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Phone: +90 (332) 241 50 00-44513
Fax: +90 (332) 241 21 84
e-mail: cagdastipdergisi @gmail.com
web: http://www.jcontempmed.com

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Letter to the Editor should not exceed 500 words. Short relevant comments on medical and scientific issues, particularly controversies, having no more than five references and one table or figure are encouraged. Where letters refer to an earlier published paper, authors will be offered right of reply.

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b) All pages should be numbered consecutively in the top right-hand corner, beginning with the title page.

c) The title page should not include the names and institutions of the authors.

d) The manuscript should be presented in the following order: Title page, Abstract (English, Turkish), Keywords (English, Turkish), Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements (if present), References, Figure Legends, Tables (each table, complete with title and foot-notes, on a separate page) and Appendices (if present) presented each on a separate page.

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Abstract should be in both English and Turkish and should consist "Aim, Materials and Methods, Results and Conclusion". The purpose of the study, the setting for the study, the subjects, the treatment or intervention, principal outcomes measured, the type of statistical analysis and the outcome of the study should be stated in this section (up to 250 words). Abstract should not include reference. No abstract is required for the letters to the Editor.

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Book

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Chapter in a book

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Journal article on the Internet

Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]; 102. Available from: www.nursingworld.org/AJN/2002/june/wawatch.htm

Website

Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 Jul 2002]. Available from: www.cancer-pain.org

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The Intensive Care Society of Australia and New Zealand. Mechanical ventilation strategy in ARDS: Guidelines. Int Care J Aust 1996;164:282-4.

Acknowledgements

The source of financial grants and the contribution of colleagues or institutions should be acknowledged.

Tables

Tables should be complementary, but not duplicate information contained in the text. Tables should be numbered consecutively in Arabic numbers, with a descriptive, self-explanatory title above the table. All abbreviations should be explained in a footnote. Footnotes should be designated by symbols in the following order: *,†, ‡, §, ¶.

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All illustrations (including line drawings and photographs) are classified as figures. Figures must be added to the system as separate .jpg or .gif files (approximately 500x400 pixels, 8 cm in width and at least 300 dpi resolution). Figures should be numbered consecutively in Arabic numbers and should be cited in parenthesis in consecutive order in the text.

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All measurements must be given in metric system (Système International d'Unités, SI). Example: mg/kg, µg/kg, mL, mL/kg, mL/kg/h, mL/kg/min, L/min, mmHg, etc. Statistics and measurements should always be given in numerals, except where the number begins a sentence. When a number does not refer to a unit of measurement, it is spelt out, except where the number is greater than nine.

Abbreviations that are used should be defined in parenthesis where the full word is first mentioned. Some common abbreviations can be used, such as iv, im, po, and sc.

Drugs should be referred to by their generic names, rather than brand names.

Editorial Correspondence

Prof. Dr. Resul YILMAZ
Selçuk Üniversitesi, Tıp Fakültesi
Çocuk Yoğun Bakım Bilim Dalı
Alaeddin Keykubat Yerleşkesi Selçuklu/Konya 42075 Türkiye
Phone: +90 (332) 241 50 00-44513
Faks: +90 (332) 241 21 84

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ORIGINAL ARTICLES

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Original Article / Orijinal Araştırma

Evaluation of Apelin/APJ and Fibronectin Expression in Genitourinary Tumors: An Immunohistochemical Analysis

Genitoüriner Tümörlerde Apelin/APJ ve Fibronektin Ekspresyonunun Değerlendirilmesi: Bir İmmünohistokimyasal Analiz

Sevinç Şahin[1], Sema Avcı[2], Işıl Çiçekdağı[3], Ezgi Golal[2]

[1]Alanya Alaaddin Keykubat University Faculty of Medicine, Department of Pathology, Antalya, Turkey
[2]Alanya Alaaddin Keykubat University Faculty of Medicine, Department of Histology and Embryology, Antalya, Turkey
[3]Alanya Education and Research Hospital, Department of Pathology, Antalya, Turkey

Abstract

Aim: Cancer is a leading cause of death worldwide, making cancer research and the development of new treatment methods crucial. Bladder, endometrial, and prostate cancers are among the most prevalent forms of cancer. This study aimed to investigate the expression and distribution of endogenous apelin/APJ receptor and fibronectin in these genitourinary tumors and compare them to benign tissues to contribute new data to the literature.

Material and Method: Immunohistochemical methods were applied to 44 cases, including benign and malignant formalin-fixed paraffin-embedded tissues of the endometrium, prostate, and bladder.

Results: The findings showed a significant increase in apelin, APJ, and fibronectin expression in endometrioid adenocarcinoma, urothelial carcinoma, and prostatic adenocarcinoma compared to benign tissues. Moreover, the expression of these molecules had a direct correlation with each other in these tumors. However, as the tumor grade increased, the expression of these molecules decreased in prostatic adenocarcinoma and endometrioid adenocarcinoma.

Conclusion: This is the first study to examine the co-expression and distribution of endogenous apelin/APJ receptor and fibronectin in genitourinary tumors and compare them histologically with benign counterparts, to the best of our knowledge. This underscores the novelty and significance of our findings, providing a foundation for further exploration of the potential roles of these molecules in tumorigenesis and cancer therapies.

Keywords: Apelin, APJ, bladder carcinoma, endometrial carcinoma, fibronectin, genitourinary tumors, immunohistochemistry, prostate carcinoma

Öz

Amaç: Kanser, dünya genelindeki ölüm nedenleri arasında önde gelen bir durum olup, kanser araştırmaları ve yeni tedavi yöntemlerinin geliştirilmesi ciddi önem arz etmektedir. Mesane, endometrium ve prostat kanserleri, en yaygın kanser türleri arasında yer almaktadır. Bu çalışma, bu genitouriner tümörlerdeki endojen apelin/APJ reseptörü ve fibronektin ekspresyonunu ve dağılımını araştırmayı ve elde ettiği verileri benign dokularla karşılaştırarak literatüre katkı sunmayı amaçlamıştır.

Gereç ve Yöntem: Endometrium, prostat ve mesaneye ait formalin ile fikse parafine gömülü benign ve malign dokularını içeren 44 vakaya immünohistokimyasal analiz uygulanmıştır.

Bulgular: Endometrioid adenokarsinom, ürotelyal karsinom ve prostat adenokarsinomunda apelin, APJ ve fibronektin ekspresyonunda benign dokulara kıyasla önemli bir artış olduğu saptanmıştır. Ayrıca, bu tümörlerde bu moleküllerin ekspresyonu birbirleriyle doğrudan bir ilişki sergilemiştir. Bununla birlikte, prostat adenokarsinomunda ve endometrioid adenokarsinomunda tümör derecesi arttıkça, bu moleküllerin ekspresyonu azalmıştır.

Sonuç: Bu çalışma, literatürde genitouriner tümörlerdeki endojen apelin/APJ reseptörü ve fibronektin ekspresyonunun ve dağılımının histolojik olarak benign karşılıklarıyla kıyaslandığı ilk çalışmadır. Elde edilen bulguların, apelin/APJ reseptörü ve fibronektinin tümorigenez ve kanser tedavilerindeki potansiyel rolleri açısından planlanacak daha ileri araştırmalar için bir temel oluşturacağı kanısındayız.

Anahtar Kelimeler: Apelin, APJ, mesane karsinomu, endometrial karsinom, fibronektin, genitouriner tümörler, immünohistokimya, prostat karsinomu

Corresponding *(İletişim)*: Sevinç ŞAHİN, Alanya Alaaddin Keykubat University Faculty of Medicine, Department of Pathology, Antalya, Turkey
E-mail *(E-posta)*: sevinc.sahin@alanya.edu.tr
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INTRODUCTION

Genitourinary system cancers account for approximately 25% of all tumors worldwide.[1] Prostate, bladder, and endometrium cancers are among the most common genitourinary system cancers leading to death globally.[2] The heterogeneity and variability in treatment and survival responses of different cancer types underscore the need to elucidate the biological mechanisms of tumor formation and progression. Consequently, researchers are investigating the targeting of certain molecules in the tumor microenvironment that may be involved in the pathogenesis and prognosis of cancer to discover more effective therapies. Identifying a general and appropriate target that is oncogenic or tumor suppressive will provide an ideal basis for designing and developing cancer therapeutic strategies. Apelin, angiotensin-like receptor 1 (APJ), and fibronectin are among the most attractive molecules for current studies evaluating their association with different tumors, but there is little data available for genitourinary tumors.[3]

Apelin is a bioactive peptide that binds to a G protein-coupled receptor called APJ.[3] Apelin encodes a secreted precursor called preproapelin, consisting of 77 amino acids. The preproapelin is cleaved by endopeptidases, generating several active forms of apelin.[3] The apelin/APJ axis is widely expressed in organs such as the heart, brain, kidney, placenta, etc.[3] It has been demonstrated that the apelin/APJ system plays a role in various physiological processes such as cardiovascular regulation, angiogenesis, pain, feeding behavior, etc.[3,4] Additionally, recent studies have revealed the potential role of the apelin/APJ system in tumorigenesis and mainly adverse prognosis of various tumors, including lung, skin, breast, kidney, ovary, bladder, endometrium, and prostate cancer, with some contradictory results.[3,5-12]

Fibronectin (FN) is a ~500 kDa glycoprotein located in a polymeric fibrillar network in the extracellular matrix (ECM).[13] Fibronectin functions are mediated by insoluble polymeric fibrils. The conversion of soluble fibronectin to fibronectin fibrils in the ECM is initiated by binding to cell surface integrins, other fibronectin subunits, collagen, heparin, fibrin, matrix metalloproteinases (MMPs), and growth factors, resulting in exposure of cryptic epitopes necessary for polymerization.[13] To the best of our knowledge, no literature is currently available that evaluates the potential relationship between the apelin/APJ axis and fibronectin in genitourinary tumors. Both molecules have been implicated in a range of cellular processes, including angiogenesis, cell adhesion, migration, and signaling. While fibronectin has been identified as a potential target for anti-cancer therapies, its precise role in tumor development and progression remains unclear, particularly in genitourinary tumors.[14] Interestingly, cancerous fibronectin appears to have a tumor-suppressive role, but may also be pro-metastatic and associated with poor prognosis.[15] In contrast, fibronectin deposited in the tumor microenvironment is paradoxically associated with a better prognosis.[15] In light of these findings, it is important to better understand how fibronectin affects tumor transformation and metastatic progression.

To address this gap in the literature, our study aims to investigate the expression and distribution of endogenous apelin/APJ receptor and fibronectin in endometrium, prostate, and bladder tumors, and compare these findings to their benign counterparts histologically.

MATERIAL AND METHOD

The study was carried out with the permission of Akdeniz University Clinical Research Ethics Committee (Date: 09.11.2022, Decision No: KAEK-661). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 44 formalin-fixed paraffin-embedded tissues containing endometrial curettage (8 endometrioid adenocarcinomas, 4 secretory phase endometrium, and 3 proliferative phase endometrium), prostate needle biopsy (11 acinar adenocarcinomas intermixed with benign tissues), and bladder transurethral resection material (9 urothelial carcinomas, and 9 benign bladder tissues) from the archive of the Department of Pathology at Alanya Education and Research Hospital were evaluated. The Hematoxylin&Eosin-stained slides were examined to confirm the diagnosis and tumor grade and paraffin blocks containing both tumors with different grades and benign epithelial areas were selected, if available. Immunohistochemical staining of 5 µm thick sections prepared from the selected paraffin blocks was performed using apelin, APJ, and fibronectin primary antibodies and appropriate secondary antibodies.

The sections were placed onto a superfrost slide and incubated in an oven at 56°C overnight. They were then rehydrated by passing them through a series of xylene and alcohol to deparaffinize them. To eliminate antigenic masking, they were boiled in citrate buffer (100244; Merck) and incubated with hydrogen peroxide (18312; Sigma) to remove endogenous peroxidase activity. The sections were then treated with a UV block (TA-125-UB Thermo Scientific) to prevent nonspecific immunoglobulin binding. Primary antibodies for Apelin (Thermo; PA5-114860, 1/150), Apelin Receptor (APJ) (Thermo; PA5-114830, 1/200), and Fibronectin (Abcam; ab2413, 1/200) and their respective secondary antibodies were added. The reaction was made visible with DAB chromogen (D4168; Sigma), and the sections were counterstained with Mayer's Hematoxylin (109249, Merck). Dehydrated sections were passed through a series of alcohol and xylene, and then closed with Entellan. The protein expression levels and localizations were then determined in these sections. Finally, the sections were measured using Image J (1.52 R, National Institutes of Health, USA) after photographing them with an Olympus CX43 Microscope (Japan) to visualize the protein localization.

Immunohistochemical staining intensity of apelin, APJ, and fibronectin was scored using the following criteria: score 0 for no staining, score 1 for mild staining, score 2 for moderate staining, and score 3 for strong staining. Clinicopathologic parameters were obtained from patient records for each sample.

Statistical Analysis

Statistical analysis was performed using Image J software (version 1.52 R; National Institutes of Health) on three randomly selected photographs from each experimental group. Differences in expression between groups were calculated using ANOVA and Sidak's test for multiple comparisons. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 8. The difference between groups and the p-value summary of the difference was marked with an asterisk/asterisks.

RESULTS

Comparison of Endometrioid Adenocarcinoma and Benign Endometrial Tissues

The mean age of patients with endometrioid adenocarcinoma, secretory phase endometrium, and proliferative phase endometrium were 58.3±4.9 (range, 43-69), 43.7±2.1 (range, 40-47), and 46.3±1.4 (range, 40-50), respectively. According to the International Federation of Gynecology and Obstetrics (FIGO) system, 3 out of 8 endometrioid adenocarcinomas were grade 1 (low grade), and the remaining 5 cases were grade 2 (high grade). The expression of apelin, APJ, and fibronectin was found to be significantly higher in endometrioid adenocarcinoma than in benign tissues (p<0.05) (**Figures 1, 2, 3,** and **4**). There was a higher expression of apelin, APJ, and fibronectin in low-grade endometrioid adenocarcinoma than in high-grade tumors (p<0.05) (**Figures 1, 2, 3**, and **4**). The benign endometrial tissues exhibiting secretory phase features showed higher expression of apelin and APJ, prominent in the apical parts of the glands, compared to those exhibiting the proliferative phase (p<0.05) (**Figures 1, 2,** and **3**).

Comparison of Bladder Carcinoma and Benign Bladder Tissues

The mean age of patients with urothelial carcinoma and benign bladder tissues was 69.2 ± 8.4 (range, 56-77) and 63.6 ± 6.3 (range, 47-74), respectively. According to the WHO 2016 classification, there were 5 high-grade and 4 low-grade urothelial carcinomas. Five tumors were non-infiltrative, and 4 tumors were infiltrative. The male-to-female ratio in patients with urothelial carcinoma and benign bladder tissue was 2 (n=6/3) and 8 (n=8/1), respectively.

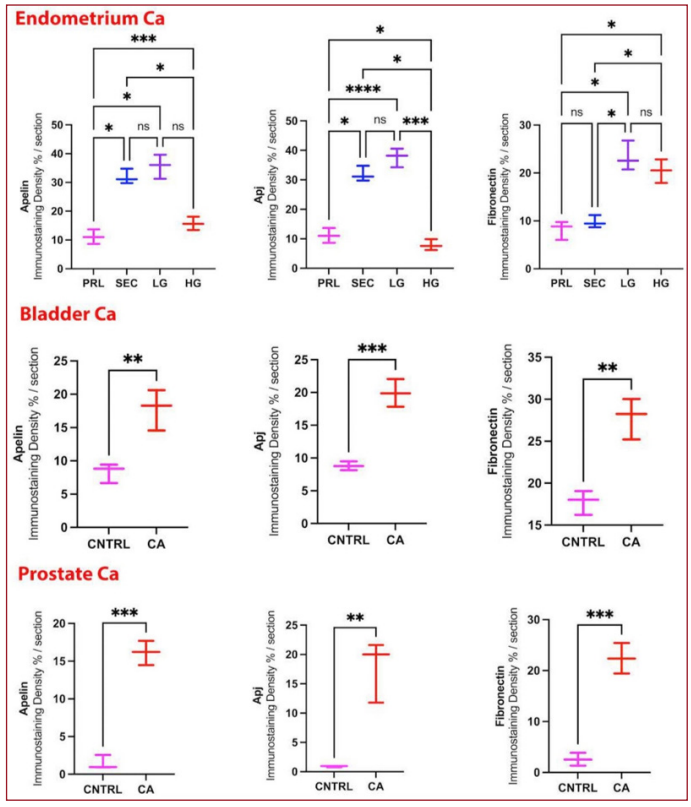

Figure 1. Immunohistochemical density of apelin, APJ, and fibronectin expression in endometrium, bladder, and prostate cancer; PRL: proliferative phase, SEC: secretory phase, LG: low-grade, HG: high-grade, CNTRL: control, Ca/CA: cancer, ns: no statistical difference, *: statistically significant difference, p<0.05.

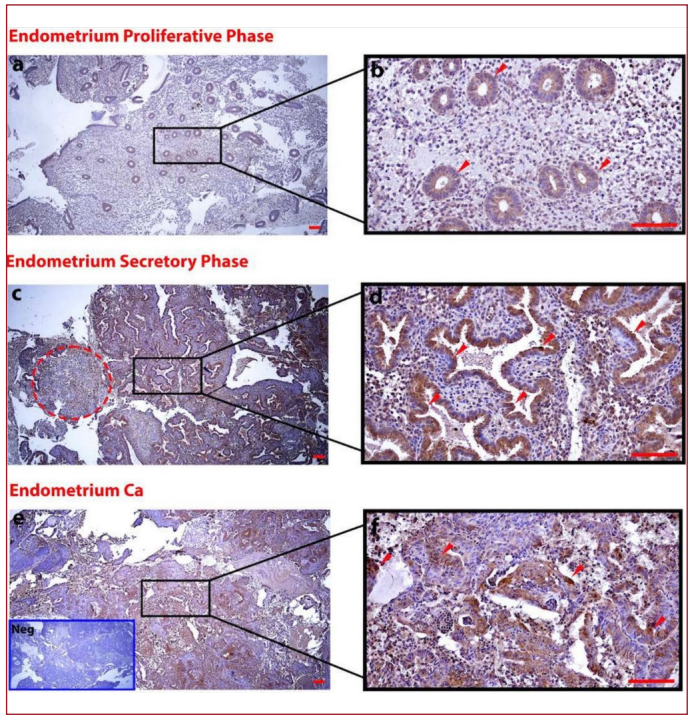

Figure 2. Immunohistochemical expression of apelin in benign endometrial tissues (a-d), and endometrioid carcinoma (e-f); red arrows indicate positive staining in endometrial glands and epithelial structures, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

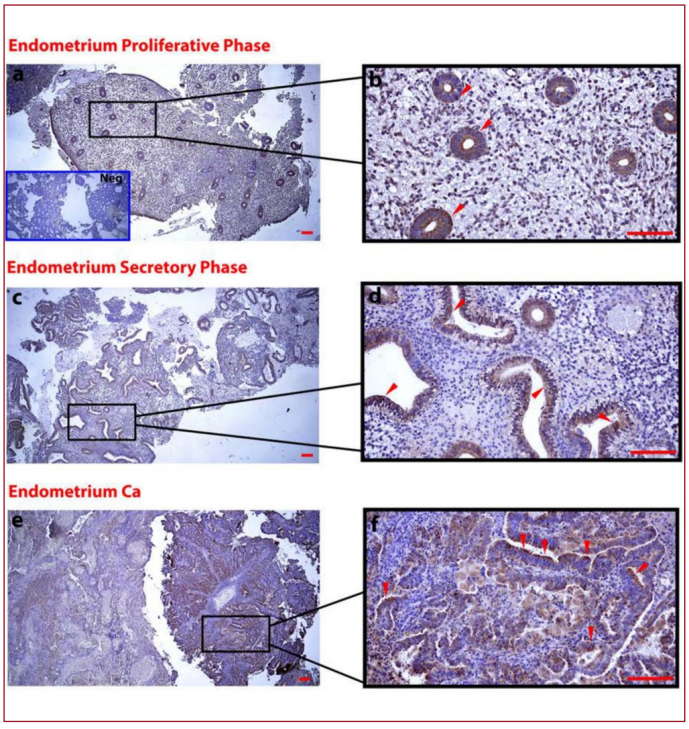

Figure 3. Immunohistochemical expression of APJ in benign endometrial tissues (a-d), and endometrioid adenocarcinoma (e-f); red arrows indicate positive staining in endometrial glands and epithelial structures, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

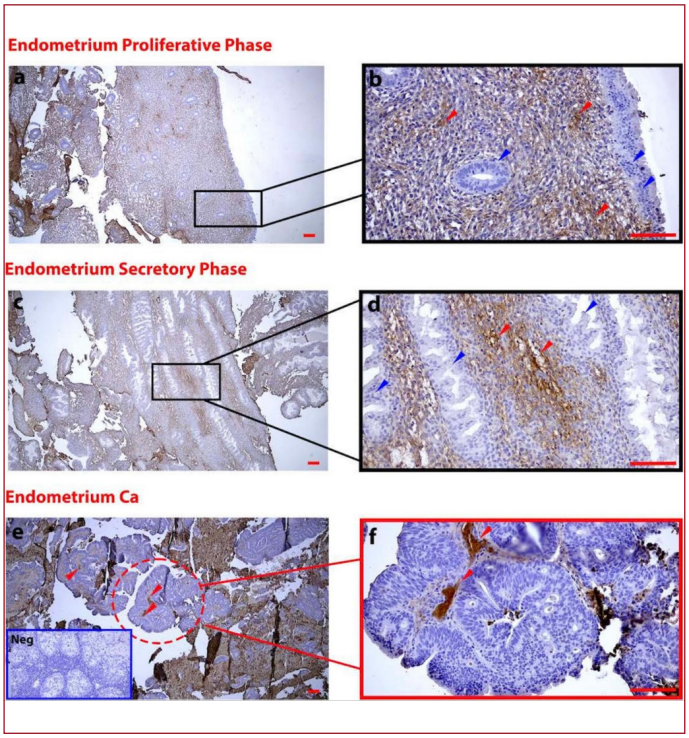

Figure 4. Immunohistochemical expression of fibronectin in benign endometrial tissues (a-d), and endometrioid carcinoma (e-f); red arrows indicate positively stained endometrial glands and epithelial structures, the blue arrows indicate negatively stained endometrial glands and epithelial structures, the red dashed circle with red arrows represents densely positively stained blood vessels in endometrial cancer, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

The expression of apelin, APJ, and fibronectin was significantly higher in urothelial carcinoma compared to benign tissues ($p<0.05$) (**Figures 1, 5, 6,** and **7**). The staining intensity of apelin, APJ, and fibronectin was higher in high-grade invasive bladder carcinomas compared to low-grade ones (**Figures 1, 5, 6,** and **7**). Invasive urothelial carcinomas exhibited higher expression of apelin, APJ, and fibronectin than non-invasive ones of the same grade. Apelin showed more intense staining in the upper 1/3 of the tumoral epithelium. In benign urothelial epithelium, apelin was intensely expressed in the plaque region of umbrella cells, whereas the plaque region was negative for apelin and thinner compared to the benign epithelium (**Figure 5**). The increase in apelin expression, especially in vessels adjacent to the tumor cells feeding urothelial carcinoma, was remarkable.

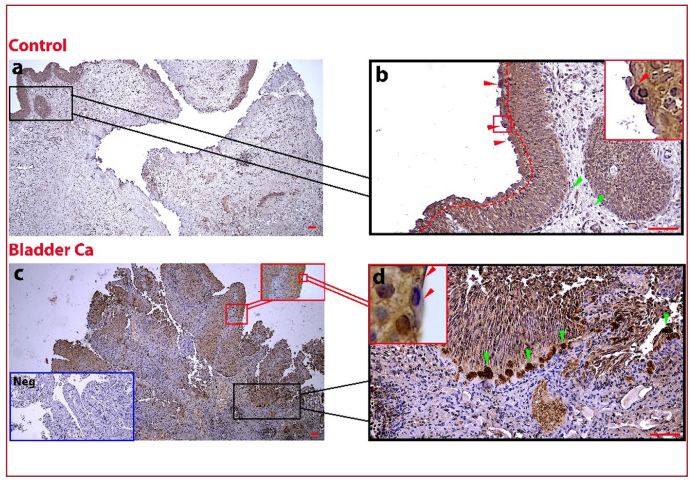

Figure 5. Immunohistochemical expression of apelin in benign bladder tissues (a-b), and urothelial carcinoma (c-d); red arrows indicate umbrella cells, green arrows indicate blood vessels, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

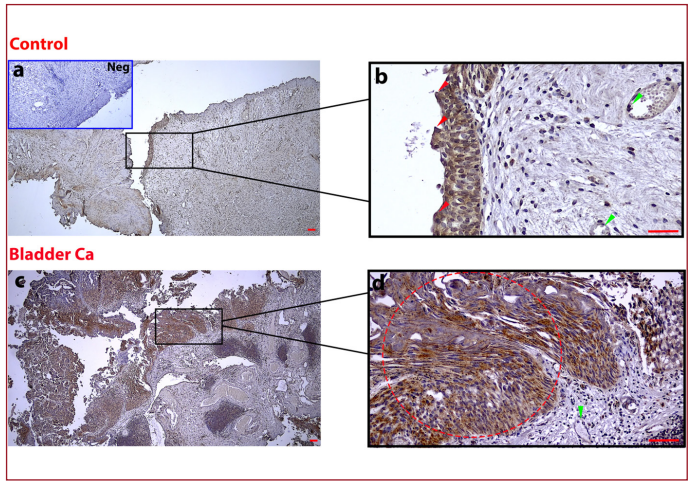

Figure 6. Immunohistochemical expression of APJ in benign bladder tissues (a-b), and urothelial carcinoma (c-d); red arrows indicate umbrella cells, green arrows indicate blood vessels, the red dashed circle represents positively stained transitional epithelium, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

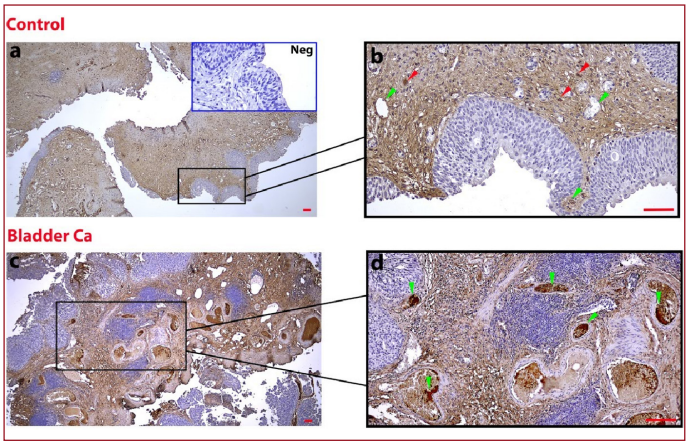

Figure 7. Immunohistochemical expression of fibronectin in benign bladder tissues (a-b), and urothelial carcinoma (c-d); red arrows indicate stromal cells, green arrows indicate blood vessels, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

Comparison of Prostate Adenocarcinoma and Benign Prostatic Tissues

The mean age of patients with prostate adenocarcinoma was 68.9±6.3 (range, 58-77). According to the WHO 2016 classification system, there were 3 acinar adenocarcinomas with Gleason's grade 3, 8 acinar adenocarcinomas with Gleason's grade 4, and 5 acinar adenocarcinomas with Gleason's grade 5. Significantly higher levels of apelin, APJ, and fibronectin were observed in prostatic adenocarcinoma compared to benign tissues (**Figures 1, 8, 9,** and **10**). In glands of prostatic adenocarcinoma and muscles, apelin and APJ were expressed intensely compared to the benign epithelium (**Figures 1, 8,** and **9**). Fibronectin expression was observed in the tumoral stroma and intra-tumoral nerves, but it was negative in the tumoral epithelium except for some tumor cells showing a cribriform pattern (**Figure 10**). The expression of apelin, APJ, and fibronectin decreased as the tumor grade increased, similar to endometrioid carcinoma.

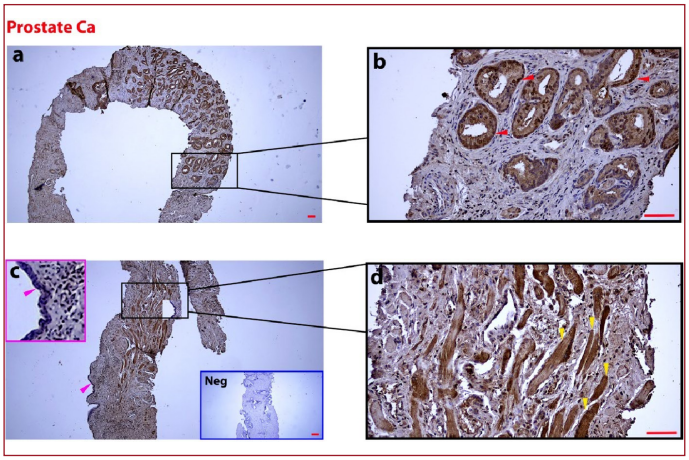

Figure 8. Immunohistochemical expression of apelin in Gleason's grade 3 prostate cancer (a-d); red arrows indicate positive staining in glands, pink arrows indicate negatively stained control epithelium, yellow arrows indicate positively stained muscles, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

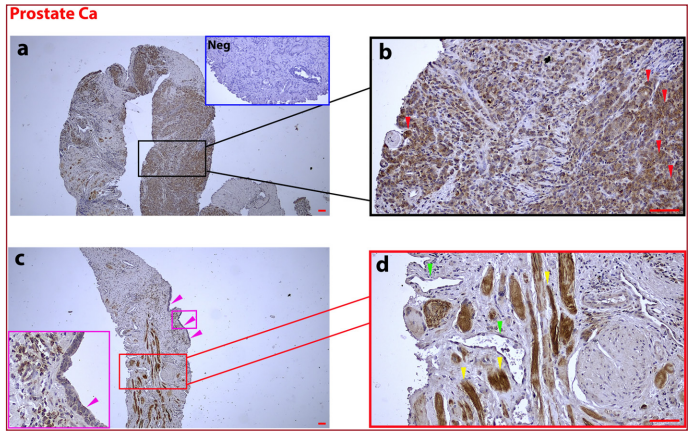

Figure 9. Immunohistochemical expression of APJ in Gleason's grade 5 prostate cancer (a-d); red arrows indicate positively stained glands and cells, pink arrows indicate negatively stained control epithelium, green arrows indicate blood vessels, yellow arrows indicate positively stained muscles, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

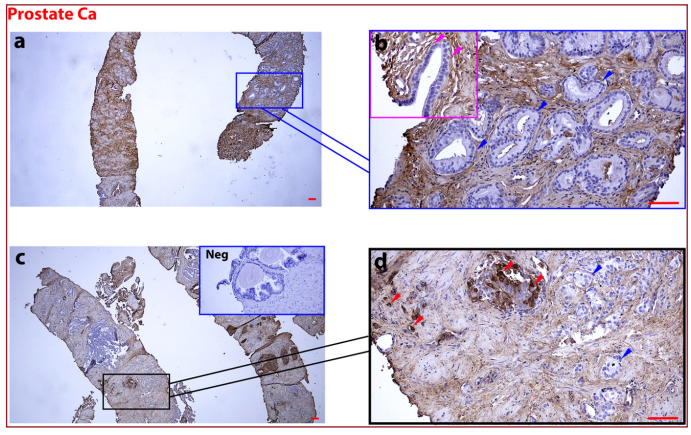

Figure 10. Immunohistochemical expression of fibronectin in Gleason's grade 3 prostate cancer (a-d); red arrows indicate positively stained glands and cells, pink arrows indicate negatively stained control epithelium, blue arrows indicate negatively stained glands, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

DISCUSSION

The literature suggests that the apelin/APJ axis is associated with a poorer prognosis in certain genitourinary cancers such as bladder cancer, based on only a few studies.[3,9,16] Overexpression of apelin has been linked to higher tumor stage, vascular invasion, and distant metastasis in bladder cancer.[3] In our study, we found that higher levels of apelin and APJ expression were present in invasive urothelial carcinomas of the same grade compared to noninvasive ones. Additionally, the staining intensity of both apelin and APJ was higher in high-grade tumors than in low-grade tumors. Yang et al. reported higher immunohistochemical expression of apelin in tumor cells compared with adjacent benign tissues in bladder cancer with muscle invasion.[3,12] Similarly, in our current study, we observed higher expression of both apelin and APJ in urothelial carcinoma compared to benign tissues. Apelin showed more intense staining in the upper one-third of the tumoral epithelium. In the benign

urothelial epithelium, apelin was intensely expressed in the plaque region of umbrella cells, while in urothelial carcinoma, the plaque region was thinner compared to the benign epithelium. Umbrella cells, along with the plaque region containing uroplakin, provide a barrier by changing shape as the bladder fills and empties.[17]

The findings mentioned in the previous paragraph may be due to either tumor-related content change, architectural deformation, or dysfunction of the plaque region. The increase in apelin expression was notable, particularly in vessels located adjacent to the tumor cells that fed urothelial carcinoma. This increase may be related to neoangiogenesis, as suggested by a study conducted by Yang et al., which proposed that apelin might function as a pro-angiogenic factor.[12,18] The authors also claim that high levels of apelin are associated with a poor prognosis due to high tumor stage, distant metastasis, and vascular invasion. Our study found higher levels of apelin and APJ expression in high-grade tumors, which are known to have an unfavorable prognosis.

Regarding prostate cancer, Wan et al. have reported that overexpression of apelin is linked to disease progression and poor prognosis and that miR-224 regulates it inversely.[10] Additionally, a few studies suggest that apelin-13 promotes the proliferation of prostate cancer cells similar to androgens, and contributes to tumorigenesis.[19,20] In our study, we found that the expression of apelin and APJ was significantly higher in prostatic adenocarcinoma glands and muscles, compared to benign epithelium, which is consistent with the report of Soylu et al. using immunohistochemistry.[18]

Altinkaya et al.[5] recently presented an association between higher serum apelin levels and an elevated risk of endometrial cancer in obese patients. They demonstrated higher serum levels of apelin compared to the controls. In the current study, the expression of apelin, along with APJ, was detected to be higher in endometrioid adenocarcinoma than in benign control tissues. The benign glands in the secretory phase showed a higher expression of apelin and APJ, mainly in the apical parts of the glands, than those in the proliferative phase. Similar to prostate cancer, apelin expression was inversely associated with tumor grade in endometrial cancer, suggesting a possible interaction of apelin with hormones such as estrogen, progesterone, or androgen.

On the other hand, fibronectin showed a parallel pattern to apelin and APJ in all 3 genitourinary tumors examined in the study.

The prostate has organ-specific ECM components such as fibronectin, laminin, chondroitin, heparan sulfate proteoglycan, etc.[21] It is claimed that prostate stroma may induce non-prostate epithelial cells to differentiate into a prostatic phenotype.[21,22] Furthermore, the prostate cancer cell line (LNCaP) is known to secrete fibronectin.[23] A study using reverse transcription-polymerase chain reaction reported higher fibronectin expression in prostate cancer cell lines than benign prostatic hyperplasia.[23] In our study, fibronectin showed abundant positivity in the stroma of benign prostate tissue rather than cancer cells, similar to other studies in the literature.[23,24] These data might indicate the up-regulation of fibronectin in the tumor foundation of prostate cancer. Interestingly, a study by Jia et al.[25] showed that fibronectin expression was focal and significantly lower in high-grade prostate cancer than in low-grade ones, which seems to point out the down-regulation, similar to the current study.

Several studies in the literature on bladder cancer have reported a correlation between high levels of fibronectin expression and poor prognosis and increased invasiveness in the disease.[19] Arnold et al.[26] have similarly shown that total fibronectin expression is significantly higher in patients with bladder cancer than in normal controls, which is consistent with our study. They have also investigated the link between urinary oncofetal ED-A fibronectin and poor prognosis in bladder cancer patients.[19] Moreover, urine fibronectin has been proposed as a potential diagnostic and prognostic biomarker in bladder cancer.[27,28] However, further research is required to ascertain the precise role of fibronectin in bladder cancer progression and its potential as a therapeutic target.

The literature contains only a limited number of studies on fibronectin expression in endometrial cancer.[29] Our study produced similar findings to a previous investigation by Futyma et al. regarding the upregulation of fibronectin genes by PCR in endometrial cancer tissues compared to normal endometrial tissues.[28,29] Similarly, another Northern blotting study found higher fibronectin expression in endometrial hyperplasia and carcinoma compared to benign endometrial specimens.[19] These results suggest a potential association between fibronectin upregulation and carcinogenesis in the endometrium.

CONCLUSION

To summarize, our study demonstrated a significant increase in apelin, APJ, and fibronectin expression in endometrioid adenocarcinoma, urothelial carcinoma, and prostatic adenocarcinoma compared to benign tissues. Furthermore, the expression of apelin, APJ, and fibronectin exhibited a positive correlation with each other in these tumors. In opposite to urothelial carcinoma, as the grade of prostatic adenocarcinoma and endometrioid adenocarcinoma increased, the expression of apelin, APJ, and fibronectin decreased, which seemed to be related to hormonal issues.

To the best of our knowledge, our study is the first to investigate the co-expression and distribution of endogenous apelin/APJ receptors and fibronectin in genitourinary tumors and compare them histologically with benign counterparts. This underscores the novelty and significance of our findings and suggests that the Apelin/APJ axis and its interaction with fibronectin could be considered useful targets for future studies to clarify their possible association with tumorigenesis and develop more effective cancer therapies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Akdeniz University Clinical Research Ethics Committee (Date: 09.11.2022, Decision No: KAEK-661).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Comparison of Length of Hospital Stay and Routine Laboratory Parameters in COVID-19 Patients with and without Serum Vitamin D Deficiency

Serum D Vitamini Eksikliği Olan ve Olmayan COVID-19 Hastalarında Hastanede Kalış Süresi ve Rutin Laboratuvar Parametrelerinin Karşılaştırılması

Ayşe Umay Çalışır[1], Birsen Aydın[2], Şirin Çetin[3], Selçuk Sezikli[4]

[1]Amasya University, Faculty of Arts and Sciences, Department of Biology, Amasya, Turkey
[2]Amasya University, Institute of Health Sciences, Department of Molecular Medicine, Amasya, Turkey
[3]Amasya University Faculty of Medicine Department of Biostatistics, Amasya, Turkey
[4]Amasya University Faculty of Medicine, Department of Internal Medicine, Amasya, Turkey

Abstract

Aim: Although some recent studies have shown that serum 25-hydroxyvitamin D (25(OH)D) may be effective on the course of COVID-19 disease, the results obtained are still controversial. Therefore, in this study, it was aimed to examine whether there are differences in terms of age, gender, length of hospital stay, biochemical and hematological parameters between those with and without serum 25(OH)D deficiency in COVID-19 patients.

Material and Method: The data of 413 patients hospitalized in Ankara Pursaklar State Hospital whose COVID-19 positivity was revealed by PCR test were evaluated retrospectively. Those with less than serum 25(OH)D (<20 ng/mL) were considered as vitamin D deficient group.

Results: It was observed that there was a significant difference between the groups with and without serum 25(OH)D deficiency in terms of biochemical parameters total bilirubin (p=0.007), potassium (<0.05) and glucose (p=0.038) values. CRP (p=0.051) and fibrinogen (p=0.048) values, which are factors of inflammation and coagulation, were found to be significantly higher in the group with 25(OH)D deficiency. Similarly, hematocrit (p<0.05) and neutrophil count (p<0.001), which are hematological parameters, increased significantly in patients with 25(OH)D deficiency. There were no differences in age, gender and length of hospital stay between the groups with and without 25(OH)D deficiency.

Conclusion: Our findings showed that 25(OH)D deficiency in hospitalized COVID-19 patients was associated with some biochemical, hematological and inflammatory factors, but not with age, gender and length of hospital stay.

Keywords: COVID-19, 25(OH)D deficiency, blood parameters

Öz

Amaç: Son zamanlarda yapılan bazı çalışmalar serum 25-hidroksivitamin D (25(OH)D)'nin COVID-19 hastalığının seyri üzerinde etkili olabileceğini göstermiş olsa da elde edilen sonuçlar halen tartışmalıdır. Bu nedenle bu çalışmada COVID-19 hastalarında serum 25(OH)D eksikliği olan ve olmayanlar arasında yaş, cinsiyet, hastanede kalış süresi, biyokimyasal ve hematolojik parametreler açısından farklılık olup olmadığının incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Ankara Pursaklar Devlet Hastanesi'nde yatan ve PCR testi ile COVID-19 pozitifliği saptanan 413 hastanın verileri retrospektif olarak değerlendirildi. Serum 25(OH)D (<20 ng/mL)'den düşük olanlar vitamin D eksikliği olan grup olarak kabul edildi.

Bulgular: Biyokimyasal parametreler total bilirubin (p=0.007), potasyum (p=0.015) ve glukoz (p=0.038) değerleri açısından serum 25(OH)D eksikliği olan ve olmayan gruplar arasında anlamlı fark olduğu görüldü. Enflamasyon ve pıhtılaşma faktörleri olan CRP (p=0.051) ve fibrinojen (p=0.028) değerleri arasında 25(OH)D eksikliği olan grupta anlamlı fark bulundu. Benzer şekilde hematolojik parametrelerden hematokrit (p<0.05) ve Nötrofil sayısı (p<0.001) 25(OH)D eksikliği olan hastalarda anlamlı olarak farklıydı. 25(OH)D eksikliği olan ve olmayan gruplar arasında yaş, cinsiyet ve hastanede yatış süresi açısından fark bulunamadı.

Sonuç: Sonuç olarak hastanede yatan COVID-19 hastalarında 25(OH) D eksikliğinin bazı biyokimyasal, hematolojik ve inflamatuar faktörlerle ilişkili olduğu ancak yaş, cinsiyet ve hastanede yatış süresi ile ilişkili olmadığı bulgulandı.

Anahtar Kelimeler: COVID-19, 25(OH)D eksikliği, kan parametreleri

Corresponding *(İletişim)*: Şirin Çetin, Amasya University Faculty of Medicine Department of Biostatistics, Amasya, Turkey
E-mail *(E-posta)*: cetinsirin55@gmail.com
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INTRODUCTION

In addition to microbiological and radiological examinations, biochemical and hematological tests are also used for the diagnosis of the disease in COVID-19 infection. The biochemical and hematological tests will be useful in the diagnosis of tissue-organ damage related to infection, in identifying the patient with a poor prognosis and in monitoring the course of the disease.[1] Some biochemical and hematological parameters such as LDH, CRP, ALT and NEU are important in determining the severity and prognosis of COVID-19[2,3] and especially increased neutrophil lymphocyte ratio (NLO) is a marker of systemic inflammation in the severity of COVID-19 disease.[4] Moreover, meta-analysis studies reveal that AST, ALT and total bilirubin levels in COVID-19 patients are important parameters that determine the course of COVID-19 and should be included in routine tests.[5]

Vitamin D deficiency is a global health problem that concerns approximately 1 billion people worldwide[6] and a meta-analysis study conducted in Turkey shows that the prevalence of vitamin D deficiency varies between 58.9% and 66.6%. [7] Recent studies showing that vitamin D reduces the risk of respiratory tract infection also suggested that it may have an impact on the course of COVID-19 disease. Vitamin D, which has many pharmacological and physiological functions, plays an important role in the immune system by regulating antiviral mechanisms and inflammatory processes.[8] Vitamin D deficiency leads to the production of proinflammatory cytokines such as TNF-α, IL-1β and IL-6, resulting in increased CRP levels and inflammation.[9] It has been suggested that adequate vitamin D levels can prevent cytokine storms and reduce the severity of the disease by regulating inflammatory marker levels in COVID-19 patients.[10]

It has been observed that the serum vitamin D level is lower in COVID-19 positive patients than in negative patients.[10,11] Studies in different European countries[12-14] show that there is a relationship between the increasing number of COVID-19 cases, mortality and severity and vitamin D deficiency. In contrast, Chen et al.[15] showed that vitamin D deficiency or insufficiency, even vitamin D supplementation was not significant in the risk of COVID-19 or susceptibility to death in a meta-analysis study involving 536,105 patients. Similarly, Hastie et al.[16] demonstrated in a study of 341,484 UK Biobank participants that vitamin D deficiency or insufficiency was not associated with the severity or mortality of COVID-19 infection.

In short, studies examining the relationship between vitamin D and viral infections show conflicting results due to differences in methodology, demographics, vitamin D levels and supplement dosages in these studies.[17] From this point of view, in this retrospective study, we aimed to show the differences in age, gender, lenth of hospital stay, biochemical, hematological and inflammation parameters between COVID-19 positive patients with (≥20 ng/mL) and without vitamin D deficiency (<20 ng/mL).

MATERIAL AND METHOD

This single-center, retrospective study was conducted on 413 COVID-19 patients hospitalized in Ankara Pursaklar State Hospital, whose diagnosis was confirmed by real-time polymerase chain reaction (RT-PCR) between March 1, 2020 and January 31, 2021. The study was conducted only with patients who were discharged from the COVID-19 service, patients who were taken to intensive care or died were not included in the study. Serum 25(OH)D levels were processed by the Alinity i commercially available immunoassay kits in an Alinity i automated Analyzer (Abbott Laboratories, Illinois, United States). COVID-19 positive patients hospitalized in the ward were divided into vitamin D deficiency group [25(OH)D >= 20 ng/mL, n=374, 57.87±16.25 years old] and vitamin D deficiency group [25(OH)D]. <20 ng/mL, n=39, 62.87±12.00 years old].

The age, gender, length of hospital stay, routine blood tests (such as hemogram, biochemistry tests, coagulation parameters, biochemical, hematological and other laboratory data) of the patients were retrospectively scanned from the hospital information operating system. Also, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLT), and CRP/lymphocyte ratio (CLR) were calculated.

Ethical Consideration

The study was first approved by the Turkish Ministry of Health (28 February-2021). The study was carried out with the permission of Amasya University Non-interventional Clinical Researches Ethics Committee (Date: 08.04.2021, Decision No: 55). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis

Data from research, IBM SPSS Version 22.0. (IBM Corp.) was analyzed using the program. The conformity of the data to the normal distribution was checked with the Kolmogorov-Smirnov / Shapiro Wilk test. Categorical variables are shown as numbers (n) and continuous variables as mean (mean). Chi-square test was used for group comparisons, Mann Whitney U Test was used for comparison of continuous data not suitable for normal distribution and two groups, and t test was used for comparison of continuous data suitable for normal distribution and two groups. Whether there is a significant relationship between biochemical parameters, inflammation and coagulation factors and hematological data and length of hospital stay was examined by looking at the correlation coefficients.

The effects of age, gender, and length of hospital stay on Vitamin D deficiency were investigated using binary logistic regression and the parameters that could be effective in predicting the diagnosis of vitamin D deficiency by Roc Curve Analysis.

RESULTS

A total of 413 COVID-19 positive hospitalized patients [male (n=233; 56.4%); female (n=180; 43.6%)] were included in the study. 25(OH)D levels were < 20 ng/ml in 39 of these patients and ≥20 ng/ml in 374 patients. There was no significant difference between the groups with and without vitamin D deficiency in terms of age (p=0.095), length of hospital stay (p=0.333) and gender (p=0.734) (**Table 1**). Similarly, as a result of Binary Logistic Regression Analysis, age(p=0.103), gender(p=0.995 and length of hospital stay (p=0.222) were not significantly associated with vitamin D deficiency, which is the dependent variable (p=0.103; p=0.995; p=0.222) (**Table 2**).

Table 1. Age, gender and length of hospital stay in groups with and without vitamin D deficiency

	Group vitamin D deficiency		Group without vitamin D deficiency		Statistics	p
	n	Mean± SD	n	Mean± SD		
Age	39	62.87±12.00	374	57.87±16.25	6110.000	0.095*
Length of hospital stay	39	11.59±7.87	374	10.03±5.58	6608.000	0.333*
Gender						
Male	21		212		0.734	
Famale	18		162	0.116**		
Total	39		374			

*Mann Whitney U test, ** Chi-Square Test Statistic, SD, Standard deviation

Table 2. Logistic Regression Analysis of the relationship between age, gender, length of hospital stay and vitamin D Deficiency

	S.E.	z	Exp(B)	95% C.I.for Exp(B)		p
				Lower	Upper	
Age	0.011	2.654	0.982	0.960	1.004	0.103
Gender	0.344	0.000	1.002	0.510	1.968	0.995
Length of hospitalization	0.024	1.490	0.972	0.928	1.018	0.222

There were significant differences in biochemical parameters such as total bilirubin (p=0.007), glucose (p=0.038) and potassium (p=0.015) between the groups with and without vitamin D deficiency. Although AST (p=0.251) and ALT (p=0.249) values were higher in the vitamin D deficient group, they were not statistically significant. Among the inflammation and coagulation factors, CRP (p=0.041) and fibrinogen (p=0.048) were found to be significantly higher in the vitamin D deficiency group. On the other hand, there were no significant differences between the levels of coagulation factor D-Dimer (p=0.332) and inflammation marker Ferritin (p=0.781), which are important parameters for COVID-19. It was observed that the levels of HCT (p=0.006) and NEU (p<0.0001) were significantly higher in the patient group with vitamin D deficiency. On the other hand, there was no significant difference between the groups in terms of NLR (p=0.119) and PLR rates (p=0.520), which are important inflammatory markers showing the severity of COVID-19 (**Table 3**).

Table. 3 Biochemical Parameters, Inflammation, Coagulation Factors and Hematological Data in the Groups with and without Vitamin D Deficiency

	Group Vitamin D Deficiency	Group Without Vitamin D Deficiency	t	p
	Mean ± SD	Mean± SD		
AST (U/L)	35.86±5.85	31.41±3.72	568.500	0.251**
ALT (U/L)	39.85±5.45	34.67±1.28	1.152	0.249*
AST/ALT	1.09±0.22	1.26±0.04	-0.713	0.486*
Total bilirubin (mg/dL)	0.63±0.02	0.47±0.03	2508.50	0.007**
Üric acid (mg/dL)	11.53±3.02	10.71±3.97	-0.464	0.643*
BUN (mg/dL)	27.63±3.18	20.86±1.15	1.518	0.131*
Creatinine (mg/dL)	7.57±0.65	7.98±0.14	4006.00	0.558**
EGFR (mL/dk/1.73 m²)	80.54±3.52	89.14±1.32	-1.882	0.061*
LDH (U/L)	340.75±40.12	266.13±8.17	1.822	0.078*
CK-MB (U/L)	16.8±3.28	20.10±2.17	211.500	0.557**
Glucose (mg/dL)	201.73±23.34	153.38±6.04	1.954	0.038*
Sodium (mmol/L)	123.91±5.63	121.72±1.91	6783.50	0.885**
Potassium (mmol/L)	4.39±0.09	4.17±0.027	2.448	0.015*
Calcium (mg/dL)	8.64±0.15	8.63±0.070	0.027	0.979*
Chlorine (mmol/L)	98.07±0.76	99.49±0.99	-0.502	0.619*
CRP (mg/dL)	19.69±2.15	15.67±0.58	1.967	0.041*
CLR (CRP/ LYM)	11.96±1.38	12.15±0.63	5521.00	0.338**
D-Dimer(mg/L)	1.28±0.38	1.33±0.17	6427.50	0.332**
Ferritin (ng/mL)	479.27±92.00	460.42±31.79	862.00	0.781**
Fibrinogen (mg/dL)	458.71±54.67	376.75±18.16	3961.50	0.048**
PCT (%)	0.24±0.017	0.21±0.004	5901.500	0.139**
INR	1.15±0.07	1.11±0.02	3799.500	0.969**
APTT (sn)	22.92±0.89	23.73±0.25	4112.000	0.085**
RBC (10^6/µL)	4.78±0.09	4.74±0.02	0.407	0.684*
HGB (g/dL)	13.83±0.29	13.50±0.08	1.108	0.268*
HCT (%)	169.74±24.41	97.13±4.02	2.934	0.006*
MCV (fL)	87.18±0.89	86.53±0.34	0.586	0.558*
MCH (pg)	28.98±0.32	28.57±0.13	0.964	0.336*
MCHC (g/dL)	33.24±0.12	32.99±0.055	5971.00	0.169**
RDW (%)	13.65±0.22	14.08±0.10	6223.500	0.313**
WCB (10^3/µL)	8.14±1.14	6.54±0.17	1833.500	0.117**
LYM (10^3/µL)	2.17±0.20	1.85±0.063	1.464	0.144*
NEU(10^3/µL)	5.27±0.37	4.21±0.14	4398.500	0.000**
NLR (NEU/ LYM)	3.35±0.43	3.27±0.17	5844.500	0.119**
MON (10^3/µL)	0.59±0.06	0.53±0.024	5670.000	0.070**
BAS(10^3/µL)	0.025±0.003	0.02±0.003	6417.000	0.453**
EOS(10^3/µL)	31.09±7.06	23.95±2.12	5836.000	0.194**
PLT (10^3/µL)	263.67±19.78	235.48±4.81	6157.500	0.269**
PLR (PLT/ LYM)	151.32±14.40	170.29±6.37	6475.500	0.520**
MPV (fL)	9.41±0.14	9.29±0.042	0.807	0.420*
PDW (%)	16.71±0.38	16.51±0.126	0.461	0.645*

*t Test, **Mann Whitney U Test

In the study, we also evaluated the correlations between laboratory parameters and length of hospital stay in patient groups with and without vitamin D deficiency. Although there was a correlation between some parameters and the length of hospital stay in the group without vitamin D deficiency, no correlation was observed with these parameters in the patient group with vitamin D deficiency. In the group without vitamin D deficiency, serum uric acid (r=0.126, p=0.027), CRP (r=0.128, p=0.014), fibrinogen (r=0.157, p=0.006) levels and length of hospital stay were found to be positively correlated at 0.05, 0.05 and 0.01 significance levels, respectively. As serum uric acid, CRP and fibrinogen levels increased, the length of hospital stay also increased. On the other hand, negative correlation were observed at 0.05 significance level between APTT (r=-0.118, p=0.27), RBC (r=-0.113, p=0.28) levels and the length of hospital stay; As APTT and RBC levels decreased, the length of hospital stay increased (**Table 4**).

Table 4. Correlations between Length of Hospital Stay and Uric Acid, CRP, Fibrinogen, APTT, RBC in the Group Without Vitamin D Deficiency

	Uric acid	CRP	Fibrinogen	APTT	RBC
r	0.126*	0.128	0.157	-0.118	-0.113
p	0.027	0.014	0.006	0.027	0.028
N	304	372	309	351	374

In the ROC analysis, it was observed that age, gender and length of hospital stay [AUC (area under curve) values of 0.514, 0.581 and 0.547, respectively] were not parameters explaining vitamin D deficiency with sufficient sensitivity. In addition, glucose, CRP and HTC (AUC values of 0.705, 0.620 and 0.682, respectively) were found to be parameters that explain vitamin D deficiency with poor sensitivity (**Figure 1**).

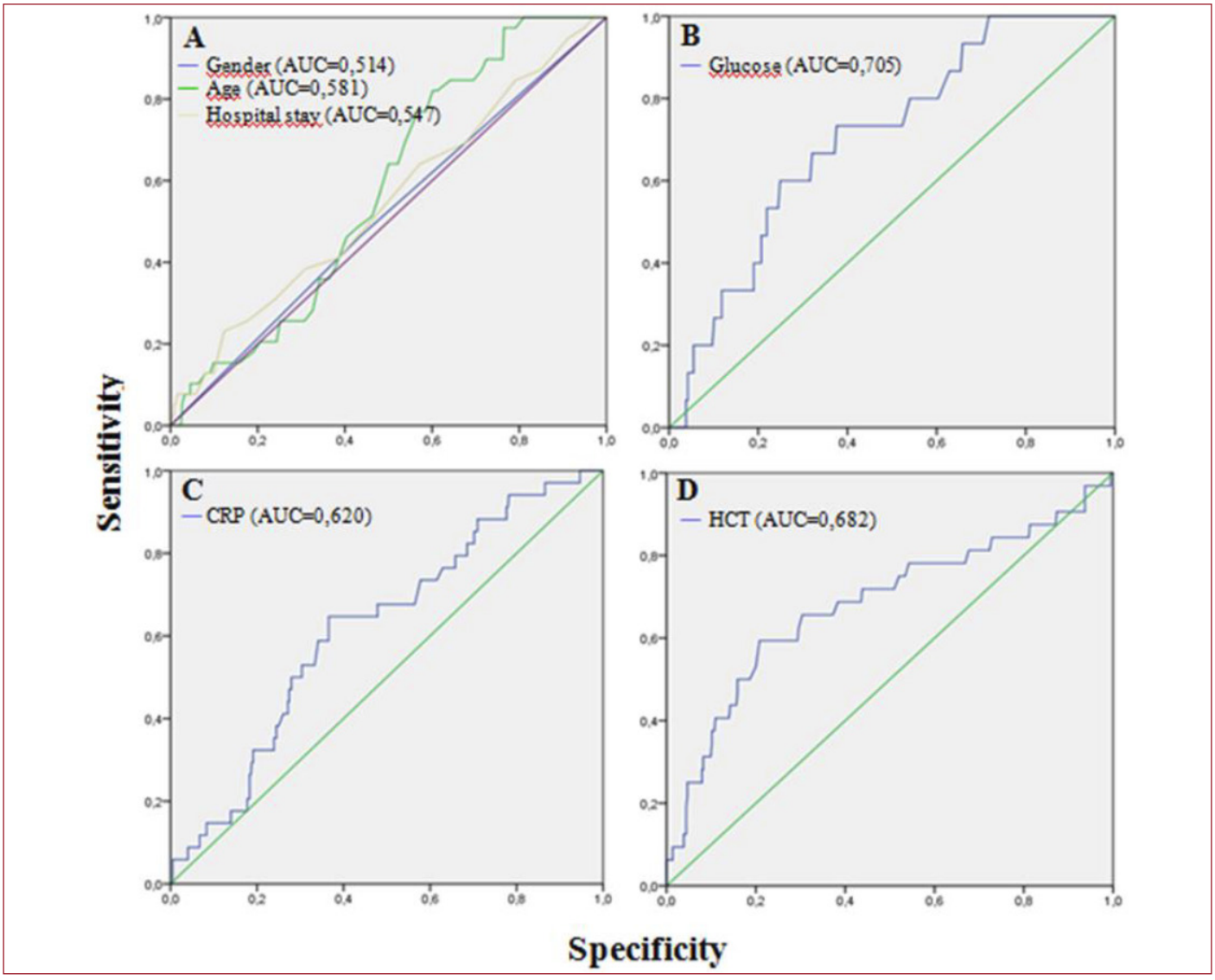

Figure 1. ROC curves analysis for vitamin D of Age, Gender, Length of Hospital Stay, Glucose, CRP and HCT Parameters in Predicting Vitamin D Deficiency in COVID-19 Patients.

ROC: receiver operating characteristic.

DISCUSSION

In our study, no significant difference was found between the groups with and without vitamin D deficiency in terms of age, length of hospital stay and gender. These findings supported by[18] suggest that COVID-19 patients with serum 25(OH)D < 20 ng/mL did not differ in age, gender, race, BMI or comorbidities compared with those with 25(OH)D ≥ 20 ng/mL. On the other hand, Demir et al.[19] stated in their study that COVID-19 patients with vitamin D >30 ng/ml had lower hospital stays than those with vitamin D levels <10 ng/mL and <20 ng/ml. Similarly, studies with COVID-19 patients serum 25(OH)D <20 ng/ml[20] and severe 25(OH)D deficiency (<10 ng/mL)[8] have found that these patients have longer hospital stays compared to patients with higher 25(OH)D concentrations.

Significant changes in heart and muscle damage (CK, CK-MB, Troponin-I, myoglobin increase), liver function (AST, ALT, total bilirubin increase and albumin decrease) and kidney function (BUN and creatinine increase) parameters in severe COVID-19 patients.[1] Similarly, higher levels of ALT, AST, GGT, and bilirubin were observed in patients with severe COVID-19 compared to those with mild.[5,22] Although not statistically significant in our study, it is seen that biochemical parameters such as ALT, AST, BUN and LDH increase in patients with vitamin D deficiency compared to patients without vitamin D deficiency. On the other hand, Demir et al.[19] showed that there was no difference between vitamin D levels of COVID-19 patients and biochemical and hematological parameters such as AST, ALT, Platelet, WBC, PLT, Urea, Creatinine, Na, K, Lymphocyte, neutrophil.

Bilirubin, which has antioxidant and anti-inflammatory effects, is the end product of heme catabolism. In our study, we observed higher bilirubin levels in the group with vitamin D deficiency compared to those without. This result is similar to studies showing that serum bilirubin levels are significantly higher in patients with severe COVID-19 symptoms such as pneumonia, ARDS, multiple organ damage, and septic shock.[22,23]

One of the statistically significant results of the current study is the presence of hyperkalemia in the vitamin D deficiency group. The serum potassium value, whose homeostasis is regulated by the kidneys, is important in the prognosis and management of COVID-19 patients. Hyperkalemia may occur in COVID-19 patients due to decreased urine output as a result of kidney failure or from acid/base imbalance caused by the side effects of the drugs that patients are treated.[24]

Vitamin D deficiency has been associated with increased infection formation, immunological disorders, cancers, obesity, insulin resistance, high fasting glucose concentrations and type 2 diabetes.[25] In our study, we observed a higher glucose level in COVID-19 patients with vitamin D deficiency compared to those without. Moreover, in ROC analysis, we determined that glucose level may be the most important parameter that can be used to predict vitamin D deficiency. In line with our findings, it was observed that blood glucose levels were high in COVID-19 emergency room patients with vitamin D deficiency and this situation increased the severity of the disease.[26] In relation to this, it has been reported that diabetic COVID-19 patients are at higher risk of hospitalization, severe lung involvement and mortality than non-diabetic patients.[27]

In our current study, in addition to biochemical parameters, hematological parameters such as CRP, fibrinogen, HCT and neutrophil count increased significantly in vitamin D deficient COVID-19 patients and these findings were found to be correlated with many studies in the literature. CRP is an acute phase protein synthesized in response to proinflammatory cytokines such as TNF-α, IL-1β and IL-6 and it appears to be elevated in COVID-19 positive cases. Similarly, vitamin D deficiency leads to the production of these proinflammatory cytokines, resulting in elevated CRP levels and inflammation.[9] Considering that C-reactive protein is an important marker for the severity of COVID-19 inflammation and cytokine storm, this result once again reveals the possible role of vitamin D in reducing the complications caused by cytokine storm.

It has been observed that COVID-19 patients with adequate vitamin D value have lower levels of inflammatory markers such as CRP and D-Dimer, have shorter hospital stays and better computed tomography results than those with low vitamin D value.[19] It has been reported that COVID-19 positive patients with serum vitamin D level >30 ng/ml have very low CRP levels moreover, vitamin D levels and ferritin, CRP and D dimer levels are inversely proportional.[10] In addition, it was found that vitamin D level was positively correlated with lymphocyte count and negatively correlated with CRP and fibrinogen levels in pediatric COVID-19 cases.[28] Compared to COVID-19 negative groups, COVID-19 patients were found to have higher levels of HCT, neutrophils and CRP but lower levels of Hb, monocytes, eosinophils and basophils.[29] Similarly, Bonetti et al.[3] reported that LDH, CRP, neutrophils, lymphocytes, albumin, APTT and age parameters are important determinants of hospital mortality in hospitalized COVID -19 patients. Pimentel et al.[30] observed that there was no significant change in CRP level but higher neutrophil count and neutrophil/lymphocyte ratio in COVID-19 intensive care patients with vitamin D deficiency compared to those who were not deficient.

In the current study, a negative correlation was observed between APTT and RBC levels and hospitalization time in the patient group without vitamin D deficiency, that is, as the level of these parameters decreased, the length of stay in hospital increased. Negative correlations between the severity of COVID-19 disease and the level of RBC can be explained in different ways. The decrease in the number of RBCs responsible for oxygen transport causes hypoxia,

which increases the severity of the COVID-19 disease or the COVID-19 virus damages the RBC structure. Thomas et al.[21] suggested that although there was no significant difference in hematological parameters such as RBC count and hematocrit between COVID-19 positive and healthy individuals, the COVID-19 virus could change the protein and lipid content of RBC membranes. Similarly, it has been reported that the virus may enter RBCs via spike and affect glycoprotein and hemoglobin function.[32] Significantly higher plasma D-dimer and APTT levels were observed in patients who died from COVID-19 infection, where APTT is one of the determining parameters in COVID-19 mortality.[33]

CONCLUSION

In the current study, higher total billurin, glucose, potassium, CRP, fibrinogen, HCT and neutrophil counts were observed in ward COVID-19 patients with serum vitamin D deficiency. In addition, in the group with a vitamin D level greater than 20 ng/ml, a positive correlation was observed with the length of hospital stay and Uric acid, CRP and fibrinogen levels and a negative correlation with APTT and RBC values. Serum vitamin D level has been effective on many biochemical, hematological and inflammation parameters in COVID-19 patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Amasya University Non-interventional Clinical Researches Ethics Committee (Date: 08.04.2021, Decision No: 55).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

The Evaluation of the Effect of the Treatments on Oxidative Stress and Inflammation in Patients Receiving Different Dialysis Modalities

Farklı Diyaliz Yöntemleri Alan Hastalarda Tedavilerin Oksidatif Stres ve İnflamasyon Üzerindeki Etkisinin Değerlendirilmesi

Sumeyra Koyuncu[1], Hilal Sipahioglu[1], Ismail Kocyigit[2], Oktay Oymak[2], Bulent Tokgoz[2], Murat Hayri Sipahioğlu[2]

[1]Health Sciences University, Kayseri Medical Faculty, Department of Internal Medicine
[1]Department of Nephrology, Erciyes Medical Faculty, Kayseri, Turkey

Abstract

Aim: To evaluate the effects of different dialysis methods on oxidative stress and inflammation in patients with end-stage renal disease (ESRD) who are newly enrolled in a routine dialysis program.

Material and Method: In this prospective study, 138 ESRD patients and 30 healthy volunteers were evaluated. Fifty-four of 73 hemodialysis (HD) patients and 51 of 65 periton dialysis (PD) patients completed the study. Other patients were excluded from the study. The levels of superoxide dismutase (SOD) and total antioxidant capacity (TAC) were measured to determine the oxidative stress status, and IL-6, IL-10, and F2 isoprostane levels were measured to determine the inflammation status, just before the start of dialysis treatment and at 6 months.

Results: At the beginning of the study, hs-CRP and IL-6 levels were significantly higher in the patient group compared to the control group (p<0.001, p<0.001, respectively), and IL-10 levels were significantly lower (p=0.008). The hs-CRP and IL-10 levels in the HD group were found to be similar at the beginning of the study and in the 6th month. On the other hand, IL-6 levels decreased significantly compared to baseline values at 6 months (p=0.016). In the PD group, no difference was observed in terms of hs-CRP and IL-10 levels at the beginning and the 6th month (p>0.05), but IL-6 levels were found to be decreased compared to baseline at the 6th month (p<0.001). When HD and PD patient groups were compared with each other, no difference was found between the groups in terms of hs-CRP and IL-10 levels in the 6th month of dialysis treatment. IL-6 levels were found to be significantly higher in the HD group (p<0.001). At the beginning of the study, the F2 isoprostane level was significantly higher in the patient group than the control group (p<0.001), whereas the SOD and TAC levels were significantly lower (p=0.001, p=0.024, respectively). In the HD group, the F2 isoprostane level was found to be significantly higher at 6 months compared to baseline (p=0.019). There was no significant change in SOD and TAC levels. There was no significant difference in F2 isoprostane and TAC levels at 6 months from baseline in the PD group, whereas SOD levels were found to be significantly lower (p=0.015).

Conclusion: The oxidative status found in ESRD patients increases with dialysis treatments. Oxidative stress increase is more prominent in HD patients. Therefore, we think that giving antioxidant treatment in patient groups undergoing dialysis treatment may benefit complications related to oxidative stress.

Keywords: Hemodialysis, peritoneal dialysis, oxidative stress

Öz

Amaç: Rutin diyaliz programına yeni başlayan son dönem böbrek yetmezliği (ESRD) olan hastalarda farklı diyaliz yöntemlerinin oksidatif stres ve inflamasyon üzerine etkilerini değerlendirmek.

Gereç ve Yöntem: Bu prospektif çalışmada 138 SDBY hastası ve 30 sağlıklı gönüllü değerlendirildi. 73 hemodiyaliz (HD) hastasının 54'ü ve 65 periton diyalizi (PD) hastasının 51'i çalışmayı tamamladı. Diğer hastalar çalışma dışı bırakıldı. Oksidatif stres durumunu belirlemek için süperoksit dismutaz (SOD) ve toplam antioksidan kapasite (TAC) seviyeleri, inflamasyon durumunu belirlemek için IL-6, IL-10 ve F2 izoprostan seviyeleri, diyaliz tedavisi başlamadan hemen önce ölçüldü ve 6. ayda ölçüldü.

Bulgular: Çalışmanın başında hasta grubunda kontrol grubuna göre hs-CRP ve IL-6 düzeyleri anlamlı olarak yüksek (sırasıyla p<0.001, p<0.001), IL-10 düzeyleri anlamlı olarak düşüktü (p=0.008). HD grubunda hs-CRP ve IL-10 düzeyleri çalışmanın başında ve 6. ayda benzer bulundu. IL-6 seviyeleri ise 6. ayda başlangıç değerlerine göre anlamlı olarak azaldı (p=0.016). PD grubunda başlangıç ve 6. ay hs-CRP ve IL-10 düzeyleri açısından fark izlenmezken (p>0,05), ancak 6. ayda IL-6 düzeylerinin başlangıca göre düştüğü saptandı. (p<0.001). HD ve PD hasta grupları kendi aralarında karşılaştırıldığında diyaliz tedavisinin 6. ayında hs-CRP ve IL-10 düzeyleri açısından gruplar arasında fark saptanmadı. IL-6 düzeyleri HD grubunda anlamlı olarak yüksek bulundu (p<0.001). Çalışmanın başında F2 izoprostan düzeyi hasta grubunda kontrol grubuna göre anlamlı olarak yüksek (p<0,001), SOD ve TAC düzeyleri anlamlı olarak düşüktü (sırasıyla, p=0,001, p=0,024). HD grubunda 6. ayda F2 izoprostan düzeyi başlangıca göre anlamlı olarak yüksek bulundu (p=0.019). SOD ve TAC düzeylerinde anlamlı bir değişiklik olmadı. PD grubunda 6. ayda F2 izoprostan ve TAC düzeylerinde başlangıca göre anlamlı fark bulunmazken, SOD düzeyleri anlamlı olarak düşük bulundu (p=0.015).

Sonuç: SDBY hastalarında bulunan oksidatif durum diyaliz tedavileri ile artmaktadır. Oksidatif stres artışı HD hastalarında daha belirgindir. Bu nedenle diyaliz tedavisi gören hasta gruplarında antioksidan tedavi verilmesinin oksidatif strese bağlı komplikasyonlara fayda sağlayabileceğini düşünüyoruz.

Anahtar Kelimeler: Hemodiyaliz, periton diyalizi, oksidatif stres

Corresponding *(İletişim)*: Eda Albayrak, Tokat Gaziosmanpasa University, Medical Faculty, Department of Radiology, Tokat, Turkey
E-mail *(E-posta)*: edalbayrak1@hotmail.com
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INTRODUCTION

Cardiovascular events are the most common cause of morbidity and mortality in patients with chronic renal failure (CRF).[1] These events include inflammation, oxidative stress, hyperhomocysteinemia, defects in bone-mineral metabolism, retention of uremic toxins, anemia, and high troponin levels.[2]

CRF is a chronic inflammatory disease. It has known that patients with end-stage renal disease (ESRD) have 10-fold higher serum proinflammatory cytokine levels compared to the normal population. Several factors are responsible for the occurrence of inflammation; the usage of catheters, the increase in adipose tissue, and impaired adipokine balance cause inflammation due to reasons such as the decrease in the clearance of proinflammatory cytokines, aggregation of uremic toxins, fluid overload, increased level of endotoxins, especially in peritoneal dialysis (PD) patients.[3]

In ESRD patients, an increase in the formation of oxygen free radicals (OFR) and a chronic decrease in major antioxidant systems was observed with dialysis. The reason for this is different in hemodialysis (HD) and PD patients. The reasons that are responsible for the increase in oxidative stress products are the type of dialysis membrane, the use of heparin, intravenous iron intake, activation of thrombocytes and leukocytes in HD patients, and the low pH of the solution, high lactate concentration, and increased osmolarity in patients with dialysis.[4]

Several studies have shown that proinflammatory cytokines (TNF-α, IL-1, IL-6, and others) and CRP levels are higher than the normal population in these patients during the predialysis period and after dialysis treatment.[5-7]

In this study, we aimed to investigate the difference between the effects of different renal replacement methods on oxidative stress and inflammation through various parameters in patients with ESRD.

MATERIAL AND METHOD

This study was carried out in University Faculty of Medicine, Department of Nephrology. Ethical approval was obtained from the Ethics Committee of University (2011/36, 04.01.2011) before the study and informed consent was obtained from all patients and healthy volunteers that were included in the study. Patients aged between 18 and 70 years with stage 5 renal failure were included in the study. Diabetic patients, patients with malignancy, active infection, severe heart failure, respiratory failure, patients that have hepatitis or that are the carriers, patients with a rheumatic disease using anti-inflammatory drugs were all excluded from the study. A total of 73 HD and 65 PD patients participated in the study. Thirteen of the HD patients died before the second evaluation, 4 patients were excluded from the study because they did not need dialysis anymore, and 2 patients could not be reached. Ten of the PD patients died before the second control, 3 of them underwent renal transplantation, and 1 of them was excluded from the study because they did not require dialysis. As a result, 54 HD and 51 PD patients completed the study. 30 healthy volunteers were taken as the control group. Socio-demographic characteristics of the patients such as age and gender were recorded.

Blood urea nitrogen (BUN) and serum creatinine, glucose, triglyceride, total cholesterol, LDL and HDL cholesterol, uric acid, alkaline phosphatase (ALP), total protein, albumin, calcium (Ca), phosphorus (P), parathyroid hormone (PTH), hemoglobin concentration, hematocrit, transferrin saturation, and ferritin levels were measured from blood samples taken from the patients.

Interleukin 6 (IL-6), interleukin 10 (IL-10), and high sensitivity C-reactive protein (hs-CRP) measurements were used in the evaluation of inflammation, whereas F2 isoprostane, Superoxide Dismutase (SOD), Total Antioxidant Capacity (TAC) measurements were used in the evaluation of the oxidative stress-antioxidant system. These measurements were performed twice, firstly when the patient was admitted to the service for renal replacement and secondly 6 months after being included in the renal replacement treatment program. Blood samples in HD patients were taken in the period right before dialysis, whereas in PD patients and healthy volunteers, they were taken on an empty stomach at 8 am. SOD level was studied in plasma, other parameters were studied in serum. The collected blood samples were stored at -80°C after centrifugation until the day of the study.

Statistical Analysis

SPSS 15.0 software was used for statistical analysis of the data. Percentages were calculated for categorical variables and the chi-square test was used for comparison. Kolmogorov-Smirnov test was used to determine the distribution of the data. Normally distributed data were expressed as mean value ± standard deviation, and data not distributing normally were expressed as median (minimum-maximum). In the comparison of HD, PD, and control groups, the Anova test was used when the group distribution was equal, and the Kruskal-Wallis test was used when the group distribution was not equal. In the one-to-one comparison of the groups; Student's t-test was used when group distribution was equal, and the Mann-Whitney U test was used when group distribution was not equal. In comparison of the baseline and 6th-month values; the paired samples test was used when group distribution was equal, and Wilson signed ranks test was used when group distribution was not equal. The relationship between inflammation and oxidant-antioxidant parameters was evaluated by Spearman correlation analysis. The statistical significance level was accepted as $p < 0.05$.

RESULTS

138 patients with ESRD who were included in this study but 105 of them completed the study. Fifty-four patients were on the HD and 51 patients were on the PD program. As a

control group, 30 healthy volunteers participated in the study. All study participants were evaluated according to demographical parameters and shown in **Table 1**. While 15 were females (50 %) in the control group, 29 were females (53%) in the HD group, and 24 were females (47%) in the PD group. Additionally, the average age of the control group was 42.6±5.0 years, it was 59.22±11.2 years HD group, and the PD group was 43.96±15.62. The inflammation parameters of all groups are shown in **Table 2** at the beginning of the study.

Table 1: Demographical characteristics of the patients in this study.

Parameters	HD group n (%)	PD group n (%)
Age (year)	59.2±11.2	43.9±15.2
Gender (female)	24 (47.1)	29 (53.7)
Etiology		
Hypertension	33 (61.1)	14 (27.4)
Cystic disease	4 (7.3)	8 (15.7)
Obstructive Uropathy	2 (3.7)	3 (5.9)
Glomerulonephritis	1 (1.9)	2 (3.9)
Amyloidosis	0 (0)	3 (5.9)
ATN*	1 (1.9)	2 (3.9)
Interstitial nephritis/ Pyelonephritis	0 (0)	3 (5.9)
Unknown	13 (24.1)	16 (31.4)

*Acute tubular necrosis

Table 2: The relationship between the control group and the patient groups in terms of inflammation and oxidant/antioxidant parameters

Parameters	Control	HD	PD	p
Hs-CRP (mg/dl)	3.2 [a,b] (3-9.3)	7.3 (3-98)	4.6 (3-203)	<0.001
IL-6 (pg/ml)	12.7 [c,d] (3.6-260)	41.8 (8.2-1824.6)	33.6 (8.2-1476.4)	<0.001
IL-10 (pg/ml)	20.9 [e,f] (2.7-137.3)	7.7 (4.6- 91.8)	7.27 (4.6-123.6)	0.028
F2 isoprostane (pg/ml)	86 [x,y] (76-149)	108 (72-4152)	109 (81-3254)	<0.001
SOD* (units/ml)	0.1 [z,t] (0.02-0.2)	0.1 (0.03-0.6)	0.1 (0.02-0.8)	0.004
TAC** (mM)	1.7 [p,r] (0.3-3.6)	2.1 (0.01-10.6)	3.4 (0.03-13)	0.012

a: Control group and HD group p<0.001, b: Control group and PD group p<0.001 c: Control group and HD group p<0.001, d: Control group and PD group p<0.001
e: Control group and HD group p=0.015, f: Control group and PD group p=0.017
x: Control group and HD group p<0.001, y: Control group and PD group p<0.001
z: Control group and HD group p=0.007, t: Control group and PD group p=0.001
p: Control group and HD group p=0.004
IL: Interleukin
* Superoxide Dismutase
**Total Antioxidant Capacity

At the beginning of the study, hs-CRP and IL-6 levels were found to be significantly higher in the patient group compared to the control group, and the IL-10 level was found to be significantly lower. In HD and PD groups, hs-CRP levels were found to be significantly higher than the control group (p<0.001, p<0.001 respectively), IL-6 levels were significantly higher (p <0.001, p <0.001 respectively), and IL-10 levels were significantly lower (p=0.015, p=0.017, respectively). There was no significant difference between PD and HD groups in terms of hs-CRP, IL-6, and IL-10 levels (p=0.236, p=0.090, p=0.584 respectively). The hs-CRP values were found to be similar at the baseline and in the 6th month. IL-6 levels were found to be significantly decreased after 6 months compared to baseline. There was no significant difference in IL-10 levels. There was no significant difference in terms of hs-CRP values. IL-6 levels were found to be decreased after 6 months compared to baseline. There was no significant difference in terms of IL-10 levels. There was no significant difference between the two groups in terms of hs-CRP values. In the 6th month of the study, IL-6 levels were found to be significantly higher in the HD group. There was no significant difference in terms of IL-10 levels. The comparison of inflammation parameters between dialysis groups in the 6th month of the study is shown in **Table 3**.

Table 3: Comparison of baseline and 6th month inflammation parameters of patients between HD and PD groups

	Parameters	Baseline	6th month	p
HD Group	hs-CRP (mg/dl)	7.3 (3-186)	11.9 (3-98)	0.224
	IL-6 (pg/ml)	41.8 (8.2-1824.5)	27.3 (8.2-1480.9)	0.016
	IL-10 (pg/ml)	7.7 (5.6-91.8)	8.2 (2.7-209.1)	0.064
PD Group	hs-CRP (mg/dl)	4.61 (3-203)	11.4 (3-102)	0.300
	IL-6 (pg/ml)	33.6 (8.2-1476.4)	16.4 (2.7-129.1)	<0.001
	IL-10 (pg/ml)	7.3 (4.6-123.6)	7.27 (4.6-180.9)	0.765

HD: Hemodialysis, PD: Periton dialysis, IL: Interleukin

Table 4: Comparison of baseline and 6th month oxidant-antioxidant parameters of patients in HD and PD groups

	Parameters	Baseline	6th month	P
HD Group	F2 isoprostane (pg/ml)	108 (72-4152)	162 (79-4866)	0.019
	SOD (units/ml)	0.1 (0.003-0.573)	0.1 (0.003-0.670)	0.476
	TAC (mM)	2.1 (0.01-10.59)	2.3 (0.01-13.0)	0.533
PD Group	F2 isoprostane (pg/ml)	109 (81-3524)	101 (75-5040)	0.099
	SOD (units/ml)	0.1 (0.02-0.8)	0.1 (0.02-0.5)	0.015
	TAC (mM)	3.4 (0.03-13)	3.4 (0.1-8.8)	0.183

SOD: Superoxide Dismutase, TAC: Total Antioxidant Capacity

The oxidant-antioxidant system parameters of all groups at the beginning of the study are shown in Tables 4. At the beginning of the study, the levels of F2 isoprostane, SOD, and TAC in the patient group were found to be significantly higher than the control group. F2 isoprostane level was higher in HD and PD groups (p<0.001, p<0.001 respectively). SOD level was found to be significantly higher in HD and PD groups compared to the control group (p=0.007, p=0.001 respectively). In the control group, the TAC level was found to be similar to the HD group (p=0.198), but lower than the PD group (p=0.004). No significant difference was found between the HD and the PD groups in terms of F2 isoprostane and SOD levels (p=0.491, p=0.274 respectively). Although the TAC level was higher in the PD group compared to the HD group, no statistically significant difference was found (p=0.059).F2 isoprostane was significantly higher in the HD group after 6 months compared to baseline. There was no significant

difference in SOD and TAC levels. There was no significant difference in terms of the F2 isoprostane level in the 6th month in the PD group compared to the baseline. SOD levels were found to be significantly lower. There was no significant difference in terms of the TAC level.

When the patient groups (54 HD patients and 51 PD patients) were evaluated and correlation analysis was made between inflammation and oxidant-antioxidant parameters at the beginning of the study; there was a directly proportional and statistically significant relationship between hs-CRP and IL-6 (r=0.224, p=0.023), between IL-6 and F2 isoprostane (r=0.233, p=0.019), between IL-10 and TAC (r=0.199, p=0.043) SOD and uric acid (r=0.252, p=0.012) levels. Besides, there was an inversely proportional but not statistically significant relationship between hs-CRP and TAC levels (r=0.192, p=0.051). No significant relationship was found between inflammation and oxidant-antioxidant parameters. In the 6th month of the study, when the patient groups (54 HD patients and 51 PD patients) were evaluated and correlation analysis was made between inflammation and oxidant-antioxidant parameters; a directly proportional and statistically significant correlation was found between IL-6 and F2 isoprostane (r=0.309, p=0.002) and between IL-10 and TAC (r=0.234, p=0.016) levels. No significant relationship was found between inflammation and oxidant-antioxidant parameters.

DISCUSSION

In our study, oxidative stress parameters F2 isoprostane, SOD, and TAC were found to be higher in patients who just started dialysis compared to the healthy control group. At the sixth month, an increase in the F2 isoprostane level was detected in HD patients, while the SOD level in PD patients increased. Considering the values in the sixth month, the F2 isoprostane level was higher in HD patients compared to PD patients.

When the inflammatory parameters were evaluated, it was observed that CRP and pro-inflammatory cytokine IL-6 was higher in dialysis patients compared to the control group, and anti-inflammatory cytokine IL-10 level was lower in dialysis patients. It was observed that IL-6 levels decreased in both HD and PD groups compared to the baseline after the six months. This decrease was more in the PD group, and the 6th-month IL-6 level was lower in the PD group compared to the HD group (p<0.001).

Inflammation is common in ESRD patients. Serological markers of acute-phase response have been found in 30-50% of them.[8] It has been known that inflammation status is increased in patients who receive renal replacement treatment. In a study by Borazan et al. comparing the inflammation status in patients who underwent HD and PD, the CRP, TNF-α, IL-6, and IL-10 levels of the patients were compared at the beginning of dialysis and after 3 months and also they were compared with the healthy control group. No significant difference was found in cytokine levels between the PD and HD groups at the beginning of the treatment and after 3 months, however, the levels of the PD and HD groups were found to be significantly higher than the control group.[9]

In this study, hs-CRP and IL-6 levels were found to be higher, and IL-10 levels were found to be significantly lower in patients with renal failure compared to the control group. The increases in CRP and IL-6 supported the general literature data showing the increase in inflammation in CRF patients. On the other hand, the low level of IL-10 in patients may indicate that the anti-inflammatory system is not effective enough in CRF patients or that there is a continuous inflammatory stimulus in such patients that requires suppression of the anti-inflammatory system. IL-6 levels measured at the 6th month of replacement treatment were found to be lower in HD and PD groups than the baseline levels. The decrease in cytokine level was more in the PD group and 6th-month values showed a significant difference in HD and PD groups. These data obtained in this study show that initiation of dialysis treatment in patients with ESRD leads to a significant improvement in inflammatory status. This improvement was more pronounced in PD patients. Removal of small and medium molecular weight toxins, which play a role in the inflammatory process, from the body along with dialysis may have played a role in the partial recovery of the inflammatory process. The higher improvement in PD patients can be partially explained by the better protection of residual renal functions and the absence of extracorporeal circulation that may cause leukocyte activation as in HD.

CRP is a strong predictor of important cardiovascular events and all-cause mortality in the dialysis population. This situation has been demonstrated in many studies.[10,11]

Even plasma IL-6 level is an independent indicator of cardiovascular events, the progression of atherosclerosis, and all-cause deaths in dialysis patients.[12-15]

The levels of CRP and IL-6 were studied in a study evaluating ESRD (stage 3-5) patients. Similarly, high levels of CRP and IL-6 were found in patients with renal failure.[16]

It is known that inflammation status is increased in patients who receive renal replacement treatment. In the study by Sundl et al. CRP and IL-6 levels were found to be significantly higher than the control group (n: 37) in PD patients (n: 37).[17]

Similarly, in another study, higher CRP and IL-6 levels were found in HD patients compared to the control group.[18]

In the study by Kim et al., TNF-α, IL-1, IL-6, IL-8, IL-10, and IL-12 levels were found higher in HD patients than in the control group.[19]

Oxidative stress increases from the early stages of renal failure. Low molecular weight toxins with prooxidant activity may accumulate in CRF and cause an increase in oxidative stress.[20]

Dialysis modalities (HD, PD) also exacerbate oxidative stress. In HD, the loss of hydrophilic free small molecular

weight substances such as vitamin C, trace elements, and regulatory enzyme compounds, thermal damage resulting from the temperature difference between dialysate and body temperature, the cytotoxic effect of chloramine in the dialysate, activation of the neutrophil and complement system from the HD membrane, the activation of the lipoprotein lipase enzyme and the increase in free fatty acids by heparin used for anticoagulation, cause lipid peroxidation and oxidant stress. Dialyzer interactions, microbial contamination or dialysate containing pyrogen substances, and the probable pro-oxidant effect of metabolites in high concentrations in the patient's plasma are seen as the major 3 causes of oxidative stress. In peritoneal dialysis, the formation of acute gastroenteritis due to heat sterilization stimulates the oxidative stress response.[4,21]

In this study, F2 isoprostane levels, which are a lipid peroxidation product and indicate the presence of oxidative stress, were found to be significantly higher in patients with ESRD. It was similar between the groups that chose HD or PD at the beginning of the study whereas, in the 6th month, it was found to be higher in the HD group compared to PD. In this study, the data we obtained with F2 isoprostane levels supported the presence of oxidative stress in patients with CRF. We showed that HD has more aspects that can activate oxidative stress than PD.

Oberg et al. examined protein carbonyl (protein oxidation product) and F2 isoprostane levels in patients with CRF (stage 3-5) and compared them with healthy subjects. The level of oxidative stress parameters was found to be higher in patients with CRF.[16]

In the study conducted by Montesa et al. with stage 4 predialysis patients (n: 32), F2 isoprostane and CRP levels were found to be significantly higher in the predialysis group than the control group. In this study, it was concluded that there is a negative correlation between F2 isoprostane and GFR.[22,23]

In the study conducted by Ramos et al., stage 3-4 CRF patients (n: 184) and healthy control group (n: 43) were compared, and F2 isoprostane and CRP levels were found to be significantly higher in CRF patients.

In our study, a directly proportional and significant relationship was found between F2 isoprostane and IL-6 levels. There appears to be a synergism between systemic inflammatory response and oxidative stress. Oxidative stress metabolites (hydrogen peroxide) can activate the NFκB pathway, which enables the synthesis of proinflammatory cytokines, resulting in amplification of the inflammatory cascade. Also, acute phase reactants can regulate the production of oxidative agents. For example, CRP has been shown to increase intracellular OFR production from IgG stimulated phagocytic cells. In these ways, events associated with increased oxidative stress may perpetuate the chronic inflammatory state.

In this study, TAC and SOD levels were found to be higher in the patient group compared to the control group. There was no significant difference between HD and PD groups at the beginning of the study and 6 months. There was a significant decrease in the PD group at 6 months compared to the beginning of the study. There are studies in the literature that have

CONCLUSION

As a result, as proved in this study, increased levels of inflammation and oxidative stress are observed in ESRD patients compared to healthy people. The initiation of dialysis treatment probably improves the level of inflammation by increasing cytokine clearance. It has been shown in this study that PD treatment provides more improvement in inflammation in the early period. Also, while HD treatment caused an increase in oxidative stress in the early period, this increase was not observed in PD treatment. The use of the peritoneal membrane as a dialysis membrane seems to be a more appropriate treatment modality in renal replacement treatments. However, we think that it would be beneficial to provide antioxidant supplements to reduce the complications that may be caused by increased oxidative stress in both patients with chronic renal failure and receiving dialysis treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of Erciyes University, Date: 04.01.2011, Decision No: 2011/36

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Determination of Awareness and Knowledge Level on Brain Death, Organ Donation and Transplantation in Intensive Careers in Konya

Konya İli Yoğun Bakım Çalışanlarında Beyin Ölümü, Organ Bağışı ve Nakli Konusundaki Farkındalık ve Bilgi Düzeyinin Belirlenmesi

Mustafa Tomruk[1], Funda Gök[2], Esma Eroğlu[3]

[1]Meram State Hospital, Department of Anesthesiology and Reanimation, Konya, Turkey
[2]Necmettin Erbakan University Meram Faculty of Medicine, Department of Anesthesiology and reanimation, Konya, Turkey
[3]Meram State Hospital, Department of Infectious Diseases and Clinical Microbiology, Konya, Turkey

Abstract

Aim: It is shown that there is a lack of social and individual information on organ transplantation and donation, and as a result, organ donation is not sufficient due to social and individual insensitivity. In our study, we aimed to evaluate the level of awareness, attitude and knowledge about brain death, organ donation and transplantation in intensive care workers of hospitals in Konya.

Material and Method: A total of 219 healthcare professionals, 144 nurses and 75 doctors, who agreed to meet face-to-face with the researcher, who were working in Konya province state hospitals, university hospitals and private hospital intensive care units, were included in the study. The data of the study were collected using the 'Data Collection Form', which was created as a result of the literature review and includes demographic information.

Results: A total of 219 health care workers participated in the study with 144 (65.8%) intensive care nurses and 75 (34.2%) intensive care physicians. There was an organ donation card of 13.2% (n: 29) of the participants. There were 16.7% (n: 24) nurses and 6.7% (n: 5) doctor donation cards in doctors. In the case of brain death, 56.9% of participants wanted their organs to be donated. In the case of brain death, 58.7 of those who did not accept organ donation did not feel ready. 21% (n: 46) previously participated in organ / tissue donation and transplantation, 16.9% (n: 37) previously participated in brain death related trainings. Among the doctors who participated in the study 37.8% (n: 28) Electroencephalography (74%) and Radionuclide Cerebral Scintigraphy (62%) are the most common supportive tests used for the diagnosis of brain death.

Conclusion: Nursing and doctors' support of tissue / organ donation and transportation does not affect having donation card. There is no significant difference between the general knowledge levels of anesthesia and other branch physicians about brain death, but anesthesia doctors have been found to be more experienced in diagnosing brain death. We believe that the positive attitudes of the intensive care workers on this subject and the sufficient level of knowledge will positively affect the amount of organ donation.

Keywords: Intensive care unit, brain death, organ donation, organ transplantation

Öz

Amaç: Organ nakli ve bağışı konusunda toplumsal ve bireysel bilgi eksikliği olduğu ve bunun sonucunda toplumsal ve bireysel duyarsızlığa bağlı organ bağışının yeterli olmadığı gösterilmektedir. Çalışmamızda Konya ilinde bulunan hastanelerin yoğun bakım çalışanlarında beyin ölümü, organ bağışı ve nakli ile ilgili olarak farkındalık, tutum ve bilgi düzeylerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Çalışmaya Konya ili devlet hastaneleri, üniversite hastaneleri ve özel hastane yoğun bakımlarında görev yapan araştırmacı ile yüz yüze görüşmeyi kabul eden 144 hemşire ve 75 doktor olmak üzere toplam 219 sağlık çalışanı oluşturmuştur. Araştırmanın verileri, literatür taraması sonucu oluşturulan ve demografik bilgilerin de yer aldığı 'Veri Toplama Formu' kullanılarak toplanmıştır

Bulgular: Çalışmaya 144 (%65,8) yoğun bakım hemşiresi ve 75 (%34,2) yoğun bakımda çalışan doktor toplam 219 sağlık çalışanı katıldı. Katılımcıların %13,2 (n:29)'sinin organ bağışı kartı vardı. Hemşirelerde %16,7 (n:24), doktorlarda %6,7 (n:5) oranında organ bağış kartı vardı. Beyin ölümü durumunda katılımcıların %56,9'sı organlarının bağışlanmasını istemiştir. Beyin ölümü durumunda organ bağışını kabul etmeyenlerin 58,7 kendini hazır hissetmediklerini belirtmiştir. Organ/doku bağışı ve nakli konusunda daha önce eğitime katılanlar %21 (n:46), beyin ölümü ile ilgili eğitimlere daha önce katılanlar %16,9 (n:37) du. Çalışmaya katılan doktorlardan haha önce apne testi uygulayan %37,8 (n:28) di. Beyin ölümü tanısı için kullanılan destekleyici testlerden en çok bilinilenleri Elektroensefalografi (%74) ve Radyonüklid Serebral Sintigrafi (%62)'dir.

Sonuç: Hemşire ve doktorların doku/organ bağışı ve naklini destekleme durumu bağış kartına sahip olma durumunu etkilememektedir. Anestezi ve diğer branş doktorlarının beyin ölümü ile ilgili genel bilgi düzeyleri arasında belirgin bir fark yoktur fakat anestezi doktorlarının beyin ölümü tanısı koyma konusunda daha deneyimli olduğu görülmüştür Yoğun bakım çalışanlarının bu konudaki tutumlarının olumlu, bilgi düzeylerinin yeterli olması organ bağışı miktarını olumlu yönde etkileyeceği kanaatindeyiz.

Anahtar Kelimeler: Yoğun bakın ünitesi, beyin ölümü, organ donör, organ bağışı, organ nakil

Corresponding *(İletişim)*: Mustafa Tomruk, Meram State Hospital, Department of Anesthesiology and Reanimation, Meram, Konya, Turkey
E-mail *(E-posta)*: mustafatomruk84@gmail.com
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INTRODUCTION

Organ donation is defined as the voluntary consent of a person to use his/her tissues and organs for the treatment of other patients while alive or after medical death.[1] Tissue and organ transplantation is one of the values that show the level of progress of countries in the field of health. Health workers providing information on this issue can positively influence the decision of potential organ donor families. Therefore, in order to increase organ donation, it is necessary to increase the knowledge of healthcare professionals on this subject.[2,3] The most important point to remember in organ transplantation is that organ transplantation cannot take place without a donor. The transplantation of organs obtained from cadavers to the most suitable recipient is another important problem. This problem necessitates organ sharing and organ harmonization. As a matter of fact, strong organizations such as Eurotransplant and UKTSSA in Europe and United Network for Organ Sharing (UNOS) in the USA are also active in organ sharing. In Türkiye, the organization is carried out according to the 'Organ and Tissue Transplantation Services Regulation' published in the official gazette dated 01/06/2000 and numbered 24066 and the National Organ and Tissue Transplantation Coordination System Directive published in January 2001.[4] When the studies conducted in our country on organ transplantation and donation are examined, it is shown that there is a lack of social and individual knowledge as well as lack of organization on the subject and as a result, organ donation is not sufficient due to social and individual insensitivity.[2] Studies have found an increase in the number of people who will accept organ donation after training on brain death and organ transplantation.[5,6]

In this study, we aimed to determine the knowledge and tendencies of intensive care unit staff (resident physicians, specialist physicians and nurses) about brain death, organ donation and transplantation, and to establish a prospective basic knowledge on this subject.

MATERIAL AND METHOD

The study was conducted between August-December 2017 in province of Konya, state hospitals, medical faculty hospitals and private hospital intensive care units. 270 healthcare workers working in intensive care were asked to participate in the questionnaire. A total of 219 (%81.1) healthcare workers, including 144 nurses and 75 doctors, who agreed to face-to-face interviews with the researcher, were included in the study. The study was initiated with the decision of Local Ethics Committee dated 16.06.2017 and numbered 2017/978. The data of the study were collected using the 'Data Collection Form', which was created as a result of the literature review and included demographic information.

Data Collection

The data of the study were collected by using the 'Data Collection Form' which was created as a result of literature review and included demographic information in order to reveal the awareness, attitude and knowledge levels of intensive care unit staff about brain death, organ donation and transplantation.[7,8]

Data Collection Form

The data collection form consists of 3 sections and 54 questions. The first section includes 10 questions to determine demographic characteristics. The second section includes 22 closed-ended, open-ended and multiple-choice questions to determine the level of knowledge, awareness and attitudes towards brain death and organ transplantation among the nurses and doctors included in the study. Questions such as Do you have relatives waiting for organ transplantation, Do you have an organ donor card, which organs and tissues can be transplanted, From whom can organ transplantation be performed, which organs and tissues can be taken without the need for family permission unless otherwise declared, Which health institutions in the province of Konya serve as transplantation centers, Is the institution you work for an organ procurement center, Has there been a case of brain death in the intensive care unit where you work, were asked to measure awareness, attitude, and knowledge level. The third section includes 22 questions about the level of brain death knowledge only for the doctors participating in the study. Questions were asked to determine the level of knowledge about the definition and diagnosis of brain death and awareness of the diagnostic methods used.

Statistical Analysis

The data obtained after the application were transferred to the computer environment and evaluated in the Statistical Packace For The Social Sciences (SPSS) for Windows 20 program. In the statistical evaluation of the data, categorical data were tabulated as numbers and percentages. Significance was evaluated at $p<0.05$ level. Continuous variables were analyzed by Chi-square test and presented as mean±standard deviation. Demographic variables were given as frequency percentages (%).

RESULTS

A total of 219 health care professionals, 144 (65.8%) intensive care nurses and 75 (34.2%) physicians working in intensive care, participated in the study. The ages of the health care professionals who participated in the study were between 19-45 years and the mean age was 28.60±5.9. The mean age of the physicians who participated in the study was 30.60±4.3, while the mean age of the nurses was 27.56±6.4. When both groups were compared in terms of age, there was a significant difference (p=0.00). Demographic data of the health personnel participating in the study are given in **Table 1**.

The answers to the questionnaire are shown in **Tables 2** and **3**. To the question "Do you have an organ donor card?" 120 (83.3%) of the nurses answered no, while 70 (93.3%) of the physicians answered no. A statistically significant difference was observed (p=0.038) (**Table 2**). When asked about the reasons for not considering organ donation, 20 out of 32 nurses (62.5%) and 7 out of 14 doctors (50%) answered "I do not feel ready". There was no statistically significant difference (p=0.381).

Table 1. Sociodemographic Characteristics of Participants

		Number	Percent
Gender			
Female		140	63.9
Male		79	36.1
Age			
Physician		30.6 ± 4.3	
Nurse		27.5 ± 6.4	
Position Status			
Physician		144	65.8
Nurse		75	34.2
Education Status			
High School		56	25.6
Associate Degree		29	13.2
Bachelor's degree		51	23.3
Master's Degree		83	37.9
Profession, if any			
Brain Surgery		5	6.7
Cardiology		3	4
Anesthesia		32	42.7
General Surgery		6	8
Emergency		11	14.7
Pediatrics		4	5.3
Internal Medicine		5	6.7
Pulmonary disease		5	6.7
Neurology		4	5.3
Your Current Institution			
Public Hospitals		34	15.5
Medical faculty hospitals		113	51.6
Private Hospitals		47	21.5
Blank		25	11.4
Number of Months If You Are a Specialization Student			
0-30		21	38.9
30-60		27	50
60 and above		6	11.1
How many years in the profession (year)			
	Nurse	1-5	63 (43.8)
		5-10	41 (28.5)
		10-15	25 (17.4)
		15-20	8 (5.6)
		20-25	5 (3.5)
		≥ 25	2 (1.4)
	Physician	1-5	51 (68)
		5-10	18 (24)
		10-15	5 (6.7)
		15-20	1 (1.3)
		20-25	-
		≥ 25	-
Duration of Work in Intensive Care Unit (year)			
	Nurse	1-5	86 (59.7)
		5-10	39 (27.1)
		10-15	12 (8.3)
		15-20	3 (2.1)
		20-25	3 (2.1)
		≥ 25	1 (0.7)
	Physician	1-5	72 (96)
		5-10	3 (4)
		10-15	-
		15-20	-
		20-25	-
		≥ 25	-

Table 2. Survey data Section 1

		N (%)
Do you have an organ donor card?		
Nurse	Yes	24 (16.7)
	No	120 (83.3)
Physician	Yes	5 (6.7)
	No	70 (93.3)
If you have not donated your organs, would you consider donating them?		
No idea		53 (27.9)
Yes		108 (56.9)
No		29 (15.2)
If no, what are the reasons? *		
I don't think organ donation is right	Nurse	2 (6.2)
	Physician	0
For religious reasons	Nurse	7 (21.9)
	Physician	5 (35.7
I don't feel ready	Nurse	20 (62.5)
	Physician	7 (50)
The idea of post-mortem surgery	Nurse	0
	Physician	1 (7.1)
Thinking that organ donation will affect my medical treatment	Nurse	3 (9.4)
	Physician	1 (7.1)
Do you know anyone waiting for an organ transplant?		
No idea		11 (5)
Yes		37 (16.9)
No		171 (78.1)
Do you have relatives who donated organs after brain death?		
No idea		33 (15.1)
Yes		25 (11.4)
No		161 (73.5)
If your 1st degree relative is brain dead, would you accept organ donation?		
Yes		153 (69.9)
No		57 (26)
No answer		9 (4.1)
If no, what is the reason?		
I don't want to take responsibility for this		26 (11.9)
I don't think organ donation is right.		5 (2.3)
I think that my environment and other family members will misunderstand me		10 (4.6)
I don't think it's religiously appropriate.		9 (4.1)
I think organ donation will affect the medical treatment of that person		6 (2.7)
Have you ever participated in training on organ/tissue donation and transplantation?		
Yes		46 (21.0)
No		173 (79.0)
Which organs and tissues can be transplanted		
Cornea		203 (92.3)
Lung		163 (74.1)
Bone marrow		167 (75.9)
Kidney		207 (94.1)
Liver		202 (91.8)
Spleen		63 (28.6)
Heart		199 (90.5)
Blood		148 (67.3)
Small intestine		59 (26.8)
Pancreas		85 (38.6)
From Whom Organ Transplantation is Performed		
No opinion		6 (2.7)
Cadaver		25 (11.4)
Live		42 (19.2)
Cadaver+Live		146 (66.7)
Which organs and tissues can be harvested without the need for family consent unless otherwise declared?		
Cornea		138 (68.7)
Kidney		2 (1.0)
Blood		32 (15.9)
Cornea+Kidney+Blood		3 (1.5)
Cornea+blood		20 (10.0)
Spleen+Kidney		1 (0.5)
Cornea + Spleen		4 (2.0)
Spleen+Kidney+Blood		1 (0.5)

*More than one option is marked.

Table 3. Survey data Section 2

	Other specialty doctors n(%)	Anesthesiologists n(%)	Total n (%)
The Regulations of the Ministry of Health of the Republic of Türkiye clearly state how to proceed in patients diagnosed with Brain Death and not accepted as organ donors			
No idea	20 (47.6)	12 (37.5)	32 (43.2)
True	21 (50)	20 (62.5)	40 (55.4)
False	1 (2.4)	0	1 (1.4)
Each country has different diagnostic criteria and requirements for the diagnosis of brain death, as set out in their own laws.			
No idea	15 (34.9)	6 (18.8)	21 (28)
True	20 (46.5)	17 (53.1)	37 (49.3)
False	8 (18.6)	9 (28.1)	17 (22.7)
In a patient considered brain dead, the diagnosis should be definitive and the disease should be incurable.			
No idea	1 (2.3)	1 (3.1)	2 (2.7)
True	37 (86)	31 (96.9)	68 (90.7)
False	5 (11.6)	0	5 (6.7)
In the presence of cardiopulmonary resuscitation or similar hypoxic ischemic acute brain injury, at least how long should elapse before evaluation of the main clinical examination finding?			
6 hours	4 (11.4)	1 (3.3)	5 (7.7)
12 hours	6 (17.1)	4 (13.3)	10 (15.4)
24 hours	14 (40)	15 (50)	29 (44.6)
48 hours	9 (25.7)	10 (33.3)	19 (29.2)
Deep tendon reflexes can be obtained in a brain dead patient.			
No idea	15 (34.9)	3 (9.4)	18 (24)
True	19 (44.2)	14 (43.8)	33 (44)
False	9 (20.9)	15 (46.9)	24 (32)
Brain dead patients may have spinal reflexes and automatisms.			
No idea	16 (37.2)	3 (9.4)	19 (25.3)
True	23 (53.5)	24 (75)	47 (62.7)
False	4 (9.3)	5 (15.6)	9 (12)
The presence of autonomic storm in a brain dead patient does not exclude brain death.			
No idea	20 (46.5)	7 (21.9)	27 (36)
True	22 (51.2)	24 (75)	46 (61.3)
False	1 (2.3)	1 (3.1)	2 (2.7)
An apnea test should be performed in the patient who is considered brain dead and a supportive test that the board of physicians deems appropriate should be added.			
No idea	2 (4.7)	2 (6.2)	4 (5.3)
True	40 (93)	30 (93.8)	70 (93.3)
False	1 (2.3)	0	1 (1.3)
I've done an apnea test before.			
Yes	6 (14.3)	22 (68.8)	28 (37.8)
No	36 (85.7)	10 (31.2)	46 (62.2)
Normothermia, normotension and normovolemia are preconditions for apnea testing.			
No idea	12 (27.9)	2 (6.2)	14 (18.7)
True	29 (67.4)	27 (84.4)	56 (74.7)
False	2 (4.7)	3 (9.4)	5 (6.7)
Before the apnea test, PaCO2 should be 35-45 mmHg and PaO2 should be above 200 mmHg with appropriate mechanical ventilation approach.			
No idea	15 (34.9)	3 (9.4)	18 (24)
True	22 (51.2)	25 (78.1)	47 (62.7)
False	6 (14)	4 (12.5)	10 (13.3)
If PaO2 cannot be brought above 200 mmHg before apnea test, apnea test should not be performed.			
No idea	22 (52.4)	3 (9.7)	25 (34.2)
True	16 (38.1)	17 (54.8)	33 (45.2)
False	4 (9.5)	11 (35.5)	15 (20.5)
An apnea test is positive if PaCO2 ≥60 mmHg at the end of the apnea test and/or if there is no spontaneous breathing despite an increase of 20 mmHg or more in PaCO2 compared to baseline.			
No idea	17 (39.5)	3 (9.4)	20 (26.7)
True	26 (60.5)	29 (90.6)	55
False	-	-	-
In patients who are considered brain dead, irreversible severe structural brain damage must be demonstrated by imaging method (CT, MRI).			
No idea	6 (14)	4 (12.5)	10 (13.3)
True	23 (53.5)	20 (62.5)	43 (57.3)
False	14 (32.6)	8 (25)	22 (29.3)
If the apnea test cannot be completed, the diagnosis of brain death can be made with supportive tests deemed appropriate by the board of physicians.			
No idea	12 (29.3)	5 (15.6)	17 (23.3)
True	28 (68.3)	24 (75)	52 (71.2)
False	1 (2.4)	3 (9.4)	4 (5.5)
Which supportive tests can be used for the diagnosis of brain death? (More than one option can be marked)			
Electroencephalography	34 (79)	22 (68)	56 (74)
Sensory evoked potentials	16 (37)	13 (40)	29 (38)
Transcranial Doppler ultrasonography	19 (44)	22 (68)	43 (57)
Radionuclide cerebral scintigraphy (SPECT)	28 (65)	19 (59)	47 (62)
CT angiography	20 (46)	23 (71)	43 (57)
Catheter cerebral angiography	16 (37)	16 (50)	32 (42)
All of them	3 (6)	6 (18)	9 (12)

DISCUSSION

The most impressive situation in our study was that although the participants viewed organ donation positively, most of them did not have an organ donation card. Considering that organ donation has become an increasingly important concept, our study also emphasizes the importance of this situation. Considering that every individual may need organ donation in their life cycle, individuals should be more sensitive.[6] Another important issue was the lack of knowledge of the participants on the subject. This unfortunately showed us that many people, except those waiting for organs and their relatives, are insensitive to the issue.

In the study by Amaral et al. 144 professors working in a university hospital in Brazil were surveyed with "yes-no" statements on organ donation, brain death and donation management. As a result of the study, it was found that 87% of the professors included in the study were willing to donate organs and 69% of them knew some of the legal conditions on organ donation in Brazil. A detailed assessment of these legal conditions showed that 79% knew the content of the diagnosis of brain death, but 44% did not know how to diagnose brain death clinically. It was observed that only 26% of the participants donated organs. It was observed that 22% of the professors considered themselves competent in making the clinical diagnosis of brain death and requested organ donation from the relatives of previously brain dead patients. As a result of this study, it was observed that although professors were enthusiastic about organ donation, those who considered themselves clinically competent in this regard were in the minority.[7] Similar to our study, the proportion of people who are willing to donate organs and the proportion of people who actually have an organ donor card are similar. The lack of a functional organ donor card in our country is another factor in this low rate. In this study, although the rate of follow-up of patients diagnosed as brain-dead was lower, in our study, a higher rate of follow-up of brain-dead patients was observed. The reason for this difference may be the high number of nurses in our study or the fact that more than one nurse followed the same patient. In our study, it was observed that the number of participants who knew the legal conditions for organ and tissue transplantation in order to diagnose brain death was low. In the study by Shabanzadeh et al. a total of 418 nurses working in 24 intensive care units in Tehran were surveyed to assess their knowledge about brain death, organ and tissue donation. Accordingly, 75.6% have a positive view of organ donation and 15% have an organ donor card. Those with a positive approach see it as "for humanity", while others have a negative approach in terms of "respect for the body". 19% have relatives waiting for organ and tissue transplantation. It was observed that 54% of the respondents were positive about organ donation by their relatives in case of death.[8] In a study conducted by Cillimoğlu et al. with 415 healthcare workers and 320 students, 44% of the participants were considering organ donation and 16.6% had organ donation cards. Among those who gave negative answers, the three most common answers were 18.5% without specifying the reason, 17.3% thinking that the death decision would be made prematurely, and 16.7% not having enough information on the subject. In this study, the rate of organ donation among relatives or close relatives was 16.6%. In addition, one third of the participants knew someone who had received an organ transplant.[1] In a study conducted by Vlaisavljevic and Milutinovic among 219 nurses, 91% would accept organ transplantation if needed, but only 32% would accept to be organ donors. Only 0.3% of the participants had an organ donor card. In this study, the negative attitude of not being a donor but accepting organ transplantation in case of need stems from mistrust and denial of health policies within the country.[9] In our study, 56.6% of the participants had a positive view of organ donation and 13.2% had an organ donation card. Of the 33 respondents who had a negative attitude towards organ donation, 81% stated that they did not feel ready and 36% cited religious reasons. Participants were more likely to be in favor of organ donation in the case of the death of a first-degree relative. In other studies, the rate of those who were in favor of donating their own organs was higher than those who were in favor of organ donation in case of the death of a relative, whereas this rate was the opposite in our study. We think that this inverse ratio is due to the fact that the majority of the participants in our study did not feel ready for organ donation, but were able to make more realistic decisions about their relatives. We also think that having relatives or close relatives who have donated organs can be important in terms of observing and empathizing with the experiences of organ donation. Melo et al. evaluated knowledge and attitudes on cadaveric organ donation and transplantation directed to 495 healthcare professionals working in hospitals in Portugal with a questionnaire-based measurement. 78% of the respondents have received training on organ donation and transplantation and 62% think that they need more training. Hospital staff with a positive attitude towards donation can positively influence the attitudes of the general public. It has been suggested that successful action by hospital staff as initiators of the organ procurement process requires more knowledge and training. Lack of adequate information and training among health professionals working in places with donation programs has negatively affected organ donation rates. In this study, it was determined that the low organ donation rates were due to the difficulties in initiating the process, diagnosing brain death and providing the necessary human resources. No difference was observed in the level of knowledge between nurses and physicians in questions on specific organ donation and transplantation.[10] In our study, 21% of healthcare professionals received training on organ transplantation. Although the proportion of employees who received training in the other study was higher than in our study, it was

observed that the knowledge levels of the participants in both studies were incomplete. In a study conducted by Yılmaz et al. at the Research and Application Hospital in Eskisehir, which included 90 physicians (specialists and assistants), 200 nurses and health officers, and 24 radiology and laboratory technicians, only 13.5% of health professionals were found to be organ donors. It was also stated that they can perform 96% kidney, 83.5% heart, 74.1% liver and 62% cornea transplantation respectively. Even though they seem to be concerned with the issue, there is a prevailing view that trainings, press announcements and announcements within health institutions are insufficient. The majority of health workers in this study stated that no one except those waiting for organs emphasized the issue. Accordingly, it is understood that kidney, liver, heart and cornea are the organs known to be transplanted the most.[11] In a study by Sarıtaş et al. involving 163 physicians, 77.6% stated that the apnea test was the basis for the diagnosis of brain death, while 67.1% stated that a confirmatory test was used together with the apnea test. However, 65.6% of participants were not familiar with the apnea test. When confirmatory tests were questioned, 46.5% responded CT angiography, 24.2% Transcranial Doppler USG, and 12.1% MR angiography.[7] In the study conducted by Vlaisavljevic and Milutinovic in which 219 nurses evaluated their attitudes and knowledge about organ donation and transplantation, 63.9% of the nurses answered EEG as the most valid method in the diagnosis of brain death.[9] In our study, 93.3% of doctors said that brain death is the complete and irreversible loss of all brain and brain stem functions. Anesthesia and other branch doctors answered yes to this question at the same rate (93%). 93.3% of all doctors said that an apnea test and a supportive test should be performed in a patient considered brain dead. It was observed that the proportion of anesthesia and other branch doctors who answered this question correctly was equal (93%). When confirmatory tests were questioned, 74% EEG, 62% SPECT, 57% CT angiography, 54% transcranial Doppler USG responses were obtained. Of all doctors, 37.8% said they had previously performed an apnea test. While this rate is 68.8% for anesthesia doctors, it is 14.3% for other branch doctors.56% said that if the relatives of the patient do not accept organ transplantation after the diagnosis of brain death, the body should be handed over to the patient's relatives. There was no significant difference between the general knowledge levels of 51 anesthesia and other branch physicians about brain death, but anesthesia physicians were more experienced in diagnosing brain death. The knowledge level of the participants in our study on apnea testing and other confirmatory tests was higher than the rates in the other study. Kocaay et al. evaluated the knowledge, awareness and attitudes of 341 participants from the departments of medicine, law, nursing and communication, who may be involved in organ transplantation processes in the future, especially regarding the process of finding a suitable organ through a questionnaire. As a result, it was determined that especially nursing and medical students wanted to be organ donors, but only 2% of them had organ donor cards. While the majority thought that organ donation was religiously appropriate, some (5%) thought that it was a sin.[12] In our study, 5.5% did not consider it religiously inappropriate.

CONCLUSION

We think that the positive attitudes and adequate level of knowledge of intensive care unit staff on this issue will positively affect the amount of organ donation because they are more together with the relatives of patients waiting for organ transplantation, transplanted patients and brain-dead patients. In-service training for healthcare professionals in intensive care units may be considered in the future.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Necmettin Erbakan University Meram Medical Faculty Local Ethics Committee (Date: 16.06.2017, Decision No: 2017/978).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Depression And Spiritual Well-Being of Hemodialysis Patients: A Sectional Study

Hemodiyaliz Hastalarında Depresyon ve Manevi İyi Oluş: Kesitsel Bir Çalışma

Aynur Bahar

Gaziantep University, Faculty of Health Sciences, Department of Psychiatric Nursing, Gaziantep, Turkey

Abstract

Aim: This study was designed to determine the relationship between depression and spirituality in hemodialysis patients in a dialysis center.

Material and Method: The research is in the type of descriptive research. The current study was conducted with 74 patients hemodialysis. The data of the study was collected by applying "Personal Information Form", "Beck Depression Inventory" and "Spiritual Well-Being Scale". The data were collected face to face using the questionnaire method. In the analysis of the data, descriptive statistics, t-test, Anova test and correlation analysis were used.

Results: More than half of the patients were 51 years and over (54.1%) and 60.8% were male. When the Beck Depression Inventory's scores were analyzed according to the cut-off point, it was found that 58.1% of the patients showed depressive symptoms. In the study, BDI mean score was 19.27±7.31, SWBS mean score was 18.74±10.8 (Subscales: Peace 5.70±3.52, Meaning 6.27±3.67, Faith 6.75±3.97). The results of this study revealed that there is a negative significant relationship between mental well-being and depression ($p<0.05$).

Conclusion: As the moral well-being level of patients receiving hemodialysis treatment increases, their depressive symptoms decrease. Therefore, assessing spiritual health and integrating spiritual care into daily practice can help improve the quality of care and achieve better health outcomes.

Keywords: Hemodialysis, depression, spiritual well-being

Öz

Amaç: Bu çalışma, bir diyaliz merkezindeki hemodiyaliz hastalarında depresyon ve manevi iyilik hali arasındaki ilişkiyi belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: Araştırma betimsel araştırma türündedir. Mevcut çalışma 74 hemodiyaliz hastası ile gerçekleştirilmiştir. Araştırmanın verileri "Kişisel Bilgi Formu", "Beck Depresyon Envanteri" ve "Manevi İyi Oluş Ölçeği" kullanılarak yüz yüze toplanmıştır. Verilerin analizinde betimsel istatistikler, t-testi, Anova testi ve korelasyon analizi kullanılmıştır.

Bulgular: Hastaların yarısından fazlası 51 yaş ve üzerindeydi (%54,1) ve %60,8'i erkekti. Beck Depresyon Envanteri puanları kesme noktasına göre incelendiğinde hastaların %58,1'inin depresif belirtiler gösterdiği saptanmıştır. Çalışmada depresyon puan ortalaması 19,27±7,31, manevi iyi oluş puan ortalaması 18,74±10,8'dir (Alt ölçekler: Huzur 5,70±3,52, Anlam 6,27±3,67, İnanç 6,75±3,97). Bu çalışmanın sonuçları manevi iyilik hali ile depresyon arasında negatif yönde anlamlı bir ilişki olduğunu ortaya koymuştur ($p<0.05$).

Sonuç: Hemodiyaliz tedavisi alan hastaların moral iyilik düzeyi arttıkça depresif belirtileri azalmaktadır. Bu nedenle, ruhi sağlığı değerlendirmek ve manevi bakımı günlük uygulamaya entegre etmek, bakım kalitesini artırmaya ve daha iyi sağlık sonuçları elde etmeye yardımcı olabilir.

Anahtar Kelimeler: Hemodiyaliz, depresyon, manevi iyi oluş

Corresponding *(İletişim)*: Aynur BAHAR, PhD, Dr. Graduate Instructor. Gaziantep University Faculty of Health Sciences, Department of Nursing, Psychiatric Nursing, Gaziantep, Turkey
E-mail *(E-posta)*: abahar@gantep.edu.tr
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INTRODUCTION

Chronic Renal Failure (CRF) is an irreversible and life-threatening chronic disease that occurs when the kidney becomes unable to perform its functions.[1] CRF affects 10-15% of adults worldwide.[2] The rate of CRF in the general adult population is similar in Turkey.[3] In the report of the European Kidney Association, it was stated that 57% of CRF patients were treated with hemodialysis, which is the most commonly used treatment method.[4,5] The Turkish Society of Nephrology reported that the most common treatment method in our country is hemodialysis with a rate of 76.9%.[6]

Hemodialysis patients have to adapt to a restrictive lifestyle that is dependent on the dialysis machine and healthcare team. [7] Patients face many serious problems such as hospitalizations, sleep disorders, psychosocial and emotional problems due to diet, fluid restriction and metabolic reasons.[8] Physical disability affects the social life of the patient, and activity restrictions cause stress. The most common psychiatric disorder in dialysis patients is depression. In the literature, it is stated that the prevalence of depression is higher in hemodialysis patients compared to the general population.[9,10]

Spirituality includes individuals' search for meaning in life, in other words, the part of the human soul that struggles for metaphysical values, concepts, and experiences. Spirituality encompasses a kind of connection between man and a divine or higher inner power.[11] Spirituality serves as a potential resource in the process of maintaining mental health and deciding on treatment and is seen as a coping mechanism in patients' struggle with stressful life events.[12-14]

Faith and religious practices are frequently used by individuals with chronic diseases to cope with the disease and provide emotional comfort.[15] The spirituality of hemodialysis patients is an important factor in reducing the burden of disease, increasing quality of life, coping and compliance.[12,13,16]

In the international literature, studies on spirituality and spiritual care of patients receiving hemodialysis treatment are in the majority, but there are limited studies on spirituality of hemodialysis patients, which are among the chronic diseases in Turkey. There is a need for studies examining the relationship between spiritual well-being and depression, which is common in hemodialysis, and incorporating spiritual well-being and spiritual care in holistic nursing practices. This study is expected to raise awareness for nurses to understand the importance of spirituality and to plan spirituality-oriented approaches in care. This study was designed to determine the relationship between depression and spirituality in hemodialysis patients in a dialysis center.

MATERIAL AND METHOD

The research is in the type of descriptive research. This study was conducted with chronic renal failure patients receiving hemodialysis treatment in a private dialysis center between December 2021 and January 2022. As a result of the power analysis, the sample size confidence interval was α=0.05, a total of 45 patients were calculated. Patients aged 18 and over, literate and able to communicate were included in the study. The number of patients registered in this center is 200. All patients were reached, but volunteers who had no communication problems and agreed to participate in the study were included in the study. The study was completed with 74 patients. Before the data were collected, the participants were informed about the research and their written consents were obtained.

Ethics of The Research

Before starting the study, approval was obtained from the Clinical Research Ethics Committee of the relevant university (Decision date: 15.12.2021; Decision no: 2021/389). Permissions were obtained from the institution where the study would be conducted and for the use of measurement tools. The sample group was informed about the study and their permission was obtained. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Instruments

Data were collected using the "Personal Information Form", "Beck Depression Inventory" and "Spiritual Well-Being Scale".

Personal Information Form: This form consists of 11 questions that include sociodemographic (age, gender, educational status, marital status, income status, employment status, number of children) and disease-related information (hemodialysis duration, other chronic diseases, status of receiving social support, support area).

Beck Depression Inventory (BDI): The scale, developed by Beck in 1961, is used to determine the risk of depression and to measure the level and severity of depressive symptoms. It was adapted into Turkish by Hisli (1989) and its Cronbach's alpha value was found to be 0.80. The BDI contains 21 self-assessment statements with four choices from less to more (0-3). The total score that can be obtained from the scale is between 0-63. The cut-off point of the scale is 17 and above. The degree of depression is minimum for 0-9 points, mild for 10-16 points, moderate for 17-29 points, and severe for 30-63 points.[17]

Spiritual Well-Being Scale (SWBS): The original name of the scale is Functional Assessment of Chronic Illness Therapy–Spiritual Well-Being Scale (FACIT–Sp). The Turkish version of the scale SWBS was made by Aktürk et al.[18] The scale with 3 subscales (peace, meaning, and faith) helps to investigate all components of spiritual well-being. The 12-item scale is scored between 0-4 (0: Never, 4: Always). It has 3 sub-categories as meaning subscale (2, 3, 5 and 8th items), peace subscale (1, 4, 6 and 7th items) and belief subscale (9, 10, 11 and 12 items), each subscale has scores between 0-16 (Total scale score is 0-48). A high score indicates the high level of spiritual well-being. The Cronbach alpha value of the Turkish version is 0.87 [18]; this value was found to be 0.78 in our study.

Data Collection

The research was conducted with patients in a private dialysis center. The data were collected by the researchers face-to-face, using the survey method. The application of the questionnaires and scales took an average of 45 minutes. The data collection process, on the other hand, covers a period of approximately two months.

Data Analysis

IBM SPSS Statistics 22.0 (IBM Corp. Armong, New York, AB) program was used in the analysis of the data. In the analysis of the data, descriptive statistics (percentage, arithmetic mean, etc.), t-test, Anova test and correlation analysis were used. The statistical significance level was accepted as 0.05 in the interpretation of the analysis results.

RESULTS

More than half of the patients were 51 years and over (54.1%) and 60.8% were male. Majority of the patients (77%) were married, 39.2% were literate, 28.4% were primary and secondary school graduates. The income of the patients was moderate (income equals expenses -63.5%) and 55.4% of them were not working in any job. Nearly half of the patients had 4 or more children (48.6%). In addition, the majority of the patients had a chronic disease other than chronic renal failure (67.6%). Most of them reported that they had been receiving hemodialysis treatment for 3-4 years. Patients (43.2%) who reported that they had the support of family and friends stated that this support was mostly in the form of meeting their physical needs (**Table 1**).

When the Beck Depression Inventory's Scores were analyzed according to the cut-off point, it was seen that 58.1% of the patients scored 17 and above. When analyzed according to the degree of depression, it was determined that 47.3% of the patients were moderately depressed (**Table 2**). In the study, BDI mean score was 19.27±7.31, SWBS mean score was 18.74±10.8, the SWBS-Peace Subscale's mean score was 5.70±3.52, the SWBS-Meaning Subscale's mean score was 6.27±3.67, and the SWBS-Faith Subscale's mean score was determined as 6.75±3.97 (**Table 3**). The correlations between the scales were in the form of a negative and highly significant correlation (p=0.001) (**Table 4**).

Table 2. The Distribution of Beck Depression Inventory's Mean Scores

Points	n	%
BDI (based on cutting score)		
0-16 points	31	41.9
17 points and more	43	58.1
BDI (according to degree)		
Minimal (0-9 points)	3	4.1
Mild (10-16 points)	28	37.8
Moderate (17-29 points)	35	47.3
Severe (30-63 points)	8	10.8

BDI: Beck Depression Inventory

Table 1. The Distribution of Descriptive Characteristics of the Patients

	n	%
Age		
18-28 age range	5	6.8
29-39 age range	8	10.8
40-50 age range	21	28.4
51 years and over	40	54.1
Gender		
Female	29	39.2
Male	45	60.8
Marital Status		
Married	57	77.0
Single	17	23.0
Educational Level		
Literate	29	39.2
Primary – Middle School Graduate	21	28.4
High School Graduate	14	18.9
University Graduate	10	13.5
Employment Status		
Employed	33	44.6
Unemployed	41	55.4
Income Level		
Income is less than expenses	12	16.2
Income is equal to expenses	47	63.5
Income is more than expenses	15	20.3
Number of Children		
No child	13	17.6
1-3 child/children	25	33.8
4 and more children	36	48.6
Hemodialysis Duration		
1 month-2 years	22	29.7
3-4 years	28	37.8
5 years and more	24	32.4
Other Chronic Diseases		
Present	50	67.6
Absent	24	32.4
Social Support		
Present	32	43.2
Absent	42	56.8
Support Area (n=32)		
Physical	14	43.8
Spiritual	11	34.3
Economic	7	21.9
TOTAL	74	100.0

Table 3. Spiritual Well-Being Scale and Beck Depression Inventory Average Scores

SCALES	Min-Max	$\bar{x}$±SD
BDI	7-44	19.27±7.31
SWBS	1-44	18.74±10.8
SWBS-Peace Subscale	0-13	5.70±3.52
SWBS-Meaning Subscale	0-15	6.27±3.67
SWBS-Faith Subscale	0-16	6.75±3.97

SWBS: Spiritual Well-Being Scale; BDI: Beck Depression Inventory

Table 4. Correlation between Spiritual Well-Being Scale and Beck Depression Inventory

	SWBS	SWBS-Peace Subscale	SWBS-Meaning Subscale	SWBS-Faith Subscale
BDI	r= -.796* p=0.001	r= -.762* p=0.001	r= -.787* p=0.001	r= -.764* p=0.001

*Correlation Analysis, SWBS: Spiritual Well-Being Scale; BDI: Beck Depression Inventory

No significant difference was found in the comparisons between age, marital status, educational level, income level, number of children, other chronic diseases, status of receiving social support and support area and scales. For this reason, it is not shown in **Table 5** where the comparisons are made.

In this study, the difference between gender and mean scores of BDI and SWBS is significant. Both BDI and SWBS scores of women were found to be higher than men. BDI mean scores of those who work in a job are lower than those who do not work, and the difference between them is significant. The mean BDI score of patients who have been on hemodialysis treatment for 5 or more years is higher than the other groups, and there is statistical significance. There is no statistical significance between the other variables and the scales (**Table 5**).

Table 5. Comparison of Mean Spiritual Well-Being Scale and Beck Depression Inventory Scores According to Some Characteristics of Patients

		n	BDI	SWBS
Gender	Female	29	21.41±7.53	24.34±10.7
	Male	45	17.88±6.89	15.13±9.30
			t= -2.070*	t= -3.906
			p=0.042	p=0.000
Employment Status	Employed	33	17.39±7.47	18.57±11.1
	Unemployed	41	20.78±6.90	18.87±10.7
			t= -2.022*	t= -0.119
			p=0.047	p=0.906
Hemodialysis Duration	1 month-2 years	22	17.04±5.09	19.77±11.4
	3-4 years	28	16.92±4.37	18.78±10.9
	5 years and more	24	24.04±9.40	17.75±10.8
			F= 9.281**	F= 0.196
			p=0.000	p=0.822

*Independent Samples T Test; **One-Way ANOVA, SWBS: Spiritual Well-Being Scale; BDI: Beck Depression Inventory

DISCUSSION

Hemodialysis causes adverse effects on patients' overall health, including mental, social, physical, emotional and spiritual aspects.[19,20] One of these negative effects is depression. Depression is one of the psychological disorders that impairs the functionality of individuals.[21]

Most of the patients participating in our study do not have social support. It has been found that the social supports given are mostly in the form of meeting physical needs. Insufficient social support in chronic diseases causes depressive disorders to develop more easily.[7] In the literature, it is emphasized that the support of family and friends is very important in the psychosocial adjustment of patients.[22] Spiritual and religious practices are closely related to the increase in life satisfaction and social support levels of patients.[11] These results may explain the high depressive symptoms of the patients.

In this study, it was found that the majority of patients showed moderate depression symptoms. The thought of losing the health, working power and being dependent on others negatively affects the development of depression.[7] In the literature, the probability of depression in hemodialysis patients is stated as 25-60%.[23-30] The present study's finding is in parallel with other studies conducted with hemodialysis patients.

It has been reported in the literature that spirituality contributes to patients' coping with serious illnesses and prevents feelings of spiritual distress, hopelessness and depression.[11,31-34] This study revealed that there is a negative and significant relationship between mental well-being and depression levels. It was found that patients with high mental well-being showed lower depressive symptoms; our results are in agreement with the sources.[21,35] The psychiatric disorder that causes the most hopelessness is known as depression. Although hope has no healing power, it encourages the patient to continue the fight and seek clinical improvement.[11] Religious beliefs as a strategy and a way of life can contribute to the development of hope and thus to the prevention of depression.

Drawing attention to the importance of searching for meaning in human life, Frankl (2017) stated that the religion believed is important in adding meaning to one's life and discovering the purpose of life. He also refers to religion as 'super meaning' to find meaning.[36] In this study, a relationship was found between the "meaning", "peace" and "belief" sub-dimensions of spiritual well-being and depression. The mean scores of the subscales of spiritual well-being, peace, and meaning were low, and the mean score of the faith subscale was high in patients with depressive symptoms. In addition, the spiritual well-being scale's total score of the patients was determined at a low level. This result is in agreement with the study finding of Musa et al (2018).[37] This situation reveals its relationship with religious practices that can strengthen spirituality in combating depressive symptoms.[38]

The depressive symptom levels detected in this study were remarkable and required psychiatric treatment. When mental problems are not treated, they decrease the quality of life.[39] In many studies with hemodialysis patients, it has been stated that spirituality, religiosity and belief are effective in reducing the risk of mental illness.[37,40,41] Patients with depression in our study had higher scores on the belief subscale. This result is important considering the studies on the protective role of belief against suicide, which is one of the symptoms of depression.

Patients undergoing hemodialysis treatment may experience negativities in their personal lives due to dependency on the machine, increased need for healthcare team and family.[42,43] Many psychological problems such as fear, anxiety, fury and depression can be seen in these patients.[44] In the literature, it has been stated that there is a negative relationship between religiosity and spirituality and anxiety, depression and stress

levels in hemodialysis patients.[45] The feeling of comfort and power that religious beliefs create in individuals can have a positive effect on mental health. In the case of chronic diseases, patients may turn to faith in God and participate in religious practices (such as congregational praying, fasting) in partnership with others in order to grasp the meaning in life.[46]

In our study, the level of depression was higher in female patients than in male patients. There are many sources that support our finding.[20,21,37] Women may be more prone to depression due to factors such as lack of social support, emotional personality structure and dependent roles. The finding that religious or spiritual beliefs are more important for female participants has been demonstrated in previous studies, both in dialysis patients and in broader defined populations,[47,48] and our study is consistent with other study results.

Being working can enable patients to tolerate dialysis symptoms better. It has been reported in the literature that there is a relationship between working status and depression.[49] The fact that patients are active and in contact with other people in their daily lives can distract attention and reduce their depression levels. In addition, in our study, it was determined that patients with hemodialysis treatment for 5 years or more were more depressed. Renal failure, which is a chronic disease, and continuous hemodialysis treatments may have reduced the patients' ability to fight and cope.

Spiritual therapies, including practices to increase patients' mental well-being and self-efficacy, and practices to strengthen spirituality should be used to reduce depression in hemodialysis patients.[12,24,25,50-52] Spiritual care initiatives that focus on promoting positive and realistic beliefs and avoiding negative ones can help hemodialysis patients avoid inappropriate emotions that may arise.

Limitations

The fact that this study was conducted in a single center and with a small sample limits the generalization of the results to the whole population. Due to the cross-sectional nature of the study, a causal relationship between mental well-being and depression cannot be established.

CONCLUSION

In the current study, the rates of depressive symptom were found to be high in hemodialysis patients. For this reason, more importance should be placed on the evaluation and improvement of the mental health of patients during the admission and treatment processes of patients to hospitals and medical centers. Based on the results of the study, it can be said that spiritual well-being can be an effective solution to reduce depression in hemodialysis patients. After the completion of this study, patients with depressive symptoms were informed and directed to go to a psychiatric examination in order to protect their mental health and take precautions.

As a result, this study can raise awareness about spiritual care practices in nurses who take care of dialysis patients. In addition, by integrating spirituality, which is an indispensable element of both the holistic approach and patient care in nursing, into clinical practices, the quality of care can be improved and health outcomes can be improved.

It is seen as a necessity to identify the psychological needs of all hemodialysis patients and to evaluate them psychiatrically. It is considered important to plan trainings to strengthen spirituality and to create environments that can facilitate the spiritual practices of patients in hemodialysis units.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gaziantep Universty Ethic Committee. (Date: 15/12/2021 Decision No: 2021/389).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Cerclage Outcome Depending On Suture Material Choice, Effects on Birth Week, Infant Weight, Intensive Care Requirement, and Infection Rates

Sütur Materyal Seçimine, Doğum Haftasına, Bebek Kilosuna, Yoğun Bakım İhtiyacı ve Enfeksiyon Oranlarına Bağlı Olarak Serklaj Sonuçları

Züat Acar[1], Fatih Omurca[2], Yunus Çavuş[3]

[1]BHT CLINIC İstanbul Tema Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey
[2]Nişantaşı University Medical School, Department of Obstetrics and Gynecology, Istanbul, Turkey
[3]Istanbul Gelisim University Vocational School of Health Services, Dicle Memorial Hospital, Diyarbakır, Turkey

Abstract

Aim: A preterm birth (PTB) is one that occurs before the full 37th week of pregnancy. Our theory is based on the possibility that braided mesh sutures made of Mersilene could lead to more complications and undesirable outcomes.

Material and Method: There were 46 pregnant women who had cervical insufficiency. Mersilene sutures were used in 26 cases and prolene sutures in 20 cases. The cervical lengths were comparable.

Results: Each case involved a pregnancy with a single fetus. Mersilene and prolene were the suture types in 26 cases and 20 cases, respectively. During the monitoring period, no problems, including infections, were noted.

Conclusion: Prolene sutures appear to be related with decreased PTB and improved neonatal results, despite the fact that mersilene sutures are the conventionally chosen material for cerclage. Prolene sutures continue to offer an option to conventional mersilene sutures, notwithstanding the need for more thorough randomized clinical trials to identify any potential associations between suture material and cerclage outcomes.

Keywords: Cerclage, suture materıal, preterm

Öz

Amaç: Preterm doğum (PTB), gebeliğin tam 37. haftasından önce meydana gelen doğumdur. Teorimiz, Mersilene'den yapılan örgülü meş sütürlerin daha fazla komplikasyona ve istenmeyen sonuçlara yol açabileceği ihtimaline dayanmaktadır.

Gereç ve Yöntem: Servikal yetmezliği olan 46 gebe vardı. 26 olguda mersilen, 20 olguda prolen sütür kullanıldı. Servikal uzunluklar benzerdi.

Bulgular: Her vakada tek fetüslü bir gebelik vardı. 26 olguda mersilen ve 20 olguda prolen sütür tipi idi. İzleme süresi boyunca enfeksiyonlar da dahil olmak üzere herhangi bir sorun kaydedilmedi.

Sonuç: Mersilen sütürlerin serklaj için geleneksel olarak seçilen materyal olmasına rağmen prolen sütürler PTB'de azalma ve neonatal sonuçlarda iyileşme ile ilişkili görünmektedir. Prolen sütürler, sütür materyali ile serklaj sonuçları arasındaki herhangi bir potansiyel ilişkiyi belirlemek için daha kapsamlı randomize klinik çalışmalara duyulan ihtiyaca rağmen, geleneksel mersilen sütürlere bir seçenek sunmaya devam etmektedir.

Anahtar Kelimeler: Serklaj, sütür materyali, preterm

Corresponding *(İletişim)*: Fatih OMURCA, Nişantaşı Üniversitesi Tıp Fakültesi, BHT CLINIC İstanbul Tema Hastanesi İstanbul Turkey
E-mail *(E-posta)*: dromurca@gmail.com
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INTRODUCTION

Preterm birth (PTB) is defined as birth before thirty-seven completed weeks of pregnancy (less than 259 days after the last menstrual period).[1] Gestational age at delivery is inversely proportional to mortality, so that the earlier the gestation at delivery the higher the mortality and requirement for intensive care admission. One of the major obstetric interventions for preventing (PTB) in women with cervical insufficiency is the cervical cerclage.[2] Cervix may shorten and dilate afterwards due to cervical insufficiency or incompetence. Acquired incompetence may be due to previous obstetrical or gynaecological procedures that distort cervical anatomy or cause trauma to the cervix. Rarely cervical weakness can be due to congenital causes leading again to anatomical or sometimes histological defect. Cervical anatomy can also be distorted by the presence of intramural pathology such as low-lying myomas or fibromas. There are no objective investigations or tests that can be performed before pregnancy to predict or diagnose subsequent cervical insufficiency. Diagnosis historically has almost always been clinical and retrospective depending on history and exclusion of other causes of the preterm birth. Hysterosalpingography and use of cervical dilators have been used in the past as diagnostic procedures prior to pregnancy but are no longer used.

Cervical cerclage, vaginal and intramuscular progesterone, a cervical pessary, or a combination of cervical pessary and vaginal progesterone have all been tried as treatments for cervical insufficiency. Still cervival cerglage seems as the most appropriate treatnent option. Despite these results, some researchers continue to search for evidence supporting those methods of PTB prevention with in a single setting, randomising patients to pessary, progesterone or cerclage arms

Cervical cerclage has been offered to treat cervical insufficiency for over 60 years. One of the reasons for its continuous use is the lack of reliable evidence to support an alternative. Several suture materials have been used to perform the cervical cerclage procedure including Mersilene 5mm tape, Mersilene silk, metal wire, human fascia lata, Prolene and Nylon. Currently the most commonly used suture material is Mersilene tape (a braided suture material/mesh) because of its perceived strength, reduced likelihood of tearing through tissues and ease of removal. While it was reported that using different suture types showed no differences with regard to extending the period of gestation,[3] it was also reported that the braided polyester thread (MersileneR) suture type was more effective for extending the period of gestation compared to other suture types in emergency or physical examination induced cerclages.[4] However in certain animal and wound site studies, based on the hypothesis that bacteria would increase more in multifilament sutures and would lead to an ascendant infection risk, it was asserted that monofilament sutures would be less correlated with infection compared to mersilene.[5,6]

Our hypothesis depends on that braided mesh suture Mersilene might cause higher comlication rates and unwanted results –like early delivery, low bir weight and intensive care unit requirement. Study was conducted in retrospective manner and included data of 46 pregnants diagnosed with cervical insufficiency. Suture material choices were decided by physician at time of cerclage procedure.

MATERIAL AND METHOD

46 pregnants with cervical insufficiency were included. All data was collected in retrospective manner. We collected written consent of all cases during data collection. Mean age was 32.5±5.1 years and mean gestational age was 18.1±5.1 weeks (Median 17, IQR 8 weeks). All cases were pregnancies with single fetus. In 26 cases suture type was mersilene and in 20 cases was prolene. Cervical lengths were similar (8.2±3.8 vs 10.6±3 cm). Data of birth week, infant weight, intensive care requirement and other complications including infections were collected retrospectively from patient charts. Patients < 18 years old, multiple pregnancies, who are unable to give informed consent, who do not want to join study group were excluded.

All surgical procedures were performed by same surgeon. The stitch was inserted using a McDonald technique. Main plan was to remove suture at 37±weeks of gestation yet as given in results some cases gestation was terminated by delivery before planned schedule.

Our study was approved as a thesis project by the Ethics Committee of Memorial Hospital in 2021, with the decision of the ethics committee numbered 003

Statistical analysis

An IBM SPSS ver 21 was used for data analysis. Kolmogorov Smirnoff test was used to define data homogentiy. Normally distributed data were expressed as mean±SD and nonnormally distributed data were expressed as median (IQR). Sudents t test and Mann Whitneyy tests were used when appropriate. Categorical variables were analyzed by chi-square and fisher tests. A p value < 0.05 was considered as statistically significant.

RESULTS

46 pregnants with cervical insufficiency were included. Mean age was 32.5±5.1 years and mean gestational age was 18.1±5.1 weeks (Median 17, IQR 8 weeks). All cases were pregnancies with single fetus. In 26 cases suture type was mersilene (Group 1) and in 20 cases was prolene (Group 2). Groups' mean ages were similar (33±5.5 vs 32±4.9 yrs). Gestational ages at cerclage were similar (18.4±3.8 vs 17.2±6.5 wks). No complications including infection was observed during follow-up period. All babies were normal vaginally delivered. Birth times were significantly shorter in group 1, (34.4±6.2 vs 37.9±4.7 weeks, p:0.02). Birth weights

were significantly lower (1839±878 vs 3257±602 grams, p:0.0001) in group 1 . Intensive care requirements were also significantly higher in group 1 (30% vs 10%, p:0000.1). All findings are summarized in **Table 1**.

Table.1 Comparison of study groups

	Mersilene group (n: 26)	Prolene group (n: 20)	P value
Age	33±5.5	32±4.9	NS
Cerclage week	18.4±3.8	17.2±6.5	NS
Birth week	34.4±6.2	37.9±4.7	0.02
Birth weight (gr)	1839±878	3257±602	0.0001
Intensive care requirement (n, %)	8, 30.7%	2, 10%	0.0001

DISCUSSION

In our study we observed that mersilene suture usage might be associated with higher preterm birth rates, lower birth weights and more requirement for intensive care unit.

Cervical cerclage has been offered to treat cervical insufficiency for over 60 years. One of the reasons for its continuous use is the lack of reliable evidence to support an alternative. It is used as an interventions to reduce rates of PTB. The placement of suture is currently only considered for singleton pregnancies and is not recommended in twin/multiple pregnancies.[7] Accoring to NICE[8] guidelines there are three main indications for cerclage insertion: elective based history, proactive based on ultrasound criteria; and emergency/rescue cerclage. MRC multicentre study[9] which recruited 1292 women who were deemed to be at high risk of cervical insufficiency and randomised them to cerclage (647 women) versus no intervention (645 women) is also an other important source for selection of patients for cerclage. Results from this trial reported that woman who had cerclage were less likely to have PTB compared to patients who didn't have any intervention (13% versus 17%). Study could not demonstarte any difference in fetal or neonatal outcome.

Various materials have been used for cerclage. Among these materials, human fascia lata, Mersilene, Prolene, Tevdek, and metal wires can be mentioned. The most commonly used ones today are non-absorbable monofilaments such as Mersileneand prolene.[10,11] Mersilene (a braided suture material) is traditionally preferred suture material because of its perceived strength, reduced likelihood of tearing through tissues and ease of removal. On the other hand braided sutures have been associated with an increased risk of infection particularly when used in potentially contaminated surgical areas

In one study, Berghella et al reported that cerclage that suture type had no effect on delivery below 35 weeks or the age of pregnancy in a group of 138 patients.[1] Similarly Stafford et al reported that suture type has no effect on pregnancy results in a group of 108 pregnant women.[12] In contradiction in a study by Kindinger et al a group of 678 pregnant women were evaluated and it was reported that the mersilene suture group had higher PTB rates and earlier birth weeks.[13] In another prospective study, the same authors applied ultrasound induced cervical cerclage to 49 patients and compared the mersilene and prolene suture usage. As a result, it was reported that the vaginal microbiome was corrupted in the mersilene suture group and that the pregnancy results were related to the corruption of the vaginal microbiome rather than the suture type.[13] Similarly Deger et al.[14] evaluated 151 pregnant women and found that pregnancy week was significantly lower in the mersilene suture group. They also reported that fetal weight, 1st and 5th minute APGAR scores in the mersilene suture group were significantly lower and intensive care requirement rates were significantly higher.

In the present study, it was observed that mersilene suture usage might be associated with higher PTB, lower birth weights and more requirement for intensive care unit. However, we did not perform any evaluation with regard to the vaginal microbiome. There are some animal models that reported monofilament sutures are associated with lower infection rates[4,6] but this association must still be be analyzed with more detailed studies. It is not surprising that infants in mersilene group had lower birth weights and required more intnsive care as their birth of week were significantly lower. The results of this study still indicate that the use of prolene sutures is recommended for better neonatal results and positive pregnancy results. Limitations of our study is small sample size and no evaluation of vaginal microbiome. More detailed randomized control studies with larger sample sizes are needed for evaluation of the effectiveness of different suture types in cerclage procedure. However, although the superiority of one suture materials over the other was not proven, the findings support the use of a thinner suture in women is correlated with lower PTB and better neonatal results.

CONCLUSION

Althought mersilene sutures are traditionaly preferred material for cerclage, prolene sutures seems to be associated with lower PTB and better neonatal results. While more detailed randomized clinical trials are needed to reveal any possible association between suture material and cerclage outcomes, prolene sutures stand still as alternative for traditional mersilene sutures.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission Memorial Hospital Ethics Committee (Date: 30.04.2021, Decision No: 003) .

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Endoscopic Treatment of Postoperative Esophageal Anastomotic Strictures: A Single Center Experience

Postoperatif Özofageal Anastomoz Darlıklarının Endoskopik Tedavisi: Tek Merkez Deneyimi

Muhammed Bahaddin Durak[1], Cem Simsek[2], Zeki Mesut Yalin Kilic [1]

[1]Department of Gastroenterology, Ankara City Hospital, Ankara, Turkey
[2]Department of Gastroenterology, Health Sciences University, Mehmet Akif Inan Training and Research Hospital, Sanliurfa, Turkey

Abstract

Aim: To evaluate the analysis, treatment methods and results of endoscopic treatments of esophagojejunostomy and esophagogastric anastomotic strictures.

Material and Method: Data from patients treated between 2009 and 2019 was collected and analyzed. The primary endpoint was defined as the absence of dysphagia for at least 6 months after the final endoscopic treatment session. The improvement in dysphagia scores at 1 and 6 months was accepted as the secondary endpoint.

Results: Of 18 patients (10 male), there were 11 patients with esophagogastric anastomotic stricture and 7 patients with esophagojejunostomy anastomotic stricture. Only balloon or bougie dilatation was applied to 13 patients, while 5 patients received a full-covered metal stent (FCMS) in addition to balloon or bougie dilatation due to persistent dysphagia symptoms. The primary endpoint was reached in 10 of the 13 patients (76.9%) who received only balloon or bougie dilatation. The secondary endpoint was reached in 3 patients. The primary endpoint was reached in 4 of the 5 patients (80%) who received a FCMS in addition to balloon or bougie dilatation. 6 patients (33.3%) had a recurrence. Major complications occurred in 4 (22.2%) patients, including perforation in 2 and stent migration in 2 patients.

Conclusion: The study demonstrated that endoscopic treatment of esophageal anastomotic strictures is a reliable and effective treatment option with a high success rate. The use of FCMS, either as a primary treatment option or in the treatment of perforation as a complication of endoscopic treatment, showed good effectiveness in our study.

Keywords: Esophagojejunostomy, esophagogastric, anastomotic stricture, full-covered metal stent

Öz

Amaç: Özofagojejunostomi ve özofagogastrik anastomoz darlıklarının endoskopik tedavilerinin analizi, tedavi yöntemleri ve sonuçlarının değerlendirilmesi.

Gereç ve Yöntem: 2009 ve 2019 yılları arasında endoskopik olarak tedavi edilen hastaların verileri toplandı ve analiz edildi. Primer sonlanım noktası, son endoskopik tedavi seansından sonra en az 6 ay süreyle disfaji olmaması olarak tanımlandı. 1. ve 6. aylarda disfaji skorlarındaki iyileşme ikincil sonlanım noktası olarak kabul edildi.

Bulgular: 18 hastanın (10 erkek) 11'inde özofagogastrik anastomoz darlığı, 7'sinde Özofagojejunostomi anastomoz darlığı vardı. 13 hastaya sadece balon veya buji dilatasyonu uygulanırken, 5 hastaya devam eden disfaji semptomları nedeniyle balon veya buji dilatasyonuna ek olarak tam kaplı metal stent uygulandı. Sadece balon veya buji dilatasyonu uygulanan 13 hastanın 10'unda (%76.9) primer sonlanım noktasına ulaşıldı. İkincil sonlanım noktasına 3 hastada ulaşıldı. Balon veya buji dilatasyonuna ek olarak tam kaplı metal stent uygulanan 5 hastanın 4'ünde (%80) primer sonlanım noktasına ulaşıldı. 6 hastada (%33.3) nüks görüldü. 2 hastada perforasyon ve 2 hastada stent migrasyonu olmak üzere 4 (%22,2) hastada majör komplikasyon gelişti.

Sonuç: Çalışmamız, özofagus anastomoz darlıklarının endoskopik tedavisinin yüksek başarı oranı ile güvenilir ve etkili bir tedavi seçeneği olduğunu göstermiştir. Tam kaplı metal stentin hem primer tedavi seçeneği olarak hem de endoskopik tedavinin bir komplikasyonu olarak perforasyon tedavisinde kullanılması çalışmamızda iyi etkinlik göstermiştir.

Anahtar Kelimeler: Özofagojejunostomi, özofagogastrik anastomoz darlığı, tam kaplı metal stent

Corresponding *(İletişim)*: Muhammed Bahaddin DURAK, Instructor in Medicine Department of Gastroenterology, Ankara City Hospital, Bilkent Avenue, 06800, Cankaya, Ankara, Turkey
E-mail *(E-posta)*: doctormbd@gmail.com
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INTRODUCTION

In a normal adult, when the esophageal luminal diameter is larger than 18 mm, those who can maintain a normal diet, dysphagia occurs when it is smaller than 13 mm.[1] Dysphagia can be caused on by a variety of conditions, including gastroesophageal reflux disease, achalasia, malignancies, corrosive esophagitis, eosinophilic esophagitis, radiotherapy-induced damage, esophageal webs, and postoperative esophageal anastomotic strictures.[2] Postoperative esophageal anastomotic strictures, which occur in 2%-30% of patients following esophageal surgery,[3] can be challenging to treat due to the fibrotic tissue, synechiae, and inherent risks associated with reoperating in these patients.[4] Endoscopic treatment options for postoperative esophageal strictures include balloon and bougie dilatation, as well as the use of full-covered metal stents.[5] However, significant esophageal stenosis or complex strictures often require multiple dilatation sessions,[6,7] and there is no consensus on the optimal endoscopic surgical method, follow-up intervals, time between sessions, or termination point.[8] In this study, we aim to evaluate the analysis, treatment methods, and outcomes of endoscopic treatments for esophagojejunostomy (EJ) and esophagogastric (EG) anastomotic strictures.

The most common cause of dysphagia is gastroesophageal reflux disease. Other causes will include achalasia, malignancies, corrosive esophagitis, eosinophilic esophagitis, failure secondary to radiotherapy, dysphagia, esophageal webs, and postoperative esophageal anastomotic strictures. Postoperative esophageal anastomotic strictures can be seen in 2%-30% of patients who have undergone esophageal surgery.[2] Strictures may form in the anastomosis or staple line following procedures such esophagogastric-esophagojejunal anastomosis, surgeries for achalasia, Nissen fundoplication, and bariatric surgery. Due to the fibrotic tissue created by these patient organs, synechiae, and the dangers of operating, surgical treatment of these strictures is an operation with substantial morbidity postoperatively. Additionally, several dilatation treatments may be necessary for the resolution of the instances following surgical intervention.[3] Balloon and bougie dilatation are used for endoscopic treatment of postoperative esophageal strictures.[4] Recently, endoscopic treatment of refractory benign esophageal strictures with a full-covered self-expandable metal stent (FC-SEMS) has also been widely used.[5]

Significant esophageal stenosis or complex stricture-sized endoscopic treatment requires multiple dilatation sessions from patients.[6,7] There is no common consensus regarding endoscopic surgical methods, follow-up times, time between sessions, and termination point.[8]

In this study, we aimed to evaluate the analysis, treatment methods and results of endoscopic treatments of esophagojejunostomy (EJ) and esophagogastric (EG) anastomotic strictures.

MATERIAL AND METHOD

Study Participants

This study included patients who had postoperative EG and EJ anastomotic stenosis and underwent endoscopic treatment at Turkey Yuksek Ihtisas Hospital between March 2009 and July 2019. During the postoperative period, an endoscopist checked each patient at the stenosis site and recommended them all to the hospital for endoscopic diagnosis and treatment of dysphagia. These patients' data, including those who were unable to complete the required interventions for the study and those who experienced a cancer recurrence, were gathered retrospectively and examined.

Study Setting and Equipment

All procedures were performed using an Olympus endoscope (Olympus, Tokyo, Japan). The endoscopic treatment utilized Micro-Tech (Micro-Tech Medical Company, Nanjing, China) dilatation balloons with lengths of 40-80 mm and diameters of 12-18 mm, Savary-Gilliard dilator system (Wilson-Cook Medical, USA) bougie dilators with lengths of 80-140 mm and diameters of 20-22 mm, and Micro-Tech (Micro-Tech Medical Company, Nanjing, China) full-covered metal stents with bell-shaped ends. The Mellow-Pinkas dysphagia score was calculated for all patients prior to the procedure (**Table 1**). All patients gave informed consent prior to the procedure, which was carried out by a single endoscopist while they were sedated with midazolam and pethidine. The operation was guided using fluoroscopy.

Table 1. Dysphagia score

Score 0	Able to tolerate normal diet.
Score 1	Can tolerate some solid foods
Score 2	Can tolerate semi-solid foods
Score 3	can only tolerate liquids
Score 4	Complete obstruction

Procedure:

The location and size of the stenosis was first evaluated using a gastroscope. Based on this evaluation, the endoscopist determined the appropriate balloon, bougie, or full-covered metal stent to use. A guide wire was advanced to the distal end of the stenosis and a balloon dilator was placed over the wire and centered on the stenosis. The balloon was inflated until the waist inside the balloon disappeared, and then the balloon was deflated and removed without waiting (**Picture 1**). This was repeated in this way, with the balloon diameter increasing in subsequent sessions (starting at 12 mm and increasing to 14-16 mm).

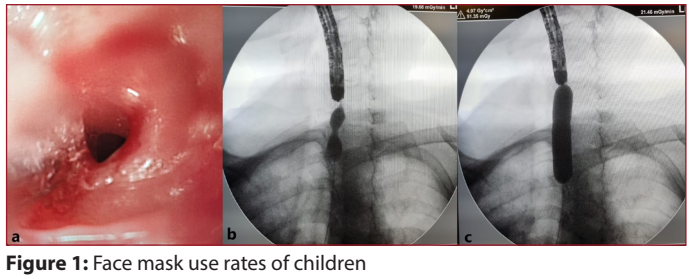

Figure 1: Face mask use rates of children

Dilatation with a bougie dilator was performed by advancing the bougie dilator up to 7 or 9 mm over the wire of the guide plate and increasing the diameter by a maximum of 3 mm. In subsequent sessions, the diameter was increased up to 15 mm. If dysphagia symptoms persisted despite the placement of a full-covered metal stent, balloon dilatation, and bougie dilatation, alternative treatment was considered. Full-covered metal stents were also used as a procedure for the treatment of perforation plates. Prior to stent placement, the stenosis site was marked visually by placing a metal object (such as a scalpel) on the tissue. The metal stent was then taken out of the scope and placed with the anastomosis line centered over the guidewire. To prevent beyond migration, hemoclips (1-4 pieces) were used to fix the metal stent's proximal end to the esophageal lining (**Picture 2**).

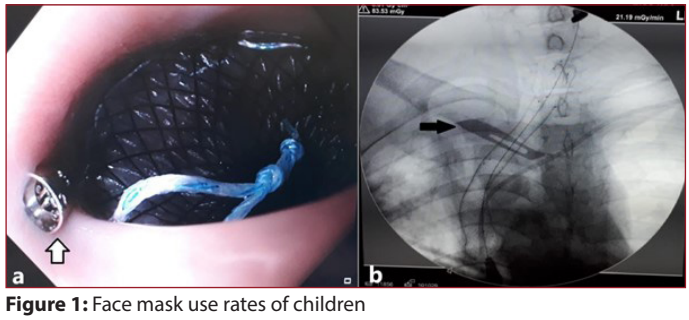

Figure 1: Face mask use rates of children

After the procedure, the presence of periesophageal free air was not evaluated using fluoroscopy. To identify subcutaneous emphysema, the neck area was consistently palpated. Any minor bleeding that occurred during the procedure was noted, and patients were closely monitored for 2 hours for major bleeding or perforation. At the end of the 2-hour period, patients without active complaints were discharged and provided with information about any complications. The dysphagia score was recalculated one week after the procedure. For patients with no significant reduction in dysphagia score, endoscopic treatment was administered at 1-2 week intervals during the first month. From the first month on, endoscopic dilatation was administered at 2-4 week intervals for 3 months, followed by 4-12 week intervals thereafter as needed. Dysphagia scores were recorded for patients at 1, 3, and 6 months.

The success of endoscopic treatment was defined as the absence of dysphagia for at least 6 months after the final endoscopic treatment session. This was the primary endpoint of our study. The improvement in dysphagia scores at 1 and 6 months was accepted as the secondary endpoint. Recurrence of dysphagia after a period of longer than 6 months without dysphagia was defined as recurrence.[9]

RESULTS

Endoscopic treatment was administered to 29 patients who developed postoperative esophageal anastomotic stricture. Adequate data was not available for 8 patients, and 3 patients were excluded from the study due to cancer recurrence at the anastomosis line, resulting in a total of 18 patients (10 male) with a median age of 64 (26-81) being included in the study. Squamous cell carcinoma of the esophagus in 6 patients, adenocarcinoma of the esophagus in 4, and adenocarcinoma of the stomach in 8 patients were the surgical indications for the formation of stricture in these individuals. Both the esophagojejunal and esophagogastric anastomotic strictures affected 11 and 7 individuals, respectively. The location of the stricture was at the lower end of the esophagus in 9 patients, at the upper end of the esophagus in 5 patients, and in the middle of the esophagus in 4 patients. Demographic data, surgical indications, type of anastomosis, and location of stricture for the included patients are listed in **Table 2**. The median time between surgery and the development of stricture was 6 months (2-16 months).

Table 2. Demographic data of patients, surgical details and stenosis localizations.

	n (%)
Median age (range)	64y (26-81)
Sex (male/%)	10 (55.5%)
Surgical indication	
Gastric adenocarcinoma	8 (44.4%)
Esophageal squamous cell carcinoma	6 (33.4%)
Esophageal adenocarcinoma	4 (22.2%)
Type of anastomosis	
Esophagogastric	11 (61.1%)
Esophagojejunal	7 (38.9%)
Stricture site	
Upper-esophagus	5 (27.8%)
Mid-esophagus	4 (22.2%)
Distal esophagus	9 (50%)

Only balloon or bougie dilatation was applied to 13 patients, while 5 patients received a full-covered metal stent in addition to balloon or bougie dilatation due to persistent dysphagia symptoms. The primary endpoint was reached in 10 of the 13 patients (76.9%) who received only balloon or bougie dilatation. The secondary endpoint was reached in 3 patients. The primary endpoint was reached in 4 of the 5 patients (80%) who received a full-covered metal stent in addition to balloon or bougie dilatation. One patient died from pulmonary embolism 3 months after the metal stent was removed.

The primary endpoint was reached in a total of 14 patients (77.7%), and the secondary endpoint was reached in 17 patients (94.4%). The mean duration to reach the primary and secondary endpoints was 8.1 months (1-25 months) and 18 days (3-34 days), respectively. The dysphagia scores, number of endoscopic treatment sessions, and clinical outcomes for the patients are listed in **Table 3**.

Table 3. Dysphagia scores and clinical outcomes of the patients.

Patient no.	Dysphagia Score			Total sessions	Duration to reach the primary endpoint (month)	Dysphagia free-period after last procedure (month)	fully covered metal stent replacements	Reaching the primary endpoint	Reaching the secondary endpoint	Recurrence
	Before the procedure	1. month	6. month							
1.	3	2	1	10	11	11		+	+	-
2.	3	1	0	13	12	12	+	+	+	-
3	3	1	0	12	25	35	+	+	+	+
4.	3	1	1	6	7	12		+	+	+
5.	2	1	0	9	7	12		+	+	-
6.	3	1	1	3	11	7		+	+	-
7.	2	0	0	12	1	39	+	+	+	+
8.	4	2	1	5	-	5		-	+	-
9.	4	1	2	5	-	3		-	-	-
10.	3	1	1	7	-	4		-	+	+
11.	3	1	0	6	7	9		+	+	-
12.	3	2	1	15	-	4	+	-	+	+
13.	3	1	0	5	4	12		+	+	-
14.	3	1	0	4	5	32		+	+	-
15.	3	2	1	15	24	12	+	+	+	+
16.	4	2	1	5	2	16		+	+	-
17.	3	2	0	5	-	22		+	+	-
18.	4	1	0	9	3	9		+	+	-

Seven patients in total received FC-SEMSs. Two patients received the stents as a result of the development of perforation associated with the treatment, whereas five patients had them for the purpose of dilatation. A total of 146 sessions of treatment were performed. During the first month, the average number of procedures was 2 (range: 1-4). The average follow-up period was 25 months (range: 8-47 months). Through the whole follow up period, the average number of procedures was 8.1 (range: 3-15).

6 patients (33.3%) had a recurrence. The recurrence occurred at an average of 10.5 months (range: 7-24 months) after treatment. Of the 4 patients with recurrence, 2 were followed asymptomatically after 2 sessions of endoscopic balloon dilatation, and 2 continue to receive endoscopic balloon dilatation sessions. In 2 patients, the cause of the stricture was cancer recurrence and total obstruction occurred, preventing endoscopic treatment. Percutaneous jejunostomy was performed surgically on both patients.

Major complications occurred in 4 (22.2%) patients, including perforation in 2 and stent migration in 2 patients. The 2 patients with perforation were treated with FC-SEMS and broad-spectrum antibiotics. After 4 weeks in one patient and 6 weeks in the other, the stents were withdrawn. There was no procedure-related mortality. During the study period, 2 patients died due to cancer recurrence and 1 patient died due to pulmonary thromboembolism.

DISCUSSION

The surgical treatment of postoperative esophageal anastomotic strictures involves surgical resection and re-anastomosis. The surgical approach is associated with perioperative morbidity and mortality due to comorbidities and secondary adhesions to previous surgery.[10] Endoscopic treatment of esophageal anastomotic strictures is an effective and safe treatment method for reducing dysphagia symptoms or significant decrease in dysphagia score, even if it requires multiple endoscopic sessions.[11] The first option in endoscopic treatment is balloon and bougie dilatation. If there is no significant symptomatic relief after multiple balloon dilatation sessions, a fully covered metal stent can be applied.[12] However, fully covered metal stents are not recommended as the first treatment option due to their high risk of migration.[13] In the study, we applied fully covered metal stents to patients who did not respond to endoscopic balloon and bougie treatment.

Studies have reported that effective endoscopic treatment of esophageal anastomotic strictures requires 2-9 dilatation sessions.[14] The average number of dilatation sessions in the present study was 8.1, which is similar to the literature.

The number of dilatation sessions administered to patients in the first month was an average of 2 (range: 1-4 months). We observed that the patients' need for dilatation decreased in subsequent months due to the increase in the diameter of the balloon and bougie sizes used in the subsequent dilatation sessions.

A total of 77.7% of patients and 94.4% of patients, respectively, reached the primary and secondary endpoints. This high percentage of success was comparable to earlier research published in the literature.[12] In a study by Lu et al.[11] the success rates of balloon dilatation and fully covered metal stent application were 70.9% and 35%, respectively. The higher success rate of endoscopic treatment in this study may be due to our combination of balloon/bougie dilatation with fully covered metal stent application.

The time to reach the primary endpoint was an average of 8.1 months. The time to reach the secondary endpoint was an average of 18 days. Dysphagia scores reduced quickly after endoscopic therapy, but it took a while for patients to reach a stage where they were no longer in need of endoscopic treatment. In this research, the recurrence rate was 33.3%. This rate can reach up to 50% in previous studies.[15] After recurrence, patients were treated endoscopically. The duration of endoscopic treatment is related to the recurrence rate. In previous studies, the recurrence rate was higher in patients treated for a shorter duration.[16] In the current study, the recurrence rate was not significantly different between the group treated for a shorter duration and the group treated for a longer duration.

The proportion of patients who reached the primary and secondary endpoint was 77.7% and 94.4%, respectively. This high success rate was similar to that in previous studies in the literature.[12] In a study by Lu et al.[11] the success rates of balloon dilatation and fully covered metal stent application were 70.9% and 35%, respectively. The higher success rate of endoscopic treatment in the present study may be due to our combination of balloon/bougie dilatation with fully covered metal stent application.

The time to reach the primary endpoint was an average of 8.1 months. The time to reach the secondary endpoint was an average of 18 days. Endoscopic treatment resulted in a rapid decrease in dysphagia scores, but it took a long time for patients to reach a period of dysphagia-free status without the need for further endoscopic treatment. The recurrence rate in our study was 33.3%. This rate can reach up to 50% in previous studies.[15] After recurrence, patients were treated endoscopically. However, there is no consensus regarding the optimal management with endoscopy. Therefore, continuing endoscopic treatment may be a reasonable option for patients in whom surgical treatment is not possible.

FC-SEMS are used not only in the treatment of esophageal anastomotic strictures, but also in the treatment of perforation, a complication of endoscopic treatment. Patients who developed perforation during endoscopic treatment were treated with FC-SEMS. The effectiveness of FC-SEMS in the iatrogenic perforation of the esophagus has been demonstrated in the literature.[16]

The limitations of our study include its retrospective design and the lack of a sufficient number of patients to compare endoscopic treatment methods. Although larger, controlled prospective studies are needed, we believe that the study can contribute to such studies.

CONCLUSION

Our study demonstrated that endoscopic treatment of esophageal anastomotic strictures is a reliable and effective treatment option with a high success rate. The success rate of endoscopic treatment increased with multiple sessions, but the recurrence rate was also high. The use of FC-SEMS, either as a primary treatment option or in the treatment of perforation as a complication of endoscopic treatment, showed good effectiveness in our study. However, further larger, controlled prospective studies are needed to confirm these findings and to compare different endoscopic treatment methods. Despite its retrospective design and the limitations in the number of patients, our study contributes to the existing literature and can provide guidance for the management of esophageal anastomotic strictures. Overall, endoscopic treatment should be considered as an option for patients with esophageal anastomotic strictures, particularly for those in whom surgical treatment is not possible or not desired.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital Clinical Researches Ethics Committee (Date: 26.05.2021, Decision No: E1/1809/2021).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Impact of COVID-19 Pandemic on Family Medicine Clinical Practices During the Second Wave

COVID-19 Pandemisinin İkinci Dalga Sırasında Aile Hekimliği Klinik Uygulamalarına Etkisi

Duygu Ayhan Başer[1], Raziye Şule Gümüştakım[2], Murat Çevik[3], Ekrem Basara[4]

[1]Hacettepe University School of Medicine Department of Family Medicine, Ankara, Turkey
[2]Sütçü İmam University School of Medicine Department of Family Medicine, Kahramanmaraş, Turkey
[3]Sokullu Family Health Center, Ankara, Turkey
[4]Ayaş Family Health Center, Ankara, Turkey

Abstract

Aim: It is seen that there are various changes brought by the process within the legislation and in various practices in primary health care services in extraordinary health situations such as pandemics. In this study, it was aimed to determine the effects of the COVID-19 pandemic on some clinical practices of family physicians in Turkey.

Material and Method: The descriptive study was applied to family physicians working actively in family health centers between February 15, 2021 and April 1, 2021. Family physicians working actively in family health centers formed the universe. Physicians were asked through online-survey questions regarding specific follow-ups and changes they made in some clinical practices, the changes in the number of applications, and the arrangements they made in this regard.

Results: 912 people participated in the study. 59.9% were male, 60.2% had been practicing family medicine for more than 10 years. 63.9% had PCR test and 26.8% had antibody test. 16.1% were diagnosed with COVID-19. 84% of them went to Family Health Center (FHC) every day during the pandemic. While 48.2% of them were doing triage, 53.6% of the participants stated that the number of applications decreased. 65.2% of them did their pregnant-baby-child follow-ups during the COVID-19 pandemic as before the pandemic, 24.7% carried out by appointment. 54.3% made a change in the FHC layout and terms. 50.2% stated that they did not follow up on chronic diseases during the COVID-19 period, 59.2% stated that there was a decrease in the number. 66.7% of them made their childhood vaccinations during the COVID-19 pandemic as before the pandemic, 24.7% carried out by appointment, and the number of applications for 73.6% did not change. 63.6% of them made adult vaccines during the COVID-19 pandemic as before the pandemic, the number of applications increased by 58.2%, and 73% were encouraging individuals. The number of those who came for general health status reports (driver's license, mental ability, job security, etc.) did not change by 53%, but increased by 23.6%.

Conclusion: The pandemic period has caused serious changes in family medicine clinical practices; family physicians have made various arrangements to carry out their current work in this period and have made an effort to convey this situation to the public.

Keywords: COVID-19 pandemic, family physicians, clinical practices

Öz

Amaç: Pandemi gibi olağanüstü sağlık durumlarında birinci basamak sağlık hizmetlerinde mevzuat ve çeşitli uygulamalarda sürecin getirdiği çeşitli değişiklikler olduğu görülmektedir. Bu çalışmada, COVID-19 pandemisinin Türkiye'deki aile hekimlerinin bazı klinik uygulamalarına etkilerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Tanımlayıcı tipteki araştırma, 15 Şubat 2021-1 Nisan 2021 tarihleri arasında aile sağlığı merkezlerinde aktif olarak görev yapan aile hekimlerine uygulandı. Evreni, aile sağlığı merkezlerinde aktif olarak görev yapan aile hekimleri oluşturdu. Hekimlere spesifik takipler ve bazı klinik uygulamalarda yaptıkları değişiklikler, başvuru sayısındaki değişiklikler ve bu konuda yaptıkları düzenlemeler online anket soruları ile sorulmuştur.

Bulgular: Çalışmaya 912 kişi katıldı. %59,9'u erkekti, %60,2'si 10 yıldan uzun süredir aile hekimliği yapıyordu. %63,9'unda PCR testi, %26,8'inde antikor testi vardı. %16,1'ine COVID-19 teşhisi kondu. Bunların %84'ü pandemi süresince her gün Aile Sağlığı Merkezi'ne (ASM) gitti. Katılımcıların %48,2'si triyaj yaparken, %53,6'sı başvuru sayısının azaldığını belirtti. %65,2'si gebe-bebek-çocuk takiplerini pandemi öncesinde olduğu gibi COVID-19 pandemisi sırasında, %24,7'si randevu ile gerçekleştirmiştir. %54,3'ü ASM düzeninde ve koşullarında değişiklik yaptı. %50,2'si COVID-19 döneminde kronik hastalık takibi yapmadığını, %59,2'si ise sayısında azalma olduğunu belirtti. %66,7'si çocukluk aşılarını pandemi öncesinde olduğu gibi COVID-19 salgını sırasında yaptırmış, %24,7'si randevu ile yaptırmış, %73,6'sı için başvuru sayısı değişmemiştir. Pandemi öncesinde olduğu gibi COVID-19 pandemisinde de %63,6'sı erişkin aşılarını yaptırırken, başvuru sayısı %58,2 arttı ve %73'ü bireyleri teşvik ediyordu. Genel sağlık durum raporu (sürücü ehliyeti, akli ehliyet, iş güvenliği vb.) için gelenlerin sayısı %53 değişmeyerek %23,6 arttı.

Sonuç: Pandemi dönemi aile hekimliği klinik uygulamalarında ciddi değişikliklere neden olmuş; aile hekimleri bu dönemde mevcut işlerini yürütmek için çeşitli düzenlemeler yapmışlar ve bu durumu topluma aktarmak için çaba sarf etmişlerdir.

Anahtar Kelimeler: COVID-19 pandemi, aile hekimleri, klinik uygulamalar

Corresponding *(İletişim)*: Duygu AYHAN BAŞER Associate Professor, M.D., Hacettepe University School of Medicine Department of Family Medicine, Ankara, Turkey
E-mail *(E-posta)*: duyguayhan@outlook.com
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INTRODUCTION

Family Health Centers (FHC) are the first step of health services in the health system. Family Health Centers have an important place in general health services due to their easy accessibility and faster delivery of services compared to other health units. Even in extraordinary health situations such as a pandemic, primary health care services are at the forefront of the fight against the epidemic. It is not possible to control and manage a pandemic in a healthy and correct manner without the effective and correct participation of primary health care services.[1] In the studies, it was stated that the task scheme of family physicians was clearly defined in pandemic plans and the COVID-19 pandemic was better managed in countries where primary health care services are strongly structured.[2-6]

The COVID-19 pandemic has caused various changes in the structuring of all health services. During some periods of the pandemic, elective operations were suspended in hospitals, and the number of patients in outpatient clinics was restricted.[7]

It is seen that there are various changes brought by the process within the legislation and in various practices in primary health care services in extraordinary health situations such as pandemics.[3-5] Primary health care has its own department at national level and is financed through a budget within the Ministry of Health in Turkey. Family health centers are institutions where primary care diagnosis, treatment and rehabilitative health services are provided as well as personal preventive health services. In addition to baby, child and adolescent follow-up, pregnant, 15-49 age follow-up, chronic disease follow-up, immunization services, presentation of various health reports are the main clinical services carried out in the family health center. Various practices have been planned by the state for the provision of these services during the pandemic period. The main of these are the provision of the prescribed drugs of people with chronic diseases directly from the pharmacy, and the monitoring of patients from home with the telemedicine method.[8] No study has been found in which both the pandemic conditions and the effects of various regulations that may affect the current service delivery on the applications made for clinical services in family health centers and the regulations made by family physicians in this regard have been evaluated. Knowing how these services are affected by pandemic conditions and evaluating the arrangements made by family physicians in their own clinical practices in this regard can provide important information in the action plan to be created for future pandemics. With this study, it was aimed to evaluate the thoughts and attitudes of family physicians about the effects of the COVID-19 pandemic on clinical practices, the status of specific follow-up applications, and the regulations they have made on clinical practices in primary health care services in Turkey.

METHOD

The descriptive study was conducted between 15 February 2021 and 1 April 2021. The universe of the study consisted of family physicians who actively work in family health centers and were members of the Family Medicine Federation (FMF). FMF is a federation that gathers voluntary family physicians in Turkey under one roof through their associations. FMF has a total of 73 provincial associations and approximately 17 thousand members. The sample size was calculated as 376 when calculated with 5% margin of error and 95% confidence interval. Physicians who did not work before pandemic in FHC were excluded from study.

For the study, ethical committee approval was obtained from Hacettepe University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Date:15.12.2020, Project no: GO20/1190).

The participants were asked questions about age, gender, years of working in family medicine, as well as the status of having COVID-19 infection. Physicians were asked through survey questions regarding specific follow-ups and changes they made in some clinical practices, the changes in the number of applications, and the arrangements they made in this regard.

A total of 31 questions were submitted online. The survey was developed by detailed literature research and observation of the national primary care guidelines for routine clinical practices. The electronic questionnaire form (Google Form) was sent to the family physicians who were active in family health centers and who were members of the Family Medicine Federation (FMF), via e-mail and social media (whatsapp and facebook websites of FMF).

SPSS 23.0 analysis program was used to evaluate the data. Descriptive statistics were presented as mean (±) standard deviation, median (min-max), frequency distribution, and percentage. Chi-Square Test or Fisher's Exact Test was used to compare categorical variables. When a significant difference was detected in comparisons with more than 2 categories for at least one variable (comparisons other than 2x2), the groups were compared in pairs to determine the source of the difference, and Bonferroni correction was applied to identify the groups with a difference. Conformity of continuous variables to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (if $n \geq 50$; Kolmogorov-Smirnov Test, if $n < 50$; Shapiro-Wilk Test). For the variable found to fit the normal distribution; Student T Test was used for statistical significance between two independent groups. One-Way ANOVA was used as a statistical method among three or more independent groups found to have a normal distribution. Tukey or Tamhane's T2 test results were used according to the homogeneity of the variances of the groups in post hoc multiple comparisons to determine the source of the significant differences between three or more independent groups. Statistical significance level was accepted as $p < 0.05$.

RESULTS

913 people participated in the study. 59.9% (n=546) of the participants were male and 40.1% (n=367) of them were female. The mean age of the participants was 44.31±8.92 (min=26; max=68). While 60.1% (n=549) of family physicians have been practicing family medicine for more than 10 years, 20.3% (n=185) for 6-10 years, 17.5% (n=160) has been working as a family physician for 1-5 years.

Table 1. Sociodemographic characteristics of family physicians

	%	n		
Sex				
Female	40.1	367		
Male	59.9	546		
The working duration as a family physician				
Between 1-5 years	19.6	179		
Between 6-10 years	20.3	185		
More than 10 years	60.1	549		
Regions of the Country				
Marmara Region	13.4	123		
Black Sea Region	9.4	85		
Aegean Region	12.9	118		
Mediterranean Region	13.5	123		
Central Anatolia Region	32.4	296		
Eastern Anatolia Region	10.3	94		
Southeastern Anatolia Region	8.1	74		
	Mean	**SD**	**Min**	**Max**
Age	44.31	8.92	26	68

64% (n=584) of the participants had PCR test for COVID-19 infection, 26.8% (n=245) had done antibody test. 16.1% (n=147) were diagnosed with COVID-19 infection, 23.1% (n=211) remained in quarantine. 67.8% (n=619) had a history of contact with someone with a diagnosis of COVID-19.

While 53.8% (n=489) had registered patients of 3500 and above, 38.3% (n=348) had registered patients between 2000-3500.

During the COVID-19 period, 84% (n=767) stated that they worked in the same way as in the pre-pandemic period and went to the FHC every day for outpatient services, 12.6% (n=115) stated that they worked flexibly.

86.4% (n=789) of family physicians stated that they admitted patients without an appointment during the pandemic period, and 10.7% (n=98) stated that they sometimes took patients with an appointment. 43.6% (n=398) of them stated that they performed triage at admissions during the pandemic. 53.6% stated that the number of visits decreased during this period, while 27.5% stated that they increased.

The views of family physicians on the change in the number of visits and the number of special follow-up visits during the second wave of the COVID-19 pandemic are given in **Table 2**.

Table 2. Views of family physicians on the change in the number of visits and the number of special follow-up visits during the second wave of the COVID-19 pandemic

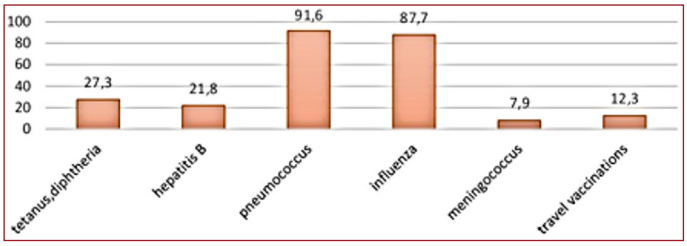
During the second wave of the COVID-19 pandemic;	Increased		Decreased		Not changed	
	n	%	N	%	n	%
Change status in primary care visit numbers	251	27.5	489	53.6	173	18.9
Change status in the number of those who come for pregnant-baby-child follow-ups	27	3.0	321	35.2	565	61.9
Change status in the number of those who come for chronic disease follow-ups	74	8.1	540	59.1	299	32.7
Change status in the number of people coming for childhood vaccination	122	13.4	119	13.0	672	73.6
Change status in the number of arrivals for adult vaccines	532	58.3	139	15.2	242	26.5
Change status in the number of visitors for general health status reports (driver's license, mental ability, job security, etc.)	215	23.5	215	23.5	483	52.9

Of the family physicians 1.9% stated that there was no change in the number of those who came for pregnant-baby-child follow-up. 65.2% (n=595) stated that they were doing their pregnant baby-child follow-ups as before the pandemic, 24.6% (n=225) by appointment, 6.4% (n=58) by telephone. 54.2% (n=495) reported that they made changes in the FHC system for pregnant-baby-child follow-ups, and 67% (n=612) reported that they had to explain the precautions they took to come to pregnant-baby-child follow-ups.

59.1% of family physicians stated that the number of people who applied for chronic disease follow-ups decreased. 50.3% (n=459) of family physicians reported that they did not follow up on chronic diseases, 29.6% (n=270) reported that they did it as before the pandemic, and 10.4% (n=95) carried out by telephone. 44.5% (n=406) reported that they had to tell the patients about the precautions they took to follow up for chronic disease.

73.6% of family physicians stated that the number of people applying for childhood vaccination did not change. 66.7% (n=609) of family physicians reported that they administered childhood vaccinations as before the pandemic, while 31.9% (n=291) reported that they carried out by appointment. 62.4% (n=570) reported that they had to explain the precautions they took to bring their children to have childhood vaccinations.

58.3% of family physicians stated that the number of people applying for adult vaccination has increased. 63.6% (n=581) of the family physicians reported that they performed adult vaccinations as before the pandemic, while 34.8% (n=318) reported that they carried out by appointment. 73.1% (n=667) reported that they encouraged their patients for adult vaccination. Graphic of recommended vaccines by family physicians is presented in **Graphic I**.



Graphic 1. Recommended vaccines during pandemic by family physicians

52.9% of family physicians stated that the number of those who came for general health status reports (license, mental ability, job security, etc.) did not change. 92.9% (n=848) of family physicians reported that they did not find it appropriate to prescribe medication or issue a report to a patient they did not see, and 68% (n=621) reported that they looked at the legislation on prescribing medication or issuing a report.

The relationship between sociodemographic features (age, sex, year in profession) and views of family physicians' about clinical practices were evaluated. There was a relationship between gender and triage practices, women physicians did triage much more than men (p=0.004). There was a relationship between gender and practice types of pregnant-baby-adolescent follow-up, chronic disease follow-up; women did telephone consultation more than men (p=0.032; p<0.001).

DISCUSSION

The current study found that family physicians' clinical practices were variably affected since the COVID-19 pandemic during second wave. Nearly half of them mentioned that the total number of applications decreased during this period. It has been reported in different studies from Turkey that there has been a decrease in applications to primary health care institutions during the pandemic period.[9,10] According to a field study conducted by Turkish Medical Association, it has been reported that a decrease in the number of patients coming for both analysis and cancer screening in 93% of FHCs.[11] In international studies, in accordance with our study, the decreased applications numbers and a dramatic shift to telehealth visits were mentioned.[12-18] Prolonging the reporting period of the drugs supplied by the Ministry of Health and ensuring that they can be obtained from pharmacies without the necessity of a prescription might be reduced the number of examinations in our country. Also, the number of telephone consultations were not high much as other countries, but in all type of practices, a little part of family physicians stated about it.

According to a guideline developed by Turkish Medical Association, triage was suggested in primary health care centers.[19] Triage means that every patient contacting the practice first provides some information on the reasons for contact and is triaged before making an appointment.[20] In our study, the biggest part of participants stated that they admitted patients without an appointment and 43.6% of them stated that they performed triage at admissions during the pandemic. In the first wave of COVID-19 pandemic, the triage was used much more than second wave, because with the time, this new disease better known by public and by health personals.[8,16,20]

During the COVID-19 period, the biggest part of the family physicians stated that they worked in the same way as in the pre-pandemic period and went to the FHC every day for outpatient services. In literature, especially in developed countries, the teleconsultation rates were stated as increased,[17-22] and with teleconsultation, some physicians worked home-office during pandemic. In our country the teleconsultation could not be used effectively by physicians because of legal issues and infrastructural problems.[23] So the working type could not be changed. In our study we found that, women physicians did triage and telephone consultation much more than men. We did not examine about reasons of these results in our survey. Maybe new researches will be planned in this context.

Biggest part of participants said that applications for chronic disease follow-ups were decreased, half of family physicians reported that they did not do chronic disease-follow up, and 10.4% carried out by telephone. According to a field study conducted by Turkish Medical Association, it has been reported that a decrease in admissions for chronic diseases was observed in 51% of the patients.[11] Prolonging the reporting period of the drugs supplied by the Ministry of Health and ensuring that they can be obtained from pharmacies without the necessity of a prescription might be reduced the number of examinations in our country. Another reason could be that from the beginning of the pandemic, the negative effect of COVID-19 on people with chronic disease was emphasized and people could be afraid from transmission of virus.

In Turkey, it has been suggested by ministry of health that the preventive health services (vaccination, infant-child follow-up, pregnant follow-up) continued in the same way in terms of the continuity of public health.[10] In accordance with this suggestion, in our study, physicians mentioned that applications for pregnant-baby-child follow-ups and childhood vaccination were not changed. Family physicians mentioned that they were doing their pregnant baby-child follow-ups as before the pandemic, however nearly half of them reported that they made changes in the FHC system for pregnant-baby-child follow-ups. Considering the literature, in a study conducted by Muhaidat et al. in Jordan, the rate of receiving antenatal care service in the pandemic period was decreased.[24] Like our study, in study of Esmeray et al., it was mentioned that, within the scope of Covid-19 measures in FHC, patients and pregnant women enter from different doors in a way that they do not come into contact with each other.[10] In this study, 66.7% of family physicians reported that they administered childhood vaccinations as before the pandemic,

while 31.9% reported that they carried out by appointment. According to data from 129 countries by UNICEF, 53% of countries reported moderate or severe disruptions in their vaccination programs.[25]

Adult vaccination applications were mentioned as increased. One third of them reported that they carried out by appointment and 73.1% reported that they encouraged their patients for adult vaccination. Pneumococcus, influenza vaccination applications were highest rate according to family physicians.

It has been suggested by ministry of health, postponing medical reports other than marriage and military service reports were among the measures taken. In our study physicians stated that applications for general health status reports (driver's license, mental ability, job security, etc.) were not changed.[9] Approximately half of family physicians stated that the number of those who came for general health status reports (license, mental ability, job security, etc.) did not change. Biggest part of them reported that they did not find it appropriate to prescribe medication or issue a report to a patient they did not see, and 68% reported that they looked at the legislation on prescribing medication or issuing a report. In a study conducted by Esmeray and colleques, the rate of driver's license examinations decreased (18.2%), while the rates of marriage (48.7%), military service (1.8%) and single physician report (31.3%) increased during pandemic.[10] According to a field study conducted by Turkish Medical Association, it has been reported that a decrease in applications for medical reports in 70% of the patients.[11]

In this study, the convenience sample was used and thus results of generalizability may be limited. The study was a cross-sectional study, in the future studies, should be employed a longitudinal design to confirm the findings and investigate the causality of relationships.

CONCLUSION

The pandemic period has caused serious changes in family medicine clinical practices, family physicians have made various arrangements to carry out their current work during this period and have made an effort to convey this situation to the public.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hacettepe University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Date:15.12.2020, Project no: GO20/1190).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Examination of Placental Dysfunction and Neonatal Outcomes in Hospitalized Patients who have Hyperemesis Gravidarum Diagnosis

Hiperemezis Gravidarum Tanılı Hastanede Yatan Hastalarda Plasental Disfonksiyon ve Neonatal Sonuçların İncelenmesi

Jule Eriç Horasanlı[1], Nurullah Şengül[2]

[1]Necmettin Erbakan University Faculty of Medicine, Department of Obstetrics and Gynecology, Konya, Turkey
[2]Dr. Ali Kemal Belviranlı Gynecology and Pediatrics Hospital, Konya, Turkey

Abstract

Aim: Evidence on the effect of hyperemesis gravidarum (HG) on pregnancy outcomes is still unclear. In this study, placenta-related dysfunctions and neonatal outcomes in patients who were hospitalized with HG were retrospectively analyzed.

Material and Method: This study was conducted retrospectively among women who had singleton deliveries in our hospital between January 2015 and January 2020. As the case group, women with singleton pregnancies who were hospitalized due to HG and delivered were included. The control group consisted of women with singleton pregnancies who delivered without hospitalization due to HG. Through the hospitalization files of the patients who were hospitalized due to HG and not hospitalized due to HG, neonatal outcomes such as placental dysfunction, including gestational diabetes, gestational hypertension, preeclampsia, or stillbirth, and low birth weight, small for gestational age (SGA), preterm birth (PTB), the necessity for neonatal intensive care, 5-min Apgar scores, were examined.

Results: The mean gestational age was determined as 37.7±1.5 weeks in the HG group and 37.8±1.4 weeks in the control group. The mean week of gestation upon hospitalization for HG was 10.6±3.6 weeks. There was no significant difference between the groups regarding preeclampsia, PTB, postpartum hemorrhage, birth weight, GDM, or neonatal intensive care unit requirement. It was found that SGA babies with abnormal birth weights were seen more frequently in the deliveries of patients hospitalized for HG (P=0.022). The 5-min Apgar scores were higher in the deliveries of patients hospitalized for HG than in the control group (P=0.004).

Conclusion: It was concluded that hospitalizations due to HG do not pose a risk of placental dysfunction. Of the neonatal outcomes, SGA was more common in the infants of mothers with HG. Contrary to the expectations herein, the 5-min Apgar scores were higher in hospitalized patients for HG.

Keywords: Hyperemesis gravidarum, Placental dysfunction, Neonatal outcomes

Öz

Amaç: Hiperemezis gravidarumun (HG) gebelik sonuçları üzerindeki etkisine ilişkin kanıtlar hala belirsizdir. Bu çalışmada HG ile hastaneye yatırılan hastalarda plasenta ile ilişkili disfonksiyonlar ve neonatal sonuçlar retrospektif olarak incelenmiştir.

Gereç ve Yöntem: Bu çalışma Ocak 2015-Ocak 2020 tarihleri arasında hastanemizde tekil doğum yapan kadınlar arasında retrospektif olarak yapılmıştır. Olgu grubu olarak HG nedeniyle hastaneye yatırılıp doğum yapmış tekil gebeliği olan kadınlar alınmıştır. Kontrol grubu, HG nedeniyle hastaneye yatmadan doğum yapan tekil gebe kadınlardan oluşturuldu. HG nedeniyle hastaneye yatırılan ve HG nedeniyle hastaneye yatırılmayan hastaların yatış dosyaları aracılığıyla, gestasyonel diyabet, gestasyonel hipertansiyon, preeklampsi veya ölü doğum dahil olmak üzere plasenta disfonksiyonu ve gebelik yaşına göre küçük düşük doğum ağırlığı (SGA) gibi yenidoğan sonuçları), erken doğum (PTB), yenidoğan yoğun bakım gerekliliği, 5 dk Apgar skorları incelendi.

Bulgular: Ortalama gebelik yaşı HG grubunda 37,7±1,5 hafta, kontrol grubunda 37,8±1,4 hafta olarak belirlendi. HG nedeniyle hastaneye yatışın ardından ortalama gebelik haftası 10.6±3.6 haftaydı. Gruplar arasında preeklampsi, PTB, doğum sonu kanama, doğum ağırlığı, GDM, yenidoğan yoğun bakım gereksinimi açısından anlamlı fark yoktu. HG nedeniyle hastaneye yatırılan hastaların doğumlarında anormal doğum ağırlığına sahip SGA bebeklerin daha sık görüldüğü saptandı (P=0,022). HG nedeniyle hastaneye yatırılan hastaların doğumlarında 5 dk Apgar skorları kontrol grubuna göre daha yüksekti (P=0.004).

Sonuç: HG nedeniyle hastaneye yatışların plasental disfonksiyon riski oluşturmadığı sonucuna varıldı. Yenidoğan sonuçlarından SGA, HG'li annelerin bebeklerinde daha yaygındı. Buradaki beklentinin aksine HG nedeniyle hastanede yatan hastalarda 5 dk Apgar skoru daha yüksekti.

Anahtar Kelimeler: Hiperemezis gravidarum, Plasenta disfonksiyonu, Neonatal sonuçlar

Corresponding *(İletişim)*: Jule ERİÇ HORASANLI, Necmettin Erbakan University Faculty of Medicine, Department of Obstetrics and Gynecology, Konya, Turkey
E-mail *(E-posta)*: juleesen@gmail.com
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INTRODUCTION

Nausea, as well as vomiting during pregnancy, are conditions that are commonly seen and have adverse physical, metabolic, psychological, and social effects on a pregnant woman.[1,2] Hyperemesis gravidarum (HG), also known as morning sickness, usually occurs between the 6th and the 16th week of pregnancy, in the first trimester of pregnancy, and occurs at any time of the day. The increase in human chorionic gonadotropin (hCG) begins within the first four weeks following the last menstrual period (LMP) and peaks at the ninth week of pregnancy, and symptoms increase in parallel.[3] The prevalence of nausea is between 50% and 90%, while it was reported as 50% for vomiting and retching.[4,5] The degree and onset of nausea are related to the hCG level. Of pregnant women with emesis, 60% recover spontaneously at the end of the first trimester, and 91% recover by the 20th week of pregnancy.[6]

'Persistent and excessive vomiting starting before the 22nd week of gestation' was defined as the International Classification of Diseases (ICD)-9 and ICD-10 in accordance with the International Statistical Classification of Disease and Related Health Problems. HG is clinically classified as mild or severe according to associated metabolic disorders, including carbohydrate restriction-induced weight loss, dehydration, ketonuria, and electrolyte imbalance. These symptoms worsen in 0.3% to 1.5% of pregnant women, so pregnant women are treated after hospitalization.[7,8] In the etiology of hyperemesis, conditions with high hCG levels, such as thyroid hormones, estrogen, leptin, fetal-maternal barrier damage, helicobacter pylori infections, multiple pregnancies, and molar pregnancies, are listed as causes.[9,10]

The relationship between placental functions and HG is not fully understood. A suggestion has been made that an increase in hCG levels in women with hyperemesis impairs trophoblast invasion and also changes placental location. Results such as abnormal placenta, preeclampsia, postpartum hemorrhage, small for gestational age (SGA), and stillbirth were found to be associated with high hCG plasma levels, especially during the second trimester.[11,12]

An American cohort study reported that the babies of women who had HG had a birth weight that was significantly lower and was more likely to have SGA.[13] A recent meta-analysis conducted on 13 case-control, 10 cohort, and one cross-sectional study on the outcomes of HG and pregnancy found that pregnant women who had undergone HG had a 30% increased risk of preterm birth (PTB) and a 40% increased risk of SGA.[14]

In the literature, limited studies could be found on placental functions and neonatal outcomes in HG patients. The placental dysfunctions and neonatal outcomes of patients hospitalized with HG were examined in the present study in order to make a regional contribution to the literature on this subject.

MATERIAL AND METHOD

This study was conducted retrospectively among women who had singleton deliveries between January 2015 and January 2020 after obtaining ethical committee approval at our university hospital. Pregnant women hospitalized due to HG and went on to deliver accepted as the case group. The control group consisted of pregnant women who delivered without hospitalization due to HG.

In both groups, the patient's medical history, aspects of pregnancy [LMP, obstetric history (delivery type, gravida, parity, stillbirth, miscarriage)], blood pressure, and proteinuria were studied. Through the hospitalization files at the time of delivery of the patients who were hospitalized due to HG and not hospitalized due to HG, neonatal outcomes including placental dysfunction (gestational hypertension, gestational diabetes (GDM), preeclampsia, stillbirth) and low birth weight (LBW), SGA, fetal growth restriction (FGR), PTB, neonatal intensive care unit (NICU) requirement, and 5-min Apgar scores were examined.

In pregnancy, the classification of hypertensive disorders is done according to the definitions of the International Society for the Study of Hypertension in Pregnancy (ISSHP).[15] The definition of gestational hypertension is having a systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg, occurring twice for a woman without hypertension before the 20th week of pregnancy. The definition of proteinuria in patients who have gestational hypertension is a dipstick reading of 2+ in randomly taken urine samples. A preeclampsia diagnosis is made if proteinuria is present in women with gestational hypertension[16] and eclampsia is defined if one or more convulsions are present[17] >140/90 mm

Fetal loss before the 20 gestational weeks and/or weighing up to 500 g is considered as a miscarriage. The definition of SGA is a birth weight at the gestational age that is less than the 10th percentile of the US National Reference for Fetal Growth[18] LBW is defined as a birth weight below 2500 g, FGR is defined as less than the 10th percentile, estimated fetal weight is determined for the gestational age, and an abdominal circumference is below the 10th percentile.[19-20] Apgar scores are measured at the 5th and 10th min post-birth.[21] The outcome reported for the Apgar score is the postpartum 5-min score. Births before 37 weeks of gestation are considered preterm births.[22] The gestational age at birth is calculated as the number of days from the first day of LMP until the date of admittance to the delivery or operating room. The American College of Obstetricians and Gynecologists defined postpartum hemorrhage as cumulative blood loss that is ≥1000 mL or bleeding, in addition to symptoms of hypovolemia, within the first 24 h after delivery, regardless of the method of delivery used.[23]

HG exposure assessment: Severe HG requiring hospitalization included women with weight loss >5%[24] compared to their weight pre-pregnancy. Women with severe hyperemesis were defined by a maternal caloric deficit, electrolyte disturbance, and ketonuria.

Inclusion criteria: Patients who were hospitalized between the 4th and 21st week of gestation due to HG and gave birth at the same hospital and those with singleton pregnancies were included.

Exclusion criteria: Multiple pregnancies, molar pregnancies, pregnant women with hyperthyroidism were not included in the study. Chronic hypertensive patients and those diagnosed with type 1 or 2 diabetes before pregnancy, as well as those who smoked cigarettes while pregnant, were excluded.

Statistical Analysis

All the data collected for statistical analysis were analyzed using IBM SPSS Statistics for Windows 23.0 (IBM Corp., Armonk, NY, USA). The descriptive characteristics of the relevant variables were calculated. The continuous and categorical variables were presented as the mean±standard deviation, median, or number (%). Evaluation of whether the data had normal distribution was conducted with the Kolmogorov-Smirnov test. A comparison of the normally distributed data was conducted with the student t-test, while the non-normally distributed data was conducted with the Mann-Whitney U test. The chi-square or the Fisher exact test was used to evaluate the categorical data. Statistical significance was accepted as $P < 0.05$.

RESULTS

The group that was hospitalized for HG and the group that was not hospitalized for HG consisted of 61 patients (a total of 122). The age, gravida, parity, number of abortions, and gestational week were similar in both groups. Mean gestational age of 37. 7±1.5 weeks was determined in the HG group and (37.8±1.4 weeks) in the control group. No significant differences were observed between the groups regarding preeclampsia, birth weight, GDM, and neonatal intensive care requirement. The mean week of gestation upon hospitalization for HG was 10.6±3.6.

While the median value of the 5-min Apgar scores was 8 (min 3, max 9) for the HG hospitalized deliveries, it was 7 (min 4, max 9) in the control group (P=0.004).

When the abnormal birth weights were compared in the groups with and without HG hospitalization, there was no difference in LGA and FGR infants; however, it was found that SGA babies were more common in the deliveries of patients hospitalized for HG (P=0.022) (**Table**). No significant differences were observed between the groups regarding the frequency of preterm delivery and postpartum hemorrhage. There was no difference in the vaginal and cesarean delivery rates.

Table. Outcomes of patients with hyperemesis

	HG (n=61)	Control (n=61)	P-value
Age, years	29.7±5.6	28.3±5.3	0.178
Gravida	3 (1, 8)	3(1, 6)	0.549
Parity	1 (0, 3)	1 (0, 5)	0.258
Abort	0 (0, 5)	0 (0, 5)	0.386
Gestational week	37.7±1.5	37.8±1.4	0.711
Preeclampsia	5 (8.2)	4 (6.6)	0.729
Birth weight, g	3201.1±523.7	3143.4±516.5	0.541
GDM	8 (13.1)	7 (11.5)	0.783
Neonatal intensive care	8 (13.1)	6 (9.8)	0.570
5-min Apgar	8 (3, 9)	7 (4, 9)	0.004
Abnormal birth weight			
SGA	8 (13.1)	2 (3.3)	0.022
LGA	4 (6.6)	0 (0.0)	
FGR	4 (6.6)	2 (3.3)	
Delivery complications			
No complication	42 (68.9)	49 (80.3)	0.120
Preterm birth	18 (29.5)	10 (16.4)	
Postpartum hemorrhage	1 (1.6)	0 (0.0)	
Delivery type			
Vaginal	17 (27.9)	23 (37.7)	0.247
Cesarean	44 (72.1)	38 (62.3)	

Data are presented as the mean±standard deviation, median (minimum, maximum), and number (%). SGA: Small For Gestational Age LGA: Large For Gestational Age, FGR: Fetal Growth Restriction, GDM: Gestational Diabetes, HG: Hyperemesis Gravidarum
Significant P-values are indicated in bold.

DISCUSSION

In this study, it was found that HG did not cause a significantly decreased birth weight but increased the birth weights of infants diagnosed with SGA. Contrary to expectations, when the patients with and without HG hospitalization were compared, the 5-min Apgar scores were also higher in the infants hospitalized for HG. No relation between hospitalization for HG and gestational hypertension, preeclampsia, and other forms of placental dysfunction was found. The main finding of the current research was that HG that required hospitalization increased the birth weights of SGA infants. Bolin et al. found that a higher risk of prematurity and preeclampsia is seen when hyperemesis occurs during the second trimester.[24,25] It was determined that women hospitalized for HG were more commonly observed to have given birth prior to 38 weeks of gestation and gave birth to a child with LBW. Among more than 500,000 live births, the aforementioned American cohort study found that HG was related to SGA and LBW.[13] In a study conducted among 1.2 million singleton births, it was stated that HG increased the risk of PTB by 18%; however, no association was found with the risk of SGA or LBW.[26,27] Studies researching the relationship between HG and PTB have thus far been conflicting. In some studies, HG has been reported as a risk factor for PTB,[28,29] while others have reported that there is no effect[30] or the diagnosis of HG may protect against PTB due to high progesterone levels.[31] In the present study, however, no correlation was found between PTB and HG.

In a Canadian study, women with HG in singleton pregnancies had a 3-fold increased risk of PTB and LBW with a pregnancy weight gain of >7 kg and a five-fold increase in the risk of a 5-min Apgar score <7.[32] In the present study, no increased risk of PTB and LBW was observed in deliveries by patients who were hospitalized for HG, while there was an increase in SGA infant births in deliveries by patients who were hospitalized for HG. While the 1-min Apgar score indicated a necessity for instant resuscitation, the 5-min Apgar score has high clinical importance for the neonatal.[33] Therefore, the 5-min Apgar scores were examined. In this study, contrary to expectations, the 5-min Apgar scores were significantly higher than the control group in deliveries by patients hospitalized for HG. This finding was considered unreliable since changes in the Apgar scores are multifactorial and depend on monitoring during labor.

Although benign nausea and vomiting early in pregnancy are associated closely with temporarily elevated hCG levels, a suggestion was put forth that persistently elevated hCG levels in women with hyperemesis disrupt the regular trophoblast migration stimulation and, as a result, alter placentation.[34,35] In conclusion, abnormal placentation can cause placental dysfunction, which manifests clinically as gestational hypertension or preeclampsia, as well as miscarriage, stillbirth, and FGR.[36,37] While the risk of preeclampsia was increased by 1.6 times in pregnant women with HG in early studies,[26] studies conducted later did not show an increased risk.[30,38,39] The results that were obtained herein were unable to prove that severe HG causes an increased risk of placental dysfunction. No significant differences were observed between the groups regarding birth complications, and no difference was observed between postpartum hemorrhage and the delivery types.

Limitations of this study: The study design was retrospective, it included a relatively small number of patients, and the treatments and duration of these treatments received by the patients were excluded from use in the study.

CONCLUSION

HG is a disease associated with hospitalization, drug use, and lower quality of life. However, the findings of the current research suggested that HG has no significant effect on placental dysfunction disorders. Of the neonatal outcomes, SGA was more common in the infants of mothers with HG. However, due to the many different results in the literature and the limitations of this study, there is a need for studies that also examine prospective, broad-based, and long-term results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission Necmettin Erbakan University Non-interventional Clinical Researches Ethics Committee (Date:18.12.2020, Decision No: 2020/29-46) .

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Does the Timing of Warfarin Ingestion Affect the Time in Therapeutic Range in Patients with Metallic Prosthetic Valve?

Varfarin Kullanım Zamanı Metalik Protez Kapaklı Hastalarda Terapötik Aralıktaki Zaman Yüzdesini Etkiler Mi?

Ömer Furkan Demir[1], Fatih Levent[1], Mustafa Ahmet Huyut[2], Özlem Arıcan Özlük

[1]University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital, Bursa ,Turkey
[2]Yeni Yuzyil University Gaziosmanpasa Hospital, Istanbul, Turkey

Abstract

Aim: Warfarin as the only approved oral agent to provide anticoagulation in patients with metallic prosthetic valves is a vitamin K antagonistt. Since effectively initiating and maintaining anticoagulation is challenging due to various factors, those patients undergo frequent periodic INR testing. We sought to investigate the effect of timing of warfarin intake on anticoagulation stability.

Material and Method: A total of 60 patients with metallic prosthetic valves were included in the study. Patients were informed to take warfarin between 7:30 and 08:00 P.M during the first month, and then to take warfarin between 09:30 and 10:00 A.M during the second month. All the patients were evaluated with INR monitoring once every 15 days during the follow-up period. The time in therapeutic range (TTR) values for the first month and second month (referred to as 'first TTR' and 'second TTR', respectively) were calculated separately using the Rosendaal method.

Results: The mean age (±SD) of the patients was 59.6±9.6 years and 36.7% (n=22) were male. There was no significant difference between the first TTR (in the month when warfarin ingested in the evening) and second TTR(in the month when warfarin ingested in the morning) values of our study group (66.23±40.7% vs 64.12±41.13%, p=0.783). The mean INR value in the first month was found to be significantly lower according to the value in the second month (2.73±0.53 vs 3.06±0.47, p=0.001).

Conclusion: The study results showed that the timing of warfarin ingestion did not affect the stability of anticoagulation despite the higher INR values achieved with a morning dose of warfarin,indicating evening warfarin ingestion is not a necessity.

Keywords: Warfarin, international normalized ratio, pharmacokinetic

Öz

Amaç: K vitamin antagonisti olan Varfarin metalik kalp kapaklı hastalarda onaylanmış oral antikoagulan tedavidir. Etkin antikoagulan tedaviyi sürdürmeyi zorlayan birçok faktör nedeniyle bu hastaların sık aralıklarla INR takibine alınması önerilmektedir. Bu çalışmanın amacı varfarin kullanma zamanının antikoagulasyona etkisi olup olmadığını araştırmaktır.

Gereç ve Yöntem: Metalik protez kapaklı toplam 60 hasta çalışmaya dahil edildi. Tüm hastalara ilk ayda varfarini saat 19:30 -20:00 arasında, ikinci ayda saat 09:30 ve 10:00 arasında alması söylendi .Hastalardan takip süresinde 15 günde bir INR ölçümü yapıldı. Birinci ve ikinci ay terapötik aralıktaki zaman yüzdesi (TTR) değerleri (sırasıyla birinci ve ikinci TTR) Rosendaal metoduyla hesaplandı.

Bulgular : Ortalama yaş 59.6±9.6 yıldı.%36,7'si (n:22) erkekti. Warfarinin gece alındığı ayda hesaplanan birinci TTR ile ve warfarinin gündüz alındığı ayda hesaplanan ikinci TTR değerleri arasında anlamlı fark bulunmadı (66.23±40.7% vs 64.12±41.13%, p=0.783). Birinci aydaki ortalama INR değeri ikinci aydakinden anlamlı olarak daha düşüktü (2.73±0.53 vs 3.06±0.47, p=0.001).

Sonuç: Çalışma sonuçları varfarin kullanma periyodunun etkin antikoagulasyon sağlanan toplam zamana bir etkisi olmadığını göstermiştir. Bunun birlikte sabah varfarin kullanımı daha yüksek INR değerlerine neden olmaktadır. Çalışmada akşam warfarin alımının bir zorunluluk olmadığına işaret edilmiştir.

Anahtar Kelimeler: Warfarin, uluslararası normalleştirilmiş oran, farmakokinetik

Corresponding *(İletişim)*: Fatih Levent M.D., University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,Bursa ,Turkey
E-mail *(E-posta)*: fatihlevent85@hotmail.com
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INTRODUCTION

Warfarin, a vitamin K antagonist, is the only approved oral agent to provide anticoagulation in patients with a metallic prosthetic valve. Warfarin significantly reduces the risk of thromboembolic events such as stroke and pulmonary embolism. The effective initiation and maintenance of anticoagulation can be challenging depending on the variability of serum warfarin concentrations that is susceptible to the person's metabolic status, diet, and additional drugs used.[1-5] Because of the metallic prosthetic valve-related problems ,e.g, lifelong treatment, absence of an alternative treatment such as non-vitamin K antagonist oral anticoagulants (NOAC), lifelong risk of fatal prosthetic valve thrombosis and thromboembolism, effective anticoagulan therapy becomes mainstay in this population.[6]

The safety and efficacy of warfarin is assessed by measuring the international normalized ratio (INR). Desired therapeutic range of INR is determined by prosthetic valve type. In current guidelines, the therapeutic range is between 2-3 for Aortic Valve Replacement (AVR) and between 2.5-3.5 for Mitral Valve Replacement (MVR).[7] The time in therapeutic range (TTR) is a parameter which defines what percentage of total follow-up time is within the therapeutic range. A TTR value of ≥70% indicates both effective and safe anticoagulation.[8-10]

The aim of this study was to investigate whether or not the time period (morning vs evening) to ingest warfarin affects TTR and INR values in patients with a metallic prosthetic valve.

MATERIAL AND METHOD

Ethics Committee Approval

The study was approved by a local clinical research ethics committee with the decision number 2011-KAEK-25 2020/02-08 dated February 5, 2020.The study was initiated just after obtaining approval from the ethics committee.

Study Design

The study was prospective in design and conducted in a single center. Patients with metallic prosthetic valves who visited the outpatients clinics for INR testing between March 1, 2020 and May 31, 2020 were identified. The study inclusion criteria were defined as age >18 years, and having undergone INR testing at least once a month during the last six months. Patients were excluded from the study if warfarin had been discontinued due to any indication during the last six months. After implementing the criteria, 70 patients were eligible for the study. Informed consent was obtained from all those patients.

As stated above, the therapeutic ranges of INR were determined as between 2 and 3 for Aortic Valve Replacement (AVR) and between 2.5-3.5 for Mitral Valve Replacement (MVR). The TTR values were calculated using the Rosendaal Method.The Rosendaal method is a computer based linear interpolation method which assumes there is a linear decrease or increase between two measured INR values. It determines an INR value for each day between those two INRs and calculates TTR using all measured and determined INRs in the follow up period.[11] Baseline TTR was calculated from the last two INR values for each patient. INR testing was performed every 15 days throughout the 2-month follow-up period. There was no patients who had a INR test before 15 days. The INR levels were measured from blood samples taken at 09:00 A.M. The patients were instructed to take warfarin between 7:30 P.M and 08:00 P.M in the first month of the study, then between 09:30 A.M and 10:00 A.M for the second month. The patients were assessed for any symptoms and newly used drugs at each INR testing. Finally, the TTR values for the first month and second month (referred to as 'first TTR' and 'second TTR') were calculated separately.

A further 10 patients were excluded from the study; 4 because the calculated TTR values were <40%, 1 because of gastrointestinal bleeding in the first week, and 5 because of a significant change in weekly tablet use. Therefore, the study was completed with 60 patients.

The INR Measurement Procedure

Blood samples were withdrawn from any vein in the forearm into 2 ml sodium citrate tubes, then the samples were centrifuged at 5000 rpm for 10 minutes. INR analysis was performed using a Sysmex CS-5100 model device with Dade Actin FS Activated PTT reagent and thromborel reagent.

Data obtained in the study were analyzed statistically using SPSS version 22.0 software (IBM-SPSS Inc., Armonk, NY, USA). The Shapiro Wilk test was used to evaluate whether the variables conformed to normal distribution. The descriptive statistics used to report demographic data were expressed as mean±standard deviation values for normally distributed continuous variables and as number and percentage for categorical variables. To conduct the comparisons between two groups based on gender (male /female), the independent sample t-test was used for continuous variables and the chi squared test was used for categorical variables. The Paired Samples t-test was used to compare the calculated variables (TTR, INR and number of tablets per week). A value of $p<0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Our group comprised 38 (63.3%) females and 22 (36.7%) males with a mean age of 59.6±9.6 years. The study population consisted of 15 (25%) patients with AVR, 35 (58.3%) with MVR, and 10 (16.7%) with AVR+MVR. In the echocardiographic examinations of the patients, left ventricle ejection fraction (LVEF) of <50% was determined in 16 (26.7%) patients. Atrial fibrillation was observed in 13 (21.7%) patients. It was stated by 55 (91.6%) patients that warfarin use was in the evening hours. The demographic and clinical characteristics of the patients, and medications used are shown in **Table 1**.

Table 1: Demographic and Clinic Characteristics of the Patients

Study population (n=60)	
Age, Years (mean±SD)	59.62±9.67
Sex, n (%)	
Male	22 (36.7%)
Female	38 (63.3%)
Diabetes, n (%)	6 (10%)
Hypertension, n (%)	15 (25%)
Smokers, n (%)	6 (10%)
Coronary artery disease, n (%)	8 (13.3%)
LVEF (%)	
>%50	44 (73.3%)
%50-%40	15 (25%)
<%40	1 (1.7%)
Atrial fibrillation, n (%)	13 (21.7%)
BMI (kg/m²) (mean±SD)	
Male	25±2.2
Female	26.6±3.9
Medications, n (%)	
Aspirin	14 (23.3%)
NSAI	8 (13.3%)
Antibiotic	3 (5%)
Basal TTR value, % (mean±SD)	69.98±24
Weekly warfarin tablet (5mg) use (mean±SD)	6.38±2.45
Duration of warfarin , year (mean±SD)	9.9±6.6

BMI: Body mass index, LVEF: Left ventricular ejection fraction, NSAI: Nonsteroidal anti-inflammatory, TTR: Time in therapeutic range

The mean number of warfarin tablets (5mg) used in a week was determined as 6.38±2.45, and the mean duration of warfarin use was 9.9±3.6 years (Table 1).

The mean age of the female patients was significantly lower than that of male patients (56.8±8.7 years vs 64.4±9.5 years, p=0.004). The female patients had lower mean systolic blood pressure values than the males (118.29±11.5 vs 128.18±13, p=0.005). The mean second TTR was higher in the male patients than in the female patients (82.68+23.0 % vs 56.71+45.5 % p:0.005). The comparisons of the characteristics based on gender are shown in **Table 2**.

The mean baseline TTR value was calculated as 69.98±24.4%. The mean baseline INR value (the mean of the last two INR values) was 2.77±0.7. There was no significant difference between the mean TTR value in the first month (first TTR) and the mean TTR value in the second month (second TTR) (64.12%±31% vs 66.23%±30%, p: 0.783). The mean INR value in the first month was determined to be significantly lower than the mean INR value in the second month(2.73±0.53 vs 3.06±0.4, p:<0.001). The number of tablets taken weekly was seen to be similar in both months (6.39±2.3 vs 6.43±2.46, p: 0.070) (**Table 3**).

Table 2. Comparisons of the Demographic and Clinic Characteristics based on gender

	Female (n=38)	Male (n=22)	p value
Age, year mean±SD	56.8±8.7	64.4±9.5	0.004
SBP, mmhg mean±SD	118.29±11.5	128.18 ±13	0.005
DBP, mmHg mean±SD	75.53 ±8.1	79.55±8.5	0.082
Heart rate bpm , mean±SD	77.7±6.9	74.3±8.5	0.007
Duration of warfarin, years (mean±SD)	9.53±6.7	7.77±6.3	0.321
HT, n (%)	9 (23.7%)	6 (27.3%)	0.757
DM, n (%)	4 (10.5%)	2 (9.1%)	0.858
CAD, n (%)	5 (13.2%)	3 (13.6%)	0.958
AF, n (%)	6 (15.8%)	7 (31.8%)	0.146
Smoking, n (%)	2 (5.3%)	4 (18.2%)	0.108
BMI kg/m2 ,mean±SD	26.6±3.9	25.0±2.2	0.056
Weekly warfarin tablet (5mg) use (mean±SD)	6.39±2.3	6.3±2.24	0.909
Baseline TTR , % mean±SD	71.6+21.1	67.9+29.5	0.529
First TTR, % mean±SD	63.37+41.8	65.41+41.2	0.855
Second TTR % mean±SD	56.71+45.5	82.68+23.0	0.005
Baseline INR mean±SD	2.78+0.74	2.75+0.86	0.884
INR in the first month mean±SD	2.73+0.5	2.74+0.5	0.942
MVR N (%)	12 (20%)	23 (38.3%)	0.215
AVR N(%)	8 (13.3%)	7 (11.7%)	0.215
MVR+ AVR N(%)	2 (3.3%)	8 (13.3%)	0.215

AF:Atrial fibrillation, AVR: Aort valve replacement ,BMI: Body mass index , CAD:Coronary heart disease ,DBP: Diastolic blood pressure , DM: Diabetes mellitus, HT:Hypertension,MVR: Mitral valve replacement SBP: Systolic blood pressure ,TTR : Time in therapeutic range

DISCUSSION

The aim of this study was to investigate the effect of timing of warfarin intake on anticoagulation stability based on TTR and INR values in patients with a metallic prosthetic valve. The mean INR values of the patients were found to be significantly higher when warfarin ingestion occured in the morning. Although there was no difference in the calculated TTR values between morning and evening warfarin ingestion, it was observed that male patients had better TTR values with morning warfarin use according to the female patients.

Since most of the patients were already taking warfarin in the evening, it was not suprising that there was no significant difference between the baseline TTR and the first TTR.

The mean baseline TTR (69.98%±24.4) in this study was higher than the mean TTR reported in the study of Yee Tan et al.[12] (57.11%) and that in the study of Boonyawat et al.[13] (54.6%). This difference could be attributed to the use of more INR values to calculate TTR than in those previous studies.

Table 3: Comparison of INR and TTR values in morning and evening ingestion

	Basal measurement	Evening ingestion	Morning ingestion	Basal measurement vs Evening ingestion (p value)	Evening ingestion vs Morning ingestion (p value)
INR, mean±SD	2.77±0.7	2.73±0.53	3.06±0.4	0.709	<0.001
TTR, % (mean±SD)	69.98±24.4	64.12±41	66.23±40	0.312	0.783
Number of tablets per week, mean±SD	6.40±2.3	6.39±2.3	6.43±2.46	0.990	0.070

TTR: Time in therapeutic range, SD: Standard deviation

The current study consisted of a younger population compared to the Influence of Duration of Warfarin Administration on Anticoagulation Stability (INRange) study designed by Garrison et al.[14] in which the mean age was 73 years. In addition, the rate of left ventricle ejection fraction (LVEF) of ≥50% was found to be higher in the current study. The fact that younger people and patients with higher LVEF values constitute the current study population, unlike other studies, may be due to rheumatic valve disease, which is one of the most common indications for prosthetic valve surgery in Turkey.[15] Acute rheumatic fever (ARF), which affects children and adolescents, remains the leading cause of rheumatic valve diseases in Turkey.[16] Since the patient population in this study was younger than the patients included in other prosthetic valve studies, the rates of hypertension, diabetes and coronary artery disease were found to be quite low.

Although patients are generally advised to take warfarin in the evening, it is not clear whether the timing of warfarin ingestion changes the level of anticoagulation. In an extensive literature search (PubMed search for warfarin [MESH] and filtering for clinical trials), none of the 1,642 articles examined the effect of warfarin administration time on TTR. In the INRange study, current warfarin users were randomly assigned to use warfarin in the evening or morning. When compared to the baseline values, the changes in the TTR values were not statistically significant in both groups defined according to warfarin timing (morning or evening). Different from our study, they included patients using warfarin for any indication. The indications for warfarin use are primarily AF, followed by deep vein thrombosis and pulmonary embolism. Since warfarin interacts with the patient's general condition and any comorbidities, such a heterogenity in the study population might have affected the results. The therapeutic INR range was determined as 2 – 3 for most of the patients in that study, which may explain the higher mean TTR value (72.2%) observed.[13] The design of the current study differed from the INR range study as both morning and evening warfarin administration was applied to a single population in order to minimize the effect of the confounding variables. Moreover, the study was completed within the 3-month period of March-April-May to minimize the effect of seasonal changes on diet and metabolism. When the demographic characteristics of the study populations were examined, no significant difference was found between the two studies in the amount of daily warfarin use and the baseline TTR values were observed to be similar in both studies.

Mean second TTR (in the month when warfarin ingested in the morning) was higher in males than the females in our study. Findings regarding the effect of gender was similar to the results of the study of Averello et al.[17] However,it should be emphasized that their study was retrospective in design including the patients taking vitamin K antagonists (VKA) - either acenocoumarol or warfarin-regardless of VKA ingesting time.

The pharmacokinetic/pharmacodynamic characteristics of warfarin such as having a very long half life (approximately 35 hours) and having very small fluctuation in plasma concentration after ingestion may explain why the change in the warfarin ingestion time did not affect TTR.[18]

There were some limitations to this study, primarily the relatively low number of participants. A second limitation was that many confounding factors such as diet were not evaluated. 2 month period may be a bit short to optimize TTR. Finally, since the Rosendaal method of TTR calculation is based on linear change between consecutive INR checks, more than two INR visits might be required to be able to obtain more reliable TTR values.

CONCLUSION

The results of this study demonstrated that INR values were significantly higher when warfarin ingestion occurred in the morning in patients with a metallic prosthetic valve. However , no significant difference was observed in TTR after a change in warfarin ingestion time. The study has highlighted that evening warfarin ingestion is not a necessity and morning warfarin ingestion may be offered to patients with INR levels below the target range. Future research is needed to confirm those results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital Ethics Committee (Date: 05.02.2020, Decision No: 2011-KAEK-25 2020/02-08) .

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Characteristics, Clinical Findings and Outcomes of 333 Pregnant Women with COVID-19 During Four Waves of Infection at a Tertiary Hospital in Turkey

Türkiye'deki Bir 3. Basamak Hastanede, Dört Enfeksiyon Dalgası Sırasında COVID-19 Olan 333 Gebe Kadının Özellikleri, Klinik Bulguları ve Sonuçları

Elif Ünlügedik Sayın, Medine Kahraman Kaya, Elif Cansu Gündoğdu, Ahmet Kale

Kartal Dr. Lütfi Kirdar City Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey

Abstract

Aim: Since the first case of SARS-CoV-2 in Turkey, it was confirmed in over 14 million people causing almost 95,000 of deaths. During the two years course of pandemic SARS-CoV-2 caused 4 waves of disease in Turkey. To enhance our knowledge on initial presentation, clinical course and severity, risk factors, and pregnancy outcomes of COVID-19 infection during the four different waves of pandemic.

Material and Method: Clinical records of 333 pregnant women with a verified positive PCR test was reviewed. The distribution of the patients during the two-year course of the pandemic was studied. Descriptive data regarding maternal age, gestational age, body mass index (BMI), education, employment status, ABO blood type, previous obstetric history, previous medical history, smoking status were collected. Maternal and immediate perinatal outcomes were examined. The primary endpoint of the study was comparison of four waves during the pandemic in terms of admission to ICU (Intensive Care Unit), use of mechanical ventilation or maternal and neonatal death.

Results: The distribution of number of the patients followed the same pattern as the general population in Turkey, except first wave, which did not seem to affect pregnant women. Most of the patients and all the maternal deaths were accumulated in the second and fourth waves. Those with more severe disease were older, at an earlier gestational age, and had a higher BMI.

Conclusion: The severity of the COVID-19 disease was strongly associated with the maternal age and gestational age. The worst maternal outcomes of the disease were detected during the second and fourth waves in Turkey.

Keywords: COVID-19, viral infection, pregnancy, risk factors, maternal outcomes

Öz

Amaç: Türkiye'de ilk SARS-CoV-2 vakası görülmesinden bu yana, 14 milyondan fazla insanda enfeksiyonun varlığı doğrulandı ve yaklaşık 95.000 ölüme neden oldu. SARS-CoV-2 pandemisi, iki yıllık seyri boyunca Türkiye'de 4 hastalık dalgasına neden oldu. Amacımız, dört farklı pandemi dalgası sırasında COVID-19 enfeksiyonunun ilk sunumu, klinik seyri ve şiddeti, risk faktörleri ve gebelik sonuçları hakkındaki bilgilerimizi geliştirmektir.

Gereç ve Yöntem: Doğrulanmış pozitif PCR testi olan 333 hamile kadının klinik kayıtları incelendi. Pandeminin iki yıllık seyri boyunca hastaların dağılımı incelendi. Anne yaşı, gebelik haftası, vücut kitle indeksi (VKİ), eğitim, çalışma durumu, ABO kan grubu, önceki obstetrik öyküsü, önceki tıbbi öyküsü, sigara içme durumu ile ilgili tanımlayıcı veriler toplandı. Maternal ve acil perinatal sonuçlar incelendi. Çalışmanın temel amacı, pandemi sırasındaki dört dalganın Yoğun Bakım Ünitesi'ne (YBÜ) kabul, mekanik ventilasyon kullanımı veya anne ve yenidoğan ölümü açısından karşılaştırılmasıydı.

Bulgular: Hasta sayısının dağılımı, hamileleri etkilemediği görülen birinci dalga dışında, Türkiye'deki genel popülasyonla aynı modeli izlemiştir. Hastaların çoğu ve anne ölümlerinin tamamı ikinci ve dördüncü dalgalarda toplanmıştır. Daha şiddetli hastalığı olanlar daha ileri yaşta kadınlar, gebeliğin daha erken aylarında ve daha yüksek vücut kitle indeksine sahipti.

Tartışma: COVID-19 hastalığının şiddeti, anne yaşı ve gebelik yaşı ile güçlü bir şekilde ilişkiliydi. Türkiye'de en kötü maternal sonuçlar, hastalığın ikinci ve dördüncü dalgalarında görüldü.

Anahtar Kelimeler: COVID-19, viral enfeksiyon, gebelik, risk faktörleri, maternal sonuçlar

Corresponding *(İletişim)*: Elif Ünlügedik Sayın, MD, PhD. Kartal Dr Lütfi Kirdar City Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey
E-mail *(E-posta)*: elif.unlugedik@gmail.com
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INTRODUCTION

The novel COVID-19 disease caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was first defined in Wuhan, China in December 2019. Since then, it triggered a tremendous global health problem. According to World Health Organization, globally over 400 million people were infected, and more than 6 million deaths were reported.[1] In Turkey, the first case of COVID-19 was identified on March 11, 2020, and since then the virus was confirmed in over 14 million people causing almost 95,000 deaths.[2] While the virus had spread dramatically around the world clinical management had also aroused a serious challenge because there was limited information about the disease and even fewer data concerning the management of obstetrical patients.

Prevalence calculation of the disease among the pregnant raised a challenge as well. The spectrum of the disease varied from asymptomatic infection, mild upper respiratory syndrome to pneumonia, severe respiratory distress, and death.[1] One study reported that asymptomatic infection rate among obstetrical patients was 15-fold higher than that of surgical patients.[3] Recently, it had been concluded that the prevalence of SARS-CoV-2 infection among pregnant women range from 2-20%[4] that is yet to be confirmed.

Current data suggest that pregnant women are not necessarily more susceptible to SARS-CoV-2 infection than the ones that are not pregnant. However, respiratory failure might increase among the pregnant patients with the existence of comorbidities such as chronic lung disease, cardiovascular disease, hypertension, immunocompromised patients, body mass index above 40 kg/m², pregestational diabetes, chronic kidney, or liver disease.[5]

In Turkey, the first case of COVID-19 infection was identified on March 11, 2020, and afterwards the disease followed a pattern characterized with a series of infection waves.[1] The first wave of the disease was observed in April 2020. With strict infection prevention policies, the number of cases remained the lowest until August 2020. In August the number of cases started to accelerate with a spike in December 2020. A surge of new cases resulted with the third wave in April 2021. The arrival of vaccines helped to decrease new infection levels through the summer of 2021. Another surge, the fourth wave, started by the end of August 2021 and had two peaks. The first peak was in September 2021, due to contagious delta variant and the second peak occurred in February 2022 attributed to the circulation of Omicron variant. In March 2022, due to decreased number of hospitalization and death, the Turkish Ministry of Health decided to cease many of the restrictions.[1]

To our knowledge, no studies have investigated the characteristics and outcomes of pregnancies during the various stages of the COVID-19 infection. In this study, we identified and reviewed the medical records of 333 pregnant women in a single tertiary center with laboratory-confirmed SARS-CoV-2 infection to enhance our understanding on the initial presentation, clinical course and severity, risk factors, and pregnancy outcomes. In addition, we aimed to compare the characteristics of the disease during the four different waves of pandemic. We hypothesized that medical background, socioeconomic level, and gestational age would have a certain impact on the maternal and neonatal outcomes.

MATERIAL AND METHOD

This study was an observational cohort of pregnant women of all gestational ages with SARS-CoV-2 infection who were admitted at a single tertiary center **KKKKKKKKKKKKKKK** Hospital in Istanbul, Turkey. The study was primarily reviewed and approved by the Ministry of Health and then by the Medical Ethics Committee of **KKKKKKKKKKKKKK** Hospital (Decision number: 2022/514/222/43. Date: 30.03.2022).

From the beginning of the pandemic, universal testing with quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay of maternal naso-pharyngeal swab specimens was required for all the hospital admissions. Pregnant patients with a positive PCR test were isolated in the COVID-19 section within the Obstetrics and Gynecology Department. During the study period, all the patients admitted to delivery were tested for SARS-CoV-2 regardless of symptoms or known exposures. We have screened the data between March 11, 2020, and March 3, 2022, which were the dates when the first case was confirmed in Turkey and many of the restrictions were ceased due to decrease in number of new cases, respectively.

Clinical records of pregnant women with a verified PCR test were reviewed. When the electronic medical records were not satisfying, researchers obtained additional data from paper files of each patient. Clinical data including epidemiological history, week of pregnancy, comorbidities, clinical and laboratory findings, treatment measures, maternal and immediate fetal outcomes were extracted. Two study investigators (EUS and MK) independently reviewed the data collection forms to verify data accuracy. All the disagreements between them were resolved by consultation with a third investigator (AK).

Distribution of pregnant patients and severity of the disease were demonstrated during the four waves of pandemic and for each spike of the disease, respectively. Descriptive data regarding maternal age, body mass index (BMI), education, employment status, ABO blood type, previous obstetric history, previous medical history, smoking status were calculated. BMI was categorized as healthy (BMI less than 25), overweight (BMI=25-30) and obese (BMI greater than 30). Education and employment status were also enumerated. Initial laboratory findings which were previously studied as possible signs of prognosis including complete blood count, neutrophil, platelet, and lymphocytes counts, and their ratio (neutrophil/lymphocytes or platelet/lymphocytes), D-dimer levels, c-reactive protein levels were collected.

Gestational age of the patients was calculated based on self-reported last day of menstruation and confirmed with previous ultrasound imaging when possible. The gestational age at the time of positive SARS-CoV-2 test was presented in the study. Patients were classified as first trimester (0-13 weeks), second

trimester (14-26 weeks) and third trimester (>27 weeks). The third trimester were further categorized into three groups: preterm (27-36 weeks), term (37-40 weeks) and post term (>40 weeks).

Based on self-reported symptoms and clinical status patients were classified as asymptomatic, mild, moderate/severe, and critical defining severity of the disease. Guidelines specified by the National Institutes of Health (NIH) was modified for this purpose.[6] Patients with non-COVID-19 relevant symptoms or no symptoms were classified as the 'asymptomatic' group. Patients mainly with upper respiratory tract symptoms formed the 'mild' group. Self-reported dyspnea without tachypnea, SpO_2 above 95%, and normal findings on chest scanning were also included in the mild group. 'Moderate-Severe' cases were defined as dyspnea with requirement of oxygen therapy, oxygen saturation below 95% and COVID-19 related findings on chest scanning (X-Ray or computerized tomography). Finally, patients admitted to critical care formed the 'critical' disease group (**Table 1**).

Table 1. SARS-CoV-2 classification based on severity of clinical presentation. Patients were divided into four groups concerning clinical course of the disease. Modified from National Institutes of Health guidance.

Asymptomatic (n=97, 29%)	Mild (n=123, 37%)	Moderate-Severe (n=98, 29%)	Critical (n=15, 4,5%)
	• Fever, cough, sore throat, fatigue, muscle pain, chills, back pain, nausea, vomiting, joint pain, nasal stuffiness, loss of smell or taste, gastrointestinal symptoms • Self-reported dyspnea with normal chest scanning and SpO_2 above 95%	• Dyspnea with requirement of oxygen therapy • Oxygen saturation below 95% • COVID-19 related findings on chest scanning	• Admission to critical care

Obstetric outcomes included maternal death, preterm birth, primary cesarean birth, placenta previa, secondary infections, chorioamnionitis, detachment of placenta, hypertensive disorders of pregnancy, existence of meconium in the amniotic fluid, venous thromboembolism, and postpartum hemorrhage. Primary cesarean birth was defined as no prior cesarean birth that included patients without prior pregnancy ahead of 20th week of gestation or history of only vaginal births. Secondary infections and chorioamnionitis were defined as infections confirmed with positive culture tests of blood, urine, and amniotic fluids, respectively. Hypertensive disorders of pregnancy were defined as preeclampsia, gestational hypertension, and chronic hypertension with superimposed preeclampsia. Venous thromboembolism was defined as deep venous thrombosis. Postpartum hemorrhages were defined as cumulative blood loss greater than 1000 mL or signs and symptoms of hypovolemia within 24 hours of birth.[7] All the pregnant patients with a positive PCR test received therapeutic doses of anti-coagulant therapy. Additional therapies included antibiotics, steroids, anti-viral therapy, and anti-fungal therapy.

Summary statistics were calculated for baseline and clinical characteristics. The mean and standard deviation (SD) for maternal age; median, first and third quartiles for BMI were calculated. Missing BMI values were not imputed. All the other variables were presented as median and percentages. Cross-tabulation was used to quantitatively analyze the relationship between severity of the disease and the baseline and clinical characteristics of the patients. Previous cesarean birth, previous preterm birth and previous hypertensive disorder of pregnancy were also included among the baseline and clinical variables.

Neonatal outcomes included miscarriage, perinatal death, preterm birth before the 37th week of gestation, birth weight, Apgar scores of 1- and 5-minute. Since the newborns were followed through the delivery hospitalization only immediate outcomes were presented in the study. Birth weight, 1-minute and 5-minute Apgar scores were presented as median, first and third quartile, miscarriage and perinatal death were expressed as number of cases and percentages.

For ordinal and nominal variables, the chi-square test and for continuous variables Kruskal-Wallis test was used to assess trends in baseline and clinical characteristics of the groups based on severity of the disease. All analyses were performed with IBM SPSS, version 20.0 and Microsoft Excel.

RESULTS

Throughout the four waves of pandemic, between March 11, 2020, and March 3, 2022, pregnant patients of all gestational ages with a positive SARS-CoV-2 test admitted to the hospital were included in the analysis. The distribution of the disease followed the general pattern in Turkey except the first wave which did not seem to affect the pregnant patients (**Figure 1**). Of 333 patients with a positive test, 97 (29%) were asymptomatic, 123 (37%) had mild illness, 98 (29%) had moderate-severe illness, and 15 (5%) had critical illness. 5 maternal deaths (1,5%) were attributed to COVID-19 infection. Distribution of the disease in terms of severity during the four waves showed that the majority of the critical group was hospitalized during the second and fourth waves (**Figure 2**) which is consistent with the distribution of the disease in the general population. Of 5 maternal deaths, one occurred during the second and the rest occurred during the fourth wave of the disease. All the maternal deaths were attributed to COVID-19 infection.

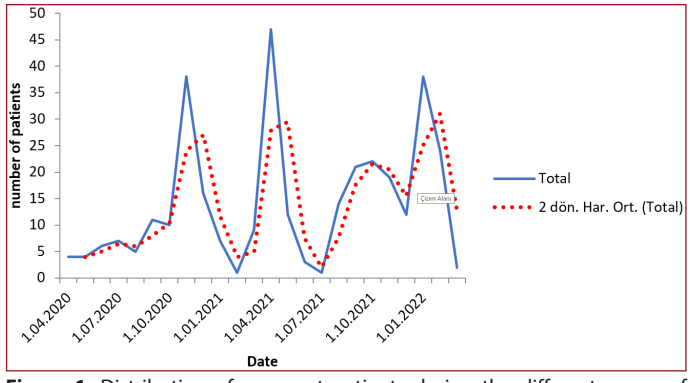

Figure 1. Distribution of pregnant patients during the different waves of infection. Continuous line describes the monthly data of COVID-19 disease among the pregnants. Dashed line shows the trend of distribution. (2 per. Mov.Avg: 2 period moving average)

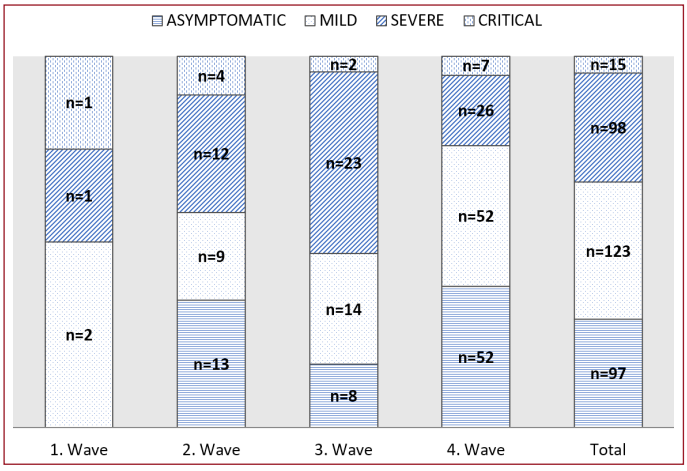

Figure 2. Grouping of the pregnants according to disease severity, during four waves of disease.

The median (interquartile range) gestational age at the time of admission to the hospital with a positive SARS-CoV-2 test result was 36 weeks of gestation (30–38). Statistical tests showed a significant difference between the groups in terms of severity according to gestational age (p<0.001) (**Table 2**). During the first trimester none of the patients had critical disease. Of 15 patients in the critical group, 8 patients were at the preterm period and 6 patients at the second trimester. There was only one patient at the term group transferred to the ICU. The majority of the severe group consisted of second trimester and preterm patients as well. Of 98 patients in the moderate-severe group, 67% of them were either at the second trimester (n=20) or preterm patients (n=46).

Table 2. Baseline Demographic and Clinical Characteristics

Characteristic	Disease Severity				p-value	Total (n=333)
	Critical (n=15)	Moderate-Severe (n=98)	Mild (n=123)	Asymptomatic (n=97)		
Gestational age					< 0,001	
1. Trimester	0	4 (4%)	7 (7 %)	3 (3%)		
2. Trimester	6 (40%)	20 (20%)	13 (10%)	2 (2%)		
Preterm	8 (53%)	46 (47%)	56 (45%)	10 (10%)		
Term	1 (7%)	25 (26%)	46 (37%)	71 (73%)		
Postterm	0	3 (3%)	1 (1%)	11 (12%)		
Age (y)	32±3,1	31±5,9	29,9±5,9	28,4±5,4	0,006	29,9±5,74
BMI (kg/m²)	28 (25,9 - 30,3)	29,8 (25,9 - 34,7)	27,8 (24,9 - 31,1)	28,9 (26,4 - 31,6)	0,02	28,70 (25,75-31,80)
Healty (BMI<25)	2 (13%)	14 (14%)	26 (21%)	10 (10%)		52
Overweight (30<BMI<25)	7 (47%)	33 (33,7%)	45 (36,5%)	35 (36%)		120
Obesity (BMI>30)	3 (20%)	44 (45%)	32 (26%)	30 (31%)		109
Previous cesarean birth	7 (47%)	34 (35%)	43 (35%)	35 (36%)	0,468	119
Previous preterm birth	0	1 (1%)	3 (2%)	1 (1%)		5
Previous hypertensive disorder of pregnancy	0	1 (1%)	4 (3%)	2 (2%)		7
Education					0,006	
İlliterate	0	8 (8%)	6 (5%)	13 (13%)		27
High School	3 (20%)	60 (61%)	77 (63%)	61 (63%)		201
University	7 (47%)	24 (24%)	23 (19%)	10 (10%)		64
Employment Status	6 (40%)	21 (21%)	24 (20%)	8 (8%)	< 0,001	61
Smoking habit	0	3 (3%)	6 (5%)	6 (6%)	0,471	15
Blood type					0,139	
0	6 (40%)	30 (31%)	37 (30%)	41 (42%)		114
A	8 (53%)	40 (41%)	52 (42%)	36 (37%)		136
B	1 (7%)	15 (15%)	27 (22%)	15 (15%)		58
AB	0	13 (13%)	7 (7%)	5 (5%)		25
Rh-positive	14 (93%)	84 (86%)	108 (88%)	82 (85%)	0,765	288
Asthma or chronic obstructive pulmonary disease	2 (13%)	7 (7%)	11 (9%)	2 (2%)	0,14	22
Chronic cardiovascular disease	0	3 (3%)	11 (9%)	3 (3%)	0,102	17
Thyroid disease	3 (20%)	13 (13%)	18 (15%)	17 (18%)	0,806	51
Pregestational diabetes	2 (13%)	8 (8%)	11 (9%)	8 (8%)	0,924	29
Chronic hematological	0	2 (2%)	2 (2%)	2 (2%)	0,947	6
Chronic neurological	0	0	3 (2%)	2 (2%)	0,447	5
Chronic other diseases	0	2 (2%)	5 (4%)	0	0,194	7

BMI= body mass index. Data are mean±SD, median (interquartile range), or n (%) unless otherwise specified. Number of missing values: BMI (n=52), Education (n=41), Employment status (n=36), Smoking habit (n=26)

The distribution of the blood type among the patients showed the same pattern with the general population of Turkey 8. Interestingly, we did not find any relation between the severity of the disease and medical comorbidities including asthma or chronic obstructive pulmonary disease, chronic cardiovascular disease, thyroid disease, pregestational diabetes, hematological, neurological or other chronic diseases (**Table 2**). Within the groups there was a difference in terms of education and employment status. However, regression analysis did not allow us to draw a conclusion about a relation between education or employment status and severity of the disease.

The most common patient-reported symptoms were cough (26%), dyspnea (23%), fever (11%), fatigue (%), and myalgia or body aches (6%) (**Table 3**). 46 patients had COVID-19 related findings on chest imaging (chest X-ray or computed tomography scan) that were in either critical or severe-moderate group.

Vital sign and laboratory findings by COVID-19 severity are described in **Table 4**. On admission, critical group had a higher heart rate, higher body temperature, lower hematocrit levels, lower white blood cells count, higher rates of neutrophil/lymphocyte and platelet/lymphocyte, and higher C-reactive protein (CRP) levels.

34 of the patients refused to use any medication other than anticoagulants and anti-inflammatories. Therapeutic doses of anticoagulant therapy were used for 306 patients which consists of 92% of the patients. Antibiotics, steroids and immunomodulators were used for 17% (n=56), 16% (n=54) and 1% (n=3) of the patients, respectively. Only 2 of the patients admitted to the ICU received anti-fungal therapy.

Table 3. Coronavirus Disease 2019 (COVID-19) Disease Severity Classification for Study Cohort

Data Through Delivery Hospitalization	Critical (n=15)	Moderate-Severe (n=98)	Mild (n=123)	Asymptomatic (n=97)	Total (n=333)
Death due to COVID-19	5 (33%)				5 (1.5%)
SpO_2 less than 94%	11 (73%)	53 (54%)			64 (19%)
Abnormal chest imaging results	10 (66%)	36 (37%)			46 (13.8%)
Documentation of self-reported dyspnea	9 (60%)	39 (40%)	28 (23%)		76 (22.8%)
Any other self-reported symptoms (excluding dyspnea)					
Cough	6 (40%)	38 (39%)	43 (35%)		87 (26%)
Myalgia or body aches	1 (7%)	8 (8%)	10 (8%)		19 (5.7%)
Fever	4 (27%)	15 (15%)	20 (16%)		39 (11.7%)
Nasal stuffiness or rhinorrhea	0	2 (2%)	2 (1.6%)		4 (1.2%)
Headache	0	5 (5%)	4 (3.2%)		9 (2.7%)
Anosmia or loss of smell/Ageusia or loss of taste	0	1 (1%)	0		1 (0.3%)
Fatigue	2 (1%)	8 (8%)	13(10%)		23 (69%)
Sore throat	0	2 (2%)	3 (2.4%)		5 (1.5%)
Nausea or vomiting	2 (1%)	4 (4%)	3 (2.4%)		9 (2.7%)
Back or joint pain	0	0	3 (2.4%)		3 (1%)
Diarrhea	0	1 (1%)	0		1 (0.3%)
Other symptoms	3 (20%)	22 (22%)	64 (52%)		89 (26.7%)
No symptoms				97 (100%)	97 (29%)

Data was presented as number and percentage within the group.

Table 4. Vital Signs and Laboratory Values by COVID-19 Severity Classification

	Disease Severity				p-value
	Critical (n=15)	Moderate-Severe (n=98)	Mild (n=123)	Asymptomatic (n=97)	
Highest heart rate (beats/minute)	88 (80 -94)	86 (80-97)	84 (80-88)	80 (80-86)	0,016
Lowest SpO_2 (%)	88 (79-91)	93 (90-95)	96 (95-97)	97 (96-98)	< 0,001
Highest temperature (Celsius)	37,1 (36,7-37,5)	36,9 (36,7-39,5)	36,8 (36,6-37,2)	36,8 (36,6-36,9)	0,005
Lowest platelet count ($\times 10^3$/mm3)	194 (173-249)	192 (164-234)	203 (168-241)	214 (169-257)	0,28
Diastolic BP	60 (60-70)	70 (60-70)	70 (60-70)	70 (60-70)	0,386
Systolic BP	105 (100-110)	110 (100-110)	110 (100-110)	110 (100-110)	0,095
Lowest hematocrit (%)	32,1 (30,9-34,5)	34,9 (31,8-37)	34,2 (31,5-37)	35 (33,1-37,4)	0,033
Lowest hemoglobin (g/dL)	10,8 (9,5-11,5)	11,55 (10,7-12,6)	11,5 (10,2-12,4)	11,6 (10,9-12,5)	0,141
Lowest WBC ($\times 10^3$/mm3)	8,8 (6,22-11,76)	7,57 (5,9-9,28)	8,15 (6,44-10,33)	9,8 (8,11-11,81)	< 0,001
Neutrophil/Lymphocytes	9,42 (8-11,77)	4,88 (3,9-7,03)	4,44 (3,43-6,42)	3,62 (2,88-5,36)	0,016
Platelet/Lymphocytes	227,63 (217,98-415,91)	169,78 (125-227,5)	155,06 (117,8-219,33)	122,38 (90,36-169,6)	< 0,001
Highest D-dimer (ng/mL)	2110 (1120-4040)	1635 (1020-3220)	1790 (1090-2845)	2170 (1370-4400)	0,046
CRP	86 (40-121)	41 (19-71)	14,5 (6-35)	9,5 (5-24,5)	< 0,001

Data are median (interquartile range). Number of missing values: hearth rate (n=32), SpO_2 (n=30), temperature (n=28), D-dimer (n=9), CRP (=11).

Maternal and immediate perinatal outcomes are presented in **Table 5**. Among 333 patients, 6 of them had abortion and 108 patients had no record of delivery. In the critical group, 1 patient died before delivery, and 3 of them were at the earlier stages of second trimester and had no records of delivery. Therefore, maternal-perinatal outcomes in the critical group were calculated for 11 patients only. Only one patient revealed thromboembolic event, subacute deep vein thrombosis (DVT).

DISCUSSION

Principal Findings

We described the effect of COVID-19 disease on pregnancy throughout the different stages of pandemic in 333 hospitalized patients from March 2020 to March 2022, in a tertiary center. Among these patients, 15 of them were transferred to the ICU and 5 of them died because of systemic effects of the virus. The highest number of patients and maternal deaths were detected during the second (n=1) and fourth waves (n=4) of the COVID-19 infection in Turkey. These results are also consistent with the distribution of total patients in the general population.

We found that gestational age is an essential risk factor that influences the severity of the COVID-19 disease. In the critical group, 14 out of 15 patients (%93) were at the second trimester or preterm stages of the pregnancy. Maternal death was also detected within this group of pregnants.

Clinical and Research Implications

US center for Disease Control and Prevention (CDC) data showed that the pregnants are at an increased risk of death (1.5 per 1000) and ICU admission (10.5 per 1000).[4] Metz et al.[9] reported 4.8 % ICU admission rate and 0.3 % maternal death. Our maternal death rate was 1.5% (5/333 patients with COVID-19), and the ICU admission rate was 4.5 % (15/333). Both rates in our study were higher. This might be partially attributed to the admission of critical patients from other health care facilities to the tertiary care center. In addition, different from the previous study,[9] we included all the hospital admissions of all gestational ages. Moreover, inclusion of the data from the fourth wave, a subsequent period, did also have a certain impact on difference of our results as 4 of the maternal deaths occurred during the fourth wave.

Older maternal age, increased body mass index and pre-existing comorbidities were defined as risk factors for severe COVID-19 infection.[9,10] Consistent with previous studies, in our study risk factors for severity of the disease also included older age and increased BMI. Interestingly, we did not find any association between comorbidities and the severity of the disease.

Initial clinical and laboratory findings were worse in the critical group. However, we were unable to evaluate the effect of treatments on outcomes of the disease except anticoagulant therapy. We used therapeutic doses of anticoagulant therapy for each patient. Whether this treatment had an additional prevention on previously reported complications of pregnancy such as hypertensive disorder of pregnancy or postpartum hemorrhage,[4,11] is yet to be confirmed.

Strengths and limitations

The strength of this study includes the standardized care of patients and the wide range of time including four different

Table 5. Perinatal Outcomes by Coronavirus Disease 2019 (COVID-19) Severity

Outcome	Critical (n=11)	Moderate-Severe (n=49)	Mild (n=71)	Asymptomatic (n=88)	p-value	Total (n=219)
Maternal death*	4 (36%)					4 (1.8%)
Cesarean birth	11 (100%)	31 (63%)	44 (62%)	49 (56%)	0,042	135 (61.6%)
Primary Cesarean birth	6 (55%)	13 (27%)	21 (30%)	15 (17%)		55 (25.1%)
Postpartum hemorrhage	1 (9%)	1 (2%)		1 (1%)		3 (1.4%)
Hypertensive disorders of pregnancy		1 (2%)	2 (3%)			3 (1.4%)
Preterm birth	8 (73%)	12 (24%)	17 (24%)	6 (7%)		43 (19.6%)
Live birth	11 (100%)	46 (94%)	70 (98.6%)	88 (100%)		216 (98.6%)
Fetal death		3 (6%)	1 (1.4%)		0,199	11 (5%)
Birth weight (g)	2031,8±839,7	2894,4±835	3050,5±702,1	3521,2±497,7	< 0,001	
Plasenta previa		2 (4%)	1 (1.4%)			3 (1.4%)
Secondary infection		3 (6%)				3 (1.4%)
IUGR				1 (1%)		1 (0.5%)
Meconium		2 (4%)	1 (1.4%)	5 (6%)		8 (3.7%)
Kolestase		1 (2%)	3 (4%)			4 (1.8%)
Detachment of placenta			3 (4%)			3 (1.4%)
Polyhidramniosis			3 (4%)			3 (1.4%)
APGAR-1	5±3	7±2	7±2	8±1	0,001	
APGAR-5	7±3	8±2	8±2	9±1	< 0,001	
Koryoamniosis			1 (1.4%)			

* Of 5 maternal deaths, one did not give birth Data are n (%) or mean±SD.

waves of pandemic. We were able to conclude that severity of the disease was strongly associated with the gestational age and different waves of the disease. As one of the biggest healthcare units in Istanbul, a city with the highest population in Turkey, our results might also reflect the general population in this country.

Limitations of this study include the fact that we only had information of inpatient settings, which prevents to draw a conclusion about the prevalence of the disease among pregnants in Turkish society. Moreover, due to descriptive nature of data, analyses were not adjusted for multiple comparisons.

CONCLUSION

These results suggest that the severity of COVID-19 infection is related to the gestational age, maternal age and BMI levels of the individuals. Given the universality and severity of the COVID-19 crisis, an important focus should be placed on planning for additional measures to reduce the number of severe cases and deaths in the more vulnerable populations. Collection of tremendous amounts of information still did not seem to prevent the destructive effect of the disease during the last wave, in Turkey. Consequently, it is of vital importance to keep collecting information to develop a road map for immediate global actions against possible new infection threats.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission Kartal Dr. Lütfi Kirdar City Hospital Clinical Researches Ethics Committee (Date: 30.03.2022, Decision No: 2022/514/222/43) .

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Original Article / Orijinal Araştırma

Lymphadenopathies Associated with Cat-Scratch Disease in Turkiye

Türkiye'de Kedi Tırmığı Hastalığı ile İlişkili Lenfadenopatiler

Aysun Yahşi[1], Bahadır Yazıcıoğlu[2]

[1]Department of Pediatric Infectious Diseases, Samsun Training and Research Hospital, Samsun, Turkiye
[2]Department of Family Medicine, Samsun Training and Research Hospital, Samsun, Turkiye

Abstract

Aim: *Bartonella henselae* is the etiologic agent of cat-scratch disease. The disease affects children and young adults. The objective of this study is to analyze the epidemiology, clinical features, and course of this disease in Turkiye.

Material and Method: Children without immunodeficiency, with relevant clinical signs and symptoms, and positive serology were included in the study. Clinical, demographic and laboratory data of patients diagnosed with cat-scratch disease between October 2018 and February 2021 were evaluated retrospectively.

Results: A total of 46 patients were included. The mean age was 132 months (IQR 90- 153 months), and 69.6% (n=32) were male. There was a history of cat contact in 73.9% of the patients and 43.5% of these were with household cats. Most of the lymphadenopathies were axillary (73.9%), and generalized lymphadenopathy was not observed. The median duration of symptoms before admission was 4 weeks. The median ultrasonographic lymphadenopathy size was 4 cm. The median clinical improvement time was 6.5 weeks. Azithromycin was given in 18 patients and intravenous and/or oral beta-lactamase inhibitor combination therapy in 18 patients while no treatment was necessary in 10 patients.

Conclusion: The typical presentation is a solitary, enlarged lymph node, mostly in the axillary region. *Bartonella henselae* IgG level should be studied prior to invasive procedures. It should be known that cat- scratch disease can develop without a history of cat contact. Meanwhile, it should be known that contact with a house cat may cause disease more often than contact with street cats.

Keywords: *Bartonella henselae*, cat-scratch disease, children, lymphadenopathy

Öz

Amaç: *Bartonella henselae*, kedi tırmığı hastalığı etkenidir. Hastalık çocukları ve genç yetişkinleri etkiler. Bu çalışmanın amacı, Türkiye'de bu hastalığın epidemiyolojisini, klinik özelliklerini ve seyrini analiz etmektir.

Gereç ve Yöntem: İmmün yetmezliği olmayan, hastalık bulgu ve semptomlarına sahip ve pozitif serolojisi olan çocuklar çalışmaya dahil edildi. Ekim 2018 ile Şubat 2021 arasında kedi tırmığı hastalığı tanısı alan hastaların klinik, demografik ve laboratuvar verileri retrospektif olarak değerlendirildi.

Bulgular: Toplam 46 hasta çalışmaya dahil edildi. Ortalama yaş 132 ay (IQR 90-153 ay) ve %69,6'sı (n=32) erkekti. Hastaların %73,9'unda kedi teması öyküsü mevcuttu ve bunların %43,5'i evcil kediydi. Lenfadenopatilerin çoğu aksiller (%73,9) idi ve jeneralize lenfadenopati izlenmedi. Başvurudan önceki medyan semptom süresi 4 haftaydı. Medyan lenfadenopati boyutu 4 cm idi. Medyan klinik iyileşme süresi 6.5 haftaydı. 18 hastaya azitromisin, 18 hastaya intravenöz ve/veya oral beta-laktamaz inhibitör kombinasyon tedavisi verilirken, 10 hastaya tedavi gerekmedi.

Sonuç: Tipik prezentasyon, çoğunlukla aksiller bölgede soliter, büyümüş bir lenf nodudur. *Bartonella henselae* IgG düzeyi, invaziv prosedürlerden önce çalışılmalıdır. Kedi tırmığı hastalığının kedi teması öyküsü olmadan da gelişebileceği bilinmelidir. Aynı zamanda ev kedisi ile temasın sokak kedileriyle temastan daha sık hastalığa neden olabileceği akılda tutulmalıdır.

Anahtar Kelimeler: *Bartonella henselae*, kedi tırmığı hastalığı, çocuklar, lenfadenopati

Corresponding *(İletişim)*: Aysun YAHŞİ, Department of Pediatric Infectious Diseases, Samsun Training and Research Hospital, Samsun, Turkiye
E-mail *(E-posta)*: aysunyahsi@yahoo.com
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INTRODUCTION

Bartonella henselae is an uncommon, intracellular, slow-growing, Gram-negative bacillus and is the most common agent of cat-scratch disease (CSD).[1] Kittens, stray cats, and cats infested with fleas represent the main vectors for human infection. Transmission to humans can result from the scratch or bite of a cat infected with *B. henselae*, as well as from exposure to cat fleas infected with the microorganism. Transmission can also occur following the contact of cat saliva with broken skin or mucosal surfaces (e.g., the mouth and eyes).[2,3] A low percentage of healthy dogs have been found to be asymptomatic carriers of *Bartonella henselae* but their role in CSD remains unclear. However, the absence of a cat in the environment or the lack of a history of being scratched does not exclude the diagnosis of CSD. [4] No evidence of person-to-person transmission exists. The disease is especially prevalent in children and young adults.[2] CSD begins with a primary inoculation lesion, which typically persists for one to three weeks. Enlarged lymph nodes appear next to the inoculation site two weeks later. The adenopathy evolves to a suppurative phase in about 10-15% of the cases .[5,6] It's typical presentation is a self-limiting lymphadenitis of the regional lymph nodes that can be accompanied by constitutional symptoms (e.g., fever, malaise, headache, nausea, abdominal pain).[7-10] CSD sometimes presents atypical signs (such as fever of unknown origin, osteomyelitis, optic neuritis, encephalitis, and endocarditis) and can rarely become a disseminated disease.[2] The manifestations of CSD can include visceral organ (especially hepatosplenic), neurological, and ocular involvement (e.g., Parinaud's oculoglandular syndrome).[7] We conducted a retrospective study of lymphadenopathy caused by *B. henselae*, using the data from a region with unknown prevalence. The objective of this study was to analyze the epidemiology, clinical features, and course of this disease in a tertiary children's care hospital in Turkey.

MATERIAL AND METHOD

Study Design and Population

This retrospective study was conducted in the 2018-2021 period at the Samsun Training and Research Hospital, a tertiary pediatric care hospital in Samsun, Turkey. A total of 46 patients diagnosed with lymphadenopathy associated with CSD and referred to the Pediatric Infectious Diseases Clinic of the Samsun Training and Research Hospital from Samsun and neighboring provinces were analyzed retrospectively. The *Bartonella henselae* indirect immunofluorescence assay (IFA) for IgG was sent to the national reference laboratory (Republic of Turkey Public Health General Directorate, Microbiology Reference Laboratories and Biological Products Department, High Risk Pathogens Reference Central Laboratory) at least once in all patients with a pre-diagnosis of CSD. The demographic-clinical characteristics, contact history, laboratory and radiological findings, *Bartonella henselae* IFA IgG values, and response to treatment were statistically evaluated. Biochemical tests, acute phase reactants, and lymphadenopathy imaging were performed in all patients. In addition, the toxoplasma, cytomegalovirus, rubella, Epstein-Barr virus, and Francisella tularensis serology was evaluated together with the Brucella Coombs test regarding the etiology of the lymphadenopathy. The Mantoux test, abdominal ultrasound, and chest X-ray examinations were performed when necessary.

The diagnosis of CSD is based on a characteristic clinical presentation, history, and supportive serological and/or nonserological tests. A positive serological test titer is defined as ≥1:256 in CSD-associated lymphadenopathies. Titers of 1:64 or 1:128 represent possible Bartonella infection, and repeat testing should be performed in 14-21 days (Ig G seroconversion). Nonserological tests include tests such as histopathology, polymerase chain reaction (PCR), and blood or tissue cultures (2,3,5). In our study, the diagnosis was made by clinical and serological tests (*Bartonella henselae* IFA IgG).

Patients with systemic involvement and/or immunodeficiencies were excluded, and patients with isolated lymphadenopathy or lymphadenitis were included. Clinical improvement was defined as greater than 80% reduction in lymph node size, normalization of C reactive protein (CRP), and normalization of leukocytosis (in patients with this finding). Enlargement of the lymph nodes together with pain, skin changes, edema, fever, and/or purulent exudate was accepted as lymphadenitis.

Patients who were clinically and/or ultrasonographically compatible with lymphadenitis were given beta-lactamase inhibitor therapy until their serological tests were concluded. Oral azithromycin treatment was given to patients whose serology results, clinical history and examination findings were compatible with CSD. Patients diagnosed with CSD by serology tests were followed without treatment if they achieved clinical improvement in this period.[2,5]

Patients with and without treatment, patients with and without a history of contact, and patients with head-neck and axillary lymphadenopathy were compared with each other in terms of clinical and laboratory findings and treatment response. In addition, those with a history of cat contact were divided into those in contact with a household cat and those in contact with a stray cat, and clinical and laboratory findings and treatment responses were compared. Patient data were also compared in patients with and without signs of lymphadenitis, based on laboratory data and clinical responses.

Ethics Committee Approval

The study was carried out with the permission Samsun University Clinical Researches Ethics Committee (Date: 23.11.2022, Decision No: 2022/12/6). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analyses

All statistical analyses were conducted using SPSS software (version 25; IBM, Chicago, IL). The data of the patients were collected retrospectively from the hospital records. Normality of distribution of the continuous variables was measured with the one-sample Kolmogorov-Smirnov test. The continuous variables with a normal distribution were expressed as mean±SD and compared using Student's T-test, while the variables with a non-normal distribution were expressed as median (min-max) and compared using the Mann-Whitney U test. Categorical variables were compared using the ⍰ 2 test or Fisher's exact test. A p value < 0.05 was considered to indicate statistical significance for all analyses.

RESULTS

A total of 46 patients were included. The median age was 132 months (IQR 90- 153 months), and 69.6 % (n=32) were male. The median duration of symptoms before admission was 4 weeks (3.75-6.5 weeks). Most lymphadenopathies (95.6%) were in a single region of the body, mainly the axillary (73.9%), while generalized lymphadenopathy was not observed. The demographics, clinical and laboratory findings, treatment, outcome, and exposure findings of the children with lymphadenopathies associated with CSD are shown in **Table 1**.

Table 1. Demographics, Clinical and Laboratory Findings, Treatment, Outcome, and Exposure among Children with Lymphadenopathies Associated with CSD

Characteristics	n=46	%
Age, months (median, IQR1)	132 (90-153)	
Age Groups		
0–6 years	8	17.4
6–12 years	12	26
13–18 years	26	56.5
Sex		
Female	14	30.4
Male	32	69.6
Cat Exposure		
Household cats	20	43.47
Stray cats	14	30.4
None	12	26
Location of solitary lymphadenopathies		
Axillary	34	73.9
Cervical	10	21.7
Submandibular	2	4.3
Presence of lymphadenitis signs		
Yes	18	39.1
No	28	60.9
Reason for prescribing antibiotic treatment		
Protracted lymphadenopathy	38	82.6
Lymphadenopathies associated with fever	8	17.4
Presence of leukocytosis		
Yes	18	39.1
No	28	60.9
Antibiotic prescribed		
Azithromycin	18	39.1
Azithromycin plus beta-lactamase inhibitor	18	39.1
None	10	21.7

1IQR: Interquatile range

Eight of the patients with lymphadenopathies were admitted and received treatment while being evaluated for the etiology as an inpatient. In the whole patient group (inpatient and outpatient), pathological, non-infectious ultrasound findings were detected in eight (17.3%) patients along with the lymphadenopathy while abscess findings were not detected in any of the patients on ultrasonography.

A lymph node biopsy was performed in four patients when the lymphadenopathy and/or lymphadenitis demonstrating pathological dimensions and ultrasonography findings did not improve with nonspecific treatment. These patients were referred to the Pediatric Infection Department when the pathology result was consistent with granulomatous inflammation. PCR hybridization tests were not evaluated for Bartonella in lymph node biopsies. One of these patients had a history of contact with a house cat. The granulomatous infectious causes were investigated in this patient and *Bartonella henselae* IgG titers were found to be high (1/512). The other three children did not have a history of cat contact but had high *Bartonella henselae* IgG values.

Eighteen (39.1%) of the patients had lymphadenitis findings. The lymphadenopathy was found to be larger (p:0.043) and the CRP value to be significantly higher (p:0.000) in this group when compared to the other patients. All patients with lymphadenitis had leukocytosis (p<0.001). The median CRP level was 10 mg/L (5-80). There was a positive correlation between the increase in lymphadenopathy size and the CRP level (p:0.003, r:0.424).

The median lymphadenopathy size (ultrasonographic measurement) was 4 cm (3-4 cm). The median clinical improvement time was 6.5 weeks (3-12 weeks). Increased lymphadenopathy size indicated a longer time until clinical improvement (p:0.023, r:0.335).

There was a history of cat contact in 73.9% of the patients, and 43.5% of these were with household cats. None of the patients had a history of dog bite or contact. Only one of the patients had a history of a papule at the bite or/and scratch site. The mean clinical improvement time following a scratch by a household cat was shorter than that of stray cat contact (p:0.004). Axillary lymphadenopathy was more common in those with a history of cat contact, while cervical and submandibular lymphadenopathy was more common in those without a contact history (p:0.001). There was no significant correlation in lymphadenopathy size, clinical improvement time, duration of symptoms before admission, *Bartonella henselae* (IFA) IgG titer, CRP levels, location of lymphadenopathy, and history of azithromycin therapy between the patients with and without a contact history (**Table 2**).

Table 2. The clinical and laboratory characteristics of the patients according to cat contact history

	Patients with cat contact history	Patients without cat contact history	p
Lymphadenopathy size (cm)	4 (3-4)	4 (4-4)	0.423
Time to clinical improvement (weeks)	6 (6-8)	5 (4-8)	0.304
Duration of symptoms before admission (weeks)	4 (3-8)	4 (4-4)	0.539
Location of lymphadenopathy Axillary Cervical and submandibular	 30 4	 4 8	0.001
CRP1 (mg/L)	8 (4.75-74)	44 (6-80)	0.269
Azithromycin treatment Yes No	 28 6	 8 4	0.416

1CRP: C reactive protein

Hepatomegaly was noticed on the physical exam in 5 of the 46 (10.8%) patients and on ultrasonography in 7 (15.2%) patients. Splenomegaly was not detected in any patient. None of the patients had microabscesses in the liver and/or spleen on ultrasonography.

Time of symptom onset was most commonly the autumn (43.5%), followed by the winter (26%), summer (21.7%), and spring (8.7%). Most of the diagnoses were made during October (n=8, 17.4%) (**Figure 1**).

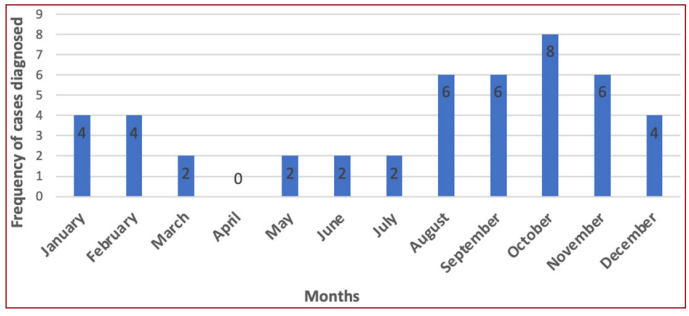

FIGURE 1- Number of lymphadenopathies associated with CSD in each month, n=46

The *Bartonella henselae* (IFA) IgG titer was 1/1024 in 6/46 patients (13%), 1/512 in 22/46 (47.8%), 1/256 in 16/46 (34.8%), and 1/128 in 2/46 (4.3%). Both of the patients with a titer of 1/128 had a history of contact and straching, and the lymphadenopathy regressed after treatment. For socioeconomic reasons, the patients did not apply for the control *Bartonella henselae* IgG test for IgG seroconversion. Clinical improvement was achieved in these two patients after treatment. There was a negative correlation between the *Bartonella henselae* (IFA) IgG titer and the duration of symptoms before admission (p:0.001, r: -0.474).

The patients received oral azithromycin 10 mg/kg on day 1 followed by 5 mg/kg for 4 days, and intolerance was not observed. Azithromycin was given to 18/46 (39.1%) patients while intravenous and/or oral combination beta-lactamase inhibitor and azithromycin therapy was given in 18/46 (39.1%) patients. Ten (21.7%) of the patients did not receive any treatment. All patients who received dual therapy had findings of lymphadenitis. No side effects related to azithromycin were observed. The dimensions of the lymphadenopathy in the follow-up of patients who did not receive antibiotics regressed until the time that results of tests such as serology and radiological imaging were obtained, and no signs of disseminated disease developed in the follow-up. Azithromycin treatment was given more frequently to patients with axillary lymphadenopathy (p:0.012). Those who received azithromycin treatment had higher CRP values than those who did not (p:0.001). There was no statistically significant difference in time from onset of symptoms to diagnosis, the lymphadenopathy size, time to clinical improvement and *Bartonella henselae* (IFA) IgG titer between the groups that received and did not receive treatment (**Table 3**).

Table 3. The clinical and laboratory characteristics of the patients according to azithromycin treatment

	Patients with azithromycin treatment	Patients without azithromycin treatment	p
Lymphadenopathy size (cm)	4 (3-4)	4 (3-4)	0.427
Time to clinical improvement (weeks)	6 (4-8)	6 (5.25-6.5)	0.319
Duration of symptoms before admission (weeks)	4 (3.25-8)	4 (3.75-4)	0.256
Location of lymphadenopathy Axillary Cervical and submandibular	 30 6	 4 6	0.012
CRP1 (mg/L)	41 (7-80)	5 (4-6)	0.001

1CRP: C reactive protein

DISCUSSION

Infections due to *Bartonella henselae* have a worldwide distribution and affect both the adult and pediatric population. CSD can present with a broad range of clinical symptoms ranging from asymptomatic infection to disseminated disease (7-9). CSD has been found to cause solitary lymphadenopathy most commonly in pediatric patients.[7,11]

The differential diagnosis for lymphadenopathy in pediatrics is broad. Often, the history and physical examination allow the determination of the correct diagnosis and beginning the appropriate treatment quickly. However, it may be difficult to determine the cause of lymph node enlargement, especially in the case of persistent lymphadenopathy, and the exact etiology may not be determined despite extensive investigations and invasive procedures such as histopathological examination. While most studies have reported higher rates of head and neck lymphadenopathy (52-65%),[7,11] we found axillary lymphadenopathy, which generally requires further evaluation, to be more common (73.9%) with most of these patients having a history of cat contact. This provides the opportunity to follow up patients

with axillary lymphadenopathy without the need for a histopathological sample. In the present study, most of the lymphadenopathies were persistent lymphadenopathy, and the median duration of lymphadenopathy was four weeks. The *Bartonella henselae* IgG assay should be performed before invasive procedures such as pathology, as it is known that the serological response develops when symptoms begin.[5] However, four of our patients were referred to the Department of Pediatric Infectious Disease for diagnosis with the pathology results after a lymph node biopsy had been performed. In addition to the lymphadenopathy dimensions, 17.3% of the patients in our study had pathological, non-infectious USG findings. While all these reasons require the etiology to be found quickly, querying a cat contact history in the anamnesis should not be forgotten and CSD should also be considered.

There is no clear consensus regarding the necessity of treatment in lymphadenopathies due to *Bartonella henselae* in immunocompetent children. While some publications recommend follow-up without treatment,[8-13] some have demonstrated that antibiotic therapy reduces the duration of symptoms.[5,14-6] Practice guidelines for the treatment of CSD, the Italian guidelines, and the Infectious Diseases Society of America (IDSA) recommend oral therapy with azithromycin.[13,17] In some patients, the lymph nodes may be painful and there may be a protracted course with the formation of abscesses and fistulas. Some studies have highlighted the need for antibiotic therapy or even multiple drainage procedures in these patients.[5] If a suppurative process develops, evacuative aspiration is recommended but an incision and drain placement are not as a chronic fistula may develop.[6] A high percentage of patients received treatment, and azithromycin was used in all. Although lymphadenitis and/or suppuration findings were present in patients with lymphadenopathy due to CSD, beta-lactamase inhibitor combination therapy was also given to 18 of the patients due to the lymphadenitis findings. Serology tests were conducted at the national laboratory and the results were delayed, and some patients did not have a history of cat contact. Azithromycin treatment was not given to ten patients due to regression of lymphadenopathy dimensions before the serology and laboratory results were obtained. No progression to abscess formation or disseminated disease was observed in the follow-up of the patients.

The infection is transmitted by direct inoculation through the scratch or bite of the reservoir, especially cats, although exposure to dogs and flea bites have also been linked to this infection. Some publications report that the disease can be transmitted to humans through the bite of a flea (Ctenocephalides felis), the vector responsible for horizontal transmission of the disease among cats. It is also mentioned that tick bites could transmit the bacteria.[18] A contact history with an animal is reported with varying frequencies in different studies (68.7%-92.4%).[7,19] In our study, a cat contact history was present in 73.9% of the cases, and axillary lymphadenopathy was more common in such patients. However, the absence of this epidemiological history in children with a clinically and serologically diagnosed disease may be due to sporadic contact unrecognized by the family or to another type of infection that has not been determined yet.

Although *Bartonella henselae* can be found in up to 71.4% of cats, the infected cats generally do not show any sign of disease.[20] The prevalence in cats varies depending on geographic location, the climate, the cat population, flea infestations, and age. *Bartonella henselae* can found in both household and stray cats. It is more common in cats under the age of one.[21-24] Contrary to publications showing that contact with stray cats more commonly leads to the disease,[23,25,26] contact with household cats was more frequent in our study, similar to the study of Derebegius et al.[11] The reason may be that household cats are mostly domestic cats that are allowed to go outdoors, and their interaction with the environment could have resulted in their becoming infected. The mean time to clinical improvement following a scratch by a household cat was shorter than that of stray cat contact. The reason could be fewer bacteria, or fewer fleas that carry these bacteria, in household cats. In other words, even if CSD is more common following contact with a household cat, recovery was achieved in a shorter time. A negative correlation was found between the *Bartonella henselae* (IFA) IgG titer and the duration of symptoms before admission in this study. This may be associated with the low serological response that occurs with low bacterial load in immunocompetent subjects, as evaluated in this study, and the prolonged time required for clinical signs to appear.

Bartonella infection has a clear affinity for the pediatric population and temperate regions with a greater incidence in the late summer, autumn, and winter seasons.[5,7,19,24,27] Some authors believe that seasonal changes in animal reproductive behavior or flea seasonality may be an explanation for this seasonal preference.[19,24,28,29] Cats usually become pregnant in the spring or early autumn, and the gestation lasts about nine weeks. Contact of kittens with children, which is a common cause of the disease, and possible transmission of CSD usually occur in the late summer and mid-winter.[19,29] The link between seasons and CSD incidence has been described in the USA, Japan, and France with increased incidence in the autumn and a decrease in the spring.[29] The patients who presented at our hospital lived in the Black Sea region, with a temperate and humid climate. As with other studies, we found that most cases were diagnosed in the autumn.[5,7,19,24,27]

The current study has the highest number of lymphadenopathy cases due to CSD in pediatric patients in Turkey, as far as we are aware, and confirms that CSD is not rare in Turkey. Most of the lymphadenopathies were persistent cases in the current study, and the median duration of lymphadenopathy was four weeks. Clinicians should have a high degree of suspicion for CSD as early diagnostic tests can

accelerate the diagnosis and facilitate patient care, especially in patients presenting from a temperate climate zone and in autumn. The *Bartonella henselae* IgG assay should be performed before invasive procedures. The treatment rate was high in the current study. None of the patients with persistent lymphadenopathy developed disseminated disease, an abscess, or fistula during follow-up, and no treatment-related side effects were observed. Querying cat exposure will facilitate the diagnosis. However, it should be known that CSD can develop without a history of cat contact, and contact with a household cat may also cause the disease (even more commonly). We should accept that this is not a rare condition, as CSD can vary from harmless to very serious forms and the diagnosis and treatment should be quickly and carefully performed. Studies showing disease prevalence rates in cats, and clinical and therapeutic studies of CSD are needed.

Infections due to *Bartonella henselae* have a worldwide distribution and affect especially children. CSD can present with a broad range of clinical symptoms ranging from asymptomatic infection to disseminated disease and solitary lymphadenopathy most commonly in pediatric patients. In the present study, lymphadenopathies were commonly axillary lymphadenopathy. CSD can mostly develop after animal contact, also it can occur without contact, while contact with household cat may also cause the disease, even more frequently than contact with stray cats. There is no clear consensus regarding the necessity of treatment in lymphadenopathies due to *Bartonella henselae* in immunocompetent children. A high percentage of our patients received treatment, and azithromycin was used in all. The diagnosis is based on a clinical suspicion for lymphadenopathies associated with CSD, as early diagnostic tests can accelerate the diagnosis and facilitate patient care, especially in patients presenting from a temperate climate zone and in autumn.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission Samsun University Clinical Researches Ethics Committee (Date: 23.11.2022, Decision No: 2022/12/6) .

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Bibliometric and Visual Analysis of the Literature on Electroconvulsive Therapy in Psychiatry between 2000-2023

2000-2023 Yılları Arasında Psikiyatride Elektrokonvülsif Tedavi ile İlgili Literatürün Bibliyometrik ve Görsel Analizi

Ali Emre Şevik

Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Psychiatry, Çanakkale, Turkey

Abstract

Aim: This study aims to give a bibliometric overview of the electroconvulsive therapy (ECT) literature in the psychiatry research area, published between 2000 and 2023.

Material and Method: The data was retrieved on June 1, 2023, in accordance with the set search strategy, from the Web of Science Core Collection, which served as the data source. For the analysis of published literature, the bibliometrics tool VOS Viewer was employed.

Results: According to the search strategy, this study included 1909 articles regarding ECT. With 567 publications and 12632 citations, the United States was determined to be the country with the greatest importance in this study. Additionally, more than 100 publications on ECT were published in Germany (n=171), Australia (n=148), China (n=127), the Netherlands (n=124), and Japan (n=110). Based on the volume of published records, the Mayo Clinic in the United States was the most significant institution in ECT research. The bulk of publications (39.9%) were published in the Journal of ECT.

Conclusions: The bibliometric analysis in this study provided information on recent developments in publications on ECT from 2000 to 2023. The results can act as the basis for further field research.

Keywords: Bibliometric analysis, electroconvulsive therapy, electroshock therapy

Öz

Amaç: Bu çalışma, psikiyatri araştırma alanında 2000-2023 yılları arasında yayınlanan elektrokonvülsif tedavi (EKT) literatürüne bibliyometrik bir bakış sunmayı amaçlamaktadır.

Gereç ve Yöntem: Veriler, belirlenen arama stratejisine uygun olarak 1 Haziran 2023 tarihinde veri kaynağı olarak kullanılan Web of Science Core Collection'dan alınmıştır. Yayınlanan literatürün analizi için bibliyometri aracı VOS Viewer kullanılmıştır.

Bulgular: Arama stratejisine göre, bu çalışma EKT ile ilgili 1909 makaleyi içermektedir. Amerika Birleşik Devletleri 567 yayın ve 12632 atıf ile bu çalışmada en büyük öneme sahip ülke olarak belirlenmiştir. Ayrıca, Almanya (n=171), Avustralya (n=148), Çin (n=127), Hollanda (n=124) ve Japonya'da (n=110) EKT ile ilgili 100'den fazla yayın yayımlanmıştır. Yayınlanan kayıtların hacmine göre, Amerika Birleşik Devletleri'ndeki Mayo Kliniği EKT araştırmalarında en önemli kurumdu. Yayınların büyük kısmı (%39,9) Journal of ECT'de yayımlanmıştır.

Sonuç: Bu çalışmadaki bibliyometrik analiz, 2000'den 2023'e kadar EKT ile ilgili yayınlardaki son gelişmeler hakkında bilgi sağlamıştır. Sonuçlar, daha ileri saha araştırmaları için temel oluşturabilir.

Anahtar Kelimeler: Bibliyometrik analiz, elektrokonvülsif tedavi, elektroşok tedavisi

Corresponding *(İletişim)*: Ali Emre ŞEVİK, Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Psychiatry, Çanakkale, Turkey
E-mail *(E-posta)*: aliemresevik@gmail.com
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INTRODUCTION

Electroconvulsive therapy (ECT) or electroshock therapy is a psychiatric treatment for mental illnesses, which is based on inducing artificial epileptic seizures by passing an electric current through the brain.[1] ECT can be used to treat major psychiatric diseases like mood disorders and schizophrenia. It can benefit greatly from this safe, well-tolerated, and very successful therapy, especially when catatonic symptoms or an abrupt aggravation of psychotic symptoms are prevalent.[2] Also, Parkinson's disease, mania, catatonia, delirium, neuroleptic malignant syndrome, autism, agitation, and depression in demented individuals are among those successfully treated by ECT.[3,4] This method has long been used to treat psychiatric disorders when pharmacological treatments are inoperative.[4] But this method was first introduced in the year 1938, by Cerletti.[5] Patients with schizophrenia were the first patients to receive ECT.[6] ECT therapy was the most popular one for treating acute psychosis up until 1952 when pharmaceutical treatment completely replaced it.[7] Nevertheless, as to its effectiveness in treating resistant schizophrenia, its use has recently attracted a lot of interest from specialists.[8] The decrease in adverse effects has been facilitated by technical developments that have improved this process. Therefore, modified ECT is currently regarded as an efficient and secure kind of therapy, especially in populations that are more susceptible, including geriatric patients, youths, and pregnant women.[2] In addition, catatonia and patients with serious suicidal ideation or intent benefit greatly from ECT treatment. ECT is particularly essential in the treatment of acute illnesses that require quick recovery. Elderly patients benefit from ECT even more than adult populations of mixed ages. Medically speaking, there aren't many reasons not to use ECT as a treatment.[9] The only early biological treatment still in use today among those first offered in psychiatry is ECT.[2] In order to identify when this technique should be utilized, the Spanish Society of Psychiatry created a consensus in 2018.[10]

Today, bibliometric analyses have been conducted in many fields, including psychiatry.[11-14] However, there is no bibliometric study aiming to analyze the literature on general use practices as a whole system of ECT in psychiatry. This research aimed to point out ECT usage in psychiatry over the previous 23 years using bibliometrics. This article attempts to give psychiatrists a thorough and impartial summary of current research on ECT procedures and supporting data. Additionally, it seeks to offer a thorough analysis of a variety of factors, including target publications for academics to publish their articles in and connections between institutions and nations.

MATERIAL AND METHOD

In this bibliometric study, the data was retrieved on June 1, 2023, using the set search approach from the Web of Science Core Collection.

The search parameters were: (electroconvulsive therapy OR electroshock therapy); time span: 2000-30 May 2023; language type: all; literature type: Article; index: Sci-Expanded (SCIE), Social Sciences Citation Index (SSCI) and research areas: Psychology OR Psychiatry OR behavioral sciences)

Articles that were not devoted to the study of psychology or psychiatry were excluded. False positives were removed from the initial sample, and it was then enlarged with pertinent missed papers.

Bibliographic data should be standardized before processing due to a researcher may use several different names.[15] After normalization, descriptive statistics, bibliometric analysis, and co-word analysis were used to examine the bibliographic data. The h-index was used to determine the articles with the greatest impact. According to the definition of the h-index, "a research area has index h when h of its the number articles have at least h citations each, and the remaining number-h articles have less than or equal to h citations each." The classics of the field are those articles that have more citations than or equal to the h-index.[16] The published literature was analyzed using the bibliometrics program VOSViewer (Leiden University, van Eck NJ).[17]

The co-word analysis aids in the identification of the most pertinent research topics and their relationships by calculating the co-occurrence frequency of keyword pairs in an article.[19-20] The most prolific publications' cooperation networks were scrutinized using co-word analysis, and a graph showing the researchers and the number of articles they co-authored as a consequence was produced. The co-word analysis was done with the VOSViewer tool.

For quantitative and qualitative analysis, the TXT format files were imported into Microsoft Excel 2019 (Microsoft Corporation). Journal Citation Reports (JCR) category was utilized for the quality assessment of the data as indications of the impact of the articles. It represents the average number of citations received by each article published in this journal over the previous two years. The JCR assigns each scientific journal to its associated IF and ranks them according to particular fields as a sign of scientific "prestige." JCR data categories from the JCR of 2021 were used in this investigation.[21]

RESULTS

According to the research methodology, 1909 research articles published in the field of psychiatry/psychology and behavioral sciences published in SCIE and SSSI indexes between 2000 and 2023 were reached. Each publication received an average of 17.97 citations each year and 34,314 citations overall. 11,418 articles in total cited these articles.

When the publication languages of these publications were analyzed, English (n=1840, 96.386%) was the main publication language. German (n=25, 1.310%), French (n=12,0.629%), Spanish (n=12, 0.629%), Turkish (n=12, 0.629%), Polish (n=7, 0.367%), and Danish (n=1, 0.052%) were other publication languages. 24.777% of them were published as open access. 2020 and 2021 were the years with the highest number of

publications with 138 articles each. Although the number of publications was irregular, it did not fall below 100 articles per year between 2018-2022. In 2023, 43 articles were published, but this number may be misleading as it reflects the first 5 months of this year.

Authors from 66 countries contributed to the literature on ECT. The United States was the leading country with 570 (29.859%) articles on ECT. Germany (n=171), Australia (n=148), China (n=127), Netherlands (n=124), and Japan (n=110) published more than 100 articles on ECT. **Figure 1** shows the global distribution of the ECT publications. **Figure 1** was produced with the Excel program.

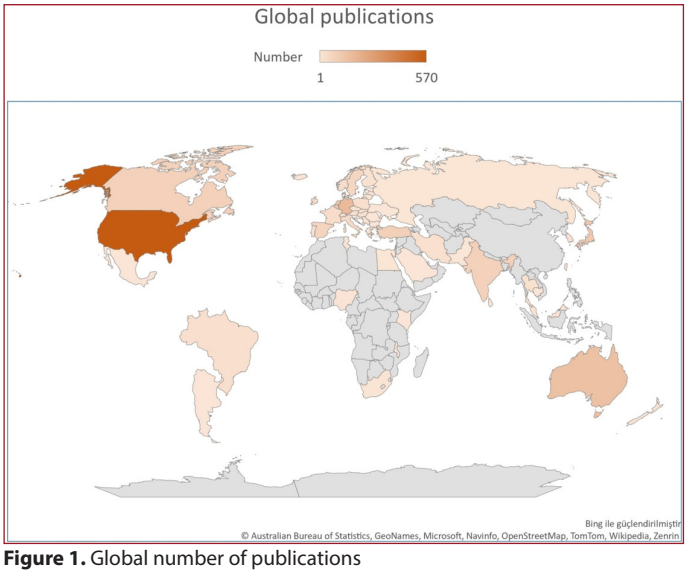

Figure 1. Global number of publications

In total, researchers from 1,918 institutions contributed to the ECT literature. The Mayo Clinic (the United States) (n=78), KU Leuven (Belgium) (n=66), Harvard University (the United States) (n=58), Columbia University (the United States) (n=52), and the National Institute of Mental Health and Neuro-Sciences (India) (n=50) were the leading institutions in the field.

Publications on ECT were published in 141 different journals. The Journal of ECT published the majority of articles (39.9%) on ECT (**Table 1**).

Table 1. Mostly publishing journals on ECT

Publication Titles	n	%	Journal Impact Factor ™ (Five year)
Journal of ECT	760	39.811	3.868
Journal of Affective Disorders	101	5.291	6.569
Journal of Clinical Psychiatry	46	2.410	5.626
Psychiatry Research	39	2.043	6.381
Journal of Psychiatric Research	38	1.991	5.959
Acta Psychiatrica Scandinavica	34	1.781	7.466
Frontiers in Psychiatry	31	1.624	5.556
European Archives of Psychiatry and Clinical Neuroscience	29	1.519	4.871
Neuropsychiatrıc Disease and Treatment	28	1.467	3.482
Psychiatry and Clinical Neurosciences	27	1.414	7.918

The majority of the ECT studies were funded by the National Institutes of Health, the National Institute of Mental Health, and the United States Department of Health and Human Services (**Table 2**).

Table 2. Top funding agencies on ECT

Funding Agencies	n	%
United States Department of Health Human Services	150	7.858
National Institutes of Health	142	7.438
National Institute of Mental Health	108	5.657
National Natural Science Foundation of China	55	2.881
German Research Foundation	29	1.519
Eli Lilly	22	1.152
Ministry of Education Culture Sports Science and Technology Japan	22	1.152
Johnson Johnson	21	1.100
Janssen Biotech Inc	20	1.048

In ECT articles, researchers used 2594 keywords. 204 of these keywords were used more than 5 times. A visualization of the keyword co-occurrence analysis with Vosviewer is given in **Figure 2**. Apart from ECT and its equivalent words[(electroconvulsive therapy, electroconvulsive therapy (ECT)], the most frequently used keywords were depression, major depressive disorder, major depression, schizophrenia, bipolar disorder, catatonia, and cognition (**Table 3**).

Table 3. Keyword analysis

Keyword	Occurrences	Total link strength
Anesthesia	38	69
Attitudes	27	30
Bipolar disorder	59	93
Catatonia	69	96
Cognition	58	118
Depression	334	458
Depressive disorder	33	47
ECT	400	385
Elderly	29	53
Electroconvulsive therapy	1139	977
Electroconvulsive therapy (ECT)	86	66
Ketamine	34	82
Major depression	79	112
Major depressive disorder	122	164
Memory	42	86
Propofol	45	74
Psychosis	27	40
Relapse	30	52
Schizophrenia	111	152
Seizure duration	26	36
Seizure threshold	30	36
Survey	30	41
Treatment-resistant depression	31	47

The United States, Germany, and Netherlands' publications had the highest h indexes 52, 33 and 30 respectively (**Table 4**).

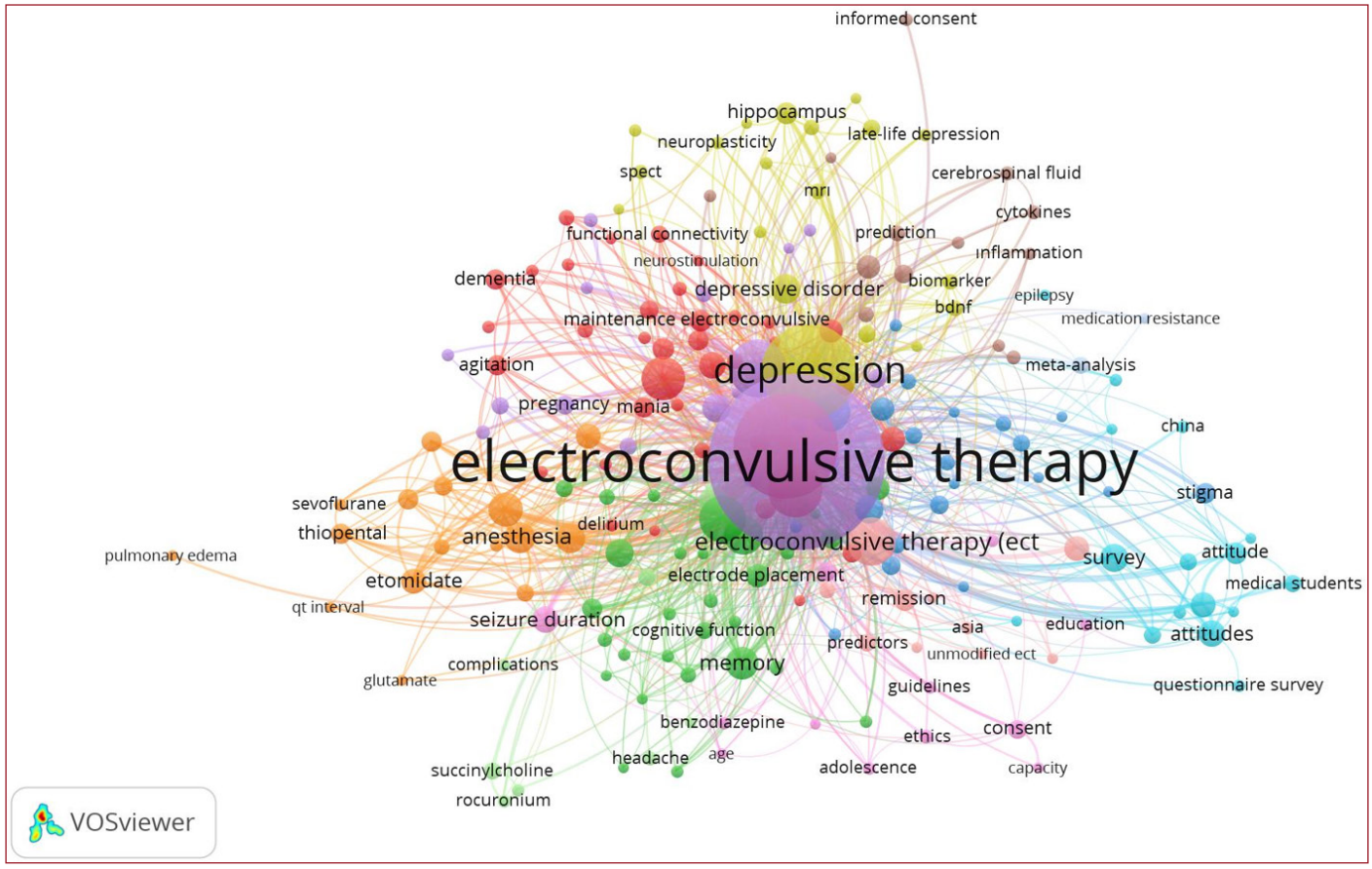

Figure 2. Keyword analysis

Table 4. Top published countries and number of citations

Country	Number of documents	Number of citations	Total link strength	H index
Australia	148	1849	112129	24
Belgium	80	1303	91894	18
Canada	81	1154	53660	18
Denmark	51	1687	51744	18
England	84	1637	58485	21
Germany	170	3729	131526	33
Japan	110	1580	66089	23
Netherlands	124	2681	131304	30
China	127	1525	76673	21
Spain	66	842	58315	16
Sweden	57	1580	54074	17
Turkey	88	771	34928	17
The United States	567	12632	279977	52*
India	83	1202	34182	18

According to the bibliographic coupling between countries analysis, the largest double indicates the country with the highest number of publications, the thickest lines indicate total link strength and the same colors indicate collaborations. This graph was made with the VosViewer tool. The United States (12632 citations), Germany (3729 citations), and the Netherlands (2681 citations) were the most cited countries. Also, Columbia University (2661 citations), Mayo Clinic (1441 citations), and Duke University (785 citations) were the most cited institutions (**Figure 4**). **Figure 5** shows the co-authorship analysis between organizations that published more than 10 articles.

The study published by Sackeim et al.[22] in 2000 was the most cited article with 578 citations. The article published by Madsen et al.[23] in 2000 was the 2nd most frequently cited article with 577 citations. This study was an experimental rat study.[23]

2021 was the most cited year with 4025 citations. Figure 6 depicts the citation numbers between 2000-2023. This graph was downloaded from the WOS website.

The article 'Increased neurogenesis in a model of electroconvulsive therapy' published by Madsen et al.[23] in 2000 was the most cited article on ECT with 579 citations.

DISCUSSION

This study aimed to examine the developments of ECT studies in the last 23 years, bibliometric parameters such as the most productive countries, institutions, and target journals for researchers. The findings may offer advice to patients, academics, physicians, funding organizations, and policymakers. The WOS database was used to retrieve the ETC publications, as this database offers top-notch publications on various scientific fields from around the world.[19,20]

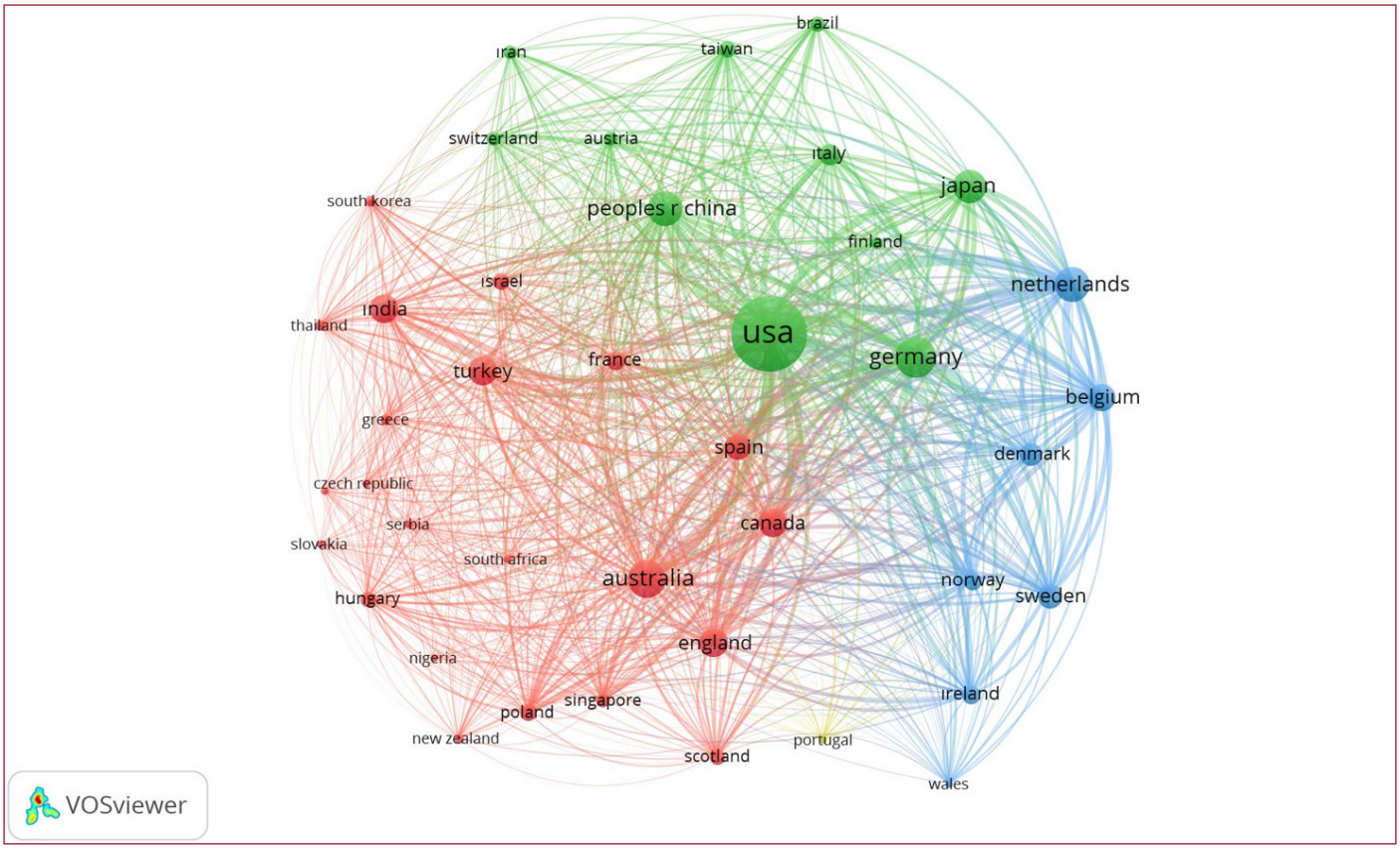

Figure 3. The bibliographic coupling between countries

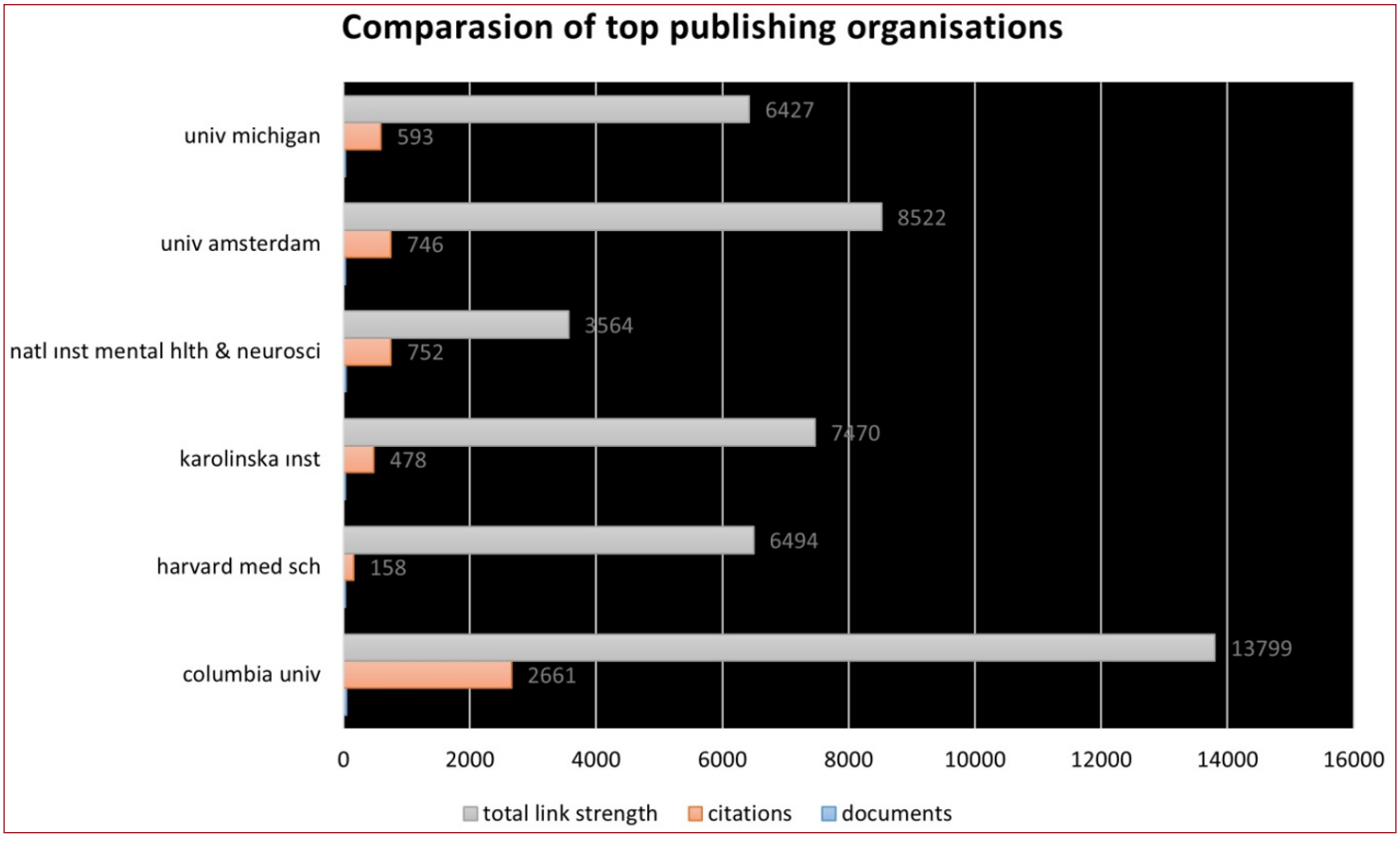

Figure 4. Comparasion of most publishing organisations

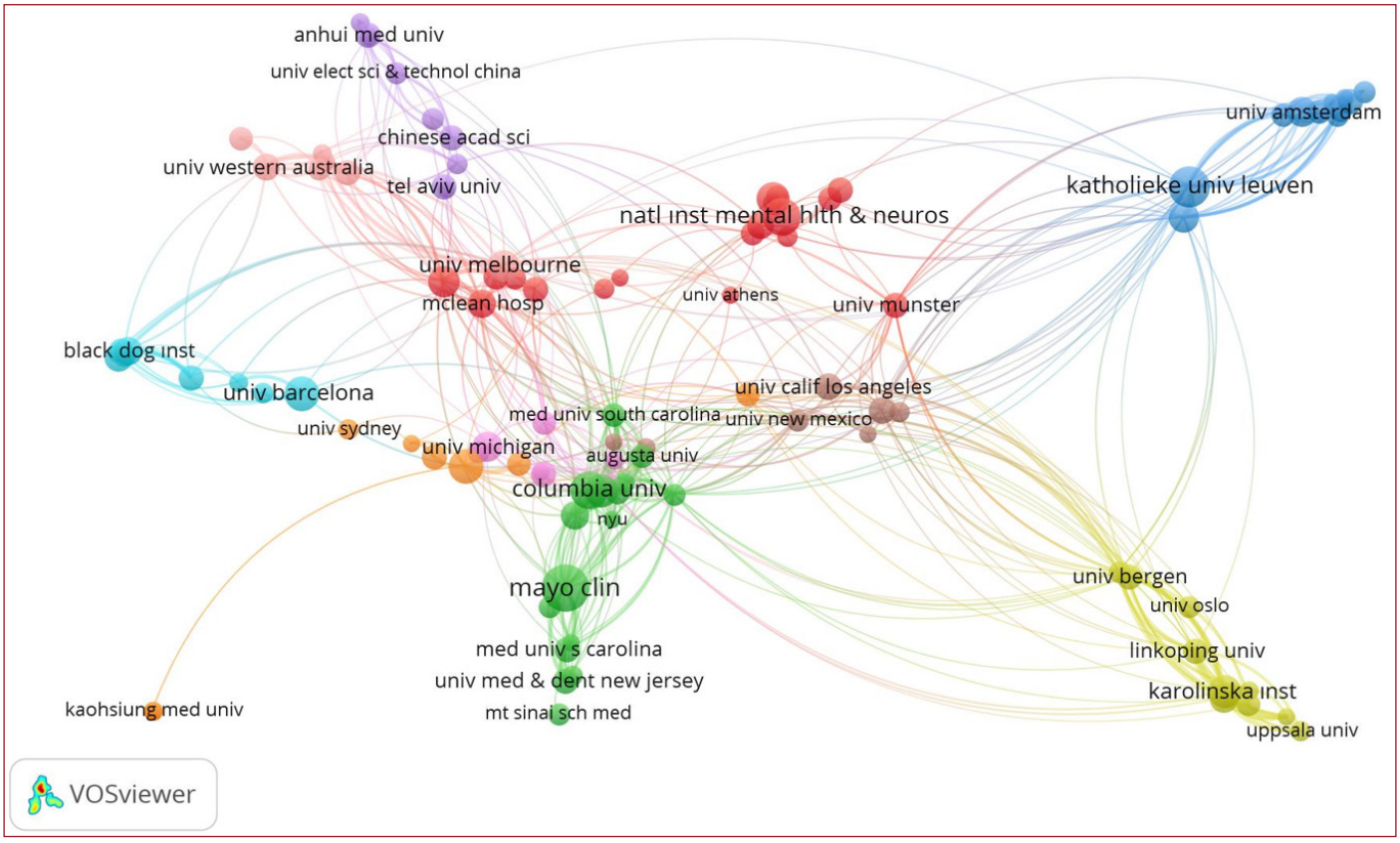

Figure 5. Co-authorship analyisis between organisations

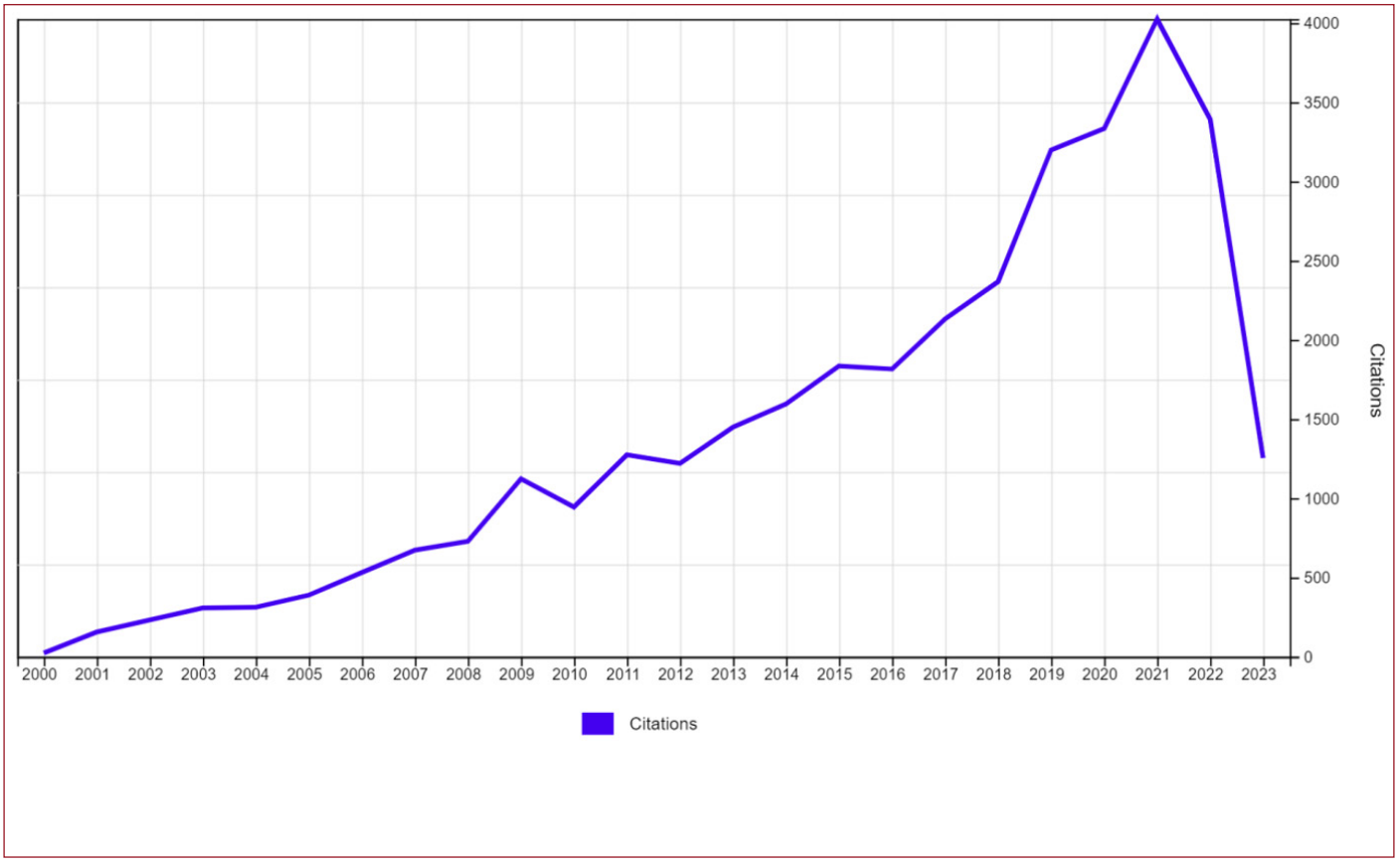

Figure 6. Citation numbers between 2000-2023

Several freeware visualization tools have also been created to assist researchers in creating knowledge network maps, tracing scientific advances, and identifying developing hotspots in a research field. These tools include VOSViewer, CiteSpace, Pajek, and BibExcel. Co-authorship, co-citation, and co-occurrence analysis are the three approaches that are most frequently utilized.[24-30] Scientific mapping offers a dataset visualization to show the connections between various countries and documents.[28] In this study, the VosViewer tool was used for creating network maps and identifying developing keywords.

By utilizing bibliometrics, this study aims to highlight the 23-year history of ECT use in psychiatry. According to this research, 1909 articles on ECT were written by 6528 researchers. The objective of this article is to provide psychiatrists with a complete and unbiased summary of the most recent research on ECT techniques and supporting evidence. Thus, it seems reasonable to assume that this field experienced its peak between 2018 and 2022. However, 43 articles were published in 2023, albeit the count may be confusing given it only includes the first five months of the year.

Based on how often two items are cited together, co-citation analysis shows the relationship between them. An article's citations might offer important information about what is currently known about a particular study area. Researchers can determine the intellectual foundation and research horizons within the area, significant authors, and other bibliometric data by looking at the co-citation relationship's strength.[16]

The relationships between the cited items are captured by citation analysis. The quantity of items that they cited one another determines how closely related the two items are. It was frequently done to determine the significant countries or institutions.[16]

Also, each node in the VOSViewer maps stands for a particular parameter, such as nations/regions, organizations, or keywords. The weight of the characteristic, such as the number of publications, number of citations, or frequency of occurrence, determines the size of the nodes. The size of the nodes increases with weight. The cluster to which the nodes and lines belong determines their color. Links are depicted by the line connecting the nodes. The indicator of TLS, which may be scaled up to reflect the total co-authorship and co-citation link strength across nations, institutions, or authors, was used to evaluate the strength of the relationships.[16]

The quantity and similarity of the authors' citations of the same research papers in the analysis of the bibliographic coupling reveal the relationship between the two pieces of work. The degree of relatedness between unrelated authors is influenced by how often they cite a particular article; the more citations, the more related the authors are.[31]

In a similar study, Cai et al.[11] examined the relationship between ECT and depressive disorder from 2012 to 2021 with the bibliometric method. 2,184 publications were included in Cai et al.[11] study. According to this study, there have been more publications on ECT and depressive disorder since 2012. The United States has made a sizable contribution to the field, and the majority of the top 10 institutions in terms of publications were also American institutions. The United States, Germany, China, Australia, and Canada were the top five nations or regions in terms of publications. The University of Toronto, Duke University, and the University of California are the three institutions with the most publications in the field over the past ten years, each with 104. The current study' findings were similar to Cai et al.[11] study according to most productive institutions, but the Mayo Clinic (the United States) (n=78), KU Leuven (Belgium) (n=66), Harvard University (the United States) (n=58) were the three institutions with the most publications in the field of ECT. This difference may be due to the fact that the study of Cai et al.[11] was on ECT and depression.

In this study also the h indexes of the top publishing countries were analyzed. The United States, Germany, and Netherlands' publications had the highest h indexes 52, 33, and 30 respectively. The number of works where they appear together, weighted by frequency of occurrence, is referred to as a keyword co-occurrence analysis. Researchers may be able to find research hotspots and trends in a field and even get ideas for new research projects if they can identify two terms that commonly appear together in a publication.[16] 'Electroconvulsive therapy, treatment-resistant depression, bipolar disorder, the hippocampus, efficacy, and electrode placement' were all identified as current research hotspots by co-occurrence analysis in Cai et al.[11] study. Although the current study's topic is similar to Cai et al.[11] study, the current study is related to the general use of ECT in psychiatry. In the current study, the most frequently used keywords were depression, major depressive disorder, major depression, schizophrenia, bipolar disorder, catatonia, and cognition. This difference highlights the different uses of ECT in psychiatry.

In terms of publications, the Journal of ECT is both the most widely read and cited journal in the area in both Cai et al.[11] study and the current study. In this study, the number of articles and the JCR of 2021 levels were used to rank the journals. This is a finding that shows the importance of ECT in the field of psychiatry. In addition, when the two most cited articles were reviewed, it was found that the first one was a double-blind clinical study and the second one was an experimental study. Both of these most cited articles were published in 2000. The high number of citations may be related to the fact that the year of publication was 23 years, or it is also possible that these were articles that made a splash at the time of publication.

The Journal of ECT covers all facets of modern ECT and reports on significant clinical and scientific advancements around the globe. It publishes on the effects of induced seizures on behavior and organ systems, review significant research findings on the mode of induction, occurrence, and propagation of seizures, and examine the challenging

sociological, ethical, and legal issues surrounding the use of ECT. Since the subject of the present study was ECT, it was considered an expected result that the highest number of publications were published in this specific journal.

The article 'Increased neurogenesis in a model of electroconvulsive therapy' published by Madsen.[32] in 2000 was the most cited article on ECT with 579 citations. This article published in Biological Psychiatry journal. This journal is Q1 category quartile.

The article 'A prospective, randomized, double-blind comparison of bilateral and right unilateral electroconvulsive therapy at different stimulus intensities' published by Sackeim et al.[33] in 2000 was the second most cited article on ECT with 578 citations. This article was published in Archives of General Psychiatry journal. This journal is Q1 category quartile, too. But in this study the content analysis and most cited articles analyses were not performed.

Limitations

This study used a single database and a single bibliometric tool. Content analysis was not performed. The time span was the last 23 years. In addition, only the literature related to the field of psychiatry searched in SSSI and SCIE indexes was analyzed. For all these reasons, it does not reflect the entire literature on ECT. For this reason, further studies can be planned in which content analyses will also be conducted using different bibliometric methods and different data sets.

CONCLUSION

The bibliometric analysis in this study gave information on new patterns in publications on ECT from 2000 to 2023. The findings might serve as a useful starting point for additional field studies. The United States has been the largest contributor to ECT research. Germany, Australia, China, China, the Netherlands, and Japan have also contributed to ECT research. However, the number of publications on ECT from developing countries was very limited.

ETHICAL DECLARATIONS

Ethics Committee Approval: No subjects, either human or animal, were used in the present study. Since this study was a primarily bibliometric study, ethical approval was not required.

Informed Consent: Informed consent was not required.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Oncological Outcomes in Geriatric Patients with Early Stage Gynecological Cancer who Underwent Surgery and Radiotherapy

Erken Evre Jinekolojik Kanserli Geriatrik Hastalarda Onkolojik Sonuçlar

Necla Gurdal, Berna Akkus Yildirim

Prof. Dr. Cemil Tascioglu City Hospital, Department of Radiation Oncology, Istanbul, Turkey

Abstract

Aim: Evaluation of oncological results obtained with surgery and adjuvant radiotherapy (RT) in geriatric patient group with early stage gynecological cancer.

Material and Method: 31 patients aged 65 years and older who were operated for early stage gynecological cancer and had adjuvant RT were included in the study. All patients were evaluated in terms of general characteristics, local and systemic treatments, and oncological outcomes.

Results: The percentages of patients diagnosed with endometrium cancer and cervix cancer are 80.6% and 19.4%, respectively. The median age of the patients was 69 (range, 65-86). All patients underwent total abdominal hysterectomy and bilateral salpingo oophorectomy, followed by adjuvant pelvic radiotherapy. Intracavitary vaginal brachytherapy was applied to 90.3% of the patients. At a median follow-up of 69 months, 3.2% of patients had local recurrence and 3.2% had distant metastases. The five-year DFS and OS rates were 93% and 80%, respectively

Conclusions: Treatment planning in geriatric patient group should be shaped according to prognostic factors, age group, and comorbidity. Remarkable oncological results can be obtained with multimodality treatments in the selected patient group.

Keywords: Gynecologic cancer, radiotherapy, geriatric patients

Öz

Amaç: Erken evre jinekolojik kanserli geriatrik hasta grubunda cerrahi ve adjuvan radyoterapi ile elde edilen onkolojik sonuçların değerlendirilmesidir.

Gereç ve Yöntem: 65 yaş ve üzeri, erken evre jinekolojik kanser nedeni ile opere edilmiş ve adjuvan radyoterapi uygulanmış 31 hasta çalışmaya dahil edilmiştir. Tüm hastalar genel özellikler, uygulanan lokal ve sistemik tedaviler, ve onkolojik sonuçlar açısından değerlendirimiştir.

Bulgular: Hastaların tanısına bakıldığında %80,6'sının endometrium kanseri %19,4'ünün serviks kanseri olduğu gözlenmiştir. Hastaların ortanca yaşı 69 (65-86) idi. Tüm hastalara total abdominal histerektomi ve bilateral salpingo ooferektomi uygulandı. Hastaların tamamına eksternal pelvik RT uygulanmıştı. Intrakaviter vaginal brakiterapi hastaların %90,3'sine uygulanmıştı. Hastaların %3,2'sinde lokal rekürrens ve yine %3,2'sinde uzak metastaz gözlenmişti. Beş yıllık hastalıksız sağkalım ve genel sağkalım oranları sırasıyla %93 ve %80 idi.

Sonuç: Geriatrik hasta grubunda tedavi planlaması prognostik faktörler, yaş grubu ve komorbidite ye göre uygun olarak şekillendirilmelidir. Seçili hasta grubunda multimodalite tedaviler ile gayet iyi onkolojik sonuçlar elde edilebilmektedir.

Anahtar Kelimeler: Jinekolojik kanser, radyoterapi, geriatri

Corresponding *(İletişim)*: Necla Gurdal, Prof.Dr.Cemil Tascioglu City Hospital ,Department of Radiation Oncology , Şişli, 34384,Istanbul, Turkey
E-mail *(E-posta)*: gurdalnecla@hotmail.com
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INTRODUCTION

Although there are different definitions in the literature for the definition of geriatric patients, such as 65, 70, 75 years and older, patients aged 65 and over are considered as the geriatric patient group according to World Health Organization (WHO).[1] With today's technology and knowledge, we are encountering more and more geriatric patients thanks to early diagnosis in cancer, richer treatment options and multidisciplinary approaches.[2] It is known that both palliative and curative radiotherapy (RT) is needed at a rate of approximately 50% nowadays, where increasingly older cancer patients are treated on the basis of factors such as the prolongation of the average human lifespan and the aging of the world population.[3,4] The geriatric patient group needs more physical and social support due to reasons such as increasing burden of comorbidity, decrease in organ function capacities, and difficulty in accessing the hospital, so this group of patients has difficulty in receiving all the planned treatments. This situation may negatively affect the success of oncological outcomes in patients diagnosed at an advanced age compared to those who receive treatment at a young age.[5] Therefore, it has been reported in many studies that cancer-specific survival rates are lower in geriatric patients than in younger patients.[6]

When deciding on oncological treatment in geriatric patients, detailed geriatric evaluation including performance, basic care needs, weakening of the immune system, physical and mental health condition, can be used to predict treatment tolerance and predict overall survival.[7,8] A Norwegian cluster-randomised controlled pilot study was designed to observe the contribution of a specific geriatric assessment and process management to oncogeriatric patients scheduled for RT. The results of the study, which is aimed to evaluate the contribution of special evaluation and treatment planning strategy in making RT decision in geriatric patients aged 65 and over, are awaited.[9]

MATERIAL AND METHOD

Study Population

Thirty one patients aged 65 and over who were treated with surgery and adjuvant RT for gynecological cancer between 2007 and 2021 were included in the study. All patients were evaluated in terms of general characteristics of the patient, tumor stage and pathological findings, local and systemic treatments applied, disease control, recurrence, distant metastasis and survival results.

Statistical Analysis

The descriptive statistics of the numerical variables obtained in the study are given as the median (range) value. The descriptive statistics of the categorical variables are given as numerical values and percentages. Data distribution was assessed by the Kolmogorov–Smirnov test. In consideration of the sample size, the non-normal distribution of variables was assumed, and nonparametric tests were used for between-group comparisons. So the categorical and numerical variables were compared using the chi-square test and Mann–Whitney U-test, respectively. Kaplan–Meier curves were generated for overall survival (OS) and disease-free survival (DFS) and significance was assessed using the log-rank test . Statistical analyses were performed using SPSS 25 software (SPSS Inc., Chicago, IL, USA). A probability value of $p<0.05$ was considered significant.

RESULTS

Patient Characteristics

The diagnosis of 80.6% of the patients included in the study was endometrium ca, and the diagnosis of 19.4% was cervix ca.The median age of the patients was 69 (range, 65-86). Median follow-up was 69 (range, 8 -219) months. 26% of the patients were grade 3, 51.5% were grade 2. 61% of the patients were between 65 and 70 years of age; 39% were 71 years or older. The median tumor size was 4 cm (range: 1,5-9). Lymphovascular space invasion (LVSI) was present in 64.5% of patients. All patients underwent total abdominal hysterectomy and bilateral salpingo oophorectomy (TAH+BSO). Lymphadenectomy was applied to 67.5 % of the patients. All patients underwent external pelvic RT. Intracavitary vaginal brachytherapy (VBT) was applied to 90.3% of the patients. The median dose of RT administered was 46 Gy (45-50 Gy) and median dose of VBT was 18 Gy (15-27.5 Gy). Chemotherapy (CT) was performed in only 4% of patients. Local recurrence was observed in 3.2% of the patients and distant metastasis was observed in 3.2% of the patients. The baseline characteristics of the patients are presented in **Table 1**. When patients aged 71 years and older were compared with patients aged 65-70 years, no difference was observed in terms of LVSI involvement, whether pelvic lymphadenectomy was performed or the number of lymph nodes dissected, disease-free survival, recurrence, and mortality. The five-year DFS and OS rates were 93% and 80%, respectively (**Figure 1-2**).

Table 1: Patient and tumor characteristics

	Patients (n:31,%)
Age	Median; 69 (range 65-86)
65-70 yr	19 (61.3%)
≥71 yr	12 (38.7%)
Comorbidity	
Yes	15 (48.4%)
No	16 (51.6 %)
Tumor Location	
Endometrium	25 (80.6%)
Uterin cervix	6 (19.4 %)
T Stage	
T1	5 (16.1%)
T2	26 (83.9 %)
Lymph node metastasis	
Yes	0 (0%)
No	31 (100%)
Surgical margin	
Positive	1 (3.2%)
Negative	30 (96.8%)
LVSI	
Yes	20 (64.5 %)
No	11 (35.5 %)
Tumor grade	
Grade 1	7 (22.6%)
Grade 2	16 (51.6 %)
Grade 3	8 (25.8%)

LVSI:Lymphovascular space invasion

Table 2: Treatment Details

	Patients (n:31,%)
Lymphadenectomy	
Only pelvic	13(42 %)
Pelvic + paraaortic/ Paraaortic sampling	8 (25.8 %)
None	10 (32.2 %)
Number of LNs removed	
Number of pelvic LNs removed	Median; 16 (range 5-64)
Number of paraoartic LNs removed	Median; 7 (range 2-30)
Chemotherapy	
No	27 (87.1 %)
Yes	4 (2.9 %)
Local recurrence	
Yes	1 (3.2%)
No	30 (96.8%)
Distant metastasis	
Yes	1 (3.2%)
No	30 (96.8%)

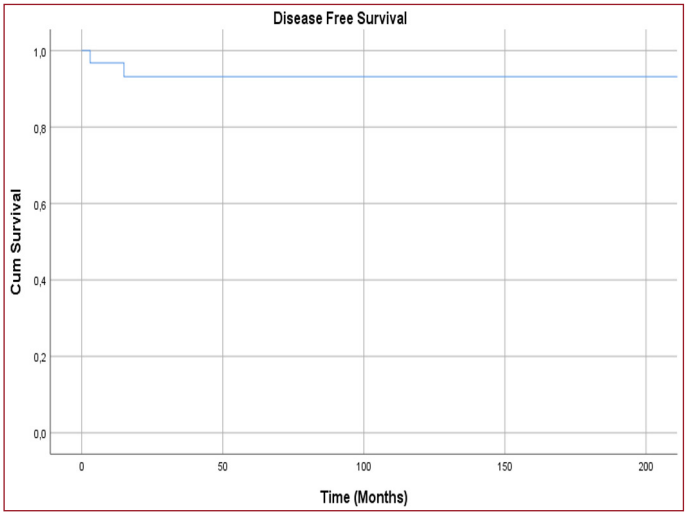

Figure 1. Kaplan-Meier plots of disease free survival.

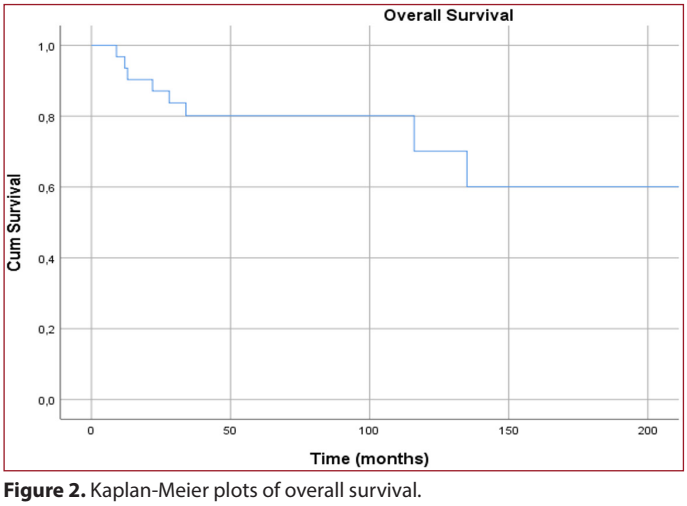

Figure 2. Kaplan-Meier plots of overall survival.

DISCUSSION

Fragility in the geriatric population reflects damage to the functionality of biological systems and it is important in the geriatric population to cope with the side effects of treatments such as surgery, CT and RT.[10,11] Each geriatric patient may have different characteristics from each other in terms of comorbidity, need for care, physical and mental health status, etc. Therefore, it would be beneficial to develop specific assessment tools for different cancer types, as opposed to a single assessment method.[12]

Sourdet et al.[13] established a geriatric oncology treatment evaluation team at Toulouse University Hospital consisting of medical oncology, radiation oncology, surgeon and nurse who are experts in the field of geriatrics. Patients aged 65 years or older diagnosed with cancer were evaluated by this team during the treatment planning phase. In 16.7% of 384 patients, the treatment plan was changed as a result of the geriatric team evaluation. It was observed that the most effective factors in the change of treatment plan were cognitive impairment (p=0.020), malnutrition (p=0.023), and low physical performance (p=0.010).

Racin et al.[14] evaluated geriatric patients with high intermediate risk and higher risk endometrial cancer according to whether lymphadectomy was performed. Adjuvant therapy was observed to be similar between the two groups. In this study, where the median age of the patients was 76.9, it was observed that the rates of DFS, cancer specific survival (CSS), and OS were statistically significantly lower in the group that did not undergo lymphadenectomy (p=0.076, p<0.001, and p<0.001, respectively). As a result of the study, it was emphasized that lymphadenectomy should be performed in geriatric patients with indications. However, in this study, the rate of adjuvant RT was only 45% in the group of patients who did not undergo lymphadectomy, and perhaps higher local control and survival rates could have been achieved if a higher rate of adjuvant RT had been applied to these patients.

Xie et al.[15] examined 36,816 uterine cervical cancer patients in their review on the Surveillance, Epidemiology, and End Results Program (SEER) database covering the years 2004-2015. When the two groups under 65 years of age and over were compared, it was observed that the 1- and 5-year CSS in the geriatric group was statistically significantly worse than the younger group. It was observed that patients who received surgery, radiotherapy or chemotherapy in the geriatric group had better survival outcomes than patients who did not receive any treatment. In the subgroup analyzes, it was observed that even in early stage geriatric patients, the group that received inadequate treatment or did not receive treatment had a statistically significant worsening course.

Cushman et al.[16] compared postoperative chemoradiotherapy (CRT) with RT alone in 166 geriatric uterine cervical cancer patients over 70 years of age with high risk factors such as parametrial invasion, positive surgical margins, or lymph node metastasis. No difference in OS was observed between the two groups, despite the number of accompanying risk factors or the evaluation of each factor separately. As a result of the study, it was emphasized that treatment selection can be made for geriatric patients on a patient basis, considering patient performance, toxicity and tolerability.

In our study, 39% of the patients Were 71 years or older. 67.5% of the patients had lymphadenectomy and external pelvic RT was applied to all of the patients and intracavitary vaginal brachytherapy (VBT) was applied to 90.3% of them. Only 3.2% of the patients had local recurrence and 3.2% had distant metastases. However, as seen in the above-mentioned studies, many factors seem to be effective on survival success while forming multidisciplinary treatments in the geriatric patient group. It seems useful to decide on a patient basis how surgical treatment, RT and CT should be performed and to evaluate the geriatric patient in detail.

CONCLUSION

Uterine cancers are among the most common cancers in women. Surgery followed by adjuvant RT also has tolerable and excellent oncological results in early stage geriatric uterine cancer patients. In order to increase the success of oncological treatment in the geriatric patient group, multimodality randomized studies shaped according to the patient group-specific prognostic factors, comorbidity and age group are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Istanbul Prof. Dr. Cemil Tascioglu City Hospital Ethics Committee (Date: 20.10.2022, No: E-48670771-514.99)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Original Article / Orijinal Araştırma

Changes in the Ubiquitination System in Children with Cerebral Palsy

Serebral Palsili Çocuklarda Ubiqütinasyon Sisteminde Değişiklikler

Ayşe Gülşen Doğan[1], İhsan Çetin[2]

[1]Hitit University Erol Olçok Training and Research Hospital, Department of Physical Medicine and Rehabilitation, Çorum, Turkey
[2]Hitit University, Faculty of Medicine, Department of Medical Biochemistry, Çorum, Turkey

Abstract

Aim: We aimed to investigate the levels of Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme (UHC-L1), Transactive Response DNA Binding Protein-43 (TDP-43) and Cullin-3 in peripheral blood associated with ubiquitination processes in children with cerebral palsy (CP).

Material and Method: We included 50 children with CP in the first patient group. In the control group, there were 30 healthy children who were matched with the patient groups in terms of age and gender. We also recorded risk factors for CP, CP type, botox application, orthosis use, maternal age at birth, and additional problems. Patients aged 6-10 years, diagnosed with CP, without genetic, metabolic disease or mental retardation history were included in this study.

Results: There were 32 female and 18 male patients in the CP group, while there were 19 female and 11 male volunteers in the control group. Maternal age was significantly higher in the CP group (p=0.002). In our study, as a result of the comparison between the control group and the CP group in terms of UCH-L1, TDP-43 and Cullin 3 levels; the levels of UCH-L1 (p=0.048), TDP-43 (p=0.028) and Cullin 3 (p=0.042) in the CP group were found to be statistically significantly lower than the levels of the control group.

Conclusion: The low serum concentrations of UCHL-L1, Cullin 3 and TDP-43 molecules in the CP group and the statistically positive correlation of these molecules with each other may help to understand the neuronal pathophysiology after disruption of the ubiquitination system.

Keywords: Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme, Transactive Response DNA Binding Protein-43, Cullin-3, cerebral palsy

Öz

Amaç: Serebral palsili (SP) çocuklarda ubikitinasyon süreçleri ile ilişkili periferik kanda Ubiquitin Karboksi Terminal Hidrolaz-L1 enzimi (UHC-L1), Transaktif Yanıt DNA Bağlayıcı Protein-43 (TDP-43) ve Cullin-3 düzeylerini araştırmayı amaçladık. .

Gereç ve Yöntem: Birinci grubuna SP'li 50 hasta dahil edildi. Kontrol grubunda yaş ve cinsiyet açısından hasta grupları ile eşleşen 30 sağlıklı çocuk vardı. SP, SP tipi, botoks uygulaması, ortez kullanımı, annenin doğum yaşı ve ek sorunlar için risk faktörlerini kayddildi. Bu çalışmaya 6-10 yaş arası, SP tanısı almış, genetik, metabolik hastalık veya mental retardasyon öyküsü olmayan hastalar dahil edildi.

Bulgular: SP grubunda 32 kadın ve 18 erkek hasta bulunurken, kontrol grubunda 19 kadın ve 11 erkek gönüllü vardı. Anne yaşı SP grubunda anlamlı olarak yüksekti (p=0,002). Çalışmamızda kontrol grubu ile SP grubunun UCH-L1, TDP-43 ve Cullin 3 düzeyleri açısından karşılaştırılması sonucunda; SP grubunda UCH-L1 (p=0,048), TDP-43 (p=0,028) ve Cullin 3 (p=0,042) düzeyleri kontrol grubuna göre istatistiksel olarak anlamlı düşük bulundu.

Sonuç: SP grubundaki UCHL-L1, Cullin 3 ve TDP-43 moleküllerinin düşük serum konsantrasyonları ve bu moleküllerin birbirleriyle istatistiksel olarak pozitif korelasyonu, ubiquitination sisteminin bozulmasından sonra nöronal patofizyolojinin anlaşılmasına yardımcı olabilir.

Anahtar Kelimeler: Ubiquitin Karboksi Terminal Hidrolaz-L1 enzimi, Transaktif Yanıt DNA Bağlayıcı Protein-43, Cullin-3, serebral palsi

Corresponding *(İletişim)*: Ayşe Gülşen DOĞAN, Hitit University Erol Olçok Training and Research Hospital, Department of Physical Medicine and Rehabilitation, Çorum, Turkey
E-mail *(E-posta)*: drmdagu@gmail.com
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INTRODUCTION

The ubiquitin-proteasome system is an important proteolytic pathway in eukaryotic cells. The ubiquitin-proteasome system is involved in numerous important processes required for cellular homeostasis, such as cell cycle regulation, apoptosis, receptor signaling, endocytosis, and others.[1,2] Cell growth and proliferation; It is controlled by ubiquitin-mediated degradation of tumor suppressors, protooncogenes and signal transduction components.[3,4] As a natural consequence of these, disorders in one or more of the ubiquitin-proteasome system components are considered to be one of the important causes of human diseases.[5,6]

Cerebral Palsy (CP) is the most common pediatric neurological disorder that occurs in the developing fetal or infant brain and is characterized by limitation due to developmental movement and posture disorders. CP is a lifelong neurodevelopmental disease that begins in early childhood.[7,8] In clinical and preclinical studies, neurodegenerative diseases have higher oxidative stress marker levels in the brain and peripheral tissues; antioxidant defense marker levels were shown to be lower. This indicates that oxidative stress is an important factor potentially playing a role in the pathogenesis of many different diseases affecting the brain such as mitochondrial disorders, cerebral ischemia and epilepsy.[9,10] It has been proven that oxidative stress markers increase and antioxidants decrease in CP, and as a result, the oxidative/antioxidant balance shifts to the oxidative side in children with cerebral palsy.[11,12] We think that disruptions in ubiquitination processes, which are caused by oxidative stress and ischemia, may also occur in children with cerebral palsy. In order to investigate this hypothesis, we aimed to investigate the levels of Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme (UHC-L1), Transactive Response DNA Binding Protein-43 (TDP-43) and Cullin-3 in peripheral blood associated with ubiquitination processes in children with cerebral palsy.

MATERIAL AND METHOD

This study was planned retrospectively. The study was carried out with the permission of Hitit University Medical Faculty Clinical Researchs Ethics Committee (Date: 05.07.2022, No: 2022/17). The sample was selected from children between the ages of 6-10 who applied to our physical therapy and rehabilitation outpatient clinic. Accordingly, we included 50 children with CP in the first patient group. In the control group, there were 30 healthy children who were matched with the patient groups in terms of age and gender. We also recorded risk factors for CP (premature, prolonged delivery, etc.), CP type, botox application, orthosis use, maternal age at birth, and additional problems. Informed consent was obtained from parents and children for voluntary participation in the study. Patients aged 6-10 years, diagnosed with CP, without genetic, metabolic disease or mental retardation history were included in this study.

Blood Collection

The blood samples of the children with cerebral palsy and the control group were taken into 8 mL capacity clot activator tubes by the blood collection nurse in our polyclinic between 08.00 and 10.00 after 12 hours of fasting. The blood, which was kept in tubes with clot activator at room temperature for half an hour, was centrifuged at 4,000 g for 10 minutes and then 4 mL of serum was obtained. Serum separated into Eppendorfs were stored at -70°C until analysis.

Biochemical Measurements

Measurement serum levels of Cullin-3, UCHL1 and TDP-43 were determined with enzyme linked immunosorbent assay method (SUNRED Biotechnology CO. LTD China; catalog number is 201-12-3552 for Cullin-3, catalog number is 201-12-2329 for UCHL1, and catalog number is 201-12-0334 for TDP-43, according to the manufacturer's instructions. These kits use a double-antibodys and wich ELISA to assay the level of Cullin-3, UCHL1 and TDP-43 in samples. Briefly, samples were added to wells which were pre-coated with monoclonal antibody and incubated; then, antibodies labeled with biotin were added, and combined with Streptavidin-HRP to form immune complex; then incubation and washing were carried out. Then chromogen solutions were added, and at the effect of stop solution, the color finally became yellow. We measured the optical density of each well under 450 nm wave length within 10 minutes after having added stop solution. According to standard concentrations and corresponding optical density values, we calculated the linear regression equation of the standart curve and we determined Cullin-3, UCHL1 and TDP-43 concentration of samples.

Statistical Methods

Frequency analysis was used for nominal and ordinal parameter description; mean and standard deviation were used for scale parameter description. Chi-Square, Likelihood Ratio and Fischer's Exact test were used for differences between categorical variables. Kolmogorov Smirnov test was used for normality test of scale parameters. Kruskal Wallis and Mann Whitney U tests were used for non-normally distributed variables. Independent Samples t-test and one Way ANOVA were used for normally distributed variables. Spearman's eho correlation analysis was used for relationship analysis. Multinominal logistic regression was used for multivariate analysis. All analysis was performed at SPSS 17.0 for windows, at 95% confidence interval and 0.05 significance level.

RESULTS

In our study, there were 32 female and 18 male patients in the CP group, while there were 19 female and 11 male volunteers in the control group. There was no significant difference between the groups in terms of age and gender (p=0.110, p=0.102). More spastic diplegic type (42%) pattern was observed in the CP group. Maternal age was significantly higher in the CP group (p=0.002). In our study, as a result of

the comparison between the control group and the CP group in terms of UCH-L1 levels; The levels of UCH-L1 (p=0.048), TDP-43 (p=0.028) and Cullin 3 (p=0.042) in the CP group were found to be statistically significantly lower than the levels of the control group (**Table 1**). In the correlation results, a positive correlation was observed between UCH-L1 and TDP-43 and Cullin-3 (**Table 2**).

Table 1. Baseline and research parameter differences between groups

	CP (n=50)	Control (n=30)	p value
Age, mean ± SD	8.00±1.66	8.32±1.36	0.110[a]
Gender, n (%)			
Female	32 (64.0)	19 (63.3)	0.102[b]
Male	18 (36.0)	11 (36.7)	
CP Type, n (%)			
Spastic tetraplegic	15 (30.0)		
Spastic diplegic	21 (42.0)		
Hypotonic	5 (10.0)		
Ataxic	4 (8.0)		
Diskinetic	5 (10.0)		
Risk factor, n (%)			
Premature	38 (76.0)		
Prolonged birth	8 (16.0)		
Postnatal throid	4(8.0)		
Mental retardation, n (%)	12 (24.0)		
Hearing disorder, n (%)	9 (18.0)		
Vision defect, n (%)	20 (40.0)		
Epilepsy, n (%)	28 (56.0)		
Device usage, n (%)			
None	10 (20.0)		
SOLID AFO	33 (66.0)		
KAFO	4 (8.0)		
PAFO	3 (6.0)		
Maternal age	31.11±4.89	26.54±4.39	0.002[c]
Cullin-3	6.17±4.17	6.33±5.20	0.042[a]
UCH-L1	4.28±2.99	5.56±4.93	0.048[a]
TDP-43	1077.38±769.39	1085.59±623.26	0.028[a]

a. Kruskal Wallis Test, b. Chi-Square Test, c. One Way ANOVA, d Independent Samples t-test, SD: Standard Deviation, CP: Cerebral palsy, AFO: Ankle foot orthosis, KAFO: Knee-ankle-foot orthosis, UCH-L1: Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme, TDP-43: Transactive Response DNA Binding Protein-43

Table 2: Spearman's rho correlation analysis between UCH-L1, Cullin-3 and TDP-43 for CP group

UCH-L1	CP (n=50)
Cullin 3	0.426*
TDP 43	0.463*

*p<0.05, UCH-L1: Ubiquitin Carboxy Terminal Hydrolase-L1 enzyme, TDP-43: Transactive Response DNA Binding Protein-43, CP: Cerebral palsy

DISCUSSION

In our study, as a result of the comparison of the control group and the CP group in terms of UCH-L1, TDP-43 and Cullin 3 levels; It was found that the levels of all three molecules in the CP group were statistically significantly lower than in the control group.

Ubiquitination, the covalent attachment of ubiquitin to a target protein, regulates most cellular processes and is involved in several neurological disorders.[13,14] The process of ubiquitination is reversible and the reverse process is called deubiquitination which is accomplished by deubiquitinating enzymes.[15] The UCH-L1 molecule is highly and specifically expressed in neurons and plays a role in the ubiquination process of proteins to be degraded via the proteasomal pathway, and plays a role in clearing oxidized or misfolded proteins that occur in normal and pathological processes.[14,16] In the study conducted by Linrui et al.[17] in Parkinson's patients, they found a low level of UCH-L1 in the patient group and associated it with cognitive dysfunction. UCH-L1 concentration has been reported to be elevated in a number of neurological diseases including aneurysmal subarachnoid hemorrhage, traumatic brain injury, stroke and neonatal hypoxic-ischemic encephalopathy.[18-23] In the studies performed, it was observed that the levels of UCH-L1 in the cerebrospinal fluid and plasma after the seizure were increased in patients with epileptic disorders, and when the patients with recurrent seizures were compared with the patients with a single seizure, the UCH-L1 levels were higher in the group of patients with recurrent seizures than in the other group.[24,25] There is only one study in the literature measuring the level of UCH-L1 in patients with CP. In the clinical efficacy study of scalp acupuncture in 45 patients with spastic CP, researchers concluded that scalp acupuncture can effectively treat spastic CP, improve cerebral hemodynamics and gross motor function, reduce muscle tension and spasticity, and improve daily living ability. In this study, they found the UHC-L1 level to be significantly higher in the treatment group compared to the control group.[26] Similar to this study, in our study, the UCH-L1 level in patients with CP was found to be significantly lower than in the healthy group. This result may show us that there is a disruption in the ubiqunitation system in SP.

TDP-43 is a 43 kDa heterogeneous nuclear ribonuclear protein (hnRNP) composed of 414 amino acids.[27] TDP-43 is synthesized in the cytoplasm and shuttled into the nucleus where it primarily resides to perform its physiological functions.[28] Pathological forms of TDP-43 were first identified in 2006 when Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration patients were found to have tau-negative, ubiquitin-positive cytoplasmic inclusion bodies. The pathogenic mechanisms in these brains ultimately result in TDP-43 depletion from the nucleus, TDP-43 mislocalization into the cytoplasm, and the formation of insoluble aggregates that contain TDP-43 with multiple posttranslational modifications including ubiquitination, phosphorylation, and truncation. These TDP-43 inclusion bodies found in neurons, neuronal cell processes, and glia are now characteristic of the pathology in the most common forms of Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration.[29,30] In the study of Meneses et al30, they found that TDP-43 pathology is a risk factor for the development of Alzheimer's type dementia and that TDP-43 pathology increases the rate of hippocampal atrophy

in AD. Aging is considered a risk factor for developing TDP-43 pathology in neurologically normal individuals. From 286 consecutive autopsy brains, Uchino and coworkers[31] reported that 40% of control elderly individuals (78.5 ± 9.7 years) with minimal senile plaques had TDP-43 pathology. TDP-43-positive individuals were reported to be significantly older than those without TDP-43 pathology from a study investigating TDP-43 in the anterior temporal pole cortex. [32] TDP-43-positive dystrophic neurites have been found in patients with the parkinsonism-dementia complex on Guam. [33] In the literature, we could not find any study conducted in patients with CP with TDP-43. In our results, lower TDP-43 levels in the patient group compared to the health group can be considered as an indicator of the deterioration in the ubiquitination mechanism.

Cullins are proteins that play a role in post-translational modification of proteins, including ubiquitination that confer substrate specificity to multimeric complex of E3 ligases acting as scaffold proteins. So far, seven members of the cullin family of proteins have been identified. Cullin 3 has begun to emerge as a protein involved in the etiopathology of multiple diseases.[34,35] The deregulation of Cullin 3 activity could be a mechanism involved in pathologies mainly associated with oxidative stress and cell cycle deregulation. Therefore, strategies oriented against Cullin 3 activity comprise a high therapeutic potential for regulation of cellular processes related to the development of several pathologies.[35]

Limitation

The biggest limitation of this study is that we looked at the levels of UCH-L1, TDP-43 and Cullin 3 molecules in the cerebrospinal fluid. The reason is that we cannot compare our results with other comparison results, as there is no other study including all parameters.

CONCLUSION

The results obtained from our study, the difference in the molecular levels of the patients with CP and the control group show that the neuronal structure is impaired in CP. The low serum concentrations of UCHL-L1, Cullin 3 and TDP-43 molecules in the CP group and the statistically positive correlation of these molecules with each other may help to understand the neuronal pathophysiology after disruption of the ubiquitination system. We believe that these results will contribute to the literature and will be a good reference for future studies. In order for these parts to be used in CP pathogenesis and treatment approaches, arrangements with more patient groups are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Medical Faculty Clinical Researchs Ethics Committee (Date: 05.07.2022, No: 2022/17).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Comparative Evaluation of the Effects of Sevoflurane and/or Dexmedetomidine on Behavior, Neuro-inflammation and Apoptosis in Pups Rat

Sevofluran ve/veya Deksmedetomidin'in Sıçan Yavrularında Davranış, Nöroinflamasyon ve Apoptoz Üzerindeki Etkilerinin Karşılaştırmalı Değerlendirilmesi

Semin Turhan[1], Berrin Işık[2], Mehmet Arda İnan[3]

[1]Hitit University Erol Olcok Training and Research Hospital Department of Anesthesia and Reanimation, Çorum, Turkey
[2]Gazi University Faculty of Medicine Department of Anesthesia and Reanimation, Ankara, Turkey
[3]Gazi University Faculty of Medicine Department of Pathology, Ankara, Turkey

Abstract

Aim: Developing brain is vulnerable to side effects of anesthetics. Neurotoxic and cognitive alterations have been documented in several species, and there is concern that small children could be affected adversely if they are exposed for long periods or recurrently to inhalation anesthesia In this experiment we aim to evaluate behavioral and neurotoxic effects of sevoflurane (SEVO) and/or dexmedetomidine (DEX) exposure in pup rats.

Material and Method: Postnatal 21 days old 36 rat were randomly divided into 6 groups (Group I (control); Group II:2.5% SEVO for 4 hours; Group III:2.5% SEVO for 4 hours+intraperitoneal (i.p.) 0.5 µg.kg-1 DEX; Group IV:2.5% SEVO for 4 hours+i.p. 5 µg.kg-1 DEX; Group V: i.p. 0.5 µg.kg-1 DEX; Group VI: i.p. 5 µg.kg-1 DEX was given). Behavior of the rat were examined with the modified Radial Arm Maze test. Histopathological evaluation of the pups' rat brain for neuroinflammation and apoptosis was performed. Statistical evaluation was carried out using the SPSS 20.0, P value <0.05 was considered statistically significant.

Results: Single 2.5% SEVO exposure for 4 hours during early life period in rats is although not show neuroinflammation signs the brain tissue histologically but impaired learning and memory in behavior test (P<0.05). In CA3 stage of the brain tissue apoptosis percentage was diminished in SEVO+DEX groups for comparison with control and single SEVO groups (P<0.05).

Conclusions: Adding DEX to SEVO caused less impairment in memory and learning function. But single 5 µg.kg-1 DEX negatively affected learning and memory function but not locomotor activity and anxiety.

Keywords: Sevoflurane, dexmedetomidine, pup rats, nteurotoxic, cognitive alterations

Öz

Giriş: Gelişen beyin anesteziklerin yan etkilerine karşı savunmasızdır. Birçok türde nörotoksik ve bilişsel değişiklikler belgelenmiştir ve küçük çocukların uzun süreler veya tekrar tekrar inhalasyon anestezisine maruz kalmaları durumunda olumsuz etkilenebileceği endişesi vardır. Bu deneyde sevofluranın (SEVO) davranışsal ve nörotoksik etkilerini değerlendirmeyi amaçlıyoruz. ve/veya yavru sıçanlarda deksmedetomidin (DEX) maruziyeti.

Gereç ve Yöntem: Postnatal 21 günlük 36 rat rastgele 6 gruba ayrıldı (Grup I (kontrol); Grup II: 4 saatlik %2,5 SEVO; Grup III: 4 saatlik %2,5 SEVO+intraperitoneal (i.p.) 0,5 µg). kg-1 DEX; Grup IV: 4 saat için %2,5 SEVO+i.p. 5 µg.kg-1 DEX; Grup V: i.p. 0.5 µg.kg-1 DEX; Grup VI: i.p. 5 µg.kg-1 DEX verildi) . Sıçanların davranışları modifiye Radial Arm Maze testi ile incelendi. Yavruların sıçan beyninin nöroinflamasyon ve apoptoz için histopatolojik değerlendirmesi yapıldı. İstatistiksel değerlendirme SPSS 20.0 kullanılarak yapıldı, P değeri <0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Sıçanlarda erken yaşam döneminde 4 saat boyunca tek seferlik %2,5 SEVO maruziyeti histolojik olarak beyin dokusunda nöroinflamasyon belirtileri göstermemekle birlikte davranış testinde öğrenme ve hafızada bozulmaya yol açmıştır (P<0,05). Beyin dokusunun CA3 aşamasında apoptoz yüzdesi SEVO+DEX gruplarında kontrol ve tek SEVO grupları ile karşılaştırıldığında azaldı (P<0.05).

Sonuç: SEVO'ya DEX eklenmesi, hafıza ve öğrenme işlevinde daha az bozulmaya neden oldu. Ancak tek 5 µg.kg-1 DEX, öğrenme ve hafıza işlevini olumsuz etkiledi, ancak lokomotor aktiviteyi ve kaygıyı etkilemedi.

Anahtar Kelimeler: Sevofluran, deksmedetomidin, yavru fareler, nörotoksik, kognitif değişiklikler

Corresponding *(İletişim)*: Semin TURHAN, Çepni, İnönü Cd. No:176, 19040 Merkez/Çorum, Türkiye
E-mail *(E-posta)*: smnondr@hotmail.com
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INTRODUCTION

Diagnostic and therapeutic procedures are becoming more common worldwide as technology advances. This development has increased anesthesia exposure for people of all ages, including fetuses. More fetuses and children under three are expected to be subjected to general anesthesia each year. During their early years, children are more vulnerable than adults to the side effects of volatile anesthetics. Neurotoxic and cognitive changes have been observed in several species, raising concerns that small children may be harmed if exposed to inhalation anesthesia for prolonged periods or regularly.[1,2]

Sevoflurane (SEVO) is a GABAergic inhalational anesthetic agent commonly used for pediatric anesthesia due to its rapid onset of action, short recovery time, and non-irritation of the upper airway.[3] Today, GABA and NMDA-mediated neuronal activity play a crucial and well-documented role in cognitive processes.[4] On the other hand, dexmedetomidine (DEX) is a relatively new agent that is a selective agonist of α2-receptors with sedative, anxiolytic, analgesic, and anesthetic properties. [5] In addition, the neuroprotective effects of DEX have been reported in different animal models.[6]

People are becoming increasingly interested in the relationship between early anesthesia exposure and cognitive function. The primary emphasis of experimental pediatric anesthesia research is on the effects of anesthetics on the brain during the fetus's rapid neuromotor development and shortly after birth. Experimental studies in recent years suggest that inhalation anesthetics may have long-lasting and permanent effects in neurodevelopmental stages by increasing neuronal cell death (apoptosis) and decreasing neurogenesis.[7]

During synaptogenesis, human brain tissue is vulnerable to neurotoxic agents, particularly during the third trimester of pregnancy and the first 2–3 years of life.[8] Therefore, for the sake of public health, it is crucial to determine whether anesthetic toxicity manifests itself during the rapid neurodevelopmental period. As a result, more research is required to determine the potential neurotoxic effects of various anesthetic agents at different developmental stages, the factors that increase and reduce anesthesia-induced neurotoxicity, and possible mechanisms.

This experimental research aims to evaluate pup rats exposed to SEVO and DEX anesthesia during their early life stage by analyzing the immediate histological damage and behavioral changes in the pups.

MATERIAL AND METHOD

In this experiment, 36 Wistar albino rat pups (postnatal 21 days old [PD21]) were used (sample size calculated by power analyses). The rats were housed under standard laboratory conditions (12-hour daytime lighting [lights on from 07:00 to 19:00] and 12-hour nighttime lighting, 20–22°C room temperature, 50–60% humidity, ad libitum feeding) from birth. The day of birth was designated as post-natal day zero (P0). All pups were carefully monitored throughout the experiment for weight and general appearance. The rats were randomly assigned to the anesthesia or control groups (n=6 per group).

Olton and colleagues introduced the radial maze task in 1976 as a measure of working memory for spatial information.[9] This experiment was performed on an eight-arm maze apparatus. To determine behavioral manifestations of any possible acute developmental deficiency in the brain, behavioral parameters were collected for each animal before sacrifice, before, and on day three after anesthetic exposure.

PD21 infant rats were exposed to study drugs with compatible groups. A 2.5% SEVO concentration and 4 h of SEVO exposure were chosen based on previous studies for an induced apoptotic response without mortality.10 Effective anesthesia level was measured by the tail pinch test, with 3–4 lb pressure on the tail root for 15 seconds.

Group I: Control Group, 100% O_2 3L/min, 0.1 mL serum saline three times at the zeroth, second and 4th hour(h) time points intraperitoneally (IP) for determination of placebo effects (n= 6); Group II: 4 h 2.5% SEVO, (AbbVie Tıbbi Ilaclar San. ve Tic. Ltd. Sti. Istanbul-Türkiye) in 100% O_2 3L/min-1 (n=6); Group III: 4 h 2.5% SEVO in 100% O_2 3L/min-1 + 0.5 µg/kg-1 DEX, (Precedex-Abbott-USA) three times at 0., 2.,4. h time points IP (n=6); Group IV: 4 h 2.5% SEVO in 100% O_2 3L/min-1 + 5 µg/kg-1 DEX three times at 0., 2.,4. h time points IP (n=6); Group V: 100% O_2 3L/min-1 + 0.5 µg/kg-1 DEX three times at 0., 2.,4. h time points IP (n=6); Group VI: 100% O_2 3L/min-1 five µg/kg-1 DEX three times at the zeroth, second and 4th hour h time points IP (n= 6).

Each rat in six groups in every test received a 10-minute session on the RAM platform. A modified RAM test was performed twice during the pre-anesthesia and post-anesthesia periods. During this process, video recordings were taken by the researcher. All behavioral testing was conducted during the light cycle by an experimenter. Video records were analyzed blinded to the group allocation of the assessed animal. Pellets were placed on RAM platform arms 1, 2, 3, and 6, and the rats were then placed on the RAM setup to evaluate the hippocampal destruction.

The rats' learning (Reference Memory Error [RME]), memory (Working Memory Error [WME]), locomotor activity (Total Distance [TD]), and anxiety behavior (Rearing [R]) were evaluated respectively by utilizing the total number of the entries to the arm, number of the entries to the baited arm.

In the first and second experiment, the number of the entries to the arm and the number of the entries to the baited arm, respectively, were noted down. At the second experiment, if the rats entered the non-baited arm more than once, their rate was recorded as WME. Finally, the number of entries for the arm and arm length (number of arms entered × 2 × arm length) were calculated and recorded as TD. Rats' behavior, like standing on their rear limbs, was recorded as R. Supported rearing behavior, in which the animal rears against the arena walls, could also be observed in this test but was ignored in this experiment.

The survival rate and time of death at the time of exposure to anesthesia were recorded before the trial ended.

Tissue Sampling and Histopathological Evaluation

After the second RAM test, the rat pups were sacrificed while being put to sleep with ketamine (Alfamine 10%; Ata Fen Veteriner Malzemeleri, Izmir/Türkiye) and 5 mg/kg xylazine (Xylazinbio 2%; Intermed Ecza Deposu, Ankara/Türkiye) by intracardiac blood aspiration. Neonatal rat bilateral hippocampal brain tissues were harvested for neuroinflammation and apoptosis tests. Their brains were extracted from their bodies and coded at random. A 10% formol solution was used to protect the coded brain tissues. The brain samples were paraffin-embedded and stained with hematoxylin and eosin. (H&E). Taking Bregma Point into account, the rat brain tissue was coronally cut 4 µm thick. CA1, CA2, and CA3 sectors are given granular lamel neurons and were evaluated for inflammation, degenerative changes (necrosis, hyperemia, gliosis, and spongy changes), and apoptosis in the hippocampus and cornu ammonia. (×400 Olymphos). Apoptotic cells were counted and examined blindly at ×400 magnification.

Ethics

On February 21, 2020, Gazi University Local Ethics Committee for Animal Experiments in Ankara, Turkey, granted ethics clearance for this research (G.Ü. ET. 20.013 code number). The trial was carried out at Gazi University Experimental Research Center between the dates of 07.27.2020 and 08.21.2020. The experiments were carried out per the "Guide for the Care and Use of Laboratory Animals"

Statistical Analyses

Statistical analysis was performed in the SPSS 20.0 program. Statistical analysis data are expressed as mean±standard deviation (SD), median, minimum, maximum, and n (%). The p-value used to define statistical significance was $p<0.05$. The Shapiro–Wilk test was applied to the measurable parameters (number of entries to the arm and number of entries to the baited arm at the RAM setup) to determine whether the distribution was normal or not. The one-way ANOVA test in independent groups was used to determine whether there was a significant difference between groups. In case of a significant difference, a comparison was made between groups with Bonferroni correction for the post hoc analysis test. A Greenhouse–Geisser correction was used for sphericity analysis in repeated measurements. Statistical evaluations of histopathological data were made with the Kruskal–Wallis test. A post hoc analysis with Mann–Whitney U was performed, which obtained statistically significant differences between groups. Apoptotic cell density data are presented as mean±standard error of the mean and comparison between means was determined using one-way ANOVA followed by the Bonferroni post hoc test for all statistical tests.

RESULTS

All the rats in the experiment were PD21. Their mean weights±SD were as follows: Group I: 31.4±2.04 g, Group II: 31.4±1.7 g, Group III: 30.6±1.7 g, Group IV: 30.05±1.34 g, Group V: 31.3±2.1 g, Group VI: 29.7±1.5 g, and no statistically significant difference was found (p=0.370). No rats perished during anesthesia exposure on the third day. One rat in Group IV died before the second RAM test on the day of the trial. As a result, the data from only 35 rodents was included in this study.

The RAM test experiment for the rats was assessed before and after anesthesia. The number of entries to the arm, the number of non-baited arms, the number of re-entries to baited arms, and the TD and R numbers were all recorded during the first and second experiments. The number of entries to the non-baited arm and the number of re-entries to the baited arm were quantified as RME and WME during the second experiment.

The RME/TEN ratio was then determined. The values in each category were similar, but they had significant differences. For example, when the RME ratio was compared between the groups, there were substantial differences between Group II and Groups I, III, and IV (P=0.015, P=0.004, and P= 0.001). There was also a variation between Groups IV and VI (P=0.028). **Table 1** displays the RME value to TEN percentage (**Figure 1**).

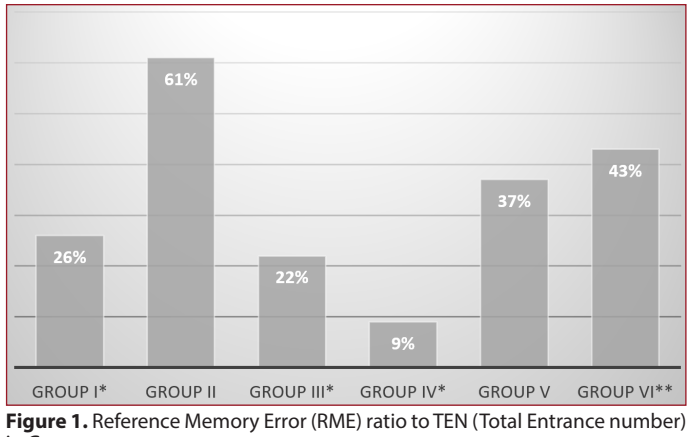

Figure 1. Reference Memory Error (RME) ratio to TEN (Total Entrance number) in Groups
*p<0,05 Comparison with Group II, **p<0,05 Comparison with Group IV
Comparison with Group I to Group II (p=0,015), Comparison with Group II to Group III (p=0,004), Comparison with Group II to Group IV (p<0,001), Comparison with Group IV to Group VI (p=0,028).

Table 1. Reference Memory Error (RME) in Groups (Mean±SD)

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
TEN	14,67±7,91	19,33±7,528	18,50±6,71	12,37±12,3	10,17±3,601	15,83±2,229
RME	2,67±1,211	10,67±2,503	3,50±1,975	1,60±1,67	3,83±2,041	7±2
%RME	%25,80	%60,40	%21,30	%9,50	%37,26	%43,60

n: Rat number, RME: Referance Memory Error, TEN: Total Entrance Number (Mean) Values, %RME: Ratio of RME to TEN

Working memory errors were determined using the number of re-entries to the baited arm and TEN variables (**Figure 2**). The percentage of WME to TEN was calculated, and there was a significant variation between groups (p=0.001). Furthermore, a significant difference in WME was observed when comparing Group II to Groups I, III, IV, V, and VI (respectively, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.017, p=0.030). In addition, the comparison of Group IV to Group VI's variables revealed significant variations (p=0.039).

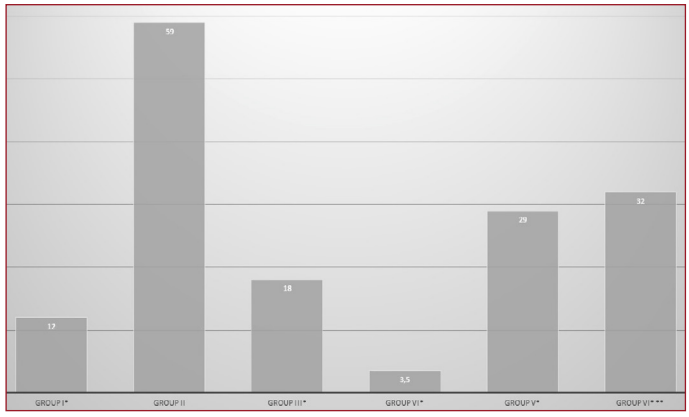

Figure 2. Working memory error (WME) ratio to total entrance number (TEN), (%)
*p<0,05 Comparison with Group II, **p<0,05 Comparison with Group IV
*(Comparison for Group II with Group I (p=0<0.001), Group III (p<0,001), Group IV (p<0,001), Group V (p=0.017), and Group VI (p=0.030)
**(Comparison for Group IV with Group VI (p=0,039)

TEN variables were used to evaluate learning behavior before and after anesthesia. Group II had a considerably higher total entrance number at pre-anesthesia versus post-anesthesia (p=0.045). Although TEN was was significantly higher (p=0.05) in Group VI, it was not in Groups I, III, IV, or V (p=0.671, p=0.590, p=0.278, and p=0.395) (**Table 2**).

Group VI had substantially longer pre-anesthesia and post-anesthesia TDs (p=0.009) than the other groups (**Table 3**) (**Figure 3**). TD was greater in some groups but not statistically significant in others, with the exception of Group VI (p=0.009). To evaluate anxiety, the rats' rearing number was measured during the post-anesthesia interval. On the second try, the rats were expected to exhibit less anxiety and increased R behavior (increase in R number). Heightening behavior, as if on two limbs, was commonly observed in Group IV but was not identified as R behavior. Groups V and VI saw an increase in the number of rearings. Group VI saw a significant increase (p=0.032) (**Table 4**).

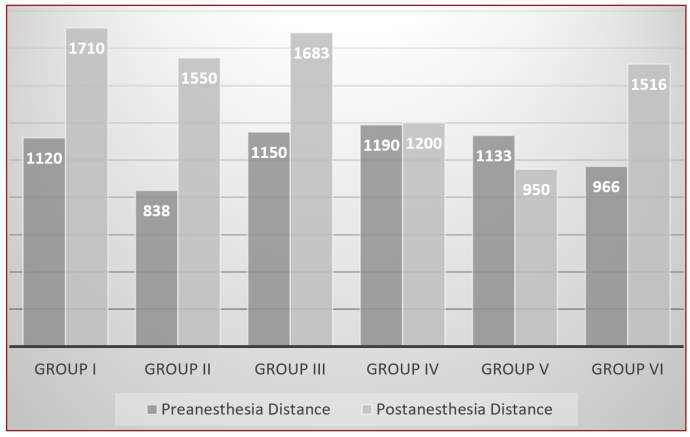

Figure 3. Preanesthesia and postanesthesia total distance variables in groups (cm)

Table 2. Comparison of preanesthesia and postanesthesia TEN values in Groups (Mean±SD).

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
TEN Preanesthesia	16,17±7,25	10,17±3,971	20±3,162	17,8±5,167	14±8,602	11,5±2,950
TEN Postanesthesia	14,67±7,91	19,33±7,528	18,50±6,71	12,37±12,3	10,17±3,601	15,83±2,229
P	0,671	0,045*	0,59	0,278	0,395	0,05*

n: Rat number, TEN: Total Entrace Number (Mean) *p≤0.05 *(Preanesthesia versus Postanesthesia is significantly different in Group II, and Group VI)

Table 3. Preanesthesia and postanesthesia total distance (Mean±SD).

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
Preanesthesia distance	1120±461,7	838±373,9	1150±384,7	1190±469,5	1133±753,4	966±273,2
Postanesthesia distance	1710±980	1550±788	1683±756	1200±1141,8	950±387	1516±248,3
p	0,171	0,137	0,146	0,982	0,673	0,009*

*p<0.05

Table 4. Rearing Number of the Groups (Mean)

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
Rearing number Preanesthesia	1,83±1,6	0,83±0,75	3±3,6	1,2±1,7	4,33±2,7	0,83±0,73
Rearing number Postanesthesia	0,5±0,8	0,50±0,8	2,67±2,42	0	6±5,02	4±2,2
P	0,121	0,576	0,75	0,208	0,352	0,032*

Preanesthesia versus postanesthesia (Mean±SD), n= rat number, * p=0,05

No signs of neuro-inflammation were found in the histopathological evaluation (H&E staining) of the brain tissues in the groups. There was no statistically significant difference in apoptosis percentages between the hippocampus's CA1 (p=0.122) and CA2 (p=0.121) stages. However, in the CA3 stages, apoptosis percentages showed a significant difference among the groups (p=0.015) (**Figure 4**). CA3 region apoptosis ratio was higher in Group III than in Groups I, II, and IV and higher in Group IV than in Groups I, II, and VI. The significant difference was determined for comparison between Group III and Groups I (p=0.017), II (p=0.041), and VI (p=0.049), and for comparison between Group IV and Groups I (p=0.004), II (p=0.01), and VI (p=0.013) as a result of the post hoc analysis. Histological images of brain slices for apoptosis in groups are shown in **Figure 4**.

DISCUSSION

Pre-anesthetic and post-anesthetic cognitive functions (learning, memory, locomotor activity, and anxiety) of rats were measured in this experiment (PD21). An eight-arm RAM platform was used to assess RME, WME, TD, and R behavior in Wistar albino rats. Histopathologic techniques were also used to examine pup rat brain slices for neuro-inflammation and apoptosis after H&E staining.

There were more impairments in memory and learning in the experimental group than in the control group. Additionally, memory and learning ability impairments were reduced when DEX was added to SEVO. However, a single large dose of DEX (5 g/kg-1) had a more negative impact on learning and memory function than in the control group but not on locomotion or anxiety. The apoptosis ratio in the CA1 and CA2 regions of the hippocampus did not vary significantly between the groups when histopathological characteristics of the brain tissue were compared. However, it varied significantly between the groups in the CA3 region during the same period. Compared to the control, one 2.5% SEVO, and five g/kg-1 DEX applied groups, two groups exposed to SEVO with DEX showed reduced levels of apoptosis.

It is well known that Wistar albino rats can be used in studies performed to reveal neuro-apoptosis and cognitive tests. Both their genetic similarities to humans and their ability to perform in behavioral tests create a suitable basis for them to be used in studies of this nature. The brain in rats is structurally similar to that of humans (prosencephalon, mesencephalon, rhombencephalon-hindbrain). There are also similarities in terms of the development of neurological tissues. Synaptogenesis in rats develops between 7 and 30 days after birth.[11,12] One human year equals approximately two rat weeks (365÷26.7=13.8 rat days).[13] In this experiment, 21-day-old rats with the highest synaptogenesis were preferred to evaluate the rapid brain development phase.

Hemodynamic stability during anesthesia exposure is an important issue. Studies have shown that 3% SEVO exposure induces hypoxia or respiratory depression in rats.[14,15] Additionally, it is reported that the motor functions were not impaired in a study conducted with 30-day-old rats given SEVO for 4 h. However, in terms of learning and memory, SEVO caused cognitive deficits. Therefore, in this experiment, rats were exposed to 2.5% SEVO for 4 h to avoid hypoxia and respiratory depression.

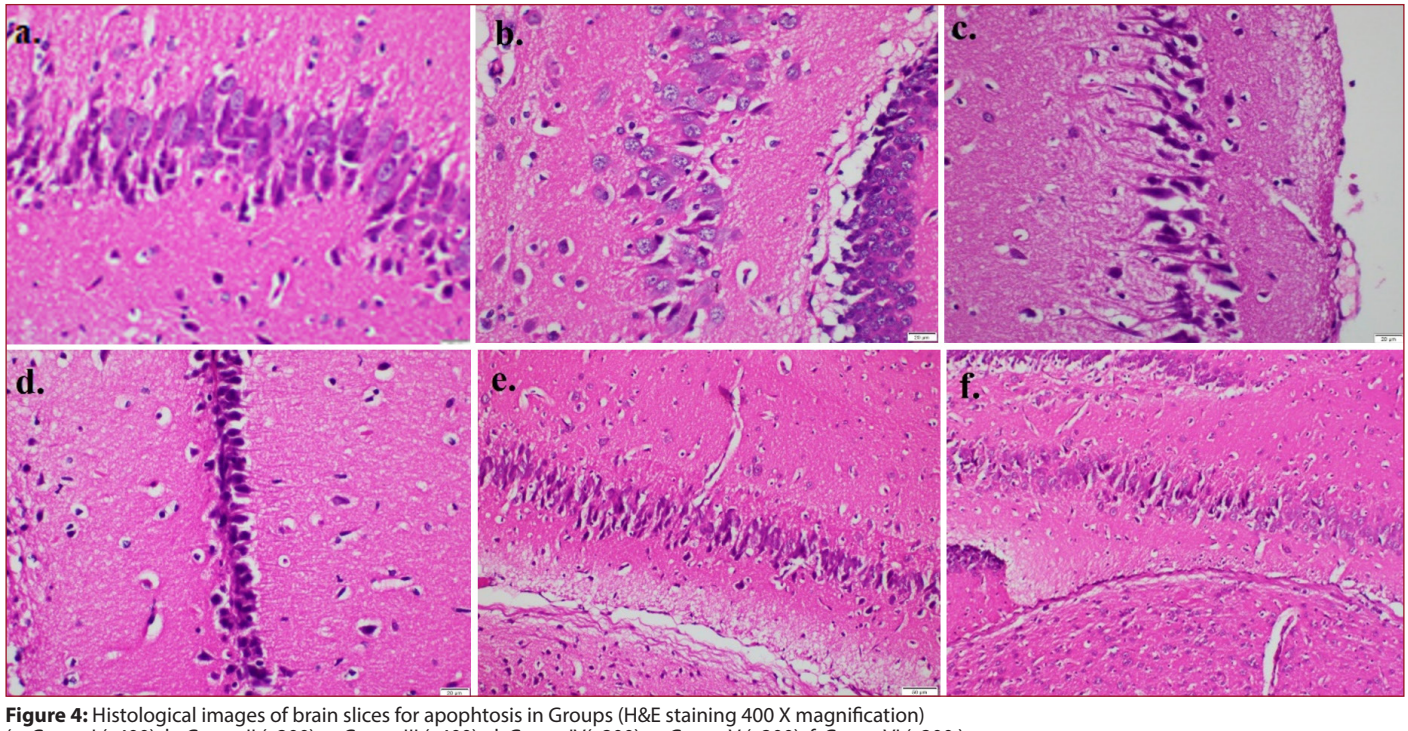

Figure 4: Histological images of brain slices for apophtosis in Groups (H&E staining 400 X magnification)
(a. Group I (x400), b. Group II (x200), c. Group III (x400), d. Group IV(x200), e. Group V (x200), f. Group VI (x200)

Reference memory error and WME are evidence of learning and memory problems. Learning behavior can be assessed with ten arms.[16,17] In addition, TD was measured to reveal locomotor activity before and after anesthesia. Pirke et al. also evaluated locomotor activity using a similar method.[18] Sagvolden et al. have also associated these behaviors with hyperactivity.[19]

Apoptosis is triggered by extrinsic and/or intrinsic cellular pathways. The central role of mitochondria in the intrinsic apoptotic death pathway has been established. Hippocampal neural apoptosis is related to the mitochondrial pathways, and this effect could be dose-dependent; however, studies on the pathways involved in the SEVO-induced development of brain apoptosis are limited. Shen et al. demonstrated that a higher dose of SEVO exposure at PD7 in Sprague Dawley rats leads to histopathological changes and apoptosis in the neonatal rat hippocampus and temporal neuro-cognition deficits.[20]

The hippocampus is a brain area that has an important role in spatial learning and memory functions.[21] Studies show that interrupting the hippocampal pathways causes significant memory deficits in the radial arm labyrinth test.[22] Recently, the CA3 region has attracted major attention for its specific role in memory processes and neuro-degeneration. CA1 region neurons receive and process information from the entorhinal cortex or CA3 region. A solid CA3 and CA1-CA3 connection is necessary for the reference memory. CA3 subfields have richer internal connectivity in hippocampal regions. CA3 pyramidal cells make excitatory contacts with neighboring inhibitory and excitatory neurons. This circuit is implicated in episodic memories and encoding spatial representations. The CA3 region receives inputs from the entorhinal cortex via mossy fiber connections. These connections are essential for memory formation.[23] In this study, baby rats' CA1, CA2, and CA3 brain regions were evaluated histologically.

Neurons are particularly sensitive during the synaptic plasticity phase. Inhalation anesthetics can induce neuronal apoptosis or programmed cell death in the developing brain, leading to long-term cognitive impairments. Exposure to SEVO during the early life period induces neuronal apoptosis, and cognitive dysfunction in a dose-recurrence and time-dependent manner in baby rats has been shown experimentally.[24] Furthermore, it has been demonstrated that higher doses of SEVO can cause histopathological changes and induce apoptosis in the neonatal rat hippocampus.[25] In our study, the number of RME and WME increased significantly in a single SEVO-exposed rat group compared to the control and other groups. But the evaluation time of the rats' RAM experiment was relatively earlier than in Li et al.'s experiment. It was revealed that the administration of a single dose of 2.5% SEVO for 4 h negatively affected cognitive functions in terms of behavior. This result is consistent with the research conducted by Perez-Zoghbi et al.[26]

A decrease in the number of neuronal cells can lead to decreased brain functions. Our experiment shows that a single 2.5% SEVO exposure for 4 h during the early stages of life in rats does not show histologically detectable neuroinflammation signs in the brain tissue. But, in the CA3 stage of the brain tissue, the apoptosis percentage was diminished in the SEVO+DEX groups compared to the control and single SEVO groups.

Not all experiments on rats report behavioral deficits after exposure to SEVO. For example, Chen et al. found that a lower dose of SEVO promotes hippocampal neurogenesis in neonatal rats and facilitates their experiment in dentate gyrus-dependent learning tasks.[27] In our experiment, post-anesthetic learning, evaluated on the RAM platform, was decreased in rats exposed solely to SEVO or SEVO + high-dose DEX.

Dexmedetomidine is a potent α2-adrenergic receptor agonist that is an adjunct to general anesthesia, reduces anesthetic doses, and provides analgesia and sedation in the perioperative period.

The findings from the published animal research on the comparative effects of SEVO and/or DEX exposure in the early life period in rats are contradictory. Goyagi reported that SEVO-dependent neurodegeneration decreased with DEX.[28] However, even DEX has been reported to decrease neuron apoptosis, and cognitive decline is caused by ketamine, isoflurane, and propofol.[29] In addition, DEX has been reported to have a protective effect in hypoxic-ischemic neonatal brains.[30] Perez-Zoghbi et al. reported that DEX reduces SEVO-induced apoptosis in several brain regions when used at 1 μg/kg^{-1} doses. But co-administration of DEX at 5 μg/kg^{-1} during SEVO anesthesia increased mortality.[31]

In this study, 0.5 μg/kg^{-1} and 5 μg/kg^{-1} DEX doses were used, and DEX added to SEVO caused less memory and learning function impairment. However, high doses (5 μg/kg-1) of DEX exposure negatively affected the learning and memory functions of the rats but not their locomotor activity or anxiety. In addition, one rat died just before the second RAM test experiment in the 5 μg/kg^{-1} DEX with SEVO group (Group IV).

The hippocampus is crucial for cognitive functions such as learning and memory in humans and animals. Several experimental studies have indicated that exposure to DEX ameliorates oxidative stress-induced cognitive deficits and restorative abnormal hippocampal synaptic plasticity.

The solely 2.5% SEVO-exposed group in this research had a higher RME and WME than the other groups and a higher WME than the high-dose DEX+SEVO group. Although there was no statistically significant difference between the DEX-only groups (Groups V and VI), they performed better regarding RME and WME than the control group. However, only the 5 g/kg^{-1} DEX group outperformed the 2.5% SEVO+5 g/kg^{-1} DEX groups regarding RME and WME.

The results of our experiment agreed with those of another study, which found that DEX reversed the negative effects of isoflurane and ketamine on learning. This implies that the negative impacts of SEVO can be mitigated with DEX.

Several pieces of literature have only used DEX to judge how well cognitive functions work.[16] According to, DEX enhances learning and spatial memory at a dose of 20 g/kg^{-1}. Another study in rats with increasing DEX doses found cerebral blood flow decreased, arterial blood pressure rose (with 10 g/kg^{-1} DEX), and cerebral vascular resistance rose.[32] An increase in RME and WME percentages in rats given five g/kg^{-1} DEX alone may be due to its impact on cerebral blood flow in our experiment. The healing impact of DEX, when coupled with SEVO, led us to believe that this effect was caused by compensation of cerebral perfusion pressure. According to Goyagi et al., 6.6, 12.5, and 25 g/kg-^{1} DEX heal long-term memory deficits and neuro-degeneration induced by SEVO in rats.[28] According to Perez-Zoghbi et al.[31] SEVO enhances brain cell apoptosis. Nevertheless, co-administration of SEVO with a low dose of DEX (1 g/kg^{-1}) did not affect the animal's reaction to external stimuli or apoptosis. However, larger doses of DEX (5–25 g/kg^{-1}) combined with SEVO increased brain cell apoptosis.

Rearing behavior in rats is related to anxiety control; therefore, anxiety was expected to diminish in rats that came to the RAM platform for the second time.[33] This research found diminished anxiety in solely DEX-administered rat groups (Groups V and VI). Morena et al.[34] reported that rat anxiety was increased with IP 300 mg/kg-1 propofol and 100–125 mg/kg^{-1} ketamine but decreased with 0.4 mg/kg^{-1} DEX. In our experiment, rats' anxiety was diminished in solely DEX- administered groups.

A low dose of SEVO (1.1%) did not cause apoptosis, but a high dose (2.5%) and long exposure time (6 h) were related to an intense apoptosis rate.[26] In our experiment, neuro-inflammation was not observed with H&E staining in groups. In the CA3 region, the apoptosis rate was lower (p=0.015) in DEX plus SEVO performed groups than in solely SEVO, control, and solely high DEX performed groups. This result was compatible with the conclusions of previous studies.[35,36] During the experiment, in the groups with SEVO plus DEX, exposure resulted in a lower apoptosis rate than in control and solely SEVO groups. Only a little research has examined a single DEX's apoptotic effect. Hoffmann et al.[37] reported that DEX improved neurological return in rats following transient brain ischemia. In addition, DEX can reduce neuroinflammation by diminishing the release of the pro-inflammatory cytokines IL1β and IL-6. Except for this, studies show that DEX decreases perinatal hypoxic brain damage by increasing neurotrophic factor expression. However, normally developing brain tissue was not examined in these studies. Based on this research, it might not be a safe anesthetic for babies, but rather only a safe anesthetic dose concentration and exposure time.

Limitations of the study

There were a number of limitations in this study. Initially, only the tail-pinch test was done under deep anesthesia, but studies indicate that the anesthesia level is adequate if it achieves immobility and unresponsiveness to the tail pinch test during anesthesia.[38] Furthermore, in rat experiments, the tail-clamp technique can be used to measure the minimum alveolar concentration. In a recent experiment, no delayed recovery and an absence of rat mortality during exposure to anesthesia was interpreted as indicating the proper depth of anesthesia. Second, to better comprehend drug effects, the blood level of DEX or inspiratory or expiratory SEVO concentration could have been measured. Unfortunately, a more sophisticated technique of histopathological evaluation could have been used. We were unable to assess this due to technical constraints. Finally, while all groups were exposed to the same oxygen concentration, a high (100% O_2) concentration may have impacted the findings.

CONCLUSION

The results depended on the properties of the chosen anesthetic agent, the doses of the agent, the time course of the application, the length of exposure, the cognitive experiment tests chosen, and the length of time these anesthetics are used. So, it can be said that SEVO and high-dose DEX, whether temporary or not, are not healthy for a rat's brain as it grows.

Cognitive functions are considerably impaired after SEVO anesthesia. DEX changes the effect of SEVO on cognitive functions in a dose-dependent manner, and DEX may cause increased locomotor activity at a high dose (5 µg/kg^{-1}). Since the negative effects are reduced using SEVO and DEX together, mechanisms other than apoptosis and inflammation should be kept in mind.

More experimental and clinical research can be done to fully understand how this effect happens, find ways to reduce it, and find drugs that are less likely to cause clinical neurotoxicity. First, we must look at other causes and mechanisms of the learning problems, such as how the blood flows through the hippocampus, parthenogenesis, and synaptogenesis.

ETHICAL DECLARATIONS

Ethics Committee Approval: On February 21, 2020, Gazi University Local Ethics Committee for Animal Experiments in Ankara, Turkey, granted ethics clearance for this research (G.Ü. ET. 20.013 code number). The trial was carried out at Gazi University Experimental Research Center between the dates of 07.27.2020 and 08.21.2020. The experiments were carried out per the "Guide for the Care and Use of Laboratory Animals"

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

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Comparison of the Effects of Clinical Outcomes on the Number of Attacks in the Course of Ulcerative Colitis: A Single Center Study

Klinik Parametrelerin Ülseratif Kolit Seyrinde Atak Sayılarına Etkisinin Karşılaştırılması: Tek Merkezli Çalışma

Hakan Sivgin[1], Abdurrahman Şahin[2], Şirin Çetin[3]

[1]Department of Internal Medicine, Faculty of Medicine, Tokat Gaziosmanpasa University, Turkey
[2]Department of Gastroenterology, Faculty of Medicine, Tokat Gaziosmanpasa University, Turkey
[3]Department of Biostatistics, Faculty of Medicine, Amasya University, Turkey

Abstract

Aim: The aim of this study was to find the factors affecting the frequency of attacks of patients diagnosed with ulcerative colitis (UC).

Material and Method: In our single-center retrospective descriptive study, 40 UC patients who admitted to our hospital for follow-up from January 2021 to December 2022. The baseline demographic and clinical characteristics, laboratory values and, treatments during the disease course were compared among patients with number of attacks < 2 per year (Group1) and number of attacks ≥2 per year (Group 2).

Results: There were 25 (male/female:11/14) patients in Group 1 and 15 (male/female:7/8) patients in Group 2. No statistically significant difference was found between the mean age, sex, age of diagnosis, duration of disease, co-morbidities and extraintestinal involvement of both groups. The median CRP values were significantly higher in the Group 2, compared to the group 1 (P=0.04). There was statistically significant differences between groups in terms of endoscopic severe activity (12% vs, 40%; p<0.001), left-sided involvement(60% vs, 40%; p=0.02), pancolonic involvement (8% vs, 33%; p<0.001), the presence of blood in stool at the time of diagnosis (80% vs 100%; p=0.03), use of biologic agents (16% vs 40%; p< 0.001) and steroids (16% vs 33%; p=0.02).

Conclusion: In UC patients who have ≥ 2 attacks per year presented with more advanced clinical features at onset and more severe outcomes than the number attacks <2 per year. Symptoms and clinical parameters at the time of diagnosis are important criteria to be considered in determining the number of attacks in follow-up.

Keywords: Ulcerative colitis, Attack, Clinical feature, Outcomes

Öz

Amaç: Bu çalışmanın amacı, ülseratif kolit (UC) tanısı alan hastaların atak sıklığını etkileyen faktörleri bulmaktır.

Gereç ve Yöntem: Tek merkezli retrospektif tanımlayıcı çalışmamızda, Ocak 2021-Aralık 2022 tarihleri arasında takip için hastanemize başvuran 40 UC hastası dahil edildi. Demografik ve klinik özellikler, laboratuvar değerleri ve hastalık seyri sırasındaki tedaviler, atak sayısı yılda < 2 (Grup 1) ve atak sayısı ≥ yılda 2 (Grup 2) olan hastalar arasında karşılaştırıldı.

Bulgular: Grup 1'de 25 (erkek/kadın:11/14) hasta ve Grup 2'de 15 (erkek/kadın:7/8) hasta vardı. Her iki grubun yaş ortalaması, cinsiyeti, tanı yaşı, hastalık süresi, yandaş hastalıkları ve ekstraintestinal tutulumu arasında istatistiksel olarak anlamlı fark saptanmadı. Median CRP değerleri Grup 2'de grup 1'e göre anlamlı derecede yüksekti (P=0,04). Endoskopik şiddet aktivitesi (%12'ye karşı %40; p<0,001), sol taraflı tutulum (%60'a karşı %40; p=0,02), pankolonik tutulum (%8'e karşı %33; p<0,001), tanı anında dışkıda kan varlığı (%80'e karşı %100; p=0,03), biyolojik ajan kullanımı (%16'ya karşı %40; p<0,001) ve steroidler (%16'ya karşı %33; p=0,02) açısından gruplar arasında istatistiksel olarak anlamlı farklılıklar vardı.

Sonuç: Ülseratif kolit seyrinde yılda ≥ 2 atak olanlarda; yılda < 2 atak olanlara göre; tanı anında daha şiddetli semptomlar ve klinik parametreler ön plandadır. İlk tanı anındaki semptomlardan bilhassa dışkıda kan ve klinik parametrelerden şiddet indexi, ülseratif kolitin takip sürecinde atak sayısının belirlenmesinde ve öngörülmesinde göz önünde bulundurulması gereken önemli kriterlerdir.

Anahtar Kelimeler: Ülseratif Kolit, Atak, Klinik Parametreler

Corresponding *(İletişim)*: Hakan SIVGIN, Assistant Professor, M.D., Gazıosmanpasa University School of Medicine Department of İnternal Medicine, Tokat, Turkey
E-mail *(E-posta)*: sivginhakan@gmail.com
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INTRODUCTION

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory disease of unknown cause that primarily affects the mucous membranes of colon that often forming erosions and ulcers. [1] The mucosal inflammation, extend from the rectum to proximal segments of the colon in an interrupted pattern. Symptoms of UC include rectal bleeding, bloody diarrhea and abdominal pain. The diagnosis of ulcerative colitis is based on a combination of clinical symptoms, endoscopic findings, histopathologic findings, and with the exclusion of alternative diagnoses. At diagnosis, most patients have mild to moderate symptoms, and less than 10% have severe disease.[2]

Ulcerative colitis is characterised by remission and exacerbations in most of the patients. On the other hand, some patients have chronic continuous activity. A subgroup of patients with UC has also been described to have lifelong remission after the induction treatment. A first flare after the diagnosis which less than 2 years, the presence of symptoms at diagnosis like fever or weight loss, and active disease in the previous year increase the risk of subsequent relapse.[3]

At diagnosis, 30-50% of patients have distal colitis that confined to rectum or sigmoid colon, 20-30% have left-sided colitis, and about 20% have pancolitis. Extension of colonic disease can occur over time. Ulcerative colitis can progress proximally in 10-19% of patients after 5 years, and in up to 28% of patients at 10 years.[4] Disease flares associated with progression of anatomic extent usually follow a severe course and require more intensive therapy like immunosuppressants, biological agents or surgery. [2] Risk factors for progressive or complicated disease include a diagnosis of the disease less than 40 years, pancolitis, lack of endoscopic healing while in clinical remission, deep ulcerations, concomitant primary sclerosing colangitis and perinuclear antineutrophil cytoplasmic antibody positivity.[5]

Determining the severity and extent of ulcerative colitis is important for selecting the most appropriate treatment. In clinical practice, disease activity is assessed by the combination of clinical symptoms, endoscopic findings, histopathology, biomarkers and quality of life. Currently, complex indices that include clinical symptoms, endoscopic findings, patient's self assessment of quality of life and the physician's global assessment have been used in the assessment of disease activity such as the Mayo score, Lichtiger score, and Simple Clinical Colitis Activity Index.[6] Considering the complexity of these indexes, it is aimed in the current study to determine simple clinical and endoscopic findings at the time of diagnosis for predicting attack frequeny. The efficiacy of treatments were also investigated to prevent subsequent attacks. Thus, we aimed to determine which patients should be followed closely and treated more intensively according to the current study findings.

MATERIAL AND METHOD

The study was carried out with the permission of Tokat Gaziosmanpaşa University Clinical Non-interventional Clinical Researches Ethics Committee (Date: 08.09.2022, Decision No: 21-KAEK-079). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. This retrospective study was carried out among UC patients who followed in the Gastroenterology Clinic of Tokat Gaziosmanpaşa University, Faculty of Medicine between January 2021 and December 2022. Patients were diagnosed as UC according to clinical, radiological, and endoscopic examinations, as well as histolopatological findings. A total of 63 patients aged 18 years and older UC patients were recruited. After the assessment of hospital records and database, it was determined that 6 of the 63 patients had psoriasis, 4 had chronic alcohol use, 3 had malignancies (2 patients lung cancer, 1 patient breast cancer), 2 had the diagnosis of autoimmune hepatitis, and 8 had missing the data required for the study. Thus, twenty-tree patients were excluded from the study.

A total of forty patients were included in the study and their data were retrospectively analyzed. Clinical symptoms and endoscopic findings including location and activity at diagnosis were recorded. All patients were classified according to the Montreal Classification of UC to determine disease extent as proctitis, left-sided colitis and pancolitis. Truelove- Witts (TW) Severity index was used to evaluate clinical activation as mild, moderate and severe ulcerative colitis. The treatments other than mesalazine, corticosteroids, immunomodulators, biological agents including anti-tumor necrosis factor (TNF)-α antibody and anti-integrins were recorded. Laboratory parameters at diagnosis which including hemogram, full biochemisty, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), were also recorded.

Patients were divided into 2 groups according to their annual attack counts per year; less than two attacs per year (Group 1) and, two or more attacks per year (Group 2). A comparison was performed between the two groups using clinical, endoscopicand laboratory findings at diagnosis and tratments.

Statistical Analysis

All statistical analyses were carried out using SPSS version 25.0 (IBM Corp. Released 2017. Armonk, NY). The normality of variables was tested by the Shapiro Wilk test. Categorical measurements were summarized as numbers and percentages, and numerical measurements with a normal distribution were represented as mean±SD; those with a non-normal distribution and ordinal variables were described as median (25%-75%). The differences were compared using either the Chi-squared test or Fisher's exact test for categorical variables. Parametric tests (t test) were used for numerical measurements with normal distribution, and nonparametric tests (Mann-Whitney U Test) were used for numerical measurements with normal distribution.

Univariate logistic regression modeling was used to measure the effect of independent variables on the dependent variable. In statistical tests, results were evaluated at 95% confidence interval and significance was evaluated at $p<0.05$.

RESULTS

Among 40 patients with UC, 24 (60%) of the patients were female and 16 (40%) were female. The mean age of ulcerative colitis patients was 48.3±17.1 years. The mean age of diagnosis was 42.1±16.8 years. The median disease duration was 7.2 (4.2-10.8) years. The most common presenting symptom was diarrhea in 36 patients (%90), blood in the stool in 35 patients (%87.5), and fever in 10 patients (%25) at the time of diagnosis. Accoding to the extent,12 patients (30%) had proctitis, 21 (52.5%) had left colitis and, 7 patients (17.5%) had pancolitis. Activity at the time of diagnosis were as follows; 10 patients (25%) mild, 21 patients (52.5%) moderate and 9 patients (22.5%) severe. All of the patients (% 100) were receiving mesalazine. Thirty-one patients (77.5%) were on immunomodulatory treatment and, 10 patients (%25) on biologic agent treatment. Nine (22.5%) patients received corticosteroids during the follow-up period. No one of our ulcerative colitis patients needed surgical treatment. Demographic and clinical features of patients and treatments were given in **Table 1**.

Table 1. Demographic characteristics and clinical data of patients

	n (%)
Gender, Male/Female	16 (40) /24 (60)
Age, year,mean±SS	48.3±17.1
Age of diagnosis,year, mean±SS	42.1±16.8
Duration of illness, years, median (%25-%75)	7.2 (4.2 -10.8)
Activity, active/remission	9 (22.5)/31 (77.5)
Disease activity at diagnosis, n(%)	
Mild, n (%)	10 (25)
Moderate, n (%)	21 (52.5)
Severe, n (%)	9 (22.5)
Montreal classification extent of UC, n(%)	
Proctitis	12 (30)
Left Side Colitis	21 (52.5)
Pancolitis	7 (17.5)
Comorbidities	
Diabetes	10 (25)
Hypertension	6 (15)
Hyperlipidemia	4 (10)
No Additional Diseases	20 (50)
Extraintestinal organ involvement	
Musculoskeletal system	6 (15)
Mucocutanöz	5 (12.5)
Ocular	2 (5)
Hepatobiliary	2 (5)
Treatment	
Mesalazine	40 (100)
Corticosteroid	9 (22.5)
Immunomodulator	31 (77.5)
Biologic agent	10 (25)

In the comparison of groups, the mean number of attacks was 2.11±0.79 per year in females and, 2.07±0.82 per year in males. There were 25 patients (M/F:11/14) in Group 1 and 15 patients (M/F:7/8) in Group 2. The clinical and laboratory features of the groups were given in **Table 2**. The mean age of the patients in the Group 1 was 50.1±7.5 years, and 56 percent of the patients were female. The mean age of the Group 2 was 49.4±7.7 years, and 53% of the patients were female. No statistically significant difference was found between the mean age, sex, age of diagnosis, duration of disease, co-morbidities and extraintestinal involvement of both groups (for all, p>0.05) (**Table 2**). Laboratory parameters of the groups was presented in **Table 3**. he median CRP values were significantly higher in the Group 2, compared to the Group 1 (p= 0.04). Other laboratory parameters did not significantly differ between the groups (for all, p>0.05).

Table 2. According to the number of attacks, the clinical features of the groups at the time of diagnosis

	N. of attacks < 2/ per year (Group 1)	N. of attacks ≥ 2/ per year (Group 2)	p
Gender, Male/Female, n (%)	11 (44)/14 (56)	7 (47)/8 (53)	0.26
Age, year, mean±Sd	50.1±7.5	49.4±7.7	0.29
Age of diagnosis, year,mean±Sd	43.9±9.2	42.9±10.5	0.22
Duration of illness, years, median (%25-%75)	7.2 (3.6 -10.8)	7.1 (4.8 -8.4)	0.39
Comorbidities, n(%)			
Diabetes	7 (28)	6 (40)	0.42
Hypertension	4 (16)	3 (20)	0.57
Hyperlipidemia	3 (7.5)	2 (13)	0.18
No additional diseases	11 (44)	4 (27)	0.46
Disease activity at diagnosis, n(%)			
Mild	7 (28)	3 (20)	0.96
Moderate	15 (60)	6 (40)	0.89
Severe	3(12)	6 (40)	<0.001*
Montreal classification of extent of UC, n (%)			
Proctitis	8 (32)	4 (26)	0.54
Left Side Colitis	15 (60)	6 (40)	0.02*
Pancolitis	2 (8)	5 (33)	<0.001*
Symptoms n(%)			
Diarrhea	21 (84)	15 (100)	0.16
Blood in the stool	20 (80)	15 (100)	0.03*
Weight Loss	9 (36)	6 (40)	0.13
Fever	6 (24)	4 (26)	0.31
Extraintestinal involvement, n(%)	9 (36)	6 (40)	0.47
Musculoskeletal system	4 (16)	2 (13)	0.56
Mucocutanos	3 (12)	2 (13)	0.77
Ocular	1 (4)	1 (6)	0.89
Hepatobiliary	1 (4)	1 (6)	0.89
Treatment , n(%)			
Mesalazine	25 (100)	15 (100)	1
Corticosteroid	4 (16)	5 (33)	0.02*
Immunomodulator	19 (76)	12 (80)	0.66
Biologic agent	4 (16)	6 (40)	<0.001*

*:p<0.05, OR: Odds ratio, CI: Confidence interval, 1: Reference value
Values are presented as mean±SD, median (%25-%75) or number (%). Patients with missing values were not included. Data are expressed as raw numbers with proportions

The comparison of symptoms and clinical charactaristics at disease onset between the groups was presented in **Table 3**. Patients who had severely active at the time of diagnosis were significantly higher rates in Group 2 (12% vs 40%; p<0.001). According to the extent, there was no difference between the two groups for the ration of proctitis (32% vs 26%; p=0.54). There left-sided colitis was more commo in Group 1 than in Group 2 (60% vs 40%; p=0.02). Likewise, pancolitis was more common in Group 2 than in Group 1 (8% vs 33%; p<0.001). There was no difference between the groups in terms of symptoms, diarrhea, weight loss and fever. Blood in stool was statistically significantly higher in Group 2 than in Group 1 (80% vs 100%; p=0.03). No statistically significant difference was found between the extraintestinal involvement of both groups (p > 0.05). When the treatment methods were compared, it was seen that the use of biologic agents and steroids was significantly higher in group 2 than in group 1 (16% vs 33%; p=0.02), (16% vs 40%; p< 0.001).

Table 3. According to the number of attacks, laboratory parametres of the groups at the time of diagnosis

Variables	Group 1	Group 2	p
Leukocyte (10^3/mm³), median (%25- %75)	9410 (6670-10610)	9720 (6030-11520)	0.22[b]
Neutrophil (10^3/mm³), median (%25- %75)	5230 (4015-6610)	5160 (4060-6020)	0.25[b]
Lymphocyte (10^3/mm³), median (%25- %75)	2015 (1580-2660)	2080(1600-2670)	0.13[b]
Hemoglobin, median (%25- %75)	12.5 (10.8-13.9)	13.7 (11.9-14.8)	0.12[b]
Platellet,median (%25- %75) (10^3/mm³)	305 (242-395)	306 (259-347)	0.9[b]
ESR, median (%25- %75)	23 (10-41)	21.5 (8-30)	0.19[b]
CRP(mg/dl), median (%25- %75)	22 (3-17.5)	28.7 (1.5-15)	0.04[b]
BUN(mg/dl), mean±Sd	12.4±4.8	12.8±4.7	0.23[a]
Creatinine, mean±Sd	0.80±0.28	0.80±0.22	0.83[a]
Sodium,mean±Sd	139.0±2.8	139.3±3.1	0.67[a]
Potassium,mean±Sd	4.34±0.23	4.45±0.43	0.16[a]
Calcium,mean±Sd	9.18±0.45	9.19±0.39	0.95[a]
ALT(IU/L), median (%25- %75)	13 (10-19)	14 (11-21)	0.55[b]
AST(IU/L), median (%25- %75)	16 (13-20)	17 (14-21)	0.52[b]
GGT(IU/L), median (%25- %75)	17 (12-28)	19 (13-27)	0.51[b]
ALP(IU/L), median (%25- %75)	79.4 (23-114)	80.3 (43- 128)	0.44[b]
T. Protein (mg/dl), mean±Sd	7.1±0.9	7.14±0.8	0.31[a]
Albumin (mg/dl), mean±Sd	4.1±0.8	4.2±0.6	0.29[a]

*p<0.05, a:Student's t- Test , b:Mann-Whitney U test, Sd:Standard deviation

Univariate analysis for the identification of the annual attack frequency demonstrated that the presence of blood in stool and endoscopic severe activity at diagnosis were statistically significant independent predictive factors. The results of univariate logistic regresiion were given in **Table 4**. Endoscopic severe activity at diagnosis had 6 times higher risk for two or more attacks per year (OR:6.01, CI:1.66-23.56, p=0.007). Similarly, the presence of blood in the stool at onset was related with 5.71 times higher risk in more frequent attack (OR: 5.71, CI: 1.06-30.66, p=0.034).

Table 4. Factors related to ≥ 2 attacks per year of ulcerative colitis (univariate logistic regression analysis results)

Variables	n	(%)	OR	95%CI	P
Severe UC activity (severe/non severe)	9	22.5	6,01	1.66-23.56	0.007*
Blood in Stool (Yes/No)	35	87.5	5.71	1.06-30.66	0.034*
Use of Biologic agent (Yes/No)	10	25	0.99	0.64-1.57	0.91
Use of Steroid (Yes/No)	9	22.5	1.14	0.74-1.76	0.547
Pancolitis (Yes/No)	7	17.5	1.07	0.66-6.33	0.58
Left Side Colitis (Yes/No)	21	52.5	1.09	1.66-19.33	0.56

*:p<0.05, Multiple Logistic regression, Method=Enter, OR: Odd Ratio, CI: Confidence Interval, R2 (Nagelkerke)=0.10, Model χ2=52.78, p<0.001, Dependent variable: number of attacks per year ≥2 (1=yes, 0=no), Correct classification probability of the model:78%, 1: Reference value

DISCUSSION

Ulcerative colitis is a chronic inflammatory bowel disease that involves the mucosa and submucosa of the colon diagnosed and treated with clinical presentation, endoscopic appearance and laboratory values, and may also present with extraintestinal findings with attacks and remissions.[7,8] Since UC is a chronic and lifelong disease, to predict the course of the disease at the time of diagnosis or in the early stages is very important because it will change the approach to the patient and the treatment. In the literature, many predictor factors have been identified for UC based on both clinical (age, gender, involvement status, etc.) and laboratory values. These prognostic predictors were: diffuse disease, proximal extension of lesions during the course of the disease, extraintestinal findings, young age of disease onset, severity of inflammation, and poor response to treatment.[9-15] Ulcerative colitis is a lifelong disease that is generally diagnosed in young adulthood, often at the age of 30-40 years. Although the female-to-male ratio has different results in different studies, it is generally accepted as equal. In accordance with the literature, the median age of diagnosis was 42 years in our study and the female patient rate was 60%. In the comparison of the two groups, there was no difference in terms of age and gender. Most clinicians have been using the partial Mayo score or the Truelove and Witts severity index for deciding treatments. This index is primarily based on the symptom category to assess the disease activity of UC at diagnosis and subsequent follow-up. Those in remission were categorized by less than 3 defecations per day, while those in severe ones were categorized by more than 6 bloody defecations per day and the presence of systemic symptoms. In our study in accordance with the literature, it was found that there was a positive significant relationship between the presence of bloody defecation and the frequency of attacks during diagnosis. The frequency of attacks was 5.7 times higher in patients with bloody defecation than in other patients.

Ulcerative colitis presents approximately 45% proctitis, 35% left side colitis and 20% pancolitis, and colectomy rates and proximal extension rates vary between 10-30% throughout the course.[16,17] In our study, 12 patients (30%) with ulcerative colitis had proctitis, 21 patients (52.5%) had left colitis and 7 patients (17.5%) had pancolitis. In the comparison of the two groups, the rate of pancolitis at the time of diagnosis was statistically higher in the group with a high number of attacks. Interestingly, those who had left-sided colitis at the time of diagnosis were found to be more in the group with a low number of attacks. According to the disease activity at the time of diagnosis, it was seen that the severe ones between the two groups were statistically higher in the group with the highest number of attacks. The frequency of attacks was 6.1 times higher in severe cases than in other patients. In the studies carried out, values such as ESR, CRP, Hb, leukocytes, albumin, which are called inflammatory markers, are used. In particular, there are studies showing that CRP is associated with UC disease activity and severity of activity.[11,12,18-20] In our study, CRP values were found to be significantly higher at the time of initial diagnosis of those with a high number of attacks between the two groups. In other inflammatory and biochemical parameters, there was no difference between the two groups.

Studies have reported cumulative colectomy rates of 20% at 5 years and 25% at 10 years.[21] The rate of UC-related operation are lower in Asian patients than they are in Western patients, with a rate of 5.9% at 5 years and 10% of 10 years.[22] One Western study showed that the age of onset was younger in patients who had undergone previous operation.[23] In our study, there was no colectomy. This finding may reflect the fact that a top-down strategy including biologic agents was more frequently used in patients because of their more severe disease state upon diagnosis. The administration of immunosuppressants and/or biologic therapy early in the course of the disease has been shown to be superior to conventional treatment in terms of better mucosal recovery, induction of steroid-free remission, and prevention of hospitalization in patients with recent IBD.[24-26] In cases with steroid dependence or steroid refractory, azathioprine (2.5 mg/kg/day) or 6-mercaptopurine (1.5 mg/kg/day) should be added to the treatment to achieve and maintain remission.[24-26] In steroid-refractory cases, in cases that are dependent on steroids even though they have taken enough tiopurine, or in cases that cannot tolerate tiopurine treatment, infliximab is an effective treatment option as a tumor necrosis factor alpha (TNF-α) blocker. A meta-analysis of 9 randomized controlled trials involving a total of 1226 patients with moderate to severe ulcerative colitis showed better short-term response, short-term improvement, long-term response, and long-term improvement in patients receiving biologic agents. In our study, it was seen that all patients in two groups used mesalazine. In the group with a high number of attacks, the use of biological agents and steroids was found to be statistically significantly higher. This study has several limitations. There were only 40 UC patients because data were collected from medical records had to include the number of attacks. Despite the small number of patients; however, there were significant differences in the severity of clinical features and outcomes between the two groups in the present study. In addition, the results of outcomes analysis using patient history including UC-related admission and steroid or biologic agents use during follow-up after diagnosis of UC were limited because of inconsistent reference points. Further study is needed to confirm the associations and trends observed here.

CONCLUSION

Consequently, UC is a relapsing and remitting disease, currently with no cure. The purpose of treatment UC is to maintain remission and prevent complications. Long-standing mucosal inflammation can lead to complications and colorectal carcinoma. It is possible that severe outcomes including symptoms such as blood in stool, disease severity, using steroid or biologic agents may be higher in more attacks patients compared to in less attacks patients with prolonged follow-up. Intensive care and uninterrupted follow-up are especially important for patients with more attacks per year..

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tokat Gaziosmanpaşa University, Noninvasive Clinical Researches Ethics Committee (Date: 18.03.2021, Decision No: 21-KAEK-079)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Relationship Between Prolonged Jaundice and Vitamin B12 Levels in Term Newborns

Term Yenidoğanlarda Uzamış Sarılık ile Vitamin B12 Düzeyleri Arasındaki İlişki

Emine Esin Yalinbas[1], Yaşar Durmaz[2], Fezan Mutlu[3]

[1]Department of Neonatology, Eskisehir City Hospital, Eskisehir, Turkey
[2]Kutahya Health Sciences University Evliya Celebi Training and Research Hospital, Pediatrics, Kutahya, Turkey
[3]Eskisehir Osmangazi University Medical Faculty, Department of Biostatistics, Eskisehir, Turkey

Abstract

Aim: Vitamin B12 deficiency, when observed during the neonatal period, can generally be traced back to maternal causes. In cases of vitamin B12 deficiency, hyperbilirubinemia may occur due to hem overproduction as the result of erythrocyte lysis. Prolonged jaundice is common during the neonatal period, and its underlying etiological causes should be analyzed. The aim of this study was to analyze whether vitamin B12 deficiency has any effect on prolonged jaundice formation in infants.

Material and Method: The study examined 89 infants; this included 45 that had been diagnosed with prolonged jaundice and 44 in the control group. Their clinical and demographic characteristics were recorded. Patient group was formed with term infants with prolonged jaundice excluding possible etiological causes of prolonged jaundice. Both the maternal and infant vitamin B12 levels were analyzed.

Results: An indirect bilirubin level of 11.8 mg/dl in the prolonged jaundice group and 3.16 mg/dl (p<0.001), a hemoglobin level of 14.18 gr/dl and 15.7 gr/dl (p=0.005), and infant vitamin B12 level of 168 pg/ml and 205.2 pg/ml (p=0.013) in the patient and control groups, respectively, showed significant differences between the two. Maternal vitamin B12 levels were found to be similar in the patient and control groups (p=0.315), and there was no significant correlation between the vitamin B12 levels of the infants and mothers (r=0.278, p=0.064).

Conclusion: Vitamin B12 deficiency can be related to prolonged jaundice in neonatals, and an early diagnosis of vitamin B12 deficiency in high risks group in the neonatal period can be useful in terms of potentially identifying and controlling related conditions.

Keywords: Jaundice, newborn, vitamin B12 deficiency

Öz

Amaç: Yenidoğan döneminde görülen vitamin B12 eksikliği genellikle maternal nedenlerle ilişkilidir. Vitamin B12 eksikliğinde yenidoğan döneminde eritrosit lizisi sonucunda aşırı hem üretimine bağlı hiperbilirubinemi gelişebilmektedir Uzamış sarılık ise yenidoğan döneminde yaygın görülen bir durumdur, allta yatan etiyolojik nedenler araştırılmalıdır. Bu çalışmanın amacı, bebeklerde uzamış sarılık gelişiminde vitamin B12 eksikliğinin etkisinin olup olmadığını araştırmaktır.

Gereç ve Yöntem: 45 uzamış sarılık tanısı alan yenidoğan ile 44 kontrol grubunun yer aldığı toplam 89 bebek çalışmaya dahil edildi. Klinik ve demografik özellikleri kaydedildi. Hasta grubu uzamış sarılığa neden olabilecek etiyolojik nedenler dışlanmış, uzamış sarılığı olan term bebeklerden oluşturuldu. Anne ve bebeklerden vit B12 düzeyleri ile çalışıldı.

Bulgular: Uzamış sarılık grubunda indirekt bilirubin düzeyi 11.8 mg/dl, kontrol grubunda 3.16 mg/dl (p<0.001), hemoglobin düzeyi hasta grubunda 14.18 gr/dl, kontrol grubunda 15.7 gr/dl (p=0.005), bebek vitamin B12 düzeyleri hasta grubunda 168 pg/ml (p=0.013) olmak üzere kontrol grubuna 205.2 pg/ml (p=0.013) göre anlamlı farklılık vardı. Anne vitamin B12 düzeyleri hasta ve kontrol grubunda benzer bulundu (p=0.315). Bebek ve annelerden bakılan vitamin B12 düzeyleri arasında anlamlı bir korelasyon olmadığı saptandı (r=0.278, P=0.064).

Sonuç: Yenidoğanlarda vitamin B12 eksikliğinin uzamış sarılık ile ilişkili olabileceği ve yenidoğan döneminde riskli grupta erken konulan vitamin B12 eksikliği tanısının buna bağlı gelişebilecek durumların kontrol altına alınmasında fayda sağlayabileceğini düşünmekteyiz

Anahtar Kelimeler: Sarılık, yenidoğan, vitamin B12 eksikliği

Corresponding *(İletişim)*: Emine Esin YALINBAŞ, Eskisehir City Hospital, Department of Neonatology, Eskisehir, Turkey
E-mail *(E-posta)*: esinylnbs@gmail.com
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INTRODUCTION

Vitamin B12 deficiency is an important global public health problem, and its deficiency is known to cause various hematological and neurological diseases. Vitamin B12 (cobalamin), a water-soluble vitamin, plays a significant role, especially in tissues with high cellular circulation. [1] Vitamin B12 functions in two coenzyme forms, namely methylcobalamin (MeCbl) and adenosilcobalamin (AdoCbl). MeCbl functions as a cofactor for methionine synthase and plays a role in its transformation into tetrahydrofolate, which is necessary for both DNA synthesis and the maturation of red blood cells. Thus, MeCbl deficiency results in megaloblastic anemia.[2] Varying degrees of hyperbilirubinemia can manifest depending on the lysis in eryhtocytes due to ineffective erythropoesis.[3]

Vitamin B12 deficiency in newborns, which mostly originates from their mothers, is a treatable condition. In a previous study, neonatal screenings for this condition presented a deficiency rate of between 3.3-18.7 in 100.000 infants in Germany [4.5] and 0.88 in 100.000 in the USA. [6] Conversely, low B12 levels were detected in 30-40% of the infants in countries like Nepal,[7] Mexico[8] and India.[9] A study from 2019 in Germany reported a predicted birth prevalence of vitamin B12 deficiency as 26 in 100.000; the same study detected previously undetected vitamin B12 deficiency in the mothers of affected neonatal (81%) and emphasized that they were given specific treatment afterwards.[10]

Prolonged jaundice is a common condition in infancy; tests of the transcutaneous bilirubin (TcB) value after week two should be ≥5 mg/dL in term infants.[11] 2-15% of all newborns and 40% of breast-fed infants were reported to be affected.[12] This study aimed to investigate the effect of vitamin B12 deficiency in prolonged jaundice formation in newborns by checking vitamin B12 levels in newborns with prolonged jaundice and their mothers.

MATERIAL AND METHOD

Newborns suffering from jaundice for a minimum period of 37 weeks in the 2nd-3rd level Neonatal Intensive Care Unit and the general pediatrics polyclinics at Kütahya Health Sciences University Evliya Çelebi Training and Research Hospital between January 2021 and February 2022 were included in this prospective study after receiving parental consent. Infants with jaundice lasting for more than two weeks and a total bilirubin level ≥ 5 mg/dl were included in the patient group. A total of 89 infants, 45 in the prolonged jaundice group and 44 in the control group, were included in the study. Hemogram, reticulocyte, maternal and infant blood group, direct Coombs test, urinary reducing substance, urine culture, glucose-6-phosphate dehydrogenase (G6PDH), free thyroxine (fT4), thyroid-stimulating hormone (TSH), maternal and infant vitamin B12 levels, lactate dehydrogenase (LDH), hemogram, and reticulocyte were planned to be taken from the patient group with prolonged jaundice and hemogram, reticulocyte, total and direct bilirubin, and maternal and infant vitamin B12 levels were planned to be taken from the control group. Infants with ABO and Rh incompatibility, abnormality in thyroid function tests, urinary culture positivity, reducing substance positivity in urine and G6PDH enzyme deficiency were not included in prolonged jaundice group. Children of mothers who were mostly on vegetarian diet, had a chronic disease, and took vitamin B12 supplements during pregnancy were not included in the study. Infants who had only been fed formula and those who suffered from additional diseases (cardiac disease, congenital abnormalities, etc) were excluded from the study. Demographical characteristics, such as admission day, sex, gestational week, delivery method, birth weight, height, head circumference, maternal age, APGAR score at first and fifth minutes, and weight at the time of admission were recorded.

After storing venous blood samples at -20°C, vitamin B12 levels were analyzed through an electrochemiluminance method using a Roche brand kit (07212771 190) on a Roche-Hitachi E411 hormone device. The study was approved by the Kütahya Health Sciences University Clinical Researches Ethics Board (Decision no: 11466, Date: 11.12.2020).

Statistical Analysis

The distribution of each continuous variable was tested for normality using the Shapiro-Wilk test. An unpaired t-test was used for normally distributed variables while a Mann Whitney U test was used for non-normally distributed variables; the results were expressed as a median value [25%-75%] and a mean±standard deviation (SD). The Pearson's Chi-square test was used to compare categorical variables, which were expressed in frequencies and percentages. Spearman's rho correlation coefficient was used for correlation analysis. Receiver Operating Characteristic (ROC) Curve analysis was used to determine the cut-off value of B12 level and the area under the curve (AUC). A p-value <0.05 was considered significant. All analyses were performed using the SPSS version 25.0 software (SPSS Inc., Chicago, IL, USA) and Medcalc Software 15.8 (Oostende, Belgium)

RESULTS

45 infants with prolonged jaundice were included in the study, and the control group consisted of 44 healthy infants. In terms of clinical and demographical characteristics, no difference was detected among the two groups in terms of gestational week, sex, delivery method, Apgar score, and admission day. Birth weight was found to be lower in the prolonged jaundice group (p=0.043) (**Table 1**). The mean admission day was 20.42±4.59 days in prolonged jaundice group.

Table 1: Comparison of patient and control groups based on demographical characteristics

	Patient (n=45)	Control (n=44)	P values
Gestational age (weeks)	37(37-38)	38(37-39)	0.116
Sex (%)			
Female	15 (45.5%)	18 (54.5%)	0.603
Male	30 (53.6%)	26 (46.4%)	
Birth weight (g)	2875±582	3105±466	0.043
Delivery method (%)			
C/S	32 (50.8%)	31 (49.2%)	1.000
VD	13 (50.0%)	13 (50.0%)	
Apgar score 1st (min)	8 (7-9)	8(7-9)	0.914
Apgar score 5th (min)	9 (9-10)	9(9-10)	0.947
Age (days)	20.42±4.59	21.06±1.02	0.602

Based on the laboratory values of the groups, the total bilirubin level was 11.8 mg/dL in the prolonged jaundice group and 3.16 mg/dL (p<0.001) in the control group, and the hemoglobin (Hb) level was 14.18±2.60 g/dL in the patient group and 15.7±2.35 g/dL (p=0.005) in the control group. No difference was detected in reticulocyte values among the groups. LDH level was detected as 361.38±134.45 U/L in the prolonged jaundice group. Vitamin B12 levels were detected as 168 (120-202) pg/mL in the prolonged jaundice group and were significantly lower than in the control group (p=0.013). Although maternal vitamin B12 levels were lower in the prolonged jaundice group, the difference was statistically insignificant (p=0.315) (**Table 2**).

Table 2: Comparison of patient and control groups based on laboratory characteristics

	Patient (n=45)	Control (n=44)	P values
Total bilirubin (mg/dL)	11.8 (10.75-13.75)	3.16 (2.57-3.50)	<0.001
Hemoglobin (g/dL)	14.18±2.60	15.7±2.35	0.005
Reticulocyte count (%)	1.49 (1.14-2.37)	1.59 (1.10-2.12)	0.992
Infant B12 levels (pg/mL)	168 (120-202)	205.2 (143.4-266)	0.013
Maternal B12 levels (pg/mL)	202 (160-258)	219.5 (189.3-255.3)	0.315

The mean vitamin B12 level was 185.66±68.33 pg/mL for 89 infants and 217.17±64.89 pg/mL for the mothers included in our study. The ROC curve analysis revealed that the best cut-off value for Vitamin B12 level for detecting jaundice was 207 (p=0.010), with 80% sensitivity, 50% specificity, 62% positive predictive value (PPV), 71% negative predictive value (NPV), and 65% accuracy. This analysis showed an AUC of 0.65 (**Figure 1**).

There was no significant correlation between the vitamin B12 levels of the infants and their mothers in our study (r=0.278, p=0.064). We also detected no correlation between vitamin B12 levels and reticulocyte (r=0.160, p=0.293), LDH (r=0.145, p=0.344), and indirect bilirubin (r=0.036, p=0.812) levels in the patient group.

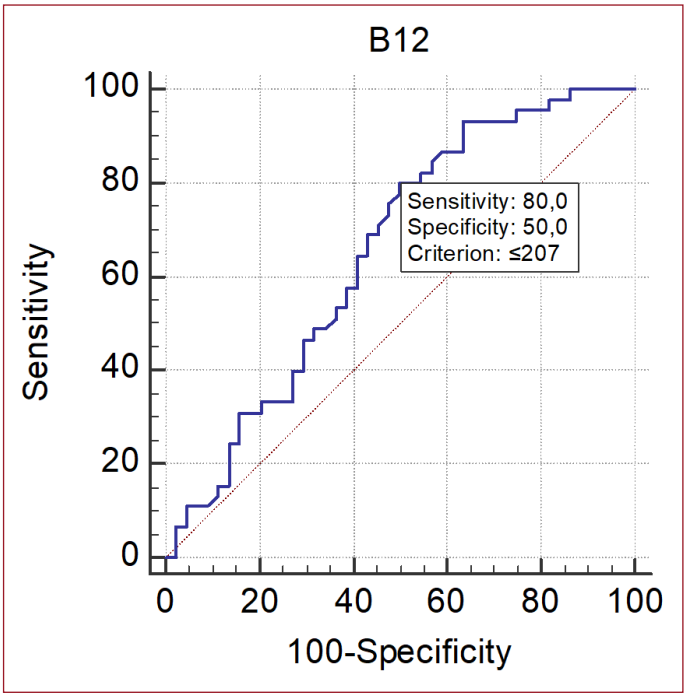

Figure 1. ROC curve analysis for the cut-off value of B12 level in detecting jaundice

DISCUSSION

B12 vitamin also plays an important role in cellular DNA synthesis and efficient erythropoesis.[13] Vitamin B12 deficiency is a common problem across all age groups in most societies; this deficiency leads to an increase in indirect bilirubin levels, often mildly, due to hemolysis.[14]

Jaundice occurs in nearly 60-80% of the infants and is very common in neonatal period. It is more common in breast-fed infants, and jaundice continues in 10% of these infants when they are one month old.[15] Prolonged neonatal jaundice is defined as a TcB value ≥5 mg/dL and persistent jaundice lasting more than two weeks in term infants. The National Institute for Health and Clinical Excellence (NICE) recommends the analysis of direct bilirubin, urine culture, glucose-6-phosphate dehydrogenase, complete blood count, and blood group in the evaluation of prolonged jaundice.[16]

The mean indirect bilirubin level was detected as 11.8 mg/dL in the prolonged jaundice group and as 3.16 mg/dL in the control group in this study. Gundur et al.[17] detected a mean total bilirubin level of 11.6±3.7 mg/dL in their study on 71 infants with prolonged jaundice. In our group with prolonged jaundice, the mean Hb level was 14.1 g/dL and the reticulocyte count was 1.49%. Considering these levels, although the Hb level was low in the patient group, no difference was found between that and the control group in terms of reticulocyte levels. LDH level was also normal in the prolonged jaundice group. The lack of high LDH and reticulocyte levels shows that there was no significant hemolysis in prolonged jaundice group.

Different values are available to define vitamin B12 deficiency. In some studies, vitamin B12 levels lower than 160 pg/mL are accepted as deficiency and those lower than 120 pg/mL are considered severe deficiency for both pregnancy and infants.[18] With regard to the definitions of the World Health Organization, children and mothers have "vitamin B12 deficiency" at <148 pmol/L; children and mothers are in the category of "low vitamin B12" at 148-221 pmol/L; and children and mothers show "vitamin B12 adequacy" at >221 pmol/L.[19] We accepted 207 pg/mL for vitamin B12 as cut-off point.

The mean vitamin B12 level was 168 pg/mL in neonatals with prolonged jaundice and 205.2 pg/mL in the control group; this is a significant difference in vitamin B12 levels. The cut-off value for the vitamin B12 level was determined as 207 pg/mL in our study. Studies showed that vitamin B12 levels in neonatals change between countries. These levels have been reported at 404 pg/mL in Norway, 357 pg/mL in Sweden, 240 pg/mL in China, and 201 pg/mL in India.[20-23] A study by Koç et al.[24] in Turkey detected a vitamin B12 value of 207 pg/mL in the umbilical cord.[23] The same study took the cut-off value for vitamin B12 as 160 pg/mL. Karademir et al.[25] detected serum vitamin B12 level as 236 pg/mL in their study on 204 term infants. It has been suggested that, by causing erythropoesis, vitamin B12 deficiency causes the premature death of both the erythroblasts in bone marrow and the macrocytes in peripheral circulation, leading to thus anemia. Vitamin B12 deficiency is particularly prevalent in India, and a study conducted there reported that B12 and folate deficiency and high homocysteine is related to low birth weight and neonatal hyperbilirubinemia.[23] Eroglu et al.[26] detected mean vitamin B12 levels of 119.9 ng/L in the patient group and as 286.17 ng/L in the control group in their study investigating the relationship between vitamin B12 deficiency and neonatal hyperbilirubinemia. The literature reported on indirect hyperbilirubinemia with severe vitamin B12 deficiency (<30 pg/mL) in a 12 year old female patient.[27] Our study did not detect a significant relationship between vitamin B12 levels and indirect bilirubin values in the prolonged jaundice group. Erdol et al.[28] reported no relationship between total bilirubin levels and B12 deficiency, which is in line with our study. Clinical findings have a mild course in vitamin B12 deficiency, and may cause growth retardation as well as hematological and neurological problems, especially in infants with untreated, severe deficiencies. Vitamin B12 deficiency has been detected in infants due to neonatal screening programs, indicating that it may be mainly maternal-sourced.[6,10,29] Prospective studies have shown that maternal vitamin B12 levels during pregnancy and B12 levels in either the cord blood or serum of the infant at birth are related.[29,30] Balcı et al.[31] reported a significant correlation between B12 levels in the mother and those in the cord blood. Hay et al.[20] showed that maternal vitamin B12 level is an important determinant of the vitamin B12 level of infants. Our study did not detect a significant correlation between maternal and infant vitamin B12 levels in either the patient group or the control group. Reischl-Hajiabadi et al.[32] found results similar to those of this study. In our case, we thought that it may be due to the fact that the blood was obtained from the cord in infants in studies that correlated B12 levels between mothers and infants, and that in our study, it was taken at an average of 20.42 days postnatal.

Some of the limitations of this study included a limited sample size and an analysis of only one biomarker.

CONCLUSION

In this study, it was determined that vitamin B12 deficiency in the infant led to the development of jaundice. We thought that the development of hyperbilirubinemia due to hemolysis might be more pronounced, especially in severe vitamin B12 deficiency. In investigating the etiology of prolonged jaundice, we believe that monitoring vitamin B12 levels, especially in developing countries such as ours and in regions with low socioeconomic status and high incidence of vitamin B12 deficiency, will provide early diagnosis and treatment and will be beneficial for maternal and infant health.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Kütahya Health Sciences University Clinical Researches Ethics Board. (Decision no: 11466, Date: 11.12.2020).

Informed Consent: Informed consent forms were obtained from the parents of all patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Association of Toll-Like Receptor 9 Expression with Prognosis in Breast Carcinoma

Meme Karsinomlarında Toll-Like Reseptör 9 Ekspresyonunun Prognozla İlişkisi

Zeliha Esin Çelik[1], Fatih Demir[2], Harun Yonar[3], Murat Çelik[1] , Orhan Onder Eren[4]

[1]Selcuk University Faculty of Medicine, Pathology Department, Konya, Turkey
[2]Ankara Etlik State Hospital, Ankara, Turkey
[3]Selcuk University Faculty of Veterinary Medicine, Department of Biostatistics, Konya, Turkey
[4]Selcuk University, Faculty of Medicine, Oncology Department, Konya, Turkey

Abstract

Aim: Breast cancer (BC) is the most common malignancy in women. Some molecules including TLR9 are still under investigation as potential prognostic factors in BC. In the present study, we aimed to determine the relation between TLR9 expression and clinicopathological prognostic parameters and survival in BC.

Material and Method: One hundred and thirty nine patients diagnosed as BC included the present study. Immuno-reactivity scoring (IRS) system was used to reveal the tissue TLR9 expression levels.

Results: We found higher TLR9 expression in tumors diagnosed as invasive carcinoma NOS, grade 3 tumors, tumors with necrosis, ER negative and Her2 positive tumors and triple negative molecular subtype. Furthermore, tumors with low TLR9 scores showed increased overall survival compared to tumors with high TLR9 scores.

Conclusion: TLR9 overexpression in BC is associated with some prognostic parameters including histologic type, tumor grade, tumor necrosis, ER and Her2 status and molecular subtype as well as overall survival. Further studies with larger patient series are needed to shed light on the use of TLR9 as a clinical and therapeutic target in BC.

Keywords: Breast cancer, pathology, TLR9, immunohistochemistry, prognosis

Öz

Amaç: Meme kanseri (MK) kadınlarda en sık görülen malignitedir. Toll-like reseptör 9 (TLR9) dahil bazı moleküller, MK'de potansiyel prognostik faktörler olarak halen araştırılmaktadır. Bu çalışmada, MK'de TLR9 ekspresyonu ile klinikopatolojik prognostik parametreler ve sağkalım arasındaki ilişkiyi belirlemeyi amaçladık.

Gereç ve Yöntem: Bu çalışmaya MK tanısı konulan 139 hasta dahil edildi. Doku TLR9 ekspresyon seviyelerini ortaya koymak için immüno-reaktivite skorlama (IRS) sistemi kullanıldı.

Bulgular: İnvaziv karsinom NOS tanısı alan tümörlerde, derece 3 tümörlerde, nekrozlu tümörlerde, Östrojen reseptörü (ER) negatif ve Human epidermal growth factor 2 (Her2) pozitif tümörlerde ve üçlü negatif moleküler alt tipte TLR9 ile yüksek düzeyde ekspresyon tespit edildi. Ayrıca, TLR9 skoru düşük olan tümöre sahip hastaların genel sağkalımı, yüksek TLR9 skoru olan tümörlü hastalara kıyasla daha fazla idi.

Sonuç: MK'de TLR9 aşırı ekspresyonu, genel sağkalımın yanı sıra histolojik tip, tümör derecesi, tümör nekrozu, ER ve Her2 durumu ve moleküler alt tip gibi bazı prognostik parametrelerle ilişkilidir. TLR9'un MK'de klinik ve terapötik bir hedef olarak kullanımına ışık tutmak için daha geniş hasta serileri ile daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Meme kanseri, patoloji, TLR9, immunohistokimya, prognoz

Corresponding *(İletişim)*: Zeliha Esin ÇELİK

E-mail *(E-posta)*: dresincelik@hotmail.com
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INTRODUCTION

Breast cancer (BC) is the most common malignancy in women with increased mortality rates in the last 50 years worldwide.[1,2] Though well-known prognostic parameters exist in BC, it is difficult to predict the biological behavior since BC is a heterogeneous disorder consists of complex pathologic entities.[3]

Toll-like receptors (TLRs) are pattern recognition receptors primarily expressed by cells of the immune system as well as epithelial tumor cells.[4] Recent studies have also demonstrated expression of TLR9, one of the TLRs, in various normal epithelial cells and in cancer cells, including breast, brain, gastric, lung and prostate cancers.[5,6]

TLRs activate the production of many biological factors, causing an inflammatory response and inducing type 1 interferons and other cytokines that activate the adaptive immune system.[3,7]

TLRs are like a double-edged sword that exhibits both antitumor and protumor activities with many features, and further studies are needed to understand their effects in the tumor mechanism. A better understanding of the working mechanism and effects of this promising structure will enable us to use the relevant existing treatments in more appropriate combinations and in appropriate cases, to prevent future undesirable effects and wrong treatments, and to better plan new treatments to be developed.

The purpose of this study is to investigate the relationship of TLR9, which has a remarkable place in cancer researches recently due to its relationship with tumor progression, with prognostic parameters in BC.

MATERIAL AND METHOD

The study was approved by Selçuk University Ethics Committee (Date: 13.01.2021, Decision no:2021/13). One-hundred and thirty nine consecutive patients with BC who underwent modified radical mastectomy between January 2009 and December 2018 were included in this study. Relevant data such as age, tumor diameter, multifocality, tumor stage, lymph node metastasis, distant metastasis, survival, and hormone receptor status were obtained from patient records. Hematoxylin-eosin stained pathology preparations were examined by 2 pathologists to evaluate pathological prognostic parameters such as histological subtype, tumor grade and presence of tumor necrosis, and to determine the appropriate tissue block for immunohistochemical staining.

The clinical and pathological staging of the cases was re-evaluated according to the American Joint Cancer Committee (AJCC) 2018 TNM BC staging system.

The molecular subtypes of the cases were determined according to the ki67 proliferation index, Her2 and hormone receptor status of the tumors. Accordingly, five different subtypes were obtained. Luminal subtype (A and B) is hormone receptor positive BC. The most common type is luminal A, which is low grade, has a low ki67 score, and has a good prognosis. Luminal B subtype expresses more proliferation and Her2 gene and less ER related gene. This subtype can be divided into two categories based on Her2 positivity or negativity. Her2 and triple-negative BC subtypes are high-grade and more aggressive types and show a high risk of systemic and local recurrence.[1]

To evaluate the association of TLR9 expression with progression-free survival (PFS) and overall survival (OS), tumors were analyzed in 6 categories, 1, 2, 3, 4, 6 and 9 based on IRS scores. Progression-free survival was defined as the interval from the date of completion of primary therapy to the date of clinical or radiological evidence of recurrent disease (confirmed by biopsy). Overall survival (OS) was accepted as the time from the date of diagnosis to death or last follow-up, without any restriction on the cause of death. These were calculated from follow-up records and the National Death Registry, last checked on December 20, 2018.

Immunohistochemical Staining Procedure

Sections were deparaffinized for 1 hour in an oven at 60°C. Immunohistochemical staining was performed using an automatic staining machine (Sequenza Immunostaining Center Each 73300001 Shandon / Thermo). For antigen retrieval, 1/10 diluted EDTA Buffer (PH: 8) (AP-9004-999 Thermo scientific) was applied in the PT Module (A80400012 Lab Vision). Sections were washed with PBS for 5 minutes.

Endogenous peroxidase activity was blocked by applying 3% hydrogen peroxide (TA-125-HP ThermoScientific). After washing with PBS for 10 minutes, protein blocking (TA-125-PBQ ThermoScientific) was performed and then sections were incubated for 60 minutes with a 1:100 dilution of primary antibody: anti-TLR9 antibody (ab37154, Abcam). Then the sections were incubated with Amplifier Quanto (TL-125-QPB ThermoScientific) for 20 minutes and with HRP Polymer Quanto (TL-125-QPH ThermoScientific) for 30 minutes, respectively. Washing was done with PBS at each step. To identify positive cells, staining was performed with DAB chromogen and finally counterstained with hematoxylin for 30 seconds. Spleen tissue was considered as positive control.

Evaluation and Scoring of Immunohistochemically Stained Slides

Preparations stained with immunohistochemical TLR9 were evaluated and scored by a pathologist who was blinded to the patients' data. In the light microscopic evaluation, the entire cross-section of the glass was reviewed at 100X magnification. The IRS system was used to determine TLR9 expression levels. This system,[8]

previously used by Wang et al., was dependent on staining intensity and percentage of positive cells. The IRS system is explained in **Table 1**.

Table 1: Immunorectivity scoring (IRS) system of TLR9

Percentage of Positive Cells (A)	Staining Intensity (B)
0: No positive cells	0: Negative
1: 1% to 33% positive cells	1: Mild
2: 34% to 66% positive cells	2: Moderate
3: 67% to 100% positive cells	3: Severe

Statistical Analysis

SPSS v25.0 package program was used for statistical analysis. The Kolmogorov-Smirnov Z test was used to test the normality of the distribution. Parametric analysis methods were used for normal distributions, non-parametric tests were preferred for abnormal distributions. Independent t-test was used to determine statistical significance when two groups were compared. Analysis of Variance (ANOVA) and non-parametric Kruskall-Wallis tests were used to compare the mean scores of more than two groups. Statistical significance was considered as $p<0.05$. PFS (progression-free survival) was defined as the time from diagnosis to recurrence or progression. OS (disease-free survival) was measured from the date of enrollment to the date of death from any cause. OS and PFS were estimated using the Kaplan-Meier method.

RESULTS

One-hundred and thirty nine patients diagnosed with BC were included in this study. All patients were female and the mean age of the patients was 55 (24-88).

We observed cytoplasmic staining in tumor cells with TLR9 (**Figure 1, 2**). TLR9 expression was reduced in normal acini compared to tumor cells (**Figure 1**).

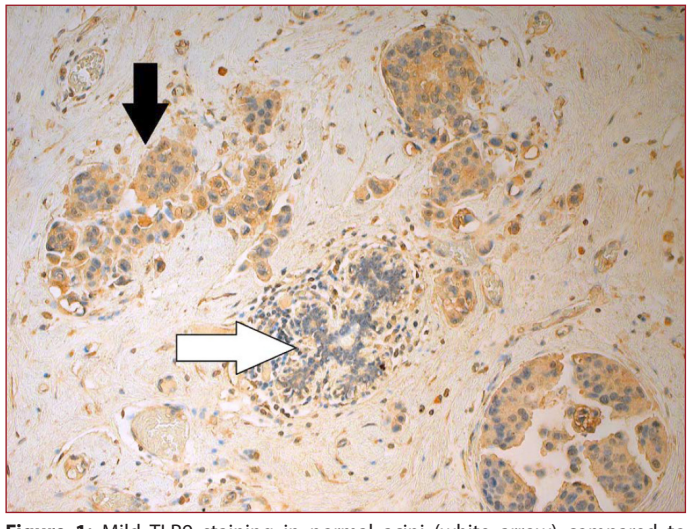

Figure 1: Mild TLR9 staining in normal acini (white arrow) compared to adjacent tumor tissue (black arrow) (TLR9, Original Magnification, X200).

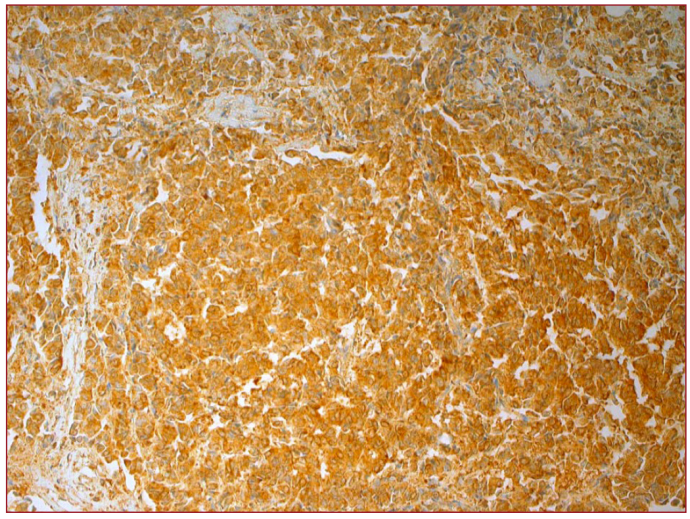

Figure 2: Strong and diffuse cytoplasmic TLR9 staining in a grade 3 tumor (TLR9, Original Magnification, X200).

Clinicopathological features of the patients are summarized in **Table 2**. One-hundred and seventeen cases were diagnosed with invasive carcinoma, not otherwise specified (NOS) and its variants. Of the variants, 4 (3.4%) were carcinoma with apocrine differentiation, 2 (1.7%) were invasive carcinoma with neuroendocrine differentiation. Other invasive carcinoma category included 13 (11.1%) invasive lobular carcinoma, 2 (1.7%) invasive papillary carcinoma, 2 (1.7%) mucinous carcinoma, 1 (0.8%) secretory carcinoma, 2 (1.7%) medullary carcinoma and 2 (1.7%) tubular carcinoma cases. A statistically significant difference was found between the histological subtypes of breast carcinoma in terms of TLR9 expression, and TLR9 expression was found to be significantly higher in patients with invasive carcinoma (NOS) than other invasive carcinomas (p=0.027).

Thirty-six (25.8%) of the cases were grade 1, 68 (48.9%) grade 2, 36 (25.8%) grade 3. TLR9 expression was found to be significantly higher in grade 3 tumors than in grade 1 and grade 2 tumors (p=0.001).

Twenty tumors had necrosis. Tumors with necrosis showed higher TLR9 expression than tumors without necrosis (p=0.03)

Twenty-seven (19.4%) of the cases were estrogen receptor (ER) negative, 27 (19.4%) were under 50% expression with ER, and 85 (61.1%) were over 50% with ER. A statistically significant correlation was found between the ER status of the cases and TLR9 expressions. TLR9 expression was found to be significantly higher in ER negative group than in the other two groups (p=0.004).

Seventy-eight (56.1%) of the cases were Her2 negative and 61 (43.9%) were Her2 positive. A statistically significant correlation was found between Her2 status and TLR9 expressions of the cases, and TLR9 expression was found to be significantly higher in the Her2 positive group than in the Her2 negative group (p=0.015).

Table 2: Relation of TLR9 expression with clinicopathological characteristics

Parameter	n	Mean IRS (±std)	P-value
Age (years)			0.131
<55	72	4.83±2.188	
≥55	67	5±2.146	
Histologic subtype			0.027
Invasive carcinoma (NOS) and variants	118	5.09±2.192	
Other invasive carcinomas	21	3.90±1.7	
Tumor grade			0.001
1	36	4.58±1.746	
2	68	4.28±2.094	
3	35	6.49±1.931	
DCIS			0.078
Absent	72	5.24±2.236	
Present	67	4.57±2.039	
Pathological T Stage			0.102
T1	45	4.3±0.286	
T2	73	5.30±0.257	
T3	19	4.68±0.502	
T4	2	6.5±2.50	
Pathological N Stage			0.345
N0	49	4.69±0.293	
N1	40	5.4±0.387	
N2	29	4.45±0.353	
N3	21	5.14±0.459	
Distant metastasis			0.962
M0	122	4.91±0.198	
M1	17	4.94±2.015	
Clinical stage			0.053
1	78	4.55±2.043	
2	26	6±2.4	
3	24	4.83±1.926	
4	11	5.09±2.3	
Tumor diameter			0.1
<2 cm	25	4.24± 1.832	
≥2 cm	114	5.06±2.207	
Molecular subtype			0.018
Luminal A	41	4.73±2.0.37	
Luminal B (Her2 negative)	31	3.94±1.999	
Luminal B (Her2 positive)	47	5.34±2.150	
Her2-rich	14	5.64±2.134	
Triple negative	6	6.17± 2.483	
Tumor focality			0.740
Unifocal	121	4.93±2.186	
Multifocal	18	4.78±2.045	
Tumor necrosis			0.030
Absent	20	4.73±2.04	
Present	119	6±2.406	
Vascular invasion			0.292
Absent	42	4.62±2.141	
Present	97	5.04±2.169	
Extracapsular extension			0.175
Absent	101	4.76±0.219	
Present	38	5.32±0.327	
Estrogen receptor			0.004
Negative	27	5.78±2.082	
<%50	25	5.58±2.120	
≥%50	85	4.44±2.084	
Progesteron receptor			0.148
Negative	45	5.18±2.149	
<%50	36	5.22±2.044	
≥%50	58	4.5±2.226	
Her2			0.015
Negative	78	4.53±2.118	
Positive	61	5.41±2.132	
Ki67			0.310
<%14	45	4.62±2.208	
%14-49	27	4.48±1.929	
≥%50	67	4.70±2.263	

n: Number of cases, Std: Standart deviation, NOS: Not otherwise specified, DCIS: Ductal carcinoma in situ

A statistically significant relationship was also found between molecular subtypes and TLR9 expressions (p=0.018). The lowest TLR9 expression was found in the luminal B Her2 negative group, and the highest expression was found in the triple-negative group. Furthermore, TLR9 expression was found to be significantly higher in the luminal B Her2 positive group and group 4, Her2 positive groups, compared to the luminal B Her2 negative group.

No statistically significant relationship was determined between TLR9 expression and tumor diameter and pathological tumor stage (p=0.1 and 0.102, respectively).

Table 3 summarizes the association of TLR9 expression with the site of metastasis. No statistically significant correlation was determined between TLR9 expression and metastasis site (p> 0.05).

Table 3: Relation of Site of Metastasis With TLR9 Expression

Parameter	n	Mean IRS (±std)	P- value
Bone metastasis			0,501
Absent	128	4,88±2,163	
Present	11	5,36±2,203	
Lung metastasis			0,739
Absent	135	4,93±0,187	
Present	4	4,5±0,866	
Liver metastasis			0,828
Absent	137	4,92±2,170	
Present	2	4,5±2,121	
Adrenal gland metastasis			0,290
Absent	136	4,89±2,180	
Present	3	6±0,01	

n: Number of cases, Std: Standart deviation.

Median follow-up was 54 (1-120) months. To evaluate the relationship of TLR9 expression with PFS and OS, cases were categorically evaluated as IRS 1, 2, 3, 4, 6 and 9. Accordingly, the mean OS in the groups with TLR9 scores 1 and 2 was significantly higher than those with TLR9 scores 3, 4, 6 and 9 (p=0.014). In terms of PFS, no difference was observed between TLR9 scores (p> 0.05) (**Table 4**, **Figure 3, 4**).

Table 4: Comparison of PFS and OS of patients in different TLR9 categories.

	TLR9 Score	Mean	Std. Error	Median	Min	Max	IQR	P*
PFS	1	99,17	22,931	121,50	6	147	101	0,166
	2	108,00	9,256	117,00	63	146	67	
	3	81,97	5,691	80,00	0	143	46	
	4	86,27	15,716	109,00	5	139	88	
	6	84,02	4,289	67,00	12	150	56	
	9	80,87	9,937	98,00	0	135	43	
OS	1[a]	120,50	13,635	129,50	56	147	42	0,014
	2[a]	112,92	9,063	128,00	63	146	61	
	3[b]	82,53	6,495	66,50	14	148	49	
	4[ab]	83,00	15,356	66,00	2	139	88	
	6[b]	82,65	3,767	68,00	5	148	48	
	9[ab]	85,87	5,872	97,00	54	121	38	

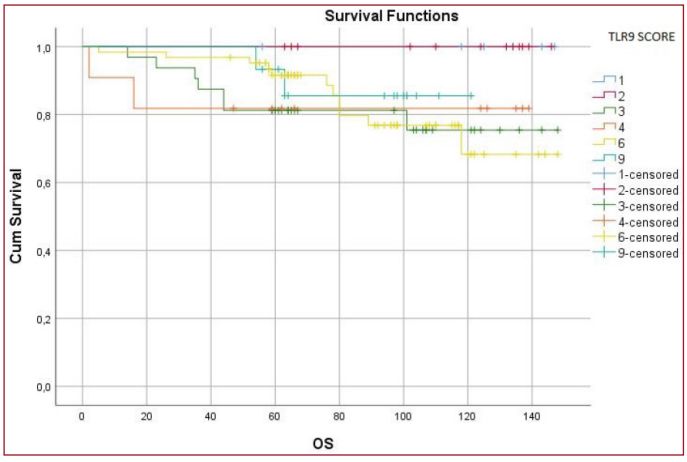

Figure 3: Kaplan Meier curves for overall survival (OS) of TLR9 expressions.

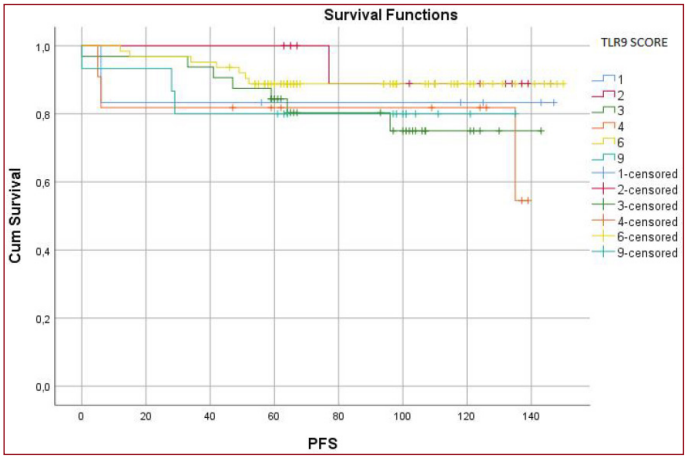

Figure 4: Kaplan Meier curves for progression free survival (PFS) of TLR9 expressions.

DISCUSSION

In the present study, we analyzed immunohistochemical expression of TLR9 in 139 BC patients, which is one of the largest patient series in the literature, and revealed that TLR9 expression is associated with some prognostic parameters such as histologic type, tumor grade, presence of tumor necrosis, ER and Her2 status, molecular subtype as well as overall survival in BC.

It is well known that cancer and inflammation are related entities, consequently, persistent inflammatory events can lead to carcinogenesis.[3,9-12] In the current literature, several studies exist to investigate relation of TLRs with both inflammation and cancer. TLRs are important components of the innate immune system not only defense host against several infectious agents, but also shown to be expressed by many epithelial tumor cells including BC.[4,11]

Among TLRs, TLR9 has shown to be highly expressed in BC cells and capable of promoting cellular invasion in vitro by increasing matrix metalloproteinase activity.[13,14] It was revelad that TLR9 assists in the progression of BC and patients with BC have higher circulating levels of TLR9 compared to normal.[11,13]

In their study, González-Reyes et al.[3] suggested that BCs with high TLR9 expression by fibroblast-like cells were associated with low probability of metastasis. In contrast, Jukkola-Vuorinen et al.[5] revealed that BCs metastasized to axillar lymph nodes at the time of diagnosis had slightly higher TLR9 expression compared with tumors with no axillary lymph node metastasis. Similarly, Qiu et al.[13] found that TLR9 expression was significantly higher in patients with BC displaying lymph node metastasis or advanced pathological stage. In our study, no relation was detected between TLR9 expression and lymph node metastasis status and stage, as well as distant metastasis.

We also evaluated the possible relationship between TLR9 expression and tumor grade, histologic and molecular subtypes, and hormone receptor status. Invasive carcinoma (NOS) subtype, formerly known as invasive ductal carcinoma, showed higher TLR9 expression compared to other subtypes. This finding differed from Jukkola-Vuorinen et al. study, who revealed the highest TLR9 expression levels in tumors with mucinous morphology.[5] In the same study, grade 3 tumors had higher TLR9 expressions than lower grade tumors as revealed by Meseure et al.[4] Our results regarding to relation of TLR9 expression with tumor grade was consistent with the literature and highest in grade 3 tumors.

Although very little is known about the relationship between TLR9 expression and ER function, previous studies revealed that estradiol and especially progesterone inhibits TLR9-mediated inflammation in both human and mouse plasmocytoid dendritic cells.[5,15] It was also suggested that these steroids could affect downstream signaling proteins in the TLR pathway.[15] In a previous study, it was shown that ER-negative tumors had higher expression with TLR9 compared to ER-positive tumors.[5] In another study, high TLR9 levels were associated with favorable outcome in triple-negative tumors.[4] In our study, similarly, higher TLR9 expression was detected in ER-negative tumors compared to ER-positive groups but on the contrary, the highest TLR9 expression was present in triple-negative subgroup, supporting that high TLR9 expression is a poor prognostic parameter in BC.

According to the current literature, there is also a relationship between TLR9 expression and the survival of patients with BC. Tuomela et al.[16] suggested that low tumor TLR9 expression is associated with significantly shortened disease-specific survival in patients with triple-negative BC. Meseure et al.[4] revealed better metastasis free survival in triple-negative BC patients with higher TLR9 levels. However, in the present study, low TLR9 expression was also found to be associated with better overall survival, supporting the association of other favorable prognostic parameters with low TLR9 expression in BC.

There are also some limitations to our study. This study was conducted in a single center with patients who did not have uniform or randomized treatment or follow-up. Prospective studies with larger patient series are needed to use TLR9 as a prognostic biomarker in BC.

CONCLUSION

TLR9 expression in BC is associated with some prognostic parameters such as the histologic type of tumors, histologic grade, presence of tumor necrosis, ER and Her2 status, molecular subtype as well as overall survival. Thus, we can conclude that low TLR9 expression is associated with better prognosis in patients with BC. Further studies with larger patient series are needed to shed light on the use of TLR9 as a clinical and therapeutic target in BC.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by Selçuk University Ethics Committee (Date: 13.01.2021, Decision no:2021/13)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Does Platelet Distribution Width Correlate with Acute Coronary Syndrome Severity and Coronary Thrombus Burden?

Trombosit Dağılım Genişliği Akut Koroner Sendrom Şiddeti ve Koroner Trombüs Yükü ile İlişkili mi?

Hızır Okuyan

University Of Health Sciences, Konya Beyhekim Training And Reseasrch Hospital, Department Of Cardiology, Konya, Turkey

Abstract

Aim: Platelets play a pivotal role in the pathogenesis of acute coronary syndrome (ACS), and platelet distribution width (PDW) shows the activities of platelets in circulation. The purpose of this study was to determine the association of PDW with the ACS severity and angiographic intracoronary thrombus burden and thrombolysis in myocardial infarction (TIMI) flow grade.

Material and Method: One hundred forty-nine consecutive patients who were diagnosed as having ACS were retrospectively evaluated. Global Registry of Acute Coronary Events (GRACE) scores were calculated from the clinical and laboratory data for ACS severity. TIMI flow grades and thrombus grades of the culprit coronary arteries were calculated for each patient. Patients were grouped into two groups according to their PDW values: high PDW and low PDW. The SPSS 17.0 software was used for statistical analysis.

Results: PDW was found to be correlated with GRACE scores ($p<0.01$). The high PDW group had higher GRACE scores (139 vs. 126, $p<0.05$). Lower TIMI flow grades (0 and 1) were seen in the high PDW group (39 vs. 33, $p<0.05$). No association between intracoronary thrombus load and PDW was found in our study ($p=0.082$).

Conclusion: In our study, patients with higher PDW levels were older and had higher GRACE scores and lower TIMI flow grades. PDW levels correlate with the ACS severity and TIMI flow grade.

Keywords: Platelet distribution width, coronary thrombus, TIMI flow, acute coronary syndrome

Öz

Amaç: Trombositler, akut koroner sendromun (AKS) patogenezinde önemli bir rol oynar ve trombosit dağılım genişliği (PDW), trombositlerin dolaşımdaki aktivitelerini gösterir. Bu çalışmanın amacı, PDW'nin AKS şiddeti, anjiyografik intrakoroner trombüs yükü ve Miyokard Enfarktüsünde Tromboliz (TIMI) akım derecesi ile ilişkisini belirlemektir.

Gereç ve Yöntem: AKS tanısı alan ardışık 149 hasta geriye dönük olarak değerlendirildi. Akut Koroner Olayların Global Kayıtları (GRACE) skorları, AKS şiddeti için klinik ve laboratuvar verilerinden hesaplanmıştır. Suçlu koroner arterlerin TIMI akım dereceleri ve trombüs dereceleri her hasta için hesaplandı. Hastalar PDW değerlerine göre yüksek PDW ve düşük PDW olmak üzere iki gruba ayrıldı. İstatistiksel analiz için SPSS 17.0 programı kullanıldı.

Bulgular: PDW, GRACE puanları ile korele bulundu ($P<0,01$). Yüksek PDW grubu daha yüksek GRACE skorlarına sahipti (139'a karşı 126, $p<0,05$). Yüksek PDW grubunda daha düşük TIMI akış dereceleri (0 ve 1) görüldü (39'a karşı 33, $p<0,05$). Çalışmamızda intrakoroner trombüs yükü ile PDW arasında ilişki bulunmadı ($p=0,082$).

Sonuç: Çalışmamızda PDW düzeyi daha yüksek olan hastalar daha yaşlıydı ve GRACE skorları daha yüksek ve TIMI akış dereceleri daha düşüktü. PDW seviyeleri, AKS şiddeti ve TIMI akış derecesi ile ilişkilidir.

Anahtar Kelimeler: Trombosit dağılım genişliği, koroner trombüs, TIMI akışı, akut koroner sendrom

Corresponding *(İletişim)*: Hızır OKUYAN, M.D., University Of Health Sciences, Konya Beyhekim Training And Research Hospital, Department Of Cardiology, Selçuklu, Konya, Turkey
E-mail *(E-posta)*: hizirokuyan@gmail.com
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INTRODUCTION

Acute coronary syndrome (ACS) includes a spectrum of clinical presentations that result from partial or complete acute obstruction of a coronary vessel. Vulnerable atherosclerotic plaque rupture and resulting thrombus formation is well-known pathophysiology. Clinical presentations vary from unstable angina pectoris (USAP) to sudden cardiac death.[1] Platelets play a major role in the pathogenesis of ACS. Besides thrombus formation, they are also involved in inflammation and immune system modulation.[2] Platelets are enlarged after they are activated. Mean platelet volume (MPV) and platelet distribution width (PDW) are the laboratory parameters that show the sizes, in other words, the "activities" of platelets in circulation.[3] A link between coronary thrombus formation and larger platelet volume in patients with ACS has been shown previously.[4] In a recent study, an association between PDW and major adverse cardiac events was demonstrated.[5] In another study, the association between PDW and coronary artery disease (CAD) severity was shown.[6] The severity of acute coronary events and also intracoronary thrombus load may be related to PDW. In this study, we aimed to determine the association between PDW, ACS severity, angiographic intracoronary thrombus burden, and thrombolysis in myocardial infarction (TIMI) flow grade.

MATERIAL AND METHOD

The clinical, angiographic, and laboratory data of 149 consecutive patients who were diagnosed as having ACS [11.4% USAP, 56.4% ST-elevation myocardial infarction (36.3% anterior myocardial infarction, 20.1% inferior myocardial infarction), 32.2% non-ST elevation myocardial infarction (NSTEMI)] were evaluated in this study. The Global Registry of Acute Coronary Events (GRACE) score for in-hospital mortality was calculated.[7] GRACE scoring estimates the probability of in-hospital death in patients with ACS. All patients' angiographic records were evaluated by an interventional cardiologist who was blinded to their clinical and demographic data. TIMI flow grades and thrombus grades of the culprit coronary arteries were calculated for each patient.[8-10] TIMI flow grades were defined as TIMI 0 Flow=No penetration of contrast beyond stenosis (100% stenosis, occlusion); TIMI 1 Flow=Penetration of contrast beyond stenosis but no perfusion of distal vessels (99% stenosis, sub-total occlusion); TIMI 2 Flow=Contrast reaches distal vessels but either at a decreased rate of filling or clearing when compared with the other coronary arteries (partial perfusion); TIMI 3 Flow=Contrast reaches the distal bed and clears at the same rate when compared with the other coronary arteries (complete perfusion). TIMI thrombus grades were defined as Grade 0: No visible thrombus; Grade 1: Angiographic features suggesting the presence of thrombus; Grade 2: Definite thrombus in angiographic views with the greatest dimension of <1/2 vessel diameters; Grade 3: Definite thrombus in angiographic views with the greatest dimension from >1/2 to <2 vessel diameters; Grade 4: Definite thrombus in angiographic views with the greatest dimension of >2 vessel diameters; Grade 5: Complete thrombotic occlusion of a vessel. Thrombus grades of patients with a TIMI thrombus grade 5 were reclassified from angiographic views after the removal of total occlusion after a guidewire advancement of balloon angioplasty.[11] The patients were divided into two groups according to the 50% percentile of PDW values, patients with high PDW and patients with low PDW. Patients with malignant disease, renal or hepatic failure, acute or chronic infection, inflammatory disease, and any hematologic disease including anemia, were excluded from the study. Immediately after emergency department admission, venous blood samples were drawn for blood chemistry analysis and complete blood counts, which were performed using a Sysmex counter XT 1800i (Sysmex). The study was carried out with the permission of KTO Karatay University NonInterventional Clinical Researches Ethics Committee (Date: 20.12.2021, Decision No: 2021-0199). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical analysis

The SPSS 17.0 software was used for statistical analysis. Spearman's rank correlation test was performed to determine the association between PDW and GRACE risk score. The Kruskal-Wallis test was used to determine any difference between the PDW values of each TIMI flow grade and each thrombus grade subclass. The t-test and Mann-Whitney test were used to test the difference between continuous variables of the high-PDW and low-PDW groups. The Chi-square test was used to test the difference between categorical variables of the high and low-PDW groups.

RESULTS

The demographic, clinical, laboratory, and angiographic data of the patients are presented in **Table 1**. Our study group consisted of mostly male patients. Most of the patients were aged around the sixth decade. Acute anterior myocardial infarction was the most frequently seen presentation.

We found that PDW ($r=0.304$, $p<0.001$) was significantly positively correlated with GRACE scores. When the analysis was performed among each TIMI thrombus grade alone, PDW values showed no differences ($p=0.187$). Patients were grouped into two groups according to their angiographic thrombus size: low thrombus burden (TIMI thrombus grade 0 and TIMI thrombus grade 1) and high thrombus burden (TIMI thrombus grade 2, 3, and 4) and PDW values did not differ between these groups [11.6 (8.9-19.9) vs. 11.9 (9.3-22.0), $p=0.40$].

Table 1. Demographic, clinical, laboratory, and angiographic data of the patients.

Variable		
Age	60.1±12.4	
Sex (Male %)	117 (78.5%)	
Diabetes mellitus	28 (18.8%)	
Hypertension	61 (40.1%)	
Hyperlipidemia	65 (43.6%)	
Current smokers	50 (33.5%)	
Creatinin (mg/dL)	0.9 (0.4-3.0)	
PDW (fL)	11.8 (8.9-22.0)	
Presentation		
USAP	17 (11.4%)	
NSTEMI	48 (32.2%)	
Anterior MI	54 (36.3%)	
Inferior MI	30 (20.1%)	
GRACE score	134.4 (68-334)	
TIMI thrombus grade	**Number of patients**	**Median PDW**
0	36 (24.2%)	11.45 (8.9-15.2)
1	27 (18.1%)	11.8 (9.4-19.9)
2	37 (24.8%)	12.5 (9.6-22.0)
3	40 (26.8%)	11.5 (9.3-16.3)
4	9 (6%)	12.4 (9.7-16.2)
TIMI Flow Grade	**Number of patients**	**Median RDW**
0	52 (34.9%)	11.75 (9.3-22.0)
1	13 (8.7%)	12.7 (9.7-18.8)
2	37 (24.8%)	13.5 (9.4-20.1)
3	47 (31.5%)	11.4 (8.9-19.9)

PDW values according to angiographic TIMI flow grades were different between the groups (p<0.01). It was seen that patients with TIMI-3 flow grade had lower PDW values than other patients. A comparison of demographic, clinical, laboratory, and angiographic characteristics of the high-PDW and low-PDW patient groups is given in **Table 2**. Patients in the high PDW group were older. Female sex predominance was present in the high PDW group. Patients in the high PDW group had higher GRACE scores. Lower TIMI flow grades (0 and 1) were seen in patients in the high PDW group. The coronary thrombus burden of the high-PDW and low-PDW groups was not different.

Table 2. Comparison of demographic, clinical, laboratory, and angiographic characteristics of the high-PDW and low-PDW patient groups.

	Low-PDW (n=78)	High-PDW (n=71)	P-value
Age	57.5±12.4	62±11.8	<0.01
Sex (Male %)	68 (87.2%)	49 (69%)	<0.01
Diabetes mellitus	13 (16.6%)	15 (21.1%)	0.05
Presentation			
USAP	8 (10.2%)	9 (12.7%)	
Anterior MI	26 (33.3%)	28 (39.4%)	
Inferior MI	19 (24.4%)	11 (15.5%)	
NSTEMI	25 (32.1%)	23 (32.4%)	
Creatinin (mg/dL)	0.7 (0.4 – 0.9)	1 (0.9 – 3.0)	0.93
GRACE score	126 (68 – 192)	139 (75 – 334)	<0.05
Thrombus grade			
0 and 1	36 (46.1%)	27 (38.0%)	
2, 3, and 4	42 (53.8%)	44 (61.9%)	0.08
TIMI flow grade			
0 and 1	33 (42.3%)	39 (54.9%)	
2 and 3	45 (57.6%)	32 (45.9%)	<0.05

DISCUSSION

In our study, PDW was found to be higher, especially in women and the elderly. High PDW may be one of the reasons why cardiovascular adverse events are more frequent in these special groups. PDW can be used as an important parameter to indicate adverse cardiac events in these patient groups. In our study, we also found a significant relationship between PDW levels and GRACE scores, and TIMI flow grade in patients with ACS. The GRACE risk scoring system has been shown to accurately predict mortality for patients with ACS, including ST-elevation MI, non-ST-elevation MI, and unstable angina pectoris.[12] In other words, GRACE scores indicate the severity of ACS. In addition to thrombus formation, platelets also play a role in inflammation and the immune system. During acute myocardial infarction, they activate leukocytes by secreting inflammatory mediators and form aggregates with leukocytes.[13,14]

Platelets are heterogeneous in size. When they pass from the bone marrow to the peripheral circulation, they are in large volume and this is suitable for their reactivity.[15,16] During the time that platelets are in circulation, their volume gradually decreases.[17] In the case of stress, platelet production is stimulated and large platelets are released into the circulation.[18] PDW is a reflection of this. Increased megakaryocyte heterogeneity as a result of increased thrombopoietic activity in the bone marrow results in an increase in PDW in peripheral blood count.[19] The growth of platelets in ACS indicates increased thrombopoietic activity in the bone marrow.[20] PDW is also a reflection of this. Increased megakaryocyte heterogeneity as a result of increased thrombopoietic activity in the bone marrow results in an increase in PDW in peripheral blood counts.[19] The growth of platelets in ACS indicates increased thrombopoietic activity in the bone marrow.[20] Thus, increased PDW in patients with ACS may be a marker of increased thrombopoietic activity driven by increased cytokines.

In our study, the relationship between GRACE risk scores and PDW can be explained by the increased thrombopoietic activity caused by increased cytokines, and it can be said that the cause of ACS severity is correlated with increased stress. In addition, we aimed to evaluate the relationship between coronary thrombus and PDW according to the degree of TIMI thrombus in our study. We could not find a similar study in the literature. A study in which only the presence of thrombi was evaluated was found, and a relationship was shown between the presence of thrombi and PDW.[22] However, in our study, no correlation was found between coronary thrombi and PDW according to the TIMI thrombus grade.

In our study, the mean PDW value was also found to be associated with TIMI flow grade. There was an inverse correlation between TIMI flow grade and PDW. It was thought that this result might be a reflection of the relationship between low TIMI flow grades and the severity of ACS. In a study performed on patients with ST-elevation MI[12] after

primary PCI, when no-reflow was defined as group 1 (TIMI flow grade 0, 1, or 2) and angiographic success was defined as group 2 (TIMI flow grade 3), PDW was found to be higher in patients without reflow, similar to our study. As a result, PDW, a laboratory marker of "stress platelets," was thought to be related to the severity of ACS and TIMI flow grades. In addition, PDW was found to be higher, especially in women and the elderly, and could be used as an important parameter to show adverse cardiac events in these groups.

Limitations

The main limitation of this study was its single-center and retrospective design. We demonstrated an association between PDW levels and ACS severity but found no association between intracoronary thrombus load and PDW in our study.

CONCLUSION

The reason for this result could be the small study population. Nevertheless, this study can provide a basis for clinical studies with larger study populations. In addition, our study population consisted of patients with acute coronary syndrome, including those with ST-elevation myocardial infarction, non-ST elevation myocardial infarction, and unstable angina pectoris, and these subgroups were not evaluated in detail due to the low number of patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of KTO Karatay University NonInterventional Clinical Researches Ethics Committee (Date: 20.12.2021, Decision No: 2021-0199).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluation of Urine Cotinine Levels in Children with Chronic Cough

Kronik Öksürüklü Çocukların İdrar Kotinin Düzeyleri İle Değerlendirilmesi

Yesim Ozdemir[1,2], Ilknur Bostanci[3], Cigdem Ozdilekcan[3,4], Serap Ozmen[3]

[1]Health Sciences University Dr. Sami Ulus Obstetrics, Children and Diseases Training and Research Hospital Department of Pediatrics, Ankara, Turkey
[2]Eskişehir City Hospital, Department of Pediatric Nephrology, Eskişehir, Turkey
[3]Health Sciences University, Dr. Sami Ulus Obstetrics, Children's Health and Diseases Training and Research Hospital, Department of Pediatric Immunology and Allergy, Ankara, Turkey
[4]Health Sciences University, Dr. Abdurrahman Yurtarslan Hospital, Department of Pulmonology, Ankara, Turkey

Abstract

Aim: Chronic cough is defined as a type of cough that exists more than four weeks. This study was planned to reveal the objective relation between urine cotinine level and tobacco smoke exposure in children suffering from chronic cough.

Material and Method: Between 01.01.2012 and 01.07.2012, children aged 5-18 years who applied to the Pediatric Allergy Department with chronic cough without any etiology (patient group) and healthy volunteers of the same age group (control group) were included in the study.

Results: A total of 112 children, 58 with chronic cough and 54 healthy, were included in the study. There was a statistically significant difference between urine cotinine levels of cases of those who were exposed and unexposed (based on the declaration of parents) to tobacco smoke ($p<0.05$). The highest mean value of cotinine (41.3±73.7) was detected in the cases of the chronic cough group exposed to smoke. The best "cut-off value" for discriminating between exposed and unexposed groups was 12.15 ng/ml with ROC analysis. The smoking ratio during pregnancy was higher in the group with chronic cough ($p<0.05$). FEV1/FVC ratio was lower in patients with chronic cough who are passive smokers compared with the cases in the other groups ($p<0.05$).

Conclusions: This is the first study objectively evaluating the chronic cough effect of passive smoking with urine cotinine level. Since there is a meaningful decrease in FEV1/FVC ratio, close follow-up is needed in children, especially those diagnosed with chronic cough who are exposed to tobacco smoke.

Keywords: biomarkers, children, chronic cough, environmental tobacco smoke, passive smoking, urine cotinine level

Öz

Amaç: Dört haftadan uzun süre devam eden öksürük kronik öksürük olarak tanımlanır. Bu çalışma, nedeni saptanamayan kronik öksürüklü çocuklarda idrar kotinin düzeyi ile sigara dumanı maruziyetini objektif olarak göstermek için planlandı.

Gereç ve Yöntem: 01.01.2012 – 01.07.2012 tarihleri arasında Çocuk Alerji Polikliniği'ne başvuran 5-18 yaş arası, kronik öksürüğü olup herhangi bir etiyoloji saptanamayan çocuklar (hasta grubu) ve aynı yaş grubu sağlıklı gönüllüler (kontrol grubu) çalışmaya dahil edildi. **Bulgular**: Kronik öksürüğü olan 58 ve 54 sağlıklı olmak üzere 112 çocuk çalışmaya dahil oldu. Ebeveyn beyanına göre sigara dumanına maruz kalan ve kalmayan olguların idrar kotinin düzeyleri arasında istatistiksel olarak anlamlı fark vardı ($p<0,05$). En yüksek ortalama kotinin düzeyi (41,3±73,7) kronik öksürüğü olup sigaraya maruz kalan grupta saptandı. Sigara dumanına maruz kalan ve kalmayan grupları ayırt etmek için en iyi "cut-off" değeri (kesim noktası) ROC analizi ile 12,15 ng/ml olarak bulundu. Gebelikte sigara içme oranı kronik öksürüğü olan ve sigara dumanına maruz kalan grupta daha yüksekti ($p<0,05$). Pasif içici olan kronik öksürüğü olan çocuklarda FEV1/FVC oranı diğer gruplara göre daha düşüktü ($p<0,05$).

Sonuç: Bu çalışma, pasif içiciliğin çocuklarda kronik öksürük üzerine etkisini idrar kotinin düzeyi ile objektif olarak değerlendiren ilk çalışmadır. FEV1/FVC oranında anlamlı düşme nedeniyle, tütün dumanına maruz kalan kronik öksürüklü çocukların yakın takibi gereklidir.

Anahtar Kelimeler: Biyobelirteçler, çevresel tütün dumanı, çocuk, kronik öksürük, pasif içicilik, idrar kotinin düzeyi

Corresponding *(İletişim)*: Yesim ÖZDEMİR ATİKEL, Eskişehir City Hospital, Department of Pediatric Nephrology, Odunpazarı, Eskişehir, Turkey
E-mail *(E-posta)*: yesim81ozdemir@outlook.com
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INTRODUCTION

Chronic cough is a type of cough that exists every day with a duration of more than four weeks.[1,2] Passive smoking is the exposure to tobacco smoke of a nonsmoking person through close contact or by sharing a common household.[2] In the literature, instead of passive smoking, the terms 'exposure of environmental tobacco smoke (ETS)', 'second-hand smoking', 'involuntary smoking', and 'exposure of side stream smoke' are commonly used.[1]

There is a growing amount of evidence concerning significant harms on respiratory system health about the exposure to ETS for children.[3-5] It has been reported that sharing the household with especially smoking mothers, related to the number of cigarettes smoked per day; causes recurrent respiratory system infections, wheezing, and chronic cough starting from early infancy.[6,7]

The reliability of parent declarations concerning the frequency of exposure to passive smoking is lower than objective measurements to understand the frequency of passive smoking exposure. Therefore, objective measures of biochemical markers are needed since the estimation of the prevalence of tobacco smoke exposure is probably low according to questionnaire data.[8,9] Various biomarkers are used to evaluate both active and second-hand smoking, but the most popular, specific and reliable marker is cotinine.[9-11]

Cotinine is the metabolite of nicotine, the major component of tobacco smoke.[12] Cotinine has a longer half-life than nicotine.[13] Since cotinine values in biological fluids (blood, saliva, semen, urine) are highly consistent, blood cotinine can also be accurately estimated by measuring the cotinine level in urine.[14] It is thought that the kidney concentrates cotinine, increasing the concentration in urine to levels 5-6 times higher than the concentration in plasma and saliva.[15] Various foods (such as cauliflower, eggplant, tomato, tea) contain low amounts of nicotine; however, dietary nicotine intake may be ignored in studies since it has been determined that daily intake does not cause a significant difference in cotinine levels.[14] Since urine cotinine is completely specific to cigarettes and a product of internal metabolism, the possibility of changes in cotinine levels with external environmental conditions during the collection of samples is also low.[16]

This study aimed to reveal the objective relationship between urine cotinine level and passive smoking or ETS exposure in children suffering from chronic cough.

MATERIAL AND METHOD

Selection and Collection of Study Cases

The including criteria for volunteers:

1. Children aged between 5-18 years admitted to the Pediatric Allergy and Immunology Outpatient Clinic of Dr. Sami Ulus Obstetrics, Children and Diseases Training and Research Hospital with chronic cough (duration of more than four weeks) without underlying any specific cause were included in the study (patient group). This group was also divided into two according to parents' declarations of the presence or absence of ETS (exposed/unexposed).
2. The similar age group of cases, healthy, without a history of chronic cough, and presence or absence of ETS according to parents' declarations (control group).

The exclusion criteria for volunteers:

History of prematurity, underlying chronic diseases such as chronic cardiac, renal, pulmonary diseases, chest deformities, neuromotor development retardation, malnutrition, obesity, and immune system disorder, those who lead to recurrent infections and active smokers were excluded from the study.

Study Protocol

The study design was planned as a real-life, cross-sectional trial. After getting the approval of the Ministry of Health Ankara Kecioren Education and Research Hospital's Ethics Committee on 27.06.2012 and with approval number B.10.4.İSM.4.06.68.49, the study was initiated. Written informed consent was taken from children and their parents. Detailed information was given. A standardized questionnaire form including social and demographic properties and smoking habits was applied for both children and their parents. Data was collected by the investigator by using face to face technique (by YO).

The evaluation form included the presence of cough in children, duration of cough, indoor smoking habits of parents, the part of the house parents using to smoke, the number of cigarettes per one day, smoking habit during pregnancy, education status of parents, monthly income of the family, the type of heat resources of house and number of rooms, number of individuals living in the same house and disease history of children. If indoor smoking in any area of the house is declared, this situation was considered passive smoking/ETS exposure. A family's monthly income level was evaluated by dividing it into two groups regarding the Turkish Statistical Institute Income Distribution and Living Conditions Statistics data at the time of the study: low income and normal or high income.

The parents were asked about their children's wheezing and previous respiratory system infections. Over eight upper/lower respiratory tract illnesses per year were defined as 'frequently getting ill'. Lung function testing was performed using a portable spirometry device (MIR spirolab). Children were shown how to perform the test by clamping the nostrils in standing position. Among the three measurements, the best value was taken into account. Forced vital capacity (FVC), forced expiratory volume during the first second (FEV1), FEV1/FVC ratio, forced expiratory flows between 25% and 75% of FVC (FEF 25-75%), and peak expiratory flow (PEF) were measured. Reference values were evaluated in accordance with the age of the children.[17]

Collection of Urine Samples and Laboratory Analysis

Samples of the morning's first urine were collected from the subjects included in the study. At least 5 ml of urine collected in sterile and capped urine containers with no preservatives was placed in a refrigerator at +4 °C without waiting and stored at

-20 °C within one hour at the latest. On the working day, urines were dissolved at room temperature, centrifuged at 3000 rpm for 5 minutes, and the supernatant portions were separated. The remaining sediment was gently inverted and placed in the device. Urine cotinine level was measured with Immulite 2000 Analyzer Nicotine Metabolite Device (Siemens) using solid phase competitive chemoluminescence immunoassay method. Cotinine levels were calculated in ng/ml.

Statistical Analysis

Data analysis was performed using SPSS for Windows 15.00 package program. Shapiro Wilk test was used to assess the distribution of continuous variables. In determining whether the cases were exposed to smoking or not by urinary cotinine measurements was evaluated by calculating the area under the ROC curve smoke, if there is an important area under the curve. p value <0.05 was considered statistically significant.

RESULTS

The study included a total of 112 children: 58 with chronic cough (patient group) and 54 without chronic cough (control group). Cases were divided into two groups according to exposure to passive tobacco smoke and unexposed ones according to their parents' declaration. Urine collections and analyses were performed on total 114 subjects. The urine cotinine level was measured as >500 ng/ml in two children who are active smokers indicating the exclusion criteria (parents also declared their children's active smoking status). One case with a urine cotinine level of 416 ng /ml was included in the study as a passive smoker since both the parents and himself absolutely denied active smoking.

In 59.8% of total cases, urine cotinine levels were found to be above 10 ng/ml, which was described as the threshold. The subjects that were declared unexposed to ETS smoke were found to have high urine cotinine levels (shown in **Table 1** and **Table 2**). According to parents' declarations, 32% of children without the presence of household smokers were found to have urine cotinine levels >10 ng/ml. There was a significant statistical difference between the exposed and unexposed group according to urine cotinine level (p<0.001). The best "cut-off value" for discriminating between exposed and unexposed group was 12.15 ng/ml (**Figure 1**).

Table 2. The distribution of urinary cotinine levels in children with or without chronic cough, according to the declarations of smoking status of parents

	Passive smoking (+) (n,%)	Passive smoking (-) (n,%)	p
Urinary cotinine levels (ng/ml)			
<10	11 (17.7)	34 (68)	0.442*
>10	51 (82.3)	16 (32)	

*Chi-Square Test (McNemar Test)

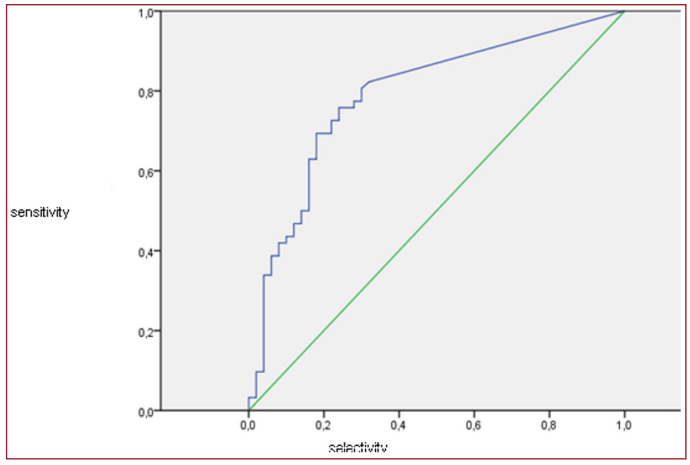

Figure 1. ROC curve of urine cotinine measurements in distinguishing children exposed and non-exposed to ETS

The demographic and social status of cases is given in **Table 3**. Considering parent's educational status, 47.5% of mothers and 32.3% of fathers were the primary school graduates. All fathers were literate and 37.4% graduated from high school. Between the groups, there was no statistically significant difference between parents' educational status (p>0.05). The cases whose mothers were primary school graduates had the most chronic cough symptom in the passive smoking group, while passive smoking rate was the least among those whose mothers were university graduates (57.1% and 7.1%, respectively). On the other hand, the most university-graduated mothers belonged to the unexposed group in the control group. However, no statistical significance was found between these two groups (p>0.05). Children with fathers of primary school graduates were the most exposed group to tobacco smoke. The mother's educational status had some influence on urine cotinine levels (p=0.042), whereas the father's educational level had none (p=0.159).

Table 1. Demographic features and urinary cotinine levels of the patients

Variables	Chronic cough group		Control group		p
	Passive smoking (+) (n=32)	Passive smoking (-) (n=26)	Passive smoking (+) (n=30)	Passive smoking (-) (n=24)	
Age (years) mean±SD	9.5±3.1	8.9±3.4	8.9±3.1	10.7±3.4	0.171*
Gender					
Male (%)	17 (53.1)	16 (61.5)	16 (53.3)	15 (62.5)	0.831**
Urinary cotinine levels(ng/ml)					
<10	6 (18.8)	15 (57.7)	5 (16.7)	19 (79.2)	<0.001***
>10	26 (81.3)	11 (42.3)	25 (83.3)	5 (20.8)	
Urinary cotinine levels (ng/ml)					
mean±SD	41.3± 73.7	13.2±5.6	23.2±13.9	17.5±25.6	<0.001*
median [min– max]	18.5[10-416]	10[10-29.6]	19.3[10-69.8]	10 [10-129]	

* Kruskal Wallis test; **Chi-Square test***;Pearson Chi-Square test

Table 3. Socio-economic characteristics of the children with and without chronic cough according to the smoking status of their parents

	Chronic cough group		Control group		p
	Passive smoking (+) (n,%)	Passive smoking (-) (n,%)	Passive smoking (+) (n,%)	Passive smoking (-) (n,%)	
Maternal education					
Illiterate	1 (3.6)	0 (0)	1 (3.7)	1 (4.8)	0.382*
Primary school	16 (57.1)	11 (47.8)	11 (40.7)	9 (42.9)	
Middle school	6 (21.4)	1 (4.3)	5 (18.5)	2 (9.5)	
High school	3 (10.7)	9 (39.1)	8 (29.6)	5 (23.8)	
University	2 (7.1)	2 (8.7)	2 (7.4)	4 (19.0)	
Paternal education					
Illiterate	0 (0)	0 (0)	0 (0)	0 (0)	0.343*
Primary school	7 (25.0)	7 (30.4)	9 (33.3)	9 (42.9)	
Middle school	9 (32.1)	3 (13.0)	3 (11.1)	2 (9.5)	
High school	10 (35.7)	10 (43.5)	12 (44.4)	5 (23.8)	
University	2 (7.1)	3 (13.0)	3 (11.1)	5 (23.8)	
The level of income					
Low	19 (63.3)	12 (48,0)	15 (55,6)	11 (50.0)	0.069*
Normal or high	11 (36.7)	13 (52,0)	12 (44,4)	11 (50.0)	
Home					
Poor housing	5 (15,6)	3 (11.5)	1 (3.4)	3 (13.0)	0.110*
Standard apartment	22 (68,8)	23 (88.5)	26 (89.7)	17 (74)	
Detached house	5 (15,6)	0 (0)	2 (6.9)	3 (13.0)	
Heating type					
Stove	10 (31.2)	5 (19.2)	2 (6.9)	5 (21.7)	0.149*
Natural gas	22 (68.8)	21 (80.8)	27 (93.1)	17 (74)	
Electricity	0 (0)	0 (0)	0 (0)	1 (4.3)	

*Chi-Square

There were no significant statistical differences between the groups regarding the duration, time differences and quantity of cough (p>0.05). When the household of cases of those exposed to tobacco smoke was evaluated, 47.5% of them had more than one smoker inside the houses. In the patient group, 40.6% of cases had both a smoker mother and father. This rate was 20.7% in the control group. Also in the control group, current father smokers' rate was 37.9%. There was no statistically significant difference between the exposed and unexposed groups, regarding active smokers (p>0.05). When classification was made according to urine cotinine levels, in the group of chronic cough and passive smoking, the rate of both mothers' and fathers' current smoking was the highest (46.2%). However, no statistical significant difference was found (p>0.05).

When comparison was made with the cases with chronic cough that have been exposed to ETS and other groups, the rate of smoking in pregnancy was found to be increased in the chronic cough group (31.3 % in the exposed chronic cough group, 16% in the unexposed chronic cough, 20.7% in the exposed control group, 0 % in the unexposed control) (p=0.005).

Eighty-four patients performed lung function tests. Patients with chronic cough, exposed and unexposed group according to urine cotinine level were classified and no statistically significant difference was found according to their lung function test measurements (FVC; p=0.780, FEV1; p=0.401, FEV1/FVC; p=0.709, PEF; p=0.559, FEF 25-75; p=0.709). When classification was made as pulmonary function tests of children with and without chronic cough according to the smoking status of their parents, there was a statistically significant difference for FEV1/FVC, PEF, and FEF25-75 values (p<0.05). However, for the values of FEV1 and FVC, no statistically significant difference was found between the four groups (p>0. 05) (**Table 4**).

Table 4. Pulmonary function tests of children with and without chronic cough according to the smoking status of their parents

Variables	Chronic cough group		Control group		p
	Passive smoking (+) (n=31)	Passive smoking (-) (n=25)	Passive smoking (+) (n=15)	Passive smoking (-) (n=13)	
FVC					
mean±SD	90.4±9.1	92.6±10.2	88.8±10.6	93.5±25.9	0.358*
median	89	91	84	87	
[min– max]	[74-111]	[71-115]	[74-112]	[74-176]	
FEV 1					
mean±SD	95.06±7.1	97.9±9.6	97.4±11.5	100.1±23	0.827*
median	95	95	94	96	
[min– max]	[79-108]	[80-118]	[80-117]	[83-172]	
FEV1 / FVC					
mean±SD	97.3±6.2	99.4±7.4	105.9±4.6	103.8±5.6	<0.001*
median	95.2	101	107	103	
[min– max]	[87-109]	[85-111]	[93-112]	[97-114]	
PEF					
Mean±SD	99.1±26,3	111±27.1	121.6±25.3	117.3±25.4	0.014*
median	96	105	116	116	
[min– max]	[69-192]	[73-208]	[91-169]	[81-164]	
FEF 25-75					
mean± SD	99.2±12.7	104.9±25.5	115.9±21.3	113.9±24.3	0.024*
median	97	93	109	111	
[min– max]	[75-136]	[79-189]	[86-161]	[81-177]	

Abbreviations: FVC: Forced vital capacity, FEF25-75%: forced expiratory flows between 25% and 75% of FVC; FEV1: forced expiratory volume during the first second, PEF: peak expiratory flow, *Kruskal Wallis test

DISCUSSION

In this study, we investigated the effect of passive smoking on chronic cough by evaluating 112 children (58 patient group and 54 control group). Exposure to environmental tobacco smoke (ETS) was detected in approximately two-thirds of our study group. In this study, the cut–off value to describe passive smoking was determined as 10 ng/ml. This value was under the reference of previous studies and recommended by the manufacturer.[18,19] In the chronic cough group, 55.1% of parents declared exposure to ETS, while 55.5% of parents declared exposure to ETS in the control group. According to parents' declaration, 32% of children without household smokers were found to have urine cotinine levels >10 ng/ml. These findings might indicate either a bias about parents' declarations or the fact that the children are sharing time in other smoke-free places or are subject to third-hand smoking. Previous studies showed that there could exist disparities between parents' declarations based on passive smoking rates and objective measurements. According to a survey, parents declared that tobacco smoke exposure for their children existed mainly in houses of relatives and friends, and shopping centers.[20] Although at least one smoker in the house was present, they thought they prevented their children from exposure by using different rooms for smoking. Apparently, this kind of preventive approach of parents is not proper when urine cotinine levels are measured.

For urine cotinine levels, the best sensitivity and specificity of cut–off value was 12.15 ng/ ml in our study. In the ETS-exposed group median urine cotinine levels were 18.5, while in the control group this value was 19.3 ng/ml. The highest mean cotinine level was detected in the chronic cough patient group who was exposed to ETS. Arvas et al. reported the mean cotinine value as 37.5 ng/ ml in their study.[21] Kahvecioglu et al. found median urine cotinine levels as 20 ng/ml.[22] A study investigating 7725 adult cases found that low socio-economic status increased the rate of passive smoking, and these societies have more death-end disease rates than high socio-economic status.[23] In our study, the 54.8% of cases had low income, and the majority had chronic cough and were in the group exposed to ETS. 63.3% of the group with chronic cough who were exposed to ETS was detected to be in low socio-economic status. This rate was 55.6% in the control group who had exposure to ETS. Being under more stress and having limited education of the families belonging to low socio-economic levels may increase their smoking frequency, and they may not be aware of the tobacco harm given to their children.

Exposure to ETS, while decreasing in FEV1, increases nonspecific bronchial reactivity leading to asthma development in some children. Fricher et al. reported a decrease in PEF rate values in primary school children who were exposed to tobacco smoke and found maternal smoking at a rate of 13.7%.[24] Another study evaluating maternal smoking exposure in children between ages 6-10 found a decrease in FEV1, FEV1/FVC, and FEF25-75 parameters.[25] It has also been shown that the non-asthmatic cases whose mothers had smoked during pregnancy had decreased FEV1, FEV1/FVC, and FEF 25-75 parameters.[26] Our study evaluated 84 out of the 112 patients' spirometry tests. When spirometry measures of chronic cough patients (classified according to urine cotinine level) were compared in our study, there was no statistically significant difference. However FEV1/FVC, FEF 25-75 and PEF values revealed significant differences between the groups with exposed or not. FEV1/FVC, FEF 25-75 and PEF values were decreased significantly in the children with chronic cough ($p<0.05$). FEV1/FVC values were found to be decreased in the children who had been detected as passive smokers according to urine cotinine levels or declared ETS exposure by the parents. Our results revealed that passive smoking affects lung functions negatively in children which comply with previous literature data.

CONCLUSION

This is the first study objectively evaluating the effect of passive smoking on urine cotinine levels in children with chronic cough who have no other specific underlying cause. In our study, the best sensitivity and specificity of cut–off value in urine cotinine levels was12.15 ng/ml. According to parents' declarations, some children without the presence of household smokers were found to have urine cotinine levels >10 ng/ml. These findings might indicate the importance of being subject to third-hand smoking besides wrong declarations or sharing smoke-free places. FEV1/FVC, FEF25-75 and PEF values were significantly lower in children with chronic cough exposed to ETS compared to the other groups. Therefore, children with chronic cough who are exposed to ETS should be followed up with lung function tests.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ministry of Health Ankara Kecioren Education and Research Hospital Ethics Committee (Date: 06.27.2012, Decision No: B.10.4.İSM.4.06.68.49).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Primary EBV Infection and Hematological Findings in Turkish Children: A Retrospective, Single-Center Study

Türk Çocuklarında Primer EBV Enfeksiyonu ve Hematolojik Bulguları: Retrospektif Tek Merkezli Bir Çalışma

Nergiz Öner[1], Gürses Şahin[1], Şule Yeşil[1], Ali Fettah[1], Fatma Nur Öz[2], Emre Çapkınoğlu[1], Azize Ceren Kılcı[1], Şeyma Ünüvar Gök[1]

[1]University of Health Sciences, Dr. Sami Ulus Maternity and Children Training and Research Hospital, Pediatric Hematology and Oncology, Ankara, Turkey
[2]University of Health Sciences, Dr. Sami Ulus Maternity and Children Training and Research Hospital, Pediatric Infectious Diseases, Ankara, Turkey

Abstract

Aim: Primary infection with EBV during childhood is usually asymptomatic. Hematologic abnormalities such as hemolytic anemia, thrombocytopenia, and neutropenia are relatively common, but aplastic anemia, severe thrombocytopenia, severe neutropenia, and lymphoid malignancies are rare. In this retrospective study, we have analyzed data from children with primary EBV infection who were examined at our center over 12 years to describe the distribution, clinical features, complications, and outcome of EBV in Turkish children.

Material and Method: Data from all children (age<18 years) with primary EBV infection who were examined in our center between 2008 and 2020 were retrospectively reviewed.

Results: In total, 120 patients were included in the study. Anemia was detected in 13 (11%) children. Mild and severe neutropenia was detected in 12 (10%) and 1 (0.8%) children, respectively. Mild thrombocytopenia without bleeding complications was present in 10 (8%) children with a mean platelet count of 250×10^9/L $\pm113\times10^9$/L. Seven patients presented with acute idiopathic thrombocytopenia (lowest platelet count, 2×10^9/L). Large diffuse cell lymphoma developed in 0.8% (n:1) patients.

Conclusion: The prognosis for infectious mononucleosis is excellent, although various acute complications may occur. However, severe complications are very rare, and most of the complications resolve spontaneously without certain therapy.

Keywords: Primary EBV infection, Turkish children, hematologic findings

Öz

Amaç: Çocukluk döneminde EBV ile birincil enfeksiyon genellikle asemptomatiktir. Hemolitik anemi, trombositopeni ve nötropeni gibi hematolojik anormallikler nispeten yaygındır, ancak aplastik anemi, şiddetli trombositopeni, şiddetli nötropeni ve lenfoid maligniteler nadirdir. Bu retrospektif çalışmada, EBV'nin Türk çocuklarındaki dağılımını, klinik özelliklerini, komplikasyonlarını ve sonuçlarını tanımlamak için 12 yıl boyunca merkezimizde izlenen primer EBV enfeksiyonu olan çocuklardan elde edilen verileri analiz ettik.

Gereç ve Yöntem: Hastanemizde 2008-2020 yılları arasında izlenen primer EBV enfeksiyonlu tüm çocuklara (18 yaş altı) ait veriler retrospektif olarak incelendi.

Bulgular: Çalışmaya toplam 120 hasta dahil edildi. 13 (%11) çocukta anemi saptandı. 12 (%10) çocukta hafif, 1 (%0,8) çocukta şiddetli nötropeni saptandı. Ortalama trombosit sayısı 250×10^9/L $\pm113\times10^9$/L olan 10 (%8) çocukta kanama komplikasyonu olmaksızın hafif trombositopeni mevcuttu. Yedi hasta akut idiyopatik trombositopeni (en düşük trombosit sayısı, 2×10^9/L) ile başvurdu. Hastaların %0,8'inde (n:1) büyük diffüz hücreli lenfoma gelişti.

Sonuç: Çeşitli akut komplikasyonlar oluşabilmesine rağmen enfeksiyöz mononükleozun prognozu mükemmeldir. Bununla birlikte, ciddi komplikasyonlar çok nadirdir ve komplikasyonların çoğu belirli bir tedavi olmaksızın kendiliğinden düzelir.

Anahtar Kelimeler: Primer EBV enfeksiyonu, Türk çocukları, hematolojik bulgular

Corresponding *(İletişim)*: Nergiz ÖNER MD, Pediatric Hematology and Oncology Specialist, University of Health Sciences, Dr. Sami Ulus Maternity and Children Training and Research Hospital, Ankara, Turkey.
E-mail *(E-posta)*: nbattaloglu@yahoo.com
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INTRODUCTION

Epstein- Barr virus (EBV) is a DNA virus that is a member of the gammaherpesviruses.[1] The reported prevalence of EBV infections varies widely depending on countries' economic status and geographic location. Infection with EBV in developing countries usually occurs during infancy and early childhood. Infectious mononucleosis (IM) is the best-known clinical syndrome caused by EBV.[2-5] Although primary infection with EBV during childhood is usually asymptomatic, adolescents and adults manifest in 30–50% of cases as the classic triad of fatigue, pharyngitis, and generalized lymphadenopathy, which constitute the major clinical manifestations of IM.[6-7] Hematologic abnormalities such as hemolytic anemia, thrombocytopenia, and neutropenia are relatively common, but aplastic anemia, severe thrombocytopenia, and severe neutropenia are rare.[8-10] EBV is associated with lymphoid malignancies, such as Burkitt lymphoma, Hodgkin lymphoma, aggressive NK cell leukemia, T- and NK cell lymphoproliferative disorder, and epithelial cell malignancies such as nasopharyngeal carcinoma.[11-15] EBV increases the risk for Hodgkin lymphoma by a factor of 2-4. The risk of developing Hodgkin lymphoma peaking at 2.1 years following infectious mononucleosis.[16-20] Hemophagocytic lymphohistiocytosis (HLH) can develop rare serious, life-threatening complications with primary EBV infection.[21-22]

A variety of neurologic conditions have been associated with EBV infection. Although headache is a common symptom, severe neurologic manifestations, such as seizures and ataxia, may occur in 1–5% of cases.[23]

In this retrospective study, we have analyzed data from children with primary EBV infection who were examined at our center over 12 years to describe the distribution, clinical features, complications, and outcome of EBV in Turkish children.

MATERIAL AND METHOD

The medical records of all children with primary EBV infection (<18 years of age) diagnosed in the Pediatric Hematology and Oncology Units of the Department of Pediatrics, Dr. Sami Ulus Maternity and Children Training and Research Hospital, between 2008 and 2020 were reviewed after our Institutional Review Board had approved the study. (date: 07.04.2021, no: E-21/04-145)

Medical records were reviewed, and 209 patients with primary EBV infection were retrospectively analyzed for initial clinical and laboratory findings, management, and outcome data. Patients were excluded if there was no regular follow-up or where analyses of anti-EBV-capsid antigen (CA)-IgM and anti-EBV-CA-IgG antibodies and EBNA were inadequately documented. In this study, we included 120 patients. In the study population, the following data were analyzed: age, sex, detailed physical examination findings, laboratory findings including complete blood count [white blood cell (WBC), neutrophil, lymphocyte, and platelet count, mean platelet volume (MPV), platelet distribution width (PDW), concentrations of hemoglobin (Hb)] and biochemical examinations [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, triglycerides, lactate dehydrogenase (LDH), C-reactive protein (CRP)] and anti-EBV-capsid antigen (CA)-IgM and anti-EBV-CA-IgG antibodies and serum concentration of immunoglobulin (Ig) A, G, and M. In addition, coagulation function test [fibrinogen (Fib), activated partial thromboplastin time (APTT), prothrombin time (PT)], bone marrow aspiration findings, parvovirus, and cytomegalovirus (CMV) infection were diagnosed with immunoglobulin M antibodies or genomic DNA copy number by polymerase chain reaction (PCR). Primary EBV infection was diagnosed based on clinical manifestations, positive IgM antibody titers, and IgG antibody titers to EBV viral capsid antigens (VCA). In addition, throat culture was performed in patients presenting with tonsillopharyngitis to exclude Group A beta-hemolytic streptococci. Neutropenia was defined as an absolute neutrophil count below 1500 per µl, thrombocytopenia as platelet count $<150\times10^9$/L and lymphocytosis as lymphocyte count above or equal to 5000 per µl or at least 50% of a total white blood cell. The following criteria were used to diagnose IM in our study: i) 3 of the following clinical symptoms: Fever, angina, large cervical lymph nodes, hepatomegaly, splenomegaly; ii) Positivity for anti-EBV-capsid antigen (CA)-IgM and anti-EBV-CA-IgG antibodies.[24]

Statistical Analysis

All reagents for testing were the original reagents of the instruments. The Kolmogorov-Smirnov normality test was used to determine if the data is normally distributed.

The Mann-Whitney U test was used to compare differences in non-parametric variables (non-normally distributed data). Categorical variables were presented as a proportion and analyzed with the Chi-squared test. Continuous data were analyzed using Student's t-test. Values were expressed as n (%), the mean ± standard deviation, or median (interquartile range). Spearman correlation analysis was used for grading variable data, whereas Pearson correlation analysis was used for continuous variable data. All statistical analyses were performed using SPSS version 22.0 (IBM Corp.). $p<0.05$ was considered to indicate statistical significance.

RESULTS

Each of the 120 Turkish children had a specific serologic profile of primary EBV infection and a clinical picture of primary EBV infection. The serologic findings of all children were positive VCA IgM and/or VCA IgG. The age of the children ranged from 1 to 17 years, with a median of 5 years. The peak incidence occurred at 3 to 5 years, followed by 8 to 11 years. The male (n: 68, 57%) female ratio was 1.3:1. No seasonal or yearly variation in the frequency of primary EBV infection was detected.

Clinical Manifestations

Fifty-seven (48%) of 120 children had fever on admission. No age difference in the magnitude and duration of the febrile response was observed. Lymphadenopathy (>2 cm) and tonsillopharyngitis were detected in 105 (88%) and 69 (58%) children, respectively. Splenomegaly and hepatomegaly were present in 55 (46%) and 39 (33%) patients, respectively. Cutaneous rashes, predominantly maculopapular, were detected in 3 children (15%), of whom all patients have antibiotics before the appearance of the skin rash. Eyelid edema was detected in 13 children (11%) and palatal petechiae in 7 (6%) children.

Laboratory Findings

Laboratory characteristics of the cases are presented in **Table 1**. Anemia was detected in 13 (11%) children. Mild and severe neutropenia was detected in 12 (10%) and 1 (0.8%) children, respectively. Mild thrombocytopenia without bleeding complications was present in 10 (8%) children with a mean platelet count of 250×10^9/L $\pm113\times10^9$/L. Seven patients presented with acute idiopathic thrombocytopenia (lowest platelet count, 2×10^9/L). Leukocytosis and lymphocytosis were detected in 68 (57%) and 73 (61%) children, respectively. ALT and AST values were elevated in 55 of 120 (46%) of all children.

Table 1: Laboratory characteristics of the cases.

	Mean±SD	Minimum-maximum
Hemoglobin (g/dl)	12.2±1.1	8-15
Mean corpuscular volume (fl)	78.1±5.6	54-89
White blood cell count (per µl)	12,554±6,656	3,600-40,750
Neutrophil count (per µl)	3,633±2,849	40-23,0000
Lymphocyte count (per µl)	6,644±3,963	1,730-25,850
Monocyte count (per µl)	1,003±1,051	140-8,760
Platelets (x109/L)	250±113	2-573
Alanine aminotransferase (U/L)	103±132	6-661
Aspartate aminotransferase (U/L)	97±110	21-569
Immune globulin M (mg/dl)	148±69	51-333
Immune globulin G (mg/dl)	1095±388	499-2280
Immune globulin A (mg/dl)	117±80	6.4-417

Complications

EBV-associated HLH was seen in a 13-year-old male patient. At the time of diagnosis, EBV PCR 12.555 copy was detected. Steroid and IVIG treatment was given and could be controlled by only steroid and IVIG treatment without any need for chemotherapy. Transient macroscopic hematuria was present early in the disease course in one child. The associated diseases at the time of diagnosis are given in **Table 2**. Large diffuse cell lymphoma developed in 0.8% (n:1) patients. In the patient whose EBNA became positive following primary EBV infection, lymphoma developed with EBV reactivation three years later.

Table 2: The associated diseases at the time of diagnosis.

Associated diseases	n	Duration of a EBNA positivity (month, mean (min-max))	Long-term complication
Selective IgA deficiency	2	1	No
Transient IgA deficiency	2	3.5 (1-6)	No
Transient hypogammaglobulinemia	2	8 (6-10)	No
Juvenile idiopathic arthritis	1	2	No
Autoimmun thyroiditis	1	4	No
Hemophagocytic lymphohistiocytosis	1	1	No

DISCUSSION

EBV affects more than 95% of the world's population. Infection with EBV in developing countries usually occurs during infancy and early childhood. In central Africa, almost all children are affected by 3 yr of age. On the contrary, two waves of primary EBV infection in childhood from 1 to 6 years and 10 years were observed in the Western communities.[25] Approximately 30% of infections occur during adolescence and young adulthood and are usually more symptomatic among more affluent populations in industrialized countries. EBV infections presented at all ages, from infants to adolescents, in our series of 120 children. The peak incidence occurred at 3 to 5 years, followed by 8 to 11 years.

The peak incidence of EBV infections in our patients was similar to the results of previous studies in Western communities, suggesting that there is no significant variation in EBV infections presentation among different ethnic populations. Hematological complications usually include anemia, thrombocytopenia, neutropenia, and lymphocytosis. Severe thrombocytopenia ($<20\times10^9$/L) is rare, while mild thrombocytopenia ($<100\text{-}150\times10^9$/L) occurs in 25-50% of patients. The mechanism involved in thrombocytopenia has been reported to occur as a result of increased peripheral destruction due to antiplatelet antibodies and splenomegaly with a normal or increased megakaryocyte count in the bone marrow. Neutropenia to less than 1,500 neutrophils per µl, typically lasting only a few days to 2 weeks, occurs in approximately 3% of cases.[9] The pathogenesis of neutropenia after EBV infection may involve decreased production or maturation of myeloid cells in the bone marrow, as a result of the direct effect of EBV or antibody-mediated peripheral destruction of myeloid cells. Anti-human neutrophil antigen-1a (anti-HNA-1a) and anti-HNA-1b antibodies are both associated with the pathophysiology in neutropenia after EBV infection, although it is unknown whether anti-neutrophil antibodies are produced in EBV-infected B cells. Autoimmune hemolytic anemia is a rare complication.

Primary EBV infection causes hemolytic anemia in approximately 3% of patients.[1,8,10] In our study, severe thrombocytopenia developed in 7 (6%) patients, hemolytic anemia developed in 1 (0.8%) patient, and neutropenia developed in 13 (11%) patients. Patients with

thrombocytopenia showed improvement after IVIG, while patients with hemolytic anemia and neutropenia recovered spontaneously. Our results were consistent with the literature.

EBV is considered the major cause of severe cases of virus-associated hemophagocytic syndrome. In previous studies, the most frequent reason for HLH was infections and the most common infectious agent was EBV. In these studies, HLH was triggered by EBV in 1/3-3/4 of the patients.[21-22,26]

The initial treatment for HLH aims to calm down the hyperactivated immune system and remedy hypercytokinemia. Some studies showed that secondary HLH might be controlled by only steroid and IVIG treatment without any need for etoposide. Similarly, in our study, HLH in a patient could be controlled by only steroid and IVIG treatment without any need for chemotherapy. Early recognition and initiation of HLH-directed therapy are important for patient survival.[27-28]

The genetic, environmental, and infectious processes, especially viral infections, appear to play a role in the etiology of rheumatic disease in children. Epstein–Barr virus is the most commonly emphasized viral agent that facilitates rheumatic diseases. Clinical and laboratory investigations have revealed that EBV triggers the development of SLE, RA, but only a limited number of published reports regarding the occurrence of EBV infection in patients with JIA and autoimmune thyroiditis.[29,30] One of our patients was diagnosed with primary EBV with JIA and another with autoimmune thyroiditis. There are different kinds of literature on whether EBV infection increases JIA frequency. EBV infection can significantly induce an immune disorder leading to an uncontrolled inflammatory process that results in JIA symptoms. The previous study observed a significantly higher level of EBV antibodies in children with autoimmune thyroid disease (study group) (n=34) than in a control group (P=0.008), suggesting that EBV infection might play a role in the pathogenesis of autoimmune thyroid disease in children. Another study further investigated this phenomenon and showed three cases with newly diagnosed autoimmune thyroid disease and primary EBV infection.[24] Notably, further studies are needed to explain the role of the immunological mechanism of EBV on various target organs.[31-32]

Selective IgA deficiency and hypogammaglobulinemia are the common immunodeficiency syndrome. The clinical and laboratory features variability suggests that IgA deficiency and hypogammaglobulinemia have multiple causes. Acquired deficiency of IgA and hypogammaglobulinemia have been linked to some drugs and infections. An infectious cause has been suggested by reports of IgA deficiency in some children with EBV.

In our study, two patients with transient IgA deficiency, two patients with selective IgA deficiency, and two patients transient hypogammaglobulinemia temporally related to an EBV infection. Thus EBV infection should be considered in the differential diagnosis of acquired IgA deficiency.[32-34]

EBV is associated with 1% of global cancers, mostly lymphomas and carcinomas; approximately 140.000 people die of EBV-associated cancers each year.[11,12] In agreement with our study, A Scandinavian study observed in 2003 an increased risk of EBV-positive HL in young adults, with an odds ratio (OR) of 2.7 [95% confidence interval (CI): 1.2 to 6.0], and a median incubation period of 4.1 years with a peak risk after 2.1 years after primary infection.[20] In later years, A British study showed similar results in two different cohorts.[35] EBV-positive DLBCL seems to affect primarily elderly patients. Studies on children are limited. In our study, large diffuse cell lymphoma developed in 0.8% (n: 1) patients. In the patient whose EBNA became positive following primary EBV infection, lymphoma developed with EBV reactivation three years later.

CONCLUSION

The prognosis for infectious mononucleosis is excellent, although various acute complications may occur. However, severe complications are very rare, and most of the complications resolve spontaneously without certain therapy.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Ankara Sami Ulus Training and Research Hospital at the University of Health Sciences Ethics Committee. (date:07.04.2021, no:E-21/04-145)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Frequency and Seasonal Distribution of Rotavirus and Adenovirus in Patients with Acute Gastroenteritis

Akut Gastroenteritli Hastalarda Rotavirüs ve Adenovirüs Sıklığı ve Mevsimsel Dağılımı

Füsun Kırca, Filiz Demirel

Ankara Bilkent City Hospital, Department of Medical Microbiology, Ankara, Turkey

Abstract

Aim: The aim of this study was to retrospectively determine the frequency of rotavirus and adenovirus and the distribution of these pathogens by age groups, gender and season in patients admitted to our hospital with gastroenteritis symptoms.

Material and Method: A total of 32755 stool samples collected from patients with gastroenteritis symptoms in the Medical Microbiology Clinic of the Ankara Bilkent City Hospital between January 1 and December 31, 2022 were evaluated retrospectively for rotavirus and adenovirus antigens. Rotavirus and adenovirus antigens were analyzed with the Rapid Cassette Test (Microcult, Biotech) kit. In addition, the distribution of pathogens by age groups, gender and season were examined. Statistical analysis was performed using Jamovi® version 2.3.21.

Results: Antigen test was positive in 9.9% of 32755 stool samples evaluated in our study. Rotavirus was detected in 8.4% and adenovirus in 1.5% of antigen positive samples. Both rotavirus and adenovirus antigen positivity were found to be statistically significantly higher in 0-1 and 2-3 age groups compared to other age groups ($p<0.001$). When the monthly distribution of rotavirus antigen positivity was examined, a statistically significant difference was found in March, April, and May ($p<0.001$).

Conclusion: It was observed that rotavirus has a significant ratio among acute gastroenteritis cases in pediatric age group in our region. Rotavirus should be considered in gastroenteritis in 0-3 age group especially in spring months, and for diagnosis, immunochromatographic-based tests can be easily applied in all health institutions. Detection of the pathogen causing gastroenteritis will contribute to the initiation of appropriate treatment, follow-up of complications, and the prevention of unnecessary antibiotic use.

Keywords: Rotavirus, adenovirus, acute gastroenteritis

Öz

Amaç: Bu çalışmanın amacı, gastroenterit semptomları ile hastanemize başvuran hastalarda rotavirüs ve adenovirüs sıklığını ve bu patojenlerin cinsiyet, yaş ve mevsimlere göre dağılımını retrospektif olarak belirlemektir.

Gereç ve Yöntem: Ankara Bilkent Şehir Hastanesi Tıbbi Mikrobiyoloji Kliniğinde 1 Ocak - 31 Aralık 2022 tarihleri arasında gastroenterit semptomları olan hastalardan alınan toplam 32755 dışkı örneği rotavirüs ve adenovirüs antijenleri açısından retrospektif olarak değerlendirilmiştir. Rotavirüs ve adenovirüs antijenleri Rapid Cassette Test (Microcult, Biotech) kiti ile analiz edilmiştir. Ayrıca patojenlerin cinsiyet, yaş ve mevsimlere göre dağılımı incelenmiştir. İstatistiksel analiz Jamovi® versiyon 2.3.21 kullanılarak gerçekleştirilmiştir.

Bulgular: Çalışmamızda değerlendirilen toplam 32755 dışkı örneğinin %9,9'unda antijen testi pozitif bulunmuştur. Antijen pozitif örneklerin %8,4'ünde rotavirüs ve %1,5'inde adenovirüs tespit edilmiştir. Hem rotavirüs hem de adenovirüs antijen pozitifliği 0-1 ve 2-3 yaş gruplarında diğer yaş gruplarına göre istatistiksel olarak anlamlı derecede yüksek bulunmuştur ($p<0.001$). Rotavirüs antijen pozitifliğinin aylara göre dağılımı incelendiğinde Mart, Nisan ve Mayıs aylarında istatistiksel olarak anlamlı bir fark bulunmuştur ($p<0.001$).

Sonuç: Bölgemizde pediatrik yaş grubunda akut gastroenterit vakaları arasında rotavirüsün önemli bir orana sahip olduğu görülmüştür. Özellikle bahar aylarında 0-3 yaş grubunda görülen gastroenteritlerde rotavirüs göz önünde tutulmalıdır. Tanı için immünokromatografik temelli hızlı kaset testler birinci basamak sağlık kuruluşları da dahil olmak üzere tüm sağlık kuruluşlarında kolaylıkla uygulanabilir. Gastroenterite neden olan patojenin tespit edilmesi, uygun tedavinin başlatılmasına, komplikasyonların takibine ve gereksiz antibiyotik kullanımının önlenmesine katkıda bulunacaktır.

Anahtar Kelimeler: Rotavirüs, adenovirüs, akut gastroenterit

Corresponding *(İletişim)*: Füsun KIRCA, Ankara Bilkent City Hospital, Department of Medical Microbiology, Ankara, Turkey
E-mail *(E-posta)*: fskirca@yahoo.com
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INTRODUCTION

Acute gastroenteritis is among the most common infectious diseases worldwide and can have serious consequences, especially in children and the elderly. The clinical aspect can range from asymptomatic infections to severe illness with dehydration which can be fatal.[1] Although acute gastroenteritis is usually a self-limiting disease, it can still lead to significant morbidity. It is a major cause of death in underdeveloped countries especially among infants. According to the Centers for Disease Control (CDC), viral gastroenteritis causes the death of more than 200,000 children worldwide annually. Viruses are the most common causative agents of acute infectious gastroenteritis.[2] More than 20 different viruses have been identified as etiologic agents of viral acute gastroenteritis, rotavirus and adenovirus being among the most common agents.[3] Rotavirus and adenovirus-associated gastroenteritis is transmitted via fecal-oral route, but it can also be easily transmitted via shared items. In addition, both viruses are non-enveloped and resistant to soap and disinfectants.[4]

Rotaviruses are double-stranded RNA viruses with a triple-layered capsid surrounding a genome with 11 RNA segments. Ten rotavirus species (A-J) have been classified, based on sequence and antigenic differences of the VP6 protein found in the inner capsid. Type A is the most common cause of infections in children.[5] The clinical picture of rotavirus-associated acute gastroenteritis includes diarrhea, vomiting, and fever and may lead to a severe condition that may require hospitalization due to fluid loss.[4]

Adenoviruses, one of the pathogens causing viral gastroenteritis, are double-stranded DNA viruses. Currently, adenovirus is divided into nine subgroups (A-I) and more than 100 genotypes have been identified. Specifically, adenovirus 40 and 41 (species F) serotypes have been classified as causative agents of pediatric gastroenteritis.[6,7] Clinical features of acute gastroenteritis caused by adenovirus include vomiting, watery diarrhea, mild fever and mild dehydration. Another feature of adenovirus 40-41 serotypes infections is prolonged diarrhea.[8]

Detection of the causative pathogen in acute gastroenteritis is important for the planning of treatment and the prevention of inappropriate antibiotic use. In the differential diagnosis of viral gastroenteritis, various epidemiological factors such as age, season, geographical region, and socioeconomic conditions in addition to clinical findings may be useful, but laboratory tests are required to confirm the diagnosis.[9]

The aim of this study was to retrospectively determine the frequency of rotavirus and adenovirus and the distribution of these pathogens by age groups, gender and season in patients admitted to our hospital with gastroenteritis symptoms.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara Bilkent City Hospital Ethics Committee (Date: 10.05.2023, No: E2-23-4081), and in accordance with the principles of the Declaration of Helsinki of the World Medical Association.

A total of 32755 stool samples collected from patients with gastroenteritis symptoms in the Medical Microbiology Clinic of the Ankara Bilkent City Hospital between January 1 and December 31, 2022 were evaluated retrospectively for rotavirus and adenovirus antigens. Patients were divided into age groups as 0-1, 2-3, 4-5, 6-10, 11-15, ≥16. The distribution of rotavirus and adenovirus by age groups, gender and season was determined.

Rotavirus and adenovirus antigens were analyzed with the Rotavirus and Adenovirus Rapid Cassette Tests (Microcult, Biotech, China) that qualitatively identify these antigens by immunochromatographic method, in accordance with the manufacturer's recommendations.

Age and gender data of the patients were obtained from the hospital information management system.

In the presence of more than one positive results on consecutive days, a single positive result was included in the evaluation.

The statistical analysis was performed using Jamovi® version 2.3.21. Normality analysis of the data was assessed by the Shapiro-Wilk test and the difference between groups was calculated by the chi-square test. The p-value of <0.05 was accepted as statistically significant.

RESULTS

In our study, a total of 32755 stool samples were examined for the presence of rotavirus and adenovirus antigens. Antigen test was positive in 9.9% (n=3239) of the patients. Rotavirus was detected in 8.4% (n=2761) and adenovirus in 1.5% (n=478) of antigen positive samples. Rotavirus-adenovirus co-infection rate was 2.4%. It was determined that 56% (n=1816) of the antigen positive patients were male and 44% (n=1423) were female, and there was no statistically significant difference between genders (p=0.853). The distribution of rotavirus and adenovirus positivity by gender is given in **Table 1**.

Table 1. Distribution of rotavirus and adenovirus positivity by gender

Gender	Rotavirus positive n (%)	Adenovirus positive n (%)	Total n (%)
Female	1220 (44)	203 (42)	1423 (44)
Male	1541 (56)	275 (58)	1816 (56)
Total	2761 (100)	478 (100)	3239 (100)

In patients with rotavirus antigen, the age range was 0-92, median age 2 [interquartile range (IQR), 4] while the age range was 0-72, median age 3 [interquartile range (IQR), 4] in patients with adenovirus antigen. The age group with the highest antigen positivity was found to be 2-3 (35%) for rotavirus and 0-1 (31%) for adenovirus. Both rotavirus and

adenovirus antigen positivity were found to be statistically significantly higher in 0-1 and 2-3 age groups compared to other age groups (p<0.001). However, there was no statistically significant difference between these two age groups in terms of positivity (p>0.05). A total of 2163 (78%) of the patients with rotavirus antigen positive and 362 (76%) of the patients who were positive for adenovirus antigen were found to be five years old or younger. The distributions of rotavirus and adenovirus positivity by age groups are given in **Figure 1** and **Table 2**.

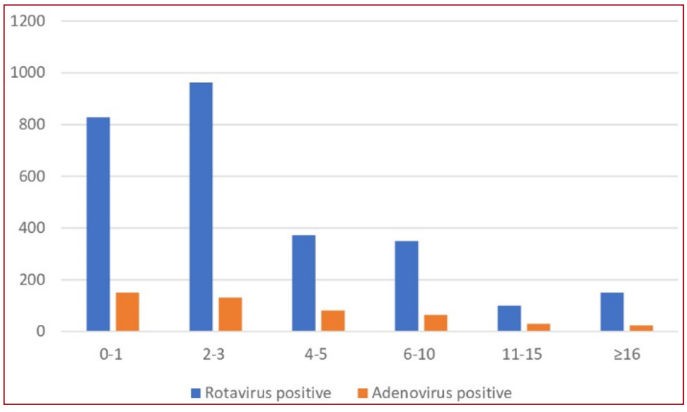

Figure 1. Distribution of rotavirus and adenovirus positivity by age groups

Table 2. Distribution of rotavirus and adenovirus positivity by age groups

Age groups	Rotavirus positive n (%)	Adenovirus positive n (%)
0-1	828 (30)	150 (31)
2-3	963 (35)	131 (28)
4-5	372 (13)	81 (17)
6-10	349 (13)	64 (13)
11-15	99 (4)	29 (6)
≥16	150 (5)	23(5)
Total	2761 (100)	478 (100)

When the monthly distribution of rotavirus antigen positivity was examined, a statistically significant difference was found in March, April and May (p<0.001). When the monthly distribution of adenovirus was assessed, no statistically significant difference was found (p= 0.154). The monthly distributions of rotavirus and adenovirus positivity are given in **Figure 2** and **Table 3**.

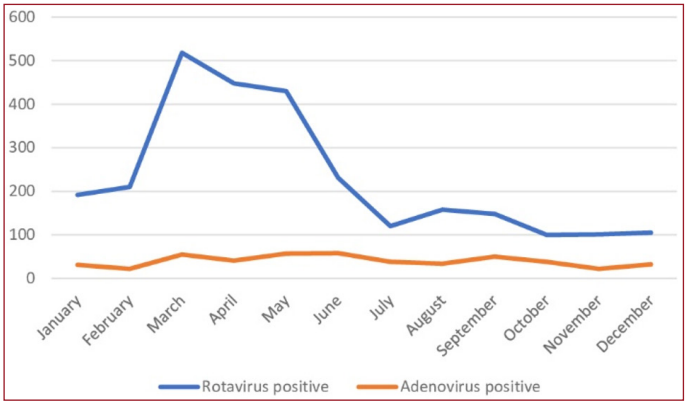

Figure 2. Monthly distribution of rotavirus and adenovirus positivity

Table 3. Distribution of rotavirus and adenovirus positivity by month

Months	Rotavirus positive n (%)	Adenovirus positive n (%)
January	192 (7)	31 (6)
February	210 (8)	22 (5)
March	518 (19)	55 (12)
April	448 (16)	41 (9)
May	430 (16)	57 (12)
June	231(8)	58 (12)
July	120 (4)	38 (8)
August	158 (5)	34 (7)
September	148 (5)	50 (10)
October	100 (4)	38 (8)
November	101 (4)	22 (5)
December	105 (4)	32 (6)
Total	2761 (100)	478 (100)

DISCUSSION

Acute gastroenteritis is a cause of morbidity and mortality in low- and middle-income countries, especially in children under 5 years of age. Viruses are the most common etiologic agents of acute infectious gastroenteritis, followed by bacteria and parasites. Among viral gastroenteritis agents, rotavirus and adenovirus are the leading causes of diarrhea worldwide.[7] The frequency of these pathogens is influenced by geographical, environmental or socio-economic factors.[3] In studies conducted in different countries, the prevalence of rotavirus was reported to vary from 9.7% to 35.9% and the prevalence of adenovirus from 2.5% to 17.1%.[3,10-15] In studies conducted in different regions in Türkiye, rotavirus and adenovirus frequency rates were reported to vary from 6.7% to 20.2% and from 1.3% to 17.6% respectively.[4,16-20] The difference in results of these studies may be due to both regional, climatic and socio-economic factors, and diagnostic methodology. In this study, rotavirus frequency was 8.4%, adenovirus frequency was 1.5%, rotavirus-adenovirus coinfection rate was 2.4% and these results are compatible with the national data. In line with previous studies conducted in our country, no statistically significant difference (p=0.853) was found in this study between genders in patients with antigen positivity.[4,9,17-21]

The age groups with the highest antigen positivity were 2-3 years (35%) and 0-1 year (30%) for rotavirus, and 0-1 (31%) and 2-3 years (27%) for adenovirus. Both rotavirus and adenovirus antigen positivity were statistically significantly higher in the 0-1 and 2-3 age groups compared to other age groups (p<0.001). Consequently, as the distribution of antigen positivity was examined according to age groups in this study, it was found that infection with both viruses was mostly observed in the age group of 3 years old and younger, which was compatible with the previous studies in our country.[16,17,19,21,22]

The epidemiology of the disease has changed drastically in countries which included rotavirus vaccine in their national vaccination programs. While rotavirus caused gastroenteritis

mostly in children under 5 years of age before it was included in the vaccination program in such countries, it was found to cause gastroenteritis in older age groups who were not vaccinated after it was included in the vaccination program.[23] In Türkiye, rotavirus vaccine has not yet been included in the national vaccination program but the vaccine is available on demand. According to the results of our study, the dramatic decrease in rotavirus infection over 5 years of age may be explained by partial immunity caused by previous rotavirus infections.

Although rotavirus is recognized as an important cause of gastroenteritis in children, it is also responsible for adult gastroenteritis. Immunity to rotavirus is not complete, and most people will have more than one infection throughout their lives. Reinfections are asymptomatic or mildly severe.[24] It has been reported that approximately 50% of parents of children with rotavirus disease develop rotavirus infection and half of them develop mild disease. This has been attributed to the fact that rotavirus-specific CD4+ T cells and neutralizing antibodies decrease over time and are insufficient to prevent reinfection.[5] In this study, the frequency of rotavirus was found to be 3.4% in adults over the age of 20, and therefore, rotavirus should be considered in adult gastroenteritis even though its incidence is low.

Despite the fact that rotavirus infections are seen all year round in tropical climates, they are seen especially in winter and early spring in countries with temperate climates including Türkiye.[19] In a national meta-analysis of 38 studies, Güzel et al.[25] found the highest incidence of rotavirus in spring and winter. In our study, consistent with the literature, we found that rotavirus infections start to increase in winter and peak in spring. A statistically significant difference was found in March, April and May ($p<0.001$). Levy et al.[26] concluded in their metanalysis of 34 epidemiologic studies from different countries that rotavirus is definitely more prevalent during the cooler months in globally temperate regions, but that seasonal peaks of infections can vary from autumn to spring. In our study, when the monthly distribution of adenovirus was examined, no statistically significant difference was found ($p=0.154$) and adenovirus did not show any seasonal characteristics.

Various methods such as Latex agglutination (LA), Enzyme-linked immunosorbent assays (ELISA), immunochromatographic method and Reverse transcriptase-polymerase chain reaction (RT-PCR) are used to detect rotavirus in stool samples. Latex agglutination is widely used for rapid testing, but its sensitivity is lower than ELISA. Immunochromatographic method has been found to have higher sensitivity and specificity compared to ELISA.[27] In a study comparing the gold standard RT-PCR with the immunochromatographic method and ELISA, the sensitivity and specificity of both methods were found to be relatively low.[28] The limitation of this study is that the virus was detected by the immunochromatographic method but RT-PCR, which is more sensitive for diagnosis, was not used. On the other hand, the immunochromatographic method has its own advantages on the grounds that it is simple to perform, faster and easy to read the results, it is useful for testing a single sample and readily available, and it is also cost-effective and does not require additional equipment. Therefore, immunochromatographic method is widely used for the diagnosis of rotavirus and adenovirus gastroenteritis in health institutions, including primary care.

CONCLUSION

It has been observed in this study that rotavirus has a significant ratio among acute gastroenteritis cases in the pediatric age group in our region. Rotavirus should be considered especially in gastroenteritis of the 0-3 age group in spring months. Immunochromatographic-based sensitivity high-speed antigen tests can be easily applied in all health institutions for diagnosis. Detection of the pathogen causing gastroenteritis will contribute to the initiation of appropriate treatment, follow-up of complications, and the prevention of unnecessary antibiotic use. Therefore, we consider that our study to determine the regional frequency and the distribution of rotavirus and adenovirus as the causative agents of gastroenteritis by age groups, gender and season is useful in terms of contributing to the epidemiological data of the country and presenting data for the national vaccination program plans.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Bilkent City Hospital Ethics Committee (Date: 10.05.2023, No: E2-23-4081).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Participation of Trainees, Trainers, and Program Directors of Anesthetists and Anesthesia Technicians in the Neonatal Resuscitation Program in Turkiye

Türkiye'deki Yenidoğan Canlandırma Programına Anestezi Uzmanları ve Anestezi Teknisyenlerinin Kursiyerler, Eğitmen ve Program Yöneticileri olarak Katılımı

Yesim Andiran Senayli[1], Başak Tezel[2]

[1]Department of Anesthesiology and Reanimation, Faculty of Medicine, Bozok University, Yozgat, Turkiye
[2]Head of Child and Adolescent Health Department of the General Directorate of Public Health, Health Ministry, Ankara, Turkiye

Abstract

Aim: The Newborn Resuscitation Program aims to ensure that healthcare professionals prevent complications that may occur in the baby, mainly due to asphyxia. Like some other health providers, anesthesia staffs also participate in this program. We aim to determine the rate of participation and contribution of anesthesiologists and anesthesia technicians to programs. We discussed why participation in this program is essential and what results in it will have when evaluated with the after-effects.

Material and Method: After the approval of the Gulhane Scientific Research Ethics Committee, the participant records of the courses, which has been organized since 1998, were examined. The records show the number of anesthesia staff according to the total number of people and their distribution by year. We compared total participants to anesthesia staff with the inclusion of trainees, trainers, and course directors in our country. The distribution of these sums and what these numbers might mean were investigated.

Results: Only one anesthetist attended the first course as a trainee in 1998. The highest participation as anesthetist and anesthesia technician was in 2010, with 494 participants, 218 and 276, respectively. Since the beginning of the course, 2392 anesthesiologists and 3124 anesthesia technicians out of 75,256 trainees have received training. The average is 3.57 percent. Sixteen out of 47 anesthesiologists and 7 out of 11 anesthesia technicians actively contribute to the programs as trainers. Only five anesthetists and two technicians determined the course directors. All of those directors are active in the program.

Conclusion: The number of trainers and course directors is relatively low compared to the total number of people working in the anesthesia branch nationwide. As anesthesia staff is critical for neonatal resuscitations, increasing anesthesia staff participation can significantly reduce neonatal mortality and morbidity.

Keywords: NRP, anesthesia, technician

Öz

Amaç: Yenidoğan Canlandırma Programı, sağlık profesyonellerinin başta asfiksi olmak üzere bebekte oluşabilecek komplikasyonları önlemesini sağlamayı amaçlamaktadır. Diğer bazı sağlık çalışanları gibi anestezi çalışanları da bu programa katılmaktadır. Bu çalışmada anestezist ve anestezi teknikerlerinin programlara katılım ve katkı oranlarını belirlemeyi amaçlıyoruz. Bu programa katılımın neden gerekli olduğunu ve sonrasındaki etkilerle birlikte değerlendirildiğinde ne gibi sonuçlar doğuracağını tartışılmıştır.

Gereç ve Yöntem: Gülhane Bilimsel Araştırmalar Etik Kurulu onayı alındıktan sonra 1998 yılından beri düzenlenen kurslara ait katılımcı kayıtları incelenmiştir. Kayıtlar, anestezi personelinin toplam kişi sayısına göre ve yıllara göre dağılımını göstermektedir. Ülkemizdeki stajyerler, eğitmenler ve kurs direktörlerini dahil ederek toplam katılımcıları anestezi personeli ile karşılaştırdık. Bu toplamların dağılımı ve bu sayıların ne anlama gelebileceği araştırıldı.

Bulgular: İlk kursa 1998 yılında sadece bir anestezist stajyer olarak katılmıştır. Anestezist ve anestezi teknikeri olarak en yüksek katılım sırasıyla 218 ve 276 olmak üzere 494 katılımcı ile 2010 yılında olmuştur. Kursun başlangıcından bu yana 75.256 kursiyerden 2392 anestezi uzmanı ve 3124 anestezi teknikeri eğitim almıştır. Katılı ortalama yüzde 3,57'dir. 47 anestezi uzmanından 16'sı ve 11 anestezi teknisyeninden 7'si programlara eğitmen olarak aktif olarak katkıda bulunmaktadır. Kurs yöneticilerini sadece beş anestezist ve iki teknisyen belirledi. Bu yöneticilerin tamamı programda aktiftir.

Sonuç: Eğitmen ve kurs yöneticisi sayısı ülke genelinde anestezi branşında çalışan toplam kişi sayısına göre görece düşüktür. Anestezi personeli yenidoğan resüsitasyonları için kritik öneme sahip olduğundan, anestezi personelinin katılımını artırmak yenidoğan ölüm ve hastalık oranlarını önemli ölçüde azaltabilir.

Anahtar Kelimeler: NRP, anesthesia, technician

Corresponding *(İletişim)*: Yeşim ŞENAYLI, Department of Anesthesiology and Reanimation, School of Medicine, Bozok University, Yozgat, Turkiye
E-mail *(E-posta)*: ysenayli@gmail.com
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INTRODUCTION

Neonates, especially preterm, are vulnerable to oxidative stress. Since their defense mechanisms against asphyxia are weak, they often need resuscitation with oxygen support during delivery.[1] Up to 10% of newborns (4-7 million/year) at birth require resuscitation assistance.[2,3] A study in the USA determined that 10% of 4 million babies born each year need various levels of resuscitation and 1% needs advanced resuscitation applications.[4] Congenital asphyxia is responsible for 19% of neonatal deaths worldwide yearly.[2] Even if death does not occur in asphyxia, it may have effects such as cognitive impairment, epilepsy, cerebral palsy, and other chronic diseases.[2]

Neonatal resuscitation can be very stressful due to inappropriate or insufficient heart rate and the color of the baby in the delivery room.[5] Recent studies, up to 10 years ago, show that young doctors are insufficient to provide ventilation with masks while standing at the head of babies and to simultaneously provide appropriate mobility of the chest wall.[5] It has also been observed that young doctors in this situation often do positive pressure breathing (PPV).[5] Despite ongoing studies and training, it is stated that there are significant difficulties in managing acute neonatal emergencies and especially intubation skills.[6] However, there is also a need for more literature that can provide information about the intubation of newborns.[6]

The Neonatal Resuscitation Program (NRP), which was initiated based on all these problems, was first established in 1987 by the American Academy of Pediatrics and the After American Heart Association, many countries started the same program quickly.[7] More than 2 million healthcare professionals worldwide received this training in 2019.[7] The result significantly improved neonatal survival.[7] Worldwide neonatal deaths decreased from 2.9% to 0.9%.[8] For instance, the NRP examination conducted in Lithuania determined that the rate of perinatal asphyxia decreased significantly.[9] Hypoxic-ischemic encephalopathy also showed a 3-fold reduction.[9]

NRP is connected with disciplines such as anesthesiology, maternal-fetal medicine, and neonatology. It has the effect of providing communication with each other by acting as a bridge between multiple disciplines.[10] In the first period when anesthesiologists started to deal with newborns, topics such as anesthesia-related airway complications, maternal aspiration and aspiration pneumonitis were primarily emphasized.[10] In addition, more attention has been paid to the adverse effects of anesthesia on lactation, maternal fever, neonatal acid-base imbalance, and its impact on cognitive functions.[10] When the American Society of Anesthesiology(ASA) evaluated how anesthetists should approach the newborn, ASA decided "An anesthetist who takes care of the mother during birth should also be able to take care of the newborn after birth.".[11] Although this decision was interpreted as the necessity of other health professionals, such as neonatologists and pediatricians, to deal with the newborn first, ASA also stated that anesthetists may be involved in neonatal resuscitation in the first place and made it a priority for anesthesiologists.[11] However, in a study conducted in the USA, it has been shown that the experience of participating in neonatal resuscitations of anesthetists is less than 20%, which can be expressed by this definition.[11] It has been determined that current resuscitation practices are mostly unstructured procedures.[11] Moreover, it has been stated that most anesthetists performing these procedures are not resuscitation certified.[11]

There are few studies on NRP in Turkiye that did not consist of anesthesia. A survey conducted in Izmir examined whether pediatric residents could benefit from NRP applications.[12] This study stated that the mortality could increase from 0.5% to 4.5% in cases where the experienced neonatal resuscitation practitioner did not perform the procedure.[12] In the international literature, the situation of anesthetists and anesthesia technicians in NRP has been examined in a few articles. Our study aims to explain the importance of the subject by revealing the NRP participation and contributions of these anesthetists and anesthesia technicians in Turkıye.

MATERIAL AND METHOD

After the approval of the Gulhane Scientific Research Ethics Committee dated 29.12.2020 and numbered 2020-516, the participant, trainers and course director data of the NRP, which has been organized regularly by the Ministry of Health since 1998, were examined. NRP organizations are carried out in our country under the responsibility of the Department of Child and Adolescent Health, General Directorate of Public Health of the Ministry of Health. It has been carried out regularly in all provinces of our country since 1998, and in some regions, this practice can be done once a month or even periodically once in 1-2 weeks. Who will be the participant is selected from the health branches in the delivery room according to the demand, that is, voluntarily. Since the administrative units of the hospitals are responsible for ensuring that at least 1 NRP-certified health personnel is present in each birth, they can make choices about the participant where necessary. However, since volunteering constitutes the majority of participation, participation rates are essential in terms of the interest shown in the subject.

The certificate period of the trainee who has received the certificate is limited to 5 years. The participation numbers of anesthetists and anesthesia technicians were examined in 4-year intervals. Since the data for 2022, which should be read in the last tranche, is not precise, the values are the sum of the three years. The results obtained are proportional to the total participation. In addition, we also examined the rate of trainers and course directors of the anesthetists and anesthesia technicians and whether they actively perform these duties.

Data Availability Statement

Data could be share if needed.

RESULTS

Table 1 shows the number of anesthetists and anesthesia technicians who had attended the courses since 1998, when NRP courses started in Turkiye, in 4-year intervals. In addition, the total number of trainees and the percentage of anesthesia technicians according to these numbers are given in **Table 1**. According to this, it is seen that there is an increase in the participation of both anesthetists and anesthesia technicians in the other 4-year periods, except for the last tranche. Anesthesia technicians participated more than experts. In the last tranche, it was decided that the low was due to the pandemic process.

Table 1: Number of Anesthetists and technicians compared to total NRP trainees.

Years/NRP Trainee	Anesthetists	Technicians	Total Trainee	Anesthetist %	Technicians %
1998-2003	168	435	7357	2.28%	5.9%
2004-2008	533	805	15572	3.42%	5.16%
2009-2013	718	812	24006	2.99%	3.38%
2014-2018	657	701	25502	2.57%	2.74%
2019-2022	316	371	14346	2.20%	2.58%
Total	2392	3124	81600	2.93%	3.82%

The number of anesthetists and technicians authorized to participate in NRP activities as trainers and course directors is given in **Table 2**. It was also examined whether these people actively contributed despite their certification. The total number of trainers and course directors is also provided in **Table 2**. It is seen that anesthesiologists are authorized to take charge as trainers and course directors. However, according to experts, although the number of anesthesia technicians is very few, it is seen that all of them take an active role.

Table 2: Registered numbers of Anesthetists and Technicians as trainer or director who are active or passive in NRP course programs.

	Total	Active	Passive	Total anesthetists	Active in course	Passive	Total technicians	Active in charge	Passive
nRP Trainer	1101	554	547	47	16	31	11	7	4
Course Director	218	145	73	5	5		2	2	

DISCUSSION

Neonatal resuscitation is an inevitable practice that should be known by professional groups such as midwives, nurses, pediatrics, anesthesia, obstetrics, and gynecologists working in delivery rooms.[13] For this reason, anesthetists dealing with obstetrics can work in neonatal intensive care units and are also involved in NRP training.[14] It has been shown that 65% of obstetric anesthetists perform neonatal resuscitations.[14] However, although it is known that anesthetists are active in practical applications, a study conducted in 2001 determined that only 16% of anesthesiologists and technicians were trained in NRP.[14] However, it has been stated that anesthetists are highly valuable practitioners in delivery and influential NRP group members in dire situations.[14] The data obtained from another study determined that the knowledge level of anesthetists was good in 2022. However, they could not act comfortably as practitioners.[14] It has been shown that the reason for this is that they do not practice regularly in newborns and, therefore, cannot reach practical comfort because they cannot increase their practice skills with their efforts.[14] Interrupted practice is a problem not only for anesthesia workers but also for other healthcare professionals. In a study conducted in India with the participation of 669 pediatricians, it was concluded that knowledge and skills should be followed continuously.[2] This study concluded that there is no uniformity in practice among pediatricians and that care is not taken.[2] Another study conducted in the USA revealed that NRP training and skill development were challenging to implement in real life. The learned information was used for 6-12 months.[4,8] It is stated that to benefit from NRP; it is necessary to apply what is learned routinely in the clinical process.[4]

Even though interventions for neonates are essential for the institution and anesthetists can do this best, active NRP training is necessary for appropriate information and application comfort.[14] However, another point that needs to be examined is that the training given to health workers who can be NRP practitioners is very different.[13] It is known that information about the quality of education of the participants is lacking.[13] However, few studies have evaluated this situation.[2,3,13] The most important reason for this is the unique difficulties of evaluating effective education.[4] Because such training sessions are held in multiple institutions at a time, also, there is no control group. However, it is possible to evaluate the training as pre- and post-education.[15] There are doubts in the studies that the NRP program gives the desired results.[15] Among the observed nonconformities were errors in self-efficacy, knowledge, and skills practices.[15] The generally accepted situation is that NRP knowledge and skills are limited.[4] A study determined that the information developed after the NRP course could have been used more effectively.[13] In addition, it was understood from the training that pediatricians, anesthetists, and obstetricians showed the most improvement.[13] It was even stated that it would be better for NRP practitioners, including anesthetists, to take NRP courses before their specialization, mainly due to their practical contributions.[8] In an event evaluation conducted at the University of Padova, Italy, in 2005, the efficiency of the NRP course was examined according to the knowledge and satisfaction levels of the participants.[3] In this study, it has been stated that the effect of the training done for up to 6 months.[3] It has been noted that this finding is consistent with the result in other studies.[3] Our study was not aimed at evaluating the quality of education, but the main reason is that it is tough to make a nutritional assessment, as stated above.

CONCLUSION

For a successful neonatal resuscitation, there is a need for a defined leader who knows the procedures to be done, can communicate effectively, can enable the team to work together, and is defined. A group of healthcare professionals in a position to lead is those who work on obstetric anesthesia. Such training is valuable in terms of strengthening clinical decision-making mechanisms. It will increase skills, decrease medical errors, increase the self-confidence of the team leader and strengthen teamwork. It can also help with overlooked and hidden problems, for example, locating necessary material. In our country, anesthesia staff seems to be performing their best as trainees, but it is evident that trainers and directors among anesthesia staff are less which means that they must be more motivated in taking part in NRP courses.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Gülhane Scientific Research Ethics Committee (Date: 29.12.2020, Decision No: 2020-516)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Effects of Caffeine Use in Hemifacial Spasm Patients

Hemifasiyal Spazm Hastalarında Kafein Kullanımının Etkileri

Mehmet Kara[1], Soner Kılıç[2]

[1]Department of Medical Pharmacology, Kayseri City Research and Training Hospital, Turkey
[2]Department of Neurology, Kayseri City Research and Training Hospital, Turkey

Abstract

Aim: Hemifacial spasm is characterized by progressive, involuntary, irregular contraction of the muscles innervated by the facial nerve. Caffeine is a phytochemical that increases muscle contraction and neurotransmitter release. The aim of this study is to evaluate the effects of caffeine-containing food consumption on disease severity and disease-related quality of life in patients with hemifacial spasm.

Material and Method: In hemifacial spasm patients who applied to the neurology outpatient clinic; hemifacial spasm quality of life scale and hemifacial spasm severity scale were evaluated prospectively. The amount of caffeine taken by the patients with daily food and beverage was determined. The relationship between the results of the scales and the amount of caffeine consumed was analyzed.

Results: A total of 60 patients, 36 women (60%) and 24 men (40%), were included in the study. It was determined that 59 of the patients (98.3%) consumed an average of 303±144 mg/day of caffeine with food. The hemifacial spasm quality of life scale score was calculated as 8.5 (4-21) and the hemifacial spasm severity scale was calculated as 3 (1-4). No significant correlation was found between the amount of caffeine consumed by gender (p=0.066). A negative correlation was found between the amount of caffeine consumed by age (r=-0.291; p=0.024). There was no significant relationship between daily caffeine consumption and hemifacial spasm quality of life scale and hemifacial spasm severity (p values 0.297 and 0.839, respectively). There is a weak positive correlation between the hemifacial spasm quality of life scale and the severity of hemifacial spasm (r=0.291; p=0.024).

Conclusion: It has been determined that the consumption of foods containing caffeine in daily life does not affect the severity of spasm and quality of life in patients with hemifacial spasm.

Keywords: Hemifacial spasm, caffeine, theophylline

Öz

Amaç: Hemifasiyal spazm, fasiyal sinir tarafından innerve edilen kasların ilerleyici, istemsiz, düzensiz kasılması ile karakterizedir. Kafein, kas kasılmasını ve nörotransmitter salınımını artıran bir fitokimyasaldır. Bu çalışmanın amacı hemifasiyal spazmlı hastalarda kafein içeren gıda tüketiminin hastalık şiddeti ve hastalıkla ilişkili yaşam kalitesi üzerine etkilerini değerlendirmektir.

Gereç ve Yöntem: Nöroloji polikliniğine başvuran hemifasiyal spazm hastalarında; hemifasiyal spazm yaşam kalitesi ölçeği ve hemifasiyal spazm şiddet skalası prospektif olarak değerlendirildi. Hastaların günlük yiyecek ve içeceklerle aldıkları kafein miktarları belirlendi. Ölçek sonuçları ile tüketilen kafein miktarı arasındaki ilişki analiz edildi.

Bulgular: Çalışmaya 36 kadın (%60) ve 24 erkek (%40) olmak üzere toplam 60 hasta dahil edildi. Hastaların 59'unun (%98,3) yemekle birlikte ortalama 303±144 mg/gün kafein tükettiği belirlendi. Hemifasiyal spazm yaşam kalitesi ölçeği puanı 8,5 (4-21), hemifasiyal spazm şiddeti ölçeği 3 (1-4) olarak hesaplandı. Cinsiyete göre tüketilen kafein miktarı arasında anlamlı bir ilişki bulunmadı (p=0,066). Yaşa göre tüketilen kafein miktarı arasında negatif korelasyon bulundu (r=-0,291; p=0,024). Günlük kafein tüketimi ile hemifasiyal spazm yaşam kalitesi ölçeği ve hemifasiyal spazm şiddeti arasında anlamlı bir ilişki yoktu (p değerleri sırasıyla 0,297 ve 0,839). Hemifasiyal spazm yaşam kalitesi ölçeği ile hemifasiyal spazm şiddeti arasında pozitif yönde zayıf bir korelasyon vardır (r=0,291; p=0,024).

Sonuç: Hemifasiyal spazmlı hastalarda günlük yaşamda kafein içeren besinlerin tüketiminin spazm şiddetini ve yaşam kalitesini etkilemediği saptanmıştır.

Anahtar Kelimeler: Hemifasiyal spazm, kafein, teofilin

Corresponding *(İletişim)*: Mehmet Kara, Department of Medical Pharmacology, Kayseri City Research and Training Hospital, Turkey
E-mail *(E-posta)*: dr.mehmetkara@hotmail.com
Received *(Geliş Tarihi)*: 19.05.2023 **Accepted *(Kabul Tarihi)*:** 26.07.2023

INTRODUCTION

Hemifacial spasm (HFS) is characterized by progressive, involuntary, irregular, clonic or tonic movements of the muscles innervated by the facial nerve (VII. cranial nerve). The distinguishing characteristic of the disease is involuntary clonic and/or tonic contraction of the facial muscles, usually unilateral.[1] Exposure of facial nerve roots to vascular compression (primary HFS) is the most common type. Excessive stimulation from the facial nerve afferent to the facial nerve nucleus and ephaptic transmissions occurring around the affected nerve region are responsible for the pathophysiology of the disease.[2] Secondary HFS causes; cerebellopontine corner tumor, aneurysms, infections (otitis media, tuberculous meningitis), epidermoid and arachnoid cysts.[3]

Although it is perceived as a benign disease, it can cause increased shame and social withdrawal for the individual. In severe cases, symptoms can affect a person's quality of life by affecting vision, speech, and mental concentration.

One of the phytochemicals found in relatively high amounts in coffee, tea and cocoa is methylxanthines. Among these methylxanthines, caffeine is the most studied substance, which has clear effects on neuronal network activity, enhances cognitive performance, and is reported to be protective against stroke, Alzheimer's disease and Parkinson's disease.[4,5] Caffeine causes muscle contraction and vasoconstriction by stimulating catecholaminergic and adenosine receptors. The net effect of caffeine in HFS patients is unknown.

The effect of caffeine, which is catabolized to dimethylxanthines by the cytochrome P450 system, disappears within a few hours.[6] The pharmacokinetics and bioavailability of caffeine are quite similar in subjects aged 20 and 71 years.[7] This age group also includes our patient group included in our study. Therefore, in our study, we can accept that the pharmacokinetics and bioavailability of caffeine do not change with age.

The aim of this study is to evaluate the effect of caffeine consumed with hot or cold beverages on the quality of life and disease severity in primary HFS patients.

During the follow-up of our HFS patients in our neurology clinic, we have patients who consume caffeine-containing foods such as tea and coffee, etc. daily. Our motivation in our study was to think that the increase in the complaints of these patients due to HFS is related to caffeine consumption.

MATERIAL AND METHOD

In primary HFS patients who applied to Kayseri City Training and Research Hospital Neurology outpatient clinic; The HFS quality of life scale and the HFS severity scale were calculated prospectively.[8,9]

Patients with intermittent, clonic and/or tonic spasm, mostly in the upper half of the face, were included in the study. Brain magnetic resonance imaging (MRI) was performed on the patients, and secondary pathologies that could cause facial nerve compression were excluded. Cases with a history of peripheral facial paralysis, trauma and infection were not included in the study. Patients using hypnotic and sedative drugs were not included in this study.

The treatments of the patients were not designed specifically for the study. They continued to receive their treatment in accordance with their routine treatment algorithms.

The amount of caffeine taken by the patients with daily food and beverage foods was determined.[10-12] The most consumed caffeine-containing food was determined as the food that contributed the most to total daily caffeine intake. In the calculation of food consumption, the statements of the patients were taken as basis. Informed consent was obtained from the patients at the time of participation in the study. No additional examination or analysis specific to the study was performed.

Patients' age, gender, daily caffeine consumption (mg/day), the most consumed caffeine-containing food (granulated coffee, black tea, green tea, Turkish coffee), HFS quality of life scale score (HFS-7) (between 0 and 28). : best, 28: worst quality of life) and HFS severity (0-4: no abnormality, 4: severe spasm).

Statistical Method

Data were analyzed with IBM SPSS V23. Kolmogorov Smirnov and Shapiro Wilk tests were used to fit the normal distribution. Descriptive statistics; number and percentage for categorical variables; For numerical variables, data that provided the normal distribution parameters were given as mean±standard deviation, and those that did not fit the normal distribution were given as median (minimum-maximum). One-way analysis of variance and independent samples t-test were used for normally distributed ones. Mann Whitney U test and Kruskal Wallis test were used for data not normally distributed. Pearson and Spearman correlation coefficients were used to analyze the relationships. Pearson chi-square test was used for categorical data. Significance level was taken as $p<0.05$.

RESULTS

Of the 60 patients, 36 (60%) were female and 24 (40%) were male. The mean age of the patients was 58.17±11.8 years. It was determined that 59 of the patients (98.3%) consumed an average of 303±144 mg/day of caffeine with food. The HFS quality of life scale score was calculated as 8.5 (4-21) and the HFS severity scale was calculated as 3 (1-4) (**Table 1**).

Table 1. General characteristics of the patients

	Average	Standard Deviation	Median	Minimum	Maximum
Age	58.17	11.814	57	39	82
Caffeine consumption, mg/day	303	144.216	301.5	0	682
Hemifacial spasm quality of life scale	9.6	4.001	8.5	4	21
Hemifacial spasm severity	2.63	0.736	3	1	4

The mean age values did not differ according to gender (p=0.843). While the mean value for women was 57.92±12.63 years, it was 58.54±10.72 years for men. Daily caffeine consumption, mg/day mean values do not differ according to gender (p=0.066). While the mean value in women was 275.06 mg, it was obtained as 344.92 mg in men. The median values of the HFS quality of life scale did not differ according to gender (p=0.778). While the median value was 8.5 (4-21) for women, it was 8.5 (5-21) for men. Median values of HFS severity do not differ according to gender (p=0.300). While the median value was 3 (2-4) in women, it was 2.5 (1-4) in men (**Table 2**).

Table 2: Comparison of quantitative data by gender

	Female		Male		p
	Mean± Standard Deviation	Median (minimum-maximum)	Mean± Standard Deviation	Median (minimum-maximum)	
Age	57.92±12.63	57 (39-82)	58.54±10,72	56.5 (42-79)	0.843[a]
Caffeine consumption, mg/day	275.06±139.52	285 (0-555)	344.92±143.79	332.5 (130-682)	0.066[a]
Hemifacial spasm quality of life scale	9.58±4.24	8.5 (4-21)	9.63±3.7	8.5 (5-21)	0.778[b]
Hemifacial spasm severity	2.72±0.51	3 (2-4)	2.5±0.98	2.5 (1-4)	0.300[b]

a Independent samples t-test; bMann Whitney U test

The distribution of the most consumed caffeine-containing foods does not depend on gender (p=0.661). While 74.3% of women consume black tea, the rate of black tea consumption in men is 79.2% (**Table 3**).

Table 3. Comparison of the most consumed caffeine-containing foods by gender

	Female	Male	P*
The most consumed caffeine-containing food			
Granular coffee	2 (5,7)	2 (8,3)	0,661
Black tea	26 (74,3)	19 (79,2)	
Green tea	2 (5,7)	0 (0)	
Turkish coffee	5 (14,3)	3 (12,5)	

*Pearson chi square

There is a negative relationship between age and only daily caffeine consumption (r=-0.291; p=0.024). As age increases, the amount of caffeine consumed decreases. There was no significant relationship between age, HFS quality of life scale and HFS severity (p values 0.892 and 0.653, respectively) (**Table 4**).

Table 4: Correlation analysis results of age and quantitative data

	Age	
	r	p
Caffeine consumption, mg/day	-0.291	0.024
Hemifacial spasm quality of life scale	-0.018	0.892
Hemifacial spasm severity	-0.059	0.653

r: Pearson correlation coefficient

While the average age of those consuming granulated coffee was 55.25 years, those consuming black tea were 58.82 years, those consuming green tea were 79 years, and those consuming Turkish coffee were 52.75 years. There is a difference between the mean ages according to the foods consumed (p=0.034). The mean age of those who consumed green tea was higher than the others (**Table 5**).

Table 5. Comparison of average age values according to consumed foods

	n	Mean± Standard Deviation	Median (minimum-maximum)	P*
Granular coffee	4	55.25±11.81[a]	51.5 (46-72)	0.034
Black tea	45	58.82±11.31[a]	58 (39-79)	
Green tea	2	79.00±4.24[b]	79 (76-82)	
Turkish coffee	8	52.75±10.38[a]	50.5 (42-72)	

*One-way analysis of variance; a-b No difference between foods with the same letter (Duncan test)

There was no significant relationship between daily caffeine consumption and HFS quality of life scale and HFS severity (p values 0.297 and 0.839, respectively) (**Table 7**).

Table 7. Correlation analysis results of caffeine consumption and quantitative data

	Caffeine consumption, mg/day	
	r	p
HFS quality of life scale	-0.137	0.297
HFS severity	0.027	0.839

r: Spearman correlation coefficient

The most consumed food was black tea (76.3%) (**Table 8**).

Table 8: Distribution of consumed foods

	n	%
Food		
Granular coffee	4	6.8
Black tea	45	76.3
Green tea	2	3.4
Turkish coffee	8	13.6

There was no difference between the median values of the HFS quality of life scale according to the foods consumed (p=0.827). The median value was determined as 8.5 (5-12) in granulated coffee, 8 (4-21) in black tea, 12.5 (6-19) in green tea and 8 (6-15) in Turkish coffee (**Table 9**).

Table 9. Comparison of HFS quality of life scale values according to consumed foods

	n	Mean± Standard Deviation	Median (minimum-maximum)	P*
Granular coffee	4	8.5±3.51	8.5 (5-12)	0.827
Black tea	45	9.56±3.82	8 (4-21)	
Green tea	2	12.5±9.19	12.5 (6-19)	
Turkish coffee	8	8.5±2.93	8 (6-15)	

*Kruskal Wallis

There was no difference between the median values of HFS severity according to the foods consumed (p=0.287). The median value was determined as 2.5 (2-4) in granulated coffee, 3 (1-4) in black tea, 3 (2-4) in green tea and 2 (2-3) in Turkish coffee (**Table 10**).

Table 10. Comparison of Hemifacial spasm severity values according to consumed foods

	n	Mean± Standard Deviation	Median (minimum-maximum)	P*
Granular coffee	4	2.75±0.96	2.5 (2-4)	0.287
Black tea	45	2.67±0.74	3 (1-4)	
Green tea	2	3±1.41	3 (2-4)	
Turkish coffee	8	2.25±0.46	2 (2-3)	

*Kruskal Wallis

There is a weak positive correlation between the HFS quality of life scale and the severity of HFS (r=0.291; p=0.024) (**Table 11**).

Table 11. Hemifacial spasm severity and Hemifacial spasm quality of life scale correlation analysis results

	Hemifacial spasm severity	
	r	p
Hemifacial spasm quality of life scale	0.291	0.024

r: Spearman correlation coefficient

DISCUSSION

Primary HFS is mostly 5th-6th grade. It occurs in the decade and is more common in females (2:1).[3] Similarly, in our study, the mean age was 58 years and the female/male ratio was 1.5.

The HFS severity scale developed by Chen et al., one of the clinical evaluation scales applied by different research groups, was applied to HFS cases. The overall mean score according to the HFS severity scale was 2.5±0.6, similar to that in our patient group.[8] In addition, the short and simple HFS quality of life scale developed by Tan et al. was applied to evaluate the health-related quality of life.[13] On this scale, 28 points are the highest and associated with being the worst in terms of quality of life.

Scores in the HFS quality of life scale may vary according to the severity of the disease, additional symptoms such as headache, and the presence of underlying comorbidities.[14] Although HFS is more common in females, gender differences in the severity of the disease and the effect of age have not been determined. Although it is thought that the symptoms may disturb female patients and younger patients more cosmetically, the effect of gender and age on quality of life and disease severity was not found in the study.

Traditionally, black tea consumption of the Turkish population is common and accordingly, black tea consumption was high in our patient group. Green tea consumption was found to be higher in older age and female gender. It has been determined that the consumption of foods containing caffeine decreases with age.

Adenosine A1 receptors are found throughout the nervous system, with the highest levels in the hippocampus, cerebellum, cerebral cortex, and thalamus. Stimulation of adenosine A1 receptors inhibits transmitter release from neurons. Adenosine A2A receptors are located in dopamine-rich areas of the brain; They are found in the striatum, nucleus acumbens, and tuberculum regions.

As a result of caffeine's inhibition of adenosine receptors, effects such as insomnia, increased locomotor activity, neurotransmitter release, and contraction of skeletal muscles occur.[15,16] In primary HFS, chronic irritation and facial demyelination develop as a result of vascular compression on the facial nerve. Abnormal overstimulation of the facial nerve leads to the development of symptoms. For this reason, it was predicted that the symptoms may increase with caffeine intake, depending on the increase in neurotransmitter release in HFS patients. However, in our study, no difference was found between caffeine intake and disease severity. The effect of caffeine on spasm severity and quality of life has not been reported in the literature.

The presence of symptoms affects the daily work of patients, causing them to feel depressed and depressed. As the severity of the disease increases, the quality of life deteriorates. In our study, a statistically significant relationship was found between the severity of the disease and the deterioration of the quality of life.

Caffeine taken into the body does not accumulate in organs or tissues. It is extensively metabolized by the liver. Patients followed by our clinic from different times were included in our study. We think that the effect of caffeine is not chronic. In this context, we aimed to determine the effect of caffeine prospectively with hemifacial spasm quality of life scale and hemifacial spasm severity scales. In our study, we showed that this effect did not exist.

It is predicted that drug groups such as Gabapentin, Baclofen, Carbamazepine, Selective serotonin reuptake inhibitors that our patients can use frequently and the caffeine dose taken with food in daily doses will not interact. Especially in our patient group with cardiovascular disease, the antihypertensive drugs they use may have a protective effect against the hypertensive effect of caffeine.

The limitation of the study was that the patients were evaluated independently of the treatment, and the treatments given could differ between the patients.

CONCLUSION

One of the results of this study is that caffeine taken with food has no effect on the severity of the disease and quality of life in HFS patients. Another result, as expected, is that the quality of life deteriorates as the severity of the disease increases. However, randomized controlled studies are needed to determine the effect of caffeine and its place in treatment modalities in HFS patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Gülhane Scientific Research Ethics Committee (Date: 08.11.2022, Decision No: 735)

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Bibliometric Approach to Total Hip Arthroplasty Literature Originating from Turkey

Türkiye Kaynaklı Total Kalça Artroplasti Literatürüne Bibliyometrik Yaklaşım

Alaaddin Oktar Üzümcügil, Mehmet Kurt, Selçuk Yılmaz

Department of Orthopedic and Traumatology, University of Kutahya Health Sciences, Turkey

Abstract

Aim: The aim of this study is to analyze research productivity in Turkey using published articles on total hip arthroplasty.

Material and Method: All scientific articles published in English in the Science Citation Index Expanded and the Emerging Sources Citation Index between 1970 and 2023 were analyzed using "Web of Science". The number of articles, authors, institutions, and the 30 most frequently cited articles were analyzed. In addition, the Scopus database was also analyzed with the same method for making comparisons. Visualization was also done with Vosviewer tool.

Results: As a result of the study, we found 190 articles in the WOS database and 485 articles in the Scopus database, until the end of March 2023. according to the WOS database (journals published in ESCI and SCIE indexes), Turkey ranked 15th among 108 countries. According to the Scopus database, Turkey ranked 22nd.

Conclusion: Scholars can help clarify the history of Turkish literature on THA by using the study's data summary. We discovered 190 articles in the WOS database and 485 articles in the Scopus database as a consequence of the research up until the end of March 2023. In comparison to other countries, the quantity of articles was remarkably low.

Keywords: Bibliometric analysis, publications, total hip arthroplasty

Öz

Amaç: Bu çalışmanın amacı, total kalça artroplastisi üzerine yayınlanmış makaleleri kullanarak Türkiye'deki araştırma verimliliğini analiz etmektir.

Gereç ve Yöntem: 1970-2023 yılları arasında Science Citation Index Expanded ve Emerging Sources Citation Index'te İngilizce olarak yayınlanan tüm bilimsel makaleler "Web of Science" kullanılarak analiz edildi. Makale sayıları, yazarları, kurumları ve en sık atıf alan 30 makalesi analiz edildi. Ayrıca karşılaştırma yapabilmek için Scopus veri tabanı da aynı yöntemle analiz edilmiştir. Görselleştirme de Vosviewer aracı ile yapılmıştır.

Bulgular: Çalışma sonucunda Mart 2023 sonuna kadar WOS veri tabanında 190, Scopus veri tabanında 485 makale bulduk. WOS veri tabanına (ESCI ve SCIE dizinlerinde yayınlanan dergiler) göre Türkiye 15. sırada yer aldı. 108 ülke arasında Scopus veri tabanına göre Türkiye 22. sırada yer aldı.

Sonuç: Bilim adamları, çalışmanın veri özetini kullanarak TKA ile ilgili Türk edebiyatının tarihini netleştirmeye yardımcı olabilir. Mart 2023 sonuna kadar yaptığımız araştırma sonucunda WOS veritabanında 190, Scopus veritabanında 485 makale tespit ettik. Diğer ülkelere kıyasla makale sayısı oldukça düşüktü.

Anahtar Kelimeler: Bibliyometrik analiz, total kalça, yayın

Corresponding *(İletişim)*: Selçuk YILMAZ, Kutahya Health Sciences University School of Medicine, Department of Orthopaedics and Traumatology, Kutahya, Turkey
E-mail *(E-posta)*: dryilmazselcuk@gmail.com
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INTRODUCTION

Total hip arthroplasty (THA) is the treatment procedure for hip arthritis in adults and has been described as one of orthopedic surgery's most effective and affordable treatments.[1] In this procedure, biocompatible materials are used to replace sections of the upper femur and acetabulum components. The major objectives of this operation are to retain hip stability while removing all discomfort and restoring complete range of motion to the joint.[2] The THA as it is known today was first developed in the 1930s utilizing metallic implants to replace bones and joints.[3] Although Wiles developed the first THA in 1938, it wasn't until Sir John Charnley from Wrightington Hospital introduced the "low-friction arthroplasty" in 1962.[4,5] The THA was considered a revolutionary surgical technique in the 1960s in the treatment of elderly arthritis patients with positive long-term results.[5] The initial success of the first implants used by Moore, Thomson, McKee-Farrar and it was short-lived, mostly due to heavy pressure from the acetabular component. With the introduction of Charnley's hip, this problem was largely resolved in the 1970s. By the 1970s, one design he produced as a result of his work on tribology almost completely replaced the others.[6] The first application of hip prosthesis in Turkey was performed by Prof. Dr. Akif Şakir Şakar in 1948. hip surgery, hip arthroscopy and hip arthroscopy in Turkey in parallel with the developments in the world preventive surgery, continues its development.[7] There are numerous surgical techniques for the THA, each with particular benefits and drawbacks. Direct posterior, direct lateral, and direct anterior routes are the most often performed techniques. When employing either procedure, a variety of technical advancements enable secure and effective femoral and acetabular reconstruction.[8] The three main treatment objectives of a THA pain relief, improved quality of life, and function restoration are the key factors that determine whether the procedure is successful.[5] THA concerns include hip dislocation, abductor dysfunction, fractures, and nerve damage, albeit the relative risks of each procedure differ. Many clinical studies show that patient-reported outcomes, complication rates, and return to function vary amongst surgical methods.[6,9]

Globally, the number of total hip replacements performed has grown exponentially over the past decade, with a sharp rise in the proportion of young patients looking to improve quality of life and return to vigorous physical activity.[1] Nowadays, life expectancy is increasing and with musculoskeletal problems (osteoarthritis, rheumatoid arthritis, traumas, etc.), the number of THA surgeries is also increasing. According to 2015 OECD Health Statistics, the highest number of THA operations were performed in Switzerland (292/100,000), Germany (283/100,000), and Austria (276/100,000 in 2013. In Turkey, this rate is 44/100,000.[10]

Bibliometric analysis is the most powerful tool available today for monitoring long-term research trends on a given topic. This approach facilitates research on online bibliometric databases that have been widely used in recent years. Through this method, the research contributions of different countries, institutions, journals and authors in the scientific field can be presented objectively, and research trends or hotspots can be identified. It can also serve as a guide for future research.[11-13] Bibliometric methods are also popular in orthopedics and have been used in previous studies.[14-21] However, no comparable studies of THA literature from Turkey were found in the existing literature.

MATERIAL AND METHOD

Although there are numerous databases that can be used for bibliometric analysis, in this study we chosed The Web of Science (WoS) Core Collection Science Citation Index Expanded (SCI-EXPANDED) and Emerging Sources Citation Index (ESCI) Indexes due to their high scientific quality. 'Article' selected as document type in search engine. To find the articles about THA between 1970 and March 2023 that originating from Turkey, the WoS database was accessed on April 1, 2023.

Search terms selected from the MESH library were as below. "arthroplasty, replacement, hip" [MeSH Terms] OR ("arthroplasty" [All Fields] AND "replacement" [All Fields] AND "hip" [All Fields]) OR "hip replacement arthroplasty" [All Fields] OR ("total" [All Fields] AND "hip" [All Fields] AND "arthroplasty" [All Fields] OR "total hip arthroplasty" [All Fields].

The literature accessed as a result of the restrictions made within the study plan was then downloaded to the computer in plain text and Excel file format.

Data extracted from these articles included bibliometric parameters such as, manuscript title, first author, total citation count, year of publication, avarage citation number since the date of publication, journal name, affiliation of origin, etc.

Graphs and tables were created using Excel files. Percentage and frequency values were used in the tables. In addition, Scopus and Wosdatabase's own graphics were also used for visualizations. The VOSviewer tool was used to generate and visualize bibliometric networks (Leiden, Leiden University, The Netherlands).[22]

In addition we selected Scopus database to search THA articles from Turkey to make comparisions with WOS database. We used same time period and same keywords.

RESULTS

13956 total global documents from 108 different countries and 11417 articles SCIE and ECSI indexed in the WOS database. There was 24 countries with more than 100 published articles on THA as shown in **Table 1**. The United States of America (USA) published most of the articles (5040 articles) on THA. China (1085 articles), Japan (1064 articles), England (926 articles) and Germany (885 articles) were the mostly publishing countries on THA.

Table 1. Mostly publishing countries on total hip arthroplasty globally according to the WOS database results

Ranking	Countries/Regions	Number of articles	% of 13.956
1	The USA	5040	36.113
2	CHINA	1085	7.774
3	JAPAN	1064	7.624
4	ENGLAND	926	6.635
5	GERMANY	885	6.341
6	CANADA	704	5.044
7	FRANCE	571	4.091
8	NETHERLANDS	399	2.859
9	SWITZERLAND	397	2.845
10	SOUTH KOREA	389	2.787
11	ITALY	385	2.759
12	SWEDEN	360	2.580
13	AUSTRALIA	349	2.501
14	DENMARK	290	2.078
15	TURKEY	190	1.361
16	AUSTRIA	183	1.311
17	SPAIN	173	1.240
18	INDIA	160	1.146
19	FINLAND	158	1.132
20	NORWAY	136	0.974
21	SCOTLAND	129	0.924
22	GREECE	127	0.910
23	TAIWAN	111	0.795
24	BELGIUM	108	0.774

Turkey ranks 15th in the number of articles published on THA. 190 articles from Turkey on THA. These articles were cited 1291 times (mean citations 6.79) with an H-index of 18. Among them, 85.789% of the articles were published in SCI-EXPANDED, and 14.211% of the articles were published in ESCI indexed journals. In terms of language of publication, these articles were published in English (94.211%), Turkish (5.263%) and Portuguese (0.526%) languages. Turkey's first article on THA was published in 1999, and 2021 is the year with the most articles published, with a total of 37 articles. The number of publications did not fall below 10 articles per year between 2014 and 2022 (**Figure 1**).

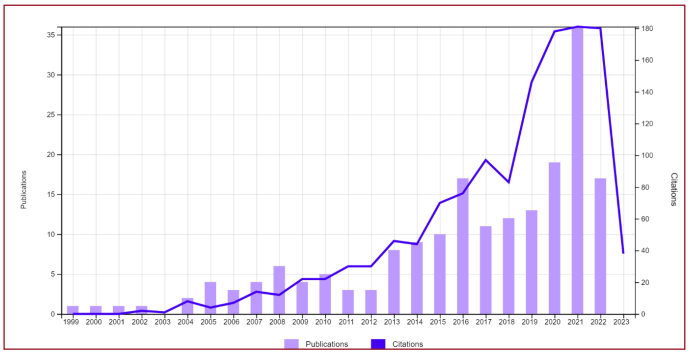
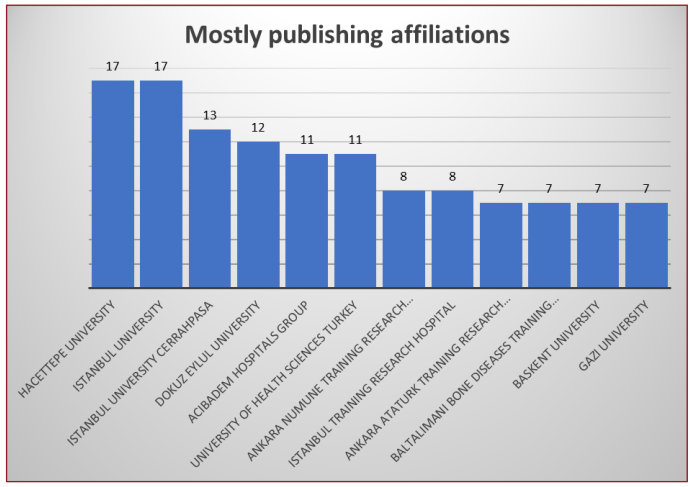


Figure 1. Citations and publications by years (WOS results)

The authors from 203 different affiliations contributed to THA literature from Turkey.

Hacettepe University (17 articles), Istanbul University (17 articles), Istanbul University / Cerrahpasa (13 articles) were the top affiliations contributed to THA literature from Turkey. The mostly publishing affiliations are listed in **Graphic 1.**



Graphic 1. Mostly publishing affiliations on total hip arthroplasty globally according to the WOS database results

The articles on THA from Turkey indexed in the WOS database were mostly published in Hip International (20 articles), Acta Orthopaedica et Traumatologica Turcica (18 articles), the Journal of Arthroplasty (17 articles) and Archives of Orthopaedic and Trauma Surgery (10 articles). **Table 2** summarizes the mostly publishing journals on THA from Turkey.

Table 2. The mostly publishing journals on total hip arthroplasty from Turkey according to the WOS database results

Publication Titles	n	% of 190
HIP International	20	10.526
Acta Orthopaedica et Traumatologica Turcica	18	9.474
The Journal of Arthroplasty	17	8.947
Archives of Orthopaedic and Trauma Surgery	11	5.789
Joint Diseases and Related Surgery	8	4.211
Eklem Hastalıkları ve Cerrahisi Dergisi (Joint diseases and related surgery)	7	3.684
Acta Orthopaedica Belgica	6	3.158
International Orthopaedics	6	3.158
Journal of Orthopaedic Surgery	5	2.632
The Indian Journal of Orthopaedics	4	2.105

*Shows10 out of 81 entries

The highly cited article on THA from Turkey according to the WOS database results published by Bilgen, et al in 2001.[23] This article was cited 107 times totally and 4.65 per year since it's publication year. **Table 3** summarizes the top 30 most cited articles on THA published in Turkey according to WOS database results.

Table 3. The top 30 most cited articles on THA published in Turkey according to WOS database results

Title	Authors	Source title	Publication year	DOI	Total citations	Average per year
C-reactive protein values and erythrocyte sedimentation rates after total hip and total knee arthroplasty	Bilgen, et al	Journal of international medical research	2001	10.1177/147323000102900102	107	4.65
Ultrasound guided Erector Spinae Plane block at L-4 transverse process level provides effective postoperative analgesia for total hip arthroplasty	Tulgar, S and Senturk, O	Journal of clinical anesthesia	2018	10.1016/j.jclinane.2017.11.006	80	13.33
Total hip arthroplasty in developmental high dislocation of the hip	Erdemli,et al	Journal of arthroplasty	2005	10.1016/j.arth.2005.02.003	64	3.37
Cementless Total Hip Arthroplasty With Modified Oblique Femoral Shortening Osteotomy in Crowe Type IV Congenital Hip Dislocation	Kilicoglu, et al	Journal of arthroplasty	2013	10.1016/j.arth.2012.06.014	51	4.64
The effect of exercise on hip muscle strength, gait speed and cadence in patients with total hip arthroplasty: a randomized controlled study	Unlu, et al	Clinical rehabilitation	2007	10.1177/0269215507077302	48	2.82
Subtrochanteric Shortening in Total Hip Arthroplasty: Biomechanical Comparison of Four Techniques	Muratli, et al	Journal of arthroplasty	2014	10.1016/j.arth.2013.09.004	43	4.3
The reliability of hip scoring systems for total hip arthroplasty candidates: assessment by physical therapists	Kirmit, et al	Clinical rehabilitation	2005	10.1191/0269215505cr869oa	35	1.84
The Effect of Relaxation Techniques and Back Massage on Pain and Anxiety in Turkish Total Hip or Knee Arthroplasty Patients	Buyukyilmaz, F and Asti, T	Pain management nursing	2013	10.1016/j.pmn.2010.11.001	32	2.91
Reliability of the six-minute walk test after total hip arthroplasty	Unver, et al	Hip international	2013	10.5301/hipint.5000073	31	2.82
Transverse Subtrochanteric Shortening Osteotomy During Cementless Total Hip Arthroplasty in Crowe Type-III or IV Developmental Dysplasia	Sofu, et al	Journal of arthroplasty	2015	10.1016/j.arth.2015.01.045	29	3.22
Late fatigue fracture of a modern cemented forged cobalt chrome stem for total hip arthroplasty - A report of 10 cases	Della et al	Journal of arthroplasty	2005	10.1016/j.arth.2005.03.038	24	1.26
Intraoperative estimation of femoral anteversion in cementless total hip arthroplasty using the lesser trochanter	Unlu, et al	Archives of orthopaedic and trauma surgery	2011	10.1007/s00402-011-1282-9	23	1.77
Cementless total hip arthroplasty with subtrochanteric transverse shortening osteotomy for severely dysplastic or dislocated hips	Yalcin, et al	Hip international	2010	10.1177/112070001002000113	22	1.57
A meta-analysis comparing the direct anterior with other approaches in primary total hip arthroplasty	Kucukdurmaz,et al	Surgeon-journal of the royal colleges of surgeons of edinburgh and ireland	2019	10.1016/j.surge.2018.09.001	21	4.2
Effects of total hip arthroplasty on spinal sagittal alignment and static balance: a prospective study on 28 patients	Eyvazov, et al	European spine journal	2016	10.1007/s00586-016-4696-9	21	2.63
Cementless total hip arthroplasty in patients with Crowe type-4 developmental dysplasia	Sofu, et al	Hip international	2013	10.5301/hipint.5000047	20	1.82
Assessment of hip abductors by MRI after total hip arthroplasty and effect of fatty atrophy on functional outcome	Kovalak, Emrah; Ozdemir, Hanife; Ermutlu, Cenk; Obut, Abdullah	Acta orthopaedica et traumatologica turcica	2018	10.1016/j.aott.2017.10.005	19	3.17
Test-retest reliability of the 50-foot timed walk and 30-second chair stand test in patients with total hip arthroplasty	Unver, et al	Acta orthopaedica belgica	2015		19	2.11

Table 3. The top 30 most cited articles on THA published in Turkey according to WOS database results

Title	Authors	Source title	Publication year	DOI	Total citations	Average per year
Total hip arthroplasty in patients with ankylosing spondylitis: Midterm radiologic and functional results	Saglam, et al	Acta orthopaedica et traumatologica turcica	2016	10.1016/j.aott.2016.06.010	18	2.25
Scintigraphic evaluation of impaction grafting for total hip arthroplasty revision	Tokgozoglu, et al	Archives of orthopaedic and trauma surgery	2000	10.1007/PL00013773	18	0.75
Two-Stage Cementless Revision Total Hip Arthroplasty for Infected Primary Hip Arthroplasties	Camurcu, Yalkin; Sofu, Hakan; Buyuk, Abdul Fettah; Gursu, Sarper; Kaygusuz, Mehmet Akif; Sahin, Vedat	Journal of arthroplasty	2015	10.1016/j.arth.2015.03.040	16	1.78
Cementless total hip arthroplasty for the management of tuberculosis coxitis	Ozturkmen, Yusuf; Karamehmetoglu, Mahmut; Leblebici, Cem; Gokce, Alper; Caniklioglu, Mustafa	Archives of orthopaedic and trauma surgery	2010	10.1007/s00402-009-0967-9	16	1.14
Long-term results of total hip arthroplasty in patients with juvenile rheumatoid arthritis	Bilsel, Nafiz; Gokce, Alper; Kesmezacar, Hayrettin; Mumcuoglu, Erhan; Ozdogan, Huri	Acta orthopaedica et traumatologica turcica	2008		16	1
Extravascular compression of the femoral vein due to wear debris-induced iliopsoas bursitis - A rare cause of leg swelling after total hip arthroplasty	Beksac, Burak; Toezuen, Remzi; Baktiroglu, Selcuk; Sener, Nadir; Della Valle, Alejandro Gonzalez	Journal of arthroplasty	2007	10.1016/j.arth.2006.04.002	15	0.88
Total hip arthroplasty in the developmental dysplasia of the hip using transverse subtrochanteric osteotomy	Ozan, Firat; Uzun, Erdal; Gurbuz, Kaan; Koyuncu, Semmi; Altay, Taskin; Kayali, Cemil	Journal of orthopaedics	2016	10.1016/j.jor.2016.06.010	14	1.75
Comparison of hemiarthroplasty and total hip arthroplasty in elderly patients with displaced femoral neck fractures	Barishan, Fatih Cansah; Akesen, Burak; Atici, Teoman; Durak, Kemal; Bilgen, Muhammed Sadik	Journal of international medical research	2018	10.1177/0300060518770354	13	2.17
RETRACTED: Femoral shortening osteotomy in total hip arthroplasty for severe dysplasia: a comparison of two fixation techniques (Retracted article. See FEB, 2023)	Catma, Mehmet Faruk; Unlu, Serhan; Ozturk, Alper; Aksekili, Atif M.; Ersan, Onder; Ates, Yalim	International orthopaedics	2016	10.1007/s00264-016-3144-0	13	1.63
Patients' Discharge Information Needs After Total Hip and Knee Arthroplasty: A Quasi-Qualitative Pilot Study	Sendir, Merdiye; Buyukyilmaz, Funda; Musovi, Duygu	Rehabilitation nursing	2013	10.1002/rnj.103	13	1.18
Gait analysis in adults with severe hip dysplasia before and after total hip arthroplasty	Marangoz, Salih; Atilla, Bulent; Gok, Haydar; Yavuzer, Gunes; Ergin, Sureyya; Tokgozoglu, A. Mazhar; Alpaslan, Mumtaz	Hip international	2010	10.1177/112070001002000409	13	0.93
Intermediate-term results after uncemented total hip arthroplasty for the treatment of developmental dysplasia of the hip	Ermis, et al	Eklem hastaliklari ve cerrahisi-joint diseases and related surgery	2010		13	0.93

Analysis Results of the WOS Database Data with VosViewer

Keyword analysis: The VOSviewer was used to find and analyze keywords that occurred more than once in a text. We classified 387 keywords into 41 study clusters, as shown in **Figure 2** using various colors. There was total 387 different keywords with minimum 1 occurrence. The lines connecting nodes depict the co-occurrence relationship, while the size of the nodes indicates frequency. The keywords with maximum occurrences were total hip arthroplasty (63 occurrences), arthroplasty (28 occurences) and hip (28 occurences) (**Table 4**).

Co Authorship Analysis

As shown in **Figure 3**, 14 countries with at least one articles were identified. The top regions and countries with the highest total link strength (TLS) were Germany (TLS=8) and the USA (TLS=7) (**Table 5**).

Table 4. The mostly occured keywords on THA published in Turkey according to WOS database results

Keyword	Occurrences	Total link strength
Arthroplasty	28	109
Cementless	10	39
Developmental dysplasia	7	24
Developmental dysplasia of the hip	10	43
Developmental hip dysplasia	5	17
Dislocation	7	24
Hip	28	110
Hip arthroplasty	11	43
Hip dysplasia	7	32
Osteotomy	8	33
Replacement	6	28
Total hip arthroplasty	63	221
Total hip replacement	10	30

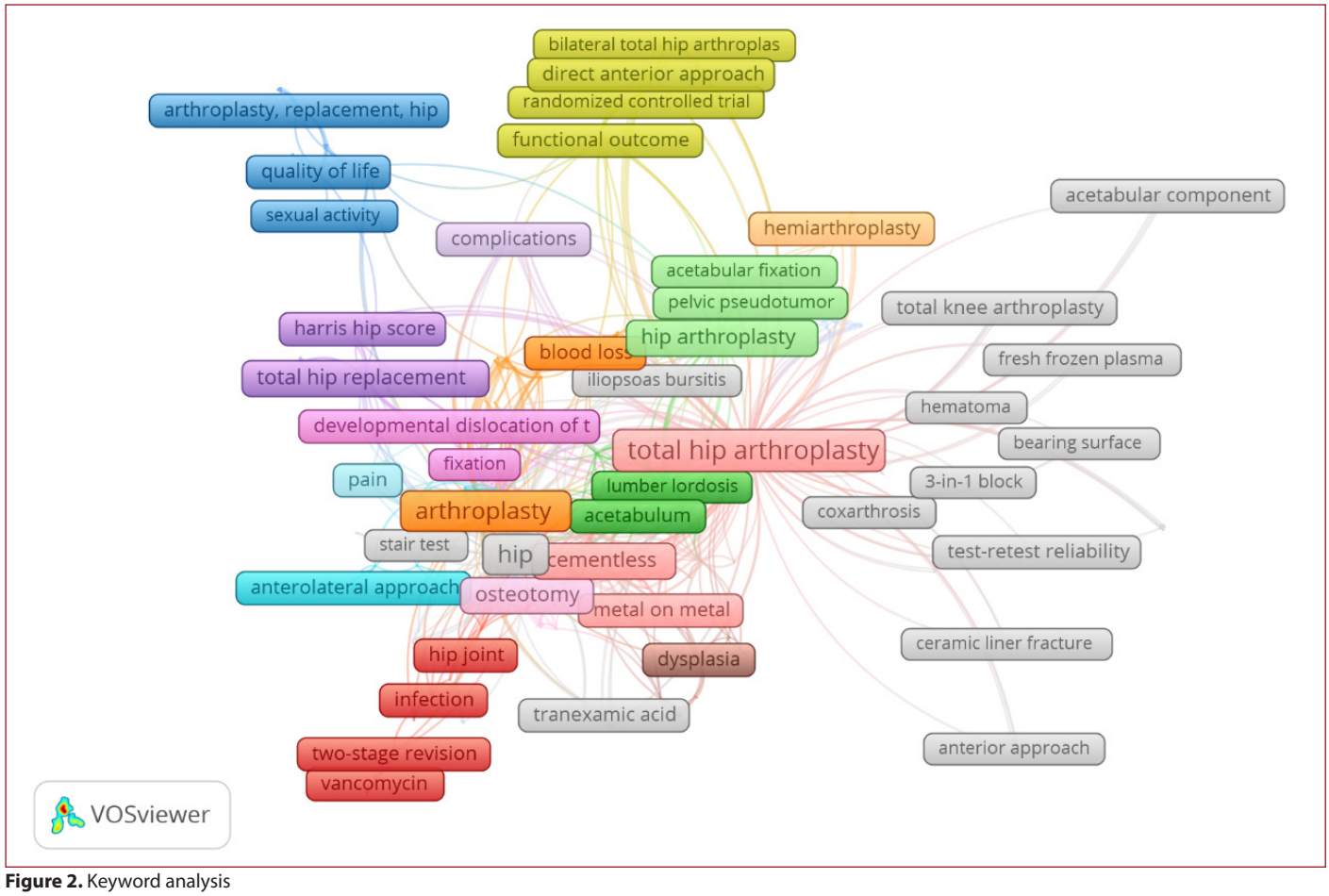


Figure 2. Keyword analysis

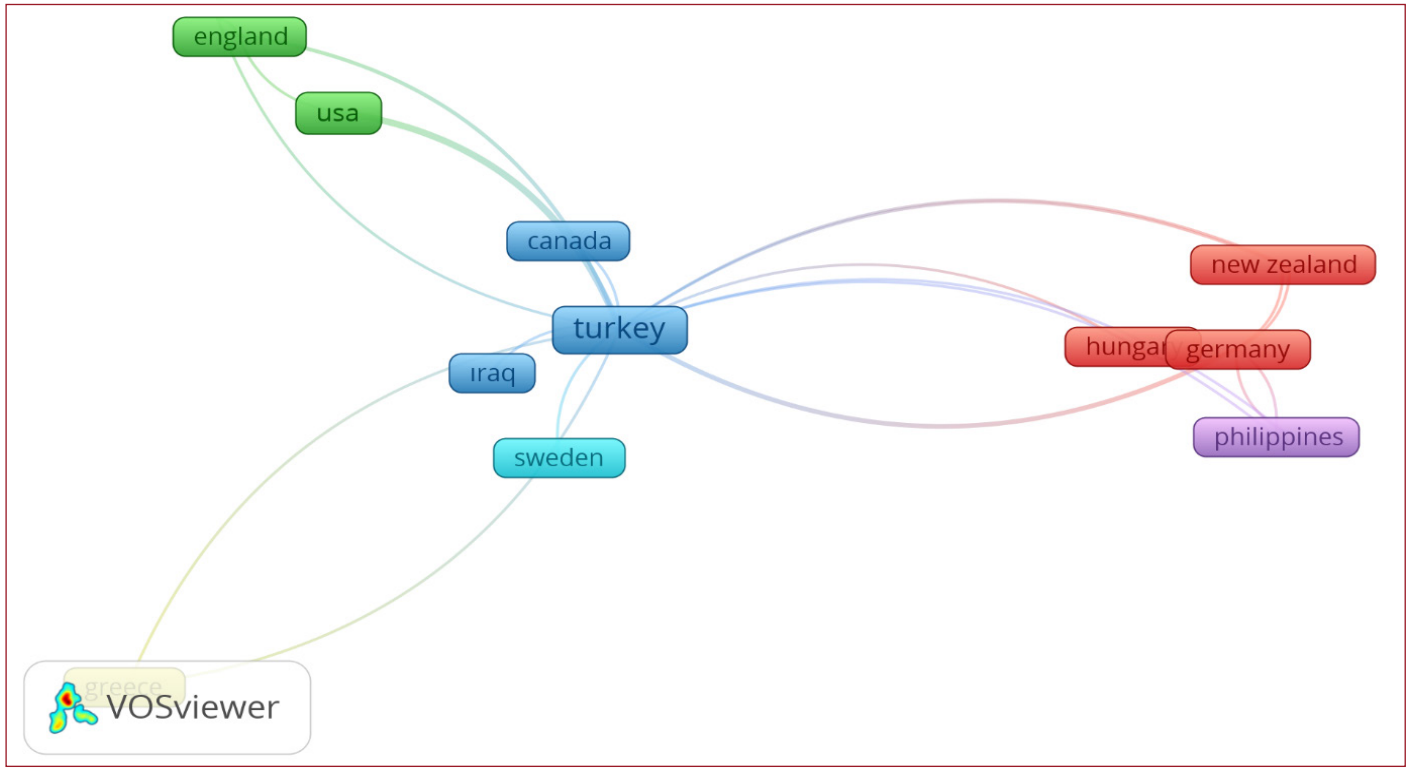


Figure 3. Co authorship analysis

Table 5. Summary of co-authorship analysis

Country	Documents	Citations	Total link strength
Azerbaijan	1	21	2
Canada	1	5	1
England	2	42	4
Germany	3	1	8
Greece	1	0	2
Hungary	1	1	2
New Zealand	1	0	3
Philippines	1	0	3
Sweden	1	2	1
Switzerland	1	0	2
Thailand	1	0	3
The USA	6	71	7
Iraq	1	0	1
Italy	1	0	3

Scopus Database Results

According to Scopus database results there was 38,366 articles, most of these articles were orginating from the USA (12,296 articles), United Kingdom (3,782 articles), Germany (3,033 articles), China (3,872 articles) and Japan (2,326 articles). Turkey ranked 22nd with 485 articles. The first article was published by Prof. Dr. Güngör Sami Çakırgil in 1972.[24]

Since the 2000s, there has been an increasing trend in the number of articles published per year, but it decreased in 2022 and 2021 was the mostly publishing year (**Figure 4**). It is seen that the number of articles on total hip arthroplasty has increased as we approach today.

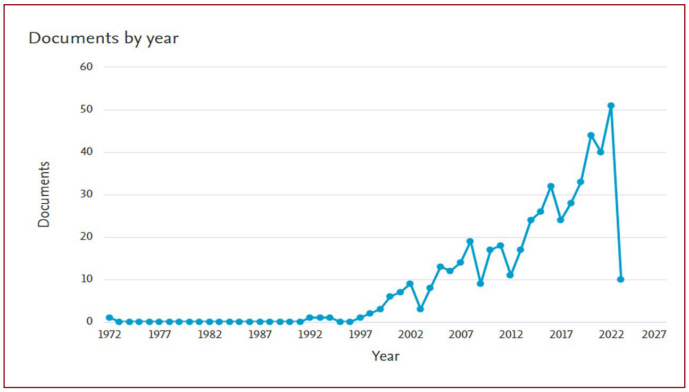
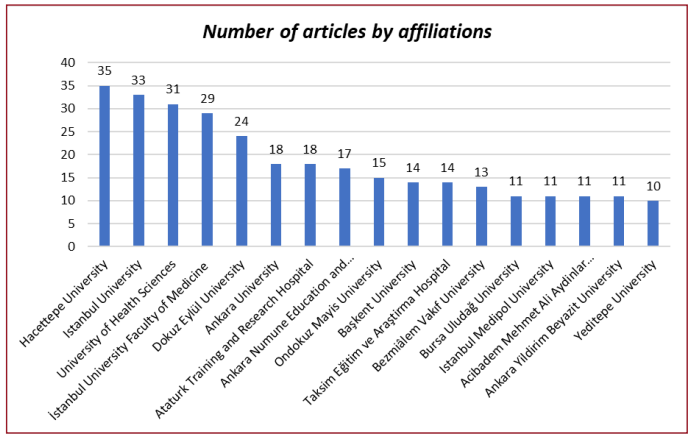


Figure 4. Documents by year. The year with the most publications is 2021

Hacettepe University (35 articles), Istanbul University (33 articles), University of Health Sciences (33 articles), Istanbul University / Cerrahpasa (29 articles) were the top affiliations contributed to THA literature from Turkey. The mostly publishing affiliations are listed in **Graphic 2**. It is seen that the number of articles on THA has increased as we approach today.



Graphic 2. Number of publications according to affiliation

The articles on THA from Turkey indexed in the Scopus database were mostly published in Acta Orthopaedica et Traumatologica Turcica (35 articles) journal. **Figure 5** summarizes the mostly publishing journals on THA from Turkey.

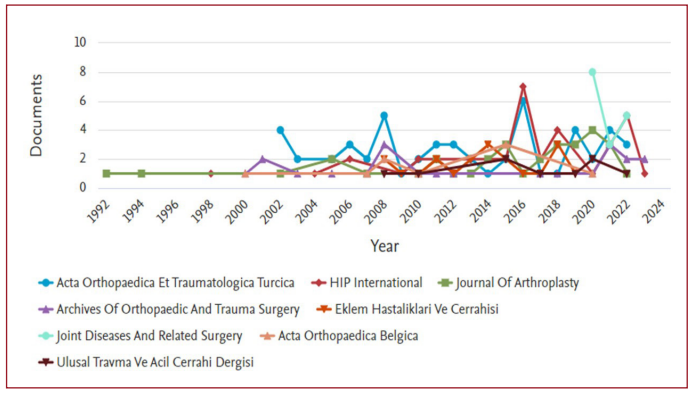


Figure 5. documents per year by source

DISCUSSION

Different databases can be selected with the bibliometric analysis method and it is possible to examine different types of publications.[16,25] Bibliometric studies examining theses and congress presentations in the field of orthopedics from our country are available in the literature.[26-28] The decision of the relevant researchers themselves is important in the selection of the reviewed literature. The WOS database, the Scopus database, PubMed, Google Scholar, ResearchGate, etc. , are usually the databases of choice. In this study, we examined the research articles published by Turkish authors on THA using the public electronic databases WOS and Elsevier's Scopus database.

With the help of bibliometric analysis, the number and quality of publications from a given country can be roughly estimated. In this study, we aimed to analyze publications on THA from Turkey using two different databases. Our study is the first bibliometric study on THA publications orginating from Turkey. In general, according to the WOS database

(journals published in ESCI and SCIE indexes), Turkey ranks 15th among 108 countries. According to the Scopus database, Turkey ranks 22nd. According to the WOS database, the first articles on THA from Turkey were published in 1999, while the first article in the Scopus database was published in 1972. 2021 was the year with the highest number of articles published in both databases. According to WOS results, the number of publications did not fall below 10 articles per year between 2014 and 2022. Since 2023 has not yet been completed, although no publications appear in 2023, it may be misleading as they may be added later.

According to WOS database results, there were 190 articles on THA from Turkey. 85.789% of these articles were published in SCI-EXPANDED and 14.211% in ESCI-indexed journals. According to the results of the Scopus database, there were 485 articles on THA from Turkey. The different results obtained in two databases may be due to the difference in the number of journals included in Scopus and ECSI/SCIE indexes. Scopus database contains more journals than WOS database.

A bibliometric analysis allowed us to provide information on the institutions that published publications, thereby identifying ancestral institutions in the field.[14-21] According to the results of our WOS database, although authors from 203 different institutions contributed to the Turkish THA literature, the institutions that contributed the most to the Turkish THA literature were Hacettepe University (17 articles), Istanbul University (17 articles) and Istanbul/Cerrahpaşa University (13 articles). In other words, the largest contributors are established universities from Turkey's two major cities (Ankara and Istanbul). According to our Scopus results, training and research hospitals under the Turkish Ministry of Health also contributed significantly to these publications, although they were also published by institutions in major cities.

Citation counts can indicate the quality of a country's publications. Until the end of 2022, the number of citations had increased especially in the last 10 years. Since 2023 has not yet been completed, the number of citations in this study should not be taken into account.

Bibliometric analyses can help us understand the journals that publish the highest proportion of publications on a topic so that researchers in this field/subject can have an idea about future journal choices.[16,18] The articles on THA from Turkey indexed in the WOS database were mostly published in Hip International (20 articles), Acta Orthopaedica et Traumatologica Turcica (18 articles), the Journal of Arthroplasty (17 articles) and Archives of Orthopaedic and Trauma Surgery (10 articles). Also the articles on THA from Turkey indexed in the Scopus database were mostly published in Acta Orthopaedica et Traumatologica Turcica (35articles) journal. The difference between the two databases may be due to the fact that these journals were indexed in different indexes in different time periods.

We also summarized the 30 most cited articles. According to our WOS database results, these articles were cited 1291 times (average number of citations 6.79) and had an H-index of 18. These numbers were also found to be low compared to previous bibliometric studies. As a result of our examination of cooperation between countries with Vosviewer, we had the most cooperation with the USA and Germany. However, both the number of collaborating countries and the level of collaborating level (TSL levels), were quite low.

Limitations

There are several limitations on this study. First of all, because the study is descriptive in nature, it can only provide a broad overview of the current level of research in the subject area and does not allow for in-depth examination of individual documents. As a result, it is impossible to understand in detail how certain papers are linked. Also, because of the search strategy's restrictions, some documents might be overlooked. Due to the existing use of keywords and subject phrases, which is highly ambiguous in our study, this also introduces additional needs for the documenting of records. There may be multiple ways to convey the same concept, which leads to inconsistent use of subject phrases and keywords and presents difficulties for thorough bibliometric analyses. Notwithstanding these drawbacks, we think our research could contribute to the THA publications originating in Turkey and offer fresh perspectives on cutting-edge developments in the field.

CONCLUSION

The data summarized in this study can help researchers understand the history of Turkish literature on THA that has been reviewed. As a result of the study, we found 190 articles in the WOS database and 485 articles in the Scopus database, published and added to the database until the end of March 2023. The number of articles was quite low compared to other countries. The publications were published by authors from big cities and centers. Since THA is an important topic in the current practice of orthopedics and the number of patients undergoing THA is expected to increase, research on THA published in our country should be supported. Also as international cooperation is limited, it should also be increased.

ETHICAL DECLARATIONS

Ethics Committee Approval: As it is not a human or animal study there is no need for ethical approval.

Referee Evaluation Process: Externally peer-reviewed.

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

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Case Report / Olgu sunumu

Cholesterol Granuloma of the Maxillary Sinus in the Patient Operated With Prediagnosis of Sinonasal Polyp: A Case Report

Sinonazal Polip Öntanısıyla Ameliyat Edilen Hastada Maksiller Sinüs Kolesterol Granülomu: Olgu Sunumu

Can Yazırlıoglu[1], Didar Gursoy Kuzuluk[1], Semsettin Okuyucu[2]

[1]Hatay Mustafa Kemal University, Faculty of Medicine, Department of Pathology, Hatay, Turkey
[2]Hatay Mustafa Kemal University, Faculty of Medicine, Department of Otolaryngology, Hatay, Turkey

Abstract

Cholesterol granuloma is a granulomatous reaction which develops against cholesterol crystals precipitating in the tissues and may be seen in any localization of the head-neck region. It has been first described by Graham and Michael in 1978 and it is rarely seen in this anatomical region. The pathogenesis of sinonasal Cholesterol granuloma is not clear. In this case report, we aimed to present a 69-year-old female patient with cholesterol granuloma originating from the maxillary sinus with clinical, radiological and pathological findings.

Keywords: Cholesterol granuloma, sinonasal polyp, maxillary sinüs

Öz

Kolesterol granülomu, dokularda çökelen kolesterol kristallerine karşı gelişen, baş-boyun bölgesinin herhangi bir lokalizasyonunda görülebilen granülomatöz bir reaksiyondur. İlk olarak 1978 yılında Graham ve Michael tarafından tanımlanmış olup nadiren bu anatomik bölgede görülmektedir. Sinonazal Kolesterol granülomunun patogenezi net değildir. Bu olgu sunumunda maksiller sinüsten köken alan kolesterol granülomlu 69 yaşındaki kadın hastayı klinik, radyolojik ve patolojik bulguları ile sunmayı amaçladık.

Anahtar Kelimeler: Kolesterol granülomu, sinonasal polip, maksiller sinüs

INTRODUCTION

Cholesterol granuloma (CG) is a granulomatous reaction which develops against cholesterol crystals precipitating in the tissues and may be seen in any localization of the head-neck region.[1,2] Even though, it is seen in the sinonasal region, it is most commonly encountered in the middle ear.[3] It is rarely found in the frontal and maxillary sinuses.[4] It has clinically and radiologically similar features with maxillary sinusitis.[5] Some mechanisms have been reported to explain the pathogenesis of cholesterol granuloma and deposition of cholesterol crystals in the sinonasal region. These mechanisms are impaired nasal drainage, impaired ventilation and haemorrhage.[1,5] It is histopathologically characterized with cholesterol crystals and surrounding giant cells, plasma cells, lymphocytes and deposition of hemosiderin.[6]

In this case report, we aimed to present a 69-year-old female patient with cholesterol granuloma originating from the maxillary sinus with clinical, radiological and pathological findings.

CASE REPORT

A 69-year-old female patient admitted to ear-nose-throat clinic with complaints of right nasal passage obstruction, lost smelling sense and nasal discharge. No feature was present in the history of the patient including no previous

Corresponding *(İletişim)*: Didar Gursoy Kuzuluk, Hatay Mustafa Kemal University, Faculty of Medicine, Department of Pathology, Hatay, Turkey
E-mail *(E-posta)*: gursoydidar@gmail.com
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surgical operation. A polypoid lesion covering the right nasopharynx was detected by anterior rhinoscopy. In the paranasal sinus computed tomography (CT) imaging performed following, soft tissue density (antrochoanal polyp?) beginning from inside maxillary sinus on the right and extending to posterior nasal passage and a defective appearance on the medial wall of maxillary sinus at this level were noticed. (**Figure 1**). Functional endoscopic sinus surgery (FESS) was performed due to prediagnosis of antrochoanal polyp. The obtained material was sent to the pathology laboratory. The material was macroscopically 6×5×4 cm in size and brown-white in color with inflammatory characteristics and an appearance of irregular tissue pieces. The tissue specimens were taken and fixed in 10% buffered formalin, routine tracking procedures were applied to the tissues, 5µ-thick sections were prepared and these sections were stained with Haematoxylin-Eosin (H&E). The microscopic examination of the sections revealed a polypoid lesion with surface covered with respiratory epithelium (**Figure 2**). The stroma of the lesion was characteristically myxoid and showed increased vascularity. Cholesterol clefts in stroma and foreign body type giant cells around those clefts were noticeable (**Figure 3** and **4**). In the light of this evidence, the case was diagnosed with cholesterol granuloma of the maxillary sinus. Anterior rhinoscopy performed in the control examination one month after the surgery demonstrated septal synechia in the right lower concha in the midline nasal septum. Our patient is being followed-up without any recurrence.

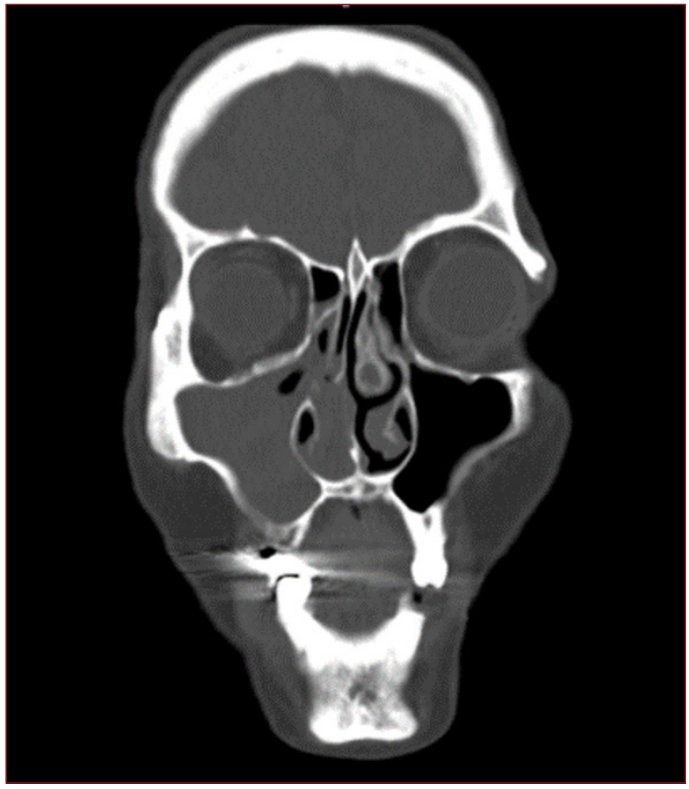

Figure 1. Coronal CT scan revealed opacification of the right maxillary sinüs and a soft tisue density mass in the nasal cavity

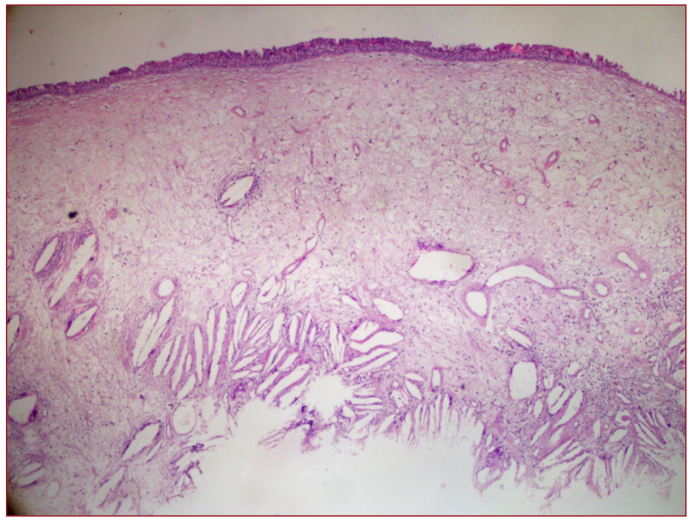

Figure 2. A polypoid lesion with surface covered with respiratory epithelium (H+E, x40)

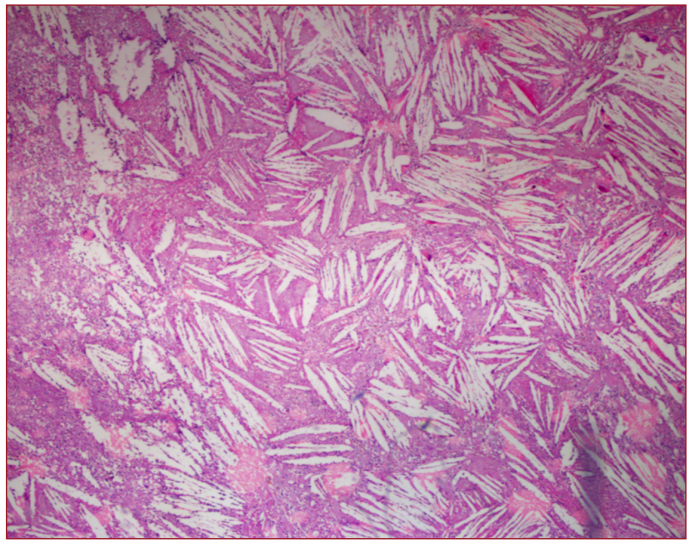

Figure 3. Cholesterol clefts in the stroma (H+E, x100)

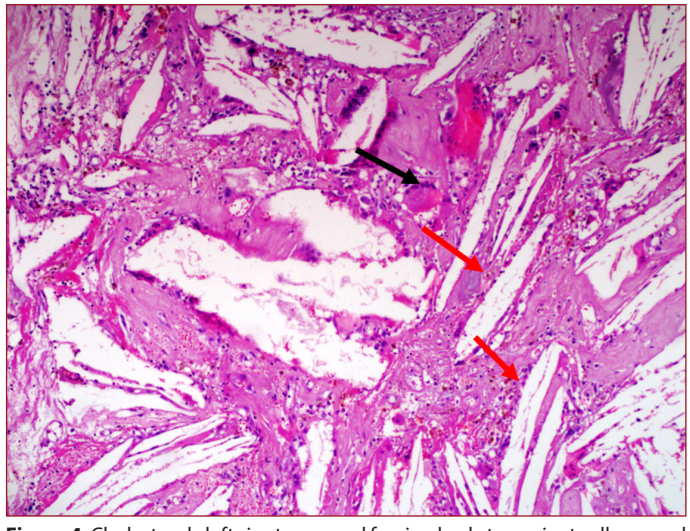

Figure 4. Cholesterol clefts in stroma and foreign body type giant cells around those clefts (black arrow giant cell, red arrow cholesterol clefts) (H+E, x400)

DISCUSSION

CG of the maxillary sinus has been first described by Graham and Michael in 1978 and it is rarely seen in this anatomical region.[3] It is discovered in a wide age range (22-85 years) and most often found in middle-aged males.[3,5,7] Male/female ratio is 3/1.[8] Although, it has no characteristic clinical and radiological symptom, the most frequent hospital admission causes of the patients are single-sided nasal obstruction, nasal discharge and pain.[3,5,7] However, its most specific symptom is clear golden-yellow nasal discharge.[7] It has been reported to be most commonly seen in frontal sinuses and followed by maxillary sinuses.[7,9] It is encountered in the right and left maxillary sinuses with similar rates.[5] Bilateral cases also have been reported in the literature.[10-12] Endoscopic findings may be similar with antrochoanal polyp, sinonasal polyp and mass.[3,7,10] Our case was a middle-aged female patient, the lesion was located in the right maxillary sinus and her complaint was nasal discharge consistently with the literature. Anterior rhinoscopy findings indicated characteristically polypoid lesion.

The pathogenesis of sinonasal CG is not clear.[7] Since sinonasal cholesterol granuloma is rarely found in the literature, it is suggested to have a similar pathophysiology with cholesterol granuloma usually found in the temporal bone, and to be granulomatous reaction against cholesterol crystals forming from the outer membrane of erythrocytes where cholesterol forms crystalline precipitation deposited in the sinus and subsequently initiating the reaction of macrophages and leukocytes.[5,13,14] Sinus obstruction may emerge due to causes such as haemorrhage, inadequate ventilation and decreased lymphatic drainage.[1,3,5,7] Direct trauma or surgery may induce events resulting in CG in the sinus.[5]

Radiographic examination is critical in the preoperative evaluation of sinonasal diseases. Sinonasal CG is radiologically encountered as cystic mass and sinus opacification in the CT. They may cause osseous erosion and opacification.[7] Magnetic resonance imaging (MRI) can provide more reliable results than CT. That results from the visibility of the characteristically increased signal activity in both T1- and T2- weighted images.[3,15,16] MRI should be performed to obtain more reliable data if CG is suspected. Mucosele, pyomucocele, cysts and neoplasms should be borne in mind in the differential diagnosis of CG because of their radiological evidence. The final diagnosis is based on histopathological evidence.[3] It is difficult to diagnose CG without histopathological examination since it manifests no characteristic clinical and radiological evidence.[7] Its characteristic histological evidence is multinuclear giant cells and cholesterol clefts surrounded by hemosiderin loaded macrophages. In also our patient, a soft tissue density suggesting image of a mass in the right maxillary sinus was detected in the CT examination and radiological prediagnosis was antrochoanal polyp. MRI was not performed in our patient.

Caldwell-Luc approach was the previously preferred method in the treatment of sinonasal CG. However, endoscopic sinus surgery is the method preferred in the first diagnosis and treatment of benign tumors in the present time.[3,7] Its availability to provide more favorable cosmetic results is the other reason for preferring FESS.[7] In also our patient, FESS was applied due to prediagnosis of antrochoanal polyp and final pathology was interpreted as CG. No recurrence was detected in the follow-ups of our patient.

CONCLUSION

It is difficult to diagnose CG preoperatively. Because CG has no characteristic clinical and endoscopic evidence and it is rarely seen in the maxillary sinus. Endoscopic approach is preferred for its treatment because of low recurrence rate, providing favorable cosmetic results and availability for complete excision.

ETHICAL DECLARATIONS

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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