1.9	5.3±0.4	<0.001[b]
Fasting blood glucose (mg/dL)	221.7±115.2	181.2±85.7	92.1±11.5	<0.001[a]
Insulin (mcU/mL)	13.9±13.6	18.3±20.9	11.8±9.5	<0.001[a]
HOMA-IR	8.5±13.8	8.2±11.2	2.3±1.7	<0.001[a]
Creatinine (mg/dL)	0.78±0.18	0.77±0.59	0.74±0.17	0.304
Systolic blood pressure mmHg	125.0±13.0	123.7±11.5	110.4±13.5	<0.001[b]
Diastolic blood pressure mmHg	76.0±8.8	76.6±7.7	68.3±10.2	<0.001[b]
Duration of diabetes (years)	10.1±5.4	9.4±6.1	-	0.063

[a]: Kruskal-Wallis tests (post-hoc: Dunn's test), [b]: ANOVA (post-hoc: Scheffé's test) were used. T2DM: Type 2 diabetes mellitus, HOMA-IR: Homeostasis model assessment of insulin resistance

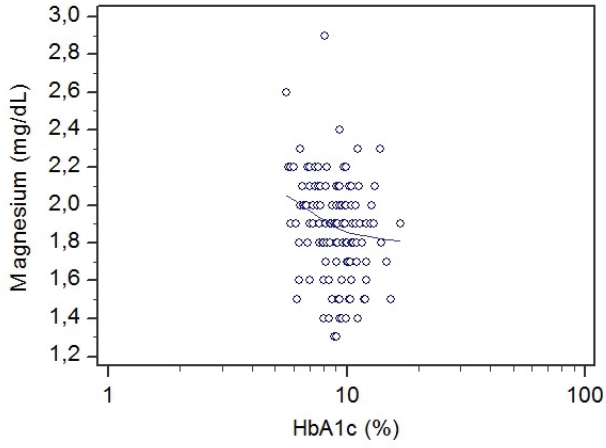

Figure 1. In a scatter diagram, the relation between magnesium and Hba1c is presented graphically

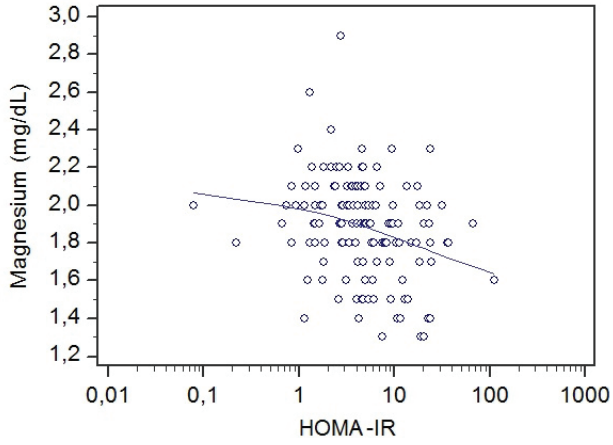

Figure 2. In a scatter diagram, the relation between magnesium and HOMA-IR is presented graphically
HOMA-IR: Homeostasis model assessment of insulin resistance

Table 2. Correlation analyses between magnesium and fasting blood glucose, HbA1c, HOMA-IR

	Fasting blood glucose	HbA1c	HOMA-IR
Magnesium	r=-0.209 p=0.005	r=-0.205 p=0.006	r=-0.284 p=0.001*

*Pearson correlation analysis was used. HOMA-IR: Homeostasis model assessment of insulin resistance

Results

The clinical and demographical data's of the study groups were shown in Table 1. Gender and age distributions were similar between the groups (p=0.798 and 0.693, respectively; Table 1).

Mean Mg levels of patients with DR were significantly lower than in those patients without retinopathy and in the healthy participants (1.88±0.25 vs 1.94±0.19 vs 2.10±0.26; p<0.001, respectively). Mean HbA1c percentages, mean serum fasting glucose levels, mean insulin levels, mean HOMA-IRs and mean BMIs were statistically different (p<0.001, respectively). Other parameters did not differ between groups. Mean systolic and diastolic blood pressures of the patients with diabetes and with or without retinopathy were higher than in those healthy controls (p<0.001) (Table 1).

Table 3. Clinical and demographical data of the subgroups

	T2DM+PR (N=81)	T2DM+NPR (N=95)	p
Age (years)	54.7±7.7	52.8±6.8	0.096
Female N (%)	42 (51.9%)	61 (64.2%)	0.132
Magnesium mg/dL	1.84±0.25	1.91±0.24	0.069
HbA1c (%)	8.9±2.0	9.5±2.1	0.063
Fasting blood glucose (mg/dL)	213.2±121.1	229.8±109.9	0.154
Insulin mcU/mL	14.1±16.2	13.8±11.0	0.428
HOMA-IR (BMI kg/m²)	8.7±17.8 29.4±5.2	8.2±9.1 31.5±6.8	0.031[a] 0.024[b]
Creatinine mg/dL	0.79±0.18	0.77±0.17	0.435
Systolic blood pressure (mmHg)	123.9±13.5	125.9±12.6	0.296
Diastolic blood pressure (mmHg)	75.7±8.8	76.3±8.8	0.640
Duration of diabetes (years)	10.9±5.8	9.4±5.4	0.077

T2DM+PR: Type 2 diabetes mellitus+proliferative retinopathy
T2DM+NPR: Type 2 diabetes mellitus+non-proliferative retinopathy
[a]: Mann Whitney U tests, [b]: T-test were used. HOMA-IR: Homeostasis model assessment of insulin resistance, BMI: Body mass index

There was a negative correlation between Mg level and serum fasting glucose (r=-0.209, p=0.005), HbA1c (r=-0.205, p=0.006, Figure 1,) and HOMA-IR (r=-0.284, p=0.001, Figure 2) (Table 2). Mg levels were found to be strongly associated with DR (OR: 2.1; 95% confidence interval: 1.2-3.6; p=0.005).

There were 95 and 81 patients with NPR and PR, respectively. In Table 3, the comparisons of these subgroups were shown. BMIs of the patients with NPR were higher than in those in patients with PR (29.4±5.2 vs. 31.5±6.8, p=0.024). Mg levels did not differ significantly in NPR and PR groups (1.91±0.24 vs 1.84±0.25; p=0.069, respectively). HOMA-IR levels of the patients with PR were higher than in patients with NPR (8.7±17.8 vs. 8.2±9.1, p=0.031). Other parameters did not differ between NPR and PR groups (Table 3).

Factors affecting HbA1C levels were evaluated by multiple linear regression analysis. The model included Mg, fasting blood glucose, HOMA-IR, age and duration of diabetes. Decreased Mg levels (β±SE=-0.92±0.40; p=0.022) and increased fasting blood glucose levels (β±SE=-0.012±0.0009; p<0,001) were determined as independent risk factors increasing HgA1C level (Table 4).

Table 4. Multiple regression analyses (backward method) were performed with HbA1c as a dependent variable and with magnesium fasting glucose. HOMA-IR, age and duration of diabetes as independent variables

Independent variables	Coefficient	Std. error	$r_{partial}$	t	p
(Constant)	7.9838	-	-	-	-
Magnesium	-0.9203	0.4020	-0.1211	-2.289	0.022
Fasting glucose	0.01200	0.0009008	0.5790	13.323	<0.001

Std. error: Standard error, HOMA-IR: Homeostasis model assessment of insulin resistance

Discussion

We showed low levels of Mg in patients with DR compared to patients without retinopathy and the healthy subjects in our study. Additionally, we found an inverse relationship between Mg and HbA1c levels. Our findings are consistent with previous studies (10-12). However, our study is the first which investigates the Mg-DR association with the largest sample size. Moreover, we also investigated the patients according to the non-proliferative and proliferative retinopathy. Similarly, the relationship between serum Mg levels and HOMA-IR in DR was investigated firstly in the current study.

Many studies have been done to evaluate the relationship between Mg and T2DM. (7,13-15) . According to those studies; we have understood that not only low serum Mg level is a risk factor for hyperglycaemia but also T2DM is one of the causes of hypomagnesemia (13). hypomagnesemia may lead to disturbances in cellular glucose transport, pancreatic insulin secretion and insulin receptor sensitivity. Tyrosine kinase activity is also negatively affected by hypomagnesemia (8,14). Furthermore, long term Mg deficiency has also been associated with high TNF-alpha levels, which is associated with post-receptor IR (16). On the other hand, T2DM leads to hypomagnesemia due to the excess renal excretion, glycosuria and insulin resistance; additionally, reduced intestinal absorption of Mg due to autonomic nerve damage (14,17).

In our study, Hba1c levels were observed to be higher in patients with DR. This result may be strongly related to uncontrolled DM due to hypomagnesemia, or IR, or hyperglycemia. It supports this hypothesis; in our study, fasting blood glucose and IR levels were higher in patients with diabetes and retinopathy. Similarly, high levels of Hba1c were reported in Kundu et al. (12) Srinivasan et al. (18) and studies.

High levels of insulin and IR were found to be a relationship with obesity and hypertension in previous studies (19,20). In the present study, we have also reported high levels of systolic, diastolic blood pressures and BMI in the study group which is more associated with IR.

Mg levels have a negative correlation with HOMA-IRs, serum fasting glucose levels and Hba1c percentages. Similar results were reported in previous studies (12,18,21). Kundu et al. (12) reported an inverse correlation between Mg levels, fasting glucose levels, and Hba1c. Similar findings have been demonstrated in the Agrawal et al. (21) study. However, the sample size of the present study is larger than in previous studies. In addition, we also analysed HOMA-IR values and showed the correlation between Mg and HOMA-IR levels in patients with diabetes and retinopathy for the first time.

Mg values were lower in patients with diabetic PR patients compared to patients with diabetic NPR. However, this result did not reach statistical significance. On the other hand, we have calculated higher HOMA-IR levels in patients with PR. This result may be related to low serum Mg levels in these patients.

Hypomagnesaemia can increase IR and worsen glycaemic control. Most of the complications of diabetes are related to uncontrolled glycemia. Malabsorption due to diabetic autonomic neuropathy and reduced insulin requirement due to nephropathy may cause weight loss (8,10,11,22). In this study, patients in both of the subgroups were overweight. However, the BMIs of the patients with PR were lower than those in patients with NPR. The lower BMIs in patients with PR can be associated with hypomagnesemia and insulin resistance.

Study Limitations

The nutrition status of the subjects was not the same in the current study; so, it was impossible to know how much Mg each subjects received daily. This can be a limitation for this study.

Conclusion

Magnesium imbalance in patients with T2DM was shown in the literature and a low Mg level can lead to vascular complications by impairing glucose homeostasis and insulin sensitivity (7,10,12,13). In patients with diabetes, serum Mg levels should be checked and Mg supplementation can be considered. Magnesium replacement decreases the risk of diabetes by improvement of IR.

Acknowledgement

The authors have declared that no conflict of interest exists.

Authorship Contributions

Concept: M.B., T.S., S.O.K., Design: M.B., T.S., S.O.K., Data Collection or Processing: M.B., S.O.K., N.S.K., S.C., Analysis or Interpretation: M.B., T.S., S.O.K., Literature Search: M.B., T.S., S.O.K., N.S.K., Writing: M.B., T.S., S.O.K.,

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. CDC 2014. Department of Health and Human Services [homepage on the Internet] National diabetes statistics report. [Accessed September 28, 2014]. Available from: http://www.cdc.gov//diabetes/pubs/statsreport14/national-diabetes-report-web.pdf
2. Diabetes Atlas. 5th ed. 2014. http://www.idf.org/diabetesatlas
3. Eszes DJ, Szabó DJ, Russell G, et al. Diabetic Retinopathy Screening in Patients with Diabetes Using a Handheld Fundus Camera: The Experience from the South-Eastern Region in Hungary. J Diabetes Res 2021;2021:6646645.
4. Barot M, Gokulgandhi MR, Patel S, Mitra AK. Microvascular complications and diabetic retinopathy: recent advances and future implications. Future Med Chem 2013;5:301-14.
5. Dedania VS, Bakri SJ. Novel pharmacotherapies in diabetic retinopathy. Middle East Afr J Ophthalmol 2015;22:164-73.
6. Piuri G, Zocchi M, Della Porta M, et al. Magnesium in Obesity, Metabolic Syndrome, and Type 2 Diabetes. Nutrients 2021;13:320.
7. Dasgupta A, Sarma D, Saikia UK. Hypomagnesemia in type 2 diabetes mellitus. Indian J Endocrinol Metab 2012;16:1000-3.
8. Pham PC, Pham PM, Pham SV, Miller JM, Pham PT. Hypomagnesemia in patients with type 2 diabetes. Clin J Am Soc Nephrol 2007;2:366-73.
9. Wilkinson CP, Ferris FL 3rd, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003;110:1677-82.
10. Sharma A, Dabla S, Agrawal RP, Barjatya H, Kochar DK, Kothari RP. Serum magnesium: an early predictor of course and complications of diabetes mellitus. J Indian Med Assoc 2007;105:16, 18, 20.
11. Hatwal A, Gujral AS, Bhatia RP, Agrawal JK, Bajpai HS. Association of hypomagnesemia with diabetic retinopathy. Acta Ophthalmol (Copenh) 1989;67:714-6.
12. Kundu D, Osta M, Mandal T, Bandyopadhyay U, Ray D, Gautam D. Serum magnesium levels in patients with diabetic retinopathy. J Nat Sci Biol Med 2013;4:113-6.
13. Keşkek SO, Kırım S, Karaca A, Saler T. Low serum magnesium levels and diabetic foot ulcers. Pak J Med Sci 2013;29:1329-33.
14. Barbagallo M, Dominguez LJ. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Arch Biochem Biophys 2007;458:40-7.
15. Esmeralda CAC, David PE, Maldonado IC, et al. Deranged Fractional Excretion of Magnesium and Serum Magnesium Levels in Relation to Retrograde Glycaemic Regulation in Patients with Type 2 Diabetes Mellitus. Curr Diabetes Rev 2021;17:91-100.
16. Rodriguez-Morán M, Guerrero-Romero F. Elevated concentrations of TNF-alpha are related to low serum magnesium levels in obese subjects. Magnes Res 2004;17:189-96.
17. Lecube A, Baena-Fustegueras JA, Fort JM, Pelegrí D, Hernández C, Simó R. Diabetes is the main factor accounting for hypomagnesemia in obese subjects. PLoS One 2012;7:e30599.
18. Srinivasan AR, Niranjan G, Kuzhandai Velu V, Parmar P, Anish A. Status of serum magnesium in type 2 diabetes mellitus with particular reference to serum triacylglycerol levels. Diabetes Metab Syndr 2012;6:187-9.
19. Ferrannini E, Buzzigoli G, Bonadonna R, et al. Insulin resistance in essential hypertension. N Engl J Med 1987;317:350-7.
20. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14:173-94.
21. Agrawal P, Arora S, Singh B, Manamalli A, Dolia PB. Association of macrovascular complications of type 2 diabetes mellitus with serum magnesium levels. Diabetes Metab Syndr 2011;5:41-4.
22. Sales CH, Pedrosa Lde F. Magnesium and diabetes mellitus: their relation. Clin Nutr 2006;25:554-62.
23. Hata A, Doi Y, Ninomiya T, et al. Magnesium intake decreases Type 2 diabetes risk through the improvement of insulin resistance and inflammation: the Hisayama Study. Diabet Med 2013;30:1487-94.

Original Article

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Could the ABO Blood Group Types be Associated with COVID-19 Mortality?

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Abstract

Aim: Currently, it is still a matter of debate whether a particular blood types is associated with an increased risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. In the present study, we aimed to evaluate the relationship between the ABO blood group types and mortality in patients hospitalized with the diagnosis of Coronavirus disease-2019 (COVID-19).

Methods: This cross-sectional study included 427 patients diagnosed with COVID-19 between March 2020 and June 2020. SARS-CoV-2 ribonucleic acid was investigated in the naso/oropharyngeal swab samples of the patients by polymerase chain reaction. The blood group types, clinical and demographic data of the cases were obtained from the hospital automation system and patient files.

Results: The age range of the patients was between 18 and 96, and 53.4% (228/427) of them were male. The ABO blood group distribution was 47.1% A, 29.5% O, 15.9% B, 7.5% AB. The overall mortality rate of COVID-19 cases was 16.9%. COVID-19 mortality risks were not significant in any of the ABO blood group types.

Conclusion: We did not find a significant relationship between blood group types and mortality in patients who were hospitalized with a diagnosis of COVID-19 and received treatment and follow-up.

Keywords: Blood groups, SARS-CoV-2, COVID-19, mortality

Introduction

Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread worldwide with increasing morbidity and mortality rates (1). More than 149 million confirmed cases and approximately 3.5 million deaths were reported by the World Health Organization on May 23, 2021 (2). COVID-19 may exhibit a wide range of clinical manifestations including respiratory, cardiovascular, hematopoietic, gastrointestinal, neurological and immune systems (3). Considering the health, social and economic impacts of the COVID-19 outbreak, understanding the susceptibility to disease and clinical outcomes is critical (4). Current clinical observations have shown that age, gender, and comorbid diseases play a role an important risk factors in the pathogenesis and prognosis of COVID-19 (5). Hematological markers and radiological findings have been associated with a worse clinical outcome (6). Hyperfibrinogenemia, lymphopenia, D-dimer elevation and leukopenia were found to be significantly different between mild/moderate and severe COVID-19 patient groups (7).

Beginning with the pioneering study of Helmbold and Vogel (8) in the early 1960s, the ABO blood group polymorphism has been investigated among infectious disease risk and susceptibility factors for many pathogens. The ABO blood group system basically includes A and B antigens and their corresponding antibodies. Differences in blood group, antigen expression can increase or decrease the susceptibility of the host to many infections. In addition, blood group antigens can alter the innate immune response to infection by facilitating intracellular uptake, signal transduction, or cell adhesion (9). Due to the variable clinical manifestations of SARS-CoV-2 infection and the severe impact of COVID-19 all over the world, intense interest in genetic and environmental factors has increased (10). During the COVID-19 pandemic, it has been hypothesized that the ABO blood group types may be associated with susceptibility to SARS-CoV-2 infection, and also that the effect of blood types on COVID-19

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severity is based on the different aggregation of receptors to SARS-CoV-2 on the host cell surface (11).

Whether a particular blood type is associated with an increased risk of SARS-CoV-2 infection remains controversial. Although studies have proven that age and comorbidities increase the risk of infection, the effect of blood types on COVID-19 severity and mortality have not yet been confirmed (12). In the present study, it was aimed to evaluate the relationship between ABO and Rh blood group types and mortality in patients hospitalized with the diagnosis of COVID-19.

Methods

Study Design and Patients

Ethics Committee approval of this study was obtained from the Istanbul Training and Research Hospital Ethics Committee with no need for consent (date: 21.05.2021, approval number: 2828). In the present study, which was designed as a cross-sectional study, 427 COVID-19 cases with positive real-time reverse transcription-polymerase chain reaction (RT-PCR) test for SARS-CoV-2 were evaluated from the hospital automation system and patient files. The study includes all adult hospitalized COVID-19 cases between March 2020 and June 2020, whose ABO blood groups were determined. The blood group types, clinical and demographic data of the cases were obtained from the hospital information system and patient files. Mortality was defined as death from all causes during hospitalization. Patients were younger than 18 years of age who were hospitalized with the diagnosis of COVID-19 but had a negative PCR test and were treated as outpatients were excluded from the study.

In vitro, the anti-A antibody found in individuals with blood type B or O appears to antagonize the interaction between SARS-CoV-1 and the angiotensin-converting enzyme 2 (ACE-2) receptor expressed by host target cells (13,14). Therefore, in addition, it was thought that it would be appropriate to examine patients with B and O blood groups and patients with A and AB blood groups separately in two different groups.

Collection, Storage and Transport of Clinical Samples

Naso/oropharyngeal swab samples were collected with dacron swabs according to current CDC-COVID-19 guidelines. Swabs were immediately placed in sterile transport tubes containing 2-3 mL of vNAT™ (Bio-Speedy® COVID-19 Transfer Tube). SARS-CoV-2 RNA in swab samples of patients was performed using one of the RT-PCR diagnostic kits (Bio-Speedy® SARS-COV-2 double gene RT-qPCR Kit) (Bioeksen, Istanbul, Turkey). Viral RNA extraction from the swap sent to the laboratory in a vNAT tube was performed according to the manufacturer's instructions. PCR mixes were prepared with the products obtained from the extraction and then amplification was performed on the CFX96 Touch System (Bio-Rad Laboratories, Inc, United States). The results were evaluated in line with the recommendations of the kit manufacturer.

Blood Group Types

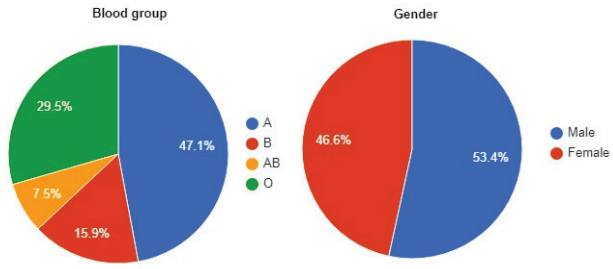
The ABO and Rh blood group types were determined using gel colon agglutination test systems (Across Gel® Gel Centrifugal Cards, Dia Pro Medical Products Inc., Istanbul, Turkey).

Statistical Analysis

Data were analyzed using SPSS 22 (IBM Corp.) package program. The compliance of the variables to normal distribution was examined using visual methods (histogram etc.) and the Kolmogorov-Smirnov test. Data are expressed as median (interquartile range). Differences between groups were compared using the non-parametric Mann-Whitney U test for continuous variables, and Pearson's chi-square or Fisher's Exact tests for categorical variables. Correlation between the ABO and Rh blood groups, age, gender, and comorbidities with mortality was analyzed by Spearman's rank correlation test. The risk prediction [Odds ratio: (OR)] for COVID-19 mortality in different ABO blood groups was calculated with a 95% confidence interval. This OR value was considered significant if the confidence intervals of the OR value did not include a value of 1. Results with a p-value of less than 0.05 were considered statistically significant.

Results

A total of 427 patients with a diagnosis of COVID-19 who were followed up in the clinical and intensive care unit and whose blood types were known were included in the study. The ages of these patients were between 18 and 96 and 53.4% (228/427) of them were males. A high proportion (47.1%) of COVID-19 cases consisted of those with A blood group. The distribution of blood groups of all cases is shown in Graphic 1. The overall mortality rate of COVID-19 cases was 16.9%. Mortality rates of patients with the O/B blood group and A/AB blood group were compared and no significant difference was found. Comorbidity was detected in 60% of the study group. Comorbidities were hypertension (36.8%), diabetes mellitus (32.8%), asthma (12.2%), coronary artery disease (12.2%), malignancies (10.8%) and chronic kidney diseases (7.5%), respectively. Hypertension and diabetes mellitus were the most common two comorbidities in both O/B and A/AB blood group patients. There was no significant difference between different blood groups in terms of pneumonia (p=0.87), length of hospital stay



Graphic 1. Distribution of COVID-19 cases by blood group and gender
COVID-19: Coronavirus disease-COVID-19

(p=0.79), and mortality rate (p=0.94). The demographic characteristics, clinical and laboratory results of the cases are presented in Table 1.

The effects of risk factors such as age, gender and comorbidity on mortality were investigated in the patient population and no statistically significant difference was found. The relationship between blood group types, age, gender, and comorbidity with mortality in COVID-19 cases is presented in Table 2. In addition, the relationship between age and mortality, independent of blood groups, was examined. The median age of patients who died was 60 years, and the median age of patients who recovered was 57 years, which was not statistically significant (p=0.14).

COVID-19 mortality risk estimates (OR) were not statistically significant for any of the ABO blood group types (Table 3).

Table 1. Demographic information, clinical and laboratory data of the COVID-19 cases

Characteristics	O/B (n=194)	A/AB (n=233)	p**
Male/female	101/93	127/106	0.61
Rh positive n (%)	177 (91.2)	212 (91)	0.93
Age*	55.5 (40-68)	59 (49-70)	**0.02**
Length of hospital stay*	9 (6-14)	9 (6-14)	0.79
Mortality rate n (%)	33 (17)	39 (16.7)	0.94
Pneumonia n (%)	179 (92.3)	216 (92.7)	0.87
Comorbidity n (%)			
Hypertension	68 (35.1)	89 (38.2)	0.50
Diabetes mellitus	54 (27.8)	86 (36.9)	**0.04**
Asthma	24 (12.4)	28 (12)	0.91
Chronic kidney disease	8 (4.1)	24 (10.3)	**0.02**
Coronary artery disease	25 (12.9)	27 (11.6)	0.68
Malignancy	16 (8.2)	30 (12.9)	0.13
Laboratory parameter values*			
Leukocyte (x10³/µL)	6.5 (4.8-8)	6.3 (4.8-8.4)	0.66
Neutrophil (x10³/µL)	4.2 (3-5.8)	4.1 (3-6)	0.99
Lymphocyte (x10³/µL)	1.3 (0.8-1.7)	1.2 (0.8-1.7)	0.45
Hemoglobin (g/dL)	13.3 (12.1-14.5)	13 (11.3-14.2)	**0.03**
Platelet (x10³/µL)	212 (166-275)	210 (170-266)	0.61
AST (U/L)	30 (21-47)	30 (24-40)	0.92
ALT (U/L)	22 (14-36)	23 (16-34)	0.52
Creatine kinase (U/L)	68 (28-135)	64 (34-121)	0.97
LDH (U/L)	250 (183-348)	244 (178-329)	0.50
Creatinine (mg/dL)	0.8 (0.6-1.1)	0.9 (0.7-1.1)	0.48
CRP (mg /L)	29.5 (7.8-99.3)	26 (6.6-85.9)	0.65
Procalcitonin (ng/mL)	0.028 (0-0.123)	0.025 (0-0.02)	0.49
D-dimer (ng/mL)	0.64 (0.33-1.33)	0.70 (0.28-1.33)	0.87

*Median (Interquartel range) **Mann-Whitney U test was used for median age, Length of hospital stay and laboratory parameters and Pearson chi-square test was used for other parameters. AST: Aspartate aminotransferase, ALT: Aspartate alanine transaminase, LDH: Lactate dehydrogenase, CRP: C-reactive protein
COVID-19: Coronavirus disease-COVID-19

Discussion

The effect of ABO blood group types on COVID-19 disease severity is still being discussed. The relationship between blood group cells and SARS-CoV-2 can be attributed to some reasons. First, some coronaviruses can attach via surface proteins to extra sugar structures on the surface of A blood group cells that are missing in blood group O. Therefore, A blood group cells are likely to come into contact with more pathogens (11,15). The other is that the relationship between the S protein of SARS-CoV-2 and the ACE-2 protein on the host cell surface has been revealed. The binding of the spike protein to the ACE-2 receptor on the host cell surface, previously predicted for SARS-CoV, can be inhibited by the presence of an anti-A antibody (15,16). It was stated that A blood group can be a risk factor for hepatitis B virus (HBV) and human immunodeficiency virus (HIV), and group O can be protective for HBV, HCV, HIV, syphilis, or malaria (17). For these reasons, the effect of blood group types on COVID-19 clinical outcomes remains a focus of interest for researchers.

In this study, we investigated the effect of blood group types on mortality in 427 hospitalized patients with COVID-19. The overall mortality rate of COVID-19 cases was 16.9%. We compared patients with blood groups O and B with anti-A antibodies and patients with blood groups A and AB without anti-A antibodies in terms of mortality risk with Spearman's correlation analysis and risk prediction analysis. We determined that there was no significant relationship between blood group types and mortality rate, mortality risk, and length of hospital stay in COVID-19 patients. In two different studies questioning the relationship between the ABO group types and the risk of increased SARS-CoV-2 infection and mortality, it was found that SARS-CoV-2 tends to infect individuals

Table 2. Relationship between blood group types, age, gender and comorbidities with mortality in the COVID-19 cases

Blood group type	Spearman's rho	p*
O/B	0.004	0.94
A/AB	-0.004	0.94
A	-0.011	0.82
B	0.026	0.59
AB	0.014	0.77
O	-0.017	0.73
Rh	0.013	0.79
Age	0.070	0.14
Gender	0.001	0.98
Comorbidity		
Hypertension	0.047	0.33
Diabetes mellitus	0.006	0.90
Asthma	-0.020	0.68
Chronic kidney disease	0.017	0.72
Coronary artery disease	-0.017	0.73
Malignancy	-0.055	0.25

*Spearman's rank correlation test, COVID-19: Coronavirus disease-COVID-19

Table 3. Evaluation of mortality risk according to blood group types of COVID-19 cases

Blood group types	Odds ratio	95% Confidence interval	p*
A	0.9	0.5-1.5	0.81
B	1.2	0.6-2.3	0.59
AB	1.1	0.4-2.9	0.76
O	0.9	0.5-1.5	0.72
O/B	1	0.6-1.6	0.94
A/AB	0.9	0.5-1.6	0.94

*Pearson's chi-square - odds ratio, COVID-19: Coronavirus disease-COVID-19

with blood type A, while individuals with blood type O have the lowest risk of infection (18,19). Zhao et al. (19) reported that the rate of those with A blood group was higher in deceased patients. Alkout and Alkout (20) found that individuals with B and O blood groups had milder COVID-19 disease. However, in subsequent studies, studies investigating the relationship between the ABO blood group types and the risk of infection or disease outcome and containing conflicting results have been published (3,21-23).

In some studies conducted in our country, it was reported that the risk of SARS-CoV-2 infection was lower in patients with O blood type (24). On the other hand, Solmaz and Araç (9) reported that blood groups did not affect the course of the disease and were not associated with mortality risk. In our study, the proportion of patients with blood group A who were hospitalized with the diagnosis of COVID-19 was higher. Many studies have shown that people with blood type A are significantly more susceptible to SARS-CoV-2 infection compared to blood type O (18,19,25). There was no control group in this study. Therefore, it could not be investigated whether people with blood group A are more prone to this disease.

Genetic-based studies have also been conducted to investigate the effect of blood group types on COVID-19 mortality, and multiple genes and loci that may affect disease severity were found in the ABO gene region (26,27). However, in a recent study in the USA, it was determined that there was no significant difference between blood groups and mortality as a result of gene analysis of COVID-19 cases that died and survived (28). It is still controversial that blood group types may be a risk factor for COVID-19 mortality.

Study Limitations

This study does not include a control group. Therefore, susceptibility to SARS-CoV-2 infection could not be investigated among people with different blood groups. However, despite this limitation, it is one of the rare studies on this subject in Turkey. Due to the large patient population, the results of the study will contribute to the literature.

Conclusion

We did not find a significant relationship between blood group types and mortality in patients who were hospitalized with a diagnosis of COVID-19 and received treatment and follow-up. Although it has been reported that susceptibility to COVID-19 is decreased in individuals with blood type O, we think that prospective and comprehensive studies are needed to determine the role of blood group types on clinical outcomes and mortality in patients with COVID-19.

Authorship Contributions

Concept: S.A., N.D.S., Design: S.A., N.D.S., Data Collection or Processing: S.A., N.D.S., Analysis or Interpretation: S.A., N.D.S., Literature Search: S.A., N.D.S., Writing: S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Tuncer G, Surme S, Bayramlar OF, et al. NEWS2 and laboratory predictors correlated with clinical deterioration in hospitalised patients with COVID-19. medRxiv 20 January, 2021.
2. Information about COVID-19- World Health Organization Available at:https://covid19.who.int. Accessed 22 May, 2021.

3. Dzik S, Eliason K, Morris EB, Kaufman RM, North CM. COVID-19 and ABO blood groups. Transfusion 2020;60:1883-4.
4. Niño M, Harris C, Drawve G, Fitzpatrick KM. Race and ethnicity, gender, and age on perceived threats and fear of COVID-19: Evidence from two national data sources. SSM Popul Health 2021;13:100717.
5. Zeng X, Fan H, Lu D, et al. Association between ABO blood groups and clinical outcome of coronavirus disease 2019: Evidence from two cohorts. Medrxiv April 17,2020.
6. Surme S, Buyukyazgan A, Bayramlar OF, et al. Predictors of Intensive Care Unit Admission or Death in Patients with Coronavirus Disease 2019 Pneumonia in Istanbul, Turkey. Jpn J Infect Dis. 2021 Feb 26.
7. Fu J, Kong J, Wang W, et al. The clinical implication of dynamic neutrophil to lymphocyte ratio and D-dimer in COVID-19: A retrospective study in Suzhou China. Thromb Res 2020;192:3-8.
8. Helmbold W, Vogel F. Correlations between ABO blood groups and epidemic diseases and their anthropological significance. Bibl Haematol 1962;13:279-80.
9. Solmaz İ, Araç S. ABO blood groups in COVID-19 patients; Cross-sectional study. Int J Clin Pract 2021;75:e13927.
10. Anderson JL, May HT, Knight S, et al. Association of Sociodemographic Factors and Blood Group Type With Risk of COVID-19 in a US Population. JAMA Netw Open 2021;4:e217429.
11. Silva-Filho JC, Melo CGF, Oliveira JL. The influence of ABO blood groups on COVID-19 susceptibility and severity: A molecular hypothesis based on carbohydrate-carbohydrate interactions. Med Hypotheses 2020;144:110155.
12. Göker H, Aladağ Karakulak E, Demiroğlu H, et al. The effects of blood group types on the risk of COVID-19 infection and its clinical outcome. Turk J Med Sci 2020;50:679-83.
13. Guillon P, Clément M, Sébille V, et al. Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. Glycobiology 2008;18:1085-93.
14. Hoiland RL, Fergusson NA, Mitra AR, et al. The association of ABO blood group with indices of disease severity and multiorgan dysfunction in COVID-19. Blood Adv 2020;4:4981-9.
15. Zaidi FZ, Zaidi ARZ, Abdullah SM, Zaidi SZA. COVID-19 and the ABO blood group connection. Transfus Apher Sci 2020;59:102838.
16. Gérard C, Maggipinto G, Minon JM. COVID-19 and ABO blood group: another viewpoint. Br J Haematol 2020;190:93-4.
17. Batool Z, Durrani SH, Tariq S. Association Of Abo And Rh Blood Group Types To Hepatitis B, Hepatitis C, Hiv And Syphilis Infection, A Five Year' Experience In Healthy Blood Donors In A Tertiary Care Hospital. J Ayub Med Coll Abbottabad 2017;29:90-2.
18. Li J, Wang X, Chen J, Cai Y, Deng A, Yang M. Association between ABO blood groups and risk of SARS-CoV-2 pneumonia. Br J Haematol 2020;190:24-7.
19. Zhao J, Yang Y, Huang H, et al. Relationship between the ABO Blood Group and the COVID-19 Susceptibility. Clin Infect Dis 2020:1150.
20. Alkout TA, Alkout AM. ABO blood groups among Coronavirus disease 2019 patients. Iberoamerican Journal of Medicine. 2020 Jun 13;2:268-74.
21. Leaf RK, Al-Samkari H, Brenner SK, Gupta S, Leaf DE. ABO phenotype and death in critically ill patients with COVID-19. Br J Haematol 2020;190:e204-e8.
22. Wu Y, Feng Z, Li P, Yu Q. Relationship between ABO blood group distribution and clinical characteristics in patients with COVID-19. Clin Chim Acta 2020;509:220-3.
23. Boudin L, Janvier F, Bylicki O, Dutasta F. ABO blood groups are not associated with risk of acquiring the SARS-CoV-2 infection in young adults. Haematologica 2020;105:2841-3.
24. Kirisci O, Ozluk S, Topalca US, Koprulu ND. Relationship of ABO Blood Groups to SARS-COV-2 Infection Causing COVID-19 Disease. Konuralp Medical Journal 2021;13:18-23.
25. Zietz M, Zucker J, Tatonetti NP. Associations between blood type and COVID-19 infection, intubation, and death. Nat Commun 2020;11:5761.
26. Ellinghaus D, Degenhardt F, Bujanda L, et al. Genomewide Association Study of Severe Covid-19 with Respiratory Failure. N Engl J Med 2020;383:1522-34.
27. Lehrer S, Rheinstein PH. ABO blood groups, COVID-19 infection and mortality. Blood Cells Mol Dis 2021;89:102571.
28. Kaiser J. Found: genes that sway the course of the coronavirus. Science 2020;370:275-6.

Case Report

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A Case of Pica with Motor-Mental Retardation and with Foreign Bodies in the Larynx and Gastrointestinal Tract: Autopsy Results and Current Literature Review

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Abstract

Pica is the continuous consumption of non-nutritive and non-food items for at least one month. In this study, a 17-year-old boy pica case diagnosed with motor-mental retardation due to cerebral palsy was presented. It was learned that the case was exhibiting object swallowing behaviour. He was found dead in the field by his relatives. At the autopsy, a 3.5x3.0x2.5 cm foreign body (stone) that completely obstructed the upper part of the larynx lumen was removed. In addition, three foreign bodies were removed from the cecum region. The death was caused by mechanical asphyxia due to foreign body aspiration.

Keywords: Pica, foreign bodies, asphyxia, intellectual disability, autopsy

Introduction

According to "DSM- 5 Diagnostic Criteria for Eating Disorders", pica is the continuous consumption of non-nutritive and non-food items for at least one month (1). The consumption of non-food products is harmful to the health of the individual and the development of children. In addition, eating non-nutritious substances is not culturally or socially appropriate. Eating behavior may be severe enough to require additional clinical intervention if it occurs due to mental disorders such as mental retardation, autism spectrum disorder, schizophrenia, or another medical condition such as pregnancy (1). The most common forms of pica are geophagia (clay, sand, mud, soil), pagophagia (ice) and trichophagia (hair) (2). Lithobezoar is the accumulation of stones and is commonly associated with a history of pica (3). Pica may be common in young children with autism spectrum disorder and intellectual disability (4). Persons with developmental and intellectual disabilities are at risk of foreign body aspiration, because a large percentage of this population is affected by dysphagia, pica (5).

In this study, a pica case diagnosed with mental retardation was presented, the importance of detailed autopsy in death cases due to foreign body aspiration was emphasized and it was aimed to draw attention to foreign body aspiration in individuals with mental retardation.

Case Report

Ethics approval for the study was obtained from Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee Approval dated May 29, 2020 and numbered 168. Permission was obtained from the father of the case for the case report.

A 17-year-old boy was found dead in the field by his relatives. It was learned that the case was receiving treatment for motor-mental retardation due to cerebral palsy, and exhibited object swallowing behavior while not using medication.

External examination of the case revealed that there was soil transmission around his mouth and nose. No traumatic lesions were detected. The pre-autopsy radiological

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examination revealed a radiopaque appearance on the upper part of the trachea and a metallic radiopaque appearance on the right lower quadrant of the abdomen (Figure 1a and 1b).

The autopsy revealed hyperemia in the epiglottis, ecchymotic areas adjacent to the epiglottis and the junction of both vocal cords. During dissection, a 3.5x3.0x2.5 cm foreign body (stone) that completely obstructed the upper part of the larynx lumen was removed (Figure 2a). In addition, one 9 cm long ice-cream stick, one 10 cm long metal object (crochet) and one 10.5 cm long branch were removed from the cecum region (Figure 2b and 2c). There were no macroscopic pathological features observed in the other organs. It was detected that the death was caused by mechanical asphyxia due to foreign body aspiration.

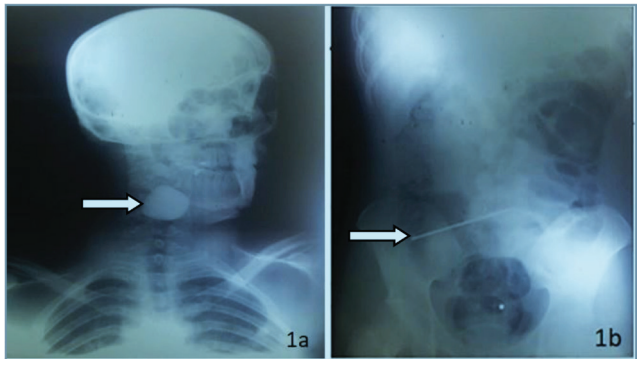

Figure 1. Radiopaque view of foreign body (stone) in neck region (1a) and metal object (crochet) in abdominal region (1b)

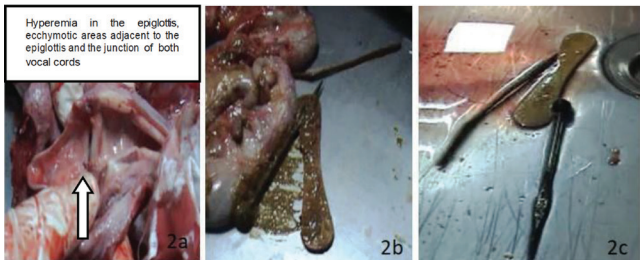

Figure 2. The appearance of the upper part of the larynx where the foreign body was removed (2a) and three foreign bodies removed from the cecum area (ice cream stick, branch piece and crochet) (2b-2c)

Discussion

A foreign body in the respiratory tract is an important and serious event. Injuries caused by foreign substances in the respiratory tract seriously threaten the health and life of children (6). In our case, who was a 17-year-old child, it was stated by his relatives that he had motor-mental developmental retardation due to cerebral palsy, and he had a habit of eating foreign bodies such as soil, stone and wood. At the autopsy, it was determined that case's death was occurred due to mechanical asphyxia caused by a stone block that completely occluded the lumen within the larynx.

One of the most serious complications that may develop in cases with pica history is an intestinal obstruction (7). Although three foreign bodies were found in the cecum region of our case, there was no history of obstruction. The risk of developing intestinal obstruction should be considered in cases with pica. Numanoğlu and Tatli (8) have reported that there was iron deficiency anemia in a 4-year-old male pica case and partial intestinal obstruction was developed. The case has been treated with the removal of foreign bodies, mostly stones via anal dilatation, enema and touch, under general anesthesia, and no laparotomy was required (8).

The presented case shows the importance of obtaining detailed information about the medical history, habits, drugs used and the development of the event from the relatives of the deceased before the autopsy. Crime scene investigation, radiological examinations of the entire body of the deceased before the autopsy and learning from the family of the deceased that the victim had a habit of eating foreign objects that were not food, provided important data for forensic medicine specialists before the autopsy. The case also emphasizes the importance of careful and detailed examination of the respiratory tract and gastrointestinal tract in order to determine whether there are other swallowed foreign bodies in corpses where a foreign body causing obstruction in the respiratory tract is detected.

In the literature, it has been reported that the morbidity rate and mortality rate are 34-40% and 11-40% respectively in pica cases with mental retardation who underwent laparotomy with gastrointestinal tract problems (9). In addition to the high morbidity and mortality rates, when respiratory aspirations are added, as in our case, serious mortality rates are encountered and treatment becomes more important in these cases. In a study, it has been stated that 80% success was achieved in the treatment of pica with cognitive-behavioral treatment together with intensifier and response reduction method in mentally retarded individuals (10).

As a result, since they cannot fully express their complaints, mental retardation cases are the patient groups that needed to be evaluated clinically in detail. Considering that the pica is more common in patients with mental retardation diagnosis, we think that laboratory and radiological examinations should be evaluated in detail in

these cases, necessary treatments should be provided, and thus possible mechanical asphyxia and intestinal obstruction and related deaths may be prevented.

Authorship Contributions

Concept: O.F.A., F.K., Design: O.F.A., F.K., Data Collection or Processing: O.F.A., F.K., M.S.Y., U.A., Z.Z.E., Analysis or Interpretation: F.K., M.S.Y., U.A., Z.Z.E., Literature Search: F.K., M.S.Y., U.A., Writing: O.F.A., F.K., M.S.Y., U.A., Z.Z.E.

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References

1. Institute for Eating Disorders. DSM- 5 Diagnostic Criteria for Eating Disorders: https://insideoutinstitute.org.au/assets/dsm-5%20criteria.pdf (Avaible on: 28.05.2021).
2. Canturk N, Ozdes T, Dogan B. Pica: a case report. J For Med 2010;24:62-7.
3. Aihole JS. Giant Colonic Lithobezoar: A Rare Case Report. J Indian Assoc Pediatr Surg 2020;25:103-5.
4. Fields VL, Soke GN, Reynolds A, et al. Pica, Autism, and Other Disabilities. Pediatrics 2021;147:e20200462.
5. Yocum AD, Dennison JL, Simon EL. Esophageal Obstruction and Death in a Nonverbal Patient. J Emerg Med 2021;60:e109-e13.
6. Liu J, Wang Y, Zhao T, Li C, Nie L. Application of 64-Slice Spiral CT Imaging Technology Based on Smart Medical Augmented Reality in the Diagnosis of Foreign Bodies in the Respiratory Tract in Children. J Healthc Eng 2021;2021:9962997.
7. Tripodi V, Caruso A, Luraghi M, et al. Paediatric gastric trichobezoar: the Rapunzel syndrome A report of two cases. Ann Ital Chir 2020;9:S2239253X20032442.
8. Numanoğlu KV, Tatli D. A rare cause of partial intestinal obstruction in a child: colonic lithobezoar. Emerg Med J 2008;25:312-3.
9. Miyakawa K, Ito M, Hatta K, Eto K, Arai H. Recurrent cholecystitis in an elderly mentally retarded patient with pica. Psychogeriatrics 2011;11:244-6.
10. Hagopian LP, Rooker GW, Rolider NU. Identifying empirically supported treatments for pica in individuals with intellectual disabilities. Res Dev Disabil 2011;32:2114-20.