HAMIDIYE MEDICAL JOURNAL

The Official Journal of University of Health Sciences Türkiye, Hamidiye Faculty of Medicine

E-ISSN: 2718-0956

August - 2023
Volume - 4
Issue - 2

ORIGINAL ARTICLES

galenos Publishing House

www.hamidiyemedj.com

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2023
Volume 4

Editorial Board

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Türkiye
Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27 **E-mail:** info@galenos.com.tr
Web: www.galenos.com.tr **Publisher Certificate Number:** 14521
Online Publication Date: August 2023 **E-ISSN:** 2718-0956
International periodical journal published three times in a year.

hamidiye med j

2023/Volume 4

HAMIDIYE
MEDICAL JOURNAL

Aims and Scope

Hamidiye Medical Journal (Hamidiye Med J) is a peer-reviewed open-access international journal that publishes conducted in all fields of medicine, interesting case reports, and clinical images, invited reviews, editorials, letters, comments and letters to the Editor including reports on publication and research ethics. The journal is the official scientific publication of the University of Health Sciences Türkiye, Hamidiye Faculty of Medicine, İstanbul, Türkiye. It is published three times a year in April, August, and December. The language of the journal is English.

The journal is based on independent and unbiased double-blinded peer-reviewed principles. Only unpublished papers that are not under review for publication elsewhere can be submitted. Hamidiye Medical Journal does not accept multiple submissions and duplicate submission even though the previous one was published in a different language. The authors are responsible for the scientific content of the material to be published. Hamidiye Medical Journal reserves the right to request any research materials on which the paper is based.

Hamidiye Medical Journal encourages and enables academicians, researchers, specialists, and primary care physicians to publish their valuable research in all branches of medicine. The primary aim of the journal is to publish original articles with high scientific and ethical quality and serve as a good example of medical publications in the Türkiye as well as in the world.

Hamidiye Medical Journal is indexed in **EBSCO Host, J-Gate** and **Ulakbim TR Index.**

English Title: Hamidiye Medical Journal

Official abbreviation: Hamidiye Med J

E-ISSN: 2718-0956

OPEN ACCESS POLICY

This journal provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Author (s) and copyright owner (s) grant access to all users for the articles published in Hamidiye Medical Journal as free of charge. Articles may be used provided that they are cited.

Open Access Policy is based on rules of Budapest Open Access Initiative (BOAI). By "open access" to [peer-reviewed research literature], we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print, search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited.

Creative Commons

A Creative Commons license is a public copyright license that provides free distribution of copyrighted works or studies. Authors use the CC license to transfer the right to use, share or modify their work to third parties. This journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC-ND 4.0) (https://creativecommons.org/licenses/by-nc-nd/4.0/) which permits third parties to share and adapt the content for non-commerical purposes by giving the apropriate credit to the original work.

Open access is an approach that supports interdisciplinary development and encourages collaboration between different disciplines. Therefore, Hamidiye Medical Journal contributes to the scientific publishing literature by providing more access to its articles and a more transparent review process.

Advertisement Policy

Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

Material Disclaimer

Statements or opinions stated in articles published in the journal do not reflect the views of the editors, editorial board and/or publisher; The editors, editorial board and publisher do not accept any responsibility or liability for such materials. All opinions published in the journal belong to the authors.

Contact
Editorial Office
Address for Correspondence

Selimiye Mah. Tıbbiye Cad. 34668 Üsküdar, İstanbul/Türkiye

Phone: +90 216 418 96 16

E-mail: tipfakultesi@sbu.edu.tr

Publisher/Issuing Body

Galenos Yayınevi Tic. Ltd. Şti.

Molla Gürani Mah. Kaçamak Sok. No: 21, 34093, Fındıkzade, İstanbul, Turkiye

Phone: +90 212 621 99 25

Fax: +90 212 621 99 27

Web page: http://www.galenos.com.tr

E-mail: info@galenos.com.tr

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2023
Volume 4

Contents

REVIEW

DOI: 10.4274/hamidiyemedj.galenos.2023.88597

Hamidiye Med J 2023;4(2):81-83

Neuroendocrine Neoplasms of the Breast

Memenin Nöroendokrin Neoplazmları

Ebru Şen[1], Şeref Oray[1], Gültekin Ozan Küçük[2], Ayşegül Akdoğan Gemici[3], Serdar Altınay[4]

[1]*University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of General Surgery, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye, Samsun Training and Research Hospital, Clinic of General Surgery, Samsun, Türkiye*

[3]*University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Radiology, İstanbul, Türkiye*

[4]*University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Pathology, İstanbul, Türkiye*

ABSTRACT

Primary neuroendocrine carcinoma of the breast is rare and consists of 0.1% of all breast cancers. In 2019, the World Health Organization newly defined this entity as neuroendocrine neoplasm of the breast and divided in two subgroups according to differantiation patern. Whether a breast lesion is primary or metastatic has crucial importance for its management and prognosis. Our aim was to briefly summarize the knowledge as to neuroendocrine neoplasm of the breast based on the current literature.

Keywords: Neuroendocrine, breast, neoplasm, carcinom

ÖZ

Memenin primer nöroendokrin karsinomu nadirdir ve tüm meme kanserlerinin %0,1'ini oluşturur. 2019 yılında Dünya Sağlık Örgütü bu antiteyi yeni memenin nöroendokrin neoplazmı olarak tanımlamış ve farklılaşma paternine göre iki alt gruba ayırmıştır. Meme lezyonunun birincil mi yoksa metastatik mi olduğu, yönetimi ve prognozu için çok önemlidir. Amacımız memenin nöroendokrin neoplazmı ile ilgili bilgileri güncel literatüre dayanarak kısaca özetlemektir.

Anahtar Kelimeler: Nöroendokrin, meme, neoplazm, karsinom

Introduction

Primary neuroendocrine carcinoma of the breast (NECB) is rare and consists of 0.1% of all breast cancers (1). Within all neuroendocrine cancers, the breast represents 1% of the origin (2). Various diagnostic criteria have been used and many classification systems have been proposed since the first introduction of neuroendocrine tumor (NET) in the breast was described.

In the previous World Health Organization (WHO) classification in 2012, these tumors were subclasssified into 3 groups: Well-differentiated NET, poorly differentiated neuroendocrine carcinoma (NEC) (or small cell carcinoma) and, invasive breast carcinoma with neuroendocrine differentiation (3). Large cell NECs were not included in this system. Recently in 2019 WHO acknowledged the term of "neuroendocrine neoplasm" (NEN) as a comprehensive definition, which includes tumors at any specific anatomical site whose morphological features and markers are in accordance with the neuroendocrine differentiation (1). In this classification, there are two subgroups: First is well-differentiated NET which is an invasive tumor characterized by low or intermediate grade and second is poorly differentiated NEC which is characterized by high grade tumor with either small or large cell neuroendocrine. The prerequisite feature of these tumors is harbouring more than 90% of neuroendocrine differentiation patern. Whereas, conventional type mammary carcinomas which are of a neuroendocrine component of 10-90% in the tumor area are called as mixed invasive and NEC.

Clinical and Radiological Characteristics

These tumors usually occur in postmenopausal women in the sixth decade of life. Similar to other breast carcinoma, breast lump is the major finding in clinical presentation. Few cases present with hormonal hypersecretion syndromes due to hormonal production (3). Interestingly, Kawasaki et al. (4) inquired 89 patients whose complaint was bloody

Address for Correspondence: Ebru Şen, University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of General Surgery, İstanbul, Türkiye
Phone: +90 505 772 83 86 E-mail: orayseref@gmail.com **ORCID ID:** orcid.org/0000-0003-2436-7133

Received: 12.04.2022 **Accepted:** 09.05.2023

nipple discharge on the admission and detected that NEC had been the ethiological cause in nearly a quarter of these patients. As for radiological evaluation, NEN is of no specific imaging characteristics compared with other types of breast cancer.

Some studies have pointed that these tumors tend to recognised larger tumor size and higher probability of axillary metastasis at the time of diagnosis (5,6,7). A population-based study from the surveillance, epidemioogy and end results database of Wang et al. (6) compared 142 patients with NEC of breast to invasive breast carcinoma, NOS. Higher histological grade and advanced clinical stage were found in NEC group and neuroendocrine differentiation was detected as an independent adverse prognostic factor. Wei et al. (7) detected lymph node metastasis in 31 of 74 patients with these tumors (42%). In a trial of 36 cases by Keten Talu et al. (8), most of the tumors were T2 and grade 2.

Diagnosis

NEN is of characteristic morphological features. Neuroendocrin tumor cells form nests, islands, alveolar-like structures surrounding by fibrovascular stroma with trabecular architecture, minimal tubular differentiation, which are characterized by uniform round or spindle-shaped cells with nuclear palisading, abundant finely granular eosinophilic cytoplasm and nuclei with "salt and pepper" chromatin distribution (1,5). These tumors express neuroendocrin markers such as chromogranin, synaptophysin, and neuron-specific enolase. In case of detection of neuroendocrin appearence on light microscopy, applying detailed immunohistochemical staining is the key point in achieving an accurate diagnosis (9). Luminal A and B phenotypes account for the vast majority of NEN (10). Most of them are HER2 negative.

After the diagnosis of NEN, metastasis to the breast should be ruled out by clinical imaging. Positron emission tomography-computed tomography is an important tool in this circumstance. Previous clinical history is also helpful. The presence of ductal carcinoma *in situ* supports the diagnosis of primary breast NEN (5). Differentiating primary NEN from metastatic one is crucial part of the management due to the diversity of treatment approaches (11). Unnecessary surgical interventions can be avoided in a metastatic setting. Neither neuroendocrin markers nor the presence of hormon receptors are useful in distinguishing these two entities. A panel of immunohistochemical site-specific leneage markers which point out its origin may be helpful in this setting. For example: TTF-1 is specific for pulmanary, CDX2 for gastrointestinal tract, mammaglobin, GATA3 and GCDFP15 for breast (2). However, the probability of an overlapping in the immunohistochemical expression analysis among NENs arising from different sites should be born in mind.

Treatment

There is no standard treatment protocol for NEN. Surgery is the first-line therapy as similar as the treatment of invasive ductal carcinoma of breast. Li et al. (12) investigated 126 cases with NECB from the Chinese population during a period between 1990 and 2015. Despite that 70% of their diseases were stage 1 or 2, mastectomy has been carried out in 79% of the patients. However, recent trials demonstrate that breast conservation surgery as well as sentinel node biopsy supplanted mastectomy in many cases.

There is no consensus on the optimal adjuvant chemotherapy protocol. Usually, NEC is treated with chemotherapeutics for small cell lung carcinoma such as platin based therapy. On the other hand, patients with NET and invasive breast carcinoma with neuroendocrin differentiation receive cytotoxic therapy similar to conventional breast cancer such as anthracycline and taxane based regimens. Some studies revealed survival advantages of radiotherapy and endocrine therapy according to hormone receptor status in patients with NET of breast (7,13). In contrast, Wang et al. (6) found that radiation therapy did not prolong survival. Along with a limited number of cases, there are conflicting results in the literature.

Prognosis

Whether NEN is of prognostic significance is remained unclear yet (14). Despite its luminal phenotype, most studies have reported an aggressive clinical course and poor outcome for patients with NECB (7,15,16). Small cell NEC is associated with a worse prognosis than other types (12). In contrast to majority ofinvasive ductal carcinoma with luminal type, NEN usually harbors a significantly lower frequency of PIK3CA mutation which is associated with favorable clinicopathological characteristics including low grade and small tumor size (16). This molecular feature may be related to their aggresive clinical behavior and worse prognosis. On the other hand, lower grade and mucin production are related to better prognosis (14). In the light of all these data and studies, it is possible to say that NETs behave similarly to other invasive breast cancers and should be managed in a clinically equivalent manner (17).

Conclusion

Neuroendocrine breast cancer types are more rare than other types of breast cancer (18). These tumors should be born in mind and appearence of neuroendocrin features should prompt the pathologist to evaluate these tumors by

special immunohistochemical markers. Treatment approach of NEN of the breast is similar to that of breast ductal carcinoma. However, prognosis has not been particularly elucidated yet except NEC which is associated with the poor prognosis.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ş., G.O.K., Concept: E.Ş., Ş.O., G.O.K., A.A.G., S.A., Design: E.Ş., Ş.O., G.O.K., A.A.G., S.A., Analysis or Interpretation: E.Ş., Ş.O., G.O.K., A.A.G., S.A., Literature Search: E.Ş., Ş.O., S.A., Writing: E.Ş.

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

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

References

1. Rakha EA, Reis-Filho JS, Sasano H, Wu Y. Neuroendocrine neoplasms. 5th ed. WHO Classification of tumors. 2019;2:155-161. [Crossref]
2. Rosen LE, Gattuso P. Neuroendocrine Tumors of the Breast. Arch Pathol Lab Med. 2017;141:1577-1581. [Crossref]
3. Bussolati G, Badve S. Carcinomas with neuroendocrine features. In: Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van der Vijver MJ. WHO Classification of Tumours of the Breast. Lyon, France: IARC Press; 2012:62-63. [Crossref]
4. Kawasaki T, Mochizuki K, Yamauchi H, Yagata H, Kondo T, Tsunoda H, et al. High prevalence of neuroendocrine carcinoma in breast lesions detected by the clinical symptom of bloody nipple discharge. Breast. 2012;21:652-656. [Crossref]
5. Marinova L, Malinova D, Vicheva S. Primary Neuroendocrine Carcinoma of the Breast: Histopathological Criteria, Prognostic Factors, and Review of the Literature. Case Rep Pathol. 2016;2016:6762085. [Crossref]
6. Wang J, Wei B, Albarracin CT, Hu J, Abraham SC, Wu Y. Invasive neuroendocrine carcinoma of the breast: a population-based study from the surveillance, epidemiology and end results (SEER) database. BMC Cancer. 2014;14:147. [Crossref]
7. Wei B, Ding T, Xing Y, Wei W, Tian Z, Tang F, et al. Invasive neuroendocrine carcinoma of the breast: a distinctive subtype of aggressive mammary carcinoma. Cancer. 2010;116:4463-4473. [Crossref]
8. Kelten Talu C, Savli TC, Huq GE, Leblebici C. Histopathological and Clinical Differences Between Primary Breast Carcinomas With Neuroendocrine Features and Primary Breast Carcinomas Mimicking Neuroendocrine Features. Int J Surg Pathol. 2019;27:744-752. [Crossref]
9. Mohanty SK, Kim SA, DeLair DF, Bose S, Laury AR, Chopra S, et al. Comparison of metastatic neuroendocrine neoplasms to the breast and primary invasive mammary carcinomas with neuroendocrine differentiation. Mod Pathol. 2016;29:788-978. [Crossref]
10. Vranic S, Palazzo J, Sanati S, Florento E, Contreras E, Xiu J, et al. Potential Novel Therapy Targets in Neuroendocrine Carcinomas of the Breast. Clin Breast Cancer. 2019;19:131-136. [Crossref]
11. Lee S, Levine P, Heller SL, Hernandez O, Mercado CL, Chhor CM. Metastatic carcinoid tumor to the breast: report of two cases and review of the literature. Clin Imaging. 2017;42:88-92. [Crossref]
12. Li Y, Du F, Zhu W, Xu B. Neuroendocrine carcinoma of the breast: a review of 126 cases in China. Chin J Cancer. 2017;36:45. [Crossref]
13. Brask JB, Talman ML, Wielenga VT. Neuroendocrine carcinoma of the breast - a pilot study of a Danish population of 240 breast cancer patients. APMIS. 2014;122:585-592. [Crossref]
14. Collado-Mesa F, Net JM, Klevos GA, Yepes MM. Primary neuroendocrine carcinoma of the breast: report of 2 cases and literature review. Radiol Case Rep. 2017;12:1-12. [Crossref]
15. Rosen LE, Gattuso P. Neuroendocrine Tumors of the Breast. Arch Pathol Lab Med. 2017;141:1577-1581. [Crossref]
16. Lavigne M, Menet E, Tille JC, Lae M, Fuhrmann L, Bonneau C, et al. Comprehensive clinical and molecular analyses of neuroendocrine carcinomas of the breast. Mod Pathol. 2018;31:68-82. [Crossref]
17. Metovic J, Cascardi E, Uccella S, Maragliano R, Querzoli G, Osella-Abate S, et al. Neuroendocrine neoplasms of the breast: diagnostic agreement and impact on outcome. Virchows Arch. 2022;481:839-846. [Crossref]
18. Qiu Y, Dai Y, Zhu L, Hao X, Zhang L, Bao B, et al. Clinicopathological Characteristics and Prognostic Profiles of Breast Carcinoma with Neuroendocrine Features. Life (Basel). 2023;13:532. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.07279

Hamidiye Med J 2023;4(2):84-91

Investigation of *PTEN* Gene Expression Levels in Patients with Different Stages and Grades of Breast Cancer

Farklı Evre ve Derecelerdeki Meme Kanserli Hastalarda *PTEN* Geninin İfadelenme Düzeylerinin Araştırılması

Hatice Arzu Özyürek[1], Evrim Suna Arıkan Söylemez[2], Murat Akıcı[3], Murat Çilekar[3], Çiğdem Tokyol[4], Yüksel Arıkan[5]

[1]*Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Genetic, Afyonkarahisar, Türkiye*

[2]*Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Biology, Afyonkarahisar, Türkiye*

[3]*Afyonkarahisar Health Sciences University Faculty of Medicine, Department of General Surgery, Afyonkarahisar, Türkiye*

[4]*Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Patology, Afyonkarahisar, Türkiye*

[5]*Park Hayat Hospital, Clinic of General Surgery, Afyonkarahisar, Türkiye*

ABSTRACT

Background: Breast cancer is the most common malignancy in women that cause death genetic and environmental factors affect the development of breast cancer. In many studies, it has been determined that *PTEN* is an important tumor suppressor gene for breast cancers and its loss induces carcinogenesis in the breast.

Materials and Methods: In our study, mRNA expression levels of *PTEN* gene were determined in tumor tissues and peripheral blood samples of 31 cases diagnosed with breast cancer, and breast tissues and peripheral blood samples of 5 healthy individuals. Real-time polymerase chain reaction method was used for the analysis.

Results: In this study, *PTEN* gene expression in tumor tissues and peripheral blood samples of breast cancer patients were determined compared to controls. The mRNA level of the *PTEN* gene in peripheral blood samples significantly downregulated (0.501-fold) ($p<0.05$), while it upregulated (1.109-fold) in tumor tissues ($p>0.05$). There was a contrast between *PTEN* gene expression behavior between blood and tissue. In addition, *PTEN* gene expression was downregulated in almost all peripheral blood samples of patients with breast cancer stage IIA. Additionally, it appears that *PTEN* gene expression was upregulated in tumor tissue in every histological grade while downregulated in peripheral blood.

Conclusion: In general, when our results are evaluated, we think that the decrease in the mRNA levels of the *PTEN* gene (down regulation) in the peripheral blood of breast cancer cases may be a potential finding in early stage diagnosis. These data will shed light on new comprehensive studies.

Keywords: Breast cancer, gene expression, different stage, biomarker, *PTEN*

ÖZ

Amaç: Meme kanseri, kadınlarda en sık görülen ve ölümle sonuçlanmasına neden olan malignitedir. Meme kanserinin gelişimine genetik ve çevresel faktörler etki etmektedir. Yapılan birçok çalışmada *PTEN*'nin meme kanserleri içinde önemli bir tümör baskılayıcı gen olduğunu ve kaybının memede karsinogenezi indüklediği belirlenmiştir.

Gereç ve Yöntemler: Çalışmamızda *PTEN* geninin mRNA ekspresyon seviyeleri, meme kanseri tanısı alan 31 olgunun tümör dokuları ve periferik kan örnekleri ile 5 sağlıklı bireye ait meme dokuları ile periferik kan örneklerinde belirlendi. Analizler için eş zamanlı-polimeraz zincir reaksiyonu yöntemi kullanıldı.

Bulgular: Bu çalışmada, meme kanserli olguların tümör dokularında ve periferik kan örneklerinde ifade edilen *PTEN* geninin, kontrol grubuna göre mRNA düzeylerindeki değişimler belirlenmiştir. Periferik kan örneklerinde *PTEN* geninin mRNA düzeyi, kontrole göre önemli derecede azalmışken (0,501 kat) ($p<0,05$), tümör dokularında artmıştır (1,109 kat) ($p>0,05$). Kan ve doku arasında *PTEN* geni ekspresyon davranışları bakımından zıtlık söz konusudur. Bunun yanı sıra, meme kanseri Evre IIA'da bulunan olguların periferik kan

Address for Correspondence: Evrim Suna Arıkan Söylemez, Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Biology, Afyonkarahisar, Türkiye

Phone: +90 532 580 36 74 E-mail: arikanmt@gmail.com **ORCID ID:** orcid.org/0000-0002-8550-793X

Received: 20.10.2022 **Accepted:** 23.02.2023

ÖZ

örneklerinin neredeyse tamamında *PTEN* gen ekspresyonu azalmıştır. İlaveten, her histolojik gradda tümör dokusunda *PTEN* gen ekspresyonu artmışken, periferik kanda azalmıştır.

Sonuç: Genel olarak sonuçlarımız değerlendirildiğinde, meme kanserli olguların periferik kanında *PTEN* geninin mRNA düzeylerindeki azalmanın (down regülasyon), erken evre teşhisinde potansiyel bir bulgu olabileceğini düşünmekteyiz. Bu veriler yeni geniş kapsamlı çalışmalara ışık tutacaktır.

Anahtar Kelimeler: Meme kanseri, gen ekspresyonu, farklı evre, biyobelirteç, *PTEN*

Introduction

Today, with the contribution of the developments in the molecular field, breast cancer is considered as a heterogeneous disease that shows differences in morphological structure, biological behavior and response to treatment. Molecular studies have gained momentum in the last 10 years, and it has become possible to evaluate thousands of genes simultaneously with the cDNA (complementary deoxyribo nucleic acid) microarray and "next generation sequencing" methods. A new molecular classification was made in breast cancers by extracting gene expression profiles from tumor tissue samples.

Tumor suppressor *PTEN* (phosphatase and tensin homolog) is a dual-specific phosphatase that acts as a negative regulator of the PI3K-AKT-mTOR pathway, thus controlling a variety of processes related to cell survival, proliferation, and growth (1).

Expression of HER family oncoproteins is increased in solid tumors compared to normal tissue. Cerb-B2 (HER2); it was found to be increased in stomach, breast, endometrium, ovarian carcinomas and vulvar Paget's disease. To show HER receptor expressions, IHC methods and fluorescent *in situ* hybridization (FISH) methods showing chromosomal amplification is used (2,3).

A gene localized to the long arm of chromosome 10 (10q 25) encodes the Ki-67 protein. This proteins expressed in all active phases (G1, S, G2 and mitosis) in the cell cycle, except the G0 phase, where cells do not proliferate (4). Positive nuclear staining for Ki-67 by immunohistochemistry is an important determinant for measuring cell proliferation. This ratio has been reported to be particularly high in aggressive tumors. High Ki-67 rate has been shown as a poor prognostic factor in many tumors (breast, lung, esophagus, kidney, prostate and endometrial cancer, malignant melanoma, non-Hodgkin lymphoma, glial tumors) (5).

Estrogen receptor (ER) and progesterone receptor (PR) evaluation is of fundamental importance for the pathological evaluation of breast carcinoma. ER is a nuclear transcription factor activated by estrogen. About 75% of breast cancers are positive for hormone receptors. Some low-grade invasive carcinomas and secretory carcinoma are typically negative. The ER does not predict the metastatic potential of the tumor but indicates that it will have a calm clinical course and a longer time to recurrence. ER positive tumors mostly metastasize to the bone, soft tissue and genitourinary system, while ER negative tumors metastasize to the visceral organs and brain. ER and PR are poor prognostic but strong predictive factors for endocrine therapy (6).

In this study, relative gene expression analysis was applied to show the *PTEN* gene behavior in the presence of breast cancer. While we were analysing the samples, we considered also both stages and grades of breast cancer.

Material and Methods

Sampling

Thirty-one breast cancer patients and 5 healthy control samples were included in our study. Tumor tissues were obtained during the breast operation performed in Department of General Surgery, Afyonkarahisar Health Sciences University, between June 2019-June 2021. Also, peripheral blood sample was taken into a 5 cc EDTA tube for RNA isolation. Patients who had previously received chemotherapy or immunotherapy for breast cancer were not included the study.

Determination of Histological Grade and Immunohistochemical Examination

Immunohistochemical examination was performed on sections prepared from paraffin blocks of tissues fixed in 10% formalin solution. Immunohistochemical application for each antibody was applied to all cases in a single session and was studied together with positive and negative controls. It was carried out by the method of detection of streptavidin-biotin-peroxidase (performed using the Leica Bond-max automated immunohistochemistry staining device). ER and PR status were determined by quick score. The quick score was calculated using a semi-quantitative method based on the percentage of nuclei that react and the intensity of immunostaining. The percentage of staining was evaluated as follows: 0= no nuclear staining, 1=<1% nuclear staining, 2=1-10% nuclear staining, 3=11-33% nuclear staining, 4=34-66% nuclear staining, 5=67-

100% nuclear staining. Staining intensity was evaluated as follows: 0= no staining, 1= weak staining, 2= moderate staining, 3= strong staining. The quick score was obtained by summing the percentage of staining and the degrees obtained from the staining intensity (7). When evaluating HER2 expression, it was scored in 4 groups (8):

Score 0: No staining in tumor cells or less than 10% of cells with membranous staining.

Score 1: More than 10% of tumor cells have pale staining on part of the cell membrane.

Score 2: More than 10% of tumor cells have weak-to-moderate staining of the entire cell membrane.

Score 3: More than 10% of tumor cells have strong staining of the entire cell membrane.

In cases with nuclear staining for p53 and Ki-67 expression, the percentage of tumor cells with nuclear staining is evaluated.

Medical pathology department routinely examines histological grade, axillary lymph node involvement and defined immunohistochemical parameters. Nottingham modification of the Bloom-Richardson system was used (9).

The staging of the patients was routinely done by the general surgery department according to the TNM staging (10).

Genetic Analysis

EZ-RNA Total RNA extraction kit (BI, Israel, Cat. No: 20-400-100) was used for RNA isolation. iScript Reverse Trancription Supermix (Biorad, USA, Cat. No: 170884) was used for reverse transcription. Genetic analysis was performed by real-time polymerase chain reaction (PCR) method using the Rotor Gene-Q (Qiagen, Hilden, Germany) and iTaq Universal SYBR Green Supermix (Biorad, USA, Kat. No: 1725122). *GAPDH* gene was used as a housekeeping gene for normalization. Oligomere Biotechnology (Ankara, Türkiye) designed oligonucleotide primers (Table 1).

Statistical Analysis

All data analyses were performed using REST 2009 V2.0.13 and SPSS v.19 software which use pairwise fixed reallocation randomization test (11) where $p<0.05$ is deemed to represent a statistically significant result. REST 2009 Software is a standalone tool for analysis of gene expression data from quantitative, real-time PCR experiments. The analysis or quantitation of relative gene expression uses expression of reference genes to normalize expression levels of genes of interest in different samples.

SPSS v19 software Pearson's correlation was used to determine linear relationship between ER/PR values and gene expression behaviour.

Results

Cases

Thirty-one breast cancer patients and 5 healthy control samples were included in our study. Table 2 shows the clinical and demographic characteristics of the breast cancer cases. Information on prognostic parameters such as, stages, histological subtype and immunohistochemical ER, PR, HER2, Ki-67 and p53 staining results obtained from patients pathology reports. Table 3 shows the surgical stage, histological grade, immunohistochemical status of all breast cancer patients.

RT-qPCR Analysis

The cDNA molecules synthesized from the RNA molecules isolated from the patients and the control group

Table 1. Primer sequences

Gene	Base sequences 5'→3'	Base lenght	Tm
***PTEN*-F**	TGGATTCGACTTAGACTTGACCT	23	59 °C
***PTEN*-R**	GGTGGGTTATGGTCTTCAAAAGG	23	61 °C
***GAPDH*-F**	CATTGCCCTCAACGACCACTTT	22	64 °C
***GAPDH*-R**	GGTGGTCCAGGGGTCTTACTCC	22	64 °C

-F: Forward, -R: Reverse, T: Tymin, G: Guanine, A: Adenine, C: Cytosine, Tm: Melting temperature

Table 2. Clinical and demographic characteristics of breast cancer patients

n		**31**			
Age, mean ± SD		53.2±12.0			
27-65, n (%) 66-90, n (%)		26 (83.9%) 5 (16.1%)			
Surgical staging		**Histological grade**		**Tumor location**	
Stage I Stage IIA Stage IIB Stage IIIA Stage IIIB	10 (32.3%) 14 (45.2%) 4 (12.9%) 2 (6.5%) 1 (3.2%)	Grade I Grade II Grade III	11 (35.5%) 13 (41.9%) 6 (22.6%)	Left breast Right breast	14 (45.2%) 17 (54.8%)

SD: Standard deviation

tissues and bloods were amplified in accordance with the relevant protocols. At the end of each reaction, melting curve analyzes were performed and it was confirmed that there was no dimerization in the primers and that the fluorescence values obtained belonged to the relevant gene region (Figure 1). Each analysis was performed in 3 replicates, both as an intraassay and an interassay. The fold change values obtained were calculated logarithmically, and their increase or decrease was prepared in graphic form.

Table 3. Surgical stage, histological grade, immunohistochemical status of all breast cancer patients

Patient no	Surgical stage	Histological grade	ER	PR	HER2	Ki-67	P53
P1	IIB	I	6	-	-	5%	10%
P2	IIA	I	-	-	-	60%	10%
P3	IIA	I	6	8	-	2-3%	10%
P4	I	II	6	8	-	3+%	10%
P5	2B	II	7	8	-	7-8+%	5%
P6	I	I	8	8	-	40%+	50%
P7	IIA	III	7	8	-	20+%	1-2%
P8	I	II	7	7	-	20%	80%
P9	IIA	III	-	-	-	80+%	100%
P10	IIA	I	6	6	-	2-3%	5%
P11	IIA	II	8	8	-	4-5%	10%
P12	IIA	II	8	5	Positive	20%	70%
P13	IIA	I	8	8	-	10%	20%
P14	I	I	8	8	-	1-2%	2-3%
P15	IIA	I	6	6	-	30%	10%
P16	IIIA	II	8	8	-	15%	1-2%
P17	IIA	III	8	-	-	30%	40%
P18	I	II	8	8	-	5%	1-2%
P19	IIA	I	8	8	-	1-2%	1-2%
P20	I	II	-	-	-	80%	60%
P21	IIA	I	7	6	-	30%	-
P22	IIIA	I	8	8	-	60%	10%
P23	2B	II	-	-	-	80%	80%
P24	I	II	7	7	-	2-3%	5%
P25	I	II	8	8	-	20%	20%
P26	IIB	III	4	4	-	60%	-
P27	IIA	II	6	8	-	5%	-
P28	IIA	III	7	7	-	15%	-
P29	I	II	-	-	-	20%	-
P30	IIIB	II	7	8	-	7-8%	-
P31	I	III	6	8	-	10%	-

mRNA Analysis of *PTEN* Gene of All Breast Cancer Patients

PTEN gene expression in tumor tissues and peripheral blood samples of breast cancer patients was determined compared to controls. The mRNA level of the *PTEN* gene in peripheral blood samples was significantly downregulated (0.501-fold) ($p<0.05$), while it was upregulated (1.109-fold) in tumor tissues ($p>0.05$) (Figure 2) (fold regulation changes are shown at Log10 level).

mRNA Analysis of *PTEN* Gene in Breast Cancer Patients at Different Stages

PTEN gene expression in tumor tissues of patients with stage I breast cancer was upregulated (1.693-fold), while it was downregulated (0.813-fold) in peripheral blood samples ($p>0.05$). *PTEN* gene expression in peripheral blood samples of patients with stage IIA breast cancer was significantly downregulated (0.813-fold) ($p<0.001$). It was downregulated in almost all patients with stage IIA (Table 4). In addition, it was downregulated (0.927-fold) in tumor tissues ($p>0.05$). *PTEN* gene expression in tumor tissues of

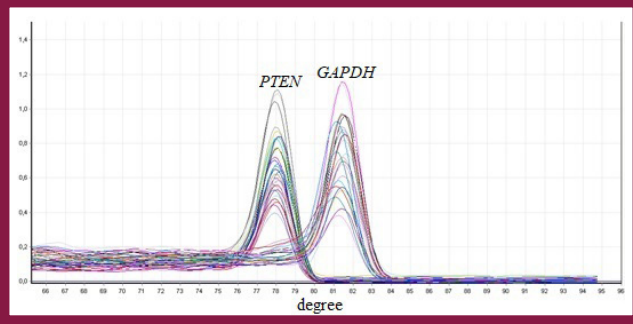

Figure 1. Example of *CDH1* and *GAPDH* melting curve

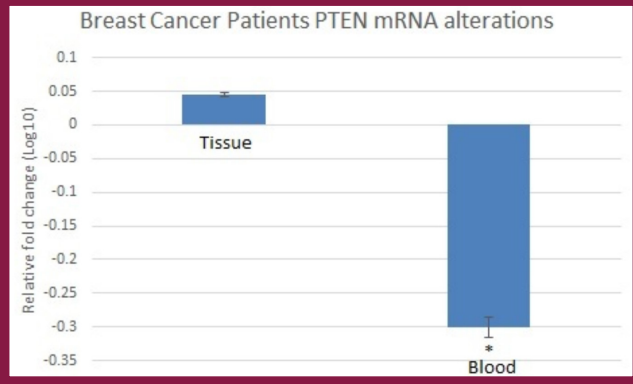

Figure 2. Alterations of *PTEN* gene expression in tumor tissues and peripheral blood samples of breast cancer patients compared to control. *GAPDH* was used as the reference gene for normalization (*$p<0.05$)

stage IIB breast cancer cases was upregulated (1.084-fold), while it was downregulated (0.937-fold) in peripheral blood samples (p>0.05). *PTEN* gene expression in tumor tissues and peripheral blood samples of patients with stage IIIA breast cancer was upregulated (1.112-fold and 1.332-fold, respectively) (p>0.05). *PTEN* gene expression in tumor tissues of stage IIIB breast cancer cases was significantly upregulated (2.231-fold) (p<0.001), while it was significantly downregulated (0.371-fold) in peripheral blood samples (p<0.05) (Figure 3, 4).

mRNA Analysis of *PTEN* Gene Expression of Breast Cancer Cases in Grade I-II-III

PTEN gene expression in tumor tissues of grade I breast cancer cases was upregulated (1.272-fold) (p>0.05), while it was significantly downregulated (0.554-fold) in peripheral blood samples (p<0.05). *PTEN* gene expression in tissue samples of grade II breast cancer cases was upregulated (1.114-fold), while it was downregulated (0.897-fold) in peripheral blood samples (p<0.05). *PTEN* gene expression in the tissue samples of grade III breast cancer cases was upregulated (1.116 fold), while it was downregulated significantly (0.302-fold) in the peripheral blood samples (p<0.05) (Figure 5, 6).

Evaluation of *PTEN* Gene Expression Changes with ER and PR Statuses

The ER and PR status of patients with breast cancer who had downregulation in tumor tissues were evaluated.

Table 4. *PTEN* gene expression levels of stage IIA breast cancer patients

Patient no	Surgical stage	*PTEN* expression blood
P2	IIA	Down
P3	IIA	Up
P7	IIA	Down*
P9	IIA	Down*
P10	IIA	Down
P11	IIA	Down
P12	IIA	Down*
P13	IIA	Down*
P15	IIA	Down*
P17	IIA	Down*
P19	IIA	Down*
P21	IIA	Down*
P27	IIA	Down*
P28	IIA	Down*

*It shows significant change in *PTEN* gene expression levels compared to control. Down means: Decreased mRNA level, Up means: Increased mRNA level

There was a significant and strong correlation between the downregulation of the *PTEN* gene in tumor tissues and the positivity of ER and PR values (Pearson correlation, r=0.770, p<0.01). Likewise, there was a significant and strong correlation between the downregulation of the *PTEN* gene in peripheral blood samples and the positivity of ER and PR values (Pearson correlation, r=0.772, p<0.01). In addition, there was a significant and strong correlation between the positivity of the ER and PR values of the cases in which the *PTEN* gene was significantly downregulated in peripheral blood samples (pearson correlation, r=0.672, p<0.01). There was one case with HER2 positive. This case was in stage IIA. *PTEN* gene expression level was downregulated in breast tumor tissue and peripheral blood samples of this patient. This downregulation was significant in peripheral blood (p<0.001). In addition, when triple negative patients ER (-), PR (-), HER2(-) were evaluated, downregulation of *PTEN* gene

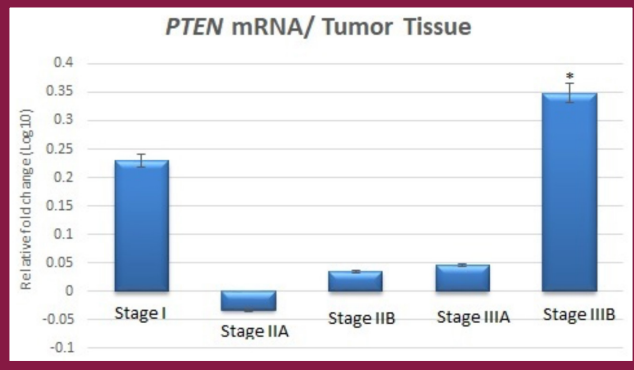

Figure 3. Alterations of *PTEN* gene expression in tumor tissues samples of breast cancer patients at different stages compared to control. *GAPDH* was used as the reference gene for normalization (*p<0.001)

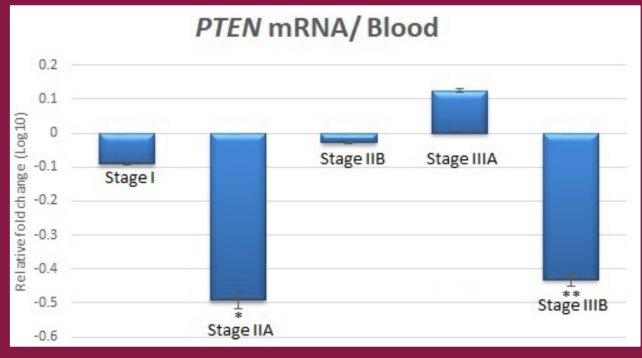

Figure 4. Alterations of *PTEN* gene expression in peripheral blood samples of breast cancer patients at different stages compared to control. *GAPDH* was used as the reference gene for normalization (*p<0.001, **p<0.05)

expression was observed in all peripheral blood samples of these patients (Table 5).

Discussion

Molecular biology and genetics represent one of the most important and interesting topics in medical oncology, thus providing a global and detailed knowledge of the molecular changes involved in tumor progression, leading to a better understanding of the carcinogenesis process, the discovery of new prognostic markers and therapeutic targets (12).

Gene expression analysis has determined global gene dosage sensitivity in cancer. Thousands of genetic variants so far. It has been associated with cancer, other diseases, and complex traits. It has been found that disease-related single nucleotide polymorphisms and somatic copy number changes frequently affect gene expression levels (13,14,15,16).

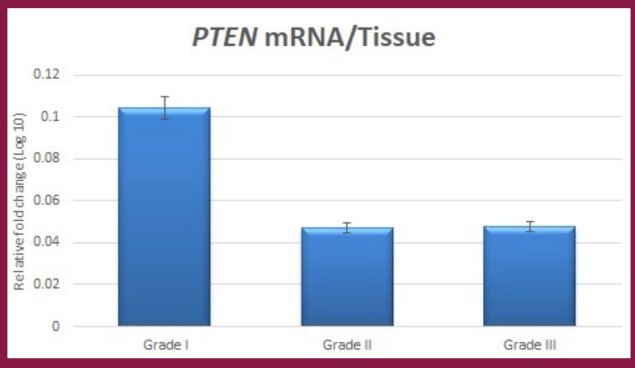

Figure 5. Alterations of *PTEN* gene expression in tumor tissues of breast cancer patients at different grades compared to control. *GAPDH* was used as the reference gene for normalization

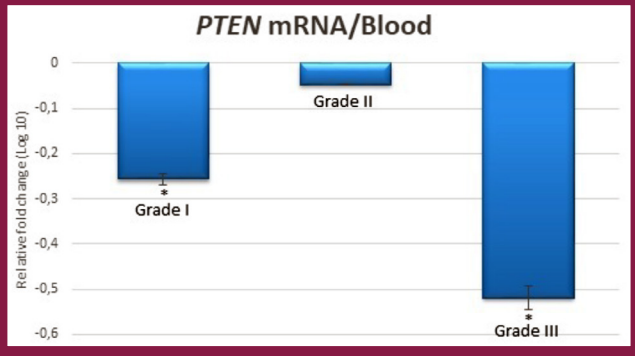

Figure 6. Alterations of *PTEN* gene expression in peripheral blood samples of breast cancer patients at different grades compared to control. *GAPDH* was used as the reference gene for normalization (*p<0.05)

Deletion and decreased *PTEN* function as a result of mutation have been detected, in sporadic cancer and hereditary cancer syndromes. An also, germline mutations causing loss of *PTEN* function have been detected in patients with Cowden's disease (17,18,19). In many studies, it has been determined that *PTEN* is an important tumor suppressor gene for breast cancers and its loss induces carcinogenesis in the breast. Similarly, in our study, *PTEN* gene expression were significantly decreased in blood samples of patients with breast cancer. Specially, this downregulation draws attention in high percentage with stage IIA patiens. Contrary to this, upregulation was detected in more samples of our tumor series, but not significant. There was a contrast between blood and tissue in terms of *PTEN* gene expression behaviors. Additionally, we found that *PTEN* gene expression was upregulated in tumor tissue at every histological grade, while it was downregulated in peripheral blood. The major differences in gene expression levels in tumor tissue samples and peripheral blood samples may reflect tissue specific regulatory mechanisms for this tumor supressor gene. Although we couldn't detect this decrease in tumor tissues, the decrease in blood was compatible with the literature. Changes of the *PTEN* gene in somatic cells are common in certain cell lines and primary tumors, including thyroid tumors, brain tumors, prostate carcinomas, endometrial carcinomas, and melanoma. However, it has been reported that such changes are less common in breast cancer, kidney carcinomas, and head and neck squamous cell carcinomas (20).

Depowski et al. (21) reported loss of PTEN protein expression in 48% of 151 breast tumors. In their analysis, they reported that loss of PTEN protein expression, node positivity, stage, tumor grade were associated with disease-related deaths. Loss of PTEN protein expression has been reported to correlate with predicted lymph node metastasis and loss of ER staining. Loss of PTEN protein expression has not been reported to correlate with stage, tumor grade,

Table 5. Surgical stage, histological grade, immunohistochemical status of breast cancer patients with triple negative

Patient no	Surgical stage	Histological grade	ER	PR	HER2	*PTEN* expression blood
P2	IIA	I	-	-	-	Down*
P9	IIA	III	-	-	-	Down*
P20	I	II	-	-	-	Down
P23	IIB	II	-	-	-	Down
P29	I	II	-	-	-	Down*

*It shows significant change in *PTEN* gene expression compared to control. Down means: Decreased mRNA level, Triple negative means: ER (-), PR (-) HER2(-)

disease relapse, or loss of PRs. These results show that the proposed *PTEN* gene is a candidate tumor suppressor in breast cancer and future studies are needed for this marker (21). Additionally, Depowski et al. (21) confirmed a significant loss of *PTEN* expression in 17 of 25 ER-negative tumors and an almost significant loss of *PTEN* in PR-negative tumors. The underlying reason for this correlation is unclear, but loss of *PTEN* expression appears to be due to an aggressive ER/PR negative phenotype (21). In addition, a significant relationship was found with PR loss (22). Contrary to this findings, in our study, there was a significant and strong correlation between the downregulation of the *PTEN* gene expression and the positivity of ER and PR values in both blood and tumor tissues. Dicrepancies may be resulted from the relatively few sample number. However, Shoman et al. (23) findings support our results. They reported strong association between downregulation of *PTEN* expression in ER-α-positive tumors (23). *PTEN* loss is shown in many cancers and in the triplet negative group of breast cancers (24). Similarly to this report, when triple negative patients [ER (-), PR (-) and HER2 (-)] were evaluated, downregulation of *PTEN* gene expression was observed in all blood samples of these patients in our study. Due to the lack of hormonal treatment and anti-HER-2 treatment alternatives in triplet negatives, drug studies have been carried out on these patients by targeting these pathway activations due to *PTEN* dysfunction. Loss of *PTEN* was observed at a rate of 66% in the triplet-negative subgroup of patients with recurrent breast cancer, while it was 28% and 22% in the hormone-positive and HER2 groups, respectively (25).

Study Limitations

The main limitation of this study was the sample size. The predicted downregulation was not observed in the tumor tissues. We think that this was due to the low number of patients with advanced stages of breast cancer.

Conclusion

When the results regarding the mRNA changes of *PTEN* in breast cancer patients were examined, the downregulation of the *PTEN* gene expression in peripheral blood samples draws attention. We think that the decrease in *PTEN* gene mRNA level in blood samples of breast cancer patients may be a potential finding of breast cancer. These data will contribute to future studies.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Afyonkarahisar Health Sciences University (04.05.2018/144).

Informed Consent: All patients provided informed consent.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

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

Conflict of Interest: The authors declare that there are no conflicts of interest.

Financial Disclosure: This work was supported by Afyonkarahisar Health Sciences University Scientific Research Projects Commission with a project numbered projected 19.LİS.002. It is accepted as a master's thesis named "Farklı Evre ve Derecelerdeki Meme Kanserli Hastalarda PTEN Geninin İfadelenme Düzeylerinin Araştırılması" in 2020.

References

1. https://www.ncbi.nlm.nih.gov/gene/999 [Crossref]
2. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. Mol Biol Int. 2014;2014:852748. [Crossref]
3. Reyes HD, Thiel KW, Carlson MJ, Meng X, Yang S, Stephan JM, et al. Comprehensive profiling of EGFR/HER receptors for personalized treatment of gynecologic cancers. Mol Diagn Ther. 2014;18:137-151. [Crossref]
4. Ambros RA. Simple hyperplasia of the endometrium: an evaluation of proliferative activity by Ki-67 immunostaining. Int J Gynecol Pathol. 2000;19:206-211. [Crossref]
5. Bai M, Agnantis NJ, Kamina S, Demou A, Zagorianakou P, Katsaraki A, et al. In vivo cell kinetics in breast carcinogenesis. Breast Cancer Res. 2001;3:276-283. [Crossref]
6. Anderson WF, Chatterjee N, Ershler WB, Brawley OW. Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database. Breast Cancer Res Treat. 2002;76:27-36. [Crossref]
7. Leake R, Barnes D, Pinder S, Ellis I, Anderson L, Anderson T, et al. Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. UK Receptor Group, UK NEQAS, The Scottish Breast Cancer Pathology Group, and The Receptor and Biomarker Study Group of the EORTC. J Clin Pathol. 2000;53:634-635. [Crossref]
8. Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Jänicke F, et al. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol. 2001;19:3808-3816. [Crossref]
9. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. Breast Cancer Res Treat. 1992;22:207-219. [Crossref]
10. Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, et al. AJCC Cancer Staging Manual, 6th ed. Chicago: Springer; 2002. [Crossref]
11. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002;30:e36. [Crossref]
12. Kheirelseid EAH. Clinical Applications of Molecular Profiling in Colorectal Cancer, Dicipline of Surgery College of Medicine, Nursing & Health Sciences National University of Ireland: Galway; 2011. [Crossref]

13. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science. 2007;315:848-853. [Crossref]
14. Fehrmann RS, Jansen RC, Veldink JH, Westra HJ, Arends D, Bonder MJ, et al. Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. PLoS Genet. 2011;7:e1002197. [Crossref]
15. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet. 2013;45:1238-1243. [Crossref]
16. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. Nat Genet. 2013;45:1127-1133. [Crossref]
17. Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet. 2001;358:1389-1399. [Crossref]
18. Murff HJ, Spigel DR, Syngal S. Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. JAMA. 2004;292:1480-1489. [Crossref]
19. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359-E386. [Crossref]
20. Tachibana M, Shibakita M, Ohno S, Kinugasa S, Yoshimura H, Ueda S, et al. Expression and prognostic significance of PTEN product protein in patients with esophageal squamous cell carcinoma. Cancer. 2002;94:1955-1960. [Crossref]
21. Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. Mod Pathol. 2001;14:672-676. [Crossref]
22. Perren A, Weng LP, Boag AH, Ziebold U, Thakore K, Dahia PL, et al. Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. Am J Pathol. 1999;155:1253-1260. [Crossref]
23. Shoman N, Klassen S, McFadden A, Bickis MG, Torlakovic E, Chibbar R. Reduced PTEN expression predicts relapse in patients with breast carcinoma treated by tamoxifen. Mod Pathol. 2005;18:250-259. [Crossref]
24. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res. 2005;65:2554-2559. [Crossref]
25. Gonzalez-Angulo AM, Ferrer-Lozano J, Stemke-Hale K, Sahin A, Liu S, Barrera JA, et al. PI3K pathway mutations and PTEN levels in primary and metastatic breast cancer. Mol Cancer Ther. 2011;10:1093-1101. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.52533

Hamidiye Med J 2023;4(2):92-102

Opinions of Physiotherapy Students on the Effect of Anatomy Education in Clinical Practice Course: A Preliminary Study

Fizyoterapi Öğrencilerinin Klinik Uygulama Dersinde Anatomi Eğitiminin Etkisine İlişkin Görüşleri: Bir Ön Çalışma

Yunus Emre Kundakcı[1], Abdülkadir Bilir[2]

[1]*Afyonkarahisar Health Sciences University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Afyonkarahisar, Türkiye*

[2]*Afyonkarahisar Health Sciences University Faculty of Health Sciences, Department of Anatomy, Afyonkarahisar, Türkiye*

ABSTRACT

Background: The aim of this study was to describe the dynamic connections between physiotherapy students' anatomy and 4th grade clinical practice courses through student perceptions.

Materials and Methods: In this cross-sectional study, a three-part questionnaire was applied to 141 fourth-year physiotherapy students. The first part included the descriptive information of the students, the second part, the perceptions of the students on anatomy knowledge during the clinical practice course using a 5-point Likert scale, and the third part, the perceptions of the students on the subjects of systematic anatomy. Descriptive statistics and the explanatory factor analysis methods was used in data analysis.

Results: According to factor analysis, a total of 21 items (Cronbach α=0.88) revealed 3 factors explaining 50.24% of the variance (p<0.001): (1) Compliance in clinical practice and anatomy courses, (2) Anatomy learning qualifications in clinical practice and (3) Importance of anatomy in clinical practice. The factors were significantly correlated with each other (p<0.05). Students who passed the anatomy course in one attempt and students who had experience working as a health professional showed high scores in factor 1 (p<0.05).

Conclusion: Findings limited to student responses provides guiding ideas to anatomists and educational physiotherapists about the effectiveness and importance of anatomy education in clinical practice course.

Keywords: Anatomy, clinical practice, education, physical therapists

ÖZ

Amaç: Bu çalışmanın amacı, fizyoterapi öğrencilerinin anatomi ve 4. sınıf klinik uygulama dersleri arasındaki dinamik bağlantıları öğrenci algıları üzerinden tanımlamaktı.

Gereç ve Yöntemler: Bu kesitsel çalışmada, dördüncü sınıftaki 141 fizyoterapi öğrencisine, üç bölümden oluşan bir anket uygulandı. Birinci bölümde öğrencilerin tanımlayıcı bilgileri, ikinci bölümde 5'li Likert ölçeği kullanılarak öğrencilerin anatomi bilgilerini klinik uygulama dersi sırasındaki algılama durumları ve üçüncü bölümde öğrencilerin sistematik anatomi konularına yönelik algıları yer aldı. Veri analizinde tanımlayıcı istatistikler ve açıklayıcı faktör analizi yöntemi kullanıldı.

Bulgular: Faktör analizine göre toplam 21 ifade, (Cronbach α=0,88) varyansın %50,24'ünü açıklayan 3 faktör ortaya koymuştur (p<0,001): (1) Klinik uygulama ve anatomi derslerindeki uyum, (2) Klinik uygulamalarda anatomi öğrenim yeterlilikleri ve (3) Klinik uygulamalarda anatominin önemi. Faktörler birbirleri ile anlamlı düzeyde ilişkiliydi (p<0,05). Anatomi dersini tekrar etmeksizin geçen öğrenciler ile bir sağlık profesyoneli olarak çalışma deneyimine sahip öğrenciler faktör 1'de yüksek puanlar gösterdi (p<0,05).

Sonuç: Elde edilen öğrenci yanıtlarıyla sınırlı bulgular, anatomi eğitiminin klinik uygulama dersindeki etkinliği ve önemi hakkında anatomistlere ve eğitici fizyoterapistlere yön gösterici fikirler vermektedir.

Anahtar Kelimeler: Anatomi, klinik uygulama, eğitim, fizyoterapist

Address for Correspondence: Yunus Emre Kundakcı, Afyonkarahisar Health Sciences University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Afyonkarahisar, Türkiye

Phone: +90 272 246 28 34 E-mail: y.emre.kndkc@gmail.com **ORCID ID:** orcid.org/0000-0002-2006-9940

Received: 17.10.2022 **Accepted:** 07.03.2023

Introduction

Anatomical knowledge and understanding are essential components of physiotherapy education and practice (1) and a path to success in further applications (2). It is also necessary to equip students with safe and effective clinical skills (3). For this reason, anatomy courses, which have tremendous impact in clinical courses and postgraduate professional practice, are an integral part of the physiotherapy education curriculum (4,5,6). In the National Core Training Program for Physiotherapy Departments in Türkiye, the importance of functional and applied/specialized anatomy knowledge focused on health or disease associated with physiotherapy practices is emphasized (7).

In the 4-year undergraduate physiotherapy education, anatomy is taught to students in their first year of university. In fact, this curriculum model is based on the assumption that further learning in later years builds on the knowledge gained in the early stages of the program (1,2). On the other hand, it has been shown that physiotherapy students cannot retain their anatomy knowledge gained in the first years in the following years (1). In the physiotherapy curriculum, applications on a real patient are only possible toward the end of undergraduate education and the clinical reasoning skills of the students develop from novice to professionalism (8). Therefore, it is necessary to clarify the effects of anatomical knowledge learned early in undergraduate education on clinical reasoning skills learned and experienced in senior clinical practice courses. In order for students to collect and interpret information from the patient, and to plan and apply treatment, they should have knowledge of the normal functions of an anatomical structures and should be able to detect possible disorders in terms of movement and participation. This model in clinical reasoning is also recommended in World Health Organization's International Classification of Functions (9,10).

Undergraduate physiotherapy students develop hypothetical-deductive reasoning skills by comparing and reflecting on different reasoning approaches (11,12). Over time, students learn to synthesize these different approaches into their individual approaches (11). Therefore, anatomy needs to be dynamically understood and evaluated in clinical contexts (12). Physiotherapists and physiotherapy students consider it extremely important to use their knowledge of anatomy to solve clinical problems and develop practical skills (4,13).

More research is needed to develop clinical reasoning skills and explore the best strategies (14). In the development of the physiotherapy profession, which has become very popular in the world in recent years, and in the education curriculum of the students, importance is given to the development of anatomy education (5,6). For this reason, in previous studies, it was tried to determine the needs of the student group by taking student feedback (4,6,15). However, to the best of our knowledge, no feedback has been received regarding the empirical impact of knowledge of anatomy on the clinical practice knowledge and experience of senior physiotherapy students. In fact, a study in this direction may contribute to the understanding of the holistic relationship between the clinical practice course, where professional skills are experienced for the last time before specialization, and anatomy education, which is one of the cornerstones of the undergraduate curriculum.

Final training of senior physiotherapy students before starting the profession as a professional physiotherapist includes clinical skills learned in 4th year clinical practice. The aim of this study is to provide a preliminary assessment of the dynamic link between clinical practice skills and anatomy learning of 4th year physiotherapy students. Emphasis on strengths or weaknesses in anatomy knowledge in clinical reasoning processes by students who experience and develop their clinical practice on patients can contribute to an improved understanding when planning anatomy and physiotherapy curriculum.

Material and Methods

Study Design, Sampling and Ethical Considerations

In this study, a cross-sectional research design was preferred using quantitative methods. The research was carried out in accordance with the Declaration of Helsinki. After the approval of Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (decision dated 05.11.2021 and numbered 2021/492), 180 students who were potential participants of the study and who were successful in the clinical practice course after the completion of the 2021 fall semester were contacted. These students were senior students enrolled in a four-year physiotherapy and rehabilitation department at Afyonkarahisar in Türkiye. Participation in the study was voluntary and all participants read and approved the informed consent form. Those who did not want to participate in the study (n=18), those who had deficiencies in their descriptive information or missing data in their answers (n=11), and those who were found to have given contradictory answers to the control question asked during the analysis (n=10) were excluded in the study (Figure 1). For the calculation of sample size we used formula [n=Nt2pq/d2(N-1)+t2pq]. The sample size for the universe of 180 physiotherapy students was determined

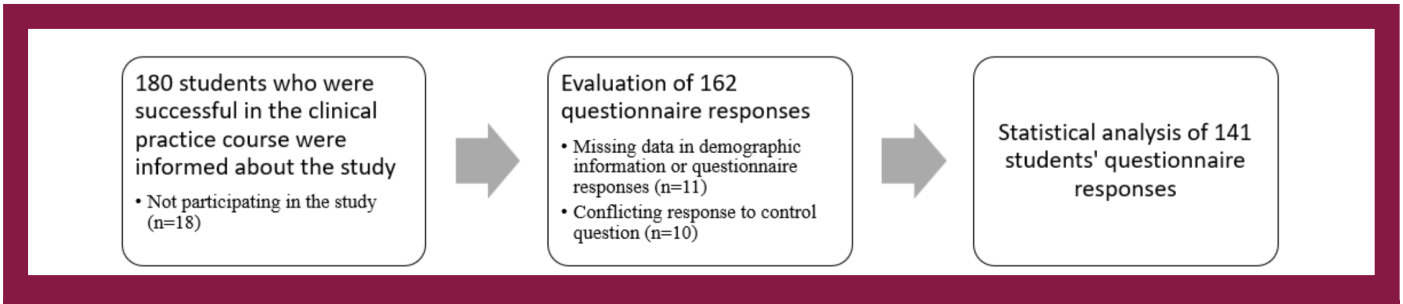

Figure 1. Flow chart and stages of the study

as 123 students (confidence level: 95% and a confidence interval: 5%). The data collection process was completed on January 10, 2021 by reaching 141 students with simple random sampling method.

Curriculum

At the university where the study was carried out, the anatomy teaching of the students who are subject to the 4-year physiotherapy undergraduate curriculum is mainly carried out in the first years of education. Anatomy teaching is offered in three different terms and with three different courses (Anatomy I and Anatomy II courses, 1st grade; Neuroanatomy course, 2nd grade). Students who successfully complete the basic sciences and physiotherapy vocational courses in the first three years take the clinical practice I and II courses in the 4th year under the supervision of physiotherapist supervisors in order to experience what they have learned in these courses in clinical settings. The information about the curriculum of the students and the purpose of the relevant courses is presented in Table 1.

Data Collection and Study Procedure

Students were motivated by the knowledge that this research would contribute significantly to the development of teaching methods. It is stated that they can stop filling out the questionnaire at any time. In this study, a questionnaire consisting of three parts and prepared within the scope of the literature (2,6,16) was used as a data collection tool. Sections in the survey form are as follows: (1) Demographic information, (2) A 5-point Likert-type, 35-question questionnaire on the relationship between anatomy education and clinical practice course and (3) 3 multiple-choice questions about participants' attitudes toward systematic anatomy issues.

Demographic information: This section included questions about participants' age, gender, employment status in a health institution, school enrollment types, clinical practice rotations, and success in anatomy courses.

Table 1. Anatomy and clinical practice training in the physiotherapy curriculum

Course and bologna knowledge	Aim of the course
Anatomy I 1st year fall semester 2 theoretical course hours per week 2 laboratory course hours per week Term ECTS: 5	Learning the anatomy of locomotor, respiratory, circulatory, digestive, urogenital, endocrine and nervous systems as well as general anatomical concepts
Anatomy II 1st year spring semester 3 theoretical course hours per week 2 laboratory course hours per week Term ECTS: 5	To understand the shape and structure of the human body in detail, to understand the structures and organs that make-up the body, to understand their functions, to understand the relationship with each other and to apply all the knowledge to the clinic.
Neuroanatomy 2nd year fall semester 2 theoretical course hours per week 2 laboratory course hours per week Term ECTS: 4	To give basic and clinical information about the structures and organs that make up the central nervous system at undergraduate level, to enable students to recognize the structures of the central nervous system theoretically and practically, and to teach the relations of these structures both within themselves and with other systems.
Clinical practice I 4th year fall semester 35 practice course hours per week Term ECTS: 30	To provide students with the opportunity to apply their treatment theoretical knowledge and skills in various clinical settings (Neurological and pediatric rehabilitation or general FTR and Orthopedic rehabilitation) under the supervision of a supervisor.

ECTS: European credit transfer and accumulation system

Survey structure: Study participants were asked whether they agreed with each item and how strongly they agreed, using a point rating scale. In this study, each item in the questionnaire was measured using a 5-point Likert scale based on positively expressed inferences (strongly disagree, 1; disagree, 2; undecided, 3; agree, 4; strongly agree, 5). Relevant survey responses for each item were

collected, and the mean and standard deviations (SD) of the responses for each item were calculated over the total scores of the respondents.

Questionnaire for the relationship between anatomy education and clinical practice course: This section was used to measure the participants' perceptions, awareness and suggestions about the information they learned in Anatomy I, Anatomy II, and Neuroanatomy courses and the experiences they gained in the clinical practice course. While preparing the questionnaire, 22 questions in the literature were included in the first stage. Sample questions were as follows: (i) "Success in anatomy theory courses is effective in success in clinical practice course." (ii) "Anatomy courses should be given again after the 1st and 2nd grades together with the vocational courses." (iii) "What I learned in the anatomy course increases my curiosity about the clinic." Afterwards, 12 more statements were added regarding the purpose and course outputs of the relevant courses, by interviewing the responsible lecturers of the anatomy and clinical practice courses. A few of these questions were: (i) "What I learned in the anatomy course allows me to be successful in clinical practice." (ii) "During clinical practice, I found that I had deficiencies in anatomy." (iii) "The knowledge achieved in anatomy lessons helps in exercise practice." In order to measure whether the students gave sincere answers when filling out the form, a statement was added to the form by the researchers as a control question and determined as an exclusion criterion. In the final form of the questionnaire, there were a total of 35 questions. In the analysis phase, the compatibility between the control questions (3rd and 27th items) was evaluated separately in each form. The forms of those with a total of 8, 9, or 10 responses to both statements were not included in the analyzes by the researchers. In addition, those with 10% or less missing data in their answers were accepted as random missing data and the mean score of the missing variable was entered instead of missing values. The flow chart of the study and at which stages and how many data losses occurred are presented in Figure 1.

Multiple choice questions about participants' attitudes towards systematic anatomy topics: This section has been prepared as multiple choice in clinical practice courses to explore the requirements of systematic anatomy knowledge. Students were asked to choose at most three among the subjects of movement (bone, joint, muscle), circulation, respiratory, urinary, digestive, genital, nervous, endocrine systems and sensory organs, according to the specified questions: (1) Most interesting to me, (2) the one I am most successful at, and (3) the most challenging for me.

Statistical Analysis

Statistical analysis was performed by using the IBM Statistics SPSS v25.0. (IBM Corp. Armonk. NY, USA). The frequency and percentage distribution of the obtained data is described. The homogeneity of the data was evaluated using the Shapiro-Wilk test, but according to the results of the parametric assumption test, the data could not meet the normality assumption. Therefore, non-parametric procedures were used for data analysis. Exploratory factor analysis was performed to investigate whether there were different structures in the data for the questionnaire items and to investigate the construct validity of the questionnaire. The number of factors to be removed by the researchers was determined by fixing it at three. The rotation method used was Varimax with Kaiser normalization. To verify that the factor analysis was appropriate for the data, the Kaiser-Meyer-Olkin sampling adequacy measure and Bartlett's test of sphericity was calculated. The mean scores of the factors were calculated for each student group variable, and the differences between the two groups were evaluated using the Mann-Whitney U test. Results were presented as means with ± SD or medians with interquartile range. Spearman correlation analysis was performed to determine the relationship between subfactors. In statistical analyzes, significance test and confidence intervals were evaluated at $p<0.05$ significance level.

Results

Demographic and Survey Completion Rate

A total of 162 (90% participation rate) students responded to the questionnaire. The study was completed with 141 (78% inclusion rate) participants after exclusion criteria. 72.3% (n=102) of the participants were female, 27.7% (n=39) were male. The mean age of the participants was 23.01±3.43. The rate of those working in any health institution before or during undergraduate education was 19.9% (n=28). The rate of those who were enrolled in the department with the post-secondary university entrance exam was 92.2% (n=130). The rate of those who received neurological and pediatric rehabilitation practices during the clinical practice course was 41.8% (n=59). The rate of those who failed any of the Anatomy I, Anatomy II and Neuroanatomy courses in the previous years and who took the course again was 37.9% (n=52) (Table 2).

Determining the Importance of Anatomy Education in Clinical Practice Courses

The statement of "Explaining the anatomy and physiotherapy clinic associating them increases the success in clinical applications. was the statement with the

Table 2. Demographic characteristics of the participants		
Demographic characteristics	**n**	**%**
Gender		
Females	102	72,3
Males	39	27.7
Type of school registration of students		
With university entrance exam	130	92.2
With external transfer exam	11	7.8
Working status as a healthcare professional of students		
Yes	28	19.9
No	113	80.1
Clinical practice course of students		
Completed general PTR and orthopedic rehabilitation practices	68	48.2
Completed pediatric and neurological rehabilitation practices	59	41.8
Completed both practices	14	9.9
Status of retaking the anatomy lesson		
None	89	63.1
Re-takers at least once	52	36.9
Age (years), mean ± SD	23.01±3.43	
N: Number of participants, %: Percentage of participants, PTR: Physiotherapy and rehabilitation, SD: Standard deviation		

highest mean (mean ± SD, 4.65±0.52), the statement of "I'm considering postgraduate education in anatomy" was the statement with the lowest mean (mean ± SD, 3.51±1.17). In Table 3, mean value, SD, frequency and percentage values are given for the answers to 35 questions.

The Cronbach α is a statistic widely cited by the authors to demonstrate the relevance of tests and scales created or adopted for research projects (17). Thirty-four statements in this study achieved a reliability coefficient of 0.88 and had high internal consistency. A limited exploratory factor analysis was performed on 3 factors to facilitate understanding and interpretation of the relationships among the questionnaire statements. A total of 21 statements (Cronbach α=0.88) diverged in 3 factors after removing the items that did not meet the sufficient threshold to enter any factor. The first factor explained 28.7% of the variance, the second 13.3% and the third 8.3%. Therefore, 50.24% of the variance was explained by these three factors. Factor 1: Students' perceptions of compliance in clinical practice and anatomy courses (10 items and Cronbach α=0.86). Factor 2: Students' perceptions of anatomy learning competencies in clinical practice (5 items and Cronbach α=0.78). Factor 3: Students' perceptions of the importance of anatomy in clinical practice (6 items and Cronbach α=0.78) (Table 4). The three factors were statistically significantly correlated among themselves (p<0.05) (Table 5).

Table 3. The questionnaire items and students' responses about anatomy knowledge and clinical applications in physiotherapy

Items	Mean ± SD	Frequency (%)				
		Strongly disagree	Disagree	Undecided	Agree	Strongly agree
Success in anatomy theory courses is effective in success in clinical practice course.	4.41±0.8	1 (0.7)	5 (3.5)	7 (5)	50 (35.5)	78 (55.3)
Success in anatomy practice courses is effective in success in clinical practice course.	4.53±0.72	2 (1.42)	1 (0.71)	4 (2.84)	47 (33.33)	87 (61.7)
Anatomy I, Anatomy II and neuroanatomy courses increase my professional skills.	4.5±0.65	1 (0.71)	0 (0)	6 (4.26)	54 (38.3)	80 (56.74)
The knowledge gained in anatomy classes is helpful in electrotherapy applications.	4.22±0.83	1 (0.71)	6 (4.26)	12 (8.51)	64 (45.39)	58 (41.13)
The knowledge achieved in anatomy lessons helps in exercise practice.	4.55±0.57	0 (0)	1 (0.71)	2 (1.42)	56 (39.72)	82 (58.16)
The knowledge gained in anatomy classes helps me during assessment and evaluation practices in physiotherapy.	4.57±0.59	0 (0)	1 (0.71)	4 (2.84)	50 (35.46)	86 (60.99)
During clinical practice, I can relate musculoskeletal structures to orthopaedic assessment and treatment practices.	4.42±0.62	0 (0)	1 (0.71)	7 (4.96)	65 (46.1)	68 (48.23)

Table 3. Continued

Items	Mean ± SD	Frequency (%)				
		Strongly disagree	Disagree	Undecided	Agree	Strongly agree
During clinical practice, I can relate neuroanatomical structures to neurological assessment and treatment practices.	4.21±0.68	0 (0)	2 (1.42)	15 (10.64)	76 (53.9)	48 (34.04)
What I learn in anatomy classes motivates me for my career.	4.25±0.84	2 (1.42)	2 (1.42)	18 (12.77)	56 (39.72)	63 (44.68)
Anatomy courses are an important topic for clinical applications in physical therapy.	4.62±0.54	0 (0)	0 (0)	4 (2.84)	46 (32.62)	91 (64.54)
Explaining the anatomy and physiotherapy clinic associated with it increases the success in clinical applications.	4.65±0.52	0 (0)	0 (0)	3 (2.13)	44 (31.21)	94 (66.67)
The anatomy course is important in choosing the appropriate treatment for the patient and pathology.	4.52±0.54	0 (0)	0 (0)	3 (2.13)	61 (43.26)	77 (54.61)
There is a relationship between the anatomy education given in the curriculum and clinical practice.	4.19±0.83	0 (0)	7 (4.96)	16 (11.35)	61 (43.26)	57 (40.43)
Before clinical applications, I need to repeat the anatomy.	4.42±0.69	0 (0)	3 (2.13)	7 (4.96)	59 (41.84)	72 (51.06)
In the exams of clinical practice courses, there are questions that question my knowledge of anatomy.	4.39±0.7	0 (0)	3 (2.13)	9 (6.38)	60 (42.55)	69 (48.94)
I watched at least one video on anatomy during clinical practice.	3.83±1.21	8 (5.67)	17 (12.06)	18 (12.77)	47 (33.33)	51 (36.17)
During clinical practice, I found that I had deficiencies in anatomy.	4.04±0.92	2 (1.42)	8 (5.67)	20 (14.18)	63 (44.68)	48 (34.04)
I reviewed my previous anatomy lecture notes during clinical practice.	4.13±0.95	4 (2.84)	7 (4.96)	12 (8.51)	64 (45.39)	54 (38.3)
Before the clinical practice course, there should be an accelerated anatomy review course.	4.09±0.95	3 (2.13)	5 (3.55)	26 (18.44)	51 (36.17)	56 (39.72)
Anatomy courses should be given again after the 1st and 2nd grades together with the vocational courses.	3.89±1.04	4 (2.84)	9 (6.38)	34 (24.11)	46 (32.62)	48 (34.04)
What I learn in anatomy, which I associate my knowledge of anatomy with clinical applications, becomes more permanent.	4.21±0.95	2 (1.42)	7 (4.96)	18 (12.77)	47 (33.33)	67 (47.52)
Increasing the theoretical course hours of Anatomy I, Anatomy II and Neuroanatomy courses improves clinical practice skills.	3.62±1.05	6 (4.26)	12 (8.51)	41 (29.08)	52 (36.88)	30 (21.28)

Table 3. Continued

Items	Mean ± SD	Frequency (%)				
		Strongly disagree	Disagree	Undecided	Agree	Strongly agree
Increasing the practical hours of Anatomy I, Anatomy II and Neuroanatomy courses improves clinical practice skills.	4.18±0.88	2 (1.42)	4 (2.84)	20 (14.18)	56 (39.72)	59 (41.84)
Seeing anatomical structures through cadavers improves my clinical practice skills.	4.28±0.86	2 (1.42)	3 (2.13)	17 (12.06)	52 (36.88)	67 (47.52)
Anatomy I, Anatomy II and Neuroanatomy course topics should be changed to be more compatible with physiotherapy clinical practice.	4.25±0.86	2 (1.42)	3 (2.13)	18 (12.77)	54 (38.3)	64 (45.39)
Topics such as radiological anatomy, cross-sectional anatomy and clinical anatomy should be added to the anatomy course content.	4.06±0.94	1 (0.71)	5 (3.55)	36 (25.53)	41 (29.08)	58 (41.13)
*Anatomy I, Anatomy II and neuroanatomy courses increase my professional skills.	1.48±0.67	86 (60.99)	45 (31.91)	8 (5.67)	2 (1.42)	0 (0)
Anatomy I, Anatomy II and neuroanatomy courses give an idea about what I can encounter in the clinic.	4.18±0.75	0 (0)	3 (2.13)	21 (14.89)	67 (47.52)	50 (35.46)
In the physiotherapy curriculum, I would like to have elective courses related to anatomy (such as anatomical palpation and anatomy of pain).	4.35±0.71	0 (0)	0 (0)	19 (13.48)	54 (38.3)	68 (48.23)
In my professional life, I would like to attend courses and training related to anatomy.	3.87±0.99	3 (2.13)	9 (6.38)	34 (24.11)	53 (37.59)	42 (29.79)
I am considering postgraduate education in anatomy.	3.51±1.17	9 (6.38)	16 (11.35)	45 (31.91)	35 (24.82)	36 (25.53)
What I learned in the anatomy course allows me to be successful in clinical practice.	4.36±0.67	1 (0.71)	0 (0)	9 (6.38)	69 (48.94)	62 (43.97)
What I learned in the anatomy course increases my curiosity about the clinic.	4.24±0.72	0 (0)	3 (2.13)	15 (10.64)	69 (48.94)	54 (38.3)
I think that anatomy is important in courses related to my profession (taping, pilates, bobath, etc.).	4.5±0.64	0 (0)	2 (1.42)	5 (3.55)	54 (38.3)	80 (56.74)
Anatomy I, Anatomy II and neuroanatomy courses give an idea about what I can encounter in the clinic.	3.53±1.26	12 (8.51)	16 (11.35)	39 (27.66)	33 (23.4)	41 (29.08)

*Duplicate control question, SD: Standard deviation

Table 4. Exploratory factor analysis results of questionnaire ıtems

Items	Factor loadings	Cronbach alpha
Anatomy I, Anatomy II and neuroanatomy courses increase my professional skills.	0.74	0.86
Success in anatomy practice courses is effective in success in clinical practice course.	0.72	
The knowledge achieved in anatomy lessons helps in exercise practice.	0.70	
There is a relationship between the anatomy education given in the curriculum and clinical practice.	0.68	
Anatomy courses are an important topic for clinical applications in physical therapy.	0.63	
Success in anatomy theory courses is effective in success in clinical practice course.	0.61	
Explaining the anatomy and physiotherapy clinic associated with it increases the success in clinical applications.	0.61	
The anatomy course is important in choosing the appropriate treatment for the patient and pathology.	0.59	
What I learn in anatomy classes motivates me for my career.	0.58	
The knowledge gained in anatomy classes helps me during assessment and evaluation practices in physiotherapy.	0.56	
What I learned in the anatomy course increases my curiosity about the clinic.	0.71	0.78
In my professional life, I would like to attend courses and training related to anatomy.	0.66	
What I learned in the anatomy course allows me to be successful in clinical practice.	0.66	
In the physiotherapy curriculum, I would like to have elective courses related to anatomy (such as anatomical palpation and anatomy of pain).	0.66	
I think that anatomy is important in courses related to my profession (taping, pilates, bobath, etc.).	0.62	
Anatomy I, Anatomy II and neuroanatomy courses give an idea about what I can encounter in the clinic.	0.62	
Before the clinical practice course, there should be an accelerated anatomy review course.	0.79	0.78
I reviewed my previous anatomy lecture notes during clinical practice.	0.75	
Anatomy courses should be given again after the 1st and 2nd grades together with the vocational courses.	0.73	
During clinical practice, I found that I had deficiencies in anatomy.	0.70	
Before clinical applications, I need to repeat the anatomy.	0.60	
Kaiser-Meyer-Olkin: 0.825; Bartlett's test approx. chi-square: 1096.26, df: 210, p<0.001		

Table 5. Spearman correlation analysis results of sub-factors

Sub-factors	1	2	3
Factor 1. Students' perceptions of compliance in clinical practice and anatomy courses (3-2-5-13-10-1-11-12-9-6)	1	0.199*	0.464**
Factor 2. Students' perceptions of anatomy learning competencies in clinical practice (33-30-32-29-34-28)		1	0.370**
Factor 3. Students' perceptions of the importance of anatomy in clinical practice (19-18-20-17-14)			1
*p≤0.05, **p≤0.01			

Difference analyzes of the sub-factors obtained from the questionnaire according to the independent variables are given in Table 6. It was determined that students who passed the anatomy course at a single time and students who had experience of working as a health professional had higher average scores in the first factor (Table 6) (p<0.05).

Table 6. The relationship of factor 1 with some participant variables

Variables	Median ± IQR	Minimum-maximum	q25–q75	p
Working status of the student in the field of health (n=141)				
Yes (n=28)	4.80±0.70	3.40-5.00	4.30-5.00	0.013*
No (n=113)	4.50±0.75	3.30-5.00	4.10-4.85	
Retaking anatomy courses (n=141)				
Yes (n=52)	4.35±0.88	3.30-5.00	4-4.88	0.041*
No (n=89)	4.70±0.70	3.60-5.00	4.2-4.9	
*p≤0.05; Mann-Whitney U test, IQR: Interquartile range				

Attitudes Towards Systematic Anatomy Topics

When the students were asked about the anatomy topics that they were successful and most interested in, the answers to these two questions were gathered under the heading of movement system. In addition, when the

students were asked about the anatomy subjects they had the most difficulty with, the answer was the nervous system.

Discussion

In this study, senior physiotherapy students who took the clinical practice course stated that the knowledge and understanding they gained in anatomy courses was an important need during clinical practice. As the answers given to the questionnaire questions reached a good level of reliability coefficient, the effect of anatomy learning on clinical reasoning skills was examined in three factors by taking the answers of the students as reference. It was determined that the students who had a professional experience in the field of health and the students who passed the anatomy course in the first semester, perceived the compliance in clinical practice and anatomy courses more positively. Considering that students with professional experience in the field of health work in health units and are familiar with the anatomy course from their previous learning or working experience, the findings were partially predictable. It has been reported in the literature that healthcare professionals see knowledge of anatomy as a basis for understanding neurological or musculoskeletal disorders (18). Therefore, it was not surprising that students with professional experience in the field of health had a high perception of the compatibility of anatomy courses and clinical practice courses. On the other hand, interestingly, the students who passed the anatomy course in the first semester and were successful perceived the compliance between clinical practice and anatomy courses more positively when compared to the students who repeated the course because they were not successful in the anatomy course. In fact, students who repeated the course because they were not successful in the anatomy course also stated the effective role of anatomy in the clinical practice course with a high average score (4.33 on a 5-point Likert scale). Therefore, although students think that anatomy has an active role in clinical practice, failing and retaking the anatomy course seems to pave the way for the connections between these courses to be perceived at different levels. Some researchers reported differences in approach and perspective to study between undergraduate students who did and did not pass anatomy course (19). In this context, the effect of course success on student perceptions in our current study is similar to the results of the previous study.

Due to the difficult nature of the anatomy course, the anatomy knowledge learned in the course can be forgotten in the months and years following the course (1,20). In our study, senior physiotherapy students stated that they benefited from anatomy knowledge in measurement, evaluation and exercise interventions in physiotherapy, that they repeated the anatomy course, and that the anatomy course should be given with an accelerated curriculum during clinical applications. These results support the need to develop educational strategies that will integrate the anatomy knowledge of the students in the first year into physiotherapy clinical practices. In this way, students' forgetting of anatomy knowledge can be reduced (1), an even anatomy knowledge can develop dynamically until the senior year. In addition, previous studies have revealed some problems in integrating the theoretical knowledge and practical skills acquired by physiotherapy students through preclinical training into the dynamic clinical reasoning process (8,11). As a matter of fact, in a qualitative study investigating the factors affecting clinical reasoning and decision-making among physiotherapists, it was stated that the basic anatomy and physiology knowledge gained in undergraduate physiotherapy studies contributed significantly to physiotherapists in clinical reasoning and solution generation for physiotherapeutic problems (21). Findings from the present study confirmed that a deep knowledge of anatomy contributes to clinical practice course success, development of clinical skills and increasing professional motivation, with the high average of the answers given by the students to the questionnaire questions in this direction.

Anatomy education is the foundation of clinical skills in health science education for treating a human population (5). Physiotherapists have anatomy books as a bedside resource to help them make decisions in certain clinical processes (22). Decisions made before an intervention are influenced by the physiotherapist's individual characteristics, knowledge, and patient perceptions (21). In a previous study, senior physical therapist undergraduates stated that they think effective clinical reasoning depends on a deep theoretical knowledge and cognitive skills, followed by clinical experience (14). In the current study, senior physiotherapy students answered some statements about anatomy knowledge on clinical practice skills with high scores. The students thought that; (1) they need the knowledge they learned in the anatomy course just before or while taking the senior year clinical practice course, (2) the relationship between anatomy course and physiotherapy clinic will increase clinical practice skills and success, (3) anatomy knowledge helps in clinical measurement, evaluation and exercise applications, (4) anatomy is important in individual treatment selection and (5) it is important in postgraduate courses and trainings. Information from the present study was similar to the literature. Physiotherapy undergraduate students are aware of the importance of anatomy education in physiotherapy education and practice (4).

The clinical reasoning process in physiotherapy students is a complex and systematic process that continues both collaboratively and interactively (10). It is also possible to see different results worldwide depending on the focus of the physiotherapy profession in these countries (5). For this reason, the current study findings were compared with the studies conducted in Türkiye. In a study conducted with 50 physiotherapists, it was reported that clinical and functional anatomy integrated with clinical physiotherapy is a necessity and 94% of physiotherapists have ideas that anatomy education should continue after graduation (13). In our study, 67% of physiotherapist students thought that attending courses and trainings related to anatomy in their professional life, and 98% thought that explaining anatomy and physiotherapy clinic by associating them would increase the success of clinical practice. In another study, physiotherapy students emphasized the importance and necessity of anatomy knowledge during electrotherapy practices (2). In our study, 87% of the students stated that what they learned in anatomy courses was effective in electrotherapy applications. The use of electrotherapy applications as a common therapeutic agent in almost every discipline of physiotherapy and student attitudes towards the need for anatomy knowledge while placing electrodes, as stated in the previous study, have been effective on the current results.

Physiotherapy curriculum generally focuses on musculoskeletal anatomy, and physiotherapists are considered key musculoskeletal-focused practitioners with knowledge of human anatomy (6,23,24). Studies have reported that physiotherapists and physiotherapist students demonstrate more knowledge of the musculoskeletal system than some of their medical colleagues (5). It was stated in a previous study conducted with students that the system that physiotherapy students in Türkiye learn best is the musculoskeletal system (6). In addition, when the effect of anatomy education on different physiotherapy disciplines in Türkiye is evaluated, it has been stated that orthopedic rehabilitation comes to the fore in this field (13). Our findings were similar to studies in the literature. Students stated that they were more successful in musculoskeletal system subjects and showed the most interest in these subjects.

This study is limited to the answers of the students collected in the questionnaire. Although the answers of the participants in the research were confirmed by the control question, it was assumed that all students gave sincere answers. Therefore, generalizability was not an aim of this study. However, the findings obtained in the study can be evaluated in terms of the connections between physiotherapy practice courses and anatomy courses. On the other hand, although the participants in this study had taken anatomy, neuroanatomy and clinical practices through face-to-face courses, they had to take vocational courses with distance education for one and a half semesters starting from the spring semester of the 2nd year due to the pandemic. This suggests that this may have had negative effects on students' professional practice skills.

The positive effects of anatomy education on clinical experience and practices are undoubtedly important for physiotherapists. This study confirms the necessity of a knowledge of anatomy in clinical experience with an actual patient, which is a transitional stage to the professional profession for physiotherapy students. Moreover, unlike previous studies, it shows that repeating anatomy courses and having past experiences in the health field can impact student perceptions.

Conclusion

This study shows that giving the anatomy course curriculum starting from the first year including the physiotherapy clinic and giving reminder, short and catchy courses about anatomy during vocational courses can contribute to the completion of an effective learning process. In addition, with this study, it was possible to reflect the contribution of anatomy knowledge on the clinical reasoning skills of senior year physiotherapy students by focusing on the interaction between physiotherapy students' anatomy learning and clinical practice courses. In addition, it should not be forgotten that students' attitudes towards anatomy may change. Attitudes and perceptions towards anatomy knowledge may vary depending on an experience in the field of health or taking the course several times. Future studies may develop new recommendations for anatomy and physiotherapy educators by considering the current results on the importance of anatomy education in clinical practice courses and the limitations of the study.

Acknowledgements

The authors acknowledge their gratitude to all of the participants in this work.

Ethics

Ethics Committee Approval: The approval of Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (decision dated 05.11.2021 and numbered 2021/492).

Informed Consent: Participation in the study was voluntary and all participants read and approved the informed consent form.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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

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

References

1. Dayal M, Owens J, Gibson W, Strkalj G. Anatomical knowledge retention in physiotherapy students: A preliminary assessment. International Journal of Anatomy and Research. 2017;5:3474-3479. [Crossref]
2. Turhan B, Kocamaz D. Importance of Anatomy Education on Electrotherapy Lectures in Physiotherapy and Rehabilitation Curricula: Student Perceptions of a Foundation University. Med Sci Educ. 2021;31:1669-1675. [Crossref]
3. Shead D, Roos R, Olivier B, Ihunwo AO. Gross anatomy curricula and pedagogical approaches for undergraduate physiotherapy students: a scoping review protocol. JBI Database System Rev Implement Rep. 2016;14:98-104. [Crossref]
4. Ojukwu CP, Anyanwu EG, Onyebuchi CB, Uchenwoke CI, Okemuo AJ, Okafor CJ, et al. Anatomy education in physiotherapy training: perceptions of Nigerian-based undergraduate physiotherapy students. Physical Therapy Reviews. 2022;27:60-67. [Crossref]
5. Simons AC, McHugh KM, Appling S, Harris SL, Burgoon JM. Instructional Approaches: Anatomy Education of Physical Therapists. Anat Sci Educ. 2022;15:102-114. [Crossref]
6. Turhan B. Physiotherapy and rehabilitation students' opinions on anatomy education: a cross-sectional survey study. Physiotherapy Quarterly. 2020;28:46-51. [Crossref]
7. Fizyoterapi ve Rehabilitasyon Çekirdek Eğitimi Programı (FTRCEP). Yüksek Öğretim Kurumu; 2016. [Crossref]
8. Christensen N, Black L, Furze J, Huhn K, Vendrely A, Wainwright S. Clinical Reasoning: Survey of Teaching Methods, Integration, and Assessment in Entry-Level Physical Therapist Academic Education. Phys Ther. 2017;97:175-186. [Crossref]
9. Brandt C. Physiotherapy and pelvic floor health within a contemporary biopsychosocial model of care: From research to education and clinical practice. S Afr J Physiother. 2021;77:1538. [Crossref]
10. Sole G, Skinner M, Hale L, Golding C. Developing a framework for teaching clinical reasoning skills to undergraduate physiotherapy students: A Delphi study. New Zealand Journal of Physiotherapy. 2019;47:49-59. [Crossref]
11. Wijbenga MH, Bovend'Eerdt TJH, Driessen EW. Physiotherapy Students' Experiences with Clinical Reasoning During Clinical Placements: A Qualitative Study. Health Professions Education. 2019;5:126-135. [Crossref]
12. Kelly D, Hoang TN, Reinoso M, Joukhadar Z, Clements T, Vetere F. Augmented reality learning environment for physiotherapy education. Physical Therapy Reviews. 2018;23:21-28. [Crossref]
13. Duman F. Importance of anatomy education in physical therapy and rehabilitation in Turkey: A qualitative study. New Trends and Issues Proceedings on Humanities and Social Sciences. 2017;4:64-71. [Crossref]
14. Cruz EB, Moore AP, Cross V. A qualitative study of physiotherapy final year undergraduate students' perceptions of clinical reasoning. Man Ther. 2012;17:549-553. [Crossref]
15. Pickering JD, Swinnerton BJ. Exploring the Dimensions of Medical Student Engagement with Technology-Enhanced Learning Resources and Assessing the Impact on Assessment Outcomes. Anat Sci Educ. 2019;12:117-128. [Crossref]
16. Melguizo C, Prados J, Rodriguez-Serrano F, Hita F, Peran M, Boulaiz H, et al. Anatomy teaching to physiotherapy students: Preliminary study in the European higher education area setting. European Journal of Anatomy. 2007;11:59. [Crossref]
17. Taber KS. The Use of Cronbach's Alpha When Developing and Reporting Research Instruments in Science Education. Research in Science Education. 2018;48:1273-1296. [Crossref]
18. Arráez-Aybar LA, Sánchez-Montesinos I, Mirapeix RM, Mompeo-Corredera B, Sañudo-Tejero JR. Relevance of human anatomy in daily clinical practice. Ann Anat. 2010;192:341-348. [Crossref]
19. Eleazer CD, Scopa Kelso R. Influence of study approaches and course design on academic success in the undergraduate anatomy laboratory. Anat Sci Educ. 2018;11:496-509. [Crossref]
20. Schutte AF. Who is repeating anatomy? Trends in an undergraduate anatomy course. Anat Sci Educ. 2016;9:171-178. [Crossref]
21. Holdar U, Wallin L, Heiwe S. Why do we do as we do? Factors influencing clinical reasoning and decision-making among physiotherapists in an acute setting. Physiother Res Int. 2013;18:220-229. [Crossref]
22. Davies C, Howell D. A qualitative study: Clinical decision making in low back pain. Physiother Theory Pract. 2012;28:95-107. [Crossref]
23. Sytsma TT, Haller EP, Youdas JW, Krause DA, Hellyer NJ, Pawlina W, et al. Long-term effect of a short interprofessional education interaction between medical and physical therapy students. Anat Sci Educ. 2015;8:317-323. [Crossref]
24. Valenza MC, Castro-Martín E, Valenza G, Guirao-Piñeiro M, De-la-Llave-Rincón AI, Fernández-de-las-Peñas C. Comparison of third-year medical and physical therapy students' knowledge of anatomy using the carpal bone test. J Manipulative Physiol Ther. 2012;35:121-126. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.95914

Hamidiye Med J 2023;4(2):103-109

Studying of Vitamin D Receptor Gene Polymorphism in Somali Population Living in Türkiye

Türkiye'de Yaşayan Somali Popülasyonunda D Vitamini Reseptör Gen Polimorfizminin İncelenmesi

Hava Yıldırım[1], Said Mohamed Mohamud[2], Şeyma Zeynep Atıcı[2], Osmanberk Çelik[2], Tuba Köse[1], Burcu Görken[2], Ender Coşkunpınar[1]

[1]*University of Health Sciences Türkiye Faculty of Medicine, Department of Medical Biology, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine, İstanbul, Türkiye*

ABSTRACT

Background: Differences in the vitamin D receptor (*VDR*) gene, which determines the vitamin D activity, have been associated with various diseases. In this study, determination, and comparison of *VDR* gene polymorphisms between Somali individuals who grew up in a different geography, have certain dietary habits and lifestyles, but have lived in Türkiye for at least 2 years and Turkish individuals born and raised in Türkiye was intended.

Materials and Methods: Fifty-five Somali individuals and 100 Turkish volunteers living in Türkiye were included in our study. 2 mL of peripheral blood samples were taken from the volunteers. Genomic DNA was isolated according to the kit protocol. Polymerase chain reaction (PCR) was performed with primers specially designed for the gene regions of interest. After PCR, restriction fragment length polymorphism was performed with appropriate enzymes to determine the genotypes. The results were statistically evaluated with the chi-square test and Student's t-test.

Results: The genotype frequencies for the *VDR* gene (ApaI rs7975232, G>T and TaqI rs731236, T> C) Somali group and Turkish group are listed. There was no significant difference between the two groups (p-value: >0.05).

Conclusion: The distribution of the *VDR* gene TaqI rs731236, T>C genotypes and alleles in the two groups was significantly different (*p-value: *0.006 and *p-value: 0.021).

Keywords: *VDR* gene polymorphism, TaqI, ApaI

ÖZ

Amaç: Bu çalışmada, farklı bir coğrafyada büyümüş, belirli beslenme alışkanlıkları ve yaşam tarzlarına sahip ancak en az iki yıldır Türkiye'de yaşayan Somalili bireyler ile Türkiye'de doğup büyümüş Türk bireyler arasında vitamin D reseptör (*VDR*) gen polimorfizmlerinin belirlenmesi ve karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmamıza Türkiye'de yaşayan 55 Somalili birey ve 100 Türk gönüllü dahil edildi. Gönüllülerden 2 mL periferik kan örneği alındı. Kit protokolüne uygun olarak alınan kan örneklerinden genomik DNA izole edildi. Polimeraz zincir reaksiyon (PCR), ilgili gen bölgeleri için özel olarak tasarlanmış primerler ile yapıldı. PCR işleminden sonra genotipleri belirlemek için uygun enzimlerle kısıtlama parçası uzunluk polimorfizmi yapıldı.

Bulgular: *VDR* geni (ApaI rs7975232, G> T ve TaqI rs731236, T> C) Somali grubu ve Türk grubu için genotip frekansları tablolarda listelenmiştir. İki grup arasında anlamlı fark bulunmadı (p-değeri: >0,05).

Sonuç: *VDR* geni TaqI rs731236, T>C genotipleri ve allellerinin iki gruptaki dağılımı anlamlı olarak farklı bulundu (*p-değeri: *0,006 ve *p-değeri: 0,021).

Anahtar Kelimeler: *VDR* gen polymorfizimi, TaqI, ApaI

Address for Correspondence: Ender Coşkunpınar, University of Health Sciences Türkiye Faculty of Medicine, Department of Medical Biology, İstanbul, Türkiye
Phone: +90 532 240 71 57 E-mail: ecoskunpinar@gmail.com **ORCID ID:** orcid.org/0000-0002-1003-5544

Received: 02.09.2022 **Accepted:** 15.03.2023

Introduction

Vitamin D, which is in the group of fat-soluble vitamins, has two forms that are metabolized in the same way, namely ergocalciferol (D2) and cholecalciferol (D3). Conversion to the active form takes place in the liver and kidney (1). The hormone form regulates the expression of related genes through its receptor. As a result, there is an increase in the level of calcium and phosphate in the serum. During the formation process of vitamin D, 25-hydroxyvitamin D form is synthesized in the liver, and 1α, 25-dihydroxyvitamin D form is synthesized in the kidney (2). There are factors that affect the production of vitamin D in the skin. To these factors, we can count the skin color of the person, the seasons, the choice of clothing, the geographical region where one lives, and even the exposure to the sun at changing times of the day. A certain vitamin D is needed to maintain bone health. Vitamin D deficiency or insufficiency is considered a global problem (3,4).

The vitamin D receptor (*VDR*) gene is located on the q arm of chromosome 12 (*12q13.1*), this gene contains nine exons and eight intron regions (5). It is known that vitamin D plays a role in activities that are not directly related to bone metabolism, such as regulating blood pressure, modulating immunological responses, regulating insulin production, and providing protection against some types of cancer. As a result of studies, it has been revealed that vitamin D is also effective on neurons during the brain development process (6). The VDR plays a role in the regulation of many genomic activities that occur in living things, and thus in the execution of metabolic events. Disruptions in vitamin D metabolism or genetic variations in its binding to the VDR pose a risk for the emergence of some diseases such as autism (6). There have been studies showing that autism rates are increasing among dark-skinned immigrants who migrated to countries located between northern latitudes (7).

Functional polymorphisms affecting gene expression have been reported in this gene (8). It has been proven rs7975232 (ApaI), rs2228570 (FokI) and 3' untranslated region (3'UTR) polymorphisms in the first codon cause epilepsy or schizophrenia, and conditions associated with neurodegenerative diseases (8,9). As a result of studies, the association of polymorphisms with various diseases draws attention to the effectiveness of these genomic changes, but the data is still very limited (10,11). In this study, our aim was to determine VDR polymorphisms in Somali individuals living in Türkiye and to compare them with the Turkish population.

Material and Methods

All procedures were followed in accordance with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was taken from all participants included to the study.

Study Groups

T.C. Ethics Committee permission was granted by Biruni University Clinical Research Ethics Committee with the date of 31.10.2018 and decision number 2015-KAEK-43-18-16. Fifty-five Somali individuals between the ages of 18-26 and 100 Turkish volunteers living in Türkiye for more than 2 years were included in the study. It was confirmed that the individuals participating in the study did not have any chronic health problems.

DNA Isolation

Blood samples were collected from Somali and Turkish participants in purple capped tubes containing EDTA. VDR (DNA) extraction was performed in accordance with the manufacturer's kit protocol (item no: 11796828001 Roche Applied Sciences, Germany). The concentration and purity of DNA obtained after isolation were measured with a spectrophotometer (Denovix DS-11 FX, USA) and DNA samples were placed in a -20 °C refrigerator for storage.

Genotyping

Genotyping processes were performed using restriction fragment length polymorphism as well as polymerase chain reaction (PCR). Two separate PCR reactions were used to detect the two polymorphisms in *VDR* gene (ApaI rs7975232, G> T and TaqI rs731236, T> C). The primers of the *VDR* gene used were as reported, ApaI: Forward 5′ CAGAGCATGGACAGGGAGCAA 3′ and Reverse 5′ GCAACTCCTCATGGCTGAGGTCTC 3′, TaqI: Forward 5′ GGGACGATGAGGGATGGATGGACAGAGC 3′ and Reverse 5′ GGAAAGGGGTTAGGTTGGACAGGA 3′. The ApaI polymorphism PCR cycle conditions were denaturation at 96 °C for 2 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min and one final cycle of extension at 72 °C for 1 min. PCR products were digested with ApaI restriction enzyme at 55 °C for 4 h. The TaqI polymorphism the PCR cycle conditions were denaturation at 96 °C for 2 min, followed by 35 cycles at 94 °C for 45 sec, 60 °C for 45 sec, 72 °C for 45 sec and one final cycle of extension at 72 °C for 10 min. PCR products were digested with Taq I restriction enzyme at 65 °C for 4 h. The digested products were analyzed after running on a 2% agarose gel stained with ethidium bromide and examined under transillumination

(Figure 1, 2). Each gel was evaluated by two observers who were unaware of the subject's condition. In the case of any conflict, the samples were repeated. The expected results after restriction for each gene are also given in Table 1.

Statistical Analysis

To analyze the data, obtained in our study; Fisher's Exact test was used to compare categorical variables and test the separation of genotype frequencies from Hardy Weinberg equilibrium. Comparison of variables between groups was performed using Student's t-test. All statistical analyzes were performed using SPSS V.20.0. (SPSS for Windows, version 20.0. Chicago, USA). A p-value of <0.05 was considered statistically significant.

Results

The gender distribution in Somalian group (60% male and 40% female) was comparable with that in Turkish group (62% male and 38% female, p-value: 0.802). Genotype frequencies for *VDR* gene (ApaI rs7975232, G> T and TaqI rs731236, T> C) Somalian group and Turkish group are listed in Table 2. The distribution of the *VDR* gene (ApaI rs7975232, G> T genotypes and alleles in two groups was not found to be significantly different (p-value: >0.05). The distribution of the *VDR* gene TaqI rs731236, T> C genotypes and alleles in two groups was found to be significantly different (*p-value: *0.006 and *p-value: 0.021).

Discussion

Vitamin D is involved in many biological processes such as bone development, metabolic functioning of the endocrine system, development of immune response, regulation of cell differentiation (12). Due to its complex role in biological processes, it has been the subject of many studies all over the world for a long time. Many variants of the *VDR* gene have been reported in association with different diseases, both due to its importance in the pathophysiology of diseases and its predisposition to many

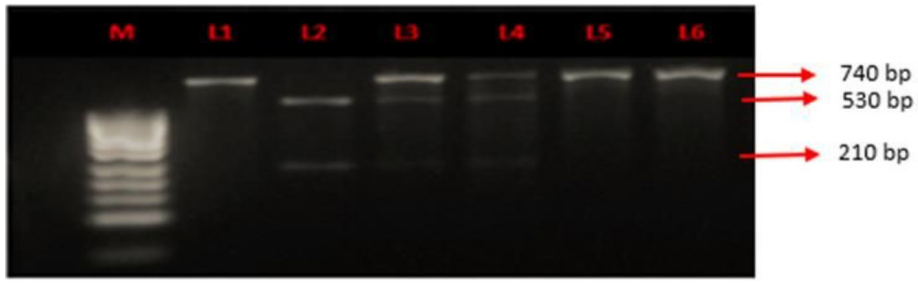

Figure 1. PCR-RFLP to detect (rs7975232, G> T) polymorphism of Apa I. Polymerase chain reaction products (740 bp) digested with restriction enzyme Apa I and analyzed by 2% agarose gel. M: Marker (Puc8X DNA ladder - MBI Fermentas); L1, L5, L6: Wild type homozygotic (GG); L3, L4: Heterozygotic (GT); L2: Homozygotic (TT)

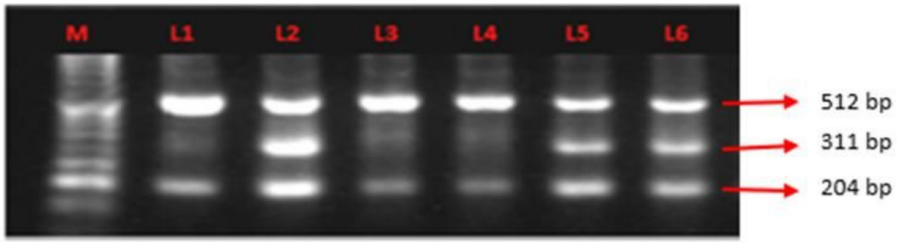

Figure 2. PCR-RFLP to detect (rs731236, T> C) polymorphism of Taq I. Polymerase chain reaction products (512 bp) digested with restriction enzyme Taq I and analyzed by 2% agarose gel. M: Marker (Puc8X DNA ladder - MBI Fermentas); L1, L3, L4: Wild type homozygotic (TT); L2, L5, L6: Heterozygotic (TC)
PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

Table 1. Genotypes and PCR-RFLP product size

Genotypes and product size			
ApaI PCR product is 740 bp	**GG (wild type)**	**GT (heterozygous)**	**TT (homozygous)**
	740 bp	740 bp	740 bp
		530 bp	
	530 bp	210 bp	
TaqI PCR product is 716 bp	**TT (wild type)**	**TC (heterozygous)**	**CC (homozygous)**
	512 bp	512 bp	311 bp
		311 bp	
	204 bp	204 bp	204 bp

RFLP: Restriction fragment length polymorphism, PCR: Polymerase chain reaction

Table 2. Genotype frequencies for *VDR* gene (ApaI rs7975232, G> T and TaqI rs731236, T> C)

	Somalian group		Turkish group			
VDR ApaI	**n**	**%**	**n**	**%**	**X^2**	**p-value**
Genotyping						
GG	29	53.33	51	51	0.545	0.761
GT	25	45.00	45	45		
TT	1	1.67	4	4		
Allele					0.141	0.706
G	83	75.45	147	73.5		
T	27	24.55	53	26.5		
VDR TaqI						
Genotyping						
TT	14	25.45	58	58	9.929	***0.006**
TC	39	70.91	33	33		
CC	2	3.64	9	9		
Allele						
T	67	60.91	149	74,5	5.262	***0.021**
C	43	39.09	51	25.5		

VDR: Vitamin D receptor

diseases. The pathophysiological processes that are aimed to explained by matching epidemiological data with genetic findings make each of these variants important for different societies and diseases. In this study, the *VDR* gene ApaI rs7975232, G> T and TaqI rs731236, T> C genotypes and allele frequencies were reported in a population of healthy Somali and Turkish individuals living in Türkiye.

As indicated in Table 3 and Table 4, the *VDR* gene ApaI rs7975232 genotype and alleles are in the line with McClure et al. (26), Garnero et al. (14), Carling et al. (16), Fountas et al. (17), Riggs et al. (23), Zmuda et al. (24), Haddad (13) findings. Our findings of the TaqI rs731236 genotype and alleles are in line with the findings reported by Kung et al. (19) and Ongphiphadhanakul et al. (21) but not with the findings reported by McClure et al. (26), Garnero et al. (14), Carling et al. (16), Fountas et al. (17), Riggs et al. (23), Zmuda et al. (24), Haddad (13). Especially TaqI rs731236 genotype difference and frequency of T allele show statistically significant difference compared to frequency of C allele (*p-value: 0.000352 *p-value: 0.0127). VDR is known to regulate cell proliferation, intestinal calcium absorption and cell differentiation. The effect of VDR can be regulated not only by vitamin D, but also by protein kinase A, parathyroid hormone, and growth factors. Based on this information, it is predicted that SNP changes in the *VDR* gene may affect calcium metabolism and may predispose to many diseases such as beta thalassemia, diabetes mellitus type-II, breast cancer, osteoporosis, allergic diseases. In addition, some neurodegenerative diseases were found associated with *VDR* gene polymorphism in several studies. According to the

result of one of these studies, reported by Cieślińska et al. (28) in 2017, Taq-I polymorphism appears to be associated with childhood autism (28). In another study by Cieślińska et al. (29) in 2018, Taq-I polymorphism was associated with the development of acute pancreatitis (29). In a study conducted by Koroglu et al. (30) in 2014, it was reported that bronchopulmonary dysplasia was associated with Taq-I polymorphism. The role of *VDR* gene polymorphisms in different diseases have not been understood yet, and the results of the research about predispositions to diseases are variable. The importance of the research about polymorphisms is rely on the genotypic and allelic differences due to the ethnic origin of healthy individuals living in the same geography. This requires genotypic and allelic comparisons of healthy and sick individuals of the same population, and then comparing populations in different geographies. The gene polymorphisms we study on this research are related with many diseases in many populations in different research so far. But there is not any research compares healthy Turkish and Somalian population. As a result of the findings obtained in this study, we believe that conducting these studies with individuals who have diseases that are thought to be related to geography will be useful in understanding the pathogenesis of these diseases. It should be considered that the effect of polymorphisms may be related to differences in the stability of RNA or even changes in a completely different gene, rather than changes in protein structure. At this point miRNAs, which can inhibit the translation of RNA even though they are not coding, and to perform this function on many genes in the genome at the same time, are remarkable. Variables such as geographical differences, lifestyle, dietary habits, and duration of daylight benefit or vice versa, cannot fully explain the personal influences associated with susceptibility to diseases and low vitamin D levels in populations. Although polymorphic alterations are associated with various diseases, these disease susceptibility studies should be continued on large populations to fully understand the cellular and molecular processes. The results of data obtained from these studies should be examined with the meta-analysis method to determine the affection of geographical features on these processes. In addition, studying allelically different haplotypes instead of a single variant may be more decisive in understanding the disease pathophysiology of the

Table 3. Genotypes and allele frequency distribution of *VDR* gene (ApaI) polymorphism in various population and p-values of different allele and genotypes in different populations

Country/ethnicity	No	Age (years)	Genotype (%)				Allele (%)			Reference
			(AA)	(Aa)	(aa)	p	(A)	(a)	p	
Europe										
North India	150	20-74	36	44	20	NS	58	42	NS	13
France	189	31-57	30	50	20	NS	54	46	NS	14
Austria	163	44-78	29	45	26	NS	52	48	NS	15
Sweden	100	70±1	27	52	21	NS	53	47	NS	16
Greece	53	20-70	36	43	21	NS	58	42	NS	17
South Pacific										
Australia	518	NR	26	51	23	NS	51	49	NS	18
Asia										
Türkiye	102		39.2	42.2	18.6	NS	60	40	NS	13
Iran	100		17	56	27	***	45	55	**	13
Japan	488	8-78	9	48	43	***	33	67	**	18
China	144	30-40	10	36	54	***	29	71	***	19
Korea	104	NR	3	28	69	***	17	83	***	20
Thailand	84	40-79	11	50	39	***	36	64	**	21
South India	80	NR	38	46	16	NS	61	39	NS	22
Americas United States										
White, Minnesota	128	>30	30	46	24	NS	53	47	NS	23
Black Pennsylvania	101	≥65	44	46	10	NS	67	33	NS	24
Mexican, California	100	7-12	21	55	24	NS	48	52	NS	25

*p≤0.05, **p≤0.01, ***p≤0.001, at 5% level of significance, NS: Not significant (p>0.05)

Table 4. Genotypes and allele frequency distribution of *VDR* gene (Taq-I) polymorphism in various population and p-values of different allele and genotypes in different populations

Country/ethnicity	No	Age (years)	Genotype (%)				Allele (%)			Reference
			(TT)	(Tt)	(tt)	p	(T)	(t)	p	
Europe										
North India	346	20-74	49	40	11	*	66	34	NS	13
France	189	31-57	33	49	18	0.05	57	43	NS	14
Austria	163	44-78	12	49	39	***	36	64	***	15
Sweden	100	70±1	34	54	12	NS	61	39	NS	16
Greece	53	20-70	38	41	21	NS	59	41	NS	17
South Pacific										
Australia	518	NR	36	48	16	NS	60	40	NS	18
Asia										
Türkiye	102		47	35.2	6.9	*	70	30	NS	13
Iran	100		18	35.5	47	***	35.5	64.5	***	13
Japan	488	8-78	77	22	1	***	88	12	***	18
China	144	30-40	90	10	0	***	95	5	***	19
Thailand	84	40-79	83	17	0	***	92	8	***	21
Americas United States										
White, Minnesota	130	≥30	41	44	15	NS	63	37	NS	23
Black Pennsylvania	101	≥65	32	53	15	0.05	58	42	NS	24
Mexican, California	101	59-84	51	40	9	NS	71	29	NS	26
White, North Carolina	162	NR	33	45	22	*	55	45	NS	27

*p≤0.05, **p≤0.01, ***p≤0.001, at 5% level of significance, NS: Not significant (p>0.05)

possible association of polymorphic changes. Therefore, the data obtained from this study can form the basis for the development of future epidemiological and clinical databases. VDR involved studies of allelic association with various chronic inflammatory and degenerative diseases are ongoing. In the long term, these studies may help to understand genetic susceptibility to diseases and improve clinical management and treatment protocols.

Conclusion

The distribution of the *VDR* gene TaqI rs731236, T> C genotypes and alleles in two groups was found to be significantly different (*p-value: *0.006 and *p-value: 0.021).

Acknowledgments

The authors thank the members of Medical Biology from the University of Health Sciences, for moral and material supports.

Ethics

Ethics Committee Approval: T.C. Ethics Committee permission was granted by Biruni University Clinical Research Ethics Committee with the date of 31.10.2018 and decision number 2015-KAEK-43-18-16.

Informed Consent: Informed consent was taken from all participants included to the study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Y., S.M.M., Ş.Z.A., O.Ç., T.K., B.G., Concept: E.C., Design: E.C., Data Collection or Processing: S.M.M., Ş.Z.A., O.Ç., B.G., Analysis or Interpretation: E.C., Literature Search: H.Y., T.K., E.C., Writing: H.Y., S.M.M., Ş.Z.A., O.Ç., T.K., B.G., E.C.

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

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

References

1. Hymøller L, Jensen SK. 25-hydroxyvitamin D circulates in different fractions of calf plasma if the parent compound is vitamin D_2 or vitamin D_3, respectively. J Dairy Res. 2016;83:67-71. [Crossref]

2. DeLuca HF. Vitamin D: Historical Overview. Vitam Horm. 2016;100:1-20. [Crossref]
3. Greenhagen RM, Frykberg RG, Wukich DK. Serum vitamin D and diabetic foot complications. Diabet Foot Ankle. 2019;10:1579631. [Crossref]
4. Al-Ghafari AB, Balamash KS, Al Doghaither HA. Relationship between Serum Vitamin D and Calcium Levels and Vitamin D Receptor Gene Polymorphisms in Colorectal Cancer. Biomed Res Int. 2019;2019:8571541. [Crossref]
5. Balabanova S, Richter HP, Antoniadis G, Homoki J, Kremmer N, Hanle J, et al. 25-Hydroxyvitamin D, 24, 25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D in human cerebrospinal fluid. Klin Wochenschr. 1984;62:1086-1090. [Crossref]
6. Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front Neuroendocrinol. 2013;34:47-64. [Crossref]
7. Cannell JJ. Autism and vitamin D. Med Hypotheses. 2008;70:750-759. [Crossref]
8. Cui X, Gooch H, Petty A, McGrath JJ, Eyles D. Vitamin D and the brain: Genomic and non-genomic actions. Mol Cell Endocrinol. 2017;453:131-143. [Crossref]
9. Jiang P, Zhu WY, He X, Tang MM, Dang RL, Li HD, et al. Association between Vitamin D Receptor Gene Polymorphisms with Childhood Temporal Lobe Epilepsy. Int J Environ Res Public Health. 2015;12:13913-13922. [Crossref]
10. Guerini FR, Bolognesi E, Chiappedi M, Mensi MM, Fumagalli O, Rogantini C, et al. Vitamin D Receptor Polymorphisms Associated with Autism Spectrum Disorder. Autism Res. 2020;13:680-690. [Crossref]
11. Zhang Z, Li S, Yu L, Liu J. Polymorphisms in Vitamin D Receptor Genes in Association with Childhood Autism Spectrum Disorder. Dis Markers. 2018;2018:7862892. [Crossref]
12. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Renal Physiol. 2005;289:F8-F28. [Crossref]
13. Haddad S. Vitamin-D receptor (VDR) gene polymorphisms (Taq-I & Apa-I) in Syrian healthy population. Meta Gene. 2014;2:646-650. [Crossref]
14. Garnero P, Borel O, Sornay-Rendu E, Delmas PD. Vitamin D receptor gene polymorphisms do not predict bone turnover and bone mass in healthy premenopausal women. J Bone Miner Res. 1995;10:1283-1288. [Crossref]
15. Boschitsch E, Suk EK, Mayr WR, Lang T, Schwartz WM, Panzer S. Genotypes of the vitamin-D-receptor gene and bone mineral density in Caucasoid postmenopausal females. Maturitas. 1996;24:91-96. [Crossref]
16. Carling T, Kindmark A, Hellman P, Holmberg L, Akerström G, Rastad J. Vitamin D receptor alleles b, a, and T: risk factors for sporadic primary hyperparathyroidism (HPT) but not HPT of uremia or MEN 1. Biochem Biophys Res Commun. 1997;231:329-332. [Crossref]
17. Fountas L, Moutsatsou P, Kastanias I, Tamouridis N, Tzanela M, Anapliotou M, et al. The contribution of vitamin D receptor gene polymorphisms in osteoporosis and familial osteoporosis. Osteoporos Int. 1999;10:392-398. [Crossref]
18. Tokita A, Matsumoto H, Morrison NA, Tawa T, Miura Y, Fukamauchi K, et al. Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. J Bone Miner Res. 1996;11:1003-1009. [Crossref]
19. Kung AW, Yeung SS, Lau KS. Vitamin D receptor gene polymorphisms and peak bone mass in southern Chinese women. Bone. 1998;22:389-393. [Crossref]
20. Park BS, Park JS, Lee DY, Youn JI, Kim IG. Vitamin D receptor polymorphism is associated with psoriasis. J Invest Dermatol. 1999;112:113-116. [Crossref]
21. Ongphiphadhanakul B, Rajatanavin R, Chanprasertyothin S, Chailurkit L, Piaseu N, Teerarungsikul K, et al. Vitamin D receptor gene polymorphism is associated with urinary calcium excretion but not with bone mineral density in postmenopausal women. J Endocrinol Invest. 1997;20:592-596. [Crossref]
22. Selvaraj P, Chandra G, Kurian SM, Reetha AM, Narayanan PR. Association of Vitamin D receptor gene variants of Bsm, Apa I and Fok I polymorphisms with susceptibility or resistant to pulmonary tuberculosis. Current Science. 2003;12:1564-1568. [Crossref]
23. Riggs BL, Nguyen TV, Melton LJ 3rd, Morrison NA, O'Fallon WM, Kelly PJ, et al. The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. J Bone Miner Res. 1995;10:991-996.
24. Zmuda JM, Cauley JA, Danielson ME, Wolf RL, Ferrell RE. Vitamin D receptor gene polymorphisms, bone turnover, and rates of bone loss in older African-American women. J Bone Miner Res. 1997;12:1446-1452. [Crossref]
25. Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V. Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. N Engl J Med. 1997;337:77-82. [Crossref]
26. McClure L, Eccleshall TR, Gross C, Villa ML, Lin N, Ramaswamy V, et al. Vitamin D receptor polymorphisms, bone mineral density, and bone metabolism in postmenopausal Mexican-American women. J Bone Miner Res. 1997;12:234-240. [Crossref]
27. Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL, Bell DA. Association of prostate cancer with vitamin D receptor gene polymorphism. Cancer Res. 1996;56:4108-4110. [Crossref]
28. Cieślińska A, Kostyra E, Chwała B, Moszyńska-Dumara M, Fiedorowicz E, Teodorowicz M, et al. Vitamin D Receptor Gene Polymorphisms Associated with Childhood Autism. Brain Sci. 2017;7:115. [Crossref]
29. Cieślińska A, Kostyra E, Fiedorowicz E, Snarska J, Kordulewska N, Kiper K, et al. Single Nucleotide Polymorphisms in the Vitamin D Receptor Gene (VDR) May Have an Impact on Acute Pancreatitis (AP) Development: A Prospective Study in Populations of AP Patients and Alcohol-Abuse Controls. Int J Mol Sci. 2018;19:1919. [Crossref]
30. Koroglu OA, Onay H, Cakmak B, Bilgin B, Yalaz M, Tunc S, et al. Association of vitamin D receptor gene polymorphisms and bronchopulmonary dysplasia. Pediatr Res. 2014;76:171-176. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.64325

Hamidiye Med J 2023;4(2):110-116

The Effect of the Level of Serum C-reactive Protein on Proteinuria and Lipid Values, Echocardiography Findings, and Clinical Course in Adult Patients with Nephrotic Syndrome

Erişkin Nefrotik Sendromlu Hastalarda C-reaktif Protein Seviyesinin Proteinüri, Lipid Değerleri, Ekokardiografi Bulguları ve Klinik Gidiş Değişkenleri Üzerine Etkisi

İdris Yıldırım[1], Zeki Kemeç[2], Mehmet Emin Yılmaz[3]

[1]*University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Gastroenterology, İstanbul, Türkiye*

[2]*Batman Training and Research Hospital, Clinic of Nephrology, Batman, Türkiye*

[3]*Dicle University Faculty of Medicine, Department of Nephrology, Diyarbakır, Türkiye*

ABSTRACT

Background: This study investigates the effect of the level of serum C-reactive protein (CRP) on proteinuria and lipid values, echocardiography (ECHO) findings, and clinical course in adult patients with nephrotic syndrome (NS).

Materials and Methods: All medical records of 1440 patients hospitalized with the NS diagnosis in the Nephrology Clinic of Dicle University between 2000 and 2010 and whose treatment was started after being followed up, were scanned within the scope of this study and files of 104 patients, who were considered to have sufficient data were reviewed retrospectively. Study variables include demographic data, physical examination findings at admission, blood count parameters, biochemical parameters, proteinuria level, ECHO findings, and length of hospital stay. According to their serum CRP level, patients were divided into two groups to evaluate the relevant variables: Patients with a CRP level above 5 mg/L were defined as group I, and patients with a CRP level equal to or above 5 mg/L were accepted as group II. The normal range of CRP in the laboratory of our hospital was 0-5 mg/L.

Results: The following results were obtained as a result of the comparison of the groups according to their serum CRP levels: 124-hour urine (volume) (p=0.003), serum calcium (p=0.001), albumin (p=0.001), total protein (p=0.035), high-density lipoprotein (p=0.038) and hemoglobin (p=0.032) levels at hospitalization were lower significantly in group I compared to group II. 2- Length of hospital stay (p=0.030), creatinine (p=0.009), lactate dehydrogenase (p=0.006), platelet (p=0.005) and spot urinary protein (p=0.038) level in group I was significantly higher than group 2.

Conclusion: In adult NS patients, an increase in proteinuria, deterioration in kidney functions, a decrease in daily urine volume, a prolonged hospitalization period, and a decrease in serum albumin levels has an association with high serum CRP levels. In this study, no significant correlation was found between CRP value and cardiac parameters left atrium dilatation, ratio of current velocities E and A, left ventricular posterior wall thickness at end diastole, ejection fraction measured in echocardiography.

Keywords: C-reactive protein, nephrotic syndrome, proteinuria, lipid values, echocardiographic findings

ÖZ

Amaç: Bu çalışmada, serum C-reaktif protein (CRP) seviyesinin erişkin nefrotik sendrom (NS) tespit edilen olgularda proteinüri ve lipid değerleri, ekokardiyografi (EKO) bulguları ile klinik gidişat değişkenlerine etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışma kapsamında, 2000-2010 yıllarında Dicle Üniversitesi Tıp Fakültesi Nefroloji Kliniği'nde NS tanısıyla yatırılmış, tedavi süreci başlamış bütün olgulara ait dosyalar (1440 hasta) taranmış, toplam 104 hastaya ait dosyalar retrospektif bir şekilde incelenmiştir. Çalışma değişkenleri, hasta demografik verileri, kliniğe yatıştaki fizik muayene bulguları, hemogram ve biyokimyasal parametreler, proteinüri düzeyi, EKO bulguları ve hastanede yatış süresi idi. Hastalar, ilgili değişkenlerin değerlendirilmesi

Address for Correspondence: İdris Yıldırım, University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Gastroenterology, İstanbul, Türkiye

Phone: +90 505 710 81 22 E-mail: idrisyildirim2000@yahoo.com **ORCID ID:** orcid.org/0000-0001-7887-2886

Received: 18.11.2022 **Accepted:** 28.03.2023

ÖZ

amacıyla serum CRP düzeyine göre iki gruba ayrıldı: CRP seviyesi 5 mg/L üzerinde olan hastalar grup I, CRP seviyesi 5 mg/L'ye eşit ve üzerinde olan hastalar grup II olarak belirlendi. Hastanemiz laboratuvarında normal CRP aralığı 0-5 mg/L idi.

Bulgular: Grupların serum CRP seviyelerine göre karşılaştırılması aşağıdaki sonuçları ortaya çıkardı: 1- Grup I'de kliniğe yatışta kontrol edilen 24 saatlik idrar (hacmi) (p=0,003), serum kalsiyum (p=0,001), albümin (p=0,001), total protein (p=0,035), yüksek dansiteli lipoprotein (HDL) (p=0,038) ve hemoglobin (p=0,032) seviyeleri, grup II'ye göre anlamlı derecede düşüktü. 2- Grup 1'de, yatış süresi (p=0,030), kreatinin (p=0,009), laktat dehidrogenaz (p=0,006), trombosit (p=0,005) ve spot idrar protein (p=0,038) seviyeleri grup 2'ye kıyasla anlamlı derecede yüksekti.

Sonuç: Erişkin NS'li hastalarda proteinüri düzeyinde artış, böbrek işlevlerinde bozulma, günlük idrar miktarında düşme, hastane yatış süresinde uzama ve serum albümin düzeyinde düşme, yüksek serum CRP seviyesi ile ilişkilidir. Araştırmamızda CRP değeri ile EKO ile ölçümlenen kardiyak parametrelerin sol atrium dilatasyonu, E ve A akım hızları oranı, dilatasyonda sol ventrikül arka duvar kalınlığı, ejeksiyon fraksiyonu arasındaki ilişkinin anlamlı olmadığı görülmüştür.

Anahtar Kelimeler: C-reaktif protein, nefrotik sendrom, proteinüri, lipid değerleri, ekokardiyografi bulguları

Introduction

Nephrotic syndrome (NS) is one of the clinical syndromes that lead to various complications, such as edema and hyperlipidemia. The definition of this syndrome includes massive proteinuria of more than 40 mg/m^2 per hour, which leads to hypoalbuminemia of less than 30 g/L, caused by increased permeability of the basal membrane damaged in the kidney glomeruli, primarily due to infectious or thromboembolic factors. It can occur primarily due to a kidney-specific disease, or may develop as a result of glomerular permeability abnormality due to diabetes, congenital infections, neoplasia, systemic lupus erythematosus, or a particular use of a drug. There is a trigger factor such as an upper respiratory tract infection in nearly 50% of the cases, an allergic reaction in one-third, and less frequently, an insect bite, treatment with psychiatric drugs, and vaccination. The first causes include focal glomerulosclerosis, minimal change nephropathy, hereditary nephropathies, and membranous nephropathy. Diabetes mellitus (DM) is the second cause. Immune causes include antibody vasculitis, systemic lupus erythematosus, Goodpasture's syndrome, Berger's disease, membranoproliferative or extramembranous glomerulonephritis, acute infectious glomerulonephritis, toxicity (non-steroidal anti-inflammatory drugs), alloantibodies due to enzyme replacement therapy, and thrombotic microangiopathy. Infectious causes include hepatitis B and C virus, hepatitis, HIV, immunodeficiency, toxoplasmosis, cytomegalovirus, and parvovirus B1. In addition, preeclampsia, paraproteinemia, and amyloidosis can be counted among the causes. The most common cause of NS in children is minimal changes in glomerulonephritis; in white adults, it is membranous nephropathy. However, in African populations, the main cause is focal segmental glomerulosclerosis. Clinical data shows that the NS could be frequently recurrent, steroid-sensitive, steroid-resistant, and steroid-dependent. The creation of phospholipase antibodies, the deposition of the immune complex, or the formation of alloantibodies are among the causes of NS syndrome (1,2,3,4,5,6).

C-reactive protein (CRP) is a major acute phase reactant that is seen to rise acutely and rapidly in case of infection and tissue damage in the human body which is among the non-specific laboratory findings. It triggers hepatic production in cases of various tissue damage, infection and inflammation. The American Heart Association recommends determining serum hs-CRP levels in all patients at risk for cardiovascular disease. Normal levels of CRP are mostly 2 mg/L or less. Standard methods make it possible to measure CRP in the range of 3-8 mg/L. It is possible to detect CRP levels below this limit with current "high sensitivity" (hs-CRP) methods. Today, hs-CRP measurements are used in risk determination. The diagnostic value of CRP, which is one of the non-specific indicators for inflammation, is quite high in many clinical situations. In line with today's accepted values; a CRP value below 1 mg/L is low risk, 1-3 mg/L is moderate risk, and above 3 mg/L is high risk. In many studies, CRP is used as an activity indicator and clinical course predictor for different diseases (7,8,9,10).

Material and Methods

A total of 1440 patient files hospitalized with the NS diagnosis and followed up and treated in the Nephrology Service of Dicle University between 2000 and 2010 were retrospectively analyzed. One hundred-four patients, with sufficient data, were included in the study. Informed consent was obtained from all patients. This research, which is a thesis study, was carried out following the Local Ethics Committee of Batman University approval dated 04.02.2021 and numbered 3671 and the Declaration of Helsinki.

Inclusion Criteria:

- Those with NS with a histopathological diagnosis based on primary or secondary causes.
- Diabetic patients with proteinuria in the nephrotic range.
- Patients with adequate parameters for the study.

Exclusion Criteria:

- Patients without histopathological diagnosis.
- Patients with malignancy.
- Patients with signs of acute coronary syndrome or heart failure.
- Patients with cerebrovascular disease.
- Patients with active connective tissue disease.
- Patients with a chronic inflammatory disease with acute exacerbation.
- Patients with signs of infection.

Study parameters include; histopathological diagnoses, demographic data, amount of fluid taken in and out from clinical admission until discharge, arterial blood pressure, blood count parameters [hemoglobin, white blood cell (WBC), platelet, hematocrit], biochemical parameters [glucose, urea, uric acid, sodium (Na), calcium (Ca), creatinine (Kr), chlorine (Cl), magnesium (Mg), potassium (K), aspartate transaminase (AST), phosphorus (P), alanine transaminase, lactate dehydrogenase (LDH), high-density lipoprotein (HDL), cholesterol, triglyceride (TG), albumin, low-density lipoprotein (LDL), CRP, total protein], fibrinogen, immunoglobulin panel [immunoglobulin M (IgM), immunoglobulin A (IgA), immunoglobulin G (IgG)], complement proteins (C3, C4), erythrocyte sedimentation rate (ESR), 24-hour urine protein, urine analysis (urine leukocytes, protein, erythrocytes and urine density), echocardiogram findings [ejection fraction (EF), left atrium thickness (LAD), interventricular septum thickness (EA), left ventricular posterior wall thickness (SVPDK)] and length of hospital stay. All study parameters are shown in Table 1.

We divided the patients into two groups: CRP level >5 mg/L in group 1 and CRP level ⩽5 mg/L in group 2. Data collected from both groups were compared and analyzed. In addition, the serum CRP and other study variables of the patients at admission to hospital and discharge were compared within each group, and the relationship between them was analyzed.

Ethical statement: The Non-Interventional Clinical Research Ethics Committee of Batman University approved the permission for this study with a letter dated 04/02/2021 and numbered 3671, and the study was carried out following the Helsinki Declaration criteria.

Statistical Analysis

Study data were analyzed in computer virtual environment with SPSS for Windows 20.0 software program. For comparisons between groups, chi-square and Student's t-tests were used for independent variables, and Wilcoxon signed-rank test (Wilcoxon signed-rank test) was used for dependent variables. Pearson correlation analysis was used to compare dependent variables and serum CRP levels. Data were expressed as mean ± standard deviation. Data were analyzed with a 95% confidence interval. The p-value <0.05 was considered statistically significant.

Results

The study participation includes a total of 104 patients. Of the study participants, 53% (55) were female, and 47% (49) were male. While the mean serum CRP level in group I was 31.70±21.75 mg/L, it was 2.21±1.58 mg/L in group II.

Table 1. Comparison of the clinical admission and discharge variables of the groups

	Group 1 (n=49)			Grup 2 (n=55)		
Variables	**Clinical admission**	**Clinical discharge**	**p**	**Clinical admission**	**Clinical discharge**	**p**
CRP (mg/L)	31.70±21.75	20.62±16.23	<0.001	2.21±1.58	1.73±1.51	0.002
SBP (mm/Hg)	126.09±18.11	113.53±17.82	<0.001	125.00±23.12	116.26±16.70	<0.001
DBP (mm/Hg)	75.36±10.20	71.58±10.51	0.018	76.11±12.55	70.95±10.07	0.001
Urea (mg/dL)	84.63±60.75	66.12±36.68	0.031	63.44±58.60	53.79±43.77	0.122
Kr (mg/dL)	2.56±2.49	2.04±1.57	0.118	1.55±1.40	1.43±1.45	0.169
Albumin (g/dL)	1.86±1.05	1.82±0.97	0.445	2.60±1.02	2.55±0.89	0.476
LDL (mg/dL)	195.29±119.01	157.21±94.07	<0.001	155.06±88.80	140.96±71.77	0.009
Cholesterol (mg/dL)	289.85±138.48	243.21±106.65	<0.001	249.60±94.31	230.82±76.75	0.002
TG (mg/dL)	238.53±126.16	192.02±91.41	<0.001	219.01±117.33	205.41±104.20	0.039
24-hour protein (mg/day)	6031.70±3367.30	4778.12±3936.80	0.001	4811.61±4079.16	3402.55±3380.13	<0.001

CRP: C-reactive protein, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Cr: Creatinine, LDL: Low-density lipoprotein, TG: Triglyceride

The difference between the groups was statistically significant (p<0.001). The age range of the patients was determined as 18-80 years. The patients' mean age with normal serum CRP level (group 2) and high CRP level (group 1) were 35.92±16.25 years and 46.68±20.34 years, respectively. It was observed that those with higher serum CRP levels were older, and the difference was statistically significant (p=0.004). Renal histopathological reports of 104 patients who were diagnosed between 2000-2010 and whose data matched the research criteria were also obtained. Distribution of cases according to the NS subtype is shown in the table (Table 2).

Table 2. Distribution of patients by nephrotic syndrome subtypes

Primary NS		Secondary NS	
	n		n
FSGS	19	DM	17
MN	14	Amiloidosis	18
MPGN	16	SLE	13
MDH	3		
IgAN	3		
RPGN	1		

n: Number of patients, FSGS: Focal segmental glomerulosclerosis, MN: membranous glomerulonephritis, MPGN: Membranoproliferative glomerulonephritis, MDH: Minimal change disease, IgAN: IgA nephropathy, RPGN: Rapidly progressive glomerulonephritis, DM: Diabetes mellitus, SLE: systemic lupus erythematosus

Daily amount of proteinuria (p=0.003), albumin (p=0.001), Ca (p=0.001), total protein (p=0.035), HGB (p=0.032) and HDL (p=0.038) serum level at hospitalization were statistically significantly lower in group 1 than in group 2 (Table 3).

Again, Length of hospital stay (p=0.030), age (p=0.004), creatinine (p=0.009), platelets (p=0.005), LDH (p=0.006), ESR (p<0.001), spot urine protein (p=0.038), C4 (p<0.001) and 24-hour proteinuria (p<0.001) levels in group I were statistically significantly higher than group II. However, although serum LDL (p=0.051) levels were high, there was no significant difference between the groups (Table 2).

Serum CRP (p<0.001), diastolic blood pressure (DBP) (p=0.018), systolic blood pressure (SBP) (p<0.001), cholesterol (p<0.001), urea (p=0.031), TG (p<0.001), LDL (p<0.001) and proteinuria (p=0.001) levels measured at hospital admission in group 1 were found to be significantly higher than the values measured at discharge (Table 3).

When the values at hospital admission were compared with values at discharge in group 2, statistically significant elevation in variables such as CRP (p=0.002), DBP (p=0.001), SBP (p<0.001), TG (p=0.039), cholesterol (p=0.002), LDL (p=0.009), daily proteinuria was found (Table 3).

In the evaluation of correlation analysis of serum CRP level with other study variables, a positive correlation was

Table 3. Comparison of group parameters

Variables	Group 1 (n=49)	Group 2 (n=55)	p
Clinical hospitalization urine amount (volume) (mL)	1162.19±693.52	1586.50±695.73	0.003
Ca (mg/dL)	8.13±0.65	8.59±0.70	0.001
Albumin (g/dL)	1.86±1.05	2.60±1.02	0.001
Total protein (g/dL)	5.19±1.04	5.68±1.19	0.035
HDL (mg/dL)	43.87±14.35	51.60±20.50	0.038
HGB (g/dL)	11.64±2.62	12.66±2.14	0.032
CRP (mg/L)	31.70±21.75	2.21±1.58	<0.001
Lenght of hospitalization (days)	11.21±10.06	7.50±7.10	0.030
Age (years)	46.68±20.34	35.92±16.25	0.004
Creatinine (mg/dL)	2.56±2.49	1.55±1.40	0.009
LDH (U/L)	314.02±152.88	248.36±87.93	0.006
Platelets ($x10^9$/L)	347.29±143.58	285.42±76.30	0.005
ESR (mm/h)	70.51±30.29	38.53±27.28	<0.001
C4 (mg/dL)	31.68±11.80	23.88±9.43	<0.001
Urine protein (mg/day)	438.41±137.85	363.88±197.55	0.038
LDL (mg/dL)	195.29±119.01	155.06±88.80	0.051
24-hour protein (mg/day)	6953.65±2791.69	3948.12±2550.09	<0.001

n: Number of patients, CRP: C-reactive protein, Cr: Creatinine, LDH: Lactate dehydrogenase, ESR: Erythrocyte sedimentation rate, C4: complement 4, LDL: Low-density lipoprotein, Ca: Calcium, HDL: High-density lipoprotein, HGB: Hemoglobin

found between CRP elevation and creatinine (p=0.003), urea (p=0.015), LDH (p=0.015), ESR (p=0.004), WBC (p=0.004), C4 (p=0.023), urinary erythrocyte (p=0.041), urinary leukocytes (p=0.032), daily proteinuria (p=0.044) levels. However, a negative correlation was found between CRP elevation and 24-hour urine (p=0.043). The relationship between echocardiography findings (EF, LAD, SVPDK, EA) and serum CRP level in patients with NS is not statistically significant (p>0.05).

Discussion

As an acute phase reactant, CRP increases secondary to infection and tissue damage. The values, which reach the peak levels within 1-2 days, decrease to the normal level with the restoration of the tissue structure and function (11). CRP is used to determine the response to treatment, to evaluate the course of the infection, and to detect the inflammatory response in chronic rheumatological diseases such as vasculitis and rheumatoid arthritis (12). High serum CRP level was associated with macro and microalbuminuria independent of hypertension, DM and other potential factors (p<0.001) (13). Our study found a positive correlation between spot urine protein and serum CRP levels (p=0.038). Similar to our study, it has been determined in another study that the increase in serum CRP level is associated with the incidence and prevalence of proteinuria (p=0.042) (14). In other studies, the rate of nephropathy was higher and the development time of nephropathy was shorter in diabetic patients with high hsCRP levels than those with normal hsCRP levels (15). High CRP levels in type 2 diabetic patients have been associated with an increased prevalence of albuminuria (16). Hs-CRP was found to be independently associated with diabetic nephropathy (17). The diabetic nephropathy patients' hs-CRP concentrations were significantly higher than the control group, which includes DM patients without nephropathy and healthy people. Moreover, hs-CRP concentration in the macro albuminuria group was significantly higher compared to the microalbuminuria group and the non-albuminuria group (18). Diabetic patients with complications had significantly higher hs CRP and microalbuminuria than uncomplicated diabetic patients and the control group (19). the results showed that patients with decreased hs-CRP have a lower risk of decline in kidney development and function of proteinuria (20). It has been understood in our study that the amount of 24-hour urine protein was significantly higher in the high CRP value group than the low CRP value group (<0.001). A positive correlation was found between CRP and proteinuria (p=0.044), urea (p=0.015), creatinine (p=0.003) levels in cases with nephrotic proteinuria. Low-grade inflammatory markers (hsCRP, IL-6) have been associated with diabetic nephropathy in type 1 diabetic patients (21). One study showed that both MBL and hsCRP concentrations are associated with progression of kidney disease in type 1 diabetes (22). The hs-CRP cumulative exposure has been associated with following CKD increased risk and is helpful in risk estimation (23). In a different study, CRP, serum amyloid A and IL-6, which are acute phase indicators, were associated with diabetic nephropathy and glomerular basement membrane thickness (p<0.005) (24). However, in a study of Tencer et al. (25), no statistically significant relationship was found between the increase in proteinuria and the CRP level in 166 cases with glomerulonephritis (MPGN, MN, IgAN) (p>0.005). hs-CRP, s-albumin, and WBC are inflammatory markers and studies have been conducted showing them to be associated with the progression of IgAGN (26). Previous studies show the significant role of inflammation in increasing the risk of cardiovascular diseases. Regarding hs-CRP, some studies show that this inflammatory index can predict long-term cardiovascular risk, enriches traditional risk assessment with prognostic information, and predict cardiovascular risk not reflected by traditional risk factors (27). In one study, high plasma hs-CRP and IL-6 levels were found to be associated with LVH and systolic dysfunction in patients with CKD (28). Independent of cardiovascular risk factors, high hs-CRP level was associated with microalbuminuria. Additionally, high hs-CRP levels were associated with an increased risk of developing microalbuminuria in people with CVD risk factors (29). The relationship between CRP and echocardiography indicators (LAD, EA, SVPDK, EF) was not statistically significant (p>0.05). In the study, it was observed that the systolic (p<0.001) and diastolic (p=0.018) blood pressure values measured before discharge in high CRP levels patients were significantly lower than the values measured during their hospitalization. It is thought that the fact that the patients are in the active phase of NS and the use of diuretic, anti-proteinuric and reno-protective drugs in addition to immunosuppressive drugs during the hospitalization period may also be effective. Ueland et al. (30) observed that the hs-CRP level is significantly increased in patients with familial hypercholesterolemia. It was observed that hs-CRP level remained high in these cases, despite the anti-hyperlipidemic treatment (pravastatin 20-40 mg/g) (30). On the other hand, it was observed in this study that cholesterol (p=0.081), TG (p=0.423), LDL (p=0.051) levels were higher in patients with high CRP levels compared to patients with normal CRP levels. However, this relationship was not found to be statistically significant. In our study, it was determined that the cholesterol, TG, LDL levels measured during the discharge process of the patients in group I were significantly lower than the values measured

during the hospitalization period (p<0.001). It is thought that this may be associated with the significant decrease in the level of CRP and proteinuria due to anti-hyperlipidemic and immunosuppressive treatment given during hospitalization. It was also determined that a positive correlation is between CRP level and the length of stay in the hospital rather than a statistically significant relationship (r=0.134, p=0.178).

Conclusion

The present study found that an increase in proteinuria, deterioration in kidney functions, decrease in daily urine volume, prolonged hospitalization and decrease in serum albumin levels in adult NS patients are associated with high serum CRP level and CRP level can be used as a fine parameter for the follow-up of patients. In our study, it was determined that the relationship between the level of CRP and cardiac parameters measured by Echocardiography (LAD, EA, SVPDK, EF) was not statistically significant.

Ethics

Ethics Committee Approval: The Non-Interventional Clinical Research Ethics Committee of Batman University approved the permission for this study with a letter dated 04/02/2021 and numbered 3671, and the study was carried out following the Helsinki Declaration criteria.

Informed Consent: Informed consent was obtained from all patients.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: İ.Y., M.E.Y., Design: İ.Y., Z.K., M.E.Y., Data Collection or Processing: İ.Y., Analysis or Interpretation: İ.Y., Literature Search: İ.Y., Z.K., Writing: İ.Y., Z.K., M.E.Y.

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

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

References

1. Tapia C, Bashir K. Nephrotic Syndrome. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023. [Crossref]
2. Hill AJ, Stone DE, Elliott JP, Gerkin RD, Ingersoll M, Cook CR. Management of Nephrotic Syndrome in the Pregnant Patient. J Reprod Med. 2016;61:557-561. [Crossref]
3. Raina R, Krishnappa V. An update on LDL apheresis for nephrotic syndrome. Pediatr Nephrol. 2019;34:1655-1669. [Crossref]
4. Dumas De La Roque C, Prezelin-Reydit M, Vermorel A, Lepreux S, Deminière C, Combe C, et al. Idiopathic Nephrotic Syndrome: Characteristics and Identification of Prognostic Factors. J Clin Med. 2018;7:265. [Crossref]
5. Dumas De La Roque C, Combe C, Rigothier C. Actualité sur les mécanismes physiopathologiques des syndromes néphrotiques idiopathiques : lésions glomérulaires minimes et hyalinose segmentaire et focale [Up to date of pathophysiology mechanism of idiopathic nephrotic syndromes: Minimal change disease and focal and segmental glomerulosclerosis]. Nephrol Ther. 2018;14:501-506. [Crossref]
6. Kodner C. Nephrotic syndrome in adults: diagnosis and management. Am Fam Physician. 2009;80:1129-1134. [Crossref]
7. Matsuo S, Tsumori M, Yamamoto Y, Takahashi H. [Clinical and laboratory correspondence to outpatients with the extreme value of C-reactive protein]. Rinsho Byori. 1992;40:1307-1311. [Crossref]
8. Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. Clin Sci (Lond). 2005;108:205-213. [Crossref]
9. Kılıçturgay K: Enflamasyonun akut faz cevabıyla izlenmesi. immunoloji, 3rd ed. Nobel kitapevleri: İstanbul; 2003;226. [Crossref]
10. Acartürk E. C-Reaktif Protein ve Koroner Arter Hastaliği [C-reactive protein and coronary artery disease]. Anadolu Kardiyol Derg. 2004;4:203-204. [Crossref]
11. Kushner I, Ballow SP. Laboratory evaluation of inflammation (erythrocyte sedimentation rate and the acute phase reactans). İn: Kelley W, Harris E, Ruddy S, editors. Textbook of Rheumatology. 5th ed. Philadelphia: W.B. Saunders Company; 1997;699-705. [Crossref]
12. Marnell L, Mold C, Du Clos TW. C-reactive protein: ligands, receptors and role in inflammation. Clin Immunol. 2005;117:104-111. [Crossref]
13. Sabanayagam C, Lee J, Shankar A, Lim SC, Wong TY, Tai ES. C-reactive protein and microalbuminuria in a multi-ethnic Asian population. Nephrol Dial Transplant. 2010;25:1167-1172. [Crossref]
14. Iseki K, Iseki C, Kinjo K. C-reactive protein is a predictor for developing proteinuria in a screened cohort. Nephron Clin Pract. 2011;117:c51-c56. [Crossref]
15. Sinha SK, Nicholas SB, Sung JH, Correa A, Rajavashisth TB, Norris KC, et al. hs-CRP Is Associated With Incident Diabetic Nephropathy: Findings From the Jackson Heart Study. Diabetes Care. 2019;42:2083-2089. [Crossref]
16. Abrahamian H, Endler G, Exner M, Mauler H, Raith M, Endler L, et al. Association of low-grade inflammation with nephropathy in type 2 diabetic patients: role of elevated CRP-levels and 2 different gene-polymorphisms of proinflammatory cytokines. Exp Clin Endocrinol Diabetes. 2007;115:38-41. [Crossref]
17. Varma V, Varma M, Varma A, Kumar R, Bharosay A, Vyas S. Serum Total Sialic Acid and Highly Sensitive C-reactive Protein: Prognostic Markers for the Diabetic Nephropathy. J Lab Physicians. 2016;8:25-29. [Crossref]
18. Liu Q, Jiang CY, Chen BX, Zhao W, Meng D. The association between high-sensitivity C-reactive protein concentration and diabetic nephropathy: a meta-analysis. Eur Rev Med Pharmacol Sci. 2015;19:4558-4568. [Crossref]
19. El Boukhrissi F, Benbella I, Ouleghzal H, Safi S, Bamou Y, Balouch L. Evaluation of ultrasensitive CRP and microalbuminuria as cardiovascular risk markers in type 2 diabetic moroccan patients. Tunis Med. 2017;95:982-987. [Crossref]
20. Liu L, Gao B, Wang J, Yang C, Wu S, Wu Y, et al. Reduction in Serum High-Sensitivity C-Reactive Protein Favors Kidney Outcomes in Patients with Impaired Fasting Glucose or Diabetes. J Diabetes Res. 2020;2020:2720905. [Crossref]
21. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH. Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients. Diabetologia. 2003;46:1402-1407. [Crossref]
22. Hansen TK, Forsblom C, Saraheimo M, Thorn L, Wadén J, Høyem P, et al. Association between mannose-binding lectin, high-sensitivity C-reactive protein and the progression of diabetic nephropathy in type 1 diabetes. Diabetologia. 2010;53:1517-1524. [Crossref]
23. Gao J, Wang A, Li X, Li J, Zhao H, Zhang J, et al. The Cumulative Exposure to High-Sensitivity C-Reactive Protein Predicts the Risk of Chronic Kidney Diseases. Kidney Blood Press Res. 2020;45:84-94. [Crossref]

24. Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, Saller A, et al. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. J Am Soc Nephrol. 2005;16:S78-S82. [Crossref]
25. Tencer J, Thysell H, Westman K, Rippe B. Elevated plasma levels of acute phase proteins in mesangioproliferative glomerulonephritis, membranous nephropathy and IgA nephropathy. Scand J Urol Nephrol. 1995;29:5-9. [Crossref]
26. Kaartinen K, Syrjänen J, Pörsti I, Hurme M, Harmoinen A, Pasternack A, et al. Inflammatory markers and the progression of IgA glomerulonephritis. Nephrol Dial Transplant. 2008;23:1285-1290. [Crossref]
27. Ridker PM. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: from concept to clinical practice to clinical benefit. Am Heart J. 2004;148:S19-S26. [Crossref]
28. Gupta J, Dominic EA, Fink JC, Ojo AO, Barrows IR, Reilly MP, et al. Association between Inflammation and Cardiac Geometry in Chronic Kidney Disease: Findings from the CRIC Study. PLoS One. 2015;10:e0124772. [Crossref]
29. Yang SK, Liu J, Yi B, Mao J, Zhang XM, Liu Y, et al. Elevated High Sensitivity C-Reactive Protein Increases the Risk of Microalbuminuria in Subjects With Cardiovascular Disease Risk Factors. Ther Apher Dial. 2017;21:387-394. [Crossref]
30. Ueland T, Vissers MN, Wiegman A, Rodenburg J, Hutten B, Gullestad L, et al. Increased inflammatory markers in children with familial hypercholesterolaemia. Eur J Clin Invest. 2006;36:147-152. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.92005

Hamidiye Med J 2023;4(2):117-122

Long-term Effects of Diving on Corneal Tomography, Anterior Chamber Depth, and Axial Length of the Eye

Sualtı Dalışın Kornea Tomografisi, Ön Kamara Derinliği ve Gözün Aksiyal Uzunluğu Üzerindeki Uzun Dönem Etkileri

Belma Kayhan, Nur Demir

Ministry of Health, İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Ophthalmology, İstanbul, Türkiye

ABSTRACT

Background: The aim of the study was to investigate the effects of long-term SCUBA and deep diving on corneal tomographic parameters, anterior chamber depth, and axial length of the eye in professional divers.

Materials and Methods: This cross-sectional, comparative study comprised military divers and non-diver military personnel. The diver group was divided into two groups, the SCUBA group, which dives up to 40 m with half-face masks, and the deep diver group, which dives deeper than 40 m with diving helmets. Healthy military personnel who didn't dive constituted the control group. A complete ophthalmological examination, corneal tomography, pachymetry, anterior chamber depth, and axial length measurements were made in all participants with a Scheimpflug camera and IOLMaster 500 partial coherence interferometry device.

Results: The SCUBA diver group consisted of 30 eyes of 15 participants, the deep diver group consisted of 60 eyes of 30 participants, and the control group consisted of 80 eyes of 40 participants. The median diving time was 10.00 years in the SCUBA diver group, while in the deep diver group, it was 10.50 years. The anterior chamber depth value showed a statistically significant decrease in the SCUBA diver group compared to the control group (p=0.018). The y-coordinate of the corneal thinnest point to the corneal apex showed a statistically significant increase in the SCUBA diver group compared to both the control group and the deep diver group (p=0.019). The differences in corneal keratometry, Q value, astigmatism, thinnest pachymetry, and axial length measurements were not statistically significant between the groups (p>0.05).

Conclusion: The significant decrease in the anterior chamber depth, together with the increase in the y-coordinate of the thinnest point suggested a positive pressure effect on the cornea in diving groups, especially in the SCUBA group. However, none of the eyes showed loss of visual acuity and/or abnormal findings on the ophthalmological examination.

Keywords: Anterior chamber depth, anterior ophthalmic parameters, cornea, deep diving, eye, SCUBA

ÖZ

Amaç: Bu çalışmanın amacı, profesyonel dalgıçlarda uzun süreli SCUBA ve derin dalışın kornea tomografik parametreler, ön kamara derinliği ve gözün aksiyel uzunluğu üzerindeki etkilerini araştırmaktı.

Gereç ve Yöntemler: Bu kesitsel, karşılaştırmalı çalışma, askeri dalgıçlar ve dalgıç olmayan askeri personeli içermekteydi. Dalgıç grubu, yarım yüz maskeleri ile 40 metreye kadar dalan SCUBA grubu ve kasklarla 40 metreden daha derine dalan derin dalgıç grubu olarak iki gruba ayrıldı. Dalış yapmayan sağlıklı askeri personel kontrol grubunu oluşturdu. Tüm katılımcılara tam göz muayenesi, Scheimpflug kamera ve IOLMaster 500 parsiyel koherens interferometri cihazı ile, kornea tomografisi, pakimetri, ön kamara derinliği ve aksiyal uzunluk ölçümleri yapıldı.

Bulgular: SCUBA dalgıç grubu 15 katılımcının 30 gözünden, derin dalış grubu 30 katılımcının 60 gözünden ve kontrol grubu 40 katılımcının 80 gözünden oluştu. SCUBA dalgıç grubunda ortanca dalış süresi 10,00 yıl iken, derin dalgıç grubunda ortanca dalış süresi 10,50 yıl oldu. Ön kamara derinlik değeri SCUBA dalgıç grubunda kontrol grubuna göre istatistiksel olarak anlamlı bir azalma gösterdi (p=0,018). Kornea en ince noktasının kornea apeksine olan y-koordinatı, SCUBA dalgıç grubunda hem kontrol grubu hem de derin dalgıç grubuna göre istatistiksel olarak anlamlı bir artış gösterdi (p=0,019). Gruplar arasında korneal keratometri, Q değeri, astigmatizma, en ince pakimetri ve aksiyel uzunluk ölçümlerindeki fark istatistiksel olarak anlamlı değildi (p>0,05).

Sonuç: Ön kamara derinliğindeki belirgin azalma, en ince noktanın y-koordinatındaki artışla birlikte dalış gruplarında, özellikle SCUBA grubunda kornea üzerinde pozitif bir basınç etkisi olduğunu gösterdi. Ancak hiçbir gözde görme keskinliği kaybı ve/veya oftalmolojik muayenede anormal bulgular saptanmadı.

Anahtar Kelimeler: Ön kamara derinliği, ön oftalmik parametreler, kornea, derin sualtı dalışı, göz, SCUBA

Address for Correspondence: Belma Kayhan, Ministry of Health, İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Ophthalmology, İstanbul, Türkiye

Phone: +90 216 690 33 34 E-mail: drbelmakayhan@gmail.com **ORCID ID:** orcid.org/0000-0003-0748-6691

Received: 25.02.2023 **Accepted:** 31.05.2023

Introduction

Diving is a profession practiced in fields such as military, scientific, search and rescue. It is also an activity done as underwater photography and sports. The number of professional and amateur divers is increasing all over the world and in Türkiye.

During diving activity, exposure to increased ambient pressure at an undersea level can cause medical problems in many organs, especially in the respiratory system and the eye (1,2,3,4). Ocular barotrauma is one of the main diving issues in ophthalmology (5,6,7,8). According to Boyle's law, the volume of a certain quantity of gas is inversely proportional to the absolute pressure (9). Air-filled organs and tissues decrease in volume as the pressure increases. The aqueous liquid and vitreous gel protect the eye against barotrauma in the absence of any mask in front of the eye. With a mask, an air-filled chamber forms in front of the eyes. This chamber is susceptible to pressure changes under the sea level. As the depth increases under the sea, the exposed pressure to the mask will also increase (9). Thus, the volume and pressure of the chamber formed by the mask will also change. It is expected that these changes will also affect the eyeballs forming a wall of the chamber. This effect results in ocular barotrauma.

Equipment technology is also being improved day by day to protect divers from both life-threatening decompression sickness and potential adverse effects on the eyes and other organs. In SCUBA diving, which is up to 40 m deep, half-face masks that cover the eyes and nose or full-face masks where the whole breathing is done inside the mask are used. However, in deeper dives, only full-face masks or diving helmets are used. Because they have different designs, they will be affected by pressure and the gas content differently. This study aimed to investigate the effects of long-term SCUBA and deep diving on corneal tomographic parameters, anterior chamber depth (ACD), and axial length (AL) of the eye in professional divers.

Material and Methods

This cross-sectional study was approved by the Ethical Committee of the University of Health Sciences Türkiye (date: 16.02.2023, number: 23-13) and conducted according to the criteria of Helsinki Declaration. Written informed consent was obtained from all participants.

The study consisted of military divers and non-diver military personnel referred to the outpatient clinic of the ophthalmology department for routine periodic eye examinations. The diver group was divided into two groups as the SCUBA group, which dives up to 40 m, and the deep diver group, which dives deeper than 40 m. Healthy military personnel who didn't dive constituted the control group. According to the criteria for being military personnel, all participants were individuals with less than 1 D of a refractive error and no other ophthalmological disorders. Individuals who had undergone any procedure including refractive surgery on the cornea and had any history of intraocular surgery were excluded from the study. Other exclusion criteria were smoking and presence or history of any systemic disease, including hypertension.

Visual acuity and intraocular pressure measurements, slit-lamp and fundus examinations were performed on each participant. Corneal tomography, pachymetry, and anterior chamber measurements were made with a Scheimpflug tomography device (Pentacam, Oculus®, Germany). IOLMaster 500 device (Carl Zeiss Meditec AG, Jena, Germany) was used for the AL measurement Keratometry (K) 1, K 2, K mean, astigmatism, maximal K (within 6 mm diameter), Q value (corneal asphericity value), thinnest pachymetry, and the y-coordinate of the thinnest point to the corneal apex were recorded from the corneal tomographic measurements (Figure 1). ACD was another parameter obtained from the Scheimpflug device (Figure 1). Corneal tomography parameters evaluated in the study are described in Table 1.

The SCUBA diver group used half-face masks that covered the nose and upper face area, but with a breathing apparatus in the mouth as a separate part from the mask. The deep diver group used diving helmets, with full breathing within the helmet, covering the entire face, including the mouth. Annual dive hours were recorded for each participant in both diver groups.

Statistical Analysis

Data were analyzed with IBM SPSS V23. The Kolmogorov-Smirnov and Shapiro-Wilk tests for conformity to normal distribution were examined. Independent two-sample t-test was used to compare normally distributed data according to paired groups, and Mann-Whitney U test was used to compare non-normally distributed data. The Kruskal-Wallis test was used to compare the data that were not normally distributed according to groups of three or more, and multiple comparisons were examined with the Dunn test. One-Way Analysis of Variance was used to compare normally distributed data according to groups of three or more, and multiple comparisons were examined with Duncan's test. The relationship between non-normally distributed data was used with Spearman's rho correlation coefficient. Analysis results were presented as mean ± standard deviation and median (minimum-maximum) for

quantitative data, and frequency (percent) for categorical data. Significance level was taken as $p<0.05$.

Results

The study compared three groups; the SCUBA diver group, the deep diver group and the control group. The SCUBA diver group consisted of 30 eyes of 15 participants, the deep diver group consisted of 60 eyes of 30 participants, and the control group consisted of 80 eyes of 40 participants. All participants were male military personnel. The mean age was 33.40±6.32 years in the SCUBA diver group, 34.30±7.95 years in the deep diver group and 33.66±6.06 years in the control group. There was no statistical difference between the ages of the groups ($p>0.05$). All participants had 20/20 of uncorrected visual acuity. The median diving time as years was 10.00 (2.00-22.00) years in the SCUBA diver group, while in the deep diver group, the median was 10.50 (2.00-30.00) years. There was no statistical difference in the diving time as years between the groups ($p=0.935$). Consistently, there was no significant difference in the total diving time as hours between groups [400.00 (20.00-950.00) hours in the SCUBA diver group and 275.00 (50.00-2000.00) hours in the deep diver group as the median] ($p=0.356$).

When the corneal K 1, K 2, K mean and K max values were compared between the groups, no statistically significant difference was observed ($p>0.05$) (Table 2). There was no statistically significant difference between the groups in the comparison of astigmatism and Q value ($p>0.05$) (Table 2).

While there was no difference between the groups in the comparison of corneal thicknesses, the y-coordinate

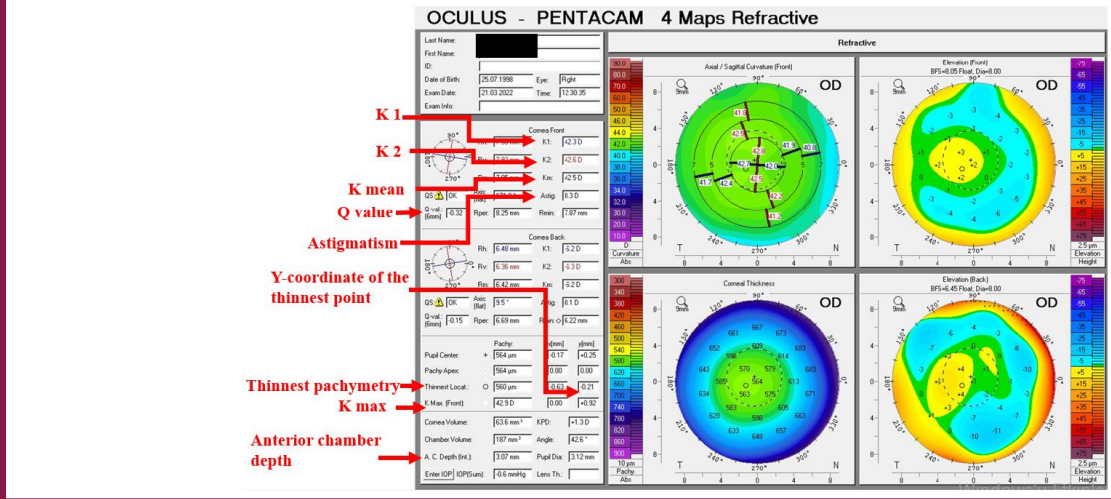

Figure 1. The image of the Pentacam 4-maps refractive screen. The data from the Pentacam measurement are displayed on the image

Table 1. Parameters obtained from the corneal tomography measurements

Parameter	Description
Keratometry 1 (K 1)	The curvature-related refractive power of the major flat meridian in the central 3 mm of the anterior cornea.
Keratometry 2 (K 2)	The curvature-related refractive power of the major steep meridian in the central 3 mm of the anterior cornea.
Mean keratometry (K mean)	The arithmetic means of the K 1 and K 2 values.
Astigmatism	The value of astigmatism in the central 3 mm of the cornea.
Q value	The asphericity value of the central 6 mm of the cornea, between -0.25 and -0.50 in normal eyes.
Maximum K (K max)	The maximum curvature-related refractive power in the any point of the central 8 mm of the cornea.
Thinnest pachymetry	The minimum value of the corneal thickness within central 8 mm of the cornea.
Y-coordinate of the thinnest point	The vertical coordinate of the thinnest point relative to the corneal apex.
Anterior chamber depth	The distance from the posterior surface of the corneal apex to the center of the anterior surface of the crystalline lens.

Table 2. Comparisons of the SCUBA diver, deep diver and control groups

	SCUBA diver group n=30		Deep diver group n=60		Control group n=80		Test st.	p
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)		
K 1 (D)	42.53±1.37	41.95 (40.60-45.70)	42.30±1.47	42.20 (38.50-45.10)	42.47±1.78	42.15 (39.40-52.50)	0.129[1]	0.937
K 2 (D)	43.16±1.49	42.80 (41.10-46.60)	43.10±1.57	42.80 (40.00-46.20)	43.12±1.43	43.00 (40.30-46.50)	0.003[2]	0.958
K mean (D)	42.83±1.42	42.35 (40.90-46.10)	42.68±1.53	42.40 (38.80-45.60)	42.72±1.41	42.55 (38.90-45.70)	0.074[2]	0.786
Q value	-0.23±0.09	-0.24 [(-0.06) - (-0.48)]	-0.23±0.13	-0.20 [(-0.04) - (-0.69)]	-0.26±0.12	0-.24 [(-0.03) - (- 0.61)]	3.266[1]	0.195
Astigmatism (D)	0.66±0.25	0.60 (0.40-1.30)	0.77±0.41	0.70 (0.10-2.00)	0.78±0.45	0.80 (0.10-2.10)	1.821[1]	0.402
K max (D)	43.66±1.73	43.25 (41.30-48.70)	43.60±1.64	43.40 (40.10-46.70)	43.67±1.48	43.40 (40.70-48.80)	0.133[1]	0.936
Thinnest pachymetry (µm)	547.03±37.21	545.00 (479.00-638.00)	537.17±30.20	537.50 (476.00-605.00)	537.35±37.19	534.00 (475.00-633.00)	1.540[1]	0.463
Y-coordinate of TP (mm)	-0.62±0.25	-0.61 [(-0.14) - (-1.19)][b]	-0.47±0.19	-0.52 [(-0.12) - (-1.05)][a]	-0.48±0.22	-0.48 [(0.00) - (-1.09)][a]	7.935[1]	0.019
ACD (mm)	3.03±0.28[b]	2.94 (2.50-3.48)	3.05±0.30[ab]	3.10 (2.47-3.78)	3.16±0.30[a]	3.14 (2.35-3.82)	5.707[2]	0.018
AL (mm)	23.67±0.61	23.54 (22.88-25.22)	23.86±0.69	23.76 (22.17-25.73)	23.83±0.65	23.91 (22.50-25.43)	3.280[1]	0.194

SD: Standard deviation, Test st.: Test statistics, K: Keratometry, D: Diopter, TP: Thinnest point, ACD: Anterior chamber depth, AL: Axial length, [1]Kruskal-Wallis H test, [2]One-Way Analysis of Variance, [a-b]There is no difference between groups with the same letter

of the thinnest point on the cornea showed a statistically significant increase in the SCUBA diver group compared to both the control group and the deep diver group (p=0.019) (Table 2).

The ACD value showed a statistically significant decrease in the SCUBA diver group compared to the control group (p=0.018). Although the ACD value in the deep diver group was much lower than the control group, there was no statistically significant difference between the deep diver group and either the control group or the SCUBA diver group (p>0.05) (Table 2).

When the AL of the eyes were compared, although they were shorter in the SCUBA diver group than in the deep diver and control groups, the differences were not statistically significant (p>0.05) (Table 2).

No significant correlation was found between the y-coordinate value of the thinnest point and the diving time (r=-0.164; p=0.122). However, a negative correlation was found significantly between diving time and ACD (r=-0.240; p=0.023).

Discussion

To the best of our knowledge, this is the first study to examine the effects of diving on the anterior segment parameters of the eye in a long-term and real-life context. While human beings live on the earth, they are exposed to the ambient pressure created by the combined dispersed gases of equal weight in the atmosphere. At sea level, this exposed pressure is called one atmosphere absolute (ATA). This is natural and ideal for human life. In the environment where diving activity takes place, human are exposed to pressure above one ATA at the undersea level and this pressure increases as the depth increases. The body's

response to pressure changes in the environment depends on the anatomy of the organ (10). The size of a liquid-filled or solid organ will not change with pressure. However, gas-filled, elastic-walled organs gradually compensate for pressure changes with volume changes, according to Boyle's law. Since the eyeballs contain aqueous and vitreous fluids, they will not be affected by changes in pressure below sea level. However, the use of masks, which is an indispensable equipment in diving, changes this situation. Masks create an air-filled space in front of the eyeballs, thus creating a new situation in which they will act from the changing pressure.

The study compared two groups of divers who dived at different depths and with different equipment to each other and to a control group of similar demographic and physiological characteristics who did not dive. The SCUBA diver group contained cases diving up to 40 m and used half-face masks covering upper half of the face with the nose and without the mouth. The deep diver group consisted of cases diving more than 40 m and used diving helmets covering the entire face including the nose and mouth. The two types of the masks form chambers with different physiological properties (11). In a half-face mask, a mouth breathing apparatus is used separately from the mask and the diver is only able to exhale into the mask (11). When the diver inhale and exhale through the separate mouthpiece during diving, negative pressure and related ocular barotrauma will occur inside the mask according to Boyle's law (11). With a diving helmet, the diver can both inhale and exhale into the helmet. Thus, it is easier to balance the gas pressure with the aid of a regulator, with a breathing environment close to normal conditions.

The present study showed that ACD of the eye was significantly reduced in the SCUBA diver group compared to the control group. ACD in the deep diver group was also lower than in the control group, higher than the SCUBA diver group, but the differences were not statistically significant. The half-face mask is expected to create negative pressure inside the mask and a vacuum effect on the eyeballs. However, the decrease in ACD revealed by the study suggests the effect of positive pressure on the cornea. A previous study stated a reduction in ACD with swimming googles but that study showed a short-term change and reversibility after the removal of the googles (12). The diver groups in the present study consisted of professional divers. Because these divers were trained in exhaling through their noses to balance the undesirable negative pressure, a negative pressure effect could not be observed in the results of the study. It can even be thought that these divers exhale more and create positive pressure in the mask to avoid negative pressure during descent. The positive pressure with diving helmets complies with the laws of physics. Prolonged exposure to the positive pressure may cause flattening of the cornea and can reduce anterior chamber depth. The other finding in the study is that ACD values are negatively correlated with the diving hours. ACD value decreased as the diver's diving time increased.

Another finding in the study suggesting the flattening effect of positive pressure on the cornea is the decrease in central K values. The median values of K mean and astigmatism were the lowest in the SCUBA diving group, the deep diving group and the control group were followed it (K mean, 42.35 D, 42.40 D, 42.55 D; astigmatism, 0.60 D, 0.70 D, 0.80 D, respectively) although the differences were statistically insignificant.

A statistically significant variable in the study was the y-coordinate of the corneal thinnest point. This data shows the distance of the thinnest point of the cornea from the geometric center. In ectatic disorders of the cornea such as keratoconus, it is seen that the thinnest point moves away from the geometric apex and shifts towards the inferior and temporal (13,14). There are many studies showing that rubbing the eyeballs, thus pressing and flattening the cornea, plays an important role in the etiology of keratoconus (15,16,17). The current study found the lowest y-coordinate of the thinnest point in the control group (-0.48), but it increased to -0.52 in the deep diver group, and -0.61 in the SCUBA diver group, demonstrating the highest shift of the corneal vertex in the last group. This finding supports the theory of positive pressure on the eye in the SCUBA diver group, in line with the reduction in ACD.

AL measurements were parallel to ACD measurements, with the highest AL in the control group and the lowest AL in the SCUBA diver group, but the differences were not statistically significant.

The results of the present study showed that, although not statistically significant, the cornea was the thickest in the SCUBA diver group, followed by the deep diver group and the control group, respectively. Deleu et al. (18) found an increase in corneal thickness after 30 and 60 min of SCUBA diving and they suggested that this may be due to ocular barotrauma. On the contrary, Maverick et al. (19) reported that although they detected a minimal decrease in corneal thickness in their measurements after 20 minutes of SCUBA diving in 12 eyes, there was no significant change. Jiménez et al. (20) in their experimental study with swimming goggles, did not detect a significant change in corneal thickness in their measurements about 5 minutes with the goggles and 2 minutes after taking them off. All these studies are on the short-term effects of SCUBA diving or swimming goggles and are not based on long-term and real-life data like the current study.

Conclusion

The results of the study revealed a significant difference in ACD and the y-coordinate of the thinnest point, with the highest difference in the SCUBA diver group. The decrease in ACD correlated with the increase in diving time. The decrease in ACD, together with the increase in the y-coordinate of the thinnest point and the decrease in the K mean values, suggest a positive pressure effect on the cornea. Because the participants in the study group were professional and well-trained, they managed to protect their eyes from the possible side effects of diving. Despite the significant differences in some variables obtained in the study, none of the participants had any visual symptoms. No abnormal findings were detected in the ophthalmologic examinations. For this reason, it is thought that the good training of divers and the use of advanced equipment minimize the effect of diving activity on the cornea and other anterior segment parameters in real-life practice.

Acknowledgement: The authors would like to thank Dr. Mertan Acar for his contribution to recruiting participants for the study.

Ethics

Ethics Committee Approval: This cross-sectional study was approved by the Ethical Committee of the University of Health Sciences Türkiye (date: 16.02.2023, number: 23-13) and conducted according to the criteria of Helsinki Declaration.

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.K., N.D., Concept: B.K., N.D., Design: B.K., N.D., Data Collection or Processing: B.K., N.D., Analysis or Interpretation: B.K., Literature Search: B.K., Writing: B.K., N.D.

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

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

References

1. Chevasutho P, Premmaneesakul H, Sujiratana A. Descriptive study of decompression illness in a hyperbaric medicine centre in Bangkok, Thailand from 2015 to 2021. Diving Hyperb Med. 2022;52:277-280. [Crossref]
2. Pollock NW, Buteau D. Updates in Decompression Illness. Emerg Med Clin North Am. 2017;35:301-319. [Crossref]
3. Casadesús JM, Nieto-Moragas J, Serrando MT, Boadas-Vaello P, Carrera A, Aguirre F, et al. Pulmonary barotrauma in SCUBA diving-related fatalities: a histological and histomorphometric analysis. Forensic Sci Med Pathol. 2023. [Crossref]
4. Goenadi CJ, Law DZ, Lee JW, Ong EL, Chee WK, Cheng J. The Effect of a Diving Mask on Intraocular Pressure in a Healthy Population. Case Rep Ophthalmol. 2016;7:328-332. [Crossref]
5. Brügger JW, Rauscher GA, Florian JP. Hyperoxic myopia: a case series of four divers. Undersea Hyperb Med. 2020;47:261-265. [Crossref]
6. Ergözen S. Preventable Diving-related Ocular Barotrauma: A Case Report. Turk J Ophthalmol. 2017;47:296-297. [Crossref]
7. Woo D, Rogers S, Leong J, Clement CI, Kourt G. Non-traumatic subperiosteal orbital hemorrhage secondary to barotrauma. Orbit. 2012;31:347-349. [Crossref]
8. González-Pastor E, Fernández-Tresguerres F, Palomares-Fernández J, Toledano N. Diplopia due to barotrauma. Arch Soc Esp Oftalmol. 2016;91:142-144. [Crossref]
9. Butler FK Jr. Diving and hyperbaric ophthalmology. Surv Ophthalmol. 1995;39:347-366. [Crossref]
10. Bosco G, Rizzato A, Moon RE, Camporesi EM. Environmental Physiology and Diving Medicine. Front Psychol. 2018;9:72. [Crossref]
11. Edmonds CB, Lippmann J, Mitchell S, editors. Diving and subaquatic medicine. Boca Raton, FL: CRC Press; 2015. [Crossref]
12. Zhang X, Wang H, Nie Y, Li W. Short-term effects of two types of goggles on intraocular pressure and anterior eye segment biometrics. BMC Ophthalmol. 2022;22:73. [Crossref]
13. Sedaghat MR, Momeni-Moghaddam H, Azimi Khorasani A, Belin MW, Monfared N, Wolffsohn JS, et al. Comparison of Keratoconus Cone Location of Different Topo/tomographical Parameters. Curr Eye Res. 2021;46:1666-1672. [Crossref]
14. Thulasidas M, Teotia P. Evaluation of corneal topography and tomography in fellow eyes of unilateral keratoconus patients for early detection of subclinical keratoconus. Indian J Ophthalmol. 2020;68:2415-2420. [Crossref]
15. Hage A, Knoeri J, Leveziel L, Majoulet A, Buffault J, Labbé A, et al. Du prurit aux frottements oculaires : une revue de la littérature [From ocular itching to eye rubbing: a review of the literature]. J Fr Ophtalmol. 2023;46:173-184. [Crossref]
16. Sahebjada S, Al-Mahrouqi HH, Moshegov S, Panchatcharam SM, Chan E, Daniell M, et al. Eye rubbing in the aetiology of keratoconus: a systematic review and meta-analysis. Graefes Arch Clin Exp Ophthalmol. 2021;259:2057-2067. [Crossref]
17. Debourdeau E, Planells G, Chamard C, Touboul D, Villain M, Demoly P, et al. New Keratoconus Risk Factors: A Cross-Sectional Case-Control Study. J Ophthalmol. 2022;2022:6605771. [Crossref]
18. Deleu L, Catherine J, Postelmans L, Balestra C. Effect of SCUBA Diving on Ophthalmic Parameters. Medicina (Kaunas). 2022;58:408. [Crossref]
19. Maverick, KJ, Conners, MS. Corneal Thickness and Intraocular Pressure Changes Associated with Scuba Diving. Investig.Ophthalmol. Vis Sci. 2003;44:2571. [Crossref]
20. Jiménez R, Molina R, Vera J, Redondo B. The short-term effects of wearing swimming goggles on corneal biomechanics. Int Ophthalmol. 2022;42:2773-2784. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.27146

Hamidiye Med J 2023;4(2):123-127

Protective Temporary Vesicostomy in Children: Evaluation of 23 Patients

Çocuklarda Geçici Koruyucu Vezikostomi: 23 Hastanın Değerlendirilmesi

Gökhan Demirtaş, Süleyman Tağcı, Bilge Karabulut, Tuğrul Hüseyin Tiryaki

University of Health Sciences Türkiye, Ankara City Hospital, Clinic of Pediatric Urology, Ankara, Türkiye

ABSTRACT

Background: Vesicostomy in children is a surgical procedure performed to temporarily empty the bladder. It is usually performed to protect upper urinary tract function in patients with neuropathic bladder, and bladder outlet obstruction. This study aims to evaluate the results of patients who had vesicostomy in the study center within ten years.

Materials and Methods: We evaluated retrospectively the treatment results and complications of vesicostomy on twenty-three children who were operated in our center from 2009 to 2019.

Results: There were sixteen (69%) boys and seven (31%) girls. Their mean age was 4.74±4.67 (1 month-16 year) years old when they underwent vesicostomy. Twelve (52.2%) of them had neurogenic bladder and one (4.3%) of them had an intact neuronal pathway which is defined as dysfunctional voiding. Six (26.1%) boys had posterior urethral valves, prune belly syndrome in two boys (8.7%) and vesicoureteral reflux in two patients. All patients had severe hydroureteronephrosis before vesicostomy. After the operation, upper urinary tract dilatation improved in nineteen patients. The creatinine level reduced after vesicostomy in 18 patients. Sixteen patients needed additional surgery. Complications after vesicostomy were stoma stenosis in two patients, mucosal prolapse in one patient (5.05%), dermatitis in two patients, and febrile urinary tract infection in two patients. Two patients needed vesicostomy revision.

Conclusion: In selected patients, vesicostomy is beneficial to prevent upper urinary tract deterioration and stabilize renal function. Nevertheless, most of children need for additional major surgery.

Keywords: Vesicostomy, renal function, children, urinary tract infection

ÖZ

Amaç: Çocuklarda vezikostomi mesanenin geçici olarak boşaltılmasını sağlamak için uygulanan bir cerrahi işlemdir. Nöropatik mesane ve mesane çıkım obstrüksiyonu olan hastalarda genellikle üst idrar yolu fonksiyonunu korumak için yapılır. Çalışmamız on yıl içinde vezikostomi uygulanan hastaların sonuçlarını değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: 2009-2019 yılları arasında merkezimizde opere edilen 23 çocukta vezikostominin tedavi sonuçlarını ve komplikasyonlarını retrospektif olarak değerlendirdik.

Bulgular: On altı (%69) erkek ve yedi (%31) kız hastanın vezikostomi yapıldığında ortalama yaşları 4,74±4,67 (1 ay-16 yaş) idi. On ikisinde (%52,2) nörojenik mesane, birinde (%4,3) nöronal yolağı sağlam olan disfonksiyonel işeme söz konusu idi. Altı (%26,1) erkek çocukta posterior üretral valv, iki erkek çocukta (%8,7) prune belly sendromu ve iki hastada vezikoüreteral reflü mevcuttu. Tüm hastalarda vezikostomi öncesi şiddetli hidroüreteronefroz vardı. Ameliyat sonrası 19 hastada üst üriner sistem dilatasyonu düzeldi. On sekiz hastada vezikostomi sonrası kreatinin düzeyi düştü. On altı hastanın ek cerrahiye ihtiyacı vardı. Vezikostomi sonrası gelişen komplikasyonlar iki hastada stoma stenozu, bir hastada (%5,05) mukozal prolapsus, iki hastada dermatit ve iki hastada ateşli idrar yolu enfeksiyonu idi. İki hastada vezikostomi revizyonu gerekti.

Sonuç: Seçilmiş hastalarda vezikostomi, üst üriner sistem bozulmasını önlemek ve böbrek fonksiyonunu stabilize etmek için faydalıdır. Bununla birlikte, çoğu çocuğun ek majör cerrahiye ihtiyacı vardır.

Anahtar Kelimeler: Vezikostomi, renal fonksiyon, çocuk, üriner sistem enfeksiyonu

Address for Correspondence: Gökhan Demirtaş, University of Health Sciences Türkiye, Ankara City Hospital, Clinic of Pediatric Urology, Ankara, Türkiye
Phone: +90 506 634 02 17 E-mail: drgokhandemirtas@gmail.com **ORCID ID:** orcid.org/0000-0003-0787-2330

Received: 26.02.2023 **Accepted:** 31.05.2023

Introduction

Vesicostomy is a simple, well-tolerated, and reversible procedure that provides temporary drainage of the bladder and is rarely used as a surgical procedure in children (1). It is usually performed to protect upper urinary tract function in patients with neuropathic bladder, and bladder outlet obstruction (1,2,3,4). Vesicostomy is not a first-line treatment modality in pediatric urological practice. If conservative treatment modality fails, vesicostomy is performed to stabilize kidney function, prevent urinary tract infection and save time for definitive surgery (1,2). Conservative treatment may include; medicine for relaxing the bladder, clean intermittent catheterization (CIC), botulinum toxin injection, subureteric injection and/or valve ablation.

Generally, after upper urinary system stabilization, vesicostomy closure is planned with a definitive surgery. There is a controversy about the effect of vesicostomy on bladder capacity and function. If drainage is performed with vesicostomy for a long time, it is seen that the bladder capacity is reduced and definitive surgery becomes very difficult or impossible (5,6). Therefore, the timing of vesicostomy closure is very important. However, some authors claim that vesicostomy has no negative effect on the bladder function and reduces the need for definitive surgery in patients (1,2,3,7,8,9).

In this retrospective study, we evaluated effect of the vesicostomy on renal function, ultrasonography appearance of the upper urinary tract, and complications of vesicostomy on children in a 10-year period.

Material and Methods

Twenty-three children underwent cutaneous vesicostomy at our clinic from January 2009 to December 2019. Medical records of the patients were evaluated the records retrospectively after the approval of the Hospital Ethics Committee of University of Health Sciences Türkiye, Ankara City Hospital (E2-21-799). The indications for vesicostomy in our clinic were bilateral severe hydroureteronephrosis with decreased renal parenchymal thickness, worsening kidney function with hydronephrosis despite optimum medical treatment using CIC and anticholinergic drugs and patients who cannot be performed CIC due to hypersensation, urethral anatomy, or age. First line therapies such as intermittent catheterization and anticholinergic drugs failed in all patients in this series.

The Blocksom technique was preferred for vesicostomy in our clinic. With this technique, a small opening is made in the lower abdomen through the bladder dome to allow the outflow of urine. Patients age, gender, vesicostomy indication, pre and postvesicostomy laboratory/radiological findings, incidence of febrile urinary tract infection, and complications were recorded from hospital records. Before deciding to close the vesicostomy, bladder capacity was measured and voiding cystourethrography was performed in all patients.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) Software Version 25.0 (SPSS Inc., Chicago, IL, USA). Results were evaluated using the Wilcoxon signed-rank test and Mann-Whitney U test. Any p-value <0.05 was considered significant.

Results

Twenty-three patients [16 (69%) boys and 7 (31%) girls] who underwent vesicostomy in our clinic included the study. The patient's age was between 1 month to 16 years mean 4.74±4.67 at the time they underwent vesicostomy. Mean length follow-up was 47.56±29.82 months with the range 11.5 months-7.8 years.

Twelve (52.2%) of them had neurogenic bladder due to neural tube defects, and one (4.3%) of them had dysfunctional voiding without neurologic problem. Six (26.1%) boys had posterior urethral valves (PUV) and two boys (8.7%) had prune belly syndrome. Two patients (8.7%) had vesicoureteral reflux (VUR). These two patients are under one year old with unremitting urinary tract infection and one of them had solitary kidney (Table 1).

Severe hydroureteronephrosis was noted in all patients before vesicostomy. The right kidney anterior posterior diameter decreased in 18 patients, stayed

Table 1. Demographic values

		n	%
Gender (n=23)	Male	16	69.5
	Female	7	30.5
Diagnosis (n=23)	Neurogenic bladder	12	52.2
	Dysfunctional voiding	1	4.3
	Posterior urethral valve	6	26.1
	Prune belly syndrome	2	8.7
	VUR	2	8.7
Postoperative complication (n=7)	Stoma stenosis	2	28.6
	Mucosal prolapsus	1	14.2
	Dermatitis	2	28.6
	fUTI	2	28.6

VUR: Vesicoureteral reflux, fUTI: Febrile urinary tract infection

stabile in five patients after vesicostomy [pre-vesicostomy mean 20.0±18.37 mm (4-90 mm); post-vesicostomy mean 11.05±5.35 (4-24 mm)] p=0.005; left kidney anterior posterior diameter stayed on stable in four patients and decreased in 19 patients [pre-vesicostomy mean 18.89±8.69 mm (9-36); post-vesicostomy mean 13.63±5.55 mm (4-29)] p=0.002. There was no significant difference between preoperative and postoperative parenchymal thinning (right kidney p=0.060, left kidney 0.161) (Table 2).

Creatinine levels decreased after vesicostomy in 18 patients. Creatinine level was stable in five patients. Mean serum creatinine levels pre and post-vesicostomy were 1.58±0.81 mg/dL (0.45-3.64 mg/dL) and 0.93±0.54 mg/dL (0.29-1.96 mg/dL), respectively. The decrease in creatinine level was significant after vesicostomy (p<0.001) and remained stable during follow-up (Table 2).

A total of seven complications were encountered in 23 patients. Stoma stenosis was noted in two patients, mucosal prolapse in one patient, dermatitis in two patients, and febrile urinary tract infection in two patients. Two patients needed vesicostomy revision.

Three patients underwent ureteroneocystostomy, six patients had augmentation with appendicovesicostomy, three patients appendicovesicostomy, three patients PUV resection, one patient nephrectomy on follow-up. Additional surgical procedure was performed in 16 (69.5%) patients. Vesicostomy was closed without any problem in 20 patients. The reason for prolonged vesicostomy in two patients is lack of social support to adequate bladder management and in one patient is waiting for definitive operation. No complication was noted after vesicostomy closure. No renal function deterioration was observed in any patient after vesicostomy.

Discussion

Sincevesicostomy was described in the 1960s (10,11), this technique has been used as a temporary diversion for limited conditions until bladder function improves. Today there is a debate going on about the role of vesicostomy for the treatment of some issue. Vesicostomy is recommended patients with bilateral severe hydroureteronephrosis with decreased parenchymal thickness, worsening kidney function and hydronephrosis despite medical treatment using CIC and anticholinergic drugs, symptomatic bilateral high-grade VUR, recurrent and symptomatic urinary tract infection or patients refusing to do CIC due to hypersensation, urethral anatomy, or age. But, since the temporary defunctionalization of the bladder will cause a decrease in bladder capacity (5,6) and is an incontinent solution, vesicostomy is not a treatment option unless it is mandatory. On the other hand, there are papers stating that vesicostomy does not affect bladder function and resulted increases bladder capacity and compliance (7,8,9).

Vesicostomy application in PUV patients after valve ablation facilitates upper urinary tract drainage, resulting in significant improvement in PUV outcome (12). On the contrary, some authors believe that although vesicostomy delays progression to end-stage kidney disease, no long-term benefit was noted in the incidence of end-stage kidney disease (13). Vesicostomy should be an option for PUV treatment. Vesicostomy has significantly better results in decreasing serum creatinine level, ore often dry and had on average higher GFR compared with valve ablation (14). In another study, primary valve ablation and vesicostomy for PUV treatment were compared. When the one-year creatinine level, glomerular filtration rate and grade of hydronephrosis were evaluated, there was no significant difference. Although there is no significant difference, they

Table 2. Comparison of the radiologic and laboratory findings

		Median	Min-max	p
Right kidney AP (mm)	Pre-vesicostomy	20.0±18.37	4-90	0.005
	Post-vesicostomy	11.05±5.35	4-24	
Left kidney AP (mm)	Pre-vesicostomy	18.89±8.69	9-36	0.002
	Post-vesicostomy	13.63±5.55	4-29	
Right kidney parenchimal thickness (mm)	Pre-vesicostomy	6.93±3.70	2-17	0.06
	Post-vesicostomy	7.97±3.87	4-14	
Left kidney parenchimal thickness (mm)	Pre-vesicostomy	18.89±5.33	3-26	0.161
	Post-vesicostomy	9.05±3.55	3-15	
Serum creatinin level (mg/dL)	Pre-vesicostomy	1.58±0.81	0.45-3.64	<0.001
	Post-vesicostomy	0.93±0.54	0.29-1.96	

Mann-Whitney U test

recommended vesicostomy due to less complication that might be a better treatment choice in newborns (15).

In our series if upper tract deterioration after PUV resection is went on and CIC is not able to use, we prefer vesicostomy for bladder drainage. Or if hydroureteronephrosis increases despite CIC and overnight catheterization, we recommend again performing vesicostomy to stabilize the renal function. Vesicostomy was performed in six patients with PUV in our series. On follow-up, vesicostomy closed in all patients with PUV. UNC was performed in a PUV patient as an additional surgical procedure. Upper urinary tracts stabilized in all PUV patients after vesicostomy. Vesicostomy was used temporarily for stabilization of renal functions before major surgery in neurogenic bladder cases in our series. Patients underwent augmentation cystoplasty and appendicovesicostomy after stabilization. In the literature, initial vesicostomy is performed in infants younger than six months old with primary bilateral high-grade VUR (16,17). In this series two patient under one year old with recurrent urinary tract infection underwent vesicostomy for primary VUR. Vesicostomy was used in these two patients, one of them had solitary kidney, which could not be stabilized with medical therapy and was closed after one year of age when UNC was performed.

The most important problem in cases with vesicostomy is the decrease in bladder capacity over time. It becomes very difficult or impossible to perform definitive surgeries to bladder with reduced capacity. Therefore, harm-benefit balance should be carefully considered in patients with vesicostomy. In our series, no reducing in bladder capacity has been noted in patients with PUV. Patients with neurogenic bladder and primary VUR, had already low bladder capacity pre-vesicostomy. There are not bladder capacity decreasing except one in our series. Additional surgical procedures were performed without problems in all patients. While low bladder capacity is not a problem with planned bladder augmentation in patients, in patients who are scheduled for antireflux surgery due to primary VUR should be very careful. Severe hydroureteronephrosis improved in 19 patients after vesicostomy, stable in four patients. Serum creatinine level decreased after vesicostomy in 18 patients. However, there is no improvement in parenchymal thinning after vesicostomy. Vesicostomy cannot reduce additional major surgeries in our series. Major surgery was required in 70% of our patients.

Conclusion

Vesicostomy should be performed as a temporary treatment option to protect the upper urinary system in a selected group of patients with neurogenic bladder and bladder outlet obstruction. This is a simple, reversible, uncomplicated, and well-tolerated procedure. Serum creatine level and upper urinary tract dilatation showed a significant improvement after vesicostomy.

Acknowledgements: This study is based on the retrospective data evaluation of patients treated in University of Health Sciences Türkiye, Ankara City Hospital Pediatric Urology Clinic.

Ethics

Ethics Committee Approval: Medical records of the patients were evaluated the records retrospectively after the approval of the Hospital Ethics Committee of University of Health Sciences Türkiye, Ankara City Hospital (E2-21-799).

Informed Consent: Retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.D., T.H.T., Concept: S.T., T.H.T., Design: B.K., T.H.T., Data Collection or Processing: G.D., S.T., T.H.T., Analysis or Interpretation: S.T., T.H.T., Literature Search: B.K., T.H.T., Writing: G.D., T.H.T.

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

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

References

1. Sharifiaghdas F, Mirzaei M, Nikravesh N. Can transient resting of the bladder with vesicostomy reduce the need for a major surgery in some patients? J Pediatr Urol. 2019;15:379.e1-379.e8. [Crossref]
2. Dönmez Mİ, Carrasco A Jr, Saltzman AF, Vemulakonda V, Wilcox DT. Long-term outcomes of cutaneous vesicostomy in patients with neuropathic bladder caused by spina bifida. J Pediatr Urol. 2017;13:622.e1-622.e4. [Crossref]
3. Krahn CG, Johnson HW. Cutaneous vesicostomy in the young child: indications and results. Urology. 1993;41:558-563. [Crossref]
4. Gobbi D, Fascetti Leon F, Gnech M, Midrio P, Gamba P, Castagnetti M. Management of Congenital Urethral Strictures In Infants. Case Series. Urol J. 2019;16:67-71. [Crossref]
5. Lome LG, Howat JM, Williams DI. The temporarily defunctionalized bladder in children. J Urol. 1972;107:469-472. [Crossref]
6. Tanagho EA. Congenitally obstructed bladders: fate after prolonged defunctionalization. J Urol. 1974;111:102-109. [Crossref]
7. Lukong CS, Ameh EA, Mshelbwala PM, Jabo BA, Gomna A, Anumah MA, et al. Role of vesicostomy in the management of posterior urethral valve in Sub-Saharan Africa. J Pediatr Urol. 2014;10:62-66. [Crossref]
8. Jayanthi VR, McLorie GA, Khoury AE, Churchill BM. The effect of temporary cutaneous diversion on ultimate bladder function. J Urol. 1995;154:889-892. [Crossref]
9. Vastyan AM, Pinter AB, Farkas A, Vajda P, Somogyi R, Juhasz Z. Cutaneous vesicostomy revisited--the second 15 years. Eur J Pediatr Surg. 2005;15:170-174. [Crossref]

10. Michie AJ, Borns P, Ames MD. Improvement following tubeless suprapubic cystostomy of myelomeningocele patients with hydronephrosis and recurrent acute pyelonephritis. J Pediatr Surg. 1966;1:347-352. [Crossref]
11. Duckett JW Jr. Cutaneous vesicostomy in childhood. The Blocksom technique. Urol Clin North Am. 1974;1:485-495. [Crossref]
12. Nanda M, Bawa M, Narasimhan KL. Mini-vesicostomy in the management of PUV after valve ablation. J Pediatr Urol. 2012;8:51-54. [Crossref]
13. Chua ME, Ming JM, Carter S, El Hout Y, Koyle MA, Noone D, et al. Impact of Adjuvant Urinary Diversion versus Valve Ablation Alone on Progression from Chronic to End Stage Renal Disease in Posterior Urethral Valves: A Single Institution 15-Year Time-to-Event Analysis. J Urol. 2018;199:824-830. [Crossref]
14. Godbole P, Wade A, Mushtaq I, Wilcox DT. Vesicostomy vs primary ablation for posterior urethral valves: always a difference in outcome? J Pediatr Urol. 2007;3:273-275. [Crossref]
15. Hosseini SM, Zarenezhad M, Kamali M, Gholamzadeh S, Sabet B, Alipour F. Comparison of early neonatal valve ablation with vesicostomy in patient with posterior urethral valve. Afr J Paediatr Surg. 2015;12:270-272. [Crossref]
16. Podesta ML, Ruarte A, Herrera M, Medel R, Castera R. Bladder functional outcome after delayed vesicostomy closure and antireflux surgery in young infants with 'primary' vesico-ureteric reflux. BJU Int. 2001;87:473-479. [Crossref]
17. Queipo Zaragozá JA, Domínguez Hinarejos C, Serrano Durbá A, Estornell Moragues F, Martínez Verduch M, García Ibarra F. Vesicostomía en la edad pediátrica. Nuestra experiencia en 43 pacientes [Vesicostomy in children. Our experience with 43 patients]. Actas Urol Esp. 2003;27:33-38. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.88700

Hamidiye Med J 2023;4(2):128-135

Quality Data and Errors in a Tertiary Microbiology Laboratory (2017-2020): "The Good, the Bad and the Ugly"

Üçüncü Basamak Bir Hastanenin Mikrobiyoloji Laboratuvarında Kalite Verileri ve Hatalar (2017-2020): "İyi, Kötü ve Çirkin"

Ali Korhan Sığ[1,2], Nermin Özen[2], Alev Çetin Duran[2], Tuğba Kula Atik[2,3]

[1]*University of Health Sciences Türkiye, Balıkesir Atatürk City Hospital, Clinic of Medical Microbiology, Balıkesir, Türkiye*

[2]*Balıkesir Atatürk City Hospital, Clinic of Medical Microbiology, Balıkesir, Türkiye*

[3]*Balıkesir University Faculty of Medicine, Department of Medical Microbiology, Balıkesir, Türkiye*

ABSTRACT

Background: In the last century, tremendous developments have happened in laboratory medicine. Even though laboratory errors have declined and quality standards have been defined concordant with technological developments, their routine and continuous monitoring has become main part of laboratory medicine. The aim of this study was to investigate contamination rates, specimen rejection and quality analysis of a microbiology laboratory of a tertiary hospital in a 4-year period.

Materials and Methods: Specimens of Balıkesir Atatürk City Hospital in 2017-2020 that were sent to microbiology laboratories were retrospectively evaluated regarding rejection rates, rejection reasons, blood culture (BC) quality and contamination rates, urine culture (UC) contamination rates. Rejection analysis and contaminations were divided according to rejection reasons and hospital services.

Results: A total of 1,862.038 samples were sent to microbiology laboratory in a 4-year period. Reasons of over 80% of specimen rejections were inappropriate specimen, inappropriate containers, insufficient specimen, and missing sample and/or test request, respectively. Outpatient and internal medicine services covered the majority of rejections, but rejections were significantly lower in intensive care units (ICUs) and surgical services ($p<0.001$). 68.5% of all UC contaminations were detected in outpatient services. The difference of UC contamination rates regarding years ($p=0.846$) and services ($p=0.182$) were not significant. 72.8% of BC contaminations were sourced from ICUs. The difference of BC contamination rates regarding years ($p=0.630$) and services ($p=0.630$) were not significant. False positivity of BCs was 1.1%, failures of first notification were ⩽0.1%, and gram staining-final identification agreement rate was 94.3%. One-vial BC rate was 3.8%, with the majority of neonatal cases (>90%).

Conclusion: Although our rejection and quality rates are below the highest thresholds of quality criteria, a need of training and organization in outpatient units was clear. Similar impropriety was observed in UC contaminations with the same units. BC contaminations in ICUs are thought to be sourced from inappropriate indwelling catheter care.

Keywords: Quality indicators, laboratory medicine, clinical laboratories, quality control, contamination

ÖZ

Amaç: Son yüzyıl laboratuvar tıbbında muazzam gelişmelere sahne olmuştur. Her ne kadar teknolojik gelişmelerle laboratuvar testlerindeki hatalar anlamlı şekilde azaltılmış ve kalite standartları belirlenmiş olsa da, bunların rutin izlemi laboratuvar tıbbının temel unsuru haline gelmiştir. Bu çalışmadaki amaç, dört yıllık süreçte üçüncü basamak bir hastanenin mikrobiyoloji laboratuvarının kontaminasyon, numune reddi ve kalite analizini değerlendirmektir.

Gereç ve Yöntemler: Balıkesir Atatürk Şehir Hastanesi'nin 2017-2020 yılları arasında mikrobiyoloji laboratuvarlarına gönderilen numunelerinin red oranı ve red sebebi ile kan kültürü kalite ve kontaminasyon oranları ve idrar kültürü numunelerinin kontaminasyon oranlarına retrospektif olarak bakılmıştır. Red analizleri ve kontaminasyon oranları red sebebine ve hastane birimlerine göre düzenlenmiştir.

Address for Correspondence: Ali Korhan Sığ, University of Health Sciences Türkiye, Balıkesir Atatürk City Hospital, Clinic of Medical Microbiology; Balıkesir Atatürk City Hospital, Clinic of Medical Microbiology, Balıkesir, Türkiye

Phone: +90 531 794 06 08 E-mail: dr_korhan@hotmail.com **ORCID ID:** orcid.org/ 0000-0003-2907-257X

Received: 27.03.2023 **Accepted:** 31.05.2023

ÖZ

Bulgular: Mikrobiyoloji laboratuvarlarına dört yıl içerisinde toplamda 1,862.038 laboratuvar numunesi ulaşmıştır. Numune redlerinin %80'inden fazlasının sebepleri sırasıyla, uygunsuz numune, uygunsuz numune kabı, yetersiz numune, kayıp numune/uygunsuz test istemi şeklindedir. Ayaktan hasta ünitelerinden ve iç hastalıkları servislerinden gelen numuneler redlerin çoğunluğunu oluştururken, yoğun bakımlardan ve cerrahi servislerden gelen numunelerde red anlamlı şekilde daha azdır (p<0,001). İdrar kültürlerinin tüm kontaminasyonlarının %68,5'i ayaktan hasta servislerinde görüldü. İdrar kültürlerinde yıllara göre (p=0,846) ve servislere göre (p=0,182) kontaminasyon oranları anlamlı bulunmadı. Toplam kan kültürü kontaminasyonlarının %72,8'i yoğun bakım ünitelerindendi. Kan kültürlerinde yıllara göre (p=0,630) ve servislere göre (p=0,630) kontaminasyon oranları anlamlı bulunmadı. Kan kültürlerinde yalancı pozitiflik %1,1, pozitifliğin ilk bildirimindeki hatalar ⩽%0,1 ve gram boya-son tanımlama uyum oranı %94,3'tü. Tek şişe kan kültürü oranı %3,8'di ve çoğunlukla neonatal olgulardı (>%90).

Sonuç: Her ne kadar numune red oranları kalite standartlarında belirtilen sınırların altında kalsa da, ayaktan hasta ünitelerinde numune yönetimi hususunda eğitim ve düzenleme gerekliliği açıktır. Aynı birimlerde benzer uygunsuzluk idrar kültürü kontaminasyonunda da görülmektedir. Yoğun bakım ünitelerindeki kan kültürü kontaminasyonunun yetersiz invazif kateter bakımından kaynaklandığı düşünülmektedir.

Anahtar Kelimeler: Kalite indikatörleri, laboratuvar tıbbı, klinik laboratuvar, kalite kontrol, kontaminasyon

Introduction

Advanced technological and scientific improvements have created a massive alteration in diagnostic methods that have directly affected the design and spectrum of clinical laboratories. Such widening landscape of clinical laboratories also caused a specific evolution in their accuracy and quality information. Introduction of computers, advanced automization and utilization of informatics have yet resulted with simplifying quality control (QC) measures, however, the need to control and improve quality in clinical laboratories has concordantly grown due to possibly increasing numbers of types of various tests. Furthermore, the parameters that have to be checked in QC measures have additionally grown with the increasing understanding of the tests due to various scientific researches (1,2). The proficiency testing/external quality assessment programs, pre/post-analytical and analytical quality specifications and internal quality measures are established-but periodically and dynamically updated- and currently in use. International/national organisations such as the Clinical and Laboratory Standards Institute (CLSI) publish and update documents to provide continuous improvement and reliability on laboratory results by establishing strict monitoring standards for QC (2,3,4,5,6). In addition, clinical laboratories that are willing to be internationally recognised try to gain international and independent approvement by accreditation certificates such as ISO 15189. Recently, in laboratory profession, global "acceptibility" has become a very important trend, since the cruciality of this point was observed in the last pandemic of Coronavirus disease-2019. Furthermore, this point actually gives a perspective of "legal responsibility" regarding the laboratory results (7).

The process control in clinical microbiology laboratories has step-by-step checkpoint data from pre- to post-analytic phases. In internationally-adapted guidelines of Turkish Ministry of Health, these data as quality measures of specimen rejection ratio, missing specimen ratio, contamination in urinary and/or blood cultures (UCs, BCs) unsuitability in internal and/or external control executions were clearly defined. In addition, measures of positivity ratio in total BCs, false positivity ratio in total BCs, time period from BC positivity to first notification to the clinic, BC ratio sampled as two or more sets, BC ratio as sampled only one bottle, compatibility ratio of gram staining result and last identification in BCs were stated (4,5,6). The aim of this study was to monitor the current condition and variations of stated measures in our clinical microbiology laboratory in 2017-2020.

Material and Methods

Ethical approval: Approved by the Ethical Board of Balıkesir University Faculty of Medicine (date: 11 Nov 2020/desicion number: 2020/203).

Materials and methods: Microbiological specimens of both inpatients and outpatients admitted to Balıkesir Atatürk City Hospital, which were obtained and sent to clinical laboratories (immunoserology, tuberculosis, bacteriology/mycology and virology) in 2017-2020, were included. The data of specimens and ratios according to the criteria of the quality of the guidelines of Turkish Ministry of Health were retrospectively investigated (4,5,6). The data were obtained by hospital sofware system and hospital quality management department. There is a software system that monitorize the sample step-by-step and the data divided to services and different laboratories. Internal medicine services (IMSs) include forensic medicine, family medicine, pediatrics, adult and pediatric psychiatry, dermatology and venereal diseases, infectious disesases, chest diseases and pulmonology, physical therapy and rehabilitation, internal medicine, cardiology, neurology, nuclear medicine, radiation

oncology, radiodiagnostics, sports medicine, underwater and hyperbaric medicine, medical genetics and medical ecology and hydroclimatology. Surgical services (SSs) include emergency medicine (ER), neurosurgery, general surgery, pediatric surgery, ophthalmology, gynecology and obstetrics, cardiac and thoracic surgery, ear-nose-throat surgery, orthopedics and traumatology, urology and plastic, reconstructive and aesthetic surgery departments. Intensive care units (ICUs) include pediatric, neonatal, surgical, cardiovascular, chest diseases and pulmonology, internal medicine, anesthesiology and reanimation and general ICUs.

The QC of all media and BC vials was performed once a month and QC of gram staining was performed once a week. In the case of a new party of any of these materials, all QC procedures according to manufacturers' recommendations were additionally performed.

Statistical Analysis

We statistically analyzed the research data using the SPSS 22.0 (SPSS INC, Chicago, IL, USA) program. Categorical variables are denoted as numbers and percentages, and we performed a chi-square test the compare the data between the independent groups. A p-value of <0.05 was considered statistically significant.

Results

In a 4-year period (2017-2020), a total of 2.181.162 tests were requested and 1.862.038 (85.4%) specimens were sent to the clinical microbiology laboratories. Specimen rejection analysis regarding services and reasons, UC and BC contaminations and various BC quality parameters are presented in Tables 1, 2, 3.

Over 80% of specimen rejections are caused because of inappropriate specimen and/or container, insufficient specimen, missing specimen and/or test requests. The majority (90.7%) of the rejected specimens were sourced from outpatient units and IMSs. However, such rejections were particularly rare in SSs and ICUs. There was statistically significant difference between polyclinics and IMSs compared with SSs and ICUs (p<0.001). The nearly half of specimen rejections (47.7%) were observed in samples that were sent to bacteriology/mycology and virology laboratories.

12.4% of all UCs were reported as contamination and outpatient services had the highest rate (68.8% of all urinary contaminations; 11.9% of all UCs from outpatient services), which was predominantly sourced by pediatrics and ER (52.2% of all urinary contaminations; 6.5% of all UCs). Regarding services, 20.2% of all UCs from inpatient services were contaminated, which's majority was again from pediatrics (10.5% of all urinary contaminations). The lowest UC contamination was from infectious diseases and urology inpatient services (1.8% of all UC contaminations). There was not any significant difference in contamination rates between services (p=0.182) and in addition, among years, UC contamination rates did not show any significant alteration (p=0.846).

22.6% of all BCs gave positive signal with a contamination rate of 5.6%, and false positivity of 1.1%. The alterations contamination rates regarding years was not significant (p=0,630). Majority of contaminations were from ICUs (72.8% of all BC contaminations, 6.7% of all BCs from ICUs). All BCs of outpatient services were obtained from ERs with a rate of 1.9% among all BC contaminations. Pediatric ICUs had the highest contamination rates (45.0% of ICU contaminations, 32.8% of all BC contaminations, 1.8% of all BCs), followed by surgical ICUs (26.1% of ICU contaminations, 18.9% of all BC contaminations, 1.1% of all BCs). The lowest BC contamination was from infectious diseases and cardiovascular surgery services (2.9% of all BC contaminations). On the other hand, all services did not significantly differ in BC contaminations (p=0.630). Failure in the first notification of positivity to the clinic was ⩽0.1%, with the Gram staining-final identification agreement as 94.3%. One vial BCs was 3.8% of BCs, which were mostly neonatal cases (>90%).

Table 1. Specimen rejection analysis regarding reasons

Specimen rejection analysis	Immunoserology	Tuberculosis	Bacteriology/mycology/ virology	Total	In total samples (%)
Improper barcoding	30	8	56	94	0.005
Missing sample and/or test request	75	54	144	273	0.015
Inappropriate specimen	45	208	268	521	0.028
Insufficient specimen	60	128	128	316	0.017
Inappropriate containers	270	8	156	434	0.023
Inappropriate transport/storage conditions	15	16	8	39	0.002
Other (intra- and post-analytical phases)	45	12	128	185	0.009
Total	540	434	888	1862	0.1

Table 2. Specimen rejection analysis regarding services

Specimen rejection analysis	Immunoserology		Tuberculosis		Bacteriology/mycology/virology		Total[1]	
	n	%	n	%	n	%	n	%
Polyclinics (outpatient services)	432	0.07	66	0.2	573	0.2	1071	0.2[a]
Internal medicine services	46	0.09	356	0.5	216	0.07	618	0.2[b]
Surgical services	31	0.6	12	0.3	40	0.05	83	0.08[c]
Intensive care units	31	0.5	0	-	59	0.04	90	0.05[d]
Total	540	0.08	434	0.4	888	0.1	1862	0.1

[a]Among outpatient, [b]Among internal medicine patients, [c]Among surgical patients, [d]Among intensive care unit patients, [1]There was a statistically significant difference between polyclinics and internal medicine services vs. surgical services and intensive care units (p<0.001)

Table 3. Contamination rates regarding urine and blood culture samples

	Years	2017		2018		2019		2020		Total[1,2]	
	Services	n	%	n	%	n	%	n	%	n	%
Urine culture (UC)	Intensive care units (ICU)	53	5.3	170	10.0	247	14.3	313	14.1	783	11.8[a]
	Inpatient services	174	19.5	359	18.5	377	21.0	408	21.0	1318	20.2[b]
	Outpatient services	1229	14.4	888	9.1	1250	11.3	1192	13.5	4559	11.9[c]
	Total	1456	13.2	1417	9.9	1874	12.3	1913	14.3	6660	12.4
	Years	2017		2018		2019		2020		Total[1,2]	
	Services	n	%	n	%	n	%	n	%	n	%
Blood culture (BC)	Intensive care units	97	4.2	389	6.8	392	6.9	415	8.3	1293	6.7[a]
	Inpatient services	59	3.1	180	4.6	125	3.6	84	3.2	448	3.8[b]
	Outpatient services	13	5.6	4	2.7	7	5.5	11	7.5	35	5.3[c]
	Total	169	3.4	573	5.9	524	5.6	510	6.6	1776	5.6

[a]Among ICU patients, [b]Among inpatients, [c]Among outpatients, [1]There was not statistically significance in differences of UC (p=0.846) and BC (p=0.630) contamination rates regarding years. [2]There was not statistically significance in differences of UC (p=0.182) and BC (p=0.630) contamination rates regarding services

Discussion

In the last decades, advances of new technologies that the caused massive automation of laboratory processes have created to an increasing trend in test demands, and accordingly rising amounts of test workload with increasingly sophisticated tests. As a result, clinical laboratories perform billions of test reports, that require maintenance of quality on not only analytical processes, but also all steps starting from test order to result interpretation. Quality indicators (QIs) are beneficial tools to enable laboratories to monitor and quantify the quality of a selected test by comparing with a pre-defined criteria in order to optimize laboratory perfomance. QI is an objective measure tool, which was defined via many scientific researches and manufacturers' investigations. Continuous monitoring, observing errors, systematic and consistent data recording and correction reports are the main goals to improve performance and patient safety. Various studies indicated the numbers of QIs, on the other hand, authorities such as CLSI, ministries of health and ISO published different but concordant guides to achieve the same goals: safety of patients, effectiveness, equity, patient-centeredness, timeliness and efficiency (1,2). In Türkiye, Turkish Ministry of Health along with board organisations declared such guides, especially in the last decade, which strongly recommend the steps stated above (4,5,6). Recently, Sciacovelli et al. (8) published update on quality specifications of the QIs, focusing on every phase of laboratory errors, which shows the levels of ranges of "qualifications".

Specimen rejection and missing specimen ratios are two of the major QIs for microbiology laboratories. In our study, a rate of 0.1% was observed in overall, with a particular rejection predominance because of pre-analytical problems (90.1%). Inappropriate specimen and/or containers and insufficient specimen held the majority, indicating mostly sampling issues prior to transport to laboratories. These results are actually compatible with many studies, since researches generally state a pre-analytical predominance in laboratory errors (8,9,10). However, regarding each individual indicators, all but one pre-analytical QIs of

our laboratory showed "medium" quality, with "improper barcoding" demonstrating "low" quality. Although these pre-analytical errors are thought to be unrelated to the laboratory, laboratory/diagnostic errors are actually accepted as in five phases including pre-pre-analytic, pre-analytic, analytic, post-analytic and post-post-analytic steps. The pre-pre-analytic phase consists of test selection and request, patient identification, sampling and transport to laboratory. This phase was reported as the most "error-tic" step, since several studies reported that over 70% of laboratory errors were sourced from this period (9,10,11). On the other hand, intra- and post-analytic phases had high quality, indicating intralaboratory perfomance was at an optimum level, in our study. Analytic phase is accepted as the least susceptible to errors (9,10). However, clinical biochemistry and microbiology laboratories differ at this point, since microbiology laboratories require relatively more manual/hand-made operations. This might elevate the numbers of errors in microbiology, but in the last decade, with the aggressive interventions in standardization of microbiology laboratories by such as publishing national microbiology standards and continuous lectures organized by public health reference laboratories, significant experience has been gained by microbiology professionals (12). We believe "well-quality" for analytic phase was a result of this, but unfortunately, we could not obtain any data to prove this hypothesis, since our facility was established in 2017. On the other hand, it was clear that bacteriology/mycology/virology laboratories, which have relatively more manual/hand-made operations than others, had the highest numbers of errors in intra- and post-analytical phases. This picture might be a clue to correctness of the hypothesis stated above. Post-post-analytic phase actually depends on interpretation of clinicans (9). In our laboratory, there is a continuous commucation line with the clinicans, and during reporting there are explanation boxes that states the "meaning(s)" of the result. We believe that this "well-quality" is caused by these applications.

Heavy workload is generally a disruptive issue against achieving quality goals. ERs are "victims" of such a condition, since in some studies it is obviously observed that majority of the errors are sourced from there. In the analysis of our study regarding services, outpatient units showed the highest number of laboratory errors (57.5% of all errors). In addition, as previously stated, specimen rejection rates were significantly higher in polyclinics (outpatient units) and IMSs than SSs and ICUs ($p<0.001$). Among outpatients, 40.3% of errors were from ERs, which coincides 23.2% of all errors. This is actually a huge amount, but common it is (13). The second problematic area was IMSs, particularly pediatrics. IMSs had 33.2% of all errors, whereas pediatrics had 21.4% (64.4% of errors in IMSs). It must be stated that in our data pediatric ER was included to ER category, so in assessment of pediatrics as a whole ER+ICU+in/outpatient services, pediatrics seems to have the majority of all errors among all services. We believe these results obviously showed that "the need for quick manipulation" causes pre/pre-pre analytical problems significantly. A non-problematic process such as blood sampling might be seriously problematic when it is applied to minors, like the implementations in the ERs. Thus, as previously reported (13), ERs and pediatric units cover nearly half of the errors (44.6% of all errors). The surprising result of our data was the ICUs (even pediatric ICUs were included in this category), since they took generally second line in previous studies (13). It must be noted that errors in ICU category were mainly sourced by pediatric ones (78.9% of ICU errors), which was totally compatible with our assessments above.

Bloodstream infections (BSIs) are serious causes of mortality and morbidity, that require immediate and accurate interpretation (14,15,16). The skin preparation with an appropriate disinfectant has a crucial role to significantly reduce contamination. There are various disinfectant solutions in use of such purpose, like alcoholic iodine, aqueous povidone-iodine, alcoholic chlorhexidine and other alcoholic antiseptics. Their superiority to each other of these solutions was also a topic of reseach, that did not indicate a consensus. However, it was strongly recommended to use sterile disposable devices and application of antiseptics also to the tops of BC vials. Independent from what kind of disinfectant used, proper training and experience (e.g.; dedicated BC collection teams) were particularly notified by researchers, which was found to have a strong reducer effect on BC contamination rates (15,16). 0% contamination is impossible and is not desired (indicates that there is an interpretative issue), while The American Society of Microbiology recommends a BC contamination rate to be ⩽3% (14,17). However, reports from Türkiye showed a dark picture, since many studies stated their BC contamination findings were above this rate. A 10-year BSIs study from a tertiary center reported a rate of 6.4%, while another center shared it as 6.5% (14,18). Similar results were stated from different centers even in wider studies (4.9-6.8%) (19,20,21). Unfortunately but similarly, in our study, BC contamination was found as 5.6%. There was not any significant reduce in contamination rates regarding years ($p=0.630$), despite our all continuous trainings in order to achieve proper sampling. In addition, there was not any significant difference in BC contamination rates between services ($p=0.630$) (possibly due to their patient load), however, most of the contaminations were caused from ICUs, particularly pediatric/neonatal ICUs (72.8% of all BC contaminations,

6.7% of all BCs from ICUs). We believe this situation is because of the lack of proper catheter care and disinfection, since blood sampling from indwelling catheters is seriously common in ICUs. High level of contaminations particularly in pediatric/neonatal ICUs, are actually an indicator of this hypothesis, since healthcare staff usually do not prefer to make further invasive interventions to minors, while a catheter is in use. Higher *Candida parapsilosis* complex isolation rates from these ICUs (unpublished data) is another clue indicating this claim, since this organism is a direct sign of catheter care (22,23).

Positivity ratio in total BCs, false positivity ratio in total BCs, period from BC positivity to first notification to the clinic, BC ratio sampled as two or more sets, BC ratio as sampled only one bottle and compatibility ratio of gram staining result and last identification in BCs are other parameters that are in routine monitoring. 22.6% of all BCs gave positive signals with a contamination rate of 5.6%, and false positivity of 1.1%. This false positivity rate is actually slightly higher than ideal (≤1%), but in our retropective analysis we found a period of malfunction of our BC device, which covers nearly 60% of our false positivities. False positivity can even as high as 20-50%, but this data includes contaminations too. False positivities without any growth of microorganisms are generally caused due to high level of leukocytes, over-filled vials or improper incubation conditions (such as overheat). In such cases vials should be re-inserted for routine incubation, with a spesific warning not to leave the vials out of the devices more than 1h (15). "First notification to the clinic" is another criteria with a huge importance, since early treatment has a crucial role in prognosis of patients (24). First notifications of our laboratory are made by direct contact with the clinic and/or hospital software that sends a panic SMS to the responsible physician. We were unable to discriminate the ways of notifications, however, our notification failures were extremely rare (≤0.1%), which were mostly because of fault SMS notifications. Obtaining two seperate sets (four vials; set: one aerobic, one anaerobic vial) is a crucial point in order to achieve optimal isolation rates of bacteremia/fungemia agent (except neonatal cases) (25). Our laboratory follows this rule very strictly (one vial 3.8%, mostly neonatal cases), since except particular rare cases, BCs without at least two vials are in rejection zone. However, in our sectional prospective observation, it was noticed that some of the clinics do not strictly follow "one set from catheter, one set from peripheral" rule in diagnosis of catheter-assosciated infections, which laboratory itself could not discriminate. We could not make any comment on the ratio of this, but it is clear that there is a need of training in sampling procedures. In routine procedures, gram staining has a crucial role in the first notification of positivity. It also guides the laboratory for positive vial to be cultivated onto additional media (25).

Compatibility of gram staining result in BCs and last identification of the causative organism is an indicator of the quality of gram staining and training of the laboratory staff. The gram staining-final identification agreement was found as 94.3%. The most incompatibility was observed with Gram-positive *cocci* that were reported as yeasts, followed by Gram-negative cocobasilli that were reported as Gram-positive *bacilli*. In 5 cases, polymicrobial infections were reported as monomicrobial cases, and in one fungemia case the organism was reported as Gram-positive *cocci*. In 8 cases no organism was detected in gram staining, while yeast and Gram-negative *bacilli* were isolated in cultivation. It was very clear that there is a lack of training and experience among laboratory staff in evaluation of gram staining. A similar but higher concordance was found by other centers (26). Some researchers claimed beter results with automated gram staining evaluation (27), however we believe this rate can be elevated by just simple training interventions without any automated system. Of note, gram staining and positivities were, of course, reported to services, so that there is a possibility that they have started an antimicrobial regimen due to these preliminary data. However, in our investigation, it was obvious that our infectious disease department approach especially to coagulase negative *staphylococci* in a huge suspicion, since "one vial Gram-positive *cocci* positivity" resulted in antimicrobial treatment with only <1%. Thus, we believe these faults caused only minor issues clinically, but maybe major issues financially.

Urinary tract infections (UTIs) are one of the most common infections in both pediatric and adult populations. The definition of UTIs consists of a wide spectrum of infections from asymptomatic cases to serious infections that may cause mortal sepsis. Gram-negative bacteria, mainly the order *Enterobacterales,* are the majority of causatives, since *Escherichia coli* causes almost 80% of UTIs (28). Catheter-associated UTIs are the most frequently encountered nosocomial infections, with an elevating risk of infection concordant with the duration of catheterization (28,29). UCs generally have the largest piece of workload in microbiology laboratories, that can be contaminated by periurethral, epidermal, perianal, and vaginal microbiota, which is a serious financial cost and delayer of diagnosis. Contamination rates differ fom center to center, that can be even below 1% to over 40%. Sampling procedure especially in outpatient services strictly related with contamination rates, alongside with pre-cultivation processes such as refrigeration (29). Studies indicated that pediatric cases, especially under 24 months of age,

have the highest contamination rates, probably due to "clean-catch" and/or "bag-catch" methodology to obtain sample (30). Furthermore, female gender, pregnancy and obesity were found to be significantly related with contamination in primary care (31). In total, our UC contamination rate was 12.4%, which can be defined as high-medium performance regarding quality (32). There was not any significant alteration in contamination rates regarding both years (p=0.846) and services (p=0.182). On the other hand, majority of the contaminations were sourced from outpatient services (68.8% of all urinary contaminations; 11.9% of all UCs), especially pediatrics and ER (52.2% of all urinary contaminations; 6.5% of all UCs). These results are actually expected, since sampling from pediatric populations is thorny and more susceptible to contamination as stated above. In addition, like in BCs, "the need for rapid sampling" and "under-elective conditions" may cause higher contaminations in ERs. Interestingly, inpatient services showed higher amounts of contamination than ICUs, that was possibly because of pediatric cases, again (10.5% of all urinary contaminations). It must be noted that 53.1% of pediatric UC orders were done as "clean-catch" or "bag-catch" in our facility. This rate is actually higher in reality, since some physicians mistakenly order UCs as in routine, but the samplings were done as "clean-catch" or "bag-catch". Accordingly, the methodology of sampling seems to be the main source of contaminations. We believe our facility struggles about the same issues with other centers worldwide, thus, a programe should be organised on spesific training of parents in obtaining proper urine samples in case of any order of culture.

Conclusion

Here we presented QI results of our clinical microbiology laboratory, and even though they are partially satisfactory, it seems some urgent actions has to be taken in particular issues. Although our rejection and quality rates are below highest thresholds of quality criteria, a need of training and organization in especially outpatient units is obviously required. Similar impropriety was observed in UC contaminations with the same units. BC contaminations in ICUs are thought to be sourced from inappropriate indwelling catheter care, which is a common problem in many facilities and yet requires an emerging intervention in our hospital.

Acknowledgements: The authors wish to declare special thanks to Onur Irmak, MD and İlkay Bozdağ, MD (Balıkesir Atatürk City Hospital, Clinic of Medical Microbiology, Balıkesir, Türkiye) for their precious support.

Ethics

Ethics Committee Approval: Approved by the Ethical Board of Balıkesir University Faculty of Medicine (date: 11 Nov 2020/desicion number: 2020/203).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.K.S., N.Ö., A.Ç.D., Concept: A.K.S., N.Ö., T.K.A., Design: A.K.S., A.Ç.D., T.K.A., Data Collection or Processing: A.K.S., N.Ö., A.Ç.D., T.K.A., Analysis or Interpretation: A.K.S., N.Ö., A.Ç.D., Literature Search: A.K.S., A.Ç.D., T.K.A., Writing: A.K.S., N.Ö., A.Ç.D., T.K.A.

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

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

References

1. Plebani M. Quality in laboratory medicine: 50years on. Clin Biochem. 2017;50:101-104. [Crossref]
2. Plebani M, Sciacovelli L, Marinova M, Marcuccitti J, Chiozza ML. Quality indicators in laboratory medicine: a fundamental tool for quality and patient safety. Clin Biochem. 2013;46:1170-1174. [Crossref]
3. The Clinical And Laboratory Standards Institute (CLSI). Developing and Using Quality Indicators for Laboratory Improvement, 2nd Edition: CLSI Guideline QMS12. Wayne, PA; 2019. [Crossref]
4. T.C. Sağlık Bakanlığı, Sağlık Hizmetleri Genel Müdürlüğü, Sağlıkta Kalite, Akreditasyon ve Çalışan Hakları Dairesi Başkanlığı. Sağlıkta Kalite Standartları, Gösterge Yönetimi Rehberi v2.1. Ankara; 2020. [Crossref]
5. T.C. Sağlık Bakanlığı, Sağlık Hizmetleri Genel Müdürlüğü, Sağlıkta Kalite, Akreditasyon ve Çalışan Hakları Dairesi Başkanlığı. Klinik Mikrobiyoloji Laboratuvarları, Kalite Yönetimi Rehberi. Ankara; 2014. [Crossref]
6. T.C. Sağlık Bakanlığı, Sağlık Hizmetleri Genel Müdürlüğü, Sağlıkta Kalite, Akreditasyon ve Çalışan Hakları Dairesi Başkanlığı. Sağlıkta Kalite Standartları (Hastane). v6.0. Ankara; 2020. [Crossref]
7. Akyar I. Klinik mikrobiyoloji laboratuvarinda ISO 15189 akreditasyonu: Genel bilgiler ve laboratuvarimizdaki durum [ISO 15189 accreditation in clinical microbiology laboratory: general concepts and the status in our laboratory]. Mikrobiyol Bul. 2009;43:683-697. [Crossref]
8. Sciacovelli L, Padoan A, Aita A, Basso D, Plebani M. Quality indicators in laboratory medicine: state-of-the-art, quality specifications and future strategies. Clin Chem Lab Med. 2023;61:688-695. [Crossref]
9. Plebani M. System-related and cognitive errors in laboratory medicine. Diagnosis (Berl). 2018;5:191-196. [Crossref]
10. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med. 2006;44:750-759. [Crossref]
11. O'Kane M. The reporting, classification and grading of quality failures in the medical laboratory. Clin Chim Acta. 2009;404:28-31. [Crossref]
12. T.C. Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü, Mikrobiyoloji Referans Laboratuvarları ve Biyolojik Ürünler Dairesi Başkanlığı. Ulusal Mikrobiyoloji Standartları, Bulaşıcı Hastalıklar Laboratuvar Tanı Rehberi. Ankara, 2014. Available at https://hsgm.saglik.gov.tr/depo/birimler/Mikrobiyoloji_Referans_Laboratuvarlari_ve_Biyolojik_Urunler_DB/rehberler/UMS_LabTaniRehberi_Cilt_1.pdf [Crossref]

13. Dikmen ZG, Pinar A, Akbiyik F. Specimen rejection in laboratory medicine: Necessary for patient safety? Biochem Med (Zagreb). 2015;25:377-385. [Crossref]
14. Mataj V, Guney M, Sig AK, Uskudar-Guclu A, Albay A, Bedir O, et al. An Investigation into Bacterial Bloodstream Infections and Antibiotic Resistance Profiles in a Tertiary Hospital for a Ten-Year Period. Clin Lab. 2020;66:1467-1477. [Crossref]
15. Lamy B, Dargère S, Arendrup MC, Parienti JJ, Tattevin P. How to Optimize the Use of Blood Cultures for the Diagnosis of Bloodstream Infections? A State-of-the Art. Front Microbiol. 2016;7:697. [Crossref]
16. De Plato F, Fontana C, Gherardi G, Privitera GP, Puro V, Rigoli R, et al. Collection, transport and storage procedures for blood culture specimens in adult patients: recommendations from a board of Italian experts. Clin Chem Lab Med. 2019;57:1680-1689. [Crossref]
17. Riedel S, Carroll KC. Blood cultures: key elements for best practices and future directions. J Infect Chemother. 2010;16:301-316. [Crossref]
18. Balıkçı A, Belas Z, Eren Topkaya A. Kan Kültürü Pozitifliği: Etken ya da Kontaminasyon mu? [Blood culture positivity: is it pathogen or contaminant?]. Mikrobiyol Bul. 2013;47:135-140. [Crossref]"
19. Berksoy E, Öncel EK, Bardak Ş, Demir Ş, Yılmaz SB, Demir G, et al. True Bacteremia or Contamination? Predictive Factors for Contamination in Blood Cultures Obtained in the Pediatric Emergency Room. Eurasian J Emerg Med 2023;22:7-13. [Crossref]
20. Başustaoğlu A, Süzük Yıldız S, Mumcuoğlu İ, Karahan ZC, Öğünç D, Kaleli İ, et al. Kan kültürü uygulamalarının değerlendirilmesi: İşletim sistemi (Epicenter) verilerinin kullanımı [Evaluation of blood culture practices: Use of system (Epicenter). Mikrobiyol Bul. 2019;53:12-21. [Crossref]
21. Arabacı Ç, Kutlu O. Evaluation of microorganisms isolated from blood cultures and their susceptibility profiles to antibiotics in five years period. J Surg Med. 2019;3:729-733. [Crossref]
22. Zuo XS, Liu Y, Hu K. Epidemiology and risk factors of candidemia due to Candida parapsilosis in an intensive care unit. Rev Inst Med Trop Sao Paulo. 2021;63:e20. [Crossref]
23. Gülmez D, Sığ AK, Akar N, Duyan S, Arıkan Akdağlı S. Enfeksiyon Etkeni Mantarların Zamana Göre Sıklık ve Tür Dağılımlarındaki Değişimler: 12 Yıllık (2008-2019) Mikoloji Laboratuvarı Verileri Ne Söylüyor? [Changing Trends in Isolation Frequencies and Species of Clinical Fungal Strains: What Do the 12-years (2008-2019) Mycology Laboratory Data Tell About?]. Mikrobiyol Bul. 2021;55:53-66. [Crossref]
24. Tamelytė E, Vaičekauskienė G, Dagys A, Lapinskas T, Jankauskaitė L. Early Blood Biomarkers to Improve Sepsis/Bacteremia Diagnostics in Pediatric Emergency Settings. Medicina (Kaunas). 2019;55:99. [Crossref]
25. Klinik Mikrobiyoloji Uzmanlık Derneği (KLİMUD). Kan Dolaşımı Örneklerinin Laboratuvar İncelemesi Rehberi v2.0. Ankara; 2022. [Crossref]
26. Öksüz Ş, Dönmez B, Keskin B, Memiş N, Karamurat ZD, Çalışkan E, et al. Evaluation of Quality Assurance Indicators and Contamination Rate in Blood Culture. Konuralp Med J. 2021;13:557-562. [Crossref]
27. Smith KP, Kang AD, Kirby JE. Automated Interpretation of Blood Culture Gram Stains by Use of a Deep Convolutional Neural Network. J Clin Microbiol. 2018;56:e01521-e01517. [Crossref]
28. Klein RD, Hultgren SJ. Urinary tract infections: microbial pathogenesis, host-pathogen interactions and new treatment strategies. Nat Rev Microbiol. 2020;18:211-226. [Crossref]
29. LaRocco MT, Franek J, Leibach EK, Weissfeld AS, Kraft CS, Sautter RL, et al. Effectiveness of Preanalytic Practices on Contamination and Diagnostic Accuracy of Urine Cultures: a Laboratory Medicine Best Practices Systematic Review and Meta-analysis. Clin Microbiol Rev. 2016;29:105-147. [Crossref]
30. Tosif S, Baker A, Oakley E, Donath S, Babl FE. Contamination rates of different urine collection methods for the diagnosis of urinary tract infections in young children: an observational cohort study. J Paediatr Child Health. 2012;48:659-664. [Crossref]
31. Hansen MA, Valentine-King M, Zoorob R, Schlueter M, Matas JL, Willis SE, et al. Prevalence and predictors of urine culture contamination in primary care: A cross-sectional study. Int J Nurs Stud. 2022;134:104325. [Crossref]
32. Bekeris LG, Jones BA, Walsh MK, Wagar EA. Urine culture contamination: a College of American Pathologists Q-Probes study of 127 laboratories. Arch Pathol Lab Med. 2008;132:913-917. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.97752

Hamidiye Med J 2023;4(2):136-141

Discharge Creatinine Level Predicts Long-term Clinical Outcomes in Patients with Infective Endocarditis

Enfektif Endokarditli Hastalarda Taburcu Kreatinin Değerinin Uzun Dönem Klinik Sonuçlardaki Öngördürücülüğü

Mehmet Bozbay[1], Mehmet Eren[2]

[1]*Private Medikar Hospital, Clinic of Cardiology, Karabük, Türkiye*

[2]*University of Health Sciences Türkiye, Dr. Siyami Ersek Cardiovascular and Thoracic Surgery Training and Research Hospital, Clinic of Cardiology, İstanbul, Türkiye*

ABSTRACT

Background: The impact of admission creatinine levels on long-term mortality in infective endocarditis (IE) is well known. The relationship between discharge creatinine levels and long-term clinical outcomes of IE has not been studied yet. The goal of the present study was to evaluate the association between discharge creatinine values and long-term unfavorable outcomes of IE.

Materials and Methods: A total of 118 IE patients were retrospectively obtained. Patients were classified two tertiles, based on discharge creatinine levels. The high discharge creatinine group (n=39) was identified as having a creatinine level in the third tertile (>0.93 mg/dL) and the low group (n=79) was identified as having a level in the lower two tertiles (≤0.93 mg/dL) to evaluate long-term follow-up.

Results: Long-term mortality was more frequent in the high group (33.3% vs. 11.3%, p=0.03). Although high discharge creatinine level (>0.93 mg/dL) was found as an independent parameter of long-term mortality (odds ratio: 2.21, 95% confidence interval: 1.34-3.65 p=0.002), estimated glomerular filtration rate did not predict long-term mortality. All-cause and heart failure-associated rehospitalization patients had higher discharge creatinine values (1.2±0.8 vs. 0.9±0.3, p=0.002 and 1.2±0.8 vs. 0.9±0.3, p=0.001, respectively).

Conclusion: High discharge creatinine level are related to long-term mortality and rehospitalization in IE.

Keywords: Endocarditis, creatinine, mortality

ÖZ

Amaç: Enfektif endokarditli (EE) hastalarda başvuru kreatinin değerlerinin uzun dönem mortaliteye etkisi bilinmektedir. EE hastalarında taburcu kreatinin değerinin uzun dönem klinik sonuçlar ile ilişkisi değerlendirilmemiştir. Bu çalışmada EE hastalarında taburcu kreatinin değerinin uzun dönem istenmeyen klinik sonuçlarla ilişkisinin değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Toplam 118 EE hastası retrospektif olarak elde edildi. Hastalar taburcu kreatinin değerlerine göre iki gruba ayrıldı. Taburcu kreatinin değeri >0,93 mg/dL olan hastalar yüksek taburcu kreatinin grubu (n=39), taburcu kreatinin değeri ≤0,93 mg/dL olan hastalar düşük taburcu kreatinin grubu (n=79) olarak tanımlandı.

Bulgular: Yüksek taburcu kreatinin grubundaki hastalarda uzun dönem mortalite artmış olarak saptandı (%33,3 vs. %11,3, p=0,03). Yüksek taburcu kreatinin değeri (>0,93 mg/dL) uzun dönem mortalitenin bağımsız belirleyicisi (olasılık oranı: 2,21, %95 güven aralığı: 1,34-3,65 p=0,002) olmasına rağmen tahmini glomerüler filtrasyon hızı uzun dönem mortalite ile ilişkili olarak değerlendirilmedi. Tüm nedenli ve kalp yetersizliği ile ilişkili hastaneye tekrar yatış oranları yüksek taburcu kreatinin grubundaki hastalarda daha sık izlendi (1,16±0,78 vs. 0,85±0,3, p=0,002 ve 1,15±0,81 vs. 0,87±0,34, p=0,001).

Sonuç: EE hastalarında yüksek taburcu kreatinin değeri artmış uzun dönem mortalite ve tekrar hastaneye yatış ile ilişkilidir.

Anahtar Kelimeler: Endokardit, kreatinin, ölüm

Address for Correspondence: Mehmet Bozbay, Private Medikar Hospital, Clinic of Cardiology, Karabük, Türkiye
Phone: +90 505 950 16 12 E-mail: mbozbay42@gmail.com **ORCID ID:** orcid.org/0000-0001-9460-2210

Received: 26.04.2023 **Accepted:** 05.06.2023

Introduction

Infective endocarditis (IE) is a condition characterized by severe morbidity and mortality. Although there have been improvements in diagnosis and medical and surgical therapy, the complications and mortality rates of the disease have remained high. Mortality rates range from 9-26% in-hospital and 10-40% for long-term period (1,2,3,4). Previous studies have reported association with admission creatinine levels and mortality from IE (5,6,7). The impact of discharge creatinine levels on long-term mortality and outcomes of IE has not yet been studied. The aim of this study was to evaluate the impact of discharge creatinine levels and long-term unfavourable outcomes of IE.

Material and Methods

A total of 171 IE patients were obtained in a retrospective study between January 2008-December 2020 at the University of Health Sciences Türkiye, Dr. Siyami Ersek Cardiovascular and Thoracic Surgery Training and Research Hospital and January 2013-January 2021 Private Medikar Hospital. The modified Duke criteria (8) used for a definitive diagnosis of IE. Forty-eight patients died in-hospital, and five patients who treated with hemodialysis were excluded. Therefore, 118 patients were evaluated. The study population was divided into tertiles, according to discharge creatinine values. The high discharge creatinine group (n=39) was classified as having a creatinine level in the third tertile (>0.93 mg/dL) and the low group (n=79) was classified as having a level in the lower two tertiles (≤0.93 mg/dL) to evaluate long-term follow-up. Estimated glomerular filtration (eGFR) was calculated by modification of diet in renal disease formula (9).

The study population was analyzed according to clinical, laboratory, and echocardiographic parameters. Blood samples were obtained three separate venipuncture sites for blood cultures. On admission and during in-hospital stay, hematologic and biochemical measurements were noted by daily. Within 72 hours of admission, each patient was evaluated through a comprehensive echocardiographic examination. Serum creatinine concentrations were calculated with the Jaffe method.

Mortality was determined as a death during follow-up. Hypertension (HT) was determined as use of antihypertensive agent, a systolic pressure >140 mm Hg, or a diastolic pressure >90 mm Hg. Diabetes mellitus was defined as the usage of insulin or antidiabetic drugs. Acute kidney injury was determined serum creatinine level rised more than 0.3 mg/dL (10). Paravalvular abscess, dehiscence of the prosthetic valve, fistula, and indication of surgical treatment were defined according to previous guidelines (2). Heart failure was defined as the having of pulmonary edema or cardiogenic shock occurring with valvular perforation, intracardiac abscess, fistula, or valvular obstruction.

Follow-up data were noted from hospital documents. The primary endpoint was all-cause mortality. All of rehospitalizations were noted.

Statistical Analysis

Quantitative variables were shown as mean value ± standard deviation, and qualitative variables were expressed as percentages. Comparisons of parametric values assessed by means of a two-tailed Student's t-test. χ^2 or Fisher's Exact test used to compare with categorical variables. A backward stepwise multivariate logistic regression analysis was used to determinate predictors of mortality. Age greater than 65 years, admission creatinine level, HT, surgical therapy, and peak and discharge creatinine levels were evaluated. The cumulative survival curves for mortality were established using the Kaplan-Meier method, with differences evaluated using the log-rank test. All statistical analyses were noted by using SPSS version 15.0 (SPSS Inc., Chicago, IL).

Results

The clinical and demographic parameters of the patients are summarized in Table 1. Mean age was 45.9±18.3. Prosthetic valve endocarditis was seen in 35 patients, and admission creatinine level was 1.0±0.7. Surgical treatment

Table 1. Clinical and demographic findings of the study patients

Age (years)	45.9±18.3	Dehiscence	11 (34.3)
Male gender	71 (60.1)	Abscess	21 (17.7)
Diabetes mellitus	9 (7.6)	Fistula	13 (11)
Hypertension	34 (28.8)	WBC (10^9 g/dL)	10.2±4.7
Mitral native	24 (20.3)	Hemoglobin (g/dL)	11.1±2.1
Aort native	22 (18.6)	Admission creatinine (mg/dL)	1.0±0.7
Mitral and aortic involvement	11 (9.3)	LVEF (%)	55±10.8
Lead endocarditis	15 (12.7)	Surgical treatment	71 (60.1)
Prosthetic valve	32 (27.1)		

Mean (SD) and n (%) are reported for continous and categorical variables, respectively.
LVEF: Left ventricular ejection fraction, WBC: White blood cell, SD: Standard deviation

was performed on 71 patients. A comparison of the clinical and demographic findings of patients with survival and non-survival is listed in Table 2. C-reactive protein levels were parallel in survival and non-survival patients (105.1±85.3 vs. 110.1±79.7, p=0.8, respectively). The causative microorganisms were *Viridans streptococci* (30 patient, 25.4%), *Staphylococcus aureus* (14 patient, 11.8%), coagulase negative *Staphylococcus* (29 patient, 24.5%) *Enterococcus* spp. (6 patient, 5.0%), culture-negative (25 patient, 21.1%) and other microorganisms (14 patient, 11.8%).

The values of admission, peak, and discharge creatinine and eGFR levels in survival and non-survival subjects are listed in Table 3. Admission, peak, discharge creatinine and eGFR levels were higher in the mortality group. Table 4 and Table 5 present admission, peak, discharge creatinine and eGFR levels with all-cause and heart failure-associated rehospitalization. Patients with rehospitalization had higher discharge creatinine and eGFR values. Multivariate logistic regression analysis indicate that age and discharge creatinine level were independent parameters of long-term mortality (Table 6).

Table 2. Comparison of the clinical and demographic findings of patients with survival and non-survival

	Survival (n=96)	Non-survival (n=22)	p
Age (years)	42.4±17.3	61.0±14.5	**<0.001**
Male gender	61 (63.5)	10 (45.4)	0.11
Diabetes mellitus	6 (6.2)	3 (13.6)	0.23
Hypertension	21 (21.8)	13 (59)	**0.001**
Dehiscence	8 (8.3)	3 (13.6)	0.44
Abscess	18 (18.7)	3 (13.6)	0.57
Fistula	10 (9.6)	3 (13.6)	0.66
WBC (10^9 g/dL)	10.3±4.0	10.1±7.1	0.85
Hemoglobin (g/dL)	11.2±2.2	10.5±1.8	0.14
LVEF (%)	55.3±11.0	53.9±10.6	0.58
Surgical treatment	62 (64.5)	9 (40.9)	**0.04**
AKI	35 (36.4)	15 (68.1)	**0.007**

Mean (SD) and n (%) are reported for continous and categorical variables, respectively.
LVEF: Left ventricular ejection fraction, WBC: White blood cell, AKI: Acute kidney injury, SD: Standard deviation

Table 3. Admission, peak and discharge levels of the survival and non-survival patients

	Survival group (n=96)	Non-survival group (n=22)	p
Admission creatinine (mg/dL)	0.9±0.4	1.4±1.3	**0.009**
Peak creatinine (mg/dL)	1.4±0.8	2.2±1.7	**0.002**
Discharge creatinine (mg/dL)	0.8±0.3	1.4±0.9	**<0.001**
Admission eGFR (mL/min)	95.5±35.0	69.7±28.4	**<0.001**
Peak eGFR (mL/min)	70.9±36.0	41.5±21.1	**<0.001**
Discharge eGFR (mL/min)	103.8±33.4	67.0±35.7	**<0.001**

eGFR: Estimated glomerular filtration rate

Table 4. Admission, peak and discharge creatinine levels of the all-cause rehospitalization and non-rehospitalization patients

	Non-rehospitalization (n=85)	Rehospitalization (n=33)	p
Admission creatinine (mg/dL)	1.0±0.4	1.19±1.1	0.1
Peak creatinine (mg/dL)	1.4±0.8	1.9±1.5	**0.04**
Discharge creatinine (mg/dL)	0.9±0.3	1.2±0.8	**0.002**
Admission eGFR (mL/min)	94.6±35.4	80.6±33.2	0.052
Peak eGFR (mL/min)	69.7±36.8	54.3±30.1	**0.03**
Discharge eGFR (mL/min)	101.7±34.5	82.7±37.3	**0.01**

eGFR: Estimated glomerular filtration rate

Discharge creatinine level 0.93 mg/dL was determined as an efficacious cut-off value for long-term mortality (area under curve=0.71; 95% confidence interval 0.58-0.84, p=0.003), the sensitivity was 59.1%, and the specificity was 72.9%. Long-term mortality was more frequent in the high-discharge creatinine group (Figure 1). The median follow-up time was 40 months and long-term mortality increased in the high discharge creatinine group. (11.3% vs. 33.3%, p=0.03).

Discussion

This is the initial study to investigate the impact of discharge creatinine levels on long-term mortality and clinical outcomes in IE. In the present study, we found that discharge creatinine level was an independent parameter of long-term mortality and rehospitalization. Although eGFR level was related to rehospitalization it was not predictor of long-term mortality.

IE is a life-threatening condition. The mortality rate of the disease was 15% in a 1980s cohort, mortality rates have remained high despite improvements in diagnosis and medical and surgical therapy (1,2,3,4,9). Many studies have shown the relationship between renal dysfunction and cardiovascular diseases (10,11,12,13,14,15,16). Shlipak et al. (10) evaluated 130.099 elderly patients hospitalized with myocardial infarction, and they found that mild and moderate renal failure (creatinine level >1.5 mg/dL) were related to increased long-term mortality. Furthermore, Santopinto et al. (12) reported that the creatinine clearance

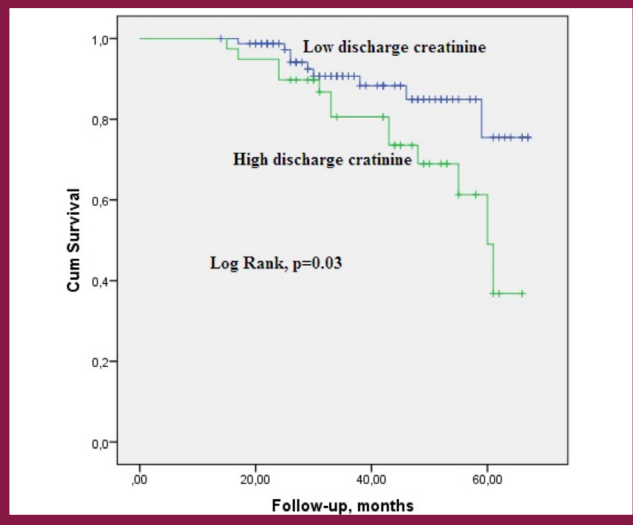

Figure 1. Long-term mortality of the low- and high-discharge creatinine groups

Table 5. Admission, peak and discharge creatinine levels of the heart failure related rehospitalization and non-rehospitalization patients

	Non-rehospitalization (n=91)	Rehospitalization (n=27)	p
Admission creatinine (mg/dL)	1.0±0.4	1.2±1.3	**0.05**
Peak creatinine (mg/dL)	1.4±0.8	2.0±1.6	**0.002**
Discharge creatinine (mg/dL)	0.9±0.3	1.2±0.8	**0.001**
Admission eGFR (mL/min)	93.6±35.3	81.0±33.7	0.1
Peak eGFR (mL/min)	68.7±36.0	54.4±32.1	0.06
Discharge eGFR (mL/min)	100.4±35.8	82.9±36.3	**0.03**

eGFR: Estimated glomerular filtration rate

Table 6. Effects of multiple variables on long-term mortality in univariate and multivariate Cox regression analysis

	Univariate OR	95% CI	p	Multivariate OR	95% CI	p
Age	1.057	1.023-1.093	0.001	1.055	1.025-1.087	**<0.001**
Admission creatinine	1.624	1.136-2.32	0.008			
HT	2.47	0.9-6.83	0.08			
Surgical treatment	0.24	0.08-073	0.01			
Peak creatinine	1.6	1.2-2.13	0.001			
Discharge creatinine	2.6	1.7-4.01	<0.001	2.29	1.339-3.647	**0.002**
Admission eGFR	0.98	0.966-0.995	0.007			
Peak eGFR	0.972	0.955-0.990	0.002			
Discharge eGFR	0.975	0.961-0.988	<0.001			

HT: Hypertension, eGFR: Estimated glomerular filtration rate, OR: Odds ratio, CI: Confidence interval

rate was related to mortality and major bleeding in acute coronary syndrome (ACS). Marenzi et al. (14) examined 2756 patients with ACS, and they showed that during treatment in the intensive cardiac care unit, creatinine level increases of more than 0.3 mg/dl were an important predictor of mortality. Cystatin C (CysC) is a useful parameter to evaluate renal function. Akgul et al. (15) showed that high CysC levels were related to the higher increased both in-hospital and one-month mortality of patients with ST-elevation myocardial infarction.

IE is associated with renal dysfunction, and previous studies have shown the impact of in-hospital creatinine levels and long-term mortality (5,6,7). There are several reasons for renal dysfunction in IE. Focal and diffuse glomerulonephritis characterized by immune complex deposition, vasculitic glomerulonephritis, acute interstitial nephritis, renal infarcts due to septic embolism or haemodynamic disturbance, and nephrotoxic antibiotics such as aminoglycosides are associated with renal failure in IE. Immune complexes have been shown in up to 90 percent of patients, and their levels were successfully lowered as a result of antibiotic therapy (17). Both focal and diffuse glomerulonephritis may occur in the same patients, and it may be present despite the absence of clinical signs of renal failure. Furthermore, vasculitic and immune complex glomerulonephritis may be associated with cell-mediated immunity (18,19). Hypotensive episodes because of heart failure, renal emboli, or severe septicaemia may even cause acute tubular damage and renal cortical necrosis (20). Microscopic hematuria, pyuria, or proteinuria occurs, and gross hematuria may be related to renal infarction. Majumdar et al. (21) investigated renal pathological findings in IE and evaluated 62 patients' renal tissue obtained by necropsy or biopsy. They found that localized infarction was the most frequent renal lesion. Acute glomerulonephritis, which was mainly vasculitic glomerulonephritis, was seen in 26% of samples.

Moreover, Erbay et al. (7) showed that a creatinine level ⩾2 mg/dL was an important risk factor for mortality from IE. Hsu et al. (22) examined 315 patients and reported that renal dysfunction (creatinine level ⩾1.5) was an independent predictor of in-hospital mortality of IE. In a related study, Wallace et al. (5) investigated 208 IE patients retrospectively within the first 48 hours after admission for clinical markers, and they showed that serum creatinine levels >133 μmol/dL were related to in-hospital and long-term mortality. In addition, Koeda et al. (23) found that the admission creatinine and eGFR values were associated with in-hospital mortality from IE. Buchholtz et al. (24) prospectively investigated 231 IE patients, and they divided patients into four groups according to their admission-estimated endogenous creatinine clearance (EECC). In this study, in which the mean follow-up was 453 days, they showed that patients in group IV (EECC <30 mL/min) were strongly related to both in-hospital and long-term mortality. In contrast, we did not found association between eGFR level and long-term mortality. Bjurman et al. (25) evaluated 125 IE patients, and they noted CysC levels at admission and after two weeks of treatment. They showed that CysC levels were important predictors of mortality.

The association between discharge creatinine levels and long-term unfavourable outcomes in IE has not yet been reported. Many studies have shown the impact of admission creatinine, ECCC, eGFR, and CysC levels and long-term mortality (5,24,25). Wallace et al. (5) noted that a cut-off creatinine level was 133 μmol/dL (1.5 mg/dL) was an important parameter for in-hospital and six-month mortality. In this study, we found that discharge creatinine cut-off value of 0.93 mg/dL was an important parameter of long-term mortality from IE. In addition, eGFR value was not a factor of long-term mortality in IE. Notably, we reported that discharge creatinine level may be associated with long-term mortality in IE.

The precise mechanism for this relationship is not known. Although successful antibiotic therapy normalizes urinary parameters in most patients, microscopic hematuria or proteinuria may continue for a long time. In these patients, extensive residual glomerular changes, such as proliferation or sclerosis of glomeruli, have been shown on postmortem examination (17). This condition may, in fact, be related to a genetic mechanism.

Study Limitations

Our study is a retrospective, non-randomized, single-centre study and may be related to selection bias. In addition, we did not look at other laboratory markers such as CysC, and we did not calculate ECCC, and the effects of diet modification on renal disease.

Conclusion

Our study showed that discharge creatinine level is an important parameter of long-term mortality and rehospitalization in IE. Creatinine is a easily available, inexpensive marker for predicting long-term mortality and clinical outcomes of IE.

Ethics

Ethics Committee Approval: The study was approved by the Scientific Research Ethics Committee of the Karabük University with the decision numbered 2023/1227 on 17/01/2023.

Informed Consent: Informed consent was obtained.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.B., M.E., Concept: M.E., Design: M.E., Data Collection or Processing: M.B., Analysis or Interpretation: M.B., M.E., Literature Search: M.B., Writing: M.B., M.E.

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

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

References

1. Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Bolger AF, Levison ME, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation. 2005;111:e394-e434. [Crossref]
2. Habib G, Hoen B, Tornos P, Thuny F, Prendergast B, Vilacosta I, et al. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): the Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society of Chemotherapy (ISC) for Infection and Cancer. Eur Heart J. 2009;30:2369-2413. [Crossref]
3. Tiryakioglu O, Tiryakioglu SK, Türk T, Ozkan H, Bozat T, Yavuz Ş. Timing for surgical therapy in active infective endocarditis. Turkish J Thorac Cardiovasc Surg. 2006;14:194-198. [Crossref]
4. Thuny F, Grisoli D, Cautela J, Riberi A, Raoult D, Habib G. Infective endocarditis: prevention, diagnosis, and management. Can J Cardiol. 2014;30:1046-1057. [Crossref]
5. Wallace SM, Walton BI, Kharbanda RK, Hardy R, Wilson AP, Swanton RH. Mortality from infective endocarditis: clinical predictors of outcome. Heart. 2002;88:53-60. [Crossref]
6. Nonaka M, Kusuhara T, An K, Nakatsuka D, Sekine Y, Iwakura A, et al. Long-term clinical outcomes and predictors of survival after prosthetic valve endocarditis surgery. J Heart Valve Dis. 2013;22:704-712. [Crossref]
7. Erbay AR, Erbay A, Canga A, Keskin G, Sen N, Atak R, et al. Risk factors for in-hospital mortality in infective endocarditis: five years' experience at a tertiary care hospital in Turkey. J Heart Valve Dis. 2010;19:216-224. [Crossref]
8. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. 2000;30:633-638. [Crossref]
9. Klahr S, Levey AS, Beck GJ, Caggiula AW, Hunsicker L, Kusek JW, et al. The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. N Engl J Med. 1994;330:877-884. [Crossref]
10. Shlipak MG, Heidenreich PA, Noguchi H, Chertow GM, Browner WS, McClellan MB. Association of renal insufficiency with treatment and outcomes after myocardial infarction in elderly patients. Ann Intern Med. 2002;137:555-562. [Crossref]
11. Gibson CM, Pinto DS, Murphy SA, Morrow DA, Hobbach HP, Wiviott SD, et al. Association of creatinine and creatinine clearance on presentation in acute myocardial infarction with subsequent mortality. J Am Coll Cardiol. 2003;42:1535-1543. [Crossref]
12. Santopinto JJ, Fox KA, Goldberg RJ, Budaj A, Piñero G, Avezum A, et al. Creatinine clearance and adverse hospital outcomes in patients with acute coronary syndromes: findings from the global registry of acute coronary events (GRACE). Heart. 2003;89:1003-1008. [Crossref]
13. Marenzi G, Moltrasio M, Assanelli E, Lauri G, Marana I, Grazi M, et al. Impact of cardiac and renal dysfunction on inhospital morbidity and mortality of patients with acute myocardial infarction undergoing primary angioplasty. Am Heart J. 2007;153:755-762. [Crossref]
14. Marenzi G, Cabiati A, Cosentino N, Assanelli E, Milazzo V, Rubino M, et al. Prognostic significance of serum creatinine and its change patterns in patients with acute coronary syndromes. Am Heart J. 2015;169:363-370. [Crossref]
15. Akgul O, Uyarel H, Ergelen M, Pusuroglu H, Gul M, Turen S, et al. Predictive value of elevated cystatin C in patients undergoing primary angioplasty for ST-elevation myocardial infarction. J Crit Care. 2013;28:882.e13-885-e20. [Crossref]
16. Ronco C, McCullough P, Anker SD, Anand I, Aspromonte N, Bagshaw SM, et al. Cardio-renal syndromes: report from the consensus conference of the acute dialysis quality initiative. Eur Heart J. 2010;31:703-711. [Crossref]
17. Neugarten J, Baldwin DS. Glomerulonephritis in bacterial endocarditis. Am J Med. 1984;77:297-304. [Crossref]
18. Cunningham MA, Huang XR, Dowling JP, Tipping PG, Holdsworth SR. Prominence of cell-mediated immunity effectors in "pauci-immune" glomerulonephritis. J Am Soc Nephrol. 1999;10:499-506. [Crossref]
19. Rennke HG, Klein PS, Sandstrom DJ, Mendrick DL. Cell-mediated immune injury in the kidney: acute nephritis induced in the rat by azobenzenearsonate. Kidney Int. 1994;45:1044-1056. [Crossref]
20. Eknoyan G, Lister BJ, Kim HS, Greenberg SD. Renal complications of bacterial endocarditis. Am J Nephrol. 1985;5:457-469. [Crossref]
21. Majumdar A, Chowdhary S, Ferreira MA, Hammond LA, Howie AJ, Lipkin GW, et al. Renal pathological findings in infective endocarditis. Nephrol Dial Transplant. 2000;15:1782-1787. [Crossref]
22. Hsu CN, Wang JY, Tseng CD, Hwang JJ, Hsueh PR, Liau CS. Clinical features and predictors for mortality in patients with infective endocarditis at a university hospital in Taiwan from 1995 to 2003. Epidemiol Infect. 2006;134:589-597. [Crossref]
23. Koeda C, Tashiro A, Itoh T, Okabayashi H, Nakamura M. Mild renal dysfunction on admission is an important prognostic predictor in patients with infective endocarditis: a retrospective single-center study. Intern Med. 2013;52:1013-1018. [Crossref]
24. Buchholtz K, Larsen CT, Hassager C, Bruun NE. In infectious endocarditis patients mortality is highly related to kidney function at time of diagnosis: a prospective observational cohort study of 231 cases. Eur J Intern Med. 2009;20:407-410. [Crossref]
25. Bjurman C, Snygg-Martin U, Olaison L, Fu ML, Hammarsten O. Cystatin C in a composite risk score for mortality in patients with infective endocarditis: a cohort study. BMJ Open. 2012;2:e000856. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2023.30592

Hamidiye Med J 2023;4(2):142-151

Factors Affecting Health Literacy and Relationship Between Health Literacy and Child Emergency Utilization: Relationship Between Health Literacy and Emergency Service

Sağlık Okuryazarlığını Etkileyen Faktörler ve Sağlık Okuryazarlığı ile Çocukların Acil Durumda Kullanımı Arasındaki İlişki

Sevgi Akova

University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Pediatric Emergency, İstanbul, Türkiye

ABSTRACT

Background: This study examines the health literacy differences between parents of patients who visited the yellow and green zones and its impact on their emergency use.

Materials and Methods: The study analyzes the participants' demographic characteristics such as age, gender, marital status, occupation, income, and education. The health literacy levels of the participants were determined using the Turkish health literacy scale-32 (TSOY-32).

Results: TSOY-32 scores of patients with a yellow zone triage scale who visited the pediatric emergency service were found to be adequate and similar. Participants with secondary and master's degrees exhibited problematic health literacy, while those with a doctoral degree showed insufficient health literacy. Participants with primary, high school, vocational school, and university degrees displayed sufficient health literacy. It was observed that parents with higher monthly incomes had higher health literacy. Regarding the parents of patients who visited the pediatric emergency green zone, significant differences were found. TSOY-32 scores of male parents were problematic, while those of female parents were adequate. According to TSOY-32 score evaluation, participants with secondary school, high school, and masters degrees exhibited sufficient health literacy. Participants with primary and vocational school degrees showed problematic health literacy, and those with doctoral degrees exhibited insufficient health literacy. Despite varying educational backgrounds, it was determined that the health literacy of parents who visited the pediatric emergency green area was similar. When comparing the yellow and green zones, it was found that parents who visited the green zone had lower health literacy levels.

Conclusion: We have concluded that having sufficient health literacy is directly related to the results of having sufficient basic health knowledge, being aware of their rights and responsibilities, and approaching all problems that may arise in the system by making the right decisions.

Keywords: Health literacy, yellow zone, green zone, parent

ÖZ

Amaç: Sarı alan ve yeşil alana başvuran hasta ebeveynlerinin arasındaki sağlık okuryazarlık farkları ile acil başvurularına olan etkisi incelenmiştir.

Gereç ve Yöntemler: Katılımcıların yaş, cinsiyet, medeni durum, meslek, gelir durumu ve eğitim durumu gibi demografik özellikleri analiz edilmiştir. Katılımcıların sağlık okuryazarlık düzeyleri Türkçe sağlık okuryazarlığı ölçeği-32 (TSOY-32) kullanılarak tespit edilmiştir.

Bulgular: Çocuk acil servisine başvuran triaj skalası sarı olan hastaların TSOY-32 puanı yeterli ve benzer bulunmuştur. Ortaokul ve yüksek lisans mezunu olanlar sorunlu, doktora mezunu olanlar yetersiz sağlık okuryazarlığı sergilemiştir. İlkokul, lise, meslek yüksekokulu ve üniversite mezunu olanlar ise yeterli sağlık okuryazarlığına sahip olmuşlardır. Aylık geliri yüksek olan ebeveynlerin sağlık okuryazarlığı daha yüksektir. Çocuk acil yeşil alana başvuran hasta ebeveynleri arasında anlamlı farklar bulunmuştur. TSOY-32 puanlarına göre erkek ebeveynlerin sağlık okuryazarlığı sorunludur, kadın ebeveynlerin ise yeterlidir. Ortaokul, lise ve yüksek lisans mezunu olanlar yeterli sağlık okuryazarlığına sahipken, ilkokul ve meslek yüksekokulu mezunu olanlar sorunlu, doktora mezunu

Address for Correspondence: Sevgi Akova, University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Pediatric Emergency, İstanbul, Türkiye
Phone: +90 533 313 74 74 E-mail: akovasevgi2000@hotmail.com **ORCID ID:** orcid.org/0000-0003-3332-6771

Received: 23.02.2023 **Accepted:** 21.06.2023

ÖZ

olanlar ise yetersiz sağlık okuryazarlığı sergilemiştir. Eğitim seviyeleri farklı olsa da çocuk acil yeşil alana başvuran ebeveynlerin sağlık okuryazarlıklarının benzer olduğu tespit edilmiştir. Sarı ve yeşil alanlar karşılaştırıldığında, yeşil alana başvuran ebeveynlerin sağlık okuryazarlık düzeylerinin daha düşük olduğu görülmüştür.

Sonuç: Yeterli düzeyde sağlık okuryazarlığa sahip olunması, yeterli temel sağlık bilgisine sahip olabilme, hak ve sorumluluklarının bilincinde olma, sistem içerisinde oluşabilecek tüm sorunlara doğru karalar vererek yaklaşabilme sonuçlarıyla doğrudan ilişkili olduğu kanaatine vardık.

Anahtar Kelimeler: Sağlık okuryazarlığı, sarı alan, yeşil alan, ebeveyn

Introduction

The paragraph you provided contains technical information and some specific details. I have made some edits to improve clarity and readability:

The concept of health literacy (HL) was first introduced by Simonds (1,2). The World Health Organization (WHO) defines HL as "the cognitive and social skills that individuals need to access, understand, and use health-related information in order to maintain and improve their own health" (3). According to the American Medical Association, HL refers to "individuals' ability to read health-related messages, understand instructions on medication labels, and comprehend and follow instructions provided by healthcare professionals" (4).

Sørensen et al. (5) analyzed various definitions of HL and concluded that it encompasses the knowledge, motivation, and skills required for individuals to access, understand, evaluate, and utilize health information to improve their health, prevent diseases, and enhance their quality of life (6).

Several scales are commonly used to determine HL levels, such as the rapid estimate of adult literacy in medicine and the test of functional HL in adults (7). Other available tests include the mini-mental state examination (8), the wide range achievement test (9,10), the medical achievement reading test (5), the newest vital sign (11), a two-question test (8), the health activity literacy scale (9), and the Turkish health literacy scale-32 (TSOY-32) (12).

TSOY-32 is a newly developed scale consisting of 32 items. It is based on the research conducted for the European health literacy survey-questionnaire (HLS-EU). Unlike the original scale, the TSOY-32 is structured as a 2x4 matrix with two basic dimensions (treatment and care, and disease prevention/health promotion) and four processes (access to health-related information, understanding of health-related information, assessment of health-related information, and use/application of health-related information), resulting in eight components (9). In the evaluation of the scale, the indices were standardized between 0 and 50, following the approach used in HLS-EU study.

The following formula was employed for this purpose:

Index = (mean - 1) x (50/3)

Similar to the HLS-EU study, the obtained index was classified into four categories. Each item was graded on a scale of 1 to 4, with 1 representing "very easy", 2 for "easy", 3 for "difficult", and 4 for "very difficult". The code 5 was used to indicate "I have no idea". Before calculating the scores, the codes were recoded as 1-4, 4-1, and the calculations were performed using a formula.

HL plays a crucial role in eliminating health inequalities, increasing lifespan, and improving the quality of life for individuals. For patients, it ensures that health information is understandable and that they are involved in the decision-making process, while for healthcare professionals, it leads to professional satisfaction, effective communication, and the acquisition of clinical skills (13). Lack of HL can create barriers to access information and services, making it important for individuals to navigate complex healthcare systems and effectively manage their own health. Differences in individuals' ability to read and understand health-related texts contribute to existing health inequalities within healthcare systems. It has been observed that individuals with low HL are more likely to experience poor health, struggle to understand their treatment, and have a higher risk of hospitalization. The annual healthcare costs for individuals with very low literacy levels may be four times higher than the general population (14,15).

When examining the relationship between HL levels and health management, it has been observed that individuals with insufficient or limited HL use preventive health services less frequently and have worse chronic disease management. Additionally, their morbidity and mortality rates are higher (16,17).

The term "triage" originates from the French word "trier", meaning to choose or separate. The main objective of triage is to prioritize the diagnosis and treatment of emergencies, thereby preventing morbidity and mortality (18). Triage systems may vary between countries and within different hospitals within the same country based on specific needs (19). In Türkiye and in our hospital, a 3-stage color scale triage system is implemented following the Ministry of

Health's recommendation. Under this system, patients are categorized as "very urgent (red)," "urgent (yellow zone)", and "not urgent (green zone)" (20).

In Türkiye and other countries, the admission of non-emergency patients to emergency departments (ED) has become a common occurrence, resulting in increased overcrowding. This overcrowding can lead to delays in the diagnosis and treatment of emergency patients. Furthermore, unnecessary admissions to the emergency department impose an avoidable economic burden on the healthcare system, as it increases the demand for staff, materials, and laboratory resources.

In this study, our primary objective was to emphasize the importance of reducing unnecessary visits to the emergency room. We sought to achieve this by assessing the level of HL among individuals and examining the relationship between HL and the frequency of emergency department visits.

Material and Methods

The materials and methods of this study involved assessing the HL levels of parents whose children sought medical care in the green and yellow zones of pediatric emergency services. We conducted a review of previous HL studies conducted in our country and compared the HL levels between these two groups. The main objective was to explore the relationship between disparities in HL, rates of ED utilization, and the potential misuse of the ED by parents in the yellow and green zones.

The participants' HL levels were assessed using TSOY-32. The scale consists of 32 items, and their responses were used to calculate an overall HL index.

The item scores were transformed to a range of 0 to 50 to facilitate comparison across the index scores. The resulting scores were then categorized into four levels:

Insufficient HL (>0-25 points),
Limited HL (>25-33 points),
Adequate HL (>33-42 points),
Excellent HL (>42-50 points).

To simplify the analysis, insufficient and limited HL levels were combined into a single category referred to as limited HL (0-33 points).

Our study was conducted as a single-center, prospective, observational survey at the pediatric ED of University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, from 2020 to 2022. The study protocol received ethical approval from the Ethics Committee on 14/01/2021, with the protocol number B.10.1.TKH.4.34.H.GP.0.01/16. The research followed the principles stated in the Helsinki Declaration and Good Clinical Practice, ensuring compliance with ethical guidelines regarding the research subject. Informed consent was obtained from each participant before they completed the questionnaire, indicating their voluntary participation in the study.

Statistical Analysis

The data collected will be analyzed using the Statistical Package for the Social Sciences (SPSS) Statistics 25.0 software package developed by IBM Corp. The normality of the data distribution will be assessed using the Kolmogorov-Smirnov test. Descriptive statistical methods, including measures such as median, quartile, and frequency, will be utilized to evaluate the study data. Parametric tests such as One-Way ANOVA will be employed for parametric data, while non-parametric tests like the Mann-Whitney U test and Kruskal-Wallis H test will be used for non-parametric data. Correlation analysis will be conducted to assess numerical variables, and the chi-square test will be applied for the analysis of categorical variables. A significance level of $p<0.05$ will be considered for all analyzes.

Results

Parents of patients who applied to the pediatric emergency yellow zone had the following characteristics:

• The average age of the applicant parents ranged from 33.48 (median: 33) to a maximum of 74.

• Out of the total, 95 were men and 281 were women. The mean TSOY-32 score for parents in the yellow zone, categorized by gender, was as follows: Male - 33.3, female - 34.72.

• Based on these findings, it was determined that the TSOY-32 score of parents of patients in the pediatric emergency service with a yellow zone triage scale was sufficient and similar, with no statistically significant difference based on gender ($p>0.05$).

• Out of the participants, 332 were married and 44 were single. The mean TSOY-32 scores for parents in the yellow zone were as follows: Married - 34.41, single - 33.36.

• In conclusion, there was no statistically significant difference in the HL of parents of patients with a yellow zone triage scale in the pediatric emergency service based on marital status ($p>0.05$). It was also observed that the TSOY-32 scores were sufficient and similar for parents who were married or single.

Regarding the participants' educational levels, 80 had completed primary school, 95 had completed secondary school, 126 had completed high school, 10 had attended vocational school, 57 had attended university, 6 had a master's degree, and 2 had a doctorate. The average TSOY-32 scores for parents who applied to the pediatric emergency yellow zone were as follows: Primary school graduate 35.19, secondary school graduate 32.79, high school graduate 34.7, vocational school graduate 35.77, university/college

graduate 34.54, graduate degree 32.60, PhD graduates were found to be 14.1.

On the other hand, TSOY-32 scores were found to be problematic for those who graduated from secondary school and master's degree, insufficient for those with a doctorate degree, and sufficient for those who graduated from primary school, high school, vocational school and university. According to these results, there was no statistically significant difference (p>0.05) in the HL education level of the parents of the patients whose triage scale was yellow zone.

When the income status of the applicant parents was analyzed, 158 of them had less than their expenses, 169 of them had an income equal to their expenses, and 49 of them had more than their income. The average TSOY-32 scores of the parents who received yellow zone according to their income; income less than expenses 33.03, income equal to expenses 35.11, income more than expenses It was 35.53.

The TSOY-32 results of parents applying for childhood emergency yellow zone were assessed as adequate according to income. HL of parents of pediatric patients who applied to pediatric emergency services was statistically significant if the monthly deviation (p<0.05) in HL of parents of patients who applied to pediatric emergency services and had a yellow zone triage scale was statistically significant It turns out that It was higher for parents with higher monthly income.

Considering the occupational status of our participants, 208 were housewives, 64 workers, 7 retired, 11 students, 24 civil servants, 6 tradesmen, 21 self-employed, 1 farmer, 34 had other occupations. The average of TSOY-32 scores of parents with yellow color according to occupational groups: Housewife 34.71, employee: 34.77, retired: 34.61, in students 27.73, officer 36.81, small business 36.31, in freelancers 32.41, in other occupational groups 31.99.

Farmer; it was detected as 32.1. According to the TSOY-32 scores of the occupational groups, it was evaluated as sufficient in all groups, and problematic in those who overlap freely with other occupations. Occupational differences (p>0.05) were not found to be statistically significant in the HL of the parents of the patients with yellow zone triage scale who applied to the pediatric emergency service (Tables 1, 2, 3).

Parents of the patients who applied to the pediatric emergency green zone;

• It was observed that the mean age was 33.8 (median: 34), the minimum age of parents was 19, and the highest age of parents was 65.

• When we analyzed in terms of gender, 153 were male and 318 were female. The TSOY-32 mean score of green zone parents by gender; male; it was 33.39 for 32.47 women.

According to these results, TSOY-3 scores of green field parents were found to be problematic in males and adequate in females. The gender difference (p>0.05) was not statistically significant in the HL of the parents of the patients whose triage scale was green zone.

• In our analysis according to education level, 102 of them had primary school, 128 had secondary school, 154 had high school, 23 had vocational school, 55 had university/school education, 8 had master's degree and 1 had doctorate education. The TSOY-32 score averages of green field parents according to their educational status: Primary school graduate 32.64, secondary school graduate 33.6, high school graduate 33.15, vocational school graduate 32.7, university/college graduate 32.66, master's degree 35.45, PhD graduate was 17.2.

According to the TSOY-32 scale, primary school, secondary school, high school, vocational school and university graduates who applied to the green space were found to be problematic, while those with a doctorate degree were found to be inadequate. Accordingly, there was no statistically significant difference between the educational status and HL of the parents who applied to the green space (p>0.05).

As a result of the occupational analysis we conducted on the applicant parents, it was determined that 207 of them were housewives, 105 were workers, 12 were retired, 33 were students, 37 were civil servants, 15 were tradesmen, 33 were self-employed, 1 was a farmer and 28 were from other occupational groups. TSOY-32 averages of green field parents: Housewife 33.02, worker 32.59, retired 34.25, student; 34.47, civil servant 32.36, artisan 30.6. Self-employed; 32.08, other professions; 36.89, farmer; 32.3 were found to be sufficient and not statistically significant (p>0.05).

When we look at the income level, we see that 232 of the parents' income is less than their expenditures, 164 of the parents' income is equal to their expenditures, and 75 of the parents' income is more than their expenditures. Mean scores of the TSOY-32 according to the income level of the parents in the green zone: Income less than expenses; 33.39. Income equal to expenses; 33.02.

Income more than expenses; 32.32. TSOY-32 assessment of green field parents was found to be problematic for those whose income was more than expenditure and adequate for those whose income was more than expenditure, and was not found to be statistically significant (p>0.05).

In our study, 378 of the parents of patients with green field practice scale were married and 93 were single. The

mean scores of the TSOY-32 according to the marital status of green field patients: Married 33.18, single 32.71.

According to the TSOY-32 scores, married green field parents were found to be adequate and single parents were found to be problematic, which was not statistically significant (Tables 1, 2, 3) (p>0.05).

There was no statistically significant difference in terms of HL index score (p=0.117) in emergency service green zone and yellow zone applications (Table 2).

According to TSOY-32 scale applied to the patients included in the study, 16.4% of the participants were excellent, 38.8% sufficient, 30.5% problematic/limited, 14.3% insufficient health literate. This distribution was 14.1% excellent, 40.1% sufficient, 29.7% problematic/limited, 16.1% insufficient for the green zone. In the yellow zone, 19.4% were found to be excellent, 37.2% sufficient, 31.4% problematic/limited, and 12.30% inadequate. In terms of distribution of HL levels according to this scale, there is no significant relationship between the emergency room green zone and emergency room yellow zone groups (p=0.075) (Table 3).

When the HL of the parents of the patients included in the study was compared in the yellow zone and the green zone, it was found that the HL of the parents was lower in the green zone applications (p<0.05, pearson coefficient: 0.125).

Discussion

When the literature was examined; two studies investigating the level of HL with the widest social cross-section were determined. These studies are studies carried out in the USA and European union countries. According to the European HL survey, 12.4% insufficient, 35.2% problematic, 36% sufficient and 16.5% excellent HL levels were determined. According to this study, there was a difference in HL levels between countries. Bulgaria (26.9%)

Table 1. Comparison of the distribution rates of demographic data between the emergency service green zone and yellow zone application groups

			Green zone		Yellow zone			Total		p
			Median (25-75%)		Median (25-75%)			Median (25-75%)		
Age			34 (28-40)		33 (27-39)			33 (28-39)		0.453
		n	Triage %	Variable %	n	Triage %	Variable %	n	%	
Gender										0.022
	Male	153	32.5	61.7	95	25.3	38.3	248	29.28	
	Female	318	67.5	53.1	281	74.7	46.9	599	70.72	
Marital status										0.002
	Married	378	80.3	53.2	332	88.3	46.8	710	83.8	
	Single	93	19.7	67.9	44	11.7	32.1	137	16.2	
Job										0.003
	Housewife	207	43.9	49.9	208	55.3	50.1	415	49	
	Employee	105	22.3	62.1	64	17	37.9	169	20	
	Retired	12	2.5	63.2	7	1.9	36.8	19	2.2	
	Student	33	7	75.0	11	2.9	25.0	44	5.2	
	Officer	37	7.9	60.7	24	6.4	39.3	61	7.2	
	Small business	15	3.2	71.4	6	1.6	28.6	21	2.5	
	Freelancer	33	7	61.1	21	5.6	38.9	54	6.4	
	Other	29	6.2	45.3	35	9.3	54.7	64	7.5	
Monthly income										0.011
	Income less than expenses	232	49.3	59.5	158	42	40.5	390	46	
	Income equivalent to expenses	164	34.8	49.2	169	44.9	50.8	333	39.3	

Table 2. Comparison of literacy index score between groups					
	Green zone		Yellow zone		p
	Median	(25-75%)	Median	(25-75%)	
Health literacy index score	33.3	28.50-38.70	33.9	29.22-40.25	0.117

Table 3. Distribution of health literacy levels among the emergency service green zone and yellow zone application groups								
		Green zone		Yellow zone		Total		p
Health literacy levels		n	%	n	%	n	%	
								0.075
	Inadequate health literacy	76	16.10	45	12.00	121	14.30	
	Troubled limited health literacy	140	29.70	118	31.40	258	30.50	
	Adequate health literacy	189	40.10	140	37.20	329	38.80	
	Excellent health literacy	66	14.10	73	19.40	139	16.40	

and Austria (18.2%) are the countries with the highest prevalence of insufficient HL. When the inadequate and problematic/limited categories were evaluated together, it was determined that the highest HL literacy rate was in Bulgaria (62.1%), followed by Austria (56.4%) and Spain (58.3%) (21). According to the US National Adult Literacy Study, it was stated that 12% had adequate HL, 53% moderate level, 22% basic level, and 14% below basic level (22).

In a study by Tanrıöver et al. (23) according to the Turkish HL survey conducted in Türkiye, 24.5% of Turkish citizens have poor HL, 40.1% have limited HL, 27.8% have adequate HL and 7.6% were found to have excellent HL [2-(101)]. According to the Turkish version of the European HL scale by Okyay and Abacıgil (12), 13.1% of the population had inadequate SHL scores and 39.6% had limited problems and 32.9=14.5% had excellent SHL scores.

In a study by Ergün (24) among health students, 25.9% had poor HL, 34.0% had limited problems, 27.0% had excellent HL, and 13.1% had very good HL. In a study by Çopurlar et al. (25) of 67 medical and nursing students, 63.6% rated their HL as adequate and 36.4% as limited. In our survey, 10.2% of students rated their HL as inadequate, 30.0% rated it as problematic, 33.0% rated it as adequate, and 26.8% rated it as excellent. I rated it as being. The results of our study are similar to those conducted in health-educated volunteers.

In our study, 248 (29.3%) of 847 people were male and 599 (70.7%) were female. When the ED clinic examined the green field and yellow field applications, it was seen that there was a statistically significant correlation between the gender of the patients who applied and the choice of triage (p=0.022). We found that the number of female parents with a yellow zone triage scale was significantly higher than that of male parents. Edirne et al. (26) similarly, in their study, 43.2% of the patients were male and 56.8% were female. Unlike our study, Bertakis et al.'s (27) study named gender differences in the use of health services in the USA found that men mostly used emergency services and 3rd level hospital services, while women used primary health care services more. They associated this situation with men wanting to reach treatment more quickly (27).

The mean TSOY-32 of the parents who applied to the pediatric ED was found to be 33,3 for males and 34.72 for female parents. With these findings, TSOY-32 score was found to be sufficient and similar, and no statistically significant difference was found in terms of gender (p>0.05). When TSOY-32 index score was examined, Okyay and Abacıgil (12) observed that there was no relationship between genders, similar to our study (12). Haerian colleagues did not find any difference in HL index scores between genders in Iran (28). In the study conducted by Tanrıöver et al. (23) it was reported that the level of HL was lower in women, unlike our results. Unlike in the study of Sørensen et al. (21), a lower level of HL was found in males. In the study of von Wagner et al. (29) conducted in England, they also reported that being male is a risk factor for low HL, which is different from the results of our study. Among the reasons for the different results in these studies; We think that there may be educational inequality and differences in regional education levels.

As a result of our evaluation according to marital status, when examined in terms of marital status, it was seen that the emergency service yellow zone application was statistically significantly related to the emergency room green zone (p=0.002). It was observed that 710 (88.3%) married parents had a higher number of yellow zone applications than 248 (29.28%) male parents.

The average of TSOY-32 scores according to marital status; married; 34.41 singles: 33.36. We did not observe a statistically significant difference in marital status (p>0.05) in HL of the parents of the patients whose triage scale was yellow zone, and we also found that the TSOY-32 scores of those whose parents were married or single were adequate and similar. When we examined the relationship between the HL index score and marital status, Rikard et al. (30) found that the HL score of people who are married or living with married people is higher than that of single people. In a local study by Deniz and Oğuzöncül (31), no relationship was found between marriage and HL. Economic stability of married people, increase in quality of life, low depression levels, and increased social connections have been shown as the reason for this situation (32).

In the analysis of the education level of the parents of the patients included in the study, it was found that there was no statistically significant relationship between the applications for the green field to the emergency service and the applications for the yellow zone to the emergency service (p=0.90). In the study of Khan et al. (33) in Canada, unlike our study, they reported that the use of emergency services decreased as the level of education increased. In the study conducted by Andrews and Kass (34), again different from our study, they found that as the education level decreases, there is an increase in unnecessary emergency applications and they consider their illness more important and urgent.

There was no statistically significant difference (p>0.05) in the HL of the parents of the patients whose triage scale was yellow zone according to their education level. There was no statistically significant difference (p>0.05) in the HL education of the parents of the patients who applied to the pediatric emergency service and whose triage scale was green. According to the results; according to TSOY-32, according to the educational status of the green field parents, those who graduated from secondary school, high school, and masters degree adequately, those who graduated from primary school, vocational school and university were found to have problems, and those who graduated from doctoral schools were observed as insufficient. Contrary to what is believed, we obtained different data from the thought that there will be an increase in the TSOY-32 ratio with the increase in education level. Similar to our study, in the study of Nakayama et al. (35), who compared the HL index and education level, no relationship was found. Similar to our study, no significant difference was found between the education level and HL index of Özdemir et al. (36). The common opinion is that there is a very close relationship between the level of HL and the level of education (36). In the study of Lee et al. (37), unlike our study, they reported that the level of HL increased with the increase in the education level of Asian immigrants in the United States. Also different from our study, Beauchamp et al. found that the level of HL was higher with the increase in education level.

In the study we conducted according to the occupational group, no statistically significant difference was found in the HL of the parents of the patients whose triage scale was yellow and green field (p>0.05). In the evaluation of the occupational groups of the parents whose triage scale was yellow zone, according to their TSOY-32 scores, they were considered adequate in all groups, and those who had free overlap with other occupations were considered problematic. Evaluation of the parents who applied to the child emergency green zone according to TSOY-32 was determined as sufficient for workers, civil servants, tradesmen, self-employed farmers, troubled housewives, retired, students and other occupational groups. We observed that studies on this subject are limited. In a study conducted by Haghdoost et al. (38) in Iran, the level of HL of fixed workers and students was observed to be significantly higher than those of non-permanent jobs.

TSOY-32 scores of the parents who applied to the pediatric emergency yellow zone according to their income were evaluated as adequate and the monthly income difference in HL (p<0.05) was found to be statistically significant. We found that it was too much. Of the parents who applied to the child emergency green zone, the TSOY-32 assessment was found to be problematic for those whose income was more than their expenses, and sufficient for those whose income was more than their expenses. The health literacy monthly income difference (p>0.05) was not statistically significant.

In our study, when we compared the HL of the parents of the patients in terms of yellow zone and green zone applications, it was found that the HL of the parents who applied to the green field service was lower. In our examination of the HL index score, the median index score of the parents who applied to the green zone was 33.3 and 33.9 for those who applied to the yellow zone. In our literature review; in a study that included 9 European countries of the European Union, 16.5% was found to be excellent, 36% sufficient, 35.2% problematic/limited, 12.4% inadequate (21). In the study of Nakayama et al. (35), 4.2% was found to be excellent, 10.4% sufficient, 35.5% problematic/limited, and 49.9% inadequate. With this study conducted in Japan, they found the opposite result in the idea that the level of HL is high in developed countries (35). In the study carried out by the Minister and his friend in Ağrı province of our country, the mean score of TSOY-32 scale was determined as 24,59, and when we analyzed the

two groups as adequate/excellent, insufficient/limited, they found the insufficient/limited ratio of the study to be 77.8, similar to our study. They found TSOY-32 score to be 33.8 (39). In TSOY-32 study of Okyay and Abacıgil (12), they found general HL to be in the problematic/limited class with a rate of 29.5. We found the general HL score of our study to be 33.6, and we determined that it was at a problematic-limited level.

Today, while the health system is getting more complex, the expectations from the people who benefit from this system are also increasing. Individuals are expected to be knowledgeable about the service required in the process of eliminating the health problems of themselves or their relatives. People can only find a solution in this complex structure if they have the ability to reach basic information about health and health service concepts, understand and fulfill the requirements. This proficiency state is defined by sufficient HL.

Inadequate HL leads to problems in the effective use of health services and an unhealthier life, while it results in low productivity, increased morbidity and mortality and cost increase at the social level. It is stated in studies conducted on this subject that among the reasons for applying to the emergency room green zone, patients want to receive service without making an appointment and waiting in line, easy access to the service, and the results of the examinations requested as a result of the examination in a short time on the same day. This suggests that patients do not perceive the concept of "urgency" correctly. Considering that the average of emergency service admissions in our country is around 30% of all patients, the low level of HL in our country is effective in the density of the emergency services.

The WHO sees HL as a key to improving health (WHO, 2012) ensuring public access to health and health services; appropriately recognizing and drawing conclusions from the messages to be conveyed in health promotion and development; communication channels for information transfer, especially screen education; addressing all parts of the world and applying effectively to health care providers at the right place, at the right time, at the right frequency, is the way to increase HL. The social determinants of health, the "education level of society" alone, is not enough. There are differences in levels of education and perceptions of topics related to education and behavioral reflection. All of them must be together for a healthy life (9).

Healthcare delivery is increasingly patient-centric and the role of patients in the system is increasing day by day. There is also a growing demand from patients who are more consciously aware of their health services. This situation draws attention to the concept of patient safety.

HL is a type of literacy that is gaining in importance. Literacy is a concept whose definition changes and develops over time. In the understanding of the modern world, where the most important fact is "change", the classical definition of literacy has remained incomplete and inadequate, and the concept of literacy has been reinterpreted from different dimensions over time in parallel with the paradigm shifts.

The social, cultural and economic changes that humanity has been in throughout history have started to occur faster than ever before, especially since the 20th century. It has become a necessity for a person to receive education throughout his life in order to keep up with the society he lives in and these rapid changes. Thus, the concept of lifelong education emerged. Healthcare delivery is increasingly patient-centric and the role of patients in the system is increasing day by day. There is also a growing demand from patients who are more consciously aware of their health services. This situation draws attention to the concept of patient safety. Patients have various duties and responsibilities in ensuring patient safety. However, patients must be in good health to actively participate in the process and fulfill their assigned roles. At this point, the concept of health literacy comes to the fore. HL should be emphasized to empower patients and make a positive contribution to patient safety by enabling them to actively participate in the process. Factors affecting HL and the relationship between HL and patient safety. Thesis is an indispensable part of the literacy learning process. In this respect, many branches of science, from sociology to psychology, from education to history, have focused on the concept of literacy and have done a lot of research on this subject both in theory and in practice. The Turkish Language Association (2011) defines the concept of literacy as "the state of being literate". Literate is defined as "an educated person who can read and write". Literacy with its simplest and general definition; It can be expressed as the ability to read written texts and create a written text (Aldemir, 2003).

According to the data of the Ministry of Health of the Republic of Türkiye, the rate of applying to the hospital at least once during pregnancy was 98% in 2013. The average number of examinations of pregnant women who applied to the hospital was 4.3 in 2013. Again in 2013, 98% of all deliveries in Türkiye were carried out in hospitals. After birth, the average number of follow-ups per infant was 8.8 and the average number of follow-ups per child was 2.2 in 2013 (Ministry of Health, 2013). These figures alone show how important health services are in our lives. Almost everyone opens their eyes to this world in hospitals, in other words, within the healthcare system. Throughout their lives, they interact with the health care system many

times, both for themselves and for their relatives. However, the system in question is often extremely complex for both those who benefit from health services and their relatives. This complex system is becoming more and more patient-centered. This approach has imposed new responsibilities on patients. Individuals are exposed to an intense flood of information by health service providers, pharmacists, as well as the internet and the media, and those who demand health care are expected to be able to make basic decisions about health. However, the fact that this information from the environment is understandable, applicable and usable in making the right decision is closely related to the HL level of the individual. The basis of making the right decisions is to have sufficient basic health information, to be aware of their rights and responsibilities and to act in this direction. This becomes possible only if people have sufficient HL (CDC, 2009).

Study Limitations

The limitation of the study is that parents admitted to the pediatric ED with their children were involved in the study.

The study was conducted on all of parents who took their children to pediatric ED. Parents preferring policlinics were not included in this study, so the parents were far away from performing triage. They all preferred urgent healthcare, mostly red zone, less preferred yellow and green zones.

Conclusion

It has been determined that having sufficient HL is directly related to the results of having sufficient basic health information, being aware of their rights and responsibilities, and approaching all problems that may arise in the system by making the right decisions.

Highlight Key Points

1. In this article, we aimed that by having sufficient health literacy, individuals will have the ability to have sufficient basic health knowledge, to be aware of their rights and responsibilities, and to approach all problems that may arise in the system by making the right decisions.

2. According to the data in the article, it has been revealed that emergency services are misused by individuals who do not have sufficient health literacy in Türkiye, as in many countries. When the patients were compared in terms of yellow zone and green zone use, we found that the health literacy of the parents who applied to the green zone was lower.

3. It was found that those who graduated from secondary school and master's degree had problems, those with doctorate degrees were insufficient, those who graduated from primary school, high school, vocational school and university were found to be sufficient. With these results, it has been revealed that the concept of emergency patient and the information is insufficient or should be emphasized more in the education system.

Ethics

Ethics Committee Approval: Our study was conducted as a single-center, prospective, observational survey at the pediatric ED of University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, from 2020 to 2022. The study protocol received ethical approval from the Ethics Committee on 14/01/2021, with the protocol number B.10.1.TKH.4.34.H.GP.0.01/16.

Informed Consent: Informed consent was obtained from each participant before they completed the questionnaire, indicating their voluntary participation in the study.

Peer-review: Internally and externally peer-reviewed.

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

References

1. Simonds SK. Health Education As Social Policy. Health Education Monographs. 1974;2:1-10. [Crossref]
2. Egbert N, Nanna KM. Health Literacy: Challenges and Strategies, The Online Journal of Issues in Nursing. 2009;14. [Crossref]
3. Track 2: Health literacy and health behaviour: World Health Organization; 2009 http://www.who.int/healthpromotion/conferences/7gchp/track2/en/. [Crossref]
4. Health literacy: report of the Council on Scientific Affairs. Ad Hoc Committee on Health Literacy for the Council on Scientific Affairs, American Medical Association. JAMA. 1999;281:552-557. [Crossref]
5. Sørensen K, Van den Broucke S, Fullam J, Doyle G, Pelikan J, Slonska Z, et al. Health literacy and public health: a systematic review and integration of definitions and models. BMC Public Health. 2012;12:80. [Crossref]
6. Berkman ND, Davis TC, McCormack L. Health literacy: what is it? J Health Commun. 2010;15:9-19. [Crossref]
7. Smith S KM. Health Literacy: a Brief Literature Review. Health Literacy Brief. 2010;3:1-30. [Crossref]
8. Deniz S. Sağlık Okuryazarlığını Anlamak. Galatasaray Üniversitesi İletişim Dergisi. 2013;73-92. [Crossref]
9. Pala E. Evaluation of the health literacy level of the patients who applied to a Tertiary Hospital Family Medicine Clinic. Journal of Health Sciences and Medicine. 2022;5:693-697. [Crossref]
10. Morris NS, MacLean CD, Chew LD, Littenberg B. The Single Item Literacy Screener: evaluation of a brief instrument to identify limited reading ability. BMC Fam Pract. 2006;7:21. [Crossref]
11. Maniaci MJ, Heckman MG, Dawson NL. Functional health literacy and understanding of medications at discharge. Mayo Clin Proc. 2008;83:554-558. [Crossref]
12. Okyay P, Abacıgil F. Türkiye sağlık okuryazarlığı ölçekleri güvenilirlik ve geçerlilik çalışması. Avrupa sağlık okuryazarlığı ölçeği Türkçe uyarlaması (ASOYTR). 2016:24-41. [Crossref]
13. Health literacy program. http://www.ama-assn.org/ama/pub/aboutama/amafoundation/our-programs/public-health/health-literacy-program.page, (Erişim), 04.09.2019. [Crossref]

14. Services H. Healthy people 2010: Understanding and improving health: Bernan Assoc; 2000. [Crossref]
15. Uğurlu Z. Sağlık kurumlarına başvuran hastaların sağlık okuryazarlığının ve kullanılan eğitim materyallerinin sağlık okuryazarlığına uygunluğunun değerlendirilmesi. Ankara: Başkent Üniversitesi; 2011. [Crossref]
16. Çopurlar CK, Kartal M. Sağlık Okuryazarlığı Nedir? Nasıl Değerlendirilir? Neden Önemli? Turkish Journal of Family Medicine and Primary Care. 2016;10. [Crossref]
17. Sadeghi S, Brooks D, Stagg-Peterson S, Goldstein R. Growing awareness of the importance of health literacy in individuals with COPD. COPD. 2013;10:72-78. [Crossref]
18. Emergency Triage Assessment and Treatment (ETAT) –Manual for Participants. World Health Organisation Geneva 2005. [Crossref]
19. Gilboy N, Tanabe P, Travers DA, Rosenau AM, Eitel DR. Emergency Severity Index, Version 4: Implementation Handbook. AHRQ Publication No.05-0046-2. Rockville, MD: Agency for Healthcare Research and Quality. May 2005 [Crossref]
20. Yataklı Sağlık Tesislerinde Acil Servis Hizmetlerinin Uygulama Usul ve Esasları Hakkında Tebliğde Değişiklik Yapılmasına Dair Tebliğ. Resmi Gazete. 2018. [Crossref]
21. Sørensen K, Pelikan JM, Röthlin F, Ganahl K, Slonska Z, Doyle G, et al. Health literacy in Europe: comparative results of the European health literacy survey (HLS-EU). Eur J Public Health. 2015;25:1053-1058. [Crossref]
22. Kutner M, Greenburg E, Jin Y, Paulsen C. The Health Literacy of America's Adults: Results from the 2003 National Assessment of Adult Literacy. NCES 2006483. National Center for Education Statistics. 2006. [Crossref]
23. Tanrıöver M, Yıldırım H, Ready N, Çakır B, Akalın E. Türkiye sağlık okuryazarlığı araştırması. Sağlık-Sen Yayınları. 2014. [Crossref]
24. Ergün S. Sağlık Yüksekokulu Öğrencilerinde Sağlık Okuryazarlığı. Kocaeli Tıp Dergisi. 2017;6:1-6. [Crossref]
25. Çopurlar CK, Akkaya K, Arslantaş İ, Kartal M. Health Literacy of Students Who Applied to Medical and Nursing Faculty in Dokuz Eylul University. Turkish Journal of Family Medicine and Primary Care. 2017;11:144-151. [Crossref]
26. Edirne T, Edirne Y, Atmaca B, Keskin S. Yüzüncü Yıl Üniversitesi Tıp Fakültesi Acil Servis hastalarının özellikleri. Van Tıp Dergisi 2008;15:107-111. [Crossref]
27. Bertakis KD, Azari R, Helms LJ, Callahan EJ, Robbins JA. Gender differences in the utilization of health care services. J Fam Pract. 2000;49:147-152. [Crossref]
28. Haerian A, Moghaddam MHB, Ehrampoush MH, Bazm S, Bahsoun MH.Health literacy among adults in Yazd, Iran. J Educ Health Promot. 2015;4:91. [Crossref]
29. von Wagner C, Knight K, Steptoe A, Wardle J. Functional health literacy and health-promoting behaviour in a national sample of British adults. J Epidemiol Community Health. 2007;61:1086-1090. [Crossref]
30. Rikard RV, Thompson MS, McKinney J, Beauchamp A. Examining health literacy disparities in the United States: a third look at the National Assessment of Adult Literacy (NAAL). BMC Public Health. 2016;16:975. [Crossref]
31. Deniz S, Oğuzöncül AF. Assessment of Health Literacy Level in Akcadag, Malatya, Turkey. Firat Tip Dergisi. 2020;25:5-13. [Crossref]
32. Williams K, Umberson D. Marital status, marital transitions, and health: a gendered life course perspective. J Health Soc Behav. 2004;45:81-98. [Crossref]
33. Khan Y, Glazier RH, Moineddin R, Schull MJ. A population-based study of the association between socioeconomic status and emergency department utilization in Ontario, Canada. Acad Emerg Med. 2011;18:836-843. [Crossref]
34. Andrews H, Kass L. Non-urgent use of emergency departments: populations most likely to overestimate illness severity. Intern Emerg Med. 2018;13:893-900. [Crossref]
35. Nakayama K, Osaka W, Togari T, Ishikawa H, Yonekura Y, Sekido A, et al. Comprehensive health literacy in Japan is lower than in Europe: a validated Japanese-language assessment of health literacy. BMC Public Health. 2015;15:505. [Crossref]
36. Özdemir S, Akça HŞ, Algın A, Kokulu K. Health Literacy in The Emergency Department: A Cross-sectional Descriptive Study. Eurasian J Emerg Med 2020;19:94-97. [Crossref]
37. Lee SY, Arozullah AM, Cho YI. Health literacy, social support, and health: a research agenda. Soc Sci Med. 2004;58:1309-1321. [Crossref]
38. Haghdoost AA, Karamouzian M, Jamshidi E, Sharifi H, Rakhshani F, Mashayekhi N, et al. Health literacy among Iranian adults: findings from a nationwide population-based survey in 2015. East Mediterr Health J. 2019;25:828-836. [Crossref]
39. Bakan AB, Yildiz M. 21-64 Yaş Grubundaki Bireylerin Sağlık Okuryazarlık Düzeylerinin Belirlenmesine İlişkin Bir Çalışma. 2019;8:33-40. [Crossref]

CASE REPORT

DOI: 10.4274/hamidiyemedj.galenos.2023.78941

Hamidiye Med J 2023;4(2):152-155

A Case Report of Primary Isolated Extrahepatic Hydatid Cyst of the Thigh

Primer İzole Uyluk Yerleşimli Ekstrahepatik Kist Hidatik Olgu Sunumu

Ramazan Serdar Arslan[1], Mete Çiçek[2], Süleyman Diker[3], Şirin Küçük[4]

[1]*Servergazi State Hospital, Clinic of General Surgery, Denizli, Türkiye*

[2]*Uşak Training and Research Hospital, Clinic of General Surgery, Uşak, Türkiye*

[3]*Uşak Training and Research Hospital, Clinic of Internal Medicine, Uşak, Türkiye*

[4]*Uşak Training and Research Hospital, Clinic of Pathology, Uşak, Türkiye*

ABSTRACT

Hydatid cysts can appear as surprise masses in various anatomical regions of the body. Thyroid, breast, musculoskeletal system can be counted as atypical localization areas. The thigh, gluteal region, and neck are the most common locations for primary isolated muscular hydatid cysts. This text discusses the diagnosis, imaging methods, treatment strategy, and patient management of a primary isolated left thigh hydatid cyst. A 32-year-old male patient presented to the outpatient clinic with a slowly growing mass on his left thigh. Physical examination revealed a well-circumscribed tender mass in the upper medial part of the left thigh that did not cause skin discoloration. Ultrasonography revealed a multiloculated complex cystic mass measuring 10x6x12 cm between the thigh subcutaneous tissue and muscle planes. A multiseptal cystic mass was discovered between the muscle planes in the anteromedial part of the left thigh using contrast-enhanced tomography. The total cyst excision was performed under spinal anesthesia on the patient. During the two-year follow-up, no recurrence was detected in the patient's physical examination or radiological imaging. Extrahepatic primary hydatid cysts are frequently found subcutaneously. Thigh, gluteal region, neck, and extremity localization is extremely uncommon. Total cyst excision is the most commonly used surgical treatment method for intramuscular hydatid cysts.

Keywords: Echinococcosis, hydatid cyst, hydatid disease, extrahepatic cyst, thigh

ÖZ

Kist hidatik vücutta farklı anatomik bölgelere yerleşip sürpriz kitleler olarak karşımıza çıkabilmektedir. Tiroid, meme, kas ve iskelet sistemi atipik yerleşim bölgesi olarak sayılabilir. Primer izole müsküler kist hidatik en sık uyluk, gluteal bölge ve boyunda görülür. Bu yazıda; primer izole sol uyluk yerleşimli kist hidatiğin tanı, görüntüleme yöntemleri, tedavi stratejisi ve hasta yönetimi sunulmuştur. Otuz iki yaşında erkek hasta sol uyluğunda yavaş büyüyen kitle şikayeti ile polikliniğe başvurdu. Fizik muayenede sol uyluk üst mediyal kısmında deride renk değişikliğine yol açmayan, düzgün sınırlı hassas kitle saptandı. Ultrasonografide uylukta subkütan doku ve kas planları arasında 10x6 x12 cm boyutunda multiloküle kompleks kistik kitle saptandı. Kontrastlı tomografide sol uyluk anteromediyal kısmında kas planları arasında yerleşmiş multiseptal kistik kitle tespit edildi. Hastaya spinal anestezi altında total kist eksizyonu uygulandı. Hastanın iki yıllık takibinde fizik muayene ve radyolojik görüntülemelerinde nüks saptanmadı. Ekstrahepatik primer kist hidatik sıklıkla subkütan yerleşimlidir. Uyluk, gluteal bölge, boyun ve ekstremite yerleşimi oldukça nadirdir. Kas içi kist hidatik cerrahi tedavisinde kistin total eksizyonu en sık kullanılan yöntemlerdir.

Anahtar Kelimeler: Ekinekok, kist hidatik, hidatik hastalık, ekstrahepatik kist, uyluk

Introduction

Hydatid disease (HD) or echinococcosis; it is an endemic, zoonotic, parasitic infection caused by *Echinococcus granulosus* (sch) seen in both developing and developed countries and is considered a serious public health problem (1). Although this parasitic disease can be seen in any part of the body, the liver (55-70%) and then the lungs (18-35%) are the most frequently affected organs (1). The brain, ovaries, pancreas, thyroid gland, breast, gallbladder, and heart are organs where echinococcus is rarely located and have a lower incidence of 8-10% (2). The rate of infection in the musculoskeletal system and primary soft tissue is extremely rare (0.5-4.7%), even in endemic areas (2,3,4).

Address for Correspondence: Ramazan Serdar Arslan, Servergazi State Hospital, Clinic of General Surgery, Denizli, Türkiye
Phone: +90 505 591 70 91 E-mail: r.serdar.arslan@gmail.com **ORCID ID:** orcid.org/0000-0002-3139-9531

Received: 30.10.2022 **Accepted:** 07.03.2023

The disease can be discovered incidentally, or the patient can come with the mass to the affected location. A lump in the form of a soft tissue tumor, such as a sarcoma, liposarcoma, or lipoma, might be found during a clinical examination. The first preferred imaging method after the examination is ultrasonography (USG) (1,2,3). In the USG, one can see daughter cysts, vesicles, septa, hydatid sand, floating membranes, and cyst membranes (5). Serology can help in the differential diagnosis of HD and other cystic diseases. Magnetic resonance imaging (MRI), which depicts the location of the cyst and its relationships to muscles and other structures, provides the best imaging data for muscular hydatid cysts (5,6). In this article, the diagnosis of a primary isolated left thigh hydatid cyst, imaging modalities, treatment strategy and patient management are discussed with a case report.

Case Report

A 32-year-old male presented to the hospital complaining of a slow-growing, painless mass in his left thigh. The size of swelling gradually increased in the following period. He was a sheepherder during his childhood. The patient's medical history was unremarkable. A sensitive lump of around 10*15 cm was discovered during a physical examination of the left upper thigh. The skin on top was normal (Figure 1). In the rest of the body, there was no more apparent or observable swelling. The lesion was detected by ultrasound as a 10x6x12 cm thick walled, multiloculated, complicated cystic mass in the medial side of the upper left thigh. Laboratory tests, including a complete blood cell count, serum chemistries, a coagulation profile, C-reactive protein, and erythrocyte sedimentation rate, showed normal results. In the serological examination, the indirect hemagglutination test was detected as 1/640 (positive >1/128). MRI revealed a large, thick-walled cystic mass lesion of approximately 18.5*15.5*8.8 cm on the anterolateral side of the left thigh, the largest of which was 4.4 cm, accompanied by multiple cysts (Figure 2). No other organs were affected by hydatid cysts, according to contrast-enhanced computed tomography (CECT) of the chest and abdomen. While CECT of the abdomen and chest did not reveal any other organ hydatid cyst involvement, but multiseptal cystic mass localized in the muscle was observed in the anteromedila part of the left thigh, measuring approximately 110*108 mm (Figure 3). The patient was treated with albendazole (400) mg in a day for two weeks before surgery. The wound was protected with a sponge dipped in hypertonic saline (3%). After injecting hypertonic saline (3%) into the cyst, a 10-minute waiting was required. Under spinal anesthesia, oblique incision anteromedially was performed. After the skin incision, it was observed that the cyst ruptured into the subcutaneous fatty

Figure 1. Swelling at the anteromedial aspect of the left thigh (the arrow)

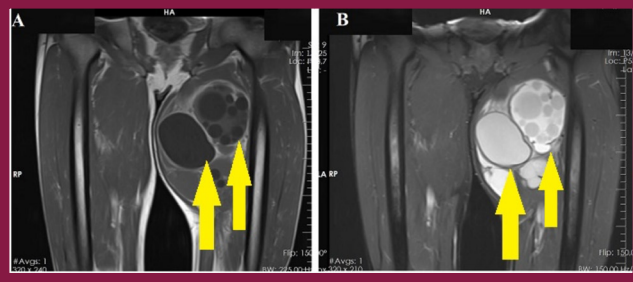

Figure 2. A. Coronal T1-weighted MRI study of the thigh demonstrate a large cystic lesion containing the smaller round cysts (arrow), **B.** Coronal T2-weighted demonstrate a large cystic lesion containing smaller round cysts (arrow)
MRI: Magnetic resonance imaging

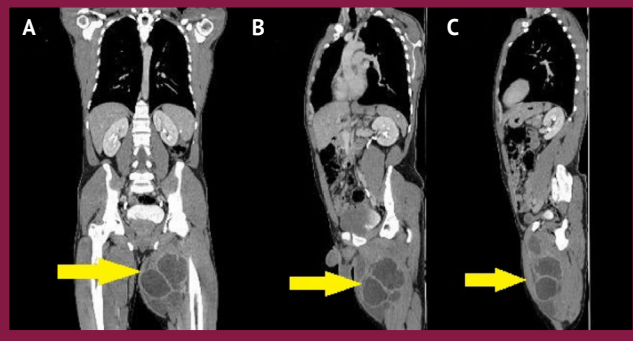

Figure 3. Large cystic lesion containing the small round cysts (computed tomography scan with contrast) **A:** Coronal, **B:** Oblique, **C:** Sagittal plane images

tissue and was self-limiting. Up to 750 mL of filthy, decaying material with daughter cysts was extracted and cystectomy was performed (Figure 4). Thorough wash was given all cavities with 10% povidone iodine. In addition to the primary closure of the incision, hemovac drains (16 Fr) were inserted. The recovery time went without incident. The drain was removed on the third day. On the fifth day, the patient was released. The physical examination was unremarkable at the 1st and 3rd month follow-ups of the patient, and no recurrence was detected in the USG. In the macroscopic examination of the specimen sent to pathology; 4-5 transparent colored walland sac-like specimens, the largest of which is 7*7*0.1 cm and the smaller one is 2*2*0.1 cm, were observed. In microscopy examination; in the examination of the section prepared from the sent material; eosinophilicacellular cuticular membrane and scolex structures were observed on the wall, covered with a germinal layer inside. The case was diagnosed as "hydatidcyst" with the present findings (Figure 5).

Conclusion

A serious health issue around the world, HD, also known as cystic echinococcosis, is an endemic parasitic disease brought on by the larval form of the echinococcus. *E. granulosus* and *E. multilocularis* cause cystic Echinococcosis and alveolar Echinococcosis in humans (1,2,3). While primary hydatid cysts of the thigh or other uncommon anatomical places have been recorded in the literature, hydatid cysts typically afflict the liver and lungs (1,2,3,4). The pathophysiology of musculoskeletal hydatid cyst disease is not clear. The two possibilities that get the most attention are subcutaneous contamination directly through damaged skin or lymphatic channel dissemination. The thigh is the most common subcutaneous location, followed by the gluteal area, the neck area, and the limbs (3,7). The location, size, and pressure of the cyst's expansion determine how the muscular hydatid cyst typically presents, but it typically takes the shape of a slow-growing, painless mass. Cyst formation is hampered by muscular lactic acidosis and muscle contraction. Parasitic cysts tend to develop around the muscles of the neck, thigh and roots of the limbs where muscle activity is lower and vascularisation is greater (8). There are serological tests available to diagnose hydatid cysts, however a negative test result does not rule them out entirely (1,2,4). Particularly in the detection of cystic membranes, septa, and hydatid sand, floating membranes, daughter cysts, and vesicles, as well as in the illness diagnosis, USG has a high accuracy rate (5,7,9). MRI is the preferred examination in case of suspected intramuscular HD, as in all muscle-joint imaging. The differential diagnosis of cystic lesions is aided by the detection of multiloculated polycystic mass lesions on MRI as well as the presence of a two-layered wall of collagen tissue and vascularized pericyst (5,8,10). The preferred method of therapy at the moment is total surgical excision of the cyst, followed by extensive irrigation of the soft tissues around it with hypertonic saline to avoid recurrence. The following surgery, these operations should be accompanied with the administration of systemic antiparasitic medications. When cystic lesions are seen on the body, the differential

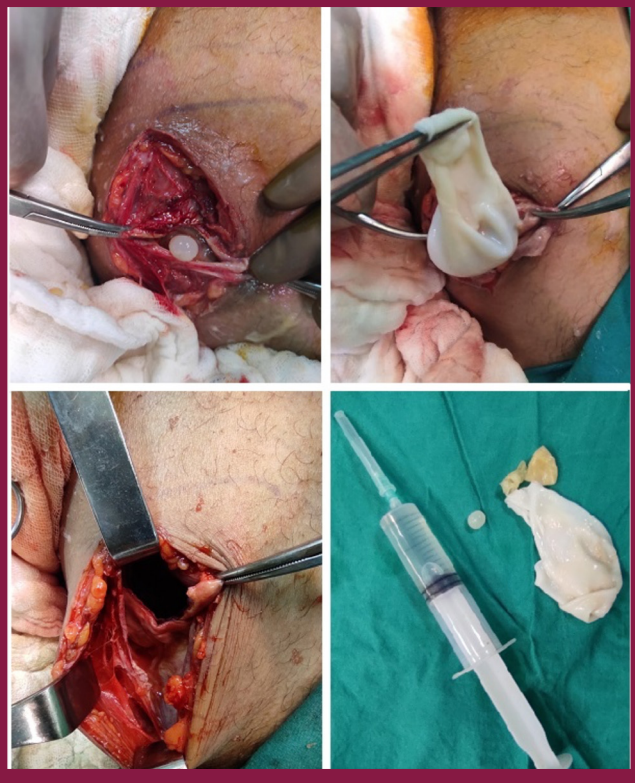

Figure 4. Peroperative images of thigh and specimens

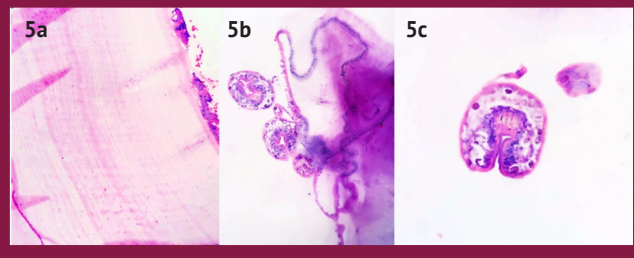

Figure 5. Pathology findings. **5a:** The appearance of eosinophilic stained, lamellar structure, acellular cuticle layer, and scolex structure in the cyst (HEx40), **5b:** The appearance of eosinophilic stained, lamellar membrane and associated scolexin the cyst (HEx20), **5c:** Scolex structures (HEx40)

diagnosis should include a hydatid cyst, especially in endemic populations. MRI is accepted as one of the best diagnostic techniques in the diagnosis and surgical planning of primary muscle localized hydatid cysts. Total excision of the cyst or pericystectomy is the most commonly used methods in the surgical treatment of intramuscular HD.

Information

This case report was published as a poster presentation at the 22nd National Surgery Congress held in Antalya between 23-27, March 2022 (EPS-0286).

Acknowledgement: We would like to thank Bugra Inal for providing language assistance and proof reading for this article.

Ethics

Informed Consent: Written informed consent was obtained from the patients for the publication of this case report.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.S.A., M.Ç., S.D., Ş.K., Concept: R.S.A., S.D., Ş.K., Design: R.S.A., M.Ç., Data Collection or Processing: R.S.A., S.D., Ş.K., Analysis or Interpretation: R.S.A., Ş.K., Literature Search: R.S.A., Writing: R.S.A., S.D., Ş.K.

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

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

References

1. Mihmanli M, Idiz UO, Kaya C, Demir U, Bostanci O, Omeroglu S, et al. Current status of diagnosis and treatment of hepatic echinococcosis. World J Hepatol. 2016;8:1169-1181. [Crossref]
2. Sezer HK, Oztas M, Ozerhan IH, Yildiz R, Can MF. Unusual presentation of a primary recurrent hydatid cyst. Grand Rounds. 2013;13:23-26. [Crossref]
3. Moravvej H, Haghighatkhah HR, Abdollahimajd F, Aref S. Primary subcutaneous hydatid cyst of the leg: An unusual location and review of the literature. Indian J Dermatol Venereol Leprol. 2016;82:558-562. [Crossref]
4. Küçük İG, KüçüK Ş. A Hydatid Cyst Case Diagnosed in Recurrent Urinary Tract Infection Examination of a Pediatric Patient. Turkish Journal of Family Medicine and Primary Care. 2020;14:492-496. [Crossref]
5. Lakhanpal V, Badgurjar M, Saxena P. An arcane case report of primary intramuscular hydatid cyst of thigh. Int J Surg Case Rep. 2021;80:105595. [Crossref]
6. Karami M, Sadatmadani SF, Kouhi H, Sadeghi B, Rostamiyan Z, Hashemzadeh M. A rare presentation of hydatid cyst, involvement of uncommon sites with sparing of typical locations. J Res Med Sci. 2021;26:1. [Crossref]
7. Vecchio R, Vecchio V, Intagliata E. Transmission ways of Echinococcus granulosus in rare muscular locations of hydatid disease. Ann Med Surg (Lond). 2020;55:332-333. [Crossref]
8. Zazo A, Zazo R, Shashaa MN, Alkarrash MS, Brimo Alsaman MZ, Niazi A. Uncommon musculoskeletal femoral hydatid cyst disturbs a female for a year: Case report. Ann Med Surg (Lond). 2020;55:30-32. [Crossref]
9. Samsami M, Qaderi S, Zebarjadi Bagherpour J, Lucero-Prisno DE 3rd. A case report of primary isolated extrahepatic hydatid cyst of the soft tissues of the breast and thigh. Int J Surg Case Rep. 2021;79:475-478. [Crossref]
10. Örmeci N, Asiller ÖÖ, Yağcı C, Örmeci T, Ellik Z, Kalkan Ç, et al. Muscle hydatid diseases: percutaneous treatment with Örmeci technique. Journal of Health Sciences and Medicine. 2022;5:732-739. [Crossref]

CASE REPORT

DOI: 10.4274/hamidiyemedj.galenos.2023.24008

Hamidiye Med J 2023;4(2):156-160

Low Grade Mucinous Appendiceal Neoplasia Presenting as Ovarian Tumor; A Rare Case Report

Over Tümörü Olarak Ortaya Çıkan Düşük Dereceli Müsinöz Apendiks Neoplazisi; Nadir Bir Olgu Raporu

Neslihan Kaya Terzi

Çanakkale Onsekiz Mart University Faculty of Health Sciences, Department of Pathology, Çanakkale, Türkiye

ABSTRACT

It can be difficult to distinguish between primary and metastases in ovarian mucinous neoplasms, both clinically and histopathologically. Especially in mucinous type ovarian neoplasia, the possibility of metastasis of a mucinous neoplasia originating from the appendix or colon should be kept in mind due to its close proximity to the ovary. Considered to be of primary ovarian origin with clinical and radiological findings; however, we present a rare case that we detected as a mucinous neoplasia metastasis originating from the appendix by histopathological and molecular analysis. Although intraoperative histopathological evaluation is important in ovarian neoplasia, a more accurate diagnosis can be made with immunohistochemical and molecular additional diagnostic methods, especially in cases with mucinous type ovarian neoplasia.

Keywords: Appendiceal mucinous neoplasm, ovary, diagnosis, molecular

ÖZ

Over müsinöz neoplazilerinde primer ve metastaz ayrımını yapmak hem klinik hem de histopatolojik olarak zor olabilir. Özellikle müsinöz tip over neoplazilerinde overe yakın komşulukları nedeniyle apendiks veya kolon kaynaklı bir müsinöz neoplazinin metastaz yapmış olma olasılığı akılda tutulmalıdır. Klinik ve radyolojik bulgular ile primer over kaynaklı olduğu düşünülen; ancak histopatolojik ve moleküler analiz ile apendiks kaynaklı bir müsinöz neoplazi metastazı olarak saptadığımız nadir bir olguyu sunuyoruz. Over neoplazilerinde intraoperatif histopatolojik değerlendirme önemli olmakla birlikte özellikle müsinöz tip over neoplazi olan olgularda ameliyat sonrasında immünohistokimyasal ve moleküler ek tanı yöntemleri ile daha doğru tanı konulabilmektedir.

Anahtar Kelimeler: Apendiks müsinöz neoplazmı, over, tanı, moleküler

Introduction

Mucinous appendiceal neoplasms are rare, sometimes found incidentally, during follow-up, or during surgery for other reasons, and histopathologically classified as low-grade mucinous appendiceal neoplasms (LAMNs), high-grade mucinous appendiceal neoplasms, and mucinous adenocarcinomas (MA) (1).

Up to 23 cases of appendiceal mucinous neoplasia have been reported till date in English medical literature. When the literature is reviewed, our case is an uniq case of primary LAMN causing ovarian metastasis and pseudomyxoma peritonei (PMP).

Case Report

Our patient, 45 years old female, was admitted to the hospital with abdominal pain and swelling. The tumor markers cancer antigen 125 (CA-125), cancer antigen 19-9 (CA 19-99) and carcinoembriyonic antigen levels rose slightly (71.5 U/mL, 48.3 ng/mL and 48.7 ng/mL, respectively). On ultrasonography (USG) imaging, a 110x105 mm multicystic mass was observed in the right ovary. A small amount of acid was detected.

Right ovarian mass was excised with laparotomy and sent to pathology for frozen study. In the macroscopic examination, a ruptured multicystic mass of 11x11x3 cm in size with mucoid material and a solid area of 4 cm in

Address for Correspondence: Neslihan Kaya Terzi, Çanakkale Onsekiz Mart University Faculty of Health Sciences, Department of Pathology, Çanakkale, Türkiye
Phone: +90 555 604 58 68 E-mail: neslihankaya88@hotmail.com **ORCID ID:** orcid.org/0000-0001-8184-4691

Received: 12.08.2022 **Accepted:** 06.04.2023

the cyst were observed. A borderline mucinous tumor was diagnosed on microscopic examination. With this result; the operation was terminated by appendectomy, bilateral salpingo-oophorectomy, hysterectomy, lymphadenectomy, omentectomy and peritoneal implant excision. Microscopically (Figure 1), the surface of the ovarian and appendiceal tumors was lined with mucinous epithelium with low-grade dysplasia. Acellular mucin was in the omentum and peritoneal tissues, and mucinous epithelium was observed in the stroma. Lymph node metastasis was not observed.

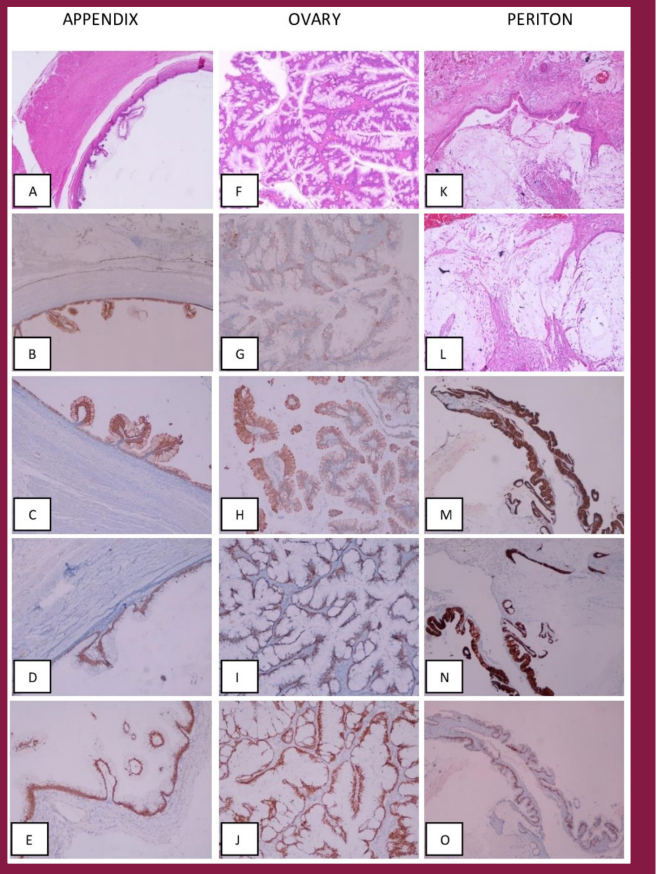

Figure 1. A-E. Low-grade appendiceal mucinous neoplasm. **(A)** Villous and flat proliferation of mucinous epithelial cells lining the appendiceal mucosa in LAMN (HX&Ex 40). Immunohistochemical positivity of CK7 **(B)**, CK20 **(C)**, CDX2 **(D)** and SATB2 **(E)** in tumor cells of the appendix mucinous neoplasm (x100). **F-J.** Low-grade appendiceal mucinous neoplasm involving the ovary. **(F)** The mucinous glands have a complex anastomosing architectural pattern (HX&Ex 40). Immunohistochemical focal positivity of CK7 **(G)** and CK20 **(H)**, diffuse positivity of CDX2 **(I)** and SATB2 **(J)** in tumor cells of the ovary (x100). **K-O.** Pseudomyxoma peritonei, low grade. **(K)** Lakes of mucin and epithelial cells (HX&E×100). **(L)** Acellular mucin in the fibrous tissue (HX&E×100). Immunohistochemical positivity of CK7 **(M)**, CK20 **(N)**, and CDX2 **(O)** in tumor cells of the periton (x100)
LAMN: Low-grade mucinous appendiceal neoplasm

Immunohistochemistry

In order to understand the origin of the tumor, 4 µm thick sections were made from the blocks of the appendix, ovary and omentum, and immunohistochemical studies were performed. The tissue was separated from paraffin with xylene and rehydrated with ethanol. Monoclonal cytokeratin 7 (CK7) (dilution 1:100), cytokeratin 20 (CK20) (dilution 1:100), CDX2 (dilution 1:250), PAX-8 (prediluted), CA-125 (dilution 1:100), and Specific AT-rich sequence binding protein 2 (SATB2) (dilution 1:100) antibodies were applied to the slides. While CK7 and CK20 stained focal positively in tumor cells of ovary, CDX2, PAX-8 and SATB2 antibodies showed diffuse nuclear positivity. On the other hand; diffuse strong positive staining was observed with CK7, CK20, CDX2 and SATB2 in the sections of tumor samples in the appendix and omentum (Figure 1).

GNAS Mutation Analysis

GNAS mutation analyzes were performed in both appendiceal and ovarian tumor tissues (Figure 2). Hotspot sites for pathogenic mutations in exons 8 and 9 of the *GNAS* gene were analyzed by polymerase chain reaction (PCR)-based direct sequencing. Tumor targets (>90% viable tumor) were manually microdissected from 10 mm thick unstained histological sections. Sections were deparaffinized. DNA was then isolated using the QIAamp DNA FFPE Tissue Kit (50) (catalog #56404) (QIAGEN, Hilden, Germany). The primers used in the amplification process are as follows: Exon 8-Forward: 5'ACTGTTTCGGTTGGCTTTGGTGA'3, exon 8-Reverse: 5'AGGGACTGGGGTGAATGTCAAGA'3, exon 9-Forward: 5'TTGACATTCACCCCAGTCCC'3, exon 9-Reverse: 5 'ACAAACACAGAAGCAAAAGCGCAAAAGCG.

The purified PCR products were submitted to direct sequencing in both directions (forward and reverse) by applying reagents from the Big Dye Terminatorv3.1 Cycle Sequencing kit. After the precipitation of alcohol, the products were run on an automatic sequencer. Bidirectional sequence traces were analyzed with SeqScape Software v3.0 and manually reviewed with the reference sequence of the *GNAS* gene.

As a result, GNAS exon 8 c.602G>A (p.R201H) mutation was identified in both appendix and ovary tumor tissues (Figure 2), while GNAS exon 9 mutation was not detected.

Follow-up

No problem was detected in the controls of the patient and he was discharged in good health. Follow-up was performed using computed tomography, USG imaging and tumor markers. No recurrence/residual tumor was detected in the first 2 years of follow-up.

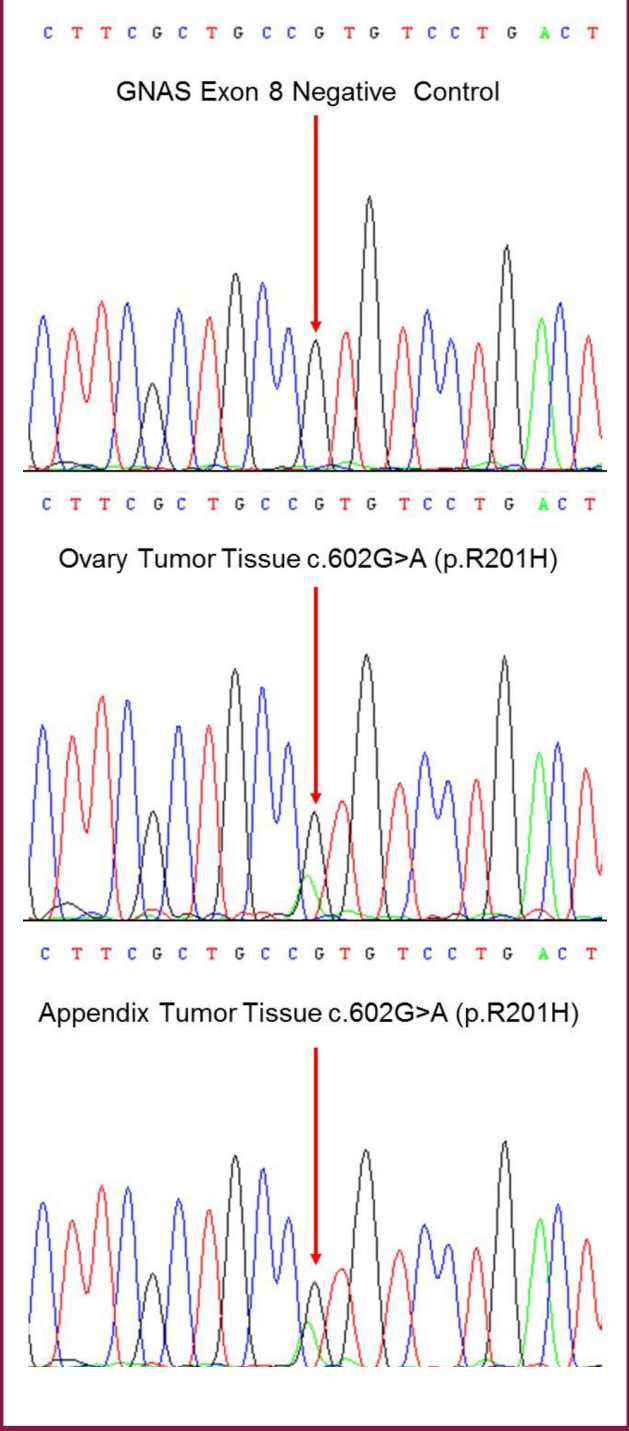

Figure 2. Representative sequencing forward electropherograms of GNAS exon 8 c.602G>A (p.R201H) mutation in both appendix and ovary tumor tissues while corresponding region of the IVS-000 polyclonal control DNA's reference sequence is shown in upper lane

Discussion

The primary/metastasis differentiation of appendiceal and ovarian mucinous neoplasms can be difficult. Only histopathological examination is not sufficient, pathological additional diagnostic methods should be applied.

Most ovarian tumors express CK7 and are CK20 negative, while tumors of colon and appendix are CK20 positive but CK7 negative. In some tumors, CDX2 will be required to exclude tumors originating from other gastrointestinal and pancreato-biliary systems.

A newly recognized marker proposed for primary/metastasis differentiation of mucinous neoplasms in the ovary is SATB2 (2). The SATB2 antibody indicates the origin of colorectal or appendiceal cancer of the lower gastrointestinal tract. Schmoeckel et al. (2) reported 7 cases of LAMN that had ovarian metastasis or caused PMP. They applied an immunohistochemical panel including SATB2 to these cases, and SATB2 showed diffuse and strong positive expression in all of them. On the other hand, it was determined that immunohistochemical SATB2 negative 40 cases had ovarian mucinous borderline tumor or ovarian mucinous carcinoma. According to this finding, SATB2 antibody can be used in the differential diagnosis of mucinous neoplasia originating from the ovary and appendix.

In the presence of 2 or more tumors in different anatomical regions, DNA sequencing can be used as a more specific indicator than immunohistochemistry for the differentiation of metastases or primary tumors (3). This approach may be useful in genetically stable tumors such as LAMN. Since genetic variants associated with LAMN are primarily confined to a few hotspot regions in GNAS, it would be appropriate to work with Sanger sequencing.

This case is the rare one in the literature with a diagnosis of LAMN metastasizing to the ovary (4) and causing low-grade PMP, and is the second one (5) without PMP. Also our case is the 4th one with simultaneous appendiceal and ovarian mass, indicating primary appendix. If we look at the other 3 literature (Table 1) from the past to the present, Klein and Rosen (6) diagnosed a postmenopausal female patient with vaginal bleeding after the operation with MA and mucinous carcinoma in bilateral ovaries. Mandai et al. (7) diagnosed appendiceal adenocarcinoid and bilateral Krukenberg tumors after the operation in a young premenopausal female patient who presented with a lower abdominal mass. Toffaha et al. (4) detected a 21 cm right adnexal complex cyst on USG imaging in a postmenopausal female patient who presented with minimal vaginal bleeding. They detected the appendix adjacent to the ovary

during the operation and considered it as primary ovarian carcinoma. As a result of histopathological examinations, it was diagnosed as primary LAMN and ovarian metastasis, as in our case.

Borges et al. (8) presented a case diagnosed LAMN by post-operative histopathological examination in a postmenopausal female patient who presented with an adnexal mass. They did not detect any other mass in the ovaries or abdomen in this patient. They searched literature in PubMed, asking if there was a case similar to this case and found 23 similar literature. Mucocele in the appendix in 9 cases, LAMN in 7 cases, mucinous cystadenoma in 6 cases

Table 1. Literature review: pre-op adnexal mass, intra-op abnormal appendix and ovary, origin in appendix[a]

Report[b]	Age (y)	Examination	Surgery	Macr. find	Micr. find	Follow-up/rec
Current case	45	21x11x3 cm	L, right adn.	Large mobile	Appendix: LAMN	No rec, 1 y
		Ruptured cystic	Mass	thin walled	ROV: LAMN met.,	
		Mass of the	Excision	multi locular	periton, omentum: PMP	
		right ovary,	with (frozen)	cystic mass		
		normal uterus,	TAH with USO,	arising from R OV.,		
		perforated	AP and periton,	N Lt OV., and U.,		
		appendix,	om., LAD	perforated appendix,		
		peritoneal and		peritoneal and omental		
		omental imp.		mass/mucus		
Toffaha A 2020	58	Soft Abd, no	L, TAH with	Large mobile thin		No Rec 3 y
		tenderness/	BSO and AP	walled multi locular	ROV: LAMN met	
		guarding; mobile		mass arising from R OV.,		
		non-tender		N. Lt OV and U.,		
		15x10 cm mass;		Small and large bowel		
		bulky U, fullness		adhesions, appendix		
		around adnexa		adherent to ovary, mucus		
				extruding through its tip		
Mandai M. 2000	35	Lower abdominal	1st Op: L, TAH,	1st Op: Large	ACC with Bil.	Died 24 m after
		mass	Lt SO, partial	Lov, N ROV.	Krukenberg	diagnosis
			Resection of	2nd Op: ROV	Tumor	
			R OV. 2nd Op:	slightly large		
			RSO, AP, Om,	and hard		
			Res of colonic			
			nodule, P&			
			para-A Lad			
Klein E. 1996	66	NR	L, TAH with	Partly exophytic	AMC, both	Death after 20 m
			BSO and	Lt OV mass,	OV involved	
			modified R H,	4 cm, distending	(ROV microscopic)	
			biopsy of peritoneal	the cecum, suspicious		
			nodules	nodule in cul-de-sac		

[a] Evidence based on case report, [b]For space considerations, only the first author is cited, AAC: Appendiceal adenocarcinoid, Abd: Abdominal, AMC: Appendiceal mucinous carcinoid, AP: Appendectomy, Bil: Bilateral, BSO: Bilateral salpingo-oophorectomy, L: Laparotomy, Lad: Lymphadenectomy, Lt: Left, Macr. find.: Macroscopic finding, Micr. find.: Microscopic finding, m: Month, N: Normal, NR: Not reported, Om: Omentectomy, Op: Operation, OV: Ovary/s P: Pelvic, Para-A: Para-aortic, R: Right, Rec: Recurrence, Res: Resection, SO: Salpingo-oophorectomy, TAH: Total abdominal hysterectomy, U: Uterus, y: Years

and MA in 1 case of these literatures was found. No tumor was detected in any other focus in any of these cases.

It is difficult to understand the origin of mucinous tumors because the ovary and appendix are close to each other anatomically, there are no specific serum markers for cancers originating from these organs, and mucinous tumors originating from the appendix can metastasize to the ovary (9).

Conclusion

Our case showed how we can distinguish between metastasis from primary appendix or ovary in a case of LAMN. Surgeons should provide the pathologist with more information about the clinical, radiological and laboratory characteristics of patients. We also found that performing frozen sections during the operation was significantly important for helping the pathologist diagnose the definitive one. In addition, the presence of PMP indicated that mucinous neoplasms of the appendix should be considered. Based on our results, a more accurate diagnosis can be made in patients with mucinous ovarian neoplasm with the diagnosis of frozen during the operation and with immunohistochemical and molecular additional diagnostic methods after the operation.

Ethics

Informed Consent: Informed consent was obtained.

Peer-review: Internally and externally peer-reviewed.

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

References

1. Aleter A, El Ansari W. Incidental appendiceal mucinous neoplasm mimicking a left adnexal mass: A case report. Int J Surg Case Rep. 2020;74:132-135. [Crossref]
2. Schmoeckel E, Kirchner T, Mayr D. SATB2 is a supportive marker for the differentiation of a primary mucinous tumor of the ovary and an ovarian metastasis of a low-grade appendiceal mucinous neoplasm (LAMN): A series of seven cases. Pathol Res Pract. 2018;214:426-430. [Crossref]
3. Matson DR, Xu J, Huffman L, Barroilhet L, Accola M, Rehrauer WM, et al. KRAS and GNAS Co-Mutation in Metastatic Low-Grade Appendiceal Mucinous Neoplasm (LAMN) to the Ovaries: A Practical Role for Next-Generation Sequencing. Am J Case Rep. 2017;18:558-562. [Crossref]
4. Toffaha A, El Ansari W, Aleter A. What you see might not be what you get: Discrepancies between intraoperative findings and preoperative diagnosis of ovarian tumors. Appendicular mucocele presenting as an adnexal mass - Case report and review of literature. Int J Surg Case Rep. 2020;75:543-549. [Crossref]
5. Perivoliotis K, Christodoulidis G, Samara AA, Sgantzou IK, Floros T, Volakakis G, et al. Low-Grade Appendiceal Mucinous Neoplasm (LAMN) Primarily Diagnosed as an Ovarian Mucinous Tumor. Case Rep Surg. 2021;2021:5523736. [Crossref]
6. Klein EA, Rosen MH. Bilateral Krukenberg tumors due to appendiceal mucinous carcinoid. Int J Gynecol Pathol. 1996;15:85-88. [Crossref]
7. Mandai M, Konishi I, Tsuruta Y, Suginami N, Kusakari T, Iwasaki T, et al. Krukenberg tumor from an occult appendiceal adenocarcinoid: a case report and review of the literature. Eur J Obstet Gynecol Reprod Biol. 2001;97:90-95. [Crossref]
8. Borges AL, Reis-de-Carvalho C, Chorão M, Pereira H, Djokovic D. Low-grade mucinous appendiceal neoplasm mimicking an ovarian lesion: A case report and review of literature. World J Clin Cases. 2021;9:2334-2343. [Crossref]
9. Pantiora EV, Massaras D, Koutalas J, Bagiasta A, Kontis EA, Fragulidis GP. Low-grade Appendiceal Mucinous Neoplasm Presenting as Adnexal Mass: A Case Report. Cureus. 2018;10:e3568. [Crossref]