e-ISSN: 2687-4717

6 cilt volume
2 sayı issue
2024 haziran june

HİTİT MEDICAL JOURNAL

HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

HİTİT ÜNİVERSİTESİ YAYINLARI

HMJ

HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024
Periyot: Yılda 3 Sayı (Şubat & Haziran & Ekim) | Period: 3 Issues per Year (February & June & October)

YAYIN DİLİ | LANGUAGE OF PUBLICATION

Türkçe & İngilizce | Turkish & English

YAZIŞMA ADRESİ | CONTACT ADDRESS

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YAYINCI | PUBLISHER

Hitit Üniversitesi Yayınları | Hitit University Press

HAKEM KURULU | REFEREE BOARD

Hitit Medical Journal Dergisi, çift taraflı kör hakemlik sistemi kullanmaktadır. Hakem isimleri gizli tutulmakta ve yayımlanmamaktadır.

Hitit Medical Journal uses a double-blind review. Referee names are kept strictly confidential.

LOCKSS: https://dergipark.org.tr/tr/pub/hititmedj/lockss-manifest

OAI: https://dergipark.org.tr/api/public/oai/hititmedj/

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Dergimizin her sayısında, tıp alanının temel, dahili ve cerrahi branşlarına dair kapsamlı ve çeşitli makaleler yayımlamaya özen gösteriyoruz. Bu sayıda da bilim dünyasına katkı sağlayacak 14 orijinal araştırma makalesi de dahil olmak üzere toplam 19 makaleyi siz değerli okuyucularımızla paylaşmanın gururunu yaşıyoruz.

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Saygılarımızla,

Doç. Dr. Abdulkerim YILDIZ
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From the Editor

Dear readers;
We are delighted to present the new issue of the Hitit Medical Journal. The increasing interest and support from our authors and readers continue to inspire us. To meet this growing interest and enhance the quality of our journal, we have strengthened our editorial board with new members. We extend our heartfelt thanks to our former team members for their contributions and dedication. We also wish our new board members success in their endeavors.

In every issue, we strive to publish a diverse range of articles covering fundamental, internal, and surgical branches of the medical field. In this issue, we are proud to present a total of 19 articles, including 14 original research papers, spanning various scientific disciplines.

We wish all our readers an enjoyable and informative reading experience.

Sincerely,

Doç. Dr. Abdulkerim YILDIZ
On behalf of the HMJ Editorial Board

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

ATR-FTIR Spectroscopic Analysis of Novel Fixatives and Their Histological Properties on Sheep Heart

Koyun Kalbinde Yeni Fiksatiflerin ATR-FTIR Spektroskopik Analizi ve Histolojik Özellikleri

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1370056
Geliş Tarihi | Received: 02.10.2023
Kabul Tarihi | Accepted: 11.01.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Özdemir F, Özkınalı S, Özyol E.N.H. ATR-FTIR Spectroscopic Analysis of Novel Fixatives and Their Histological Properties on Sheep Heart. Hitit Medical Journal 2024;6(2):98-107. https://doi.org/10.52827/hititmedj.1370056.

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Etik kurul onayı alınmasına gerek yoktur.
İntihal Kontrolleri: Evet (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: FÖ, SÖ Tasarım: FÖ, SÖ Veri Toplama/Veri İşleme: FÖ, SÖ, ENHÖ Veri Analizi: FÖ, SÖ Makalenin Hazırlanması: FÖ, SÖ, ENHÖ.
Hasta Onamı: Hasta onamı alınmasına gerek yoktur.
Finansal Destek: Finansal destek alınmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Not applicable.
Plagiarism Check: Yes (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts of interest.
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: FÖ, SÖ Design: FÖ, SÖ Data Collection/Data Processing: FÖ, SÖ, ENHÖ Data Analysis: FÖ, SÖ Manuscript Preparation: FÖ, SÖ, ENHÖ.
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

ATR-FTIR Spectroscopic Analysis of Novel Fixatives and Their Histological Properties on Sheep Heart

ABSTRACT

Objective: In this study, new fixative solutions that are odorless and non-toxic were tested on the hearts of sheep to preserve the cellular structure and tissue architecture in a life-like manner.

Material and Method: The changes of new fixatives before and after contact with tissue were detected for the first time by the ATR-FTIR spectroscopic method. After a preliminary inspection, hearts were fixed with six different solutions. It was periodically evaluated for weight, size, color, and hardness. Samples taken from the left ventricle of each heart stained with Hematoxylin eosin and examined histologically.

Results: As a result of the measurements of heart weights, reduction in Solution 1 (Standard Formaldehyde Fixative), Solution 4, and Solution 5 were detected at 5.64%, 23.70%, and 14.38%, respectively. In Solution 4 and Solution 5, it was observed that the integrity of the myocardium was protected in terms of morphology, the stiffness was suited for sectioning and the coronary vessels were preserved better than Solution 1. Further, compared to solution 1, it was determined that the endocardium and myocardium layers were well preserved in the tissues fixed in Solution 4 and Solution 5. Typical cross striation appearance in cardiac muscle tissue existed in all three Solution 1, Solution 4, and Solution 5.

Conclusion: Solution 4 and Solution 5 were found to have superior fixative properties than Solution 1 (Formaldehyde). As a result of ATR-FTIR studies, it was determined that formaldehyde was converted into toxic formic acid in Standard Solution 1, while very few harmless changes were detected in Solution 4 and Solution 5.

Keywords: Anatomy education, ATR-FTIR spectroscopy, fixative, organ preservation.

ÖZET

Amaç: Bu çalışmada, hücresel yapının ve doku mimarisinin canlı gibi korunması amacıyla, kokusuz ve toksik olmayan yeni fiksatif çözeltiler koyun kalbi üzerinde test edildi.

Gereç ve Yöntem: Yeni fiksatiflerin dokuyla temas öncesi ve sonrasındaki değişiklikleri ilk kez ATR-FTIR spektroskopik yöntemiyle tespit edildi. Ön incelemenin ardından kalpler altı farklı çözeltiyle fikse edildi. Ağırlık, boyut, renk ve sertlik açısından periyodik olarak değerlendirildi. Her kalbin sol ventrikülündenalınan ve Hematoksilen Eozin ile boyanan örnekler histolojik olarak değerlendirildi.

Bulgular: Kalp ağırlıkları ölçümleri sonucunda Çözelti 1 (Standart %10' luk Formaldehit Çözeltisi), Çözelti 4 ve Çözelti 5'te sırasıyla %5,64, %23,70 ve %14,38 azalma tespit edildi. Çözelti 4 ve Çözelti 5'te miyokardın morfolojik açıdan bütünlüğünün korunduğu, sertliğin kesit almaya uygun olduğu ve Çözelti 1'e göre koroner damarların iyi korunduğu gözlendi. Ayrıca Çözelti 1 ile karşılaştırıldığında, Çözelti 4 ve Çözelti 5'te fikse edilen dokularda endokardiyum ve miyokard katmanlarının iyi korunduğu belirlendi. Çözelti 1, Çözelti 4 ve Çözelti 5'in içerisindeki kalp kası dokularında tipik enine çizgilenme görünümü mevcuttu.

Sonuç: Çözelti 4 ve Çözelti 5'in Çözelti 1'e (Formaldehit) göre daha üstün fiksatif özelliklere sahip olduğu bulundu. ATR-FTIR çalışmaları sonucunda Standart Çözelti 1'de formaldehitin toksik formik asite dönüştüğü belirlenirken, Çözelti 4 ve Çözelti 5'te çok az ve zararsız değişiklikler tespit edildi.

Anahtar Sözcükler: Anatomi eğitimi, ATR-FTIR spektroskopisi, fiksatif, organ koruma.

Introduction

The use of embalmed specimens for three-dimensional anatomy education has been valid for years and is the most effective way to learn human anatomy (1–4). Although Cadaver dissection, a classical teaching method, has been an integral part of anatomy education for centuries, with the advancing technology in the 21st century, more effective ways of using teaching methods such as cadaver dissection are sought (2, 5–7). Anatomy education is the foundation for the successful acquisition of clinical skills and contributes significantly to students' understanding of general anatomy (2). Students working with cadaver materials is a significant part of their professional development toward becoming a doctor, dentist, or scientist (3). Especially in the surgical discipline, In-depth understanding of anatomy is indispensable for safe clinical practice (8).

Formaldehyde solution is widely used in anatomical studies as an essential component of embalming fluid in the preparation of cadavers (9). In contrast, it involves a serious health risk among students, instructors, and all parties involved, and the immediate symptoms of throat, nose, eyes, and skin irritation are the major drawbacks of formaldehyde (9). Formaldehyde has been classified as a carcinogenic substance for humans such as nasopharyngeal cancer, ocular melanoma, lung cancer, brain cancer and leukemia by the United States Environmental Protection Agency and the International Agency for Research on Cancer (9). Accordingly, the Occupational Safety and Health and Administration has set the short-term exposure limit (STEL) for formaldehyde to 2 ppm and 0.75 ppm for the time-weighted average (TWA). Hitherto, to minimize the harmful side effects of formaldehyde, alternative methods such as improving ventilation and exhausting systems, limiting the learning time in the laboratory, increasing the use of personal protective equipment or anatomical modeling such as corrosive cast and plastination have been developed (9). Although new methods such as corrosive cast and plastination are developed, they cannot replace real cadavers due to their deficiencies in teaching methods (9).

Fixation is a physicochemical process in which cells and tissues remain chemically unchanged. It is also the process of fixing tissues and cells using chemicals, with disfigurement negligible morphological. With this process, while the components in the cell become insoluble, it resists tissue destruction for the next processing and eliminates the damage caused by osmotic pressure in the tissue (10,11). Fixatives perform functions such as preventing autolysis and tissue putrefaction as well as tissue component degradation. Various stabilizing agents include formaldehyde, ethanol, osmium tetroxide, glutaraldehyde, picric acid, glyoxal, and so on (11). Temperature plays a pivotal role in increasing the fixation rate or decreasing the diffusion rate of fixative both of which affect tissue architecture (10). Generally, fixation of tissues at room temperature is appraised as the ideal temperature, except for histochemistry and electron microscopy processes that occur between 0 and 4 degrees (10).

Fourier transform infrared (FTIR) spectroscopy has significant advantages compared to many other conventional techniques as it is a dye-free, non-invasive, label-free, fast and low-cost modern method in biochemical applications (12,13). FTIR spectroscopy has attracted attention in biomedical applications in recent years due to it relies on the characteristic absorbance of the corresponding functional groups in the organic sample. Therefore, it can be a powerful diagnostic tool in anatomical studies, in the discrimination of fixatives and tissues with impaired organic structure (12–14).

The aim of this study is to find a new fixative to preserve the cellular structure and tissue architecture in a life-like manner and to detect the change in functional groups in fixatives as a result of contact with tissue by the ATR-FTIR spectroscopic method. For this, histological studies at the cellular level were performed on sheep hearts with new fixatives.

Material and Method

Tissue Collection and Fixation

Sheep hearts were obtained from a local slaughterhouse and immediately transferred to the laboratory. Hearts were rinsed with saline three times and after washing, the pericardial membrane outside the heart was removed by the Anatomy specialist. The heart was incised from the Sulcus Anterior Interventricularis using a scalpel, the mitral and tricuspid valve was ejected, but the papillary

muscles were quitted. The weights of the hearts were measured with a Precisa XB 1200 C branded weighing, their height and width were recorded, their photographs were taken and then they were placed in the prepared six fixative solutions.

Table I. The content of the prepared solutions for sheep hearts

Solutions	Contents
1	10% Formaldehyde aqua solution (200 mL) [54.05 mL Formaldehyde (37%) + 145.95 mL water]
2	1 g $MgSO_4$ + 1 g NaCl + 0.5 g $NaHCO_3$ + 50 mL Ethanol + 150 mL aqua
3	2 g Phenol + 2 g pine oil + 1.75 g Vitamin E + 5 mL Acetone + 200 mL aqua
4	50 mL glycerol + 10 mL ethoxyphenol + 10 mL Toluene + 30 mL olive oil + 50 mL Tetrahydrofuran + 50 mL Ethanol
5	2 g Phenol+ 50 mL propan-2-ol+ 50 mL aqua
6	1% Resorcinol aqua solution (200 mL)

The contents of the prepared solutions are given in Table I. All chemicals and solvents were obtained from Aldrich (U.S.). The weighted tissues were placed in six different fixative solutions prepared with polar and nonpolar properties. At the end of the 3rd day, obvious dissolution and softening of the tissues in Solution 2, Solution 3, and Solution 6 were observed and they were excluded from the study because they were unsuitable form for demonstration. At the beginning of the study, samples were stored at 4 °C in a refrigerator for 56 days. After 56 days, the hearts were allowed to stand at room temperature, and weight loss, odor, and color change were determined.

Solution 1 is the standard solution and the contents of the other five solutions are represented in Table I. In solution 2 ethanol was used due to its anti-infective agent properties, sodium bicarbonate was preferred to prevent the oxidation of ethanol to acetic acid and salts were preferred due to their antibacterial properties. In Solution 3, phenol was preferred due to its bacteriostatic properties at low concentrations, and acetone was preferred in the fixative process due to its dehydration agent feature. Pine oil was used due to it contains toluene and toluene is known to have disinfectant properties against various viruses and bacteria. In solution 4, Ethoxy phenol an aromatic compound was preferred due to as a derivative of phenol. Tetrahydrofuran is a cyclic ether structure, water-miscible organic liquid and it is preferred due to its antibacterial properties. Glycerol was preferred due to its antimicrobial properties. Isopropanol (Propan-2-ol), a better antiseptic and germicidal agent than ethanol, was used in Solution 5. In solution 6, Resorcinol is an aromatic compound and was preferred due to it being a phenol derivative.

Histological Sample Preparation

After fixation tissues were prepared with the standard histological process; dehydration, clearing, infiltration, embedding, sectioning, staining, and mounting. 5 µm thick sections were stained with Hematoxylin and eosin to assess the morphological integrity of the hearts (15). Sections were examined by a Nikon Eclipse Ni model light microscope and images were taken.

ATR-FTIR Analysis

The infrared spectra of the compounds were recorded by a Thermo Scientific Class 1 laser FT-IR (USA) spectrophotometer with ATR (Attenuated Total Reflection) mode.

Results

The first day and 3rd days later photographs of sheep hearts were seen in Figure S1 (Supplementary Data). At the end of the third day, the weight change of the hearts in six different solutions is seen in Figure 1. While an increase in weight was observed in the first three days in Solution 1, Solution 3, and Solution 6, a decrease in weight was observed in Solution 2, Solution 4, and Solution 5. The experiment was not continued, as Solution 2, Solution 3, and Solution 6 were observed dissolution in the hearts and coloration in the solutions. Deterioration of hearts and coloration of solutions were not observed in Solution 4 and Solution 5 as seen in Solution 1 (Standard Solution).

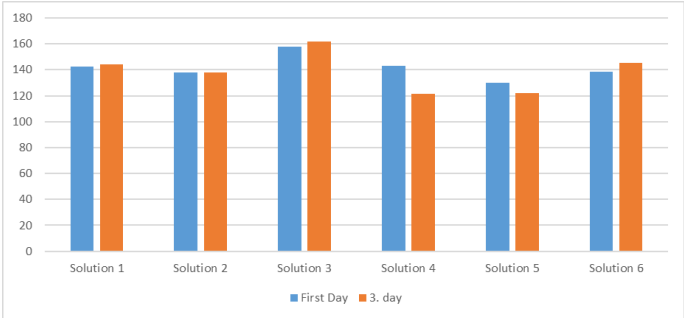

Figure I. Weight change in hearts in six different solutions over three days

Weight reduction was observed in Solution 1, Solution 4, and Solution 5 with 5.64%, 23.70%, and 14.38% respectively at the end of the 316th day (Figure 2). In Solution 4 and Solution 5, it was observed that the integrity of the myocardium was preserved in terms of morphology, its stiffness was suitable for sectioning and the overlying coronary vessels were well protected. When the tissues fixed with Solution 4 and Solution 5 were compared with the tissues fixed with formaldehyde (Solution 1), it was observed that the endocardium and myocardium layers were well preserved. There was a typical transverse striation appearance in the cardiac muscle tissue. It was preserved in all three Solutions.

Table II. Weight losses in sheep hearts

Hearts	First Day Weights	3 days Weights	10 days Weights	56 days Weights	116 days Weights	316 days Weights
1	142.62 g	143.92 g	143.11 g	141.57 g	140.78 g	134.58 g
2	138.13 g	137.98 g				
3	157.58 g	162.06 g				
4	143.22 g	121.23 g	111.32 g	111.98 g	111.53 g	109.28 g
5	130.12 g	121.90 g	117.76 g	114.64 g	113.86 g	111.41 g
6	138.60 g	145.05 g				

Except for Solution 1, the least weight reduction was observed in Solution 5 (Table II). The maximum weight change for Solution 4 and Solution 5 was observed in the first three days in Table II. After 10 days, weight reduction for Solution 4 and Solution 5 progressed very slowly, while after 116 days for Solution 1, the weight reduction increased. From this, it is seen that formaldehyde (Solution 1) causes weight loss in tissues after long periods and is less protective than Solution 4 and Solution 5. The oxidation of formaldehyde to formic acid by oxygen after long periods and the extremely toxic nature of formic acid also limit the use of this solution. The sizes of the hearts used are given in Table III. It can be seen that the dimensions are close to each other.

Table III. Size values of sheep hearts

Hearts	First Day Wide- Length	316 Days Wide-Length
1	7.5 - 8.0 cm	7.0 - 7.50 cm
2	8.0 - 9.0 cm	-
3	7.3 - 9.3 cm	-
4	8.2 - 7.9 cm	7.50 - 7.60 cm
5	7.2 - 7.9 cm	7.20- 6.90 cm
6	6.9 - 8.2 cm	-

After 316th days, Solution 4 and Solution 5 showed that the integrity of the myocardium was preserved morphologically, the stiffness was suitable for sectioning and the coronary vessels were well preserved compared to Solution 1 (Figure S2 in Supplementary Data). The tissue appearance in Solution 4 was close to the first day. For heart tissues from sheep, solution 4 and Solution 5 were found to have excellent fixative properties compared to Solution 1. Solution 4 and Solution 5 are colorless, odorless and the solutions do not deteriorate over time, making them an excellent fixative.

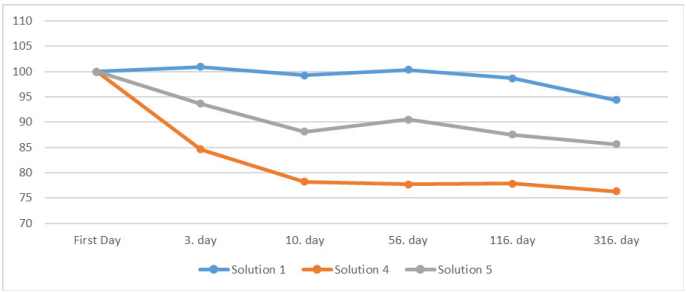

Figure II. Percentage change of heart weights in Solution 1, Solution 4, and Solution 5

Histological analysis

Hematoxylin and eosinstained sections from tissues fixed with three different fixatives were evaluated. Solution 1 fixed tissue sections are shown in Figure III. Endothelial cells in the endocardium layer were well preserved. Due to deficient washing of the fixative during tissue processing or a long fixation time, formalin pigment artifacts were detected. The nuclei of cardiomyocytes were in normal shape and position. Typical cross-striations were seen in transverse sections. In addition, erythrocytes in blood vessels were in normal appearance.

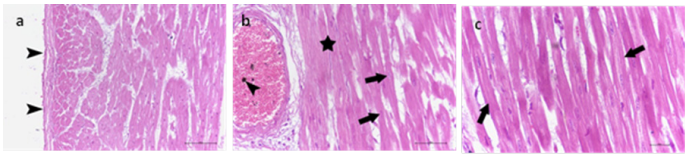

Figure III. Histology of hematoxylin and eosin-stained sections of Solution 1 fixed tissue. a) arrowheads: endocardium; b) arrowhead: formalin pigment, star: cross striations, arrows: cardiomyocyte nucleus; c) arrows: cross striations (a: 20x, b: 40x, c: 60x)

Light microscopic examination of tissues fixed in Solution 4 (Figure IV) and Solution 5 (Figure V) showed well-preserved endothelial cells in the endocardium layer. Purkinje fibers were stained paler than cardiomyocytes as it should be in both tissue samples. Their sarcoplasm was occupied by glycogen that is not preserved in most histological preparations. In our study, the tissue fixed with Solution 4 showed much better preservation of glycogen compared to Solution 5. In cross and longitudinal sections, myofibrils maintained their typical appearance better in Solution 5 fixed tissue compared to tissue fixed with Solution 4. In both samples, the nuclei were not well preserved.

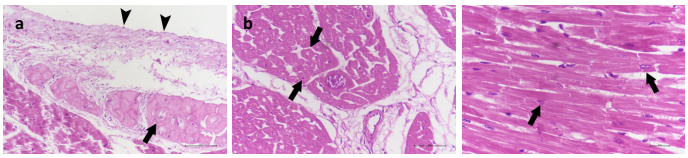

Figure IV. Histology of hematoxylin and eosin-stained sections of Solution 4 fixed tissue. a) arrowheads: endocardium, arrow: Purkinje fibers; b) arrows: cross sections of cardiac muscle fibers; c) arrows: cardiomyocyte nucleus (a: 20x, b: 40x, c: 60x)

FTIR Analyses

The FTIR spectra of the fixatives (Solution 1, Solution 4, Solution 5), initial (red color) and final (purple color) in the fingerprint region are given in Figure VI. Since changes were detected in the range of fingerprint region (1500-600 cm-1) of IR spectrums of the fixatives, the peaks in this region were investigated. The IR spectrums of the solutions obtained between 4000-600 cm^{-1} are included in Supplementary File 2. When the spectrum of 10% formaldehyde solution in red color (A) is examined, weak out-of-plane C-H bending peaks are observed at 1104 cm^{-1} and 1026 cm^{-1}. When the second spectrum in the purple color (A) is examined, the stretching peak of the strong C-O single bond seen at 1025 cm^{-1} can be attributed to the oxidation of formaldehyde to the corrosive formic acid. The strong peak of the C-O single bond seen at 1025 cm^{-1} is evidence of the presence of corrosive formic acid.

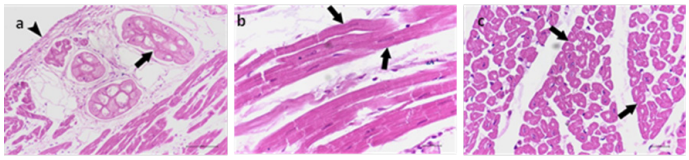

Figure V. Histology of hematoxylin and eosin-stained sections of Solution 5 fixed tissue. a) arrowhead: endocardium, arrow: Purkinje fibers; b) arrows: longitudinal sections of cardiac muscle fibers; c) arrows: cross sections of cardiac muscle fibers (a: 20x, b: 40x, c: 60x)

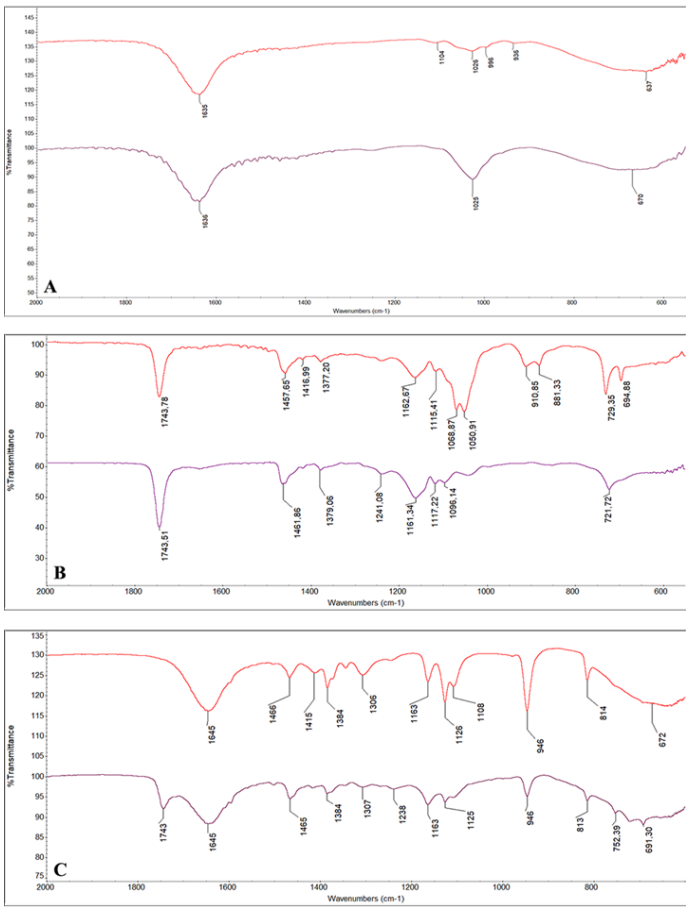

Figure VI. FTIR spectra of organic molecules used in solutions. A) Solution 1 (10% Formaldehyde aqua solution); B) Solution 4 (glycerol; ethoxyphenol; toluene; olive oil; tetrahydrofuran; ethanol); C) Solution 5 (Phenol; propan-2-ol; aqua) (red spectra: 1. st day, purple spectra: 316. days)

When the IR spectrum of fixative 4 (B) is examined, the stretching peaks of the C-O single bond (red spectrum) are seen at 1068 cm^{-1} and 1050 cm^{-1} weakened in the purple spectrum. This change indicates that some of the ethanol in the solution may have been converted to oxidation products (acetaldehyde/acetic acid). The C-O stretching peak seen at 1161 cm^{-1} in the purple spectrum belongs to the C-O single bonds of glycerin and olive oil, indicating that these compounds remain intact.

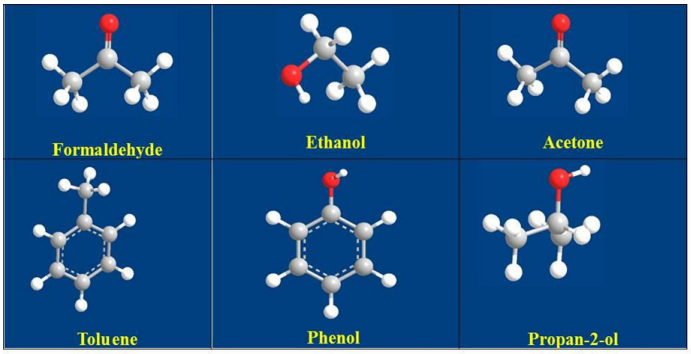

Figure VII. Structure of organic molecules used in solutions.

When the IR spectrum of Solution 5 is examined, carbonyl stretching vibration (purple spectrum) is observed at 1743 cm^{-1}, unlike the beginning (red spectrum). This may be due to the oxidation of iso-propanol to acetone. When the IR spectra at the beginning and the end of the experiment are observed, it is seen that the solutions remain the same.

Discussion

Used for the first time in 1899 for cadaver storage, formaldehyde (Figure VII) has still been used effectively for over 100 years (16). Formaldehyde is a bactericidal, fungicidal, and insecticide, although it has a decreasing effect over time. The widespread use of formaldehyde as a preservative supports that formaldehyde has perfect antiseptic properties and in this way prohibits the decay of organisms, tanning tissues without damaging the structures (16). Formaldehyde is classified as medium-high level (4-8% aqueous formaldehyde mixture) and high-level (70% alcohol and 8% formaldehyde composition) disinfectant (17). It has a wide range of effects on microorganisms. It devastates corrosive organisms when transported by a suitable means that allows them to diffuse these organisms; moreover, it reacts with proteins, forming novel chemical structures (resins) that are not durable and unsuitable as nutrients for living things (17). However, even though formaldehyde is a perfect tissue fixative, its use often results in tissues resulting in excessive stiffness, a major drawback (18). Apart from hardening, formaldehyde has other few drawbacks for preserving purposes (17). It coagulates the blood rapidly, turns the tissues gray when mixed with the blood, removes stains, dries out tissues, narrows capillaries, deteriorates over time, and has a disagreeable odor. Also, excessive formalin is shown to form a precipitate when the mummified cadaver is left unprotected for an extended range of time in the dissection laboratory (19).

Formaldehyde (Methanal), CH_2O, is an extremely reactive gas shaped by the synthesis gas of hydrocarbons or by the oxidation of methanol. Its 37% aqueous solution is known as formalin. Formaldehyde gas is further produced by the burnout of organic substances and can be generated secondary to carbon monoxide with water in the air from photochemical reactions including almost all types of hydrocarbon toxins (16). Formaldehyde is rapidly metabolized to methylene glycol and formic acid; It is generated endogenously in people and animals and further occurs owing to the metabolism of numerous xenobiotic agents (16). Due to these problems, specific biological exposure indicators like formaldehyde levels or metabolites in blood or urine have been approved to be inefficient exposure evaluations (16).

Ethanol is widely used as an alcoholic solvent and anti-infective agent in embalming processes (Figure 1). Also, in some literature, it is recommended to wash out excess formaldehyde with ethanol (16,20). There is partially uncertain literature on the effect of ethanol as a liquid preservative. Ethanol, at minimal when integrated with glycerin, the tertiary structure of the protein by affecting the hydrate coating reversible manner denatures. Hydrogen bridge bonds are broken (21). Ethanol tends to decrease the activity of the central nervous system (16).

Easily accessible isopropanol is noted as a better antiseptic and germicidal agent than ethanol (Figure 6). Isopropanol has a specific odor, but it is not unpleasant (22).

Acetone is one of the other fixative agents used in histopathology. It acts as an effective lipid solvent that causes tissue fragility. Besides tissue fixation, they are primarily used as an agent for dehydration in tissue processing. It is not recommended for use in automatic tissue processors due to their highly volatile and flammable nature (11).

In the study by Barbara M. Scholz-Böttcher on mummy residues at the Heidelberg (Germany) museum, camphor, aromatic compounds (e.g. toluene, dimethyl styrene derivatives dimethoxy benzene, tetra methoxy phenol), monoterpenes (ega-veb-pinene, cymene) and sesquiterpenes (e.g. kuparen, kadin-atrien, kadalen) were detected (23). In the study conducted by Timotty, Toluene (60 mL), Tertiary Butyl alcohol (25 mL), Ethyl alcohol (15 mL), Phenol 5 g, Naphthalene 20 g, and Canada Balsam (10 drops in Xylene) were used as embalming fluid for the storage of insects (24).

Toluene is a colorless, volatile aromatic solvent and has disinfectant properties against various viruses and bacteria (25).

Generally, 10% aqueous formalin solution is used to preserve animal and human bodies (26). Another possibility to protect cadavers in descriptive anatomy teaching is the use of glycerol, alcohol, and phenol (26). The most commonly used fixative solutions in Brazil for anatomical studies are formalin, ethyl alcohol, glycerin, and phenol (26). Phenol was first used as an anatomical embalming fluid in the mid-19th century by Laskowski (1886) (16). Initially, he used the 1:20 ratio of phenol: glycerine mixture and then the 1: 1: 4: 20 ratio of phenol: Boric acid: alcohol: glycerin mixture as the embalming liquid (16). Woodburne and Lawrence (1952) developed a new embalming formulation including phenol-formaldehyde, using isopropanol instead of alcohol and sorbitol instead of glycerin (16, 22). Erskin (1961) reported that the embalming fluid consisting of ethanol, formaldehyde, glycerin, and phenol and also containing salicylic acid, sodium arsenate, and 6-chlorophyll provides excellent dissection for cadavers over 3 years (16). In the last step of the cadaver conservation procedure developed by Logan (1983), alcohol, glycerin, phenol, and low formaldehyde were used as a preservative solution in the local injection of preservative solution for arterial protection (16). In studies conducted at Kawasaki School of Medicine on arterial patterns as embalming fluid, 95% ethyl alcohol (7.6 L) and 35% formalin (1.3 L) as fixative, diethylene glycol as protective (2.7 L), liquefied phenol as mold protective (1.3 L) and water (8.0 L) were used (16). O'Sullivan and Mitchell (1993) investigated that formaldehyde, industrial methylated spirits water, phenol, and glycerol were used in different proportions in embalming fluids obtained from 16 medical schools in the United Kingdom, and detected that phenol indicated disinfectant quality enhancing properties in all of them (27). In 2007, Silva, compared two solutions used to preserve the 112 cadavers in terms of color, odor, and flexibility in Hospital Cochin of Rene´ Descartes University, in France, a Laskowski solution with an altered Larssen solution (16). The altered Larssens (MLS) solution contained 100 mL of 10% formalin, 200 g chloral hydrate, 400 mL glycerin, 200 g sodium bicarbonate, 200 g of sodium sulfate, 180 g sodium chloride, and 2 L distilled water. The Laskowski solution consists of 200 mL ethanol, 800 mL glycerin, 50 g phenol, and 50 mg boric acid (16,26). In the study, 96.6% of the students found the Larssen solution more satisfactory in protecting cadavers for surgical training (26).

Phenol is a colorless liquid or a white solid crystal, and it was used as a disinfectant in medicine in 1867 (16). Phenol has bacteriostatic properties at a small concentration of 0.2% and is effective against various bacteria, fungi, and viruses (16). This effect occurs in the form of deactivating enzymes, effectively attacking and destroying the cell wall because of its lipophilic form, denaturing proteins and protein products. Liquefied phenol not only effectively prevents mold but also not indicate the graying effect seen in formaldehyde (16). In our study, it was determined that the colors of the heart tissues were preserved in the solutions numbered 3 and 5 including phenol.

The readily available isopropanol (Propan-2-ol) is found a better antiseptic and germicidal agent than ethanol. Isopropanol has a specific odor, but not an unfavorable one (16). In addition to formaldehyde, embalming fluids contain a variety of other chemicals such as several aliphatic alcohols (methanol, ethanol, and isopropanol), chelating agents (EDTA), surfactants

(sulfonates and sodium lauryl sulfate), deodorants (eugenol derivatives and safrole derivatives), dyes [eosin (yellowish-orange), erythrosine (red) and ponceau red] and disinfectants [salicylic acid and sulfanilamide (sulfa-base antibacterial)] (28).

In this study, solvents such as Toluene, Phenol, isopropanol, and ethoxyphenol were chosen due to their anti-infective, antiseptic, germicidal agent properties. Among them, ethoxyphenol in Solution 4 was used as a fixative for the first time and this solution was introduced to the literature as an alternative to phenol and ethyl alcohol. In addition, Solution 4 and Solution 5 were prepared for the first time in different amounts and mixtures and used as fixatives. While the deterioration of the solutions was not detected in the IR spectrum of the solutions numbered 4 and 5, the conversion to formic acid was detected in the formaldehyde solution number 1. Thus, alternative fixatives that are non-toxic and protect the tissues for a longer period, have been developed against formaldehyde which is toxic and has a limited usage time.

Conclusion

Heart tissues were evaluated for hardness, color, and odor over one-year period using new fixative solutions. As a result of the studies, it has been determined that the endocardium and myocardium layers of the heart tissues are better protected in Solution 4 and Solution 5 than in Solution 1 (formaldehyde solution). Solution 4 and Solution 5 were found to have superior fixative properties than Solution 1. From the FTIR data obtained, it was determined that solution 4 and solution 5 did not turn into harmful chemicals for health. In the IR spectrum of the 1st formaldehyde solution, the conversion of formaldehyde to formic acid was determined. As a result of this study, colorless, odorless, non-toxic fixative solutions that do not deteriorate in contact with air have been developed as an alternative to formaldehyde.

Acknowledgments

In this study, Hitit University Laboratories were used.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Effect of Vitamin D Against Progesterone-induced Effects on HepG2 Liver Cancer Cell Viability and Liver Function Tests

Progesteronun HepG2 Karaciğer Kanseri Hücre Canlılığı ve Karaciğer Fonksiyon Testleri Üzerindeki Etkilerine Karşı D Vitamininin Etkisi

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1439617
Geliş Tarihi | Received: 20.02.2024
Kabul Tarihi | Accepted: 24.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Akkuş MN, Bayram H, Sitar ME, Selam B, Cıncık M, Çakıl YD. Effect of Vitamin D Against Progesterone-induced Effects on HepG2 Liver Cancer Cell Viability and Liver Function Tests. Hitit Medical Journal 2024;6(2):108-116 https://doi.org/10.52827/hititmedj.1439617.

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Etik kurul onayı alınmasına gerek yoktur.
İntihal Kontrolleri: Evet (intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: MNA, YDC, MES, HB, FBS, MC Tasarım: MNA, YDC, MES, HB, FBS, MC Veri Toplama/Veri İşleme: MNA, YDC, MES Veri Analizi: MNA, YDC, MES, HB Makalenin Hazırlanması: MNA, YDC, MES, HB, FBS, MC.
Hasta Onamı: Gerek yoktur.
Finansal Destek: Bu çalışma Melek Naz Akkuş'un yüksek lisans projesi kapsamında Maltepe Üniversitesi Bilimsel Araştırma Projeleri tarafından desteklenmiştir.
Bilgi: Melek Naz Akkuş'un Yüksek Lisans tezinden üretilmiştir.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor
Ethical Statement: Not applicable.
Plagiarism Check: Yes (intihal.net)
Conflict of Interest: The authors declared that, there are no conflicts of interest.
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: MNA, YDC, MES, HB, FBS, MC Design: MNA, YDC, MES, HB, FBS, MC Data Collection/ Data Processing: MNA, YDC, MES Data Analysis: MNA, YDC, MES, HB Manuscript Preparation: MNA, YDC, MES, HB, FBS, MC.
Informed Consent: Not applicable.
Financial Disclosure: This study was supported by Maltepe University Scientific Research Projects within the scope of Melek Naz Akkuş's master's project.
Information: Produced from Melek Naz Akkuş's Master's thesis.

Effect of Vitamin D Against Progesterone-induced Effects on HepG2 Liver Cancer Cell Viability and Liver Function Tests

ABSTRACT

Objective: Progesterone is a signaling molecule synthesized by the adrenal glands and ovaries and is structurally the precursor of many different hormones. Vitamin D, unlike other vitamins, is a steroid hormone that can be synthesized endogenously as well as supplied exogenously. Vitamin D deficiency is a matter of considerable controversy in the world of medicine. The objective of this study was to investigate the impact of progesterone on the proliferation and liver enzyme activities of HepG2 cells, as well as to assess the potential of vitamin D in mitigating the cytotoxic effects induced by progesterone.

Material and Method: Cytotoxicity studies were conducted on HepG2 hepatocellular cancer cells to determine the appropriate doses of progesterone and vitamin D when applied alone or in combination. The hormones were administered to the experiment and control groups, either alone or in combination at specific doses. Subsequently, HepG2 cell viability, morphology and liver enzyme activities were measured comparatively between the groups.

Results: The results indicated a decrease in cell viability in the cells treated with 1 mM and 2 mM progesterone when compared to the control group. In addition, AST and LDH activity values were significantly lower with 1 mM and 2mM progesterone. Vitamin D was found not to have a cytotoxic effect on HepG2 cells between doses of 0.008 µM and 166.667 µM. Therefore, a dose of 2.5 µM was selected for further applications. No significant difference in ALT, AST, and LDH enzyme activity values was observed when only vitamin D was administered. Similar cell viability and enzyme activities were demonstrated when progesterone was administered alone or in combination with vitamin D.

Conclusion: At the doses and incubation periods used in the current study, vitamin D was found to be ineffective in preventing the cytotoxic effects caused by progesterone.

Keywords: Hepatocellular carcinoma, HepG2, liver function tests, progesterone, Vitamin D.

ÖZET

Amaç: Progesteron, adrenal bezler ve yumurtalıklar tarafından sentezlenen, yapısal olarak birçok farklı hormonun da öncüsü olan bir sinyal molekülüdür. D vitamini ise diğer vitaminlerden farklı olarak ekzojen alımın yanında endojen olarak da sentezlenebilen ancak eksiklik durumu güncel tıp dünyasında büyük tartışmalara neden olan steroid yapıda bir hormondur. Bu çalışmada amaç, progesteronun HepG2 hücre proliferasyonu ve karaciğer enzim aktivitelerine etkisini belirlemek, ayrıca D vitamininin progesteronun oluşturduğu sitotoksik etkileri engellemedeki rolünü incelemektir.

Gereç ve Yöntem: HepG2 hepatoselüler kanser hücre kültürü ortamına uygulanacak progesteron ve D vitamini dozlarının belirlenmesi için öncelikle her iki hormon için ayrı sitotoksisite çalışmaları yapılmıştır. Ardından progesteron ve D vitamini, deney ve kontrol gruplarına tek başlarına veya birlikte belirli dozlarda uygulanmıştır. HepG2 hücre canlılığı, morfolojik özellikleri ve karaciğer enzim aktiviteleri gruplar arasında karşılaştırmalı olarak değerlendirilmiştir.

Bulgular: Hücrelere uygulanan 1 mM ve 2 mM progesteron dozlarında kontrol grubuna kıyasla hücre canlılığında azalma olduğu saptandı. Ek olarak, 1 mM ve 2 mM progesteron uygulananlarda AST ve LDH aktivite değerlerinde de anlamlı olarak düşüklük bulundu. D vitamininin 0,008 µM ve 166,667 µM dozları aralığında HepG2 hücrelerinde sitotoksik bir etkiye sahip olmadığı belirlendi ve 2,5 µM dozda uygulandı. Yalnızca D vitamini uygulanan hücrelerde ALT, AST ve LDH enzim aktivite değerlerinde anlamlı bir farklılık görülmedi. Yalnızca progesteron uygulanan hücrelerle, progesteron+D vitamininin birlikte uygulandığı hücreler arasında hücre canlılığı ve karaciğer enzim düzeyleri benzerlik gösterdi.

Sonuç: Kullanılan doz ve inkübasyon sürelerinde D vitamininin progesteronun sebep olduğu sitotoksik etkileri engellemede etkili olmadığı düşünülmektedir.

Anahtar Sözcükler: D vitamini, hepatosellüler karsinom, HepG2, karaciğer fonksiyon testleri, progesteron.

Giriş

Progesteron, "gebelik hormonu" olarak bilinen ve ovaryumda yerleşim gösteren granüloza lutein hücreleri ve adrenal bezler tarafından sentezlenen bir cinsiyet hormonudur (1). Progesteron, ovülasyonu düzenleme yanında endometriyumun proliferatif aşamadan sekretuvar aşamaya geçişini sağlayıp, blastosist implantasyonunu kolaylaştırır ve hamileliğin devamı için gereklidir (2). Progesteron steroidlerin bir öncüsü olmakla birlikte, karaciğerde östradiolün metabolize edilmesi gibi inaktif hale getirilip, glukuronik asitle konjuge edilir ve idrarla atılır (3,4). Birçok meta-analizde in vitro fertilizasyon (IVF) sonrası progesteron kullanımının canlı doğum oranlarını arttırdığı ayrıca düşük tehdidi nedeniyle progesteron tedavisi alan gebelerde preterm doğumu önlemede olumlu yönde etkili olduğu bildirilmiştir (5,6). Ancak, bunun yanında ikinci ve üçüncü trimesterda vajinal progesteron tedavisi alan kadınlarda gebeliğin intrahepatik kolestazı (ICP) görülme sıklığının üç kat arttığı da gösterilmiştir (7,8). Çoğul gebeliklerde daha da sık gözlenmesi, multiparlarda tekrarlayıcı nitelik göstermesi, bazı etnik gruplarda yoğunlaşması sebebiyle, artan hormon düzeylerinin veya bunların ara metabolitlerinin, ICP etiyolojisinde önemli olduğu düşünülmektedir (9,10). Ek olarak, progesteronun kadınlarda ilaca bağlı karaciğer hasarını (Drug Induced Liver Injury, DILI) indüklediği de bilinmektedir (11). Etiyolojisinde seks hormonlarının etkili olduğu bilinen alkol dışı yağlı karaciğer hastalığı (nonalcoholic fatty liver disease)'nın en şiddetli şekli olan nonalkolik steatohepatit hastalarında progesteron kullanımının hepatik lobüler inflamasyonu indüklediği de gösterilmiştir. Ayrıca, progesteronun tümörler için elverişli bir mikroçevre oluşumuna katkıda bulunarak karaciğer kanserine neden olabileceği düşünülmektedir (4).

D vitamini, diğer tüm vitaminlerden farklı olarak kalsiyum, magnezyum, fosfat gibi minerallerin barsaktan emilimi ile kemik ve kalsiyum homeostazisinin korunmasında büyük öneme sahip olan steroid yapıda bir hormondur (12,13). Bununla birlikte güncel literatürde D vitamininin antiinflamatuar ve antifibrotik etkilerinin de olduğu, hücre farklılaşması ve proliferasyonunda önemli roller oynadığı gösterilmiştir. Bu etkileri, kronik karaciğer hastalıklarının nedenleri ve tedavisi ile ilişkilendirilmiştir (14). D vitamini seviyeleri, ICP tanısı almış gebelerde de araştırılmış ve normal gebelere göre daha düşük düzeyde olduğu görülmüştür (15–17). Ek olarak, D vitamini eksikliğinin, onkolojik, nörolojik, kardiyovasküler ve otoimmün temelde birçok farklı hastalık ile ilişkisi gösterilmiştir. Bununla birlikte, D vitamininin potansiyel malignite önleyici, iyileştirici veya süreci yavaşlatıcı etkilerini değerlendiren çok sayıda çalışma yapılmış, ancak D vitamininin kanser hücrelerindeki etkisi ile ilgili net bir fikir birliği sağlanamamıştır (18,19).

Geçtiğimiz yıllarda yapılan bir çalışma, etinil östradiol gibi kolestaza sebep olduğu bilinen maddelerin HepG2 karaciğer kanseri hücrelerinde ilgili genlerin ifadesine olan etkilerini, insan kolestaz örneklerindeki gen ifadesi profili ile karşılaştırmış ve farklı şekilde ifade edilen genlerde benzerlik bulmuştur (20). Progesteronun karaciğer hasarına yol açtığı bilinmekle birlikte, D vitamini ve progesteronun kanser ile ilişkisini bir arada inceleyen güncel bir çalışmaya rastlanmamıştır. Bu çalışma, progesteronun HepG2 hücreleri proliferasyonu ve karaciğer enzim aktivitelerine etkisini belirlemeyi, bununla birlikte progesteronun bu hücrelerde sebep olabileceği sitotoksisiteye karşı D vitamininin koruyucu rolü olup olmadığını araştırmayı amaçlamıştır. Bu doğrultuda, deney ve kontrol gruplarına progesteron ve D vitamini tek başlarına veya birlikte uygulanmış, HepG2 hücre canlılığı, morfolojik özellikleri ve karaciğer enzim aktivite düzeyleri gruplar arasında karşılaştırmalı olarak değerlendirilmiştir.

Materyal ve Metod

Hücre Kültürü

Çalışmamızda HepG2 hücre hattı (American Type Cell Culture, ATCC) kullanıldı. Dulbecco's Modified Eagle Medium (DMEM, Capricorn, Almanya), % 10 fetal bovine serum (FBS, Gibco, ABD) ve %1 Penisilin-Streptomisin (Gibco, ABD) ile besiyeri hazırlandı. HepG2 hücreleri 37°C'de %5 CO_2 içeren ortamda (Panasonic, Japonya) inkübe edildi. Ortalama %70-80 konfluent hücreler pasajlandı.

Progesteronun Eklenmesi ve Hücre Canlılığının Belirlenmesi

HepG2 hücreleri 12 kuyucuklu plakalara eşit miktarda (150.000 hücre/1mL) ekildi. Ertesi gün Depo-Provera (DP; etkin madde: Medroksiprogesteron asetat (MPA) 150 mg/mL; Pfizer, ABD) 20 µM, 50 µM,

400 µM, 1 mM, 2mM dozlarda kuyucuklara eklendi. İlaç eklenen plakalar hücre sayımı öncesi 24 saat 37°C'de, %5 CO_2 ortamında inkübatörde tutuldu. 450 nm dalga boyunda DP ışıması gözlendiği için, HepG2 hücrelerinin canlılığı Tripan mavisi (0.4%; Gibco, ABD) ile boyanıp değerlendirildi (21). Kontrol gruplarının hücre canlılığı %100 varsayıldı.

D vitamininin Eklenmesi ve Hücre Canlılığının Belirlenmesi

Öncelikle uygulanacak D vitamini dozunun belirlenmesi için hücre sayım kiti-8 (CCK-8) ile hücre canlılığı tespit edildi. Sitotoksisite deneyi için 96 kuyucuklu plaka kullanıldı. Hücre sayımı yapıldıktan sonra her kuyucukta 10.000 hücre olacak şekilde ekim yapıldı ve ilaç eklenmeden önce 24 saat inkübe edildi. Karşılaştırma amacıyla bazı kuyucuklara sadece besiyeri eklendi. D vitamini (DEVİT-3, etkin madde: vitamin D3 50.000 I.U./ 15 mL; Deva, Türkiye) 0,008-166,667 µM doz aralığında HepG2 hücrelerine eklendi ve 24 saatin sonunda her kuyucuğa 10 µL CCK-8 eklendi. 3-4 saat inkübe edilen hücreler Elisa Reader (BioTek Synergy H1) ile 450 nm dalga boyunda okundu.

D vitamininin progesteron uygulanan HepG2 hücrelerine etkisini değerlendirmek için 1 mM veya 2 mM DP, 2,5 µM D vitamini ile birlikte hücre ortamlarına eklendi. 24 saat sonra hücre canlılığı Tripan mavisi ile değerlendirildi.

Karaciğer Fonksiyon Testleri

Çalışmamızda karaciğer fonksiyon testlerinin değerlendirilmesi için 6 kuyucuklu plakalar kullanıldı. Hücre sayımı sonrası her kuyucuğa eşit olacak şekilde $5x10^5$ hücre/2 mL ekim yapıldı. Yirmi dört saat sonrasında kuyucuklara 1 mM veya 2 mM DP, 2,5 µM D vitamini ile birlikte eklendi. 24 saat sonrasında kuyucuklardaki hücreler ve besiyerleri alınıp falkon tüplere aktarıldı. Hücreler üç kere sıvı nitrojende dondurulup 37°C'de çözüldü ve 400 g'de 4 dk santrifüj edildi. Elde edilen hücre kültürü süpernatantlarından aspartat amino transferaz (AST), alanin amino transferaz (ALT) ve laktat dehidrogenaz (LDH) enzim aktiviteleri ölçümleri spektrofotometrik yöntemle otoanalizörde (Roche Cobas Integra 400) üreticinin tarif ettiği direktiflere göre yapıldı (22).

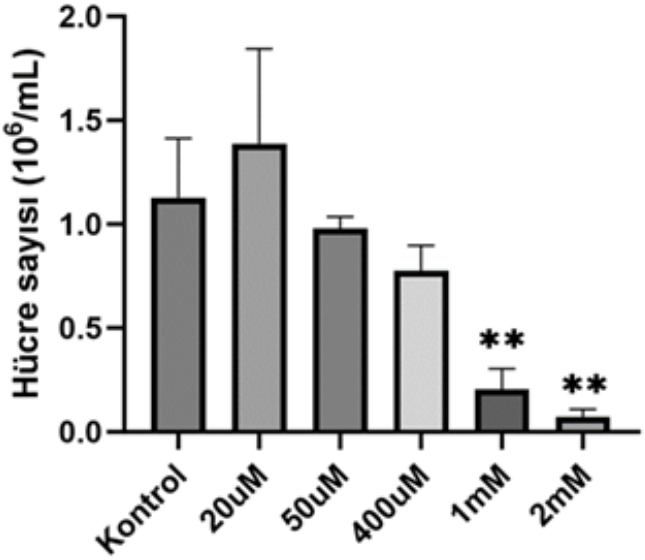
İstatistik Analizler

Verilerin tüm istatistiksel analizleri ve grafikler halinde gösterilmesi için GraphPad Prism 10.0 (San Diego, CA, USA) yazılımı kullanıldı. Değişkenlerin normallik testleri için Shapiro-Wilk W testi uygulandı. Normal dağılım gösteren veriler arasında gruplar arası karşılaştırması tek yönlü ANOVA ve Tukey post hoc testi ile yapıldı. *p<0.05* istatistiksel olarak anlamlı kabul edildi.

Bulgular

Progesteronun HepG2 Hücre Canlılığına Etkisi

HepG2 hücrelerine artan konsantrasyonlarda DP uygulandığında (20 µM, 50 µM, 400 µM, 1 mM ve 2 mM), 1 mM ile 2 mM DP uygulamalarının kontrol grubuna kıyasla hücre canlılığında anlamlı bir azalmaya neden olduğu görülmüştür (sırasıyla *p=0.0038* ve *p=0.0012*; Şekil I). Kontrol grubu ile 20 µM, 50 µM ve 400 µM DP dozları kıyaslandığında anlamlı bir farklılık bulunamamıştır (sırasıyla *p=0.7369, p=0.9640* ve *p=0.4594*).



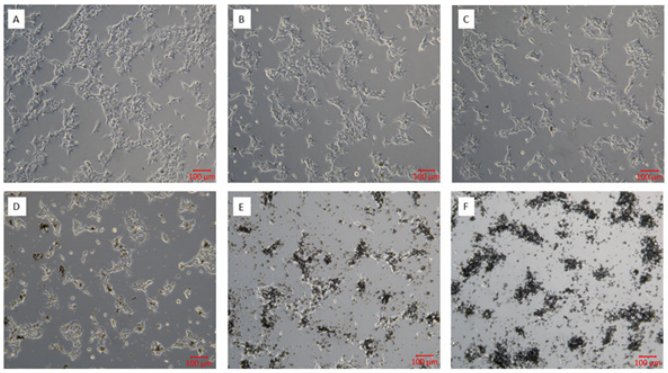
Şekil I. Kontrol grubu ve artan dozlarda DP uygulanan HepG2 hücrelerinde hücre sayıları (10^6/mL). ***p<0,01.*

24 saat progesteron uygulamaları sonunda hücreler faz-kontrast mikroskobu ile görüntülendi (10X) ve artan progesteron maruziyeti ile hücre canlılığında azalma olduğu gözlemlendi (Şekil II).

D Vitamininin HepG2 Hücre Proliferasyonuna Etkisi

D vitamini dozlarının belirlenmesi için yapılan sitotoksisite testi sonucunda, 0,008-166,667 µM doz aralığında HepG2 hücrelerinde sitotoksik bir etki bulunmadı (*p=0.1954*; Şekil III). Çalışmada kullanılacak D vitamini dozu 2,5 µM olarak belirlendi.

Progesteron ve D vitamininin HepG2 Hücre Canlılığına Etkisi

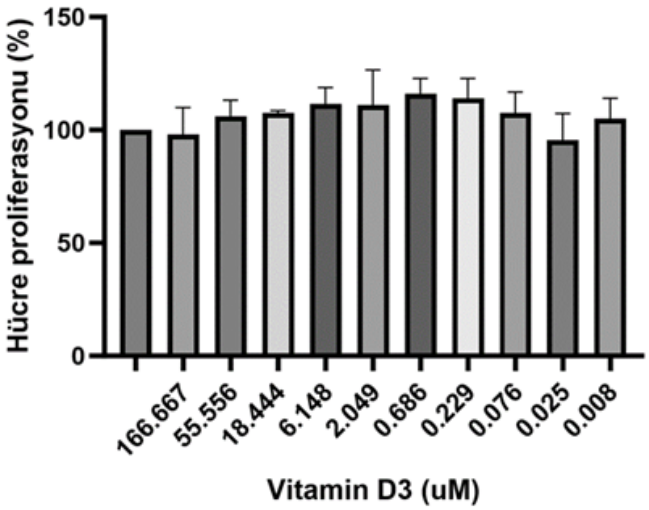


Şekil II. HepG2 hücrelerine artan dozlarda uygulanan progesteronun 24 saat sonra faz-kontrast mikroskobu görüntüleri (10X) (A. Kontrol, B. 20 µM DP, C. 50 µM DP, D. 400 µM DP, E. 1mM DP, F. 2 mM DP) (DP: Depo Provera).

Progesteronun HepG2 hücrelerinde oluşturduğu sitotoksik etkinin önlenmesinde D vitamininin olası etkileri araştırıldı. Yalnızca 2,5 µM vitamin D3 eklenen HepG2 hücrelerinin, kontrol grubuna benzer şekilde iğsi formda yüzeyde düz bir tabaka halinde yayıldığı görüldü (Şekil IV A-F). 1 mM DP uygulanan hücreler kontrol grubuyla karşılaştırıldığında hücre sayısında anlamlı bir azalma bulundu (*p=0.0005*; Şekil IV G). Benzer şekilde 1 mM DP + 2,5 µM D vitamini eklenen grup, kontrol grubu ile karşılaştırıldığında hücre sayısında anlamlı bir azalma olduğu görüldü (*p=0.0016*) (Şekil IV G). 1 mM DP ve 2,5 µM vitamin D3 birlikte uygulandığında, tek başına 1 mM DP uygulanmış gruba göre daha fazla hücre yoğunluğu gözlenmiş olmasına rağmen (Şekil IV B ve IV E), hücre sayıları arasında anlamlı bir fark bulunmamıştır (*p=0.9465*). D vitamini, 2mM DP uygulanan grupta da hücre sayısında bir farka yol açmamıştır (*p>0.999*). Her iki grupta hücreler benzer şekilde iğsi formlarını kaybetmiş ve yüzeyden ayrılmıştır (Şekil IV C ve IV F).

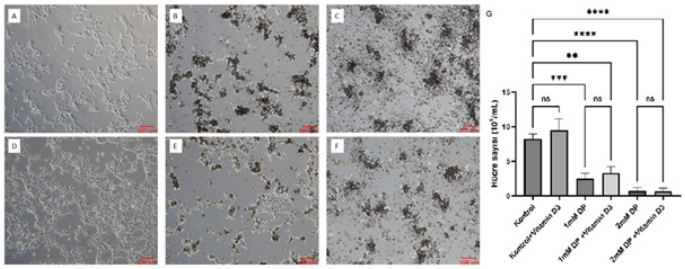
Progesteron ve D vitamininin AST, ALT ve LDH Değerlerine Etkisi

Yapılan gruplar arası karşılaştırmada AST değerleri arasında anlamlı fark bulundu (*p=0.0002*). 1mM veya 2mM DP tek başına uygulanan grupların kontrol grubuna kıyasla AST değerleri anlamlı olarak azaldı (sırasıyla *p=0.0035* ve *p=0.0029*). Bununla birlikte 1 mM ve 2mM DP uygulanan hücrelerde D vitaminin AST düzeyine bir etkisi olmadığı görüldü (1mM DP ve 1mM DP +Vitamin D3 grupları arasında *p>0.999*; 2mM DP ve 2mM DP+Vitamin D3 grupları arasında *p=0.5303*; Şekil V A).



Şekil III. Kontrol ile farklı dozlarda D vitamini uygulanan HepG2 hücrelerinin proliferasyonu (%).

ALT değerleri, D vitamini uygulanan ve uygulanmayan bütün gruplarda karşılaştırıldı ve gruplar arasında anlamlı bir farklılık saptanmadı (*p=0.4398*; Şekil V B).

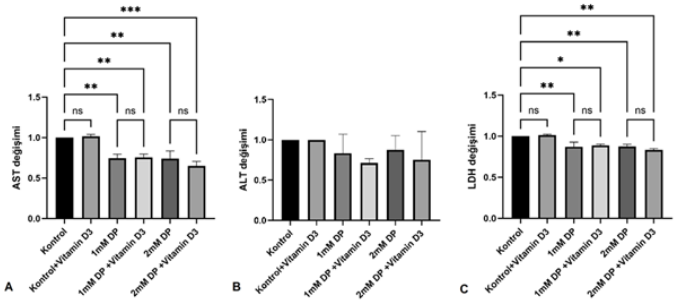


Şekil IV. Kontrol grubu ve progesteron/D vitamini uygulanan HepG2 hücre gruplarının faz-kontrast mikroskobu görüntüleri (A. Kontrol B. 1 mM DP C. 2 mM DP D. Kontrol+2.5 µM vit. D3 E. 1 mM DP+2.5 µM vit. D3 F. 2 mM DP+2.5 µM vit. D3) (DP: Depo Provera, vit. D3: D vitamini, G. Progesteron ve/veya D vitamini uygulamaları sonrası HepG2 hücre sayıları (10^5/mL). ***p<0,01, ***p<0,001 ve ****p<0,0001,* ns=anlamlılık yok).

LDH değerleri gruplar arasında karşılaştırıldı ve anlamlı bir fark bulundu (*p=0.0005*). Kontrol grubuyla karşılaştırıldığında, yalnızca 1 mM veya 2 mM DP uygulanan gruplarda LDH aktivite seviyelerinin anlamlı olarak azaldığı görüldü (sırasıyla *p=0.0054* ve *p=0.0069*; Şekil V C). DP uygulanan hücrelerde D vitaminin LDH aktivite düzeyine bir etkisi olmadığı görüldü (1 mM DP ve 1 mM DP + Vitamin D3 grupları

arasında *p=0.9910*; 2 mM DP ve 2 mM DP + Vitamin D3 grupları arasında *p=0.6390*).

Yalnızca D vitamini uygulanan hücrelerde AST, ALT ve LDH enzimleri aktivite değerleri kontrol grubu hücrelerinde elde edilen değerlere benzer bulundu.



Şekil V. A. Progesteron/D vitamini uygulanan gruplarda AST aktivite düzeylerinin karşılaştırılması. B. Progesteron/D vitamini uygulanan gruplarda ALT aktivite düzeylerinin karşılaştırılması. p>0.05. C. Progesteron/D vitamini uygulanan gruplarda LDH aktivite düzeylerinin karşılaştırılması. **p<0.05, **p<0.01, ***p<0.001*. ns=anlamlılık yok.

Tartışma

Progesteronun kendisinin ya da ara metabolitlerinin, DILI ve ICP ile ilişki olduğuna dair çalışmalar bulunmaktadır (8,9,11,23). Ayrıca dişi farelerde yapılan bir çalışmada, progesteronun inflamasyonu modüle ederek karaciğer hasarını şiddetlendirdiği bildirilmiştir (24). Son zamanlarda yapılan farklı çalışmalarda, preterm doğum riskini azaltmak için uygulanan progesteron takviyelerinin kolestaz riskini artırabileceği üzerinde durulmuştur (7,8,23).

Kalsiyum ve fosfor metabolizmasındaki önemi bilinen D vitamini yağda eriyen vitaminlerdendir ve eksikliği önemli bir küresel sağlık sorunu olarak kabul edilmektedir (25). D vitamininin karaciğer üzerinde antiinflamatuar etkisi olduğu, kronik karaciğer hastalığı ve siroz hastalarında D vitamini eksikliğinin yaygın olduğu bildirilmiştir (26,27). Klinik araştırmalarda ve kronik karaciğer hastalığının hayvan modellerinde D vitamin takviyesinin yararlı etkileri bildirilmiştir (28). Ayrıca D vitamininin insülin direnci, obezite, kanser ve kardiyovasküler hastalıkları önlemede önemli bir rol oynadığı bilinmektedir (29). Mevcut çalışmada HepG2 hücre hattı kullanılarak, progesteronun hücre canlılığı ve karaciğer enzim aktiviteleri düzeylerine etkisini belirlemek, bununla birlikte D vitamininin progesteron uygulamasının sebep olabileceği olası sitotoksik etkileri önlemedeki etkinliğini incelemek hedeflenmiştir. Kontrol grubuna göre yapılan kıyaslamalarda 1 mM ve 2 mM progesteron uygulanmış HepG2 hücrelerinin canlılığının azaldığı ve karaciğer enzimleri AST ve LDH aktivite değerlerinde düşüşe yol açtığı görülmüştür. Geçtiğimiz yıllarda yapılan bir çalışmada, benzer şekilde progesteronun NCI-H295R insan adrenokortikal karsinoma hücrelerinde ve over karsinomu primer hücrelerinde hücre canlılığında anlamlı bir azalmaya neden olduğu gösterilmiştir (30,31). HepG2 hücrelerinde epirubisin ve progesteron uygulaması sonucu sitotoksik ve apoptotik değişikliklerin gözlenmesi amaçlanan bir başka çalışmada, 50 µM progesteron ve 0,1 µg/mL epirubisinin 48 saat inkübasyon sonrası sitotoksisite ve apoptozu arttığı görülmüştür (32). Diğer yandan, düşük doz progesteron (5 nM - 1 µM aralığı) uygulanan insan over kanseri hücre hatlarında hücre canlılığında bir azalma bildirilmemiş, fakat hücre migrasyonunda anlamlı bir azalma belirlenmiştir. Bu etkinin progesteron uygulaması sonrası bozulan hücre polaritesi sebebi ile gözlendiği düşünülmektedir (33). Progesteronun antitümör etkileri esas olarak MDA-MB-468 ve SKBR3 gibi meme kanseri hücre hatlarında gösterilmiş ve apoptoz indüklenmesinin özellikle membran progesteron reseptörleri aracılığı ile olduğu bildirilmiştir (34). Benzer şekilde, pankreas adenokarsinom hücre hattında da progesteron uygulaması (1 µM and 20 µM) hücre proliferasyonunda azalmaya yol açmıştır ve membran progesteron reseptörleri aktivasyonunun bu etkiye aracılık edebileceği düşünülmektedir (35). Sistemik dolaşımda ılımlı artan karaciğer enzim aktivite düzeyleri öncelikle karaciğer hasarına işaret etmektedir. Mevcut çalışmada sitotoksik dozlarda uygulanan progesteronun karaciğer enzim düzeylerinde anlamlı olarak azalmaya yol açmasının sebebi olarak, progesteronun hücre canlılığını belirgin olarak azaltması ve sekonder aşamada enzimleri sentezleyecek yeterli fonksiyonel hepatosit olmadığı düşünülmektedir. Ancak, son yıllarda yapılan başka bir çalışmada, kadmiyum ve progesteron uygulanan sıçanlarda karaciğer enzim düzeyleri incelendiğinde, kadmiyum uygulamalarının ALT, AST ve ALP aktivite değerlerinde kontrole kıyasla anlamlı bir artışa sebep olduğu, kadmiyum ile progesteron birlikte uygulandığında bu değerlerin önemli ölçüde azaldığı, progesteronun oksidatif stresi azalttığı gözlenmiştir

(36).

D vitamininin tek başına uygulandığında HepG2 hücrelerinde sitotoksisiteye yol açmadığı ve ALT, AST ve LDH aktivite değerlerini etkilemediği görülmüştür. Ayrıca DP ile birlikte uygulandığında da hücre canlılığı ve karaciğer enzim düzeylerinde bir değişikliğe neden olmamıştır. Bu veriler ışığında, kullanılan doz ve inkübasyon sürelerinde D vitamininin progesteronun sebep olduğu sitotoksik etkileri engellemede etkili olmadığı görülmüştür.

Bu sonuçlardan farklı olarak Gocek ve ark. yapmış olduğu çalışmada, D vitamininin meme, prostat ve kolon kanseri hücrelerinde apoptozu teşvik ettiği gösterilmiştir (37). MCF-7 hücrelerine salinomisin ve/veya D3 vitamini uygulanan bir diğer çalışmada da kombinasyon uygulamasının hücre canlılığını zamana ve doza bağlı azalttığı gözlenmiştir (38). Zhang ve ark. çalışmasında da benzer şekilde over kanseri hücrelerinde D vitamininin apoptozu engellediği gösterilmiştir (39). Kısa bir süre önce yayınlanmış bir çalışmada da D vitamini uygulaması CD133+/CD44 + meme kanseri hücrelerinde SOX2 ve OCT4 kök hücre belirteçlerinin ifadesinde anlamlı bir azalma, daha düşük hücre proliferasyonu ve daha fazla apoptoz ile sonuçlanmıştır (40). Meme kanseri hücreleri ile gerçekleştirilen bir başka çalışma da benzer şekilde D vitamininin hücre canlılığını azalttığını ve bu etkiye esas olarak apoptozu ve hücre döngüsü tutulumunu indükleyerek sebep olduğunu göstermiştir (41). Diğer yandan, melatonin ve D vitamininin HepG2 ve Hep3B hücre hatlarında CCl4 kaynaklı sitotoksisite üzerindeki etkilerinin araştırıldığı çalışmada ise melatoninin ve D vitamininin hücreleri CCl4 kaynaklı hepatotoksisiteden koruduğu bildirilmiştir (42). D vitaminin, hem doğrudan neoplastik hücrelerin farklılaşmasını, çoğalmasını ve apoptozunu kontrol ederek hem de dolaylı olarak malign tümörlerin mikro çevresine ait immün hücreleri düzenleyerek kanser karşıtı etkilere sahip olduğu belirtilmektedir (43). Bununla birlikte verilen örneklerden farklı olarak D vitamininin kanser hücrelerine etkisi olmadığını gösteren çalışmalar da mevcuttur (44). Tüm bu tartışmalı sonuçlar nedeniyle D vitamininin kanser hücrelerindeki etkisi tam olarak açığa çıkarılamamıştır. Sonuç olarak, progesteron uygulamaları HepG2 hücre canlılığı ve karaciğer enzimleri AST ve LDH aktivite değerlerinde anlamlı bir azalmaya yol açmıştır. Farklı dozlarda D vitamini tek başına uygulandığında HepG2 hücrelerinde sitotoksik bir etki göstermemiş olmakla birlikte progesteronun neden olduğu sitotoksisiteyi önlemede de başarı gösterememiştir. Kullanılan doz ve inkübasyon sürelerinde D vitamininin progesteronun sebep olduğu sitotoksik etkileri engellemede rolü olmadığı düşünülmekle birlikte, farklı dozlarda ve inkübasyon sürelerinde çalışmalara ihtiyaç duyulmaktadır.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

The Comparison of Real-Time PCR and Mutation-Specific Immunohistochemistry in EGFR Mutation Analysis of Non-Small Cell Lung Carcinomas

Küçük Hücreli Dışı Akciğer Karsinomlarında EGFR Mutasyon Analizinde Real-Time PCR ve Mutasyon Özgü İmmünohistokimya Karşılaştırması

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1351295
Geliş Tarihi | Received: 08.09.2023
Kabul Tarihi | Accepted: 29.04.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Meriçöz ÇA, Güler G, Önder S. The comparison of Real-Time PCR and mutation-specific immunohistochemistry in EGFR mutation analysis of non-small cell lung carcinomas. Hitit Medical Journal 2024;6(2):117-125 https://doi.org/10.52827/hititmedj.1351295

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Hacettepe Üniversitesi Girişimsel Olmayan Araştırmalar Etik Kurulundan 08/11/2013 tarihinde onay alınmıştır (No: GO 13/519-24).
İntihal Kontrolleri: Evet (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: ÇAM, GG, SÖ Tasarım: ÇAM, GG, SÖ Veri toplama/Veri işleme: ÇAM, GG, SÖ Veri analizi: ÇAM, GG, SÖ Makalenin hazırlanması: ÇAM, GG, SÖ.
Hasta Onamı: Gerek yoktur.
Finansal Destek: Bu çalışma ile ilgili herhangi bir finansal kaynaktan yararlanılmamıştır.
Bilgi: Bu yazı Ulusal Patoloji ve Sitopatoloji Kongresi'nde sözlü bildiri olarak sunulmuştur, Bursa, 2015.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor
Ethical Statement: Etchical approval was received from Hacettepe University Ethics Committee on 07/09/2022 (No: E2-22-2356).
Plagiarism Check: Yes (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts of interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: ÇAM, GG, SÖ Design: ÇAM, GG, SÖ Data Collection/Data Processing: ÇAM, GG, SÖ Data Analysis: ÇAM, GG, SÖ Article Preparation: ÇAM, GG, SÖ.
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.
Information: This manuscript has been presented in National Pathology and Cytopathology Congress as oral presentation, Bursa, 2015.

The Comparison of Real-Time PCR and Mutation-Specific Immunohistochemistry in EGFR Mutation Analysis of Non-Small Cell Lung Carcinomas

ABSTRACT

Objective: This study aims to identify activating mutations in the epidermal growth factor receptor (EGFR) gene in patients with non-small cell lung cancer (NSCLC) and to evaluate their correlation with responses to EGFR-tyrosine kinase inhibitors (TKI) treatment. This study aims to identify activating mutations in the epidermal growth factor receptor (EGFR) gene in patients with non-small cell lung cancer (NSCLC) and to evaluate their correlation with responses to EGFR-tyrosine kinase inhibitors (TKI) treatment. We conducted a comparative analysis of Real-Time PCR and immunohistochemistry to detect EGFR mutation status in non-small cell lung cancer patients, focusing on the sensitivity, specificity, and predictive values of immunohistochemistry.

Material and Method: We evaluated 788 non-small cell lung cancer samples which were analyzed for EGFR mutation status by RT-PCR. We detected 126 EGFR mutated cases among these patients. We evaluated mutation-specific EGFR immunohistochemistry directed towards the exon 19 deletions (15 bp E746-A750) and exon 21point mutation (L858R) to the 47 EGFR mutated patients histologic material and cell blocks of cytologic specimens.

Results: 32 of the 47 cases (68%) had exon 19 deletion, 14 of them (30%) had point mutation in exon 21, and one of them (2%) showed exon 18 mutation. EGFR exon 19 (15 bp E746-A750 deletion) antibody showed a sensitivity of 100%, specificity of 40%, negative predictive value of 100%, and positive predictive value of 78%. The sensitivity of the exon 21 (L858R point mutation) antibody was 93%, specificity was 91%, negative predictive value was 97% and positive predictive value was 82%.

Conclusion: Our investigation indicates that mutation-specific EGFR immunohistochemistry has demonstrated a notable sensitivity and specificity for exon 21. However, while sensitive, the exon 19 (15 bp E746-A750 deletion) antibody lacked specificity. While positive immunohistochemical staining may suggest the presence of an EGFR mutation, making the patient potentially eligible for TKI treatment, it should not be the sole determinant. If immunohistochemistry results are negative, it is essential to resort to molecular tests to ensure accurate diagnosis and appropriate therapeutic guidance. With evolving diagnostic landscapes, it is crucial to harness both IHC and molecular techniques judiciously for optimal patient care.

Keywords: EGFR, Immunohistochemistry, Non-small cell lung cancer.

ÖZET

Amaç: Bu çalışmanın amacı, Küçük Hücreli Dışı Akciğer Kanseri (KHDAK) hastalarında EGFR genindeki aktivasyon mutasyonlarını tespit etmek ve EGFR-tirozin kinaz inhibitörlerinin (TKI) tedavi yanıtlarıyla olan ilişkisini değerlendirmektir. Küçük hücreli dışı akciğer kanseri (KHDAK) hastalarında epidermal büyüme faktörü reseptörü (EGFR) genindeki aktive edici mutasyonların belirlenmesi, EGFR-tirozin kinaz inhibitörleri (TKI) tedavi yanıtlarıyla ilişkilidir. Mutasyon spesifik antikorlar kullanılarak yapılan immünohistokimya, belirli mutant EGFR proteinlerini tespit edebilmektedir. KHDAK hastalarında EGFR mutasyon durumunu tespit etmek için Real-Time PCR ve immünohistokimyayı karşılaştırdık ve immünohistokimyanın duyarlılık, özgüllük, pozitif ve negatif öngörü değerlerini analiz ettik.

Gereç ve Yöntem: RT- PCR ile EGFR mutasyon durumu için analiz edilen 788 küçük hücreli dışı akciğer kanseri örneğini değerlendirildi. Bu hastalar arasında 126 EGFR mutasyonlu vakayı tespit edildi. 47 EGFR mutasyonlu hastanın histolojik materyali ve sitolojik örneklerin hücre bloklarına yönelik ekzon 19 delesyonları (15 bp E746-A750) ve ekzon 21 nokta mutasyonu (L858R) için mutasyon spesifik EGFR immünohistokimyası (IHK) çalışıldı ve sonuçlar boyama kuvveti ve yaygınlığına göre değerlendirildi.

Bulgular: 47 vakadan 32'si (%68) ekzon 19 delesyonuna sahipti, bunların 14'ünde (%30) ekzon 21'de nokta mutasyonu vardı ve birinde (%2) ekzon 18 mutasyonu gözlendi. EGFR ekzon 19 (15 bp E746-A750 delesyon) antikoru %100 duyarlılık, %40 özgüllük, %100 negatif öngörü değeri ve %78 pozitif öngörü değeri gösterdi. Ekzon 21 (L858R nokta mutasyonu) antikorunun duyarlılığı %93, özgüllüğü %91, negatif öngörü değeri %97 ve pozitif öngörü değeri %82 idi.

Sonuç: Araştırmamız, EGFR immünohistokimyasının, ekzon 21 mutasyonu için belirgin bir duyarlılık ve özgüllük sergilediğini göstermektedir. Ancak, duyarlı olan ekzon 19 (15 bp E746-A750 delesyon) antikoru özgüllükten yoksundur. Pozitif immünohistokimya sonucu, hastada EGFR mutasyonu olabileceğini ön görebilir ve hasta potansiyel olarak TKI tedavisine uygun olabilir; ancak bu durum tek başına belirleyici olmamalıdır. İmmünohistokimya sonuçları negatifse, doğru tanı ve uygun tedavi rehberliği için moleküler testlere başvurulması esastır.

Anahtar Sözcükler: EGFR, İmmünohistokimya, Küçük hücreli dışı akciğer karsinomu.

Introduction

Lung cancer is the most frequent cause of cancer-related mortality worldwide. Moreover, lung carcinoma is the 2nd most common cancer type by gender after prostate cancer for men and breast cancer for women. As most patients are diagnosed at advanced stages, the 5-year survival rate of people diagnosed with advanced stage non-small cell lung cancer (NSCLC) is around 6%, and of small cell lung cancer is around %3(1, 2).

Several driver mutations have been described in the pathogenesis of non-small cell lung cancers. While FGFR1 amplification and TP53 mutations are common driver events in SCC, mutations in receptor tyrosine kinases (most commonly in EGFR, ALK, ROS1, and MET) are mainly responsible for the initiation of adenocarcinoma. Today, even the detection of only a set of these mutations, such as activating EGFR mutations in lung adenocarcinomas, harbor clinical importance as targeted therapeutic agents are available (3-5).

EGFR is a transmembrane receptor protein that constitutes 486 amino acids and contains 4 extracellular and 3 intracellular domains. Approximately 25% of lung adenocarcinomas harbor an EGFR mutation where most commonly exons 18, 19, 20, and 21 are affected (6). More specifically, about 90% of EGFR mutations encountered in adenocarcinoma of the lung are found in exons 19 and 21. These activating mutations are associated with sensitivity to tyrosine kinase inhibitors (TKI). Thus, the detection of such mutations is of utmost importance in NSCLCs.

There is no approved gold-standard method to detect EGFR mutations in non-small cell lung carcinoma patients, yet real-time PCR, Sanger sequencing, Pyrosequencing, and next-generation sequencing techniques are widely used. Real-time PCR and NGS are the most commonly used methods for their specificity, sensitivity, and speed. On the other hand, monoclonal antibodies directed at the mutant protein have also been developed and considered as an alternative method.

Mutation-specific antibodies are designed to detect the two most frequent mutations; the 15-base pair deletion at exon 19 (p.Glu746_Ala750del) and the L858R point mutations at exon 21 (p.Leu858Arg). Currently, four different clones are commercially available. Antibodies directed to detect the p.Glu746_Ala750del mutation have been reported to have a range sensitivity of 47-100% in the literature (7) (8, 9). Similarly, sensitivity ranges between 36% and 100% for antibodies that detect L858R (7-10). In contrast, specificity rates are around 90-100% for exon 19 and 80-100% for exon 21 (8, 11, 12).

This study aims to compare the sensitivity and specificity of these mutation-specific antibodies with that of real-time PCR. Besides, we also aim to evaluate the utility of the detection of activating EGFR mutations by immunohistochemistry in routine practice.

Material and Methods

The principles of the Helsinki Declaration conducted this study, and after obtaining ethical approval from the Hacettepe University Non-Interventional Clinical Research Ethics Committee (decision no: Go13/519-24, at 08.11.2013), it was supported as scientific research project number 1146 by the Hacettepe University Scientific Research Projects Coordination Unit.

Patient Selection

A retrospective search was conducted to select archival cases of primary lung carcinoma whose EGFR mutation analyses were carried out using real-time PCR at the Hacettepe University Department of Pathology. The search yielded 788 such cases. The digital hospital database was used to collect data for patient age, gender, biopsy/aspiration localization, the procedure used to obtain the sample, additional techniques to aid diagnosis (histochemistry and immunohistochemistry), and survival. One hundred and twenty-six cases with EGFR mutations were selected and the remaining tissue in these blocks was reviewed for sufficiency. Cases that had insufficient tissue in blocks or cases that consisted only of aspiration cytology smears without cell blocks were excluded. The biopsy and cytology samples of the remaining 47 cases were included in the study. All H&E tumor slides were reviewed, and the block previously used for molecular analysis was preferably chosen for immunohistochemical staining.

EGFR Mutation Analysis with RT-PCR

Mutation analyses were carried out as follows: 5x8-micron thick slides were prepared from the block with the tumor. QIAamp® DNA FFPE Tissue

Kit's (QIAGEN, Hilden, Germany) protocol was followed after the deparaffinization step for DNA isolation. DNA quality was assessed by running the products in an agarose gel. The Real-time PCR EntroGen EGFR Mutation analysis kit was used according to the manufacturer's instructions using the ABI StepOnePlus Real-Time PCR platform. Every assay included appropriate controls.

Immunohistochemical Detection of Mutant EGFR

Out of 126 cases chosen for the study, 47 had sufficient tissue for immunohistochemistry. Five-micron thick slides prepared from formalin-fixed paraffin-embedded samples were stained with the primary antibody directed to the protein with p.Glu746_Ala750del mutation (clone: 6B6, dilution: 1/100, Cell Signaling Technology, Boston, MA, USA) and the primary antibody directed to the protein with L858R mutation (clone: 43B2, dilution: 1/100, Cell Signaling Technology, Boston, MA, USA). The standard streptavidin-biotin procedure was followed for the staining.

Slides were reviewed at x20 for membranous and/or cytoplasmic staining, depending on the suitable staining pattern of the antibody. Strength (intensity) and the extent (percentage) of staining were recorded for each case. Staining in >10% of tumor cells was scored as 1, and <10% was scored as 0. Cases with >10% staining were re-assessed for the intensity of staining as follows: Score 1; staining as strong as the positive control (Figure IA-B), score 2; intermediate strength of staining between scores 1 and 3 (Figure IC-D), and score 3; no staining or barely discernable staining (Figure IE-F).

Statistical Analysis

Categorical variables were described with numbers and percentages, and continuous variables were noted with medians, standard deviations, and minimum and maximum values. ROC (receiver operating characteristic) analysis was implemented for inter-variable cutoff. Scores obtained by immunohistochemical staining were compared with the RT-PCR results; sensitivity, specificity, and positive and negative predictive values were calculated. The cutoff of the p-values for statistical significance was 0.05. IBM SPSS v20 statistical analysis software package was used for these analyses.

Results

The RT-PCR results of the 47 cases that underwent immunohistochemical staining were as follows: 32 (68%) had exon 19 deletion, 14 (30%) had exon 21 point mutation and 1 (2%) had exon 18 mutation.

Thirty-seven (79%) of the samples were from the lungs while 10 (21%) were from extrapulmonary sites. Twenty-two of the pulmonary samples were small biopsies, 3 were cytology material, and 12 were resections (wedge biopsy, lobectomy, or pneumectomy). Extrapulmonary sites included 3 (7%) liver biopsies, 2 (4%) pleural biopsies, 2 (4%) lymph node biopsies, 2 (2%) soft tissue and one (2%) brain excision. Twenty-seven (57%) of the immune-stained cases were received and processed in our lab. Three of them were cell blocks prepared during the adequacy assessment in the sampling process. The remaining 20 cases (43%) were received and processed in other labs and were sent to our lab for consultation.

Table I. Staining intensity of cases with exon 19 mutation-specific immunohistochemistry

	Staining intensities in immunohistochemical exon 19 deletion specific antibody			
EGFR mutation status	Negative	Score 1	Score 2	Score 3
Exon 21 L858R mutation (n=14)	6 (%43)	1 (%7)	4 (%29)	3 (%21)
Exon 19 deletion (n=32)	-	6 (%19)	14 (%44)	12 (%37)
Exon 18 mutation (n=1)	-	-	-	1 (%100)

Immunostaining for the E746-A750 deletion at exon 19 identified diverse staining scores: 16 cases scored 3, 18 scored 2, and 7 scored 1; six were negative. Notably, a few cases with high staining scores also showed mutations in exons 21 and 18. Detailed distribution of mutations across different staining scores is presented in Table I.

When score 1 was set as a cut-off point for immunostaining intensity, the sensitivity of the immunohistochemistry assay for exon 19 mutations

was 100%, its specificity was 40%, its negative predictive value was 100% and its positive predictive value was 78%. Higher scores demonstrated varied sensitivity and specificity, indicating a trade-off between the two metrics (Table II)

Table II. Comparison of sensitivity, specificity, negative and positive predictive values when changing the threshold value in the study with mutation-specific antibodies for EGFR exon 19 and exon 21

Mutation specific antibody	IHC threshold value (positive)	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)
EGFR exon 19 E746-A750 deletion	≥ score 1	100	40	100	78
	≥ score 2	81	47	54	76
	score 3	38	73	35	75
EGFR exon 21 L858R point mutation	≥ score 1	93	64	95	52
	≥ score 2	93	91	97	82
	score 3	71	100	89	100

In the exon 21 L858R mutation-specific immunohistochemistry assay, staining intensity scores varied: 10 cases scored 3, six scored 2, and nine scored 1, with 22 cases testing negative. All cases scoring 3 harbored the exon 21 mutation. Among cases scoring 2, three had exon 21 mutations and three had exon 19 mutations. A case scoring 1 displayed an exon 18 mutation, while the rest had exon 19 mutations. Of the negative cases, 21 had exon 19 mutations and one had an exon 21 mutation, with one such case showing nuclear positivity and originating from an external institution (Table III).

Table III. Staining intensity of cases with exon 21 mutation-specific immunohistochemistry

	Staining intensities in immunohistochemical exon 21 mutation specific antibody			
EGFR mutation status	Negative	Score 1	Score 2	Score 3
Exon 21 L858R mutation (n=14)	1 (%7)	-	3 (%21)	10 (%72)
Exon 19 deletion (n=32)	21 (%66)	7 (%22)	3 (%12)	-
Exon 18 mutation (n=1)	-	1 (%100)	-	-

Comprehensive sensitivity, specificity, and predictive values for exon 21 immunostaining were derived for each score, highlighting the assay's diagnostic accuracy across different thresholds (Table III). A cut-off point of score 2 revealed the highest sensitivity, specificity, and negative and positive predictive values of 93, 91, 97, and 82%, respectively. (Table II).

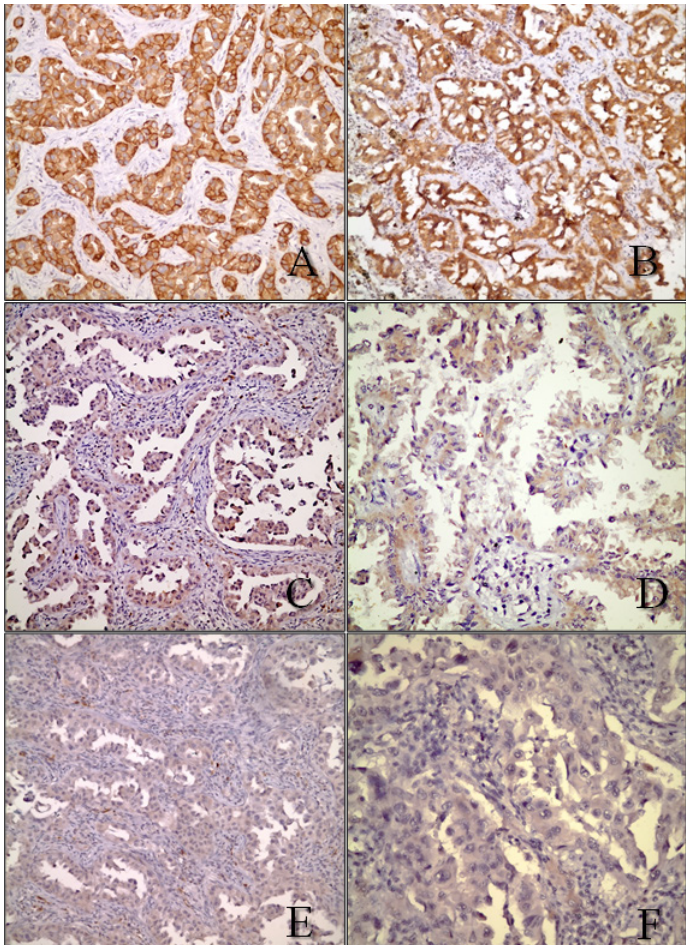

Figure I: Score 3 staining intensity in an immunohistochemical study with exon 21 mutation-specific antibody (A), Score 3 staining intensity in an immunohistochemical study with exon 19 mutation-specific antibody (B), Score 2 staining intensity in an immunohistochemical study with exon 21 mutation-specific antibody (C), Score 2 staining intensity in an immunohistochemical study with exon 19 mutation-specific antibody (D), Score 1 staining intensity in an immunohistochemical study with exon 21 mutation-specific antibody (E), Score 1 staining intensity in an immunohistochemical study with exon 19 mutation-specific antibody.

Several different staining scores were set as cut-off values in the literature. Separate ROC analyses were carried out per score to determine the ideal cut-off points. For exon 19 immunostaining, the best cut-off point was attained when scores 1 and higher were considered positive; the p-value at this point was

0.065. When scores 2 and above were considered positive in the exon 21 immunostaining, the p-value was 0.0001.

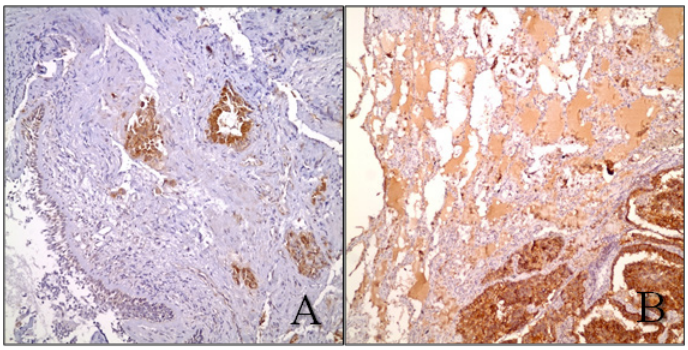

Figure II. Weak (score 1) staining in non-neoplastic bronchial epithelial cells (A), False staining in inflammatory cells and alveolar spaces (B).

A single case that harbored an exon 18 mutation revealed a score of 3 staining intensity with the exon 19 antibody and a score of 1 staining intensity with the exon 21 antibody.

In non-neoplastic tissues, both antibodies displayed weak (score 1) staining in bronchial epithelial cells, while alveolar pneumocystis were negative (Figure IIA). Weak cytoplasmic staining was occasionally encountered in alveolar macrophages, inflammatory cells, and necrotic areas (Figure IIB).

Discussion

EGFR is a transmembrane receptor tyrosine kinase involved in cell survival and development under normal conditions (13) Activating EGFR mutations are detected in 10-35% of lung adenocarcinomas (14-16). Because of the activation of the cascade, in EGFR-mutant cases, EGFR tyrosine kinase inhibitors such as erlotinib and gefitinib attain much better success in disease-free survival than carboplatin or paclitaxel (17). According to the National Comprehensive Cancer Network (NCCN) guidelines, EGFR-tyrosine kinase inhibitors are the first-line therapy in advanced, recurrent, or metastatic lung adenocarcinomas with mutant EGFR (17, 18). Therefore, EFGR mutation analysis in advanced-stage non-small cell lung carcinomas has become mandatory.

PCR-based molecular modalities for mutation detection are the most commonly implemented techniques after the discovery of the clinical significance of EGFR mutations. Most molecular techniques rely on the amplification of the mutant DNA among the wild-type DNA. In many studies, the method at hand is compared to the sequencing technique that is already known. Direct sequencing is the most common screening method; its main limitation is its low sensitivity. This method requires at least 20% of mutant DNA (19).

Macro or microdissection before DNA extraction may yield a higher tumor/non-tumor tissue ratio. These processes of sample preparation are time-consuming, labor-intensive, and require experienced/well-trained personnel. However, with its high sensitivity and easy application, RT-PCR is presently the method of choice.

As mentioned above, because EGFR mutation status depicts possible targeted therapy sensitivity, it should be determined routinely by a standard method. Yet, as most of the patients are inoperable at the time of diagnosis, small biopsies or cytology specimens are frequently the sole samples for mutation detection. Instead of techniques that require DNA isolation using a substantial amount of tissue, immunohistochemistry could be a useful method to detect mutant protein on just a single 4-um thick section.

Therefore, mutation-specific immunohistochemistry is a method that can be used to detect EFGR mutations in small samples with few tumor cells; it can be performed on intraoperative consultation slides, paraffin blocks, and even on cytology samples (cell blocks or smears). It is much less costly when compared to the other methods; results are revealed relatively faster and can be carried out in many laboratories without additional equipment. To this end, antibodies are developed to detect two of the most frequent EFGR mutations; exon 19 (15 bp, E746-A750) and exon 21 (L858R point mutation). So far, there are four commercially available mutant EGFR-specific antibody clones. These are directed at the two most frequent mutations: 6B6 and SP111 clones for exon 19 deletion at E746-A750 and 43B2 and SP125 clones for exon 21 point mutation at Leu858Arg.

In our study, the most significant ROC value for exon 19 mutation-specific immunohistochemistry was attained when the cutoff value was set at score 1, with the assay's sensitivity, specificity, and positive predictive value being 100%, 40%, and

78%, respectively. The relatively lower specificity, compared to that reported in the literature, can be attributed to the use of exon 21 or exon 18 mutant cases as negative controls, potentially leading to cross-reactivity with the exon 19 mutation-specific antibody. Despite the high specificity (98.8%) and sensitivity (100%) for detecting the 15-bp deletion in exon 19 (8, 11, 20, 21), the sensitivity for detecting various 3-8 amino acid deletions distinct from the frequent 15bp/5AA E746-A750 alteration drops to 20-67% (22, 23). According to the Catalog of Somatic Mutations in Cancer (COSMIC), these less common deletions constitute 35% of all deletions in exon 19, emphasizing the need for comprehensive molecular testing for samples that test negative with E746-A750 deletion-specific IHC to ensure no other mutations are missed. This approach aligns with the latest CAP guidelines which recommend extending molecular screenings beyond the two most frequent alterations to ensure accurate diagnosis and appropriate therapy initiation based on reliable IHC results (24).

In exon 21 L858R mutation-specific immunohistochemistry, the sensitivity and specificity values are found to be high when scores 2 and 3 are considered positive. In the present study, the sensitivity, specificity, and positive predictive values for a score 2 cutoff are 92%, 91%, and 91% respectively, showing a strong similarity to the 'perfect test' in ROC analyses with a p-value of 0.0001. Despite this high accuracy, there was a case where RT-PCR detected an exon 21 mutation that the immunohistochemistry failed to reveal, displaying only nuclear positivity and considered negative due to the criteria for positivity being membranous and/or cytoplasmic staining. This discrepancy could result from a fixation or processing artifact since the sample was processed outside our institution. Immunohistochemistry directed at the exon 21 L858R point mutation is noted to be more sensitive and specific than that for exon 19 E746-A750(8, 12, 20, 22, 25). The monoclonal antibody for exon 21 only detects the L858R mutation and misses L861Q(11), another alteration, but as per the COSMIC database, over 90% of exon 21 mutations are L858R, ensuring the antibody's effectiveness for most clinical scenarios. Therefore, cases that test positive with this antibody can typically commence tyrosine kinase inhibitor therapy without further molecular confirmation; however, negative results necessitate additional molecular diagnostics to rule out rare mutations. Moreover, the challenge of detecting less common mutations, as seen in exon 21 cases, highlights broader issues in mutation-specific testing that extend to challenges in intratumoral heterogeneity.

Intratumoral heterogeneity is defined as the presence of variable morphological and phenotypical features in different tumor cells of the same tumor. Intratumoral heterogeneity concerning EGFR mutations is a controversial issue. Some studies report up to 13% intratumoral heterogeneity of EGFR status (26). Other studies maintain that such data results from methodological disparities (27). In the present study, we had 14 cases with an extent of staining less than 90%. The areas that are devoid of EGFR expression and the positive areas do not display any morphological difference. As immunohistochemistry allows for wider areas of tissue for assessment, theoretically, mutant protein detection can be carried out with higher sensitivity and thus be less susceptible to intratumoral heterogeneity. However, at least in some cases, the possibility of staining heterogeneity being due to fixation and processing artifacts can never be fully ruled out.

Three cases included in the study had samples of cell blocks prepared in specimen adequacy assessment. Two of these cases had exon 19 and one had exon 21 mutations. All three displayed immunohistochemical positivity. Specimen adequacy assessment during sampling increases the diagnostic value of the biopsy procedure while attaining samples for further use in research or diagnostics (28). As mentioned in the literature and the CAP molecular guideline, EGFR mutation assessment cell blocks are preferred over smears (29). Immunohistochemical assays similarly yield better results with cell blocks (30). The present study does not include smear preparations so such a comparison was not attempted. Understanding all of these variations is crucial, especially when considering the overall reliability of diagnostic methods and the current guidelines that influence clinical decision-making.

If we overlook disadvantages such as the small number of patients and the absence of a true

negative group in our study, we observed that mutation-specific antibodies yield varying levels of sensitivity and specificity at different staining intensities. Furthermore, the reproducibility of such scoring assessments in routine pathological practice can be low due to variations in laboratory conditions, such as changes in the technician performing the immunohistochemistry. Current guidelines generally do not recommend the use of EGFR IHC for testing the presence of EGFR mutations due to these types of variability and the differences discussed above, like base pair differences. Despite its utility for certain molecular targets like ALK, ROS, BRAF, and PD-L1 (31-33), the role of EGFR-specific IHC is diminishing. This shift is due to the superior accuracy of newer sequencing technologies that can analyze even single cells. Given the variations in test results and reproducibility issues under different laboratory conditions, current guidelines advise against using EGFR IHC for detecting EGFR mutations. As such, we advocate for a transition to advanced genomic testing methods that provide greater precision and reliability, ensuring that our diagnostic strategies evolve to deliver the best patient outcomes.

Acknowledgment

I would like to express my gratitude to the technicians at Hacettepe University Pathology Laboratory who contributed to this study. I would also like to extend my thanks to Dr. Güneş Güner and Dr. İbrahim Kulaç for their assistance during the writing of this article.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Population Based: Evaluation of Femoroacetabular Impingement

Popülasyon Temelli: Femoroasetabular Impingement Değerlendirilmesi

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1339302
Geliş Tarihi | Received: 08.08.2023
Kabul Tarihi | Accepted: 20.03.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Balcı N, Kavak RP, Tıkman M, et al Population Based: Evaluation of Femoroacetabular Impingement. Hitit Medical Journal 2024;6(2):126-135. https://doi.org/10.52827/hititmedj.1339302.

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Ankara Bilkent Şehir Hastanesi 2 Nolu Klinik Araştırmalar Etik Kurulundan 1/3/2023 tarihinde onay alınmıştır (No: E2-23-3476).
İntihal Kontrolleri: Evet (intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: NB, RPK, MT, NK, SK, BT, SDK Tasarım: NB, RPK, MT, NK, SK, BT, SDK Veri Toplama/Veri İşleme: RPK, MT, NK, SK, BT, SDK Veri Analizi: NB, RPK, BT, SDK Makalenin Hazırlanması: NB, RPK
Hasta Onamı: Hasta onamı alınmasına gerek yoktur.
Finansal Destek: Bu çalışma ile ilgili herhangi bir finansal kaynaktan yararlanılmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Ethical approval was received from Ankara Bilkent City Hospital No. 2 Clinical Research Ethics Committee on 1/3/2023 (No: E2-23-3476).
Plagiarism Check: Yes (intihal.net)
Conflict of Interest: The authors declared that, there are no conflicts of interest.
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: NB, RPK, MT, NK, SK, BT, SDK Design: NB, RPK, MT, NK, SK, BT, SDK Data Collection/ Data Processing: RPK, MT, NK, SK, BT, SDK Data Analysis: NB, RPK, BT, SDK Article Preparation: NB, RPK
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

Population Based: Evaluation of Femoroacetabular Impingement

ABSTRACT

Objective: Femoroacetabular impingement is a dysfunction of the hip joint with the potential to cause early hip osteoarthritis. The aim of this study was to examine the Alpha Angle and Femoral Neck-Head Offset measurements of patients who underwent pelvic Computed Tomography examination for any indication with radiologists.

Material and Method: The results of 1,782 right and left hip joints of 891 individuals aged 18-45 years who underwent pelvic computed tomography examination (with or without contrast) for various reasons were evaluated retrospectively. Alpha Angle and Femoral Neck-Head Offset measurements were performed on computed tomography sections. In this study, an Alpha Angle of 55° or more and a Femoral Neck-Head Offset distance of less than 8 mm were considered abnormal.

Results: A total of 891 individuals, 758 (85.1%) males and 133 (14.9%) females, were included in this study. The mean age of the individuals was 24.65 ± 6.01 years. The mean right Alpha Angle was 45.42 ± 4.4 (min 38.00, max 62.00), the mean left Alpha Angle was 46.65 ± 5.1 (min 38.00, max 72.10), the mean right Femoral Neck-Head Offset was 10.21 ± 0.02 (min 6.2, max 11.4), and the mean left Femoral Neck-Head Offset was 10.57 ± 0.01 (min 5.6, max 12.0). The total number of individuals with Alpha Angle ≥ 55° in both hip joints was 37, 33 of whom were male and 4 were female. There were no individuals with Femoral Neck-Head Offset < 8 mm in both hip joints.

Conclusion: Imaging features known to be associated with femoroacetabular impingement are seen in asymptomatic adult hip joints, particularly in males. If anthropometric measurements are outside normal limits in asymptomatic young male and female individuals, clinical correlation of the findings of these individuals should be recommended.

Keywords: Alpha angle, femoroacetabular ımpingiment, femoral neck-head offset, hip joint, population, radiologic ımaging, young adult.

ÖZET

Amaç: Femoroasetabular sıkışma, erken kalça osteoartriti oluşturma potansiyeli bulunan kalça ekleminin disfonksiyonudur. Bu çalışma ile herhangi bir endikasyonla pelvik Bilgisayarlı Tomografi incelemesi yapılmış olan hastaların Alfa Açısı ve Femur Boyun-Baş Offset ölçümlerinin radyoloji uzmanları ile birlikte incelenmesi amaçlandı.

Gereç ve Yöntem: 18-45 yaş arası çeşitli nedenlerle pelvik Bilgisayarlı Tomografi incelemesi (kontrastlı veya kontrastsız) yapılmış olan 891 bireyin sağ ve sol 1.782 kalça eklemine ait sonuçları retrospektif olarak değerlendirildi. Bilgisayarlı tomografi kesitlerinde Alfa Açısı ve Femur Boyun-Baş Offset ölçümleri yapıldı. Bu çalışmada, Alfa Açısının 55° ve üzerinde olması, Femur Boyun-Baş Offset mesafesinin 8 mm'den küçük olan ölçüm değerleri anormal olarak değerlendirildi.

Bulgular: Bu çalışmada 758'i (%85,1) erkek ve 133'ü (%14,9) kadın olmak üzere toplam 891 birey dahil edildi. Bireylerin ortalama yaşı 24,65 ± 6,01 idi. Çalışmaya dahil edilen tüm bireylerin sağ Alfa Açısı ortalaması 45,42 ± 4,4 (min 38,00, maks 62,00), sol Alfa Açısı ortalaması 46,65 ± 5,1 (min 38,00, maks 72,10), sağ Femur Boyun-Baş Offset ortalaması 10,21±0,02 (min 6,2, maks 11,4), sol Femur Boyun-Baş Offset ortalaması 10,57 ± 0,01 (min 5,6, maks 12,0) idi. Her iki kalça ekleminde Alfa Açısı ≥ 55°bulunan birey sayısı toplam 37 olup bu bireylerin 33'ü erkek, 4'ü kadın idi. Her iki kalça ekleminde Femur Boyun-Baş Offset < 8 mm bulunan birey saptanmadı.

Sonuç: Femoroasetabular sıkışma ile ilişkili olduğu bilinen görüntüleme özellikleri özellikle erkek bireyler olmak üzere asemptomatik yetişkin kalça eklemlerinde görülmektedir. Asemptomatik genç erkek ve kadın bireylerde antropometrik ölçümlerin normal sınırlar dışında olması halinde bu bireylerin bulgularına yönelik klinik korelasyonu önerilmelidir.

Anahtar Sözcükler: Alfa açısı, femoroasetabular sıkışma, femur boyun-baş offset, genç erişkin birey, kalça eklemi, popülasyon, radyolojik görüntüleme.

Giriş

Femoroasetabular Sıkışma (FAS), genç erişkinlerde görülen kalça ve/veya kasık ağrısının nedenleri arasında yer almakta olup bu antite femur başı ve boynu ile asetabulum kenarı arasındaki anatomik-mekanik ilişkinin bozulması ile ortaya çıkan kalça ekleminin disfonksiyonudur (1-3). Femur başı ve asetabular malformasyonlar kalça ekleminin anormal temasına neden olarak kronik kalça rahatsızlığı ortaya çıkarır (4). FAS, kalça ekleminde ilerleyici kıkırdak dejenerasyonu ile erken kalça osteoartriti (OA) oluşturma potansiyeline sahiptir (2-6). Klinik olarak hastanın öyküsünde kalça ağrısı en sık görülen yakınmadır (3).

FAS'ın belirlenmesinde radyolojik görüntüleme yöntemleri ile kalça ekleminin ve femur başının morfometrik yapısının ve patolojilerin değerlendirilmesi, varyasyonların belirlenmesi için doğru görüntüleme yöntemine başvurulması oldukça önemlidir (7). Radyolojik değerlendirmede tanıyı desteklemek için anatomik yapılar arasındaki çeşitli ölçümlerden [Alfa açısı (AA), femur baş-boyun offseti (FBBO), anterior femoral uzaklık, asetabular derinlik, asetabular versiyon açısı, merkez kenar açısı, '8 işareti' pozitifliği gibi] faydalanılması önerilir. AA ve FBBO; FAS'ın tanısı ve tiplendirilmesi için kullanılan yararlı ve pratik kantitatif parametrelerdir (8, 9).

FAS, bireylerin günlük aktivitelerini etkileyerek yaşam kalitesini düşürür (10, 11). Hem yaşam kalitesinin sağlanması hem de zaman içinde gelişebilecek risklere karşı bireylerin erken ve doğru tanı alması, klinik sürecin uygun yönetimi gereklidir. Hekimlerin, FAS ile ilgili ölçüm parametrelerinin normal değerlerine aşina olması özellikle de ergen ve genç bireylerin klinik sürecinin en uygun şekilde yönetilmesine katkı sağlayabilir.(9, 12).

Genel popülasyonda FAS morfolojisinin yaygınlığına ilişkin tahminler, kullanılan FAS için örneklenen popülasyonlarda önemli heterojenlik nedeniyle büyük farklılıklar gösterebilmektedir (13). Ayrıca, Asya toplumunda yapılmış çalışma sayısının düşük olduğu belirtilmektedir (14).

Bu nedenlerle asemptomatik olan bireylerde anatomik malformasyonların görülebileceği, FAS'ın preosteoartritik olarak kabul görmesi nedeniyle erken tanı almasının sağlanmasına yönelik tüm klinisyenlerin farkındalığının artırılmasının toplum temelli FAS'ın değerlendirilmesi açısından önemli olduğunu düşünmekteyiz. FAS için anatomik ölçümleri değerlendirmek amacıyla BT en sensitif radyolojik inceleme yöntemi olarak bilinmektedir (15). Bu nedenle çalışmamız, anatomik ölçümlerin değerlendirilmesi açısından deneyimli olan radyoloji uzmanları ile birlikte yapılmıştır.

Bu çalışmada, radyolojik tetkik istem nedenleri arasında kalça ağrısı şikâyeti ile FAS ön tanısı bildirilmemiş olan hastaların haricinde herhangi bir endikasyonla pelvik Bilgisayarlı Tomografi (BT) incelemesi yapılmış olan hastaların AA ve FBBO ölçümlerinin radyoloji uzmanları ile birlikte incelenmesi amaçlandı.

Gereç ve Yöntemler

Örneklem Seçimi

Bu çalışma retrospektif kesitsel bir çalışmadır. Lokal hastane etik kurulundan izin (1.03.2023/E2-23-3476) alındıktan sonra, 3. Basamak Eğitim ve Araştırma Hastanesi Radyoloji Anabilim Dalında 01.01.2015 ve 01.12.2022 yılları arasında 18-45 yaş arası çeşitli nedenlerle (nefrolitiazis, ürolitiazis, karın ağrısı ve nedeni bilinmeyen ateş) pelvik BT incelemesi (kontrastlı veya kontrastsız) yapılmış olan 891 bireyin sonuçları retrospektif olarak değerlendirildi. BT kesitlerinde AA ve FBBO ölçümleri yapıldı.

Gelişimsel ve/veya çocukluk çağı kalça hastalıkları veya deformitesi, kalça operasyonuna ait bulguları olan, travma hikayesi olan, ölçümlerin doğruluğunu etkileyen tümöral patolojisi veya metastaz olanlar, BT incelemesi optimum olmayan hastalar çalışma dışında bırakıldı. Çalışmaya toplamda 891 bireyin sağ ve sol 1.782 kalça eklemine ait sonuçlar dâhil edildi.

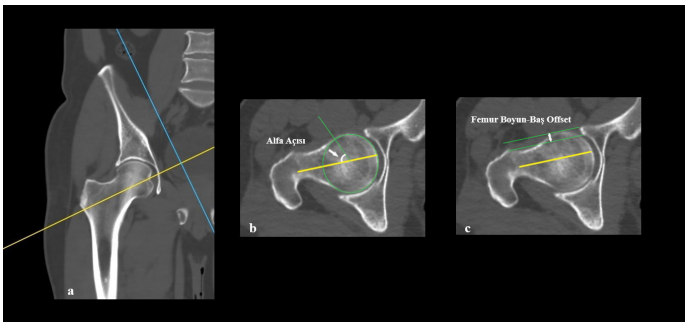
BT Protokolü

Tüm pelvik BT incelemeleri 128-kesit BT (Optima CT660, General Electric Healthcare Systems, Milwaukee, USA) ile yapıldı. Çalışmaya dahil edilen kalça eklemine ait kemik dozunda kesitsel görüntüler, femurun proksimalini anterosuperior açıdan görecek şekilde BT'nin oblik aksiyel kesiti radyoloji uzmanı tarafından reformatlanarak değerlendirildi.

Radyolojik Değerlendirme ve Analiz

BT'den elde edilen kesitsel görüntülerde 891 sağ ve sol kalça eklemi olmak üzere toplam 1.782 kalça ekleminin AA'sı ve FBBO'su ortopedi ve travmatoloji

uzmanı ile radyoloji uzmanı tarafından konsensus birliğinde ölçüm yapıldı.



Şekil I. Bilgisayarlı tomografi kesitlerinde, Alfa Açısı ve Femur Boyun Baş Offset Ölçümü.
a, Ölçümlerin yapılması için referans düzlem belirlendi. b, Alfa Açısı; femur başının oluşturduğu dairenin (Yeşil çizgi ile çizilen daire) yarıçapını oluşturan ve femur başının ortasından geçen yeşil çizgi ile femur boynunun merkez ekseninden geçen sarı çizginin kesişiminden elde edilen açı ölçüldü. c, Femur Boyun Baş Offset; femur boynunun anterior korteksinden geçen longitudinal çizgi ile femur baş-boyun bileşkesinde konturun en belirgin olduğu yer arasında kalan mesafe ölçüldü. (İki yeşil çizgi arasında kalan mesafe)

AA; femur boynunun ortası ile femur başının merkezini birleştiren longitudinal bir çizgi ile femur başının merkezinden femur başının anteriorda sferik şeklini kaybettiği noktaya çekilen çizgi arasında kalan açıdır (16). FAS'a atıfta bulunulan ortak kabul gören veya standartlaştırılmış AA değerinin halen tartışmalı olduğu söylenebilir (10, 17). Bir bireyin AA değerinin 42°'nin altında olması normal bir birey için kabul edilen ve yaygın olarak kullanılan referans değeridir (10). Kam tipi FAS morfolojisi için ise genellikle 55° ile 60°'den büyük olan AA yaygın olarak kullanılan ölçüttür (10, 17). AA'nın kantitatif değerinin açısal ölçü birimi "derece (°)" olarak belirtildi. Bu çalışmada 55° ve üzerinde olması anormal olarak değerlendirildi. FBBO veya diğer ifade ediliş biçimi olan "Anterior femoral mesafe", femur boynu anterior korteksinden geçen longitudinal çizgi ile femur baş-boyun bileşkesinde konturun en belirgin olduğu yer arasındaki mesafedir (18). Bu çalışmada bu mesafe milimetre (mm) olarak ölçüldü ve 8 mm'den küçük olan ölçüm değerleri anormal olarak değerlendirildi (Şekil 1 a,b,c).

İstatistiksel Analiz

Tanımlayıcı istatistikler vaka sayısı (n), yüzde (%), ortalama ± standart sapma ($\bar{x} \pm ss$) olarak belirtildi. Kategorik ve demografik veriler vaka sayısı (n) ve yüzde (%) olarak tablolar halinde verildi. Elde edilen verilerin dağılımı Shapiro-Wilk Testi ile değerlendirildi. Verilerin dağılım sonuçlarına göre; ikili grupların karşılaştırılması Mann Whitney-U Testi ile yapıldı. Tüm analizler, istatistik paket programı [Statistical Package for the Social Sciences (SPSS), sürüm 24.0, sürüm 2008) kullanılarak yapıldı. $p<0.05$ değeri istatistiksel olarak anlamlı kabul edildi.

Bulgular

Bu çalışmada 758'i (%85,1) erkek ve 133'ü (%14,9) kadın olmak üzere toplam 891 birey dahil edildi. Bireylerin ortalama yaşı 24,65 ± 6,01 idi. Erkeklerin (ortalama 24,72 ± 5,8) ve kadınların (ortalama 24,26 ± 6,6) yaşları arasında istatistiki olarak anlamlı bir fark yoktu.

Çalışmaya dahil edilen tüm bireylerin (n:891) AA (°) ortalamaları; sağ AA ortalaması 45,42±4,4 (min 38,00, maks 62,00), sol AA ortalaması 46,65 ± 5,1 (min 38,00, maks 72,10) idi.

Tablo I. Tüm bireylerin kalça eklemi lokalizasyonuna göre Alfa Açısı ve Femur Boyun-Baş Offset ortalama değerlerinin dağılımı

Birey Sayısı (n)	Lokalizasyon AA	Ortalama ± ss (°)	Lokalizasyon FBBO	Ortalama ±ss (mm)
Tüm Bireyler (n=891)	Sağ kalça	45,42 ± 4,4	Sağ kalça	10,21 ± 0,02
	Sol kalça	46,65 ± 5,1	Sol kalça	10,57 ± 0,01

AA: Alfa Açısı, FBBO: Femur Boyun-Baş Offset. n: sayı. ss: Standart Sapma. mm: milimetre. (°): derece

Tüm bireylerin (n:891) FBBO (mm) ölçüm sonuçlarına ait ortalamalar; sağ FBBO ortalaması 10,21±0,02 (min 6,2, maks 11,4), sol FBBO ortalaması 10,57 ± 0,01 (min 5,6, maks 12,0) idi (Tablo I).

Cinsiyete göre AA ortalamaları; erkeklerde sağ AA ortalaması 46,11 ± 3,7 ve sol AA ortalaması 47,22 ± 4,4, kadınlarda sağ AA ortalaması 41,49 ± 5,4 ve sol AA ortalaması 43,39 ± 7,0 olarak tespit edildi. Cinsiyete göre FBBO (mm) ortalamaları; erkeklerde sağ FBBO ortalaması 10,24 ± 0,59 ve sol FBBO ortalaması 10,60±0,46, kadınlarda sağ FBBO ortalaması 10,03 ± 0,80 ve sol FBBO ortalaması 10,40 ± 0,76 olarak tespit edildi. Erkeklerin AA ortalamaları ile kadınların AA ortalamaları arasında istatistiksel olarak anlamlı bir fark olduğu gözlenmiştir (Sağ kalça U=12683,500,

p<0.05 ve Sol kalça U=11579,500, *p<0.05*) Erkeklerin FBBO ortalamaları ile kadınların FBBO ortalamaları arasında istatistiksel olarak anlamlı bir fark olduğu gözlenmiştir (Sağ kalça U=43785,500, *p<0.05* ve Sol kalça U=41159,500, *p<0.05*) (Tablo II).

Tablo II. Cinsiyete göre Alfa Açısı ve Femur Boyun-Baş Offset ortalama değerlerinin kalça eklemi lokalizasyon dağılımı

Cinsiyete göre (n)	**Lokalizasyon AA**	**Ortalama ±ss (°)**	**U***	***p****
Erkek (n=758)	Sağ kalça	46,11 ± 3,7	12683,500	*0.000*
	Sol kalça	47,22 ± 4,4	11579,500	*0.000*
Kadın (n=133)	Sağ kalça	41,49 ± 5,4	12683,500	*0.000*
	Sol kalça	43,39 ± 7,0	11579,500	*0.000*
Cinsiyete göre (n)	**Lokalizasyon FBBO**	**Ortalama ±ss (mm)**	**U***	*p**
Erkek (n=758)	Sağ kalça	10,24±0,59	43785,500	*0.015*
	Sol kalça	10,60±0,46	41159,500	*0.001*
Kadın (n=133)	Sağ kalça	10,03±0,80	43785,500	*0.015*
	Sol kalça	10,40±0,76	41159,500	*0.001*

AA: Alfa Açısı, FBBO: Femur Boyun-Baş Offset. Alfa Açısının 55° ve üzerinde olması,
Femur Boyun-Baş Offset mesafesinin 8 mm'den küçük olan ölçüm değerleri anormal olarak kabul edildi.
n: sayı ss: Standart Sapma mm: milimetre. (°): derece.
*Mann Whitney-U Testi, U: Testin U değeri. *p<0.05* değeri istatistiksel olarak anlamlı kabul edildi.

AA ≥ 55°olan dağılımlar tek taraflı olarak incelendiğinde; sağ kalça için 68 (%7,6) birey , sol kalça için 86 (%9,7) birey olarak saptandı. Cinsiyete göre AA ≥55° olan dağılımlar ise; sağ kalça için 13 (%9,8) kadın, 55 (%7,3) erkek, sol kalça için ise 13 (%9,8) kadın, 73 (%9,6) erkek idi. Tüm bireylerin tek kalçada FBBO değerine göre dağılımı incelendiğinde; FBBO < 8 mm olan birey sağ kalça için 15 (%1,7), sol kalça için 4 (%0,4) olarak saptandı. Sağ kalça için; FBBO < 8 mm olanların 6'sı (%4,5) kadın iken 9'u (%1,2) erkek, sol kalça için ise 2'si (%1,5) kadın, 2'si (%0,3) erkek idi (Tablo III).

Her iki kalça ekleminde AA ≥ 55° ve FBBO <8 mm bulunan birey saptanmadı ancak AA ≥ 55° ve sağ FBBO <8 mm olan bireyler 4 kadındı ve 2'si 20 yaşında, 1'i 18 yaşında ve 1'i de 19 yaşında idi.

Tartışma

FAS, genellikle 20-45 yaş arası genç aktif kişilerde görülmektedir. Ancak kam tipi 20-30 yaş erkeklerde ve pincer tipi ise orta yaşlı kadınlarda daha sık görülmektedir (19, 20). Çalışmamızda; her iki kalça ekleminde AA ≥ 55°bulunan bireylerin yaşlarına göre dağılımlarına bakıldığında; 20 yaş altında olan bireylerin hepsi erkek olup 4 kişi idi. 20 yaş ve üstünde ise; 4 kadın, 29 erkek olduğu tespit edildi. Her iki kalça ekleminde FBBO <8 mm bulunan birey

Tablo III. Radyolojik ölçümlerin cinsiyete ve lokalizasyona göre sayısal dağılımı

Lokalizasyon	**Tüm Bireyler Ölçüm Dağılımı**	**n**	**%**	**Cinsiyet**	**Cinsiyete Göre Ölçüm Dağılımı**	**n**	**%**
Sağ kalça	AA < 55°	823	92,4	**Kadın**	AA < 55°	120	90,2
				Erkek		703	92,7
	AA ≥ 55°	68	7,6	**Kadın**	AA ≥ 55°	13	9,8
				Erkek		55	7,3
Sol kalça	AA < 55°	805	90,3	**Kadın**	AA < 55°	120	90,2
				Erkek		685	90,4
	AA ≥ 55°	86	9,7	**Kadın**	AA ≥ 55°	13	9,8
				Erkek		73	9,6
Sağ kalça	FBBO < 8 mm	15	1,7	**Kadın**	FBBO < 8 mm	6	4,5
				Erkek		9	1,2
	FBBO ≥ 8 mm	876	98,3	**Kadın**	FBBO ≥ 8 mm	127	95,5
				Erkek		749	98,8
Sol kalça	FBBO < 8 mm	4	0,4	**Kadın**	FBBO < 8 mm	2	1,5
				Erkek		2	0,3
	FBBO ≥ 8 mm	887	99,6	**Kadın**	FBBO ≥ 8 mm	131	98,5
				Erkek		756	99,7

AA: Alfa Açısı, FBBO: Femur Boyun-Baş Offset. n: sayı %: Yüzde. mm: milimetre. (°): derece.

saptanmadı. AA ≥ 55° ve FBBO < 8 mm bulunan birey saptanmadı ancak AA ≥ 55° ve sağ FBBO < 8 mm olan bireyler 4 kadındı ve 2'si 20 yaşında, 1'i 18 yaşında ve 1'i de 19 yaşında idi. Elde ettiğimiz bu sonuçlar, genç yaşlarda asemptomatik olan bireylerde herhangi bir şikâyet olmasa bile kalça ekleminde başlayan eklem değişikliklerinin FAS'ı işaret edebileceğini anımsatır. FAS'ın prevalansının %10-15 arasında değiştiği bildirilmiştir. Genç yaşta kalça OA'sına zemin hazırlayan FAS, total kalça artroplastisi açısından risk taşıması nedeniyle oldukça önemlidir (21-23).

Çalışmamızda, radyolojik tetkik istem nedenleri arasında kalça ağrısı şikâyeti ile FAS ön tanısı ile istem yapılmamış olan hastaların haricinde herhangi bir nedenle pelvik BT incelemesi yapılmış olan hastaların AA ve FBBO ölçümleri değerlendirildi. Değerlendirilen bireylerin ortalama yaşı 24,65±6,01 idi. Erkeklerin (ortalama 24,72 ± 5,8) ve kadınların (ortalama 24,26 ± 6,6) yaşları arasında istatistiki olarak anlamlı bir fark yoktu. Çalışmamızın sonuçlarında elde edilen yaş aralıkları ve yaş ortalamaları literatürde yer alan çalışmaların sonuçları ile benzer bulundu.

Çalışmamıza dahil edilen tüm bireylerin; sağ AA ortalaması 45,42 ± 4,4 (min 38,00, maks 62,00), sol AA ortalaması 46,65 ± 5,1 (min 38,00, maks 72,10), sağ FBBO ortalaması 10,21±0,02 (min 6,2, maks 11,4), sol FBBO ortalaması 10,57 ± 0,01 (min 5,6, maks 12,0) idi. Çalışmamızda; AA'nın sınır değeri olan 55°'e göre tüm bireylerin değerlendirilmesi ile AA değeri ≥ 55°olan birey sağ kalça için 68 (%7,6), sol kalça için 86 (%9,7) olarak saptandı. Cinsiyete göre AA ≥ 55° olan dağılımlar ise; sağ kalça için 13 (%9,8) kadın, 55 (%7,3) erkek, sol kalça için ise 13 (%9,8) kadın, 73 (%9,6) erkek idi. Tüm bireylerin FBBO değeri incelendiğinde; FBBO < 8 mm olan birey sağ kalça için 15 (%1,7), sol kalça için 4 (%0,4) iken cinsiyete göre dağılımları ise, sağ kalça için FBBO < 8 mm olan 6 (%4,5) kadın olup 9 (%1,2) erkek, sol kalça için ise 2 (%1,5) kadın, 2 (%0,3) erkek idi.

Kang ve ark'nın (24) yaptığı bir çalışmada; kalça ile ilgili şikâyeti olmayan yaşları 15 ile 40 arasında değişen 50 bireyin bilateral kalça eklemi incelenmiş ve eklemlerin %39'unun (kadın eklemlerinin %31'i, erkek eklemlerinin %48'i) femoroasetabular sıkışmaya zemin hazırlayan en az bir morfolojik yönü olduğu gösterilmiştir. Çalışmada, mevcut literatürde yer alan FAS için kullanılan ölçüm parametrelerine göre asemptomatik bireylerde femoroasetabular sıkışmaya yatkınlık oluşturan değişikliklerin önemli ölçüde yaygın olduğu vurgulanmıştır. Değerlendirilen bireylerin ortalama AA değeri 45,57° (min= 30°, max =70°), ortalama FBBO 9,49 mm (min=6,2 mm, max=14,7 mm), FBBO değer ≤ 8mm olan 12 kalça (%12) olduğu tespit edilmiştir. Kim ve ark. (11) tarafından FAS ile ilişkili olduğu düşünülen BT görüntüleme özelliklerine dair 18-40 yaş arasında asemptomatik erişkin bireyin 473 kalça ekleminin değerlendirildiği çalışmada; ortalama AA'nın erkeklerde 48,0°, kadınlarda 45,6° ve ortalama FBBO'nun erkeklerde 10,6 mm ve kadınlarda 10,2 mm ölçüldüğü bildirilmiştir. 292 erkek kalça ekleminin 59'unda (%20,2) ve 181 kadın kalça ekleminin 26'sında (%14,4) anormal bir alfa açısı (> 55°) bulunduğu ifade edilmiştir. 292 erkek kalça ekleminin 33'ünde (%11,3) ve 181 kadın kalça ekleminin 15'inde (%8,3) anormal FBBO (<8 mm) tespit edildiği ifade edilmiştir. Bu çalışmanın sonucunda; FAS ile ilişkili olduğu bilinen görüntüleme özelliklerinin özellikle erkek bireyler olmak üzere asemptomatik yetişkin kalça eklemlerinde yaygın olduğu vurgulanmıştır. Aytekin (25) tarafından yapılan bir çalışmada; 114 asemptomatik bireye ait 228 kalça ekleminde kam tipi FAS tanısına yönelik olarak kullanılan radyolojik ölçüm sonucu incelenmiş olup AA ortalaması 45,420 ± 0,61 ve FBBO ortalaması 10,5 mm olarak tespit edilmiştir. Tüm kalça eklemlerinin %14,4'ünde yüksek AA ve %6,5'inde düşük FBBO bulunduğu, toplumda radyolojik olarak kam tipi FAS ile uyumlu ancak asemptomatik bireylerin olabileceği ifade edilmiştir. Hack ve ark.'nın (26) asemptomatik 200 bireyde manyetik rezonans görüntüleme ile her iki kalçayı da hedefleyen incelemede; bireylerin %79'u beyaz, %55,5'i kadın olup tüm bireylerin yaş ortalaması 29,4 (min 21,4, maks 50,6) olarak tespit edilmiştir. AA değeri kam morfolojisi için >50,5° pozitif olarak kabul edilmiş ve tüm bireylerin %14' ünde kam morfolojisine sahip en az bir kalça eklem mevcut iken bunların %10,5' inde sağ veya sol tarafta AA'nın yüksek olduğu, bireylerin %3,5'inde ise her iki kalçada deformite olduğu, AA'sı yüksek olan 28 bireyin %79'u erkek, %21'i kadın olduğu tespit edilmiştir. Bizim çalışma sonuçlarına göre; AA değeri ≥ 55°olan birey sağ kalça için 68 (%7,6) iken sol kalça için 86 (%9,7) olarak saptandı. Cinsiyete dağılımında ise erkek bireylerde daha fazla görüldüğü tespit edildi. Gosvig ve ark.'nın (27), 3202

(1184 erkek, 2018 kadın) bireyin standardize AP pelvik grafilerini değerlendirdikleri çalışmada; kam tipi deformite dağılımında cinsiyete bağlı belirgin bir fark tespit edilmiş olup kam deformitesinin genel prevalansının erkeklerde yaklaşık %17 ve kadınlarda %4 olduğu bildirilmiştir. Bizim çalışmamızda da literatürle uyumlu olarak normal sınırlardan sapma gösteren bulgular erkek bireylerde daha fazladır.

Leunig ve ark.'nın (28) yaptıkları çalışmada yaş ortalaması 19,3 olan 80 asemptomatik kadının MRG sonuçları incelendi ve kam tipi deformitelere dair bazı kanıtlar 15 bireyde saptandı. Kadınlarda kam tipi deformiteler erkeklere göre nadir olmasına rağmen artmış asetabular derinlik prevalansının daha yüksek olduğu ve bunun da cinsiyete bağlı farklı biyomekanik mekanizmalar ile FAS'a yol açma olasılığını düşündürdüğü yazarlar tarafından vurgulanmıştır. Sutter ve ark. (17) sağlıklı ve hasta bireylerin AA ölçümlerinin tanıda ayırt edici olmadığını, AA'nın spesifikliğinin düşük olduğunu ve eşik değerin 60°'ye çıkarılmasının daha uygun olabileceği yönünde önerilerini sunmuşlardır. Chakraverty (29) tarafından yapılan değerlendirme ile literatür yer alan mevcut eşik değerlerin düşük olabileceği ve bu değerlerin tekrar belirlenmesi gerektiğine dair görüş bildirilmiştir. Lepage-Saucier ve ark. (30) ise FAS tanısında kullanılan parametrelerin yeniden değerlendirilmesi gerekebileceğini vurgulamışlardır.

Zhou ve ark.'nın (23) kalça ağrısı olan erkek ve kadın hastalarda FAS ile uyumlu olan radyografik bulguları değerlendirdikleri çalışmada; genel prevalans sırasıyla %61,1 ve %60,2 olarak tespit edilmiştir. Mikst tipin en yaygın olduğu, erkek hastalarda kam tipi deformite prevalansı daha yüksek iken kadın hastalarda ise kerpeten tipi deformite prevalansı daha yüksek olduğu bulunmuştur. Kam tipi FAS için ise en yaygın bulunan radyografik ölçümün >55° olan AA olduğu ifade edilmiştir.

Gosvig ve ark.'nın (31) genel popülasyon temelli 3620 kalça ekleminde anatomik malformasyonları inceledikleri çalışmada; erkek ve kadınlarda tespit ettikleri prevalans sırasıyla; derin bir asetabular yuva için %15,2 ve %19,4; kabza deformitesi için ise %19,6 ve %5,2 olarak tespit edilmiştir. Çalışma sonucunda ise derin asetabular yuvanın ve tabanca kabza deformitesinin yaygın radyografik bulgular olduğu ve artmış kalça osteoartriti riski ile ilişkili olduğu bildirilmiştir. Ochoa ve ark.'nın (32) yaptığı bir çalışmada; kalça ile ilgili şikayetler ile birinci basamak ve ortopedi kliniklerine başvuran 157 genç (ortalama yaş 32, dağılım 18-50 yaş) hastanın pelvis ve kalça radyografileri incelenmiş ve 155 hastanın 135'inde en az bir FAS bulgusu saptandığı, 487 radyografiden 413'ünün normal, birinin ise FAS olarak okunduğu bildirilmiştir. Çalışmanın sonuçlarında ise; kalça şikayetleri olan aktif hastalarda FAS'ın radyografik kanıtının bulunduğu ifade edilmiş ve birinci basamakta, radyoloji ve ortopedi kliniklerinde FAS farkındalığının artması gerektiğine vurgu yapılmıştır. Ayrıca FAS yönetiminin uzun vadeli etkilerine ilişkin ek araştırmalar yapılması önerilmiştir.

Günümüzde FAS'ın etyolojisi halen kesinlik kazanmamıştır. Pollard ve ark. (33) tarafından semptomatik FAS'lı hastaların kardeşlerinde yapılan çalışmada hem klinik bulgular hem de radyografik inceleme yapılmış ve kam tipi deformitesi olan hastaların kardeşlerinde aynı deformiteye sahip olma riski 2,8 oranında tespit edilmiştir. FAS gelişiminde kalça morfolojisini etkileyen henüz tanımlanmamış genetik faktörlerin rol oynayabileceği sonucu üzerine dikkat çekilmiş ve genetik etkilerin önemine vurgu yapılmıştır. Genetik etkilerin yanı sıra FAS başlangıcında rol oynaması muhtemel risk faktörleri arasında çocukluk yaşlarında travma geçirilmesi ve deformitenin olması, ergenlik döneminde yüksek yoğunlukta yapılan atletik aktiviteler sayılmıştır (21). Erdem Sultanoğlu ve ark'ı (34) tarafından yapılan bir çalışmada; FAS tanısı alan 104 hastanın %76'sında kalça ağrısı tespit edilmiş ve radyografik bulgulara göre tüm hastaların %80,8'inde kam tip, %13,5'inde mikst tip, %5,8'inde ise pincer tip FAS saptandığı bildirilmiştir. Nunley ve ark'ı (35) tarafından yapılan prospektif bir çalışmada; yetişkin hastalarda semptomatik asetabular displazi klinik durumu incelenmiştir ve bu çalışmaya katılan bireylerin kalça problemleri nedeniyle kesin tanı konulmadan önce ortalama olarak 3,3 sağlık hizmet sağlayıcısı (Doktorlar, kayropraktörler, fizyoterapistler ve hemşireler) tarafından görüldükleri saptanmıştır. FAS'lı hastanın semptomları deformitenin derecesi ile ilişkilidir ve yüksek düzeyde hareket gerektiren aktiviteler yapıldığında semptomlar daha erken ortaya çıkar (36). Kalça fleksiyonu ve/veya iç rotasyon gerektiren pozisyon ve aktivitelerde kalça ağrısında artış olur. Uzun süreli oturma, uzun süreli oturmadan

sonra merdiven çıkma, çömelme ve araba kullanma gibi pozisyon ve aktiviteler semptomları şiddetlendirebilir. Labral yırtık veya kondral hasar gibi yeterince eklem içi hasar meydana gelmişse, kalçada tıklama veya takılma veya ağrılı kilitlenme gibi mekanik semptomlar mevcut olabilir (37, 38).

Asemptomatik veya semptomatik bireylerde FAS'a eğilim gösteren kalça eklemi malformasyonlarının klinik bulgularla birlikte değerlendirilmesi ve izlenmesi hem koruyucu hem de tedavi edici klinik takip stratejileri oluşturmak için son derece önemlidir. Tedavi planlaması her hastaya özgü yani bireyselleştirilmiş olmalı ve hastanın yaşı, spora dönüş zamanı, tedavinin süresi, kalça morfolojisi ve kıkırdak dejenerasyonunun derecesi dikkate alınmalıdır (39).

Lin ve ark.'nın (40) FAS için kalça artroskopisi uygulanan çeşitli yaş gruplarındaki hastalar tarafından bildirilen sonuçları ve klinik başarısızlık oranlarını araştırmak amacıyla yaptıkları çalışmada; kalça artroskopisi sonuçlarına göre; genç (ortalama 27,7 yıl), orta (ortalama 41,5 yıl) ve yaşlı (ortalama 60,2 yıl) olmak üzere üç yaş gruba ayrılan hastaların incelenmesi sonucunda her ne kadar yaştan bağımsız olarak hasta tarafından bildirilen sonuçlarda iyileşmeler sağlansa da orta yaşlı ve yaşlı hastalar zaman içinde klinik sonuçlarda genç hastalara göre daha büyük düşüşler yaşandığı, hastaların total kalça artroplastisine ilerleme riskinin daha yüksek olduğu ifade edilmiştir. Hasegawa ve ark'ı (41) tarafından ≥ 50 yaş üzerinde 427 katılımcının iki taraflı kalça radyografileri (n=854) kullanılarak yapılan çalışmada; genellikle asemptomatik olan FAS ile ilişkili anatomik anormalliklerin OA için risk faktörü olduğuna vurgu yapılmıştır. Bu durum göz önünde bulundurulduğunda şikâyeti olmasa bile FAS için risk altında bulunan bireylerde veya sporcularda ve kalça ağrısı ve/veya kasık ağrısı ile sağlık hizmet sağlayıcısına başvuran genç erişkin bireylerde FAS'ın ekarte edilmesine önem verilmelidir (10).

FAS'ın doğal seyri halen araştırmalara konu olmaya devam etmektedir (7). Ancak, genel popülasyonda asemptomatik bireylerde yapılan araştırmalar OA riski nedeniyle kalça ekleminin malformasyonlarının erken teşhis edilmesine odaklanılması gerektiğini düşündürmektedir (31).

Çalışmamızın çeşitli kısıtlıkları bulunmakta olup ilki çalışmamızda değerlendirilen bireylerin yaşı ve cinsiyeti dışında FAS ile ilişkilendirilen fiziksel aktivite ve sportif faaliyetlere ilişkin sosyo-demografik özellikleri, fizik muayenede "Sıkışma (impingement) testi" ile ortaya çıkması muhtemel semptomlar veya fonksiyonel duruma ilişkin bir klinik değerlendirme ile nihai tanılara ilişkin herhangi bir bilgi yer almamasıdır ve bu konuda bir analiz yapılmamasıdır. Radyolojik incelemeler çeşitli nedenlerle (nefrolitiazis, ürolitiazis, karın ağrısı ve nedeni bilinmeyen ateş) radyoloji bölümüne pelvik BT görüntüleme yöntemi için gönderilmiş ve kalça ağrısı bu nedenler arasında yer almasa da bu hastalarda yaşadıkları süreç içinde kalça ağrısı olup olmadığını retrospektif bir çalışma olması nedeniyle bilmemekteyiz. İkincisi ise çalışmamızda radyolojik inceleme için sadece iki parametrenin değerlendirilmiş olması diğer anatomik ölçümlere ilişkin değerlendirmenin yer almamasıdır.

Sonuç

Erkeklerin AA ortalamaları ile kadınların AA ortalamaları arasında ve erkeklerin FBBO ortalamaları ile kadınların FBBO ortalamaları arasında istatistiksel olarak anlamlı bir fark olduğu gözlenmiştir. FAS ile ilişkili olduğu bilinen görüntüleme özellikleri özellikle erkek bireyler olmak üzere asemptomatik yetişkin kalça eklemlerinde görülmektedir. Asemptomatik genç erkek ve kadın bireylerde antropometrik ölçümlerin normal sınırlar dışında olması halinde bu bireylerin bulgularına yönelik klinik korelasyonu önerilmelidir.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

The Frequency of HLA-B27 Antigen Positivity in Patients with Rheumatoid Arthritis and Ankylosing Spondylitis and the Relationship between HLA-B27 Antigen and Other Autoantibodies

Romatoid Artritli ve Ankilozan Spondilitli Hastalarda HLA-B27 Antijen Pozitifliği Sıklığı ve HLA-B27 Antijeni ile Diğer Otoantikorlar Arasındaki İlişki

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1401533
Geliş Tarihi | Received: 07.12.2023
Kabul Tarihi | Accepted: 19.03.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Karakılıç GD, Bakırcı EŞ, Büyük F. The Frequency of HLA-B27 Antigen Positivity in Patients with Rheumatoid Arthritis and Ankylosing Spondylitis and the Relationship between HLA-B27 Antigen and Other Autoantibodies. Hitit Medical Journal 2024;6(2): 136-142 https://doi.org/10.52827/hititmedj.1401533

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Ankara Şehir Hastanesi 2 nolu Klinik Araştırmalar Etik Kurulundan 07/09/2022 tarihinde onay alınmıştır (No: E2-22-2356).
İntihal Kontrolleri: Evet (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: GDK Dizayn: ESB Veri toplama/Veri işleme: FB Veri Analizi: FB Makale Hazırlanması: FB
Hasta Onamı: Hasta onamı alınmasına gerek yoktur.
Finansal Destek: Bu çalışma ile ilgili herhangi bir finansal kaynaktan yararlanılmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Etchical approval was received from Ankara Bilkent City Hospital No. 2 Clinical Research Ethics Committee on 1/3/2023 (No: E3-23-3476).
Plagiarism Check: Yes (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts of interest.
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: GDK Design: ESB Data Collection/Data Processing: FB Data Analysis: FB Manuscript Preparation: FB
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

The Frequency of HLA-B27 Antigen Positivity in Patients with Rheumatoid Arthritis and Ankylosing Spondylitis and the Relationship between HLA-B27 Antigen and Other Autoantibodies

ABSTRACT

Objective: The aim of this study was to research the frequency of Human Leukocyte Antigen (HLA)-B27 antigen positivity and relationship between HLA-B27 positivity and other autoantibodies and between HLA-B27 positivity and treatment in patients diagnosed with rheumatoid arthritis (RA) and ankylosing spondylitis (AS).

Material and Method: The study is a retrospective study. Patients diagnosed with RA and AS as a result of the examinations performed at Physical Medicine and Rehabilitation outpatient clinic between January 2017 and September 2022 were retrospectively screened, and patients whose HLA-B27 antigen was tested were included in study.

Results: A total of 569 patients, 199 with RA and 370 with AS were included in study. While HLA-B27 was positive in 11% of patients with RA, it was 37.5% in patients with AS and there was a significant difference between the groups. When we analyzed the correlation of autoantibodies with HLA-B27, we found that HLA-B27 was not correlated with RF, Anti-cyclic citrullinated peptides (Anti-CCP) or Anti-nuclear antibody (ANA). When we analyzed the relationship between HLA-B27 and the treatment method, there was no significant relationship between HLA-B27 and treatment method.

Conclusion: While HLA-B27 was found to be 5% positive in the general population in the literature, we found 11% in 199 patients with RA. This study is important because it shows that HLA-B27 positivity is not very common in patients diagnosed with AS recently contrary to popular belief. More studies are needed to evaluate HLA-B27 frequency in RA and AS

Keywords: Ankylosing Spondylitis, correlation of autoantibodies, HLA-B27 antigen, Rheumatoid Arthritis.

ÖZET

Amaç: Bu çalışmanın amacı, romatoid artrit tanılı hastalarda İnsan Lökosit Antijeni (HLA-B27) antijen pozitifliği sıklığını ve romatoid artrit (RA) ve ankilozan spondilit (AS) tanılı hastalarda HLA-B27 pozitifliği ile diğer otoantikorlar ve tedavi arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntem: Ocak 2017-Eylül 2022 tarihleri arasında Fiziksel Tıp ve Rehabilitasyon polikliniğinde yapılan tetkikler sonucunda RA ve AS tanısı alan hastalar retrospektif olarak tarandı ve HLA-B27 antijeni bakılanlar çalışmaya alındı. Hastaların yaşı, cinsiyeti, HLA-B27 antijen incelemesi sırasında Sedimantasyon (SED) ve C-reaktif protein (CRP) değerleri, Romatoid Faktör (RF), Anti-CCP, Anti-nükleer antikor (ANA), HLA-B27 sonuçları, AS tanısı alan hastalarda sakroiliak Manyetik Rezonans (MR) sonuçları ve verilen ilaç tedavileri kaydedildi.

Bulgular: : Çalışmaya RA'lı 199 ve AS'li 370 olmak üzere toplam 569 hasta dahil edildi. HLA-B27 RA'lı hastaların %11'inde pozitif bulunurken AS'li hastalarda %37.5 idi ve gruplar arasında anlamlı fark vardı. Otoantikorların HLA-B27 ile korelasyonunu analiz ettiğimizde, HLA-B27' nin RF, Anti-CCP veya ANA ile korele olmadığını bulduk. HLA-B27 ile tedavi yöntemi arasındaki ilişkiyi incelediğimizde, HLA-B27 ile tedavi yöntemi arasında anlamlı bir ilişki yoktu.

Sonuç: Literatürde genel popülasyonda HLA-B27 pozitifliği %5 iken biz 199 RA hastasında %11 pozitif bulduk. Bu çalışma, sanılanın aksine yeni AS tanısı alan hastalarda HLA-B27 pozitifliğinin çok yaygın olmadığını göstermesi açısından önemlidir. RA ve AS'de HLA-B27 sıklığını ve prognoza etkisini değerlendirmek için hasta sayısının daha çok olduğu çalışmalar gereklidir.

Anahtar Sözcükler: Ankilozan Spondilit, HLA-B27 antijeni, otoantikorların korelasyonu, Romatoid Artrit.

Introduction

Rheumatoid Arthritis (RA) is a systemic autoimmune disease characterized by chronic widespread inflammation and increased morbidity and high risk of mortality. RA is a rheumatological disease from collagen vascular disease group and is recognized as an Human Leukocyte Antigen (HLA)-DR4-associated autoimmune disease. Although it can be seen at any age and in both sexes, epidemiological studies show that RA is 3 times more common in women than in men (1). The prevalence of RA varies between 0.5-1 % in Europe and North America (2). Tuncer et al. reported that the prevalence of RA in Turkey was calculated as 0.5% in men and 0.89% in women (3). Ankylosing Spondylitis (AS) is a chronic inflammatory disease that primarily affects the axial spine, causing structural damage and functional disability (4). Patients are most often diagnosed in young adulthood. In about 80% of patients, signs begin before the age of 30 (5). The prevalence of AS is about 0.9% in the world. (6) The frequency of AS in the general population in Turkey has been reported as 0.46% (3).

HLA-B27 is part of the Major Histocompatibility Complex (MHC) gene family and is the general name for a set of antigenic substances that are encoded in the B locus of chromosome 6 and used by T cells to distinguish between self and foreign cells. The prevalence of HLA-B27 antigen positivity was 4% in North Africa, 8% in Caucasian races, 2-9% in China, and 0.1-0.5% in Japan (7). This rate for Turkey was stated to be between 2.8% and 6.8% in a meta-analysis published in 2017 (8). RA was associated with HLA-DR4 and HLA-DR1 antigens, while AS was associated with HLA-B27 antigen in previous studies (9, 10). Although it was stated in previous studies that 90% of patients diagnosed with AS have HLA-B27 antigen positivity, there is also a study in our country in which this rate was found to be 70% (11). HLA-B27 antigen was found to be positive in 534 (70.1%) of 762 AS patients and 62 (63.3%) of 98 patients with a diagnosis of non-radiographic axial spondyloarthritis in another study conducted in Turkey in 2021 (12). It was stated that the relationship between RA and HLA-B27 was not significant in a meta-analysis examining the relationship between RA and HLA-B27 antigen, but HLA-B27 antigen positivity could be a potential risk factor for pharmacogenomics and personalized therapy (13).

There is no literature revealing the effect of HLA-B27 antigen positivity on treatment and its relationship with other autoantibodies in patients with RA. The aim of this study is to retrospectively show the frequency of HLA-B27 antigen positivity and the relationship between HLA-B27 antigen positivity and other autoantibodies in patients with RA and AS.

Material and methods

Study Design

This retrospective study was conducted between January 2017 and September 2022. The medical records of the patients were accessed from that date since the hospital was opened in January 2017. Ethical approval was obtained on 07.09.2022 with the number (E2-22-2356) before study.

Patients

Those who were examined in the physical therapy and rehabilitation outpatient clinic of City Hospital and were diagnosed with AS according to modified New York Criteria or Assessment in Spondyloarthritis International Society (ASAS) criteria; Patients diagnosed with RA according to the RA 2010 ACR/EULAR classification criteria and consecutively seen in the outpatient clinic were screened (14-16). Since RA and AS can be seen together and may mimic each other's symptoms, HLA-B27 antigen was examined in some patients with RA. HLA-B27 was studied in patients diagnosed with seronegative RA, especially those with inflammatory waist and hip pain. Patients between 18-65 years of age and whose HLA-B27 antigen was studied, were included in the study. Patients with uncertain RA and AS diagnoses, diagnosed with late-onset RA, with lack of clinical data, and no follow-up records were excluded from the study.

Demographic information of patients such as age and gender; and laboratory parameters such as sedimentation (SED), C-reactive protein (CRP), HLA-B27 antigen, Rheumatoid Factor (RF), Anti-cyclic citrullinated peptides (Anti-CCP), Anti-nuclear antibody (ANA) at first diagnosis entry and sacroiliac magnetic resonance imaging (MRI) of patients with AS

were recorded. HLA-B27 allele mutation screening was performed by flow cytometry from peripheral venous whole blood taken with ethylenediaminetetraacetic acid (EDTA)-containing tubes from patients.

Statistical analysis

All analyses were carried out with SPSS 26.0 (IBM, USA). The normality of the numerical data distribution was examined using the Shapiro- Wilk normality test. Continuous variables with normal distribution were presented as median± standard deviation. The categorical data were compared using the Chi-squares test. Binary logistic regression analysis was used to investigate relationship between autoantibodies. *p<0.05* was accepted for statistical significance.

Results

Nine hundred thirty patients diagnosed with RA and 635 patients diagnosed with AS were screened, and patients whose HLA-B27 was tested were included in the study. A total of 569 patients, 199 with RA and 370 with AS were included in the study. One hundred forty-five (72.9%) of the patients with RA were female; 133 (35.9%) of the patients with AS were female. There was a statistically significant difference between the groups in terms of gender and RA was more common in women (*p=0.001*). The mean age of patients with RA was 49.10±14.78 years, while the mean age of patients with AS was 39.29±1.1 years, and there was no significant difference between the groups in terms of mean age (*p=0.198*). While HLA-B27 was positive in 11% of patients with RA, it was in 37.5% of patients with AS and there was a significant difference between the groups (*p=0.001*). While 49 (17.6%) of the female patients were HLA-B27 positive; 106 (36.4%) of the male patients were HLA-B27 positive, and there was a statistically significant difference between the groups in terms of gender, and HLA-B27 positivity was more common in male patients (*p=0.001*). While 12 (8.27%) of 145 female patients diagnosed with RA were HLA-B27 positive; While 36 (27.06%) of 133 female patients diagnosed with AS were HLA-B27 positive, and 10 (18.51%) of 54 male patients diagnosed with RA were HLA-B27 positive; HLA-B27 was positive in 102 (43.03%) of 237 male patients diagnosed with AS. HLA-B27 positivity was more common in male patients in both the RA group and AS group (*p=0.001*). RF was positive in 44.7% of patients with RA, it was 10.3% in patients with AS with a significant difference between the groups (*p=0.001*). Anti-cyclic citrullinated peptides (Anti-CCP) was positive in 35.7% and 0.3% of patients with RA and AS, respectively; and there was a significant difference between the groups (*p=0.001*). Anti-nuclear antibody (ANA) was positive in 33.2% of patients with RA, it was 6.5% in patients with a significant difference between the groups (*p=0.001*). Sedimentation (SED) and C-reactive protein (CRP) levels were significantly lower in AS patients respectively (*p<0.001*), (*p=0.032*) (Table I).

Table I. Demographic features and clinical findings of the patients

		RA (N=199)	**AS** (N=370)	***p***
Age (Mean±standart deviation)		49.10±14.78	39.29±1.1	*0.198*[a]
Gender (N/%)	Female	145 (72.9)	133 (35.9)	***<0.001***[b]
	Male	54 (27.1)	237 (64.1)	
HLA-B27 (N/%)	Positive	22 (11)	138 (37.5)	***<0.001***[b]
	Negative	177 (89)	232 (62.7)	
RF (N/%)	Positive	89 (44.7)	38 (10.3)	***<0.001***[b]
	Negative	110 (55.3)	332 (89.7)	
ANTI-CCP (N/%)	Positive	71 (35.7)	1 (0.3)	***<0.001***[b]
	Negative	110 (55.3)	149 (40.5)	
ANA (N/%)	Positive	66 (33.2)	24 (6.5)	***<0.001***[b]
	Negative	133 (66.8)	100 (27)	
SED (Mean±standart deviation)		23.98±19.56	13.93±12.98	***<0.001***[a]
CRP (Mean±standart deviation)		2.5±6.58	1.60±2.35	***0.032***[a]
Sacroiliac MR (N/%)	Normal		39 (10.6)	
	Suspicious		9 (2.4)	
	Positive		322 (87)	
Medications (N/%)	NSAII	145 (72.86)	163 (44.11)	***<0.001***[b]
	NSAII+SLZ	2 (1)	70 (18.9)	***<0.001***[b]
	Methotrexate	130 (65.32)	0 (0)	***<0.001***[b]
	Other DMARDs	60 (30.15)	0 (0)	***<0.001***[b]
	Biological agent	35 (17.58)	137 (37)	***<0.001***[b]

RA: Rheumatoid Arthritis, AS: Ankylosing Spondylitis, N: number, RF: Rheumatoid Factor ANA: Anti-nuclear antibody,
SED: Sedimentation, CRP: C reactive protein, MR: Magnetic Resonance, NSAII: non-steroidal anti-inflammatory drugs,
SLZ: Salazopyrin, DMARDS: Disease-modifying antirheumatic drugs

[a] *Independent samples t-test,* [b] *Chi-square test*

Table II. Logistic regression analysis of autoantibodies with HLA-B27

	B	S.E.	Wald	df	Sig.	OR
RF	-0,390	0,634	0,379	1	0,538	0,677
Anti-CCP	-0,163	0,687	0,056	1	0,813	0,850
ANA	0,237	0,562	0,178	1	0,673	1,267

Binary logistic regression analysis OR: Odds ratio, RF: Rheumatoid Factor, Anti-CCP: cyclic citrullinated peptides,
ANA: Anti-nuclear antibody

Binary logistic regression analysis found no association between RF, Anti-CCP, and ANA with HLA-B27 positivity (Table II). When we analyzed the relationship between HLA-B27 and the treatment method, there was no significant relationship between the HLA-B27 and the treatment method (*p=0.056*) (Table III).

Table III. Analysis of the association between HLA B27 and the treatment method in patients diagnosed with RA

			Treatment		Total
			Biological agents ± DMARD	DMARD	
HLA-B27	Negative	Count	22_a	150_a	172
		Expected Count	24,7	157,3	182,0
		% within the treatment group	81,5%	93,0%	91,5%
		Residual	-2,7	2,7	
		Adjusted Residual	-2,0	2,0	
	Positive	Count	8_a	14_a	22
		Expected Count	2,3	14,7	17,0
		% within the treatment group	18,5%	7,0%	8,5%
		% of Total	2,5%	6,0%	8,5%
		Residual	2,7	-2,7	
		Adjusted Residual	2,0	-2,0	
Total		Count	27	172	199
		Expected Count	27,0	172,0	199,0
		% within the treatment group	100,0%	100,0%	100,0%
		% of Total	13,6%	86,4%	100,0%

Each subscript letter denotes a subset of treatment categories whose column proportions do not differ significantly from each other at the 0.05 levels.

Pearson Chi-square *p=.056,* DMARD: disease

Discussion

The incidence of the RA was 2-3 times higher in women in many studies conducted on patients with RA (17-19). Similar to the literature, the female/male ratio was found to be 2.68 in our study. The incidence of the AS was 2-4 times higher in men in many studies conducted on patients with AS (5, 6, 20). Similar to the literature, the male/female ratio was found to be 1.78 in our study. HLA-B27 positivity was found to be more common in male patients with AS in some studies (21, 22). Contrary to these studies, Omar et al. and Arévalo et al. did not detect a significant relationship between HLA-B27 and gender in their studies (23, 24). A statistically significant difference was found between HLA-B27 positive and HLA-B27 negative groups in terms of gender and HLA-B27 was more common in male patients in our study. The reason why AS is more common in men may be that HLA-B27 positivity is more common in male patients as seen in our study. The prevalence of HLA-B27 antigen positivity varies (25, 26). While the incidence of HLA-B27 in the unaffected people in Europe is 8%, it ranges from 3-5% in China (27). The prevalence of HLA-B27 in the general population was reported that 5-8% in studies conducted in Türkiye (28). The prevalence of HLA-B27 in AS patients was reported that 70-90% in various studies conducted in Türkiye (12, 29). While HLA-B27 is found in 5% of the population, AS is found in only 1-5% of individuals with HLA-B27 (30). The presence of AS in 5% of HLA-B27 antigen-positive individuals indicates that not only HLA-B27 but also other genes contribute to the pathogenesis of AS (31).

We found 11% positivity in 199 RA patients while HLA-B27 positivity is 5% in the general population in the literature. We could not find any article to compare our study. Some previous studies on HLA-B27 positivity in patients with RA are very old and small-scale case-control studies. Therefore, it was stated that the relationship between RA and HLA-B27 was not significant in a meta-analysis examining the relationship between RA and HLA-B27 antigen, but HLA-B27 antigen positivity could be a potential risk factor for pharmacogenomics and personalized therapy (13). In our study, unlike the literature, the frequency of HLA-B27 was found to be

37.5% in 370 AS patients. We think that this different result may be due to the difference in the number of patients, clinical variations, different ethnic origins and genetic factors.

There was no statistically significant difference in terms of medications used between the 22 HLA-B27 positive patients and the 177 negative patients in 199 patients diagnosed with RA. Similarly, there was no statistically significant difference in terms of medications used between 138 HLA-B27 positive patients and 232 negative patients in 370 patients diagnosed with AS. There was no difference in drug regimens between HLA-B27 negative and positive patients in our study and HLA-B27 positivity was used as a diagnostic tool not appear to affect the choice and change of treatment regimen. Similarly, it was determined that HLA-B27 positivity or negativity did not affect the decision (biological and non-biological) of the treatment regimen in the studies examining the role of HLA-B27 in disease activity and effectiveness of treatment in AS patients by Omar et al. (24). We could not compare the situation in which HLA-B27 positivity or negativity does not affect the treatment regimen in RA since there is no previous study examining the role of HLA-B27 in disease activity and treatment effectiveness in patients diagnosed with RA.

The lack of a healthy control group and the low number of patients with RA compared to AS were limitations of our study. Other limitations of my study were that the number of patients routinely seen in the outpatient clinic was not known and that patients whose HLA-27 levels were not tested were not included in the study which could cause bias.

Conclusion

Our findings suggest that the prevalence of HLA-B27 positive was higher in RA compared to the normal population, and lower in AS than in previous studies. More studies are needed to evaluate HLA-B27 frequency in RA and AS and the impact of HLA-B27 positive on prognosis and treatment of RA and AS.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Polypectomy Technique and Histopathological Evaluation in Colon Polyps According to Paris Classification

Paris Sınıflamasına Göre Kolon Poliplerinde Polipektomi Tekniği Ve Histopatolojik Değerlendirme

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1457092
Geliş Tarihi | Received: 22.03.2024
Kabul Tarihi | Accepted: 28.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Yakut A, Büyücek Ş, Özcan M, Nacir M. Polypectomy Technique and Histopathological Evaluation in Colon Polyps According to Paris Classification. Hitit Medical Journal 2024;6(2):143-152 https://doi.org/10.52827/hititmedj.1457092

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Bu retrospektif çalışma Dicle Üniversitesi Tıp Fakültesi Etik Kurulu tarafından onaylandı (Tarih: 09.06.2022, Sayı: 180).
İntihal Kontrolleri: Evet (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: AY, SB, MO, MN Tasarım: AY, SB, MO, MN Veri Toplama/Veri İşleme: AY, SB, MO, MN Veri Analizi: AY, SB, MO, MN Makalenin Hazırlanması: AY, SB, MO, MN.
Hasta Onamı: Hasta onamı alınmasına gerek yoktur.
Finansal Destek: Bu çalışma ile ilgili herhangi bir finansal kaynaktan yararlanılmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: This retrospective study was approved by the Dicle University Faculty of Medicine Ethics Committee (Date: 09.06.2022, Number: 180).
Plagiarism Check: Yes (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts of interest.
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: AY, SB, MO, MN Design: AY, SB, MO, MN Data Collection/Data Processing: AY, SB, MO, MN Data Analysis: AY, SB, MO, MN Manuscript Preparation: AY, SB, MO, MN.
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

Polypectomy Technique and Histopathological Evaluation in Colon Polyps According to Paris Classification

ABSTRACT

Objective: Colorectal cancers are in third place in terms of incidence and second in terms of mortality. This study aims to type the polyps detected during colonoscopy according to the Paris classification, perform polypectomy with the appropriate technique, classify them as histopathological, determine the presence of dysplasia, and review the risk status of colorectal cancer.

Material and Method: Our study is a retrospective study presented as a one-year review of 124 patients who were found to have colonic polyps due to colonoscopy, who underwent polypectomy with the appropriate technique, and whose histopathological determination was completed.

Results: The study was conducted between 2021 and 2022 with a total of 124 cases, 37.9% female and 62.1% male. The mean age of the cases was 58.58 ± 14.40 years. The way the polyps were removed was determined according to the polyp size and the Paris classification. Polypectomy was performed with biopsy forceps for <5 mm polyps. For ≥5 mm polyps, polypectomy was performed with a hotsnare after mucosal separation with saline-methylene blue-adrenaline. A piecemeal polypectomy was performed for two very large polyps. The most important factor in determining CRC surveillance and the presence of dysplasia was polyp diameter. The dysplasia rate in polyps removed with biopsy forceps was lower than in the polypectomy group with a hot snare.

Conclusion: Colorectal cancers are multifactorial, the initial architecture is polyps. The increase in the diameter of these polyps rather than the removal techniques was significant in terms of colorectal cancer risk.

Keywords: Colon polyps, colorectal cancers surveillance, presence of dysplasia in colon polyp, polypectomy, polypectomy technique.

ÖZET

Amaç: Kolorektal kanserler görülme sıklığı açısından üçüncü, mortalite açısından ise ikinci sırada yer almaktadır. Bu çalışmada kolonoskopi sırasında tespit edilen poliplerin Paris sınıflamasına göre tiplendirilmesi, uygun teknikle polipektomi yapılması, histopatolojik olarak sınıflandırılması, displazi varlığının belirlenmesi ve kolorektal kanser risk durumunun gözden geçirilmesi amaçlanmaktadır.

Gereç ve Yöntem: Çalışmamız, kolonoskopi sonucu kolonik polip saptanan, uygun teknikle polipektomi yapılan ve histopatolojik incelemesi tamamlanan 124 hastanın bir yıllık incelemesi olarak sunulan retrospektif bir çalışmadır.

Bulgular: Çalışma 2021-2022 yılları arasında %37,9'u kadın, %62,1'i erkek olmak üzere toplam 124 vaka ile gerçekleştirildi. Olguların yaş ortalaması 58,58 ± 14,40 yıldı. Poliplerin çıkarılma şekli polip boyutuna ve Paris sınıflamasına göre belirlendi. <5 mm polipler için biyopsi forsepsi ile polipektomi yapıldı. ≥5 mm'lik poliplerde salin-metilen mavisi-adrenalin ile mukoza ayrımı yapıldıktan sonra sıcak snare ile polipektomi yapıldı. İki adet çok büyük polip için parça parça polipektomi yapıldı. KRK sürveyansının ve displazi varlığının belirlenmesinde en önemli faktör polip çapıydı.

Sonuç: Kolorektal kanserler multifaktöriyeldir, başlangıç mimarisi poliplerdir. Bu poliplerin alınma tekniklerinden ziyade çaplarının artması kolorektal kanser riski açısından anlamlıydı.

Anahtar Sözcükler: Kolon polibinde displazi varlığı, kolon polipleri, kolorektal kanser sürveyansı, polipektomi, polipektomi tekniği.

Introduction

Global Cancer Statistics 2020: GLOBOCAN revealed that there may be more than 1.9 million new colorectal cancer (CRC) cases in 2020, and 935,000 patients may die due to CRC. This assumes that one in every 10 cancer cases may be a CRC patient. In general, CRC ranks third in terms of incidence and second in terms of mortality (1). In CRC screening, colonoscopy is the most appropriate method to detect both cancer and precancerous lesions directly. If there are no pathological findings in the screening colonoscopy of patients aged 50 and over, it is recommended in the guidelines to perform a colonoscopy every 10 years. However, new guidelines recently published recommend that screening colonoscopy should be performed at age 45. The sensitivity of colonoscopy in detecting CRC is >95%, while its sensitivity in detecting advanced adenomas (≥10 mm in diameter) is 88–98% (2). The well-known pathway in CRC oncogenesis is the adenoma-carcinoma sequence. Detection and removal of precursor lesions reduce the incidence and mortality of CRC (3-8). With this study, we aimed to evaluate the effect of classifying precursor lesions according to the Paris classification and removing them with appropriate techniques on the presence of dysplasia and CRC surveillance.

Material and Method

Study design and participants

The hypothesis of our study was to investigate the relationship between polyp type, size, localization, and polyp removal technique in detecting dysplastic changes when polyps are detected in patients undergoing colonoscopy and in predicting changes in screening in terms of CRC surveillance.

Colonic polyps were detected in 124 (21%) of 590 patients who applied to the endoscopy unit of a center in the Eastern Anatolia Region for colonoscopy between January 2021 and January 2022. In this retrospective study, the colonoscopy reports and pathology results of 124 patients with colon polyps were prepared by recording the data from the hospital automation system. In patients with polyps detected in the colonoscopy report, polyp characterization was performed according to the Paris classification, and polyp removal techniques were recorded separately. Patients who underwent pathological evaluation after polypectomy were included in the study. Those whose colonoscopy report was not characterized according to the Paris classification, those whose polypectomy could not be performed for various reasons, and those whose pathological evaluation was not performed were excluded from the study. The study was designed according to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. Since the study concept is retrospective, it was conducted from the hospital database with the permission and consent of the hospital, and no consent was obtained from the patients. A survey was not administered to the patients.

From colonoscopy reports, pedunculated, flat, and sessile polyps (Paris classification Ip, Isp, Is, IIa, IIa/IIc) were characterized. Again, polyps were grouped according to the data in the colonoscopy reports. Polyps smaller than 5 mm and those removed in one go with biopsy forceps were collected in the first group. Those with ≥5 mm polyp (sessile, pedunculated, and flat) and those who underwent polypectomy with a hot snare by injecting 3-4 cc of 1\10000 saline-methylene blue-adrenaline into the base were included in the other group. In our two cases that could not be completely removed with a snare, we detected one with a pedunculated polyp and the other with a sessile polyp. Since the snare could not fully grasp these two polyps, a piecemeal polypectomy was performed and 3-4 cc of 1\10000 saline-methylene blue-adrenaline was injected into the base of the stump. Those who underwent piecemeal polypectomy with a hot snare were included in the last group. These large polyps were treated with polypectomy, close to endoscopic mucosal resection, leaving normal mucosal margins in the surrounding tissue. The obtained polypectomy materials were evaluated by three pathologists with the same experience working in the center's pathology laboratory. Data on the pathological typing of polyps, the presence of dysplasia, and cancer were recorded in the hospital information system. In light of the information recommended in gastroenterology guidelines regarding the use of anticoagulants and antiplatelets, low-dose aspirin use was allowed in patients before and after the colonoscopy procedure. Low molecular weight heparin was administered

to our patients who had to receive anticoagulant treatment in the evening before the procedure. The morning dose was skipped, and the normal dose of low molecular weight heparin treatment was given again in the evening. The Boston bowel preparation scale was used for colonoscopy. While those with BBPS ≥ 6 were included in the study, those with BBPS < 6 were excluded from the study due to a lack of preparation.

Statistical analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, and maximum) were used when evaluating the research data. The suitability of quantitative data for normal distribution was tested with the Shapiro-Wilk test and graphical analysis. The Mann-Whitney U test was used to compare two groups of quantitative variables that were not normally distributed. Kruskal-Wallis test and Dunn-Bonferroni test were used for intergroup comparisons of more than two quantitative variables that were not normally distributed. Fisher-Freeman-Halton exact test was used to compare qualitative data. Statistical significance was accepted as $p<0.05$.

Ethics Statement

This retrospective study was approved by the Ethics Committee of Dicle University Faculty of Medicine (Date: 09.06.2022, Issue: 180). Guidance Recommendations for Medical Practitioners in Biomedical Research Involving Human Subjects have been prepared taking into account the Declaration of Helsinki.

Results

Colon polyps were detected in 124 (21%) of 590 adult patients who applied to the endoscopy unit of a secondary-level state hospital in the Eastern Anatolia Region between January 2021 and January 2022. This study examined a cohort of 124 patients with colon polyps. Of the 124 patients, 37.9% (n=47) were female and 62.1% (n=77) were male. The ages of the patients ranged between 16 and 86, and the average was 58.58±14.40. When the purpose of the colonoscopy of the patients included in the study was examined, 37.1% (n=46) was for screening purposes, 6.5% (n=8) was for iron deficiency anemia (IDA), and 11.3% (n=14) was for abdominal pain (Table I).

Table I. Distributions of descriptive characteristics

		n (%)
Gender	Male	77 (62.1)
	Female	47 (37.9)
Age	*Mean±Sd*	58.58±14.40
	Median (Min-Max)	60 (16-86)
Complaint	For screening purposes	46 (37.1)
	Iron deficiency anemia	8 (6.5)
	Abdominal pain	14 (11.3)
	History of colon polyp	8 (6.5)
	Constipation	5 (4.0)
	Rectal bleeding	14 (11.3)
	Malignancy examination	11 (8.9)
	Others	17 (13.7)
Technical	Hotsnare polypectomy	72 (58.1)
	Biopsy forceps	50 (40.3)
	Piecemeal polypectomy	2 (1.6)

When the cases in which polyps were detected during the colonoscopy procedure were examined according to the polypectomy technique, 58.1% (n=72) were found to have polyps larger than 5 mm. It was observed that 3-4 cc of 1\10000 saline-methylene blue-adrenaline injection was applied to the base of these polyps. 40.3% (n = 50) were diminutive polyps of <5 mm and polypectomy was performed in one go with biopsy forceps. Since 1.6% (n=2) were very large polyps, polypectomy was performed with a mucosal dissection-like hot snare by applying 1/10000 saline-methylene blue-adrenaline to the base after piecemeal polypectomy (Figure I, Table I).

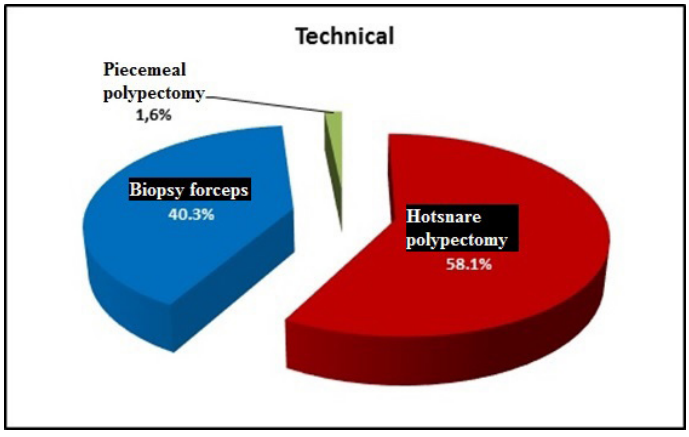

Figure I. Distribution of techniques

It was observed that polyps were detected in the ascending colon in 18.5% (n=23) of the patients who underwent polypectomy. The sizes of the polyps detected in the ascending colon ranged between 4 and 100 mm, and the average value was 15.04±23.76 mm. When ascending colon polyps were examined according to the Paris classification, 4.3% (n=1) were Ip; 73.9% (n=17) were Is; 4.3% (n=1) were IIa; 4.3% (n=1) were Ic; 4.3% (n=1) were Isp; and 8.7% (n=2) were IIa/IIc. When the pathologies of ascending colon polyps were examined, it was seen that 73.9% (n=17) were non-adenomatous polyps, 13% (n=3) were adenomatous polyps, and 13.3% (n=3) were cancer (CA). It was observed that polyps were detected in the transverse colon in 22.6% (n=28) of the patients who underwent polypectomy. The sizes of the polyps detected in the transverse colon varied between 5 and 50 mm, and the average value was 11.21 ± 11.35 mm. When transverse colon polyps were examined according to the Paris classification, 21.4% (n=6) were Ip; 53.6% (n=15) were Is; 10.7% (n=3) were IIa; and 14.3% (n=4) were Isp. When the pathologies of transverse colon polyps were examined, it was seen that 64.5% (n=16) were non-adenomatous polyps, 34.6% (n = 9) were adenomatous polyps, and 3.8% (n=1) were CA. It was observed that polyps were detected in the descending colon in 24.2% (n=30) of the patients who underwent polypectomy. The sizes of the polyps detected in the descending colon ranged between 5 and 80 mm, and the average value was 10.23±13.52 mm. When descending colon polyps were examined according to the Paris classification, 56.7% (n=17) were Is; 3.3% (n=1) were IIa; 36.7% (n=11) were Isp; and 3.3% (n=1) were IIa/IIc.When the pathologies of descending colon polyps were examined, it was seen that 54.8% (n=17) were non-adenomatous polyps, 41.9% (n=13) were adenomatous polyps, and 3.2% (n=1) were CA. It was observed that polyps were detected in the sigmoid colon in 7.3% (n = 9) of the patients who underwent polypectomy. The sizes of the polyps detected in the sigmoid colon varied between 5 and 20 mm, and the average value was 9.77 ± 6.24 mm. When sigmoid colon polyps were examined according to the Paris classification, 11.1% (n=1) were Ip; 33.3% (n=3) were Is; 33.3% (n=3) were IIa; and 22.2% (n=2) were Isp. When the pathologies of sigmoid colon polyps were examined, it was seen that 66.7% (n=6) were non-adenomatous polyps and 33.3% (n=3) were adenomatous polyps. It was observed that polyps were detected in the rectum in 56.5% (n = 70) of the patients who underwent polypectomy, and polypectomy was performed. The sizes of rectal polyps varied between 3 and 100 mm, and the average value was 16.08 ± 20.73 mm. When rectal polyps were examined according to the Paris classification, 14.3% (n=10) were Ip, 47.1% (n=33) were Is; 5.7% (n=4) were IIa; 24.3% (n=17) were Isp; and 8.6% (n=6) were IIa/IIc. When the pathologies of rectal polyps were examined, it was seen that 59.6% (n=34) were non-adenomatous polyps, 31.6% (n = 18) were adenomatous polyps, and 8.8% (n=5) were CA.

Table II. Adenomatous, dysplasia and cancer distributions

		n (%)
Adenomatous Polyp	none	76 (61.3)
	present	48 (38.7)
	VA	2 (4.2)
	TVA	4 (8.3)
	TA	42 (87.5)
Dysplasia	none	74 (59.7)
	present	50 (40.3)
	LG	47 (94.0)
	HG	3 (6.0)
CA	none	113 (91.1)
	present	11 (8.9)
	Adenocarcinoma	11 (100.0)

VA: Villous adenoma, TVA: Tubulovillous adenoma TA: Tubular adenoma, LG: Low grade, HG: High grade, CA: Cancer

Adenomatous polyps were detected in 38.7% (n=48) of the patients included in the study. When the types of adenomatous polyps are examined, 4.2% (n=2) was villous adenoma (VA), 8.3% (n=4) was tubulovillous adenoma (TVA), and 87.5% (n=42) was tubular adenoma (TA) (Table II). The presence of dysplasia was detected in 40.3% (n=50) of the polyps (Figure II). When examined according to dysplasia subtypes, it was seen that 94% (n=47) was low grade (LG) and 6% (n=3) was high grade (HG). CA was detected in 8.9% (n=11) of the cases. All CA types (n=11) were found to be malignant adenocarcinomas (Table II).

Table III. Comparison of descriptive characteristics by techniques

Hotsnare polypectomy		Techniques			p
		Hotsnare polypectomy	Biopsy forceps	Piecemeal polypectomy	
Gender	Male	45 (62.5)	32 (64.0)	0 (0)	[a]0.255
	Female	27 (37.5)	18 (36.0)	2 (100)	
Age	*Mean±Sd*	57.57±14.05	59.30±14.79	77.00±0.00	[b]0.092
	Median (Min-Max)	59 (16-86)	61.5 (19-82)	77 (77-77)	

[a]*Fisher Freeman Halton Test* [b]*Kruskal Wallis Test*

The gender and age of the cases did not show a statistically significant difference according to the polypectomy removal technique (*p>0.05*) (Table III). According to the polypectomy removal technique, the polyp pathologies of the ascending colon, transverse colon, descending colon, sigmoid colon, and rectum did not show a statistically significant difference (*p>0.05*) (Table IV). No significant difference was found between genders in terms of age (*p>0.05*). There was no significant difference between adenomatous and non-adenomatous polyps and gender (*p>0.05*).

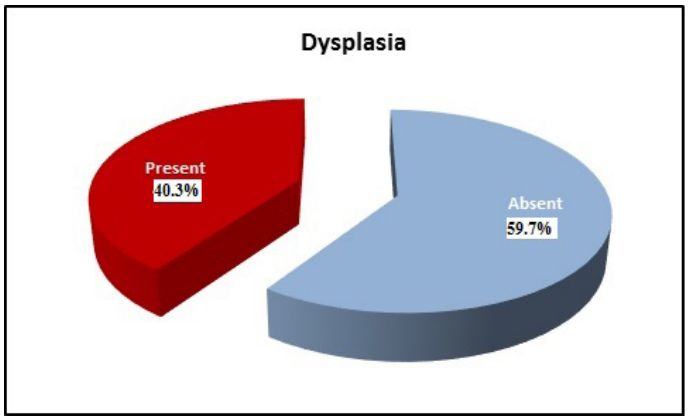

Figure II. Distribution of presence of dysplasia

A statistically significant difference was found between the polypectomy removal technique and the presence of dysplasia (*p=0.001; p<0.01*). It was observed that the dysplasia rate in polyps removed with biopsy forceps was lower than in the hotsnare polypectomy and piecemeal polypectomy groups (Table V). According to the ascending colon polyp pathologies, a statistically significant difference was found between the ascending colon polyp sizes of the cases (*p=0.007; p<0.01*). As a result of pairwise comparisons made to determine the source of the difference, the sizes of cases with malignant neoplasia in their pathology were significantly higher than those with non-adenomatous polyps (*p=0.005; p<0.001*). According to transverse colon, descending colon, and sigmoid colon polyp pathologies, no statistically significant difference was found between the polyp sizes in the same segment of the cases (*p>0.05*). A statistically significant difference was found between rectal polyp sizes according to rectal polyp pathologies (*p=0.002; p<0.01*). As a result of pairwise comparisons made to determine the source of the difference, the sizes of cases with malignant neoplasia pathology were significantly higher than those of non-adenomatous polyps (*p=0.004; p<0.01*).

Table IV. Comparison of pathologies by techniques

Pathological localizations Hotsnare polypectomy		Techniques			p
		Hotsnare polypectomy	Biopsy forceps	Piecemeal polypectomy	
Ascending Colon	Non-adenomatous Polyp	6 (60.0)	11 (84.6)	0 (0)	[a]0.150
	Adenomatous Polyp	3 (30.0)	0 (0)	0 (0)	
	Malignant Neoplasia	1 (10.0)	2 (15.4)	0 (0)	
Transverse Colon	Non-adenomatous Polyp	14 (73.7)	2 (33.3)	0 (0)	[a]0.119
	Adenomatous Polyp	4 (21.1)	4 (66.7)	1 (100)	
	Malignant Neoplasia	1 (5.3)	0 (0)	0 (0)	
Descending Colon	Non-adenomatous Polyp	11 (57.9)	6 (54.5)	0 (0)	[a]0.483
	Adenomatous Polyp	8 (42.1)	4 (36.4)	1 (100)	
	Malignant Neoplasia	0 (0)	1 (9.1)	0 (0)	
Sigmoid Colon	Non-adenomatous Polyp	3 (50.0)	3 (100)	0 (0)	[a]0.464
	Adenomatous Polyp	3 (50.0)	0 (0)	0 (0)	
Rectum	Non-adenomatous Polyp	20 (51.3)	14 (77.8)	0 (0)	[a]0.058
	Adenomatous Polyp	16 (41.0)	2 (11.1)	0 (0)	
	Malignant Neoplasia	3 (7.7)	2 (11.1)	0 (0)	

[a]*Fisher Freeman Halton Test*

Table V. Comparison of presence of dysplasia by techniques

Hotsnare polypectomy (n=75)		Techniques			*p*
		Hotsnare polypectomy	Biopsy forceps	Piecemeal polypectomy	
Dysplasia	none	35 (48.6)	39 (78.0)	0 (0)	[a]***0.001****
	present	37 (51.4)	11 (22.0)	2 (100)	

[a]*Fisher Freeman Halton Test*
***p<0,01*

Discussion

The 2010 World Health Organization (WHO) reported conventional adenomas (tubular, tubulovillous, and villous adenomas) and serrated polyps (SPs) (hyperplastic polyps [HPs], sessile serrated adenoma/polyps [SSA/Ps], and conventional serrated adenomas [TSA]) as precursors of CRC (9). The association of serrated polyps, SSA/Ps, and TSA with cancer has been discussed in many studies. For this reason, conventional adenomas and serrated polyps detected in screening colonoscopies should be removed en bloc with appropriate technique by an experienced team and examined in the pathology laboratory (10-15). Although evidence for the malignant potential of serrated polyps has not been directly demonstrated, cross-sectional studies show that dysplastic changes and malignant transformation may occur in serrated polyps (16). It is estimated that 3-22% of CRCs arise from serrated polyps (16). If the pedunculated, sessile, and flat polyps detected in our study were all <5 mm, a polypectomy was performed with biopsy forceps. Polypectomy was performed with a hot snare after mucosal removal with 3-4cc 1/10000 saline-methylene blue-adrenaline at the base of ≥ 5 mm pedunculated, sessile, and flat polyps. These polypectomy materials were sent to the pathology laboratory and examined. Again, polyps that could not be detected directly with a snare were removed by piecemeal polypectomy. After the mucosal separation process was performed with 3-4cc 1/10000 saline-methylene blue-adrenaline at the base of the stump, a mucosal resection-like polypectomy was performed, and large polyps were removed. Considering the findings of our study, there was no significant relationship between the polypectomy removal technique and the presence of dysplasia. However, it was observed that as the diameter of the polyp increased, the likelihood of dysplasia and malignancy increased. This situation we found in our study is similar to previously published articles.

Three large cohort studies in the United States found that at 10-year follow-up of patients who underwent initial screening colonoscopy, patients with advanced adenomas or large serrated polyps were more likely to develop CRC than patients without polyps (17-19). Considering the characteristics of adenomas (size, number, villous character, and presence of dysplasia), a higher risk of CRC is predicted. In contrast, the risk of CRC is lower in patients with immature adenomas, 1 or 2 SPs <10 mm. However, as the number of polyps increased, the possibility of CRC increased (17-19). Serrated polyps without atypical cells were previously called HPs. It was believed that such polyps did not have cancer potential. In 1990, Longacre et al. (20) reported serrated polyps. Torlakovic et al. (21, 22) stated that SPs should be examined as typical and atypical. They suggested that HPs have SSAs, a subtype that includes atypia. However, some studies did not approve the term adenoma, accepting that these lesions were not as oncogenic as adenomatous polyps. In the latest 2010 WHO classification, the term SSA/Ps, which includes both adenoma and polyp grades, was used (23, 24). Thus, SSA/Ps has a place in the classification among serrated polyp types in its new and standardized form. This transition has not been fully adopted, as it is widely accepted that there is no risk of developing cancer. According to the WHO classification, serrated polyps in the colorectum are generally reported as HP, TSA, and SSA/P (24). When evaluated in light of these data, every lesion seen during the endoscopy procedure should be removed by polypectomy. Similar to the literature, dysplasia was mostly detected in adenomatous polyps in our cases. We detected dysplasia in non-adenomatous polyps in two of our patients. Randomized clinical trials and the European polyp surveillance study (EPoS) recommend that patients with 1 or 2 <10 mm low-grade dysplasia and tubular adenomas should have screening colonoscopy every 5 or 10 years (25). Although surveillance recommendations in guidelines for CRC risk in conventional adenomas vary little, awareness of surveillance for CRC risk in serrated

polyps is just emerging. The view that SP\As are a different precursor lesion and a separate group for CRC has been revealed in studies with increasing evidence (26). However, SP\As remain largely unknown regarding CRC risk. SP\As, although there is limited evidence of their malignant potential, size is an important determinant. It has been determined that cases with SP\A ≥10 mm are more likely to turn into synchronous or metachronous CRCs than cases without polyps or cases with SP\A <10 mm (13, 27, 28).

We accept that every polyp/adenoma may be a precursor lesion for CRC and should be removed. Keeping this in mind, the most common polyp/adenoma group we detected were diminutive polyps with a diameter of ≤ 5 mm. Approximately 60% of polyps detected in screening colonoscopies are polyps with a diameter of ≤5 mm. The association of these diminutive polyps with CRC is low, but cannot be neglected (29, 30). In contrast, Burgess et al. (31) showed that this dimension is also important for SSA/P. The odds ratio (OR) for cytological dysplasia for every 10 mm increase in lesion size is 1.90 (32). SSA/P cytological dysplasia (SSA/P-D), presence of 0-Is according to the Paris Classification (OR=3.1); also having Kudo pitting pattern III, IV, or V (OR=3.98); and depending on increasing age (OR=1.69/decade) (32). In the literature, CRC is reported to occur in three different ways. These are the chromosomal loss of stability pathways from adenoma to carcinoma (50-70%); the other is the most mutated "Lynch syndrome" pathway (3-5%); and it consists of a serrated path (30-35%). As we mentioned above, WHO grouped serrated polyps under three headings: HP, SSA/P, and TSA. The last two types of polyps are strongly associated with the development of CRCs. HPs are less likely to become malignant than TSAs. Both HP and SSA/Ps appear morphologically similar. SSA/P is also difficult to detect (32).

Resection of premalignant serrated lesions by professionals and experienced individuals reduces the development of CRC. One of the biggest problems of inexperienced people is the inability to obtain en-bloc lesions and the difficulty of providing CRC surveillance. Unlike adenomas, not all serrated lesions are associated with CRC (33). However, when all studies are evaluated, it shows that the relationship of serrated polyps with CRC cannot be ignored. Erichsen, Rune, et al. (32) showed that patients with a history of SSA/P had an increased risk of CRC compared to patients without polyps. Although SSA/Ps have similar sizes to adenomatous polyps, the increased risk of CRC may be even higher than that of adenomatous polyps. The risk of CRC was found to be particularly high for SSA/Ps with dysplasia. A history of TSA was also associated with an increased risk of CRC, whereas patients with a history of HP had a lower risk of CRC. The estimated CRC risk after 10 years is 4.4% for SSA/P-D patients and 4.5% for TSA patients. This is the first study to quantify CRC risks for subtypes of serrated polyps with good precision (32).

As a result, in our study, increased polyp diameter and the presence of dysplasia pose a risk for CRC. This is an issue in which our study overlaps with the literature. We also found that dysplasia can be found not only in adenomatous types of polyps but also in non-adenomatous polyps. Therefore, all polyps must be removed en bloc during the colonoscopy procedure. In our study, we could not obtain sufficient information to perform CRC surveillance according to the Paris classification of polyps and polypectomy technique. The strength of our study is that, in terms of CRC, we found that, although rare, dysplastic changes may also develop in non-adenomatous polyps. Patients with CP may be overlooked in terms of CRC surveillance. Creating this awareness is very useful. On the other hand, the most important features that limit our study are that the study is retrospective, there are not enough cases, and the patients do not have long-term follow-up in terms of CRC surveillance.

Conclusion

The larger the polyp, the more likely it is to develop dysplasia, if it is adenomatous. Although we do not have enough cases, we observed that dysplasia can also develop in non-adenomatous polyps in two of our cases. According to the Paris Classification, the shape of the polyp or the technique of removing the polyp does not provide sufficient information to the

endoscopist regarding the possibility of detecting CRC and dysplasia.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

The Relationship Between Endoscopic Findings and Laboratory Results in Inflammatory Bowel Disease

İnflamatuvar Barsak Hastalıklarında Laboratuvar Sonuçları ile Endoskopik Bulgular Arasındaki İlişki

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1415074
Geliş Tarihi | Received: 06.01.2024
Kabul Tarihi | Accepted: 19.03.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Şahin M, Okcu N. The Relationship Between Endoscopic Findings And Laboratory Results İn Inflammatory Bowel Disease. Hitit Medical Journal 2024;6(2) 153-160 https://doi.org/10.52827/hititmedj.1415074

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Atatürk Üniversitesi Klinik Araştırmalar Etik Kurulundan 02/06/2015 tarihinde onay alınmıştır (No: 4/10).
İntihal Kontrolleri: Evet - (Intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: MŞ, NO Tasarım: MŞ, NO Veri Toplama/Veri İşleme: MŞ, NO Veri Analizi: MŞ, NO Makalenin Hazırlanması: MŞ, NO.
Hasta Onamı: Hasta onamı alınması gerekmemektedir.
Finansal Destek: Finansal destek alınmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Etchical approval was received from Atatürk University Clinial Research Ethics Committee on 02/06/2015 (No: 4/10).
Plagiarism Check: Yes - (Intihal.net)
Conflict of Interest: The authors declared that, there are no conflicts in interest.
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: MŞ, NO Design: MŞ, NO Data Collection/Data Processing: MŞ, NO Data Analysis: MŞ, NO Article Preparation: MŞ, NO.
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

The Relationship Between Endoscopic Findings and Laboratory Results in Inflammatory Bowel Diseases

ABSTRACT

Objective: The aim of this study was to determine the relationship between routine laboratory indicators [Including hemoglobin, white blood cells, platelets, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)] and the extent of endoscopic involvement in individuals with inflammatory bowel disease (IBD).

Material and Method: The medical records of patients who were diagnosed with Ulcerative Colitis (UC) and Crohn's Disease (CD) between 2009 and 2015 were retrospectively examined. Endoscopic findings and hemoglobin, white blood cell, platelet, ESR, and CRP values at the time of colonoscopy were analyzed. An exploratory multinomial regression model was created to examine the association of laboratory parameters and endoscopic involvement localization.

Results: In UC, a significant decrease in hemoglobin levels was present in cases with extensive colitis/pancolitis compared to distal type colitis (*p=0.02*), while no significant difference was found between left-sided colitis and distal type colitis. Elevated ESR values were notably found in left-sided colitis (*p=0.007*) and extensive colitis/pancolitis (*p=0.043*) compared to distal type colitis. CRP levels were significantly higher in cases with extensive colitis/pancolitis (*p=0.015*). No relationship was identified between laboratory parameters and the endoscopic location of involvement in CD.

Conclusion: Although hemoglobin value, ESR and CRP levels are helpful in determining the location of involvement in UC, their effects have not been observed in CD. In addition to these basic laboratory values, other parameters should also be taken into consideration in the evaluation of patients.

Key words: C-reactive protein, Crohn's Disease, Erythrocyte sedimentation rate, Ulcerative Colitis.

ÖZET

Amaç: Bu çalışmanın amacı, inflamatuvar barsak hastalığı (İBH) olan olgularda rutin laboratuvar bulguları (hemoglobin, beyaz küre, trombosit, eritrosit sedimentasyon hızı (ESH), C-reaktif protein (CRP)) ile endoskopik tutulum alanları arasındaki ilişkiyi belirlemektir.

Gereç ve Yöntem: 2009-2015 yılları arasında Ülseratif Kolit (ÜK) ve Crohn Hastalığı (CH) tanısı alan hastaların tıbbi kayıtları geriye dönük olarak incelendi. Endoskopik bulgular ile kolonoskopi sırasındaki hemoglobin, beyaz kan hücresi, trombosit, ESR ve CRP değerleri kaydedildi. Laboratuvar parametreleri ile endoskopik tutulum lokalizasyonu arasındaki ilişkiyi incelemek için multinominal lojistik regresyon modeli oluşturuldu.

Bulgular: ÜK'de; ekstensif kolit/pankolit tutulumunda distal tip kolite göre hemoglobin değerinde anlamlı derecede düşüş saptanmışken (*p=0.02*), sol taraf kolitiyle distal tip kolit arasında anlamlı fark saptanmamıştır. Sol taraf koliti (*p=0,007*) ve ekstensif kolit/pankolit (*p=0.043*) tutulumunda distal tip kolite göre ESH değerleri belirgin yüksek bulunmuştur. CRP değeri sadece ekstensif kolit/pankolit tutulumunda anlamlı yüksek çıkmıştır (*p=0.015*). CH için laboratuvar parametreleri ile tutulum yerleri arasında herhangi bir ilişki bulunamamıştır.

Sonuç: ÜK›de hemoglobin değeri, ESR ve CRP düzeyleri tutulumun yerini belirlemede yardımcı olsa da CH'de etkileri görülmemiştir. Hastaların değerlendirilmesinde bu temel laboratuvar değerlerinin yanı sıra diğer parametrelerin de dikkate alınması gerekir.

Anahtar kelimeler: C-reaktif protein, Crohn Hastalığı, Eritrosit sedimentasyon hızı, Ülseratif Kolit.

Introduction

Inflammatory bowel diseases (IBD), encompassing Ulcerative Colitis (UC) and Crohn's Disease (CD), are persistent inflammatory conditions capable of affecting any segment of the gastrointestinal tract, often manifesting through periods of remission and exacerbation. The diagnosis of these diseases relies on a comprehensive evaluation involving clinical, endoscopic, and histopathological features. However, it's crucial to note no finding is definitively diagnostic (1,2).

The incidence and prevalence of IBD vary by geographical region, ethnic group and race. The prevalence of IBD generally ranges between 0.3% and 0.5%, with women being slightly more frequently affected. It exhibits a bimodal age distribution for disease onset diagnosis. The incidence increases in young people aged 15-25, with the second peak occurring between the ages of 40-60 (3).

In UC, inflammation persists in the bowel wall and is limited primarily to the mucosa. The rectum is always involved in the disease and may progress towards the proximal segments. Pseudopolyps, polypoid tags of the mucosa, may be present on endoscopy during periods of active disease and remission. These are not true adenomas containing granulation tissue poor in epithelium. Dysplastic changes can be observed in the long term, increasing the risk of colon cancer. Macroscopically, the affected segment in CD exhibits ulcers, stenosis, fistulas, and abscesses. CD involves both segmental and full-thickness complications. A distinguishing characteristic of CD is the identification of granulomas (4-5).

The length and severity of the affected colon segment determine the clinical presentation of UC (6). If colon involvement extends up to 10-12 cm from the distal end, it is defined as distal type colitis, if it involves up to the splenic flexure, it is defined as left-side colitis, and if it extends beyond the splenic flexure, it is defined as diffuse colitis (7). CD may be inflammatory, obstructive or fibrostenotic (8). Terminal ileum involvement is present in 45-47% of patients, colon involvement in 30%, ileocolic involvement in 20%, and upper gastrointestinal tract involvement in 3-5% (9-10).

The role of laboratory findings in IBD is to identify specific forms of the disease in a less-invasive way, determine the degree of disease activity, predict the course of the disease, and predict the response to treatment interventions. The main laboratory markers in IBD are acute phase reactants including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), hemogram, fecal and serological markers.

The purpose of our study is to determine the relationship between routine laboratory findings (hemogram, ESR, CRP) and endoscopic involvement sites in cases diagnosed with IBD.

Materials and Methods

The study received ethics committee approval on 02.06.2015, with the Clinical Research Ethics Committee Service number B.30.2.ATA.0.01.00/124, which determined that there was no scientific or ethical contravention.

The records of patients diagnosed with UC and CD who applied to the the Internal Medicine Clinic of Atatürk University Faculty of Medicine between 2009 and 2015 were retrospectively examined. Hemoglobin, white blood cell, platelet, ESH, and CRP values of the patients on the date of colonoscopy were obtained from laboratory records. Patients who underwent bowel surgery, had malignancy or were pregnant, those under the age of 18, and patients receiving treatment for IBD were not included in the study.

Patients were divided into subgroups based on endoscopic findings at the time of inclusion in the study as follows: UC patients 1) Distal type colitis, 2) Left-sided colitis, 3) Extensive colitis/Pancolitis and CD patients 1) Colitis, 2) Ileitis, 3) Ileocolitis.

Statistical analysis

Descriptive statistics were performed to summarize baseline characteristics. Firstly, the minimum, maximum, mean, and median laboratory values of UC and CD and their subgroups were determined. Afterwards, the Compatibility Test, Pearson and Deviance Test, Pseudo R2 Test, Multinominal Logistic Regression Test and Classification Test were performed for the statistical validity of the model. Analysis and interpretations were made using the (Statistical Package for the Social Sciences) SPSS 18 software package.

Table I. Demographic Data of the Cohort

		Crohn's Disease (n=42)			Ulcerative Colitis (n=156)		
		Colitis	Ileitis	Ileocolitis	Distal colitis	Left-sided colitis	Extensive colitis / Pancolitis
n		7	23	12	45	46	65
Mean age (year) (minimum-maximum)	44.9 (18-83)	42.04 (19-71)			46.01 (18-80)		
Male/Female (n)	104 / 94	3 / 4	13 /10	6 / 6	25/20	26/20	36/29

Results

This retrospective study was conducted with 198 cases. Forty-two (21.2%) of them had CD and 156 (78.8%) of them were diagnosed with UC. The demographic characteristics of the cohort are summarized in Table I.

Data Analysis of Patients with Ulcerative Colitis

Hemoglobin, ESR and CRP values had a significant effect on the model; however, age, platelet, and white blood cell counts had unremarkable impact. The reference point for regression analysis in the UC model was determined as distal type colitis. Multinominal logistic regression analysis revealed that a one-unit increase in ESR value increases the probability of the disease being left-sided colitis by 1.055 times; it increases the probability of having extensive colitis/pancolitis by 1.041 times. A one-unit decrease in hemoglobin value increases the probability of the extensive colitis/pancolitis by 0.731 times. A one-unit increase in the CRP value increases the probability of the disease being extensive colitis/pancolitis by 1.064 times compared to the probability of the disease being distal type colitis (Table II).

When the variables used in the study were classified, it was seen that 61.5% of the patients were correctly classified by the analysis using independent variables. According to subgroups, 77.8% of the

Table II. Multinominal logistic regression results of Ulcerative Colitis group using distal colitis as the reference category

		B	Standard deviation	Wald	Sig.	Exp(B)	95% Confidence Interval	
							Lower limit	Upper limit
Left-sided colitis	Constant	0.369	2.475	0.022	0.881			
	WBC (10^3/L)	0.000	0.000	0.879	0.348	1.000	1.000	1.000
	HGB (g/dl)	-0.085	0.139	0.377	0.539	.918	0.699	1.206
	Platelet (x10^3/µl)	0.000	0.000	2.519	0.113	1.000	1.000	1.000
	ESR (mm/h)	0.054	0.020	7.297	0.007*	1.055	1.015	1.098
	CRP (mg/L)	0.040	0.026	2.368	0.124	1.041	0.989	1.095
	Age	0.001	0.016	0.001	0.975	1.001	0.969	1.033
Extensive/ Pancolitis	Constant	2.229	2.359	0.893	0.345			
	WBC (10^3/L)	0.000	0.000	0.704	0.401	1.000	1.000	1.000
	HGB (g/dl)	-0.314	0.135	5.385	0.020*	0.731	0.561	0.952
	Platelet (x10^3/µl)	0.000	0.000	0.326	0.568	1.000	1.000	1.000
	ESR (mm/h)	0.040	0.020	4.106	0.043*	1.041	1.001	1.081
	CRP (mg/L)	0.062	0.026	5.864	0.015*	1.064	1.012	1.118
	Age	0.002	0.017	0.011	0.916	1.002	0.970	1.035

WBC, White blood cell; HGB,Hemoglobin; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein

Table III. Multinominal logistic regression results of Crohn's Disease group using colitis as the reference category

		B	Standard deviation	Wald	Sig.	Exp(B)	%95 Confidence Interval	
							Lower limit	Upper limit
Ileitis	Constant	0.409	3.875	0.006	0.939			
	WBC (10^3/L)	-0.064	0.48	0.018	0.894	0.938	0.366	2.402
	HGB (g/dl)	0.611	2.877	0	0.985	6.62	0	
	Platelet (x10^3/µl)	0	0.003	0.029	0.865	1	0.994	1.005
	ESR (mm/h)	-0.133	9.562	0.001	0.969	0.322	2.2	4.6
	CRP (mg/L)	0.638	3.14	0.019	0.89	1.65	3	1.2
	Age	**-0.333**	0.035	0.063	.000*	0.264	0.246	0.282
Ileocolitis	Constant	2.097	3.891	0.006	0.939			
	WBC (10^3/L)	-0.064	0.48	0.018	0.894	0.938	0.366	2.4
	HGB (g/dl)	0.749	2.877	0	0.985	7.57	0	
	Platelet (x10^3/µl)	0	0.003	0.028	0.866	1	0.994	1
	ESR (mm/h)	-0.144	2.562	0.001	0.969	0.319	2.1	4.6
	CRP (mg/L)	0.646	1.14	0.019	0.89	6.016	3	1.2
	Age	-0.305	0	.	.	0.271	0.271	.271

WBC, White blood cell; HGB,Hemoglobin; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein

patients classified as distal type colitis were correctly predicted by the analysis. For other categories, these rates were 28.3% and 73.8%, respectively.

Data Analysis of Patients with Crohn's Disease

The reference point for the CD group was determined to be colitis. According to the results, no relationship was present between laboratory results and the location of disease involvement in the CD cohort. Table III summarizes the multinominal logistic regression analysis of data from the CD group.

It was observed that 76.2% of the patients were correctly classified by the analysis using independent variables. While 54.8% of the patients whose disease type was classified as ileitis were correctly predicted by the analysis, these rates for the other categories were 16.7% and 28.6%, respectively.

According to the Nagalkerke R2 evaluation, 41.9% and 80.3% of the change in the probability of the disease subtype in the UC and CD models, respectively, can be explained by the values obtained as a result of the laboratory results.

Discussion

The objective of the present study was to estimate the relationship between laboratory findings and endoscopic diagnosis in patients with IBD. Our model, based on the evaluation of laboratory parameters for IBD, is statistically significant. However, for UC, 41.9% of the change in probability regarding the type of disease can be explained by the values obtained because of the laboratory results. This indicates that existing parameters alone are insufficient to diagnose the disease and that auxiliary methods are required in addition to these parameters.

In the literature, the relationship between acute phase reactants and endoscopic involvement areas in UC patients has been evaluated in various studies using validated indices (11-18). Koçhan et al. identified statistically significant variations in ESR, CRP, platelet, and hemoglobin levels based on the Truelove-Witts index, a clinical activity index, in UC patients. However, no significant difference in leukocyte levels was noted. Additionally, a significant

distinction was observed only in leukocyte levels (*p=0.041)* between the CD and UC groups based on the disease localization. Nevertheless, the small number of patients between the groups led to the interpretation of an insufficient statistical comparison (19). In the study conducted by Önal et al. using Truelove-Witts clinical and Rachmilewitz endoscopic indices in patients with UC, an increase in CRP, ESR and white blood cell counts was found in patients with active UC compared to inactive patients. However, only statistical significance was shown in CRP values (20). In 2013, Yeşil et al. investigated whether RDW is a marker of active disease in patients with IBD. Their results revealed that the CRP and ESR levels in patients with active CD were significantly higher than those in remission or in the controls. Similar results were observed in patients with active UC versus UC patients in remission. No statistically significant differences were found between the CRP and ESR levels of UC patients in remission and CD patients in remission (21). Although we did not evaluate active and inactive patients in the present study, a negative relationship was found with hemoglobin value and a positive relationship with ESR and CRP levels in extensive/pancolitis type of UC patients with extensive/pancolitis type involvement.

The most common hematological finding in IBD is anemia (22). There are mainly two types of anemia in IBD: iron deficiency and chronic disease anemia (23). According to the results of this study, the decrease in hemoglobin value increases the possibility of involvement as extensive colitis/pancolitis, which is the more serious form of the disease. Besides, in left-sided colitis, no significant decrease in hemoglobin value was detected compared to distal type colitis. This may be attributed to the higher amount of bleeding in extensive colitis/pancolitis compared to the other types. Consistent with the present study, Kalaycı et al. reported that a significant decrease in hemoglobin value was present in patients with extensive colitis in UC (24).

ESR is widely used as a biomarker of IBD activity (25,26). It is less consistent with changes in disease activity compared to CRP. However, in the study conducted by Costa et al. in both UC and CD, it was reported that neither ESR nor CRP differed significantly between the relapsing and non-relapsing groups (27). The present study does not provide information about relapse, however supports that there is a positive relationship between the progression of the disease's involvement and the increase in ESR value.

Although CRP is not specific for IBD, its advantageous properties as a biomarker are that it can be measured easily and reliably in diagnostic laboratories and has a short half-life. Elevated CRP levels show a modest correlation with endoscopic UC and are indicative of UC clinical activity. In 2008, Lok et al. undertook a study involving 49 Chinese UC patients. They explored the sensitivity and correlation of routine serum biomarkers, such as CRP, ESR, white blood cell count, hemoglobin, platelet count, and albumin, with clinical severity and mucosal inflammation (28). Their findings indicated a strong correlation between routine serum biomarkers and endoscopic extensive colitis, but not with proctitis or left-sided colitis. These biomarkers proved beneficial in identifying patients with extensive colitis or clinically severe disease. In a cohort study of 43 UC patients conducted according to the Mayo Clinic activity index, there was a significant relationship between high CRP values and disease activity among inflammatory biomarkers other than platelets (29). They observed a significant association between elevated CRP levels in CD patients with moderate to severe clinical activity, as well as with other studied biomarkers of inflammation, active disease during ileocolonoscopy, and severely active ileitis/colitis on biopsies. In a study conducted by Chouhan et al., it was reported that the CRP value is an easy method to determine the activity and extent of the disease (30). In the present study, CRP levels were found to be significantly higher in extensive colitis/pancolitis than in distal type colitis. This may be due to widespread mucosal damage occurring in extensive colitis/pancolitis or being normal in cases of unknown cause, as in the literature mentioned. The fact that this effect was not detected significantly in left-sided colitis can be thought to be due to less mucosal damage compared to extensive colitis/pancolitis.

In this study for CD, 80.3% of the change in probability regarding the type of disease can be explained by the values obtained because of the

laboratory results. This high rate supports that the parameters used are more helpful in the diagnosis of the disease compared to UC. In the study of Yeşil et al., it was shown that the sensitivity of CRP levels in cases diagnosed with CD was 93% and the specificity was 64%. It was also stated that the sensitivity of the ESR level in showing the activity of the disease was 86% and its specificity was 58%. (21). In Sarıtaş study, a significant change was found in hemoglobin, ESR and CRP values in ileal CD (31). In the study of Koçhan et al., no significant difference was present in leukocyte, hemoglobin, platelet, ESR and CRP levels according to CD subtype (19). In the study of Nogueira et al., it was stated that ESR, platelet and white blood cell values were insufficient to determine CD activity, and a significant decrease was observed in CRP levels after 32 weeks of treatment (32). In the study conducted by Marie et al. with 28 cases diagnosed with CD, they found that CRP levels were normal only in CD with soft mucosal lesions in cases with elevated CDAI. Although this normality is estimated to be due to genetic change in CRP levels, in the study conducted by Willot et al., no relationship was found between CRP levels and genotypes (33,34). In the study conducted by Solem et al. with CD, they found that CRP values were lower in ileal lesions than in colonic lesions (29). While CRP value is considered a valuable marker in evaluating relapse, remission and treatment response in CD, the results of this study concluded that it does not play a role in predicting localization.

The present study has some limitations. Major limitations were the retrospective nature of the study, along with an inadequate number of patients in the cohort and the absence of a control group. Furthermore, UC and CD activity indexes could not be determined because the complaints at the time of colonoscopy, the presence of extraintestinal involvement, physical examination findings, complications, accompanying the disease, and the disease pattern for CD were not recorded. Despite this, the fact that it was conducted in a large cohort makes this study valuable.

In conclusion, in addition to the parameters used in the follow-up of patients with IBD, other non-invasive, more reliable parameters may be needed, especially for CD. In addition, it was concluded that it would be more appropriate to record the complaints of the patients during admission, physical examination findings and colonoscopy records and determine the activity indexes to follow the patients and determine the treatment management more accurately.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

The Relationship of Gallstone Disease with Serum RBP4 Level, Vitamin D, Lipid Profile, Insulin Resistance and Uric Acid Levels

Safra Taşı Hastalığının Serum RBP4 Düzeyi, D Vitamini, Lipid Profili, İnsülin Direnci ve Ürik Asit Düzeyleri ile İlişkisi

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1424453
Geliş Tarihi | Received: 24.01.2024
Kabul Tarihi | Accepted: 01.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
İnci BK, İnci K, Paşaoğlu ÖT, Paşaoğlu H, Dumlu GŞ. The Relationship of Gallstone Disease with Serum RBP4 Level, Vitamın D, Lipid Profile, Insulin Resistance and Uric Acid Levels. Hitit Medical Journal 2024;6(2):161-169 https://doi.org/10.52827/hititmedj.1424453

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Çalışma Gazi Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından 26/05/2014 tarihinde onaylanmıştır (Karar No:278).
İntihal Kontrolleri: Evet - (Intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: BKİ, GŞD Tasarım: BKİ Veri Toplama/Veri İşleme: BKİ, Kİ, ÖTP Veri Analizi: ÖTP, HP, BKİ Makalenin Hazırlanması: BKİ, Kİ.
Hasta Onamı: Çalışmada kullanılan hasta verileri için hastalardan bilgilendirilmiş onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Ethical approval was received from Gazi University Clinical Research Ethics Committee on 25/5/2014 (No: 278).
Plagiarism Check: Yes - (Intihal.net)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: BKİ, GŞD Design: BKİ Data Collection/Data Processing: BKİ, Kİ, ÖTP Data Analysis: ÖTP, HP, BKİ Article Preparation: BKİ, Kİ.
Informed Consent: Informed consent was obtained from the patients for the patient data used in the study.
Financial Disclosure: There are no financial funds for this article.

The Relationship of Gallstone Disease with Serum RBP4 Level, Vitamin D, Lipid Profile, Insulin Resistance, and Uric Acid Levels

ABSTRACT

Objective: The metabolic parameters associated with gallstones are the subject of numerous studies. RBP4, an adipokine, has been linked to various metabolic diseases; however, no study in the literature establishes its relationship with gallstone disease. Therefore, our study aimed to evaluate the biochemical parameters associated with gallstone disease, primarily serum RBP4.

Material and Method: Between 2015 and 2016, abdominal ultrasound, serum biochemical tests, lipid profiles, uric acid, insulin, and fasting glucose values were available for 80 patients who presented to our hospital's gastroenterology clinic, were included in the study. RBP4 levels were analyzed in the serum samples obtained from the patients.

Results: Out of the 80 participants in the study, 42 had gallstones, while 38 did not. Among the biochemical parameters, no significant difference was found between the groups in terms of total cholesterol values *(p= 0.483)*, LDL values *(p=0.224)*, and TG values *(p=0.764)*. A significant difference was observed between the two groups regarding HDL values(p=0.017). No significant difference was found between the two groups in terms of serum uric acid *(p=0.411)*, fasting glucose *(p=0.214)*, fasting insulin, HOMA-IR score *(p=0.157)*, and vitamin D levels *(p=0.340)*. The mean±SD values of the studied serum RBP-4 levels in the participants were determined as 40.24±7.12 in the control group and 39.75±8.55 in the patient group. No statistically significant difference was found between the two groups *(p=0.776)*. In correlation analyses, a significant positive correlation was found between RBP4 and vitamin D levels *(r: 0.277, p= 0.013)*, total cholesterol *(r: 0.268, p=0.016)*, triglycerides *(r: 0.387, p<0.001)*, GGT *(r: 0.294, p=0.008)*, AST *(r: 0.299, p=0.007)*, and uric acid *(r: 0.255, p=0.022)*.

Conclusion: In conclusion, our study did not find a statistically significant relationship between gallstones and RBP4, vitamin D, LDL, TG, total cholesterol, uric acid, and HOMA-IR. However, our study found a positive correlation between vitamin D levels and RBP4. This has guided future research.

Keywords: Cholelithiasis, Gallstone Disease, RBP4.

ÖZET

Amaç: Safra taşları ile ilişkilendirilen metabolik parametreler birçok çalışmanın konusunu oluşturmaktadır. RBP4, bir adipokin olup metabolik hastalıklarla ilişkilendirilmiştir; ancak bildiğimiz dahilinde, safra taşı hastalığı ile ilişkisini belirleyen bir çalışma bulunmamaktadır. Çalışmamızda, öncelikle serum RBP4 olmak üzere safra taşı ile ilişkilendirilen biyokimyasal parametreleri değerlendirmeyi amaçladık.

Gereç ve Yöntem: 2015-2016 yılları arasında gastroenteroloji kliniğine başvuran 80 hastanın abdominal ultrason, serum biyokimyasal testler, lipid profilleri, ürik asit, insulin ve açlık glukoz değerleri mevcuttu ve çalışmaya dahil edildi. RBP4 seviyeleri, alınan serum örneklerinde analiz edildi.

Bulgular: Seksen hastanın 42'sinde safra taşı bulunurken, 38'inde yoktu. Biyokimyasal parametreler arasında, toplam kolesterol değerleri *(p= 0.483)*, LDL değerleri *(p=0.224)* ve trigliserit değerleri *(p=0.764)* açısından gruplar arasında anlamlı fark bulunmamıştır. İki grup arasında HDL değerleri açısından anlamlı fark gözlemlenmiştir *(p=0.017)*. Serum ürik asit *(p=0.411)*, açlık glukozu *(p=0.214)*, açlık insulin ve HOMA-IR skoru *(p=0.157)*, vitamin D seviyeleri *(p=0.340)* açısından iki grup arasında anlamlı fark bulunmamıştır. Çalışmaya katılan kişilerin incelenen serum RBP4 seviyelerinin ortalama±SD değerleri kontrol grubunda 40,24±7,12, hasta grubunda ise 39,75±8,55 olarak belirlenmiştir. İki grup arasında anlamlı fark bulunmamıştır *(p=0.776)*. Korelasyon analizlerinde; RBP4 ile vitamin D seviyeleri *(r: 0.277, p= 0.013)*, toplam kolesterol *(r: 0.268, p=0.016)*, trigliserit *(r: 0.387, p<0.001)*, GGT *(r: 0.294, p=0.008)*, AST *(r: 0.299, p=0.007)* ve ürik asit *(r: 0.255, p=0.022)* arasında anlamlı pozitif korelasyon bulunmuştur.

Sonuç: Sonuç olarak, çalışmamızda safra kesesi taşları ile RBP4, vitamin D, LDL, TG, toplam kolesterol, ürik asit ve HOMA-IR arasında anlamlı ilişki bulunamamıştır. Ancak çalışmamızda saptanan D vitamin ve RBP4 düzeyi arasındaki pozitif korelasyonun gelecekteki çalışmalar için yol gösterici olduğu düşünülmüştür.

Anahtar Sözcükler: Kolelitiazis, RBP4, Safra Taşı Hastalığı.

Introduction

The oldest information about gallstones is based on the Egyptians and Babylonians. Four thousand years ago, the Babylonians introduced some concepts and definitions of the gallbladder into their daily medical use. However, Alexander Trallius showed in the 5th century AD that stones can form in the gallbladder. Gallstones are a common disease; in the United States, 6 percent of men and 9 percent of women have gallstones (1). For this reason, pathogenesis and the factors that cause a disease with such an old history still attract the scientific world's attention today.

The increase in obesity and accompanying diseases all worldwide have raised the interest in adipose tissue. Studies have revealed that adipose tissue is not only a simple storage site for lipids but also an important tissue that plays a key role in regulatin gendocrine, metabolic, and inflammatory processes (2). Adipose tissue cells have been shown to secrete various bioactive proteins into the circulation. These secretory proteins called adipocytokines are leptin, tumor necrosis factor-α (TNF-α), plasminogen-activator inhibitor type 1 (PAI-1), adipsin, resistin, visfatin, adiponectin, retinol binding protein-4 (RBP4) and lipocalin-2 (2).

Adipocytokines released from adipose tissue cells and adipose tissue infiltrating macrophages cause chronic inflammation, leading to insulin resistance and type 2 diabetes (3). Studies have been conducted that the level of RBP4, an adipocytokine, increases insulin resistance and is associated with metabolic syndrome, type 2 diabetes mellitus, and cardiovascular diseases (4, 5). These diseases are also risk factors for the development of cholelithiasis. Considering this cause-effect relationship, higher RBP4, chemerin, and fibroblast growth factor 21 (FGF-21) levels have been observed in children with cholelithiasis (6). Some studies found a relationship between RBP4 level (increase-decrease) and cholelithiasis, but the literature data on this subject needs to be more comprehensive, sufficient, and contradictory. Thus, we aimed to determine the relationship between RBP4 level and cholelithiasis.

Metabolic syndrome is a disease complex by elevated fasting blood glucose, blood pressure, triglycerides, low high-density lipoprotein (HDL) cholesterol, and the presence of central obesity (7). Clustering these metabolic factors increases the risk of cardiovascular disease and type 2 diabetes (8). Previous studies have shown a significant association between cholelithiasis and metabolic syndrome (9,10). It was thought that insulin resistance and hyperlipidemia, which are the components of metabolic syndrome, may be associated with cholelithiasis is independent of diabetes and obesity. Therefore, as one of the aims of the study, it was planned to examine the relationship between cholelithiasis insulin resistance and hyperlipidemia.

Vitamin D inhibits lipogenesis, induces insulin synthesis, preserves islet cells, decreases insulin resistance, and reduces appetite, favoring obesity and T2DM control (11). Vitamin D deficiency is associated with stasis in the gallbladder, and a role for vitamin D supplementation is thought to have the potential to prevent gallstones, especially in pregnant women (12). Considering the effects of vitamin D on metabolism, it aims to reveal whether there is a change in vitamin D levels in patients with cholelithiasis compared to the average population. High uric acid plays a role in inflammation, insulin resistance, diabetes mellitus, hyperlipidemia, and hypertension. Based on this data in the literature, the relationship between uric acid level and cholelithiasis was examined, and conflicting data were obtained (13).

In light of the literature data, we aimed to examine the relationship between gallstone disease and serum RBP4 levels, vitamin D levels, and metabolic parameters in our patients.

Material and Methods

Patients aged 20-75 years who applied to the gastroenterology department of Gazi University between 2015-2016 and had upper abdomen imaging were included in our study. Our significance level (alpha) was set at 0.05. To determine the minimum required sample size, we utilized an effect size of 0.6, derived from a study by Wang et al. (14) investigating the relationship between RBP-4 concentrations and cholesterol gallstone disease. This effect size served as a benchmark for comparing means between the two groups. Following calculations using G Power software (15), we determined that a minimum of

72 subjects would be needed for our study. When the imaging was evaluated, gallstone disease was detected in 42 patients, and 38 patients without gallstone disease were included in the comparison group. Our study's ethics approval was obtained from the Gazi University Faculty of Medicine Clinical Research Ethics Committee with document number 278 dated May 26, 2014. Informed voluntary consent forms have been obtained from all patients. The blood fasting glucose, fasting insulin, liver function tests, kidney function tests, lipid profiles, and vitamin D levels of the patients included in the study were retrospectively screened. Our hospital's biochemistry laboratory studied the serum RBP4 levels of the patients. The informed consent form was obtained from all participants included in the study.

Inclusion criteria

a- The ages between 20-75 years,

b- Abdominal imaging performed within the last two years,

c- Not taking antihyperlipidemic treatment and vitamin D replacement therapy in the last year,

d- No diagnosis of chronic liver disease, stage 3-4-5 renal disease, diabetes mellitus, or an additional systemic disease

Exclusion criteria:

a- Pregnancy, lactation, obesity,

b- Those who do not fill out an informed consent form

Biochemical tests

Approximately 10 ml of peripheral blood sample from the forearm of all individuals included in the study was taken into separator tubes that did not contain any anticoagulant or other additives and centrifuged at 1000xg for 20 minutes after waiting for 30 minutes at room temperature to ensure coagulation. After centrifugation, approximately 1.5 ml samples from the separated serum samples were transferred to Eppendorf tubes and stored at -80^{0}C until the study day. Laboratory investigations of the research were carried out in the Biochemistry Laboratory of Gazi University Hospital Central Laboratory. RBP4 levels in serum samples were determined by the Enzyme-Linked Immunosorbent Assay (ELISA) method using the Human RBP4 ELISA Kit (BioCER). Each serum sample was detected twice.

Statistical analysis:

SPSS version 22.0 was used to analyze the variables (IBM Corporation, Armonk, New York, United States). The conformity of the data to the normal distribution was evaluated with the Shapiro-Wilk test, homogeneity of variance was evaluated with the Levene test, and the Independent-Samples T-test, one of the parametric methods, was used together with the Bootstrap results, while the Mann-Whitney U test, which is one of the nonparametric methods, was used with the Monte Carlo simulation technique in the comparison of the two independent groups according to the quantitative data. Spearman's rho test was used to examine the correlations of the variables with each other. In a comparison of categorical variables with each other, Pearson Chi-Square and Fisher Exact tests were tested with the Monte Carlo Simulation technique. While quantitative variables are shown as mean ± std. (Standard deviation) and median Range (Maximum-Minimum) and categorical variables as n (%) in the tables; the variables were analyzed at a 95% confidence level, and a p-value less than 0.05 was considered significant.

Results

Out of the 80 participants in the study, 42 had gallstones, while 38 did not. Among the 38 patients without gallstones, 26 (68.4%) were female and 12 (31.6%) were male. Of the 42 patients with gallstones, 29 (69%) were female, and 13 (31%) were male. No significant difference in terms of gender was observed between the two groups (*p=1*). The average age in the group without gallstones was 41.82±14.10, ranging from 20-64. In the group with gallstones, the average age was 49.00±14.32, with a range of 24-75. A significant age difference was found between the two groups, with the group with gallstones having a higher average age (*p=0.033*) (Table I).

There was no significant difference between the groups among the biochemical parameters in terms of total cholesterol values (*p= 0.483*), LDL values (*p=0.224*), and TG values (*p=0.764*) (Table II). However, a significant difference was observed between the two groups regarding HDL values, with lower HDL values in the cholelithiasis group (*p=0.017*) (Table II).

Liver function tests were evaluated, and significant differences were observed between the groups in

ALT (*p=0.021*), GGT (*p=0.018*), and ALP (*p=0.033*) values, with these parameters being higher in patients with cholelithiasis (Table II).

Table I. Age and gender characteristics of the participants

	Gallstone Disease (-)	Gallstone Disease (+)	Total	*p value*
Gender				
Female	26 (68.4%)	29 (69%)	55 (68.8%)	*1.0*
Male	12 (31.6%)	13 (31%)	25 (31.3%)	
Age				
Mean±STD (Min-max)	41.82 ± 14.10 (20-64)	49.00 ± 14.32 (24-75)	45.59 ± 14.58 (20-75)	*0.033*

Serum uric acid levels did not significantly differ between the two groups (*p=0.411*) (Table II). HOMA-IR scores were calculated by examining the participants' fasting glucose and simultaneous fasting insulin levels. No significant differences were found between the two groups in terms of fasting glucose (*p=0.214*), fasting insulin, and HOMA-IR score (*p=0.157*) (Table II).

Table II. Biochemical analysis of participants

	Gallstone Disease (-)	Gallstone Disease (+)	Total	*p value*
	Median (max-min)	Median (max-min)	Median (max-min)	
Total cholesterol	183.5 (123-391)	190 (101-337)	186.5 (101-391)	*0.483*
LDL	103.5 (35-250)	116 (64-222)	110.5 (35-250)	*0.224*
Triglyceride	111.5 (30-265)	106 (36-499)	109 (30-499)	*0.764*
HDL	49.5 (30-143)	44.5 (5.5-71)	46 (5.5-143)	*0.017**
ALT	18 (1-87)	21.5 (12-588)	20 (1-588)	*0.021**
AST	19 (5-111)	21 (1-471)	20 (1-471)	*0.512*
GGT	19 (7-99)	28 (9-369)	21 (7-369)	*0.018**
ALP	71 (39-118)	81.5 (34-337)	74 (39-337)	*0.033**
Total Bilirubin	0.455 (0.18-2)	0.545 (0.22-4.4)	0.148 (0.18-4.4)	*0.139*
Direct Bilirubin	0.165 (0.04-0.66)	0.2 (0.09-3.2)	0.185 (0.04-3.2)	*0.143*
Uric acid	4.35 (2.2-8.7)	4.65 (2-9.2)	4.45 (2-9.2)	*0.411*
HOMA-IR	1.445 (0.58-6.8)	1.725 (0.33-12.93)	1.56 (0.33-12.93)	*0.157*

* statistically significant, HDL (High-Density Lipoprotein), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), GGT (Gamma-Glutamyl Transferase), ALP (Alkaline Phosphatase).

No significant difference was found between the two groups regarding vitamin D levels (*p=0.340*) (Table III).

Table III. Vitamin D and Serum RBP4 analysis of participants

	Gallstone Disease (-)	Gallstone Disease (+)	Total	*p value*
Vitamin D Median (max-min)	19.95 (5-44)	15.85 (4-71)	17.75 (4-71)	*0.340*
RBP4 Mean ± STD	40.24 ± 7.12	39.75 ± 8.55	39.98 ± 7.86	*0.776*

The mean±SD values of the studied serum RBP4 levels in the participants were determined as 40.24±7.12 in the control group and 39.75±8.55 in the patient group. No statistically significant difference was found between the two groups (*p=0.776*) (Table III). In correlation analyses, a significant positive correlation was found between vitamin D levels and RBP4 (*r: 0.277, p= 0.013*). Significant positive correlations were also observed between RBP4 levels and total cholesterol (*r: 0.268, p=0.016*), triglycerides (*r: 0.387, p<0.001*), GGT (*r: 0.294, p=0.008*), AST (*r: 0.299, p=0.007*), and uric acid (*r: 0.255, p=0.022*) (Table IV).

Table IV. Correlation Analysis

	VITD		RBP4	
	r	*p value*	*r*	*p value*
Age	*-0.085*	*0,454*	*0,049*	*0,665*
Total cholesterol	*0.124*	*0,272*	*0,268**	*0,016**
LDL	*0.042*	*0,713*	*0,183*	*0,105*
Triglyceride	*0.169*	*0,133*	*0,387**	*<0,001**
HDL	*0.025*	*0.829*	*-0.081*	*0.476*
ALT	*-0.128*	*0.258*	*0.070*	*0.539*
AST	*0.038*	*0.740*	*0.299**	*0.007**
GGT	*0.207*	*0.065*	*0.294**	*0.008**
ALP	*0.018*	*0.874*	*0.152*	*0.178*
Total bilirubin	*-0.088*	*0.440*	*0.045*	*0.692*
Direct bilirubin	*-0.136*	*0.229*	*0.081*	*0.475*
Uric acid	*0.210*	*0.062*	*0.255**	*0.022**
Insulin	*-0.128*	*0.258*	*-0.083*	*0.465*
Fasting Glucose	*0.076*	*0.502*	*0.110*	*0.330*
HOMA-IR	*-0.099*	*0.380*	*-0.070*	*0.536*
RBP4	*0.277**	*0,013**		

*statistically significant, HDL (High-Density Lipoprotein), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), GGT (Gamma-Glutamyl Transferase), ALP (Alkaline Phosphatase).

Discussion

The diagnosis and treatment of gallbladder stone disease are crucial due to the potential complications and symptoms that can significantly impact the quality of life and lead to morbidity. Gallbladder Stones have both preventable and non-preventable etiological factors. Ethnic origin, advanced age, female gender, family history, and genetics are considered non-preventable etiological factors, whereas preventable factors include obesity, rapid weight loss, and a sedentary lifestyle (16). Understanding the relationship between gallbladder Stones and other diseases and illuminating relevant laboratory parameters is essential for identifying preventable factors.

Gender and age are among the most significant non-preventable risk factors for gallbladder stone disease. The female gender, especially in the pre-menopausal age group, has a two-fold higher risk compared to males. The underlying mechanisms for this include factors such as fertility, hormones, oral contraceptive treatments, and estrogen replacement therapies (16). For instance, in a study conducted in Germany 2005 involving 4,202 individuals aged between 20 and 74, the prevalence of gallbladder stones was twice as high in women compared to men (17). Similarly, in a study conducted in Nepal 2012, the female-to-male ratio was determined to be 7 to 1 (18).

In selecting our study participants, we aimed for no significant gender difference between the two groups, particularly considering that levels of vitamin D, RBP4, and lipid profiles may vary between female and male individuals. The study includes 80 patients, with 55 being female and 25 males. No significant difference was observed in gender distribution between patients with gallbladder stones and those without ($p=1.0$).

The frequency of gallbladder stones increases with age, particularly showing a 4-10-fold increase after age 40 (19). In a study conducted in the United States, it was demonstrated that the prevalence of gallbladder stones with age increased in both Mexican American men and women, reaching 44.1% in women aged 60-74 (20). In a study by Katsuroni Sekine and colleagues published in August 2015 in the Journal of Gastroenterology and Hepatology, a total of 717 patients were prospectively examined, and the group with gallbladder stones had a significantly higher mean age (21). In a study by Tazumo and colleagues in May 2015, the expected age range for 612 gallbladder stone patients was 60-70, with a mean age of 63 (22). In our study, the mean age of individuals with gallbladder stones was significantly higher than those without stones. Although the data obtained in our study is similar to literature data, the lower mean age values in our study are thought to be due to the exclusion criterion of patients with chronic diseases in the design of our study.

Dyslipidemia, insulin resistance, and obesity are significant components of metabolic syndrome, and the level of RBP4 is associated with these components. In light of this data, there might be variability in the RBP4 levels in gallbladder stone disease, which includes components of metabolic syndrome in its etiology (23,24). Our study was designed with this purpose in mind. The first study related to our hypothesis was conducted in 2009 by Shen-Nien Wang and colleagues, where a negative correlation between gallbladder stones and RBP4 was identified (14). Another study in 2013 by Han T. and colleagues found that elevated RBP4 levels were associated with metabolic syndrome and cholesterol gallstone formation (25). In another study conducted in 2021 on a pediatric patient group, higher serum RBP4 levels were found in children and adolescents with high body weight and gallbladder stones (6). Our study found no significant difference in RBP4 levels between the two groups with and without gallbladder stones. The limited number of studies on this topic with a small number of patients in the literature and conflicting results emphasize the need for more extensive and diverse studies with a larger number of patients.

Cholesterol-associated gallstones are the most common type of gallbladder stone. The high prevalence of cholesterol-associated gallstones has raised the possibility of a relationship between lipid profiles and gallstones in patients, leading to numerous studies on this topic (26-28). While hypertriglyceridemia and low HDL levels are associated with gallstone formation, the relationship with hypercholesterolemia has not been clearly defined (29, 30). Some studies have shown elevated triglyceride levels to reduce gallbladder motility (31). Our study, consistent with

the literature, did not find a significant relationship between total cholesterol levels, LDL, and gallbladder stones. However, HDL levels were significantly lower in the group with gallstones, supporting the existing literature. The literature contains conflicting data regarding the impact of triglyceride levels on gallstone formation. Our study observed no significant difference in triglyceride levels between the included patient and control groups. However, to enhance their liability of such a study, controlling and equalizing other parameters associated with hypertriglyceridemia between the control and patient groups would be necessary to eliminate differences. Since our study excluded obese patients, a one-to-one match in terms of BMI could not be achieved between the patient and control groups. This could contribute to our study's statistically insignificant association between triglyceride levels and gallbladder stones.

Insulin resistance, another diagnostic criterion for metabolic syndrome, has been the subject of many studies in relation to gallbladder stones. A study conducted by Atamer and colleagues in 2013 and a study published in 2011 shows the relationship between insulin resistance and gallbladder stones (32,33). However, some studies published in the same years with very large patient groups did not find a significant relationship between HOMA-IR and gallbladder stones. The variability in the results among studies highlights the need for further research with diverse patient populations to understand better the relationship between HOMA-IR and gallbladder stones (34). Our study examined serum fasting insulin and fasting glucose levels in non-diabetic patients with gallbladder stones or in the control group. Using these parameters, HOMA-IR levels were calculated in patients. No statistically significant differences were found between the two groups regarding fasting glucose, insulin, and HOMA-IR.

Uric acid is influential in developing many diseases associated with inflammation and accumulation. Among the most significant are components of metabolic syndrome, including insulin resistance, diabetes mellitus, hyperlipidemia, and hypertension (13), as well as non-alcoholic fatty liver disease (35). An epidemiological study in Taiwan investigated the relationship between uric acid levels and gallbladder stones, but no significant association was found. However, in some studies, a relationship between high uric acid levels and gallbladders tones has been identified in women (36). In light of these data, we planned to include patients' serum uric acid levels in our study. However, in our study, no significant difference was found in serum uric acid levels between the groups with and without gallbladder stones. Variability in results among studies regarding uric acid levels has been attributed to the difficulty in effectively controlling both modifiable and non-modifiable metabolic factors, making standardization challenging.

The effects of vitamin D on hormone secretion, regulation of immune functions, and the modulation of cell proliferation and differentiation are now well-known (37). The association between vitamin D deficiency and obesity, insulin resistance, and metabolic syndrome continues to be the subject of numerous studies (38). Our study also investigated the relationship between vitamin D and gallbladder stones based on its metabolic effects. No statistically significant difference was found between the patient and control groups in vitamin D levels. However, correlation analyses revealed a positive correlation between vitamin D deficiency and RBP4 values. This result can guide future studies in determining the correlation between vitamin D and RBP4 levels in metabolic syndrome, a complex endocrine issue with multiple parameters.

The strength of our study is that it is one of the few studies on this subject in the literature and increases the need for new studies due to conflicting results with these studies. Limitations of our study are that it was performed on a heterogeneous and small number of patients.

In conclusion, our study did not find a statistically significant relationship between gallbladder stones and RBP4, vitamin D, LDL, TG, total cholesterol, uric acid, and HOMA-IR. However, a negative association was observed between gallstones and HDL levels, and an increase in the frequency of gallstones with age was demonstrated. The conflicting and limited literature on the relationship between gallstones and RBP4 adds value to our study and guides future research. Nevertheless, the limited number of participants poses a disadvantage in determining correlations between parameters. The absence of

other literature publications on the relationship between gallstones and vitamin D suggests that our study could Pioneer new research. Despite various studies on lipid levels, uric acid, and HOMA-IR in the literature, the existence of conflicting data emphasizes the need for more extensive retrospective or prospective studies involving many patients.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Effects of Atrial Fibrillation on Cognitive Functions in Patients Between 65-75 Years of Age

65-75 Yaş Arası Hastalarda Atriyal Fibrilasyonun Kongnitif Fonksiyonlar Üzerine Etkileri

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1445822
Geliş Tarihi | Received: 01.03.2024
Kabul Tarihi | Accepted: 01.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Yiğit E, Okşen D. Effects of Atrial Fibrillation on Cognitive Functions in Patients Between 65-75 Years of Age. Hitit Medical Journal 2024;6(2):170-177 https://doi.org/10.52827/hititmedj.1445822

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: İstanbul Medipol Üniversitesi Klinik Araştırmalar Etik Kurulu'ndan onay alındı (Etik Kurul Tarih/No: 28.09.2023/784).
İntihal Kontrolleri: Evet - (ithenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: EY Tasarım: EY Veri Toplama/Veri İşleme: EY, DO Veri Analizi: DO Makalenin Hazırlanması: EY, DO. Hasta Onamı: Çalışmada kullanılan hasta verileri için hastalardan bilgilendirilmiş onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Approval was granted by the Istanbul Medipol University Clinical Research Ethics Committee (Ethics Committee Date / Number: 28.09.2023 / 784).
Plagiarism Check: Yes - (ithenticate)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: EY Design: EY Data Collection/Data Processing: EY, DO Data Analysis: DO Article Preparation: EY, DO.
Informed Consent: Informed consent was obtained from the patients for the study.
Financial Disclosure: There are no financial funds for this article.

Effects of Atrial Fibrillation on Cognitive Functions in Patients Between 65-75 Years of Age

ABSTRACT

Objective: Atrial fibrillation (AF) is the most common arrhythmia in the elderly population and also the most common cause of ischemic stroke. Ischemic stroke is directly related to cognitive decline. The relationship between atrial fibrillation and cognitive decline has long been associated with stroke. This study aimed to reveal whether the mere presence of atrial fibrillation, independent of stroke, has negative effects on cognitive functions.

Material and Method: Male and female patients between the ages of 65 and 75 with no chronic diseases other than known hypertension were included in the study. They were divided into two groups according to electrocardiography findings: the group with newly diagnosed atrial fibrillation and the group with normal sinus rhythm (NSR). To evaluate cognitive functions, the Montreal Cognitive Assessment (MoCA) was applied to both groups and then the groups were compared in terms of scores.

Results: No statistically significant difference was observed between the groups in terms of age, patient characteristics, educational status, or laboratory findings. MoCA scores were significantly lower in the AF group than in the NSR group (p=0.001). Multivariable linear regression analysis demonstrated lower age and higher education status were independently associated with high MoCA scores (β: 3.392, 95% CI: 2.375 - 4.410, p<0.001 / β: -0.478, 95% CI: -0.664 - -0.292, p<0.001, respectively). In addition, AF was independently associated with low MoCA scores after adjustments by age and education status (β:-2.463, 95% CI: -3.448 - -1.478, p<0.001).

Conclusion: AF is a risk factor for cognitive decline regardless of the presence of ischemic stroke.

Keywords: Arrhythmia, atrial fibrillation, cognitive decline.

ÖZET

Amaç: Yaşlı popülasyonda en sık görülen aritmi olan atriyal fibrilasyon (AF), aynı zamanda iskemik inmenin de en sık nedenidir. İskemik inme bilişsel gerilemeyle doğrudan ilişkilidir. Atriyal fibrilasyon ile bilişsel gerileme arasındaki ilişki yıllardır inme ile ilişkilendirilmiştir. Bu çalışma, inmeden bağımsız olarak sadece atriyal fibrilasyon varlığının bilişsel işlevler üzerinde olumsuz etkilerinin olup olmadığını ortaya koymayı amaçlamıştır.

Gereç ve Yöntem: Çalışmaya bilinen hipertansiyon dışında kronik hastalığı olmayan, 65-75 yaş arası erkek ve kadın hastalar dahil edildi. Elektrokardiyografi bulgularına göre hastalar yeni tanı alan AF grubu ve normal sinüs ritmi (NSR) grubu olmak üzere ikiye ayrıldı. Bilişsel işlevleri değerlendirmek amacıyla her iki gruba da Montreal Bilişsel Değerlendirme (MoCA) uygulandı ve gruplar sonuçlar açısından karşılaştırıldı.

Bulgular: Gruplar arasında yaş, hasta özellikleri, eğitim durumu ve laboratuvar bulguları açısından istatistiksel olarak anlamlı fark yoktu. MoCA skoru AF grubunda NSR grubuna göre anlamlı derecede düşüktü (p=0,001). Çok değişkenli doğrusal regresyon analizi, düşük yaş ve yüksek eğitim durumunun bağımsız olarak yüksek MoCA puanıyla ilişkili olduğunu gösterdi (β: 3,392, %95 GA: 2,375- 4,410, p<0,001 / β: -0,478, %95 GA: -0,664 - -0,292, p<0,001, sırasıyla). Ayrıca AF, yaş ve eğitim durumundan bağımsız olarak düşük MoCA puanıyla ilişkili bulundu (β:-2,463, %95 GA: -3,448 - -1,478, p<0,001).

Sonuç: AF, iskemik inmenin varlığından bağımsız olarak bilişsel gerileme için bir risk faktörüdür.

Anahtar Sözcükler: Aritmi, atriyal fibrilasyon, bilişsel gerileme.

Introduction

Preserving cognitive functions in elderly patients is important in terms of both maintaining quality of life and preventing morbidity and mortality. Any undesirable event that may disrupt cranial vascularization poses a threat to brain functions. Ischemic stroke is the most important condition that threatens cerebral health and atrial fibrillation is the most common cause of ischemic stroke in patients over 65 years of age (1,2). Most studies in the literature have shown a linear relationship between AF and cognitive dysfunction (3). This relationship between AF and cognitive decline has been linked to the relationship between AF and ischemic stroke for years.

However, some studies have suggested that cognitive functions may decline in AF patients without a history of stroke (4). Silent cerebral infarctions, cerebral hypoperfusion resulting from fluctuations in ventricular rate, systemic inflammation, and changing hemodynamic conditions may have an impact on cognitive functions (5,6).

The Montreal Cognitive Assessment (MoCA) is one of many screening tests used in clinical practice to evaluate cognitive functions. This test, developed for the detection of moderate-mild cognitive impairment, is a rapid mini-mental test with high sensitivity and is easy to apply (7).

Clearly identifying risk factors is important to prevent cognitive decline. In the present study, cognitive functions in AF patients without a history of ischemic stroke were evaluated with the MoCa mini-mental test and compared with patients in normal sinus rhythm. The aim was to reveal whether the presence of AF without a history of ischemic stroke regresses cognitive functions.

Material and Method

This study was a double-center, case-control study which included male and female patients aged 65-75 who applied to the Istanbul Medipol University Hospital internal medicine and Altınbaş University cardiology outpatient clinic between September 2023 and February 2024 and were not diagnosed with chronic diseases other than hypertension.

Detailed anamnesis of the patients was taken, and physical examinations were performed. Blood pressure measurements were taken from both arms and the average was calculated (Omron M3 Upper Arm Blood Pressure Monitor). Electrocardiograms were examined (EDAN SE1200 12-channel ECG device). Patients who were diagnosed with AF (first diagnosis) according to electrocardiography findings and volunteers with normal sinus rhythm in the similar age group were included in the study. The initial diagnosis of AF was made by detecting a heart rhythm with no visible repeating P waves and irregular RR intervals on the standard 12-lead ECG recording. The first diagnosis of AF was defined as the first detection of AF, regardless of the presence/severity of symptoms and duration. To exclude patients with paroxysmal AF, patients whose ECG findings were compatible with AF were called for control after 10 days and electrocardiography was repeated. Echocardiography was performed on all patients (Philips Envisor HD Ultrasound Device). Patients with left ventricular ejection fraction ≥ 50% and echocardiography findings within normal limits were included in the study.

Demographic characteristics of the patients were recorded. Peripheral blood samples were taken after a 12-hour fast. Hemogram and simple biochemical analysis were performed.

The patients were subjected to a mini-mental test (MoCA) study under the supervision of a clinician. MoCA, which takes approximately 10 minutes to administer evaluates different cognitive functions including attention and concentration, executive functions, memory, language, visual structuring skills, abstract thought, calculation, and orientation. The highest total score that can be obtained from the test is 30. Accordingly, 21 points and the score obtained in the MoCA are considered normal (8). The atrial fibrillation group and the control group were compared in terms of MoCa test results. CHA2DS2-VASC score of patients with atrial fibrillation was calculated. It was examined whether there was a correlation between this score and MoCa.

Patients with any rheumatic valve diseases, moderate or severe valve insufficiency, mild or more severe valve stenosis and underwent valve repair or valve replacement treatment, patients with congenital heart disease, ischemic heart disease, structural heart disease, patients with a neurological disease such as

dementia/Alzheimer's, a history of ischemic stroke/transient ischemic attack, patients with psychiatric diseases, and patients with a metabolic abnormality that could cause cognitive dysfunction in blood tests were excluded from the study. Additionally, since the test may be affected by education level, only high school and university graduate patients were included. Only patients diagnosed with hypertension and who did not use any medication other than antihypertensive agents were included in the study. In addition to patient statements, all examination information and prescription details were reviewed from personal health record systems, and the information was confirmed.

All subjects received information about the content of this study and signed a written consent form before participating. All procedures complied with the Declaration of Helsinki and approval was granted by the Istanbul Medipol University Clinical Research Ethics Committee (Ethics Committee Date / Number: 28.09.2023 / 784).

According to descriptive statistics (effect size=0.739) in the study by Dautzenberg et al. (9) a sample size of 40 for each group (80 in total) was used to achieve 90% power at the two-sided 0.05 significance level. The sample size was calculated by using two-sample t test power analysis (Hintze, J. (2011). PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com).

Statistical Analysis

IBM SPSS Statistics for Windows Version 25.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Histograms and Q-Q plots were used to examine the conformity of the variables to normal distribution. Descriptive statistics were presented by using mean ± standard deviation for normally distributed continuous variables, median (25th percentile - 75th percentile) for non-normally distributed continuous variables, and frequency (percentage) for categorical variables. Normally distributed continuous variables were analyzed with the independent samples t test. Non-normally distributed continuous variables were analyzed with the Mann-Whitney U test. Categorical variables were analyzed using the chi-square test. Relationship between MoCA score and CHA2DS2-VASC score was evaluated using Spearman correlation coefficient. Linear regression analyses were performed to determine significant factors independently associated with the Montreal Cognitive Assessment score. Variables were analyzed using univariable linear regression analysis and statistically significant variables were included in the multivariable linear regression analysis. Two-tailed p-values of less than 0.05 were considered statistically significant.

Results

Included in the study were 44 individuals with atrial fibrillation (AF) and 57 individuals with normal sinus rhythm (NSR). The median age of all individuals (49 females and 52 males) was 69 (interquartile range 66 - 71, range 65 - 75) years. There was no significant difference between the AF and NSR groups in terms of age, gender, educational status, systolic blood pressure, diastolic blood pressure, and blood results including fasting blood sugar, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), thyroid stimulating hormone (TSH), and hemogram parameters.

Table I. Summary of variables and analysis results with regard to groups

	AF (n=44)	NSR (n=57)	p value
Age (year)	69 (66 - 71.5)	69 (67 - 71)	*0.815*
Sex			*0.387*
Female	24 (54.55%)	25 (43.86%)	
Male	20 (45.45%)	32 (56.14%)	
Education status			*0.718*
High school	23 (52.27%)	33 (57.89%)	
University	21 (47.73%)	24 (42.11%)	
Systolic blood pressure (mmHg)	128.5 (121- 133)	128 (120- 134)	*0.658*
Diastolic blood pressure (mmHg)	76.55 ± 7.31	75.47 ± 7.89	*0.486*
Fasting blood glucose (mg/dl)	84.66 ± 7.65	83.19 ± 8.40	*0.368*
Urea (mg/dl)	27 (21.5- 30)	24 (19- 29)	*0.268*
Creatinine (mg/dl)	0.60 ± 0.15	0.61 ± 0.13	*0.788*
ALT (U/L)	28 (23- 29.5)	28 (19- 30)	*0.675*
AST (U/L)	23.98 ± 5.20	23.84 ± 6.37	*0.909*
TSH (µIU/Ml)	2.8 (1.65- 3.9)	2.8 (1.8- 4.2)	*0.891*
Hemoglobin (g/dL)	12.96 ± 0.64	13.17 ± 0.63	*0.103*
Montreal Cognitive Assessment score	22.80 ± 3.13	25.12 ± 3.37	*0.001*
Normal (≥21)	34 (77.27%)	51 (89.47%)	*0.164*
Low (<21)	10 (22.73%)	6 (10.53%)	

Descriptive statistics were presented by using mean ± standard deviation for normally distributed continuous variables, median (25th percentile - 75th percentile) for non-normally distributed continuous variables and frequency (percentage) for categorical variables.

MoCA scores of all individuals were 24.11 ± 3.45.

Table II. Association between variables and Montreal Cognitive Assessment score, linear regression analysis results.

	Univariable			Multivariable		
	Unstandardized coefficients (95% CI)	Standardized coefficients	*p value*	Unstandardized coefficients (95% CI)	Standardized coefficients	*p value*
Age (years)	-0.477 (-0.687 - -0.267)	-0.413	*<0.001*	-0.478 (-0.664 - -0.292)	-0.414	*<0.001*
Sex, Male	0.013 (-1.356 - 1.383)	0.002	*0.985*			
Education status, University	3.210 (1.991 - 4.430)	0.465	*<0.001*	3.392 (2.375 - 4.410)	0.491	*<0.001*
Systolic blood pressure (mmHg)	-0.033 (-0.102 - 0.037)	-0.093	*0.355*			
Diastolic blood pressure (mmHg)	-0.101 (-0.189 - -0.013)	-0.223	*0.025*	0.002 (-0.067 - 0.070)	0.004	*0.960*
Fasting blood glucose (mg/dl)	-0.010 (-0.095 - 0.076)	-0.022	*0.825*			
Urea (mg/dl)	0.034 (-0.054 - 0.122)	0.077	*0.446*			
Creatinine (mg/dl)	1.810 (-3.146 - 6.766)	0.073	*0.470*			
ALT (U/L)	-0.016 (-0.126 - 0.093)	-0.030	*0.767*			
AST (U/L)	0.026 (-0.091 - 0.143)	0.044	*0.662*			
TSH (µIU/Ml)	-0.535 (-1.010 - -0.059)	-0.219	*0.028*	0.005 (-0.389 - 0.400)	0.002	*0.978*
Hemoglobin (g/dL)	0.831 (-0.234 - 1.896)	0.154	*0.125*			
Atrial fibrillation	-2.327 (-3.627 - -1.027)	-0.336	*0.001*	-2.463 (-3.448 - -1.478)	-0.356	*<0.001*
Adjusted R^2	-			0.492		
Regression model	-			f=20.396, *p<0.001*		

CI: Confidence interval

MoCA scores were significantly lower in the AF group than in the NSR group (*p=0.001*) (Figure I). Overall, 16 (15.84%) individuals had low (<21) MoCA scores. Low (<21) MoCA scores were detected in 10 patients (22.73%) in the AF group and 6 patients (10.53%) in the NSR group (Table I).

Table III. Correlation between Montreal Cognitive Assessment score and CHA2DS2-VASC score

	Overall	AF group	NSR group
r	*0.026*	*0.130*	*0.031*
p	*0.797*	*0.399*	*0.817*

r: Spearman correlation coefficient

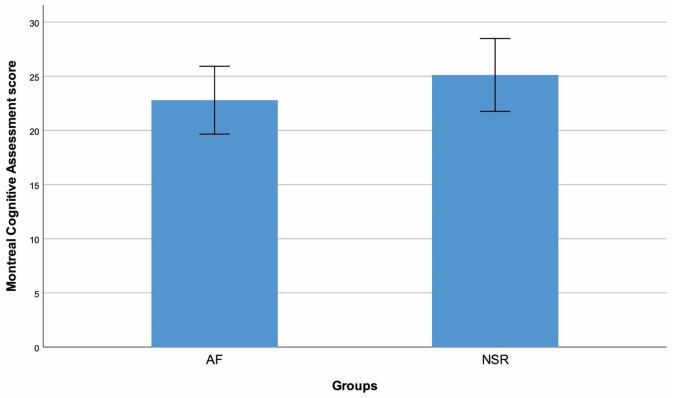

Figure I. Comparison of Montreal Cognitive Assessment (MoCA) score in AF and NSR groups (AF: Atrial Fibrillation, NSR: Normal Sinus Rhythm)

According to the multivariable linear regression analysis results, lower age (β: -0.478, 95% CI: -0.664 - -0.292, *p<0.001*) and higher education status (β: 3.392, 95% CI: 2.375 - 4.410, *p<0.001*) were independently associated with high MoCA scores. In addition, AF (β: -2.463, 95% CI: -3.448 - -1.478, *p<0.001*) was independently associated with low MoCA scores after adjustment for age and education status (Table II). We found no correlation between MoCA score and CHA2DS2-VASC score in all individuals (*r=0.026, p=0.797*), in the AF group (*r=0.130, p=0.399*) and in the NSR group (*r=0.031, p=0.817*) (Table III).

Discussion

The elderly population is increasing due to longer life spans. Age is a risk factor in itself that affects cognitive functions. Identifying and taking precautions for additional risk factors can help preserve cognitive function. In this study, it was determined that cognitive functions were significantly worse in patients with AF without a history of ischemic stroke than in patients with NSR. In other words, AF is a risk factor for cognitive decline, regardless of whether there is a history of ischemic stroke or not.

Silent cerebral infarctions, cerebral hypoperfusion resulting from fluctuations in ventricular rate, systemic

inflammation, and changing hemodynamic conditions are the main hypotheses explaining the relationship between atrial fibrillation and cognitive decline. Microemboli were detected at a rate as high as 30% by transcranial doppler ultrasonography in patients diagnosed with AF (10). To wit, microemboli, which have no obvious clinical consequences and cause silent infarctions, are found at a high rate in patients with AF. These silent infarcts may be the cause of cognitive decline (11).

The effect of hypoperfusion, another hypothesis, is explained as follows: the atrium systoles at the end of passive diastole and creates end-diastolic pressure in the ventricle. In AF, the atrium cannot perform systolic function, causing a negative effect on diastolic functions. In AF, cardiac output decreases with the withdrawal of atrium systolic support. It may cause hypoperfusion of the brain, leading to a progressive decrease in cognitive functions (12).

AF is a disease that originates from the atria but has widespread systemic effects. Fibroblast proliferation and differentiation increases in AF. Oxidative stress pathways are triggered. It may affect cognitive functions by creating localized but systemic proinflammatory effects (13).

Additionally, AF has been shown to be associated with endothelial damage. Platelet dysfunction may occur due to the pathophysiology of the disease, or the effect of the agents used (14,15).

It has been shown by different imaging methods that AF causes decline in cognitive functions. In a magnetic resonance study performed on individuals without a history of stroke, volume loss was observed in white and gray matter in the frontal cortex and cerebellum in patients with AF (16).

Various studies have been carried out around the world on this subject. In a cross-sectional study including 952 male individuals aged between 69 and 75 years, the adjusted mean cognitive score was lower in individuals with AF (+0.14+/-0.03; P=0.0003) when comorbidities and patient characteristics were adjusted (17). In a study conducted by Bunch et al, in which 37,025 cases were prospectively evaluated, a strong correlation was observed between all types of dementia, including Alzheimer's, senile and vascular dementia, and AF. Additionally, monitoring dementia in AF patients has predictive importance in terms of mortality (4). In recent studies, AF patients are divided into two: chronic and paroxysmal. The cognitive functions of both the chronic and paroxysmal AF group were found to be lower than those with normal sinus rhythm. When the chronic and paroxysmal AF groups were compared among themselves, the scores of the chronic AF group were found to be lower. This suggests that longer duration for AF is related to more decline in cognitive functions (6). A recently published systematic review reported that AF increases the impairment of cognitive functions by 1.7 to 3.3 times, independent of stroke (11).

Like these studies, in the present study, the MoCA scores, through which cognitive functions were evaluated, were significantly lower in the atrial fibrillation group. Additionally, patients with scores below normal (<21) were 22.73% in the AF group and 10.53% in the NSR group. Multivariate regression analyses also revealed that older age and the presence of AF were independently associated with lower MoCA score.

Some studies in the literature could not detect a significant relationship between AF and cognitive decline. A prospective study conducted by Park et al. on 74 newly diagnosed AF and 86 control patients, could not detect a relationship between AF and cognitive decline (18). However, in this study, the average age was 75.6 years and 59% of the patients died during the 36-month follow-up period. The reason for not finding a significant relationship may be that the remaining patients were healthy, which in turn affected the result. In another study conducted by O'Connell et al., although differences were detected between the AF group and the control group in terms of some verbal and non-verbal memory tests and some attention tests, no significant difference could be detected between the two groups in terms of cognitive functions when examined cumulatively. The reason for this may be that the number of patients diagnosed with AF included in the study consisted of a very low number of 27 (19). In a study including 533 patients, no relationship was found between AF and cognitive decline, but all patients included in this study were 85 years of age and older. Researchers interpreted this to mean that AF may not have made a significant difference because cognitive decline in very old people is affected by many other major

factors (20).

As can be seen, in cross-sectional, case-control, and cohort studies designed in different ways in the literature, patients with atrial fibrillation and normal sinus rhythm have been compared in terms of tests evaluating cognitive functions. Although there are conflicting results, results similar to those achieved in the present research have been reported more frequently.

The current study was a small-scale study conducted on 101 patients. Multicenter studies with a larger patient population are needed. Since all the patients included in this present study were newly diagnosed with atrial fibrillation, the duration of AF is unknown. Rhythm holter could be installed in patients to distinguish paroxysmal / permanent atrial fibrillation, but it could not be done due to cost. Additionally, heart failure with preserved ejection fraction was ruled out based on the fact that the patients did not have symptoms and signs of heart failure and their echocardiograms were normal, but NT-pro BNP could not be measured due to high cost.

Conclusion

The current study determined that patients with AF had more impairment in cognitive functions than individuals in sinus rhythm with similar demographic characteristics. Knowing the effects of AF on cognitive functions is especially valuable for close follow-up of elderly individuals. Early detection and treatment of AF is important to preserve cognitive functions in elderly individuals.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Evaluation of Blood Product Requests in the Emergency Department: A Prospective Observational Study

Acil Serviste Kan Ürünü İstemlerinin Değerlendirilmesi: Prospektif Gözlemsel Bir Çalışma

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1399940
Geliş Tarihi | Received: 04.12.2023
Kabul Tarihi | Accepted: 13.03.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Özdamar Y, Demir MC, Ağaçkıran İ, Aksu NM. Evaluation of Blood Product Requests in the Emergency Department: A Prospective Observational Study. Hitit Medical Journal 2024;6(2):178-185 https://doi.org/10.52827/hititmedj.1399940

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Hacettepe Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu, onay numarası: GO 18/498-21, tarih 5 Haziran 2018.
İntihal Kontrolleri: Evet - (ithenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: YÖ, NMA, MCD Tasarım: YÖ, NMA, MCD Veri Toplama/Veri İşleme: YÖ, MCD, İA, NMA Veri Analizi: YÖ, MCD, İA, NMA Makalenin Hazırlanması: YÖ, NMA, İA, MCD.
Hasta Onamı: Hastan onamı alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Hacettepe University Non-Invasive Clinical Research Ethics Committee, approval ID: GO 18/498-21, dated June 5, 2018.
Plagiarism Check: Yes - (ithenticate)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: YÖ, NMA, MCD Design: YÖ, NMA, MCD Data Collection/Data Processing YÖ, MCD, İA, NMA Data Analysis: YÖ, MCD, İA, NMA Article Preparation: YÖ, NMA, İA, MCD.
Informed Consent: Consent has been obtained from the patient
Financial Disclosure: There are no financial funds for this article.
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This study was the doctoral thesis of one of the authors and was approved by the local non-interventional ethics committee with approval ID: GO 18/498-21, dated June 5, 2018.

Evaluation of Blood Product Requests in the Emergency Department: A Prospective Observational Study

ABSTRACT

Objective: Emergency department (ED) blood product requests are rising, but it is unclear if they are excessive. We aimed to examine the blood product requests and usage rates made by emergency physicians to determine whether the requests for blood products are excessive.

Material and Method: This prospective, observational, and single-center study analyzed demographic data indications for blood transfusion, and transfusion rates of patients aged 18 years and older admitted to a tertiary adult emergency department in five months.

Results: One thousand four hundred seventy-five blood product requests (with a mean of 6.92 units per patient) were examined. Of 63.1%, these requests were not used. The transfusion rates were 40.89 % for erythrocyte suspension, 25.61% for thrombocyte suspension, and 44.34% for fresh frozen plasma. The main indications for requesting blood products were gastrointestinal bleeding and anemia. Specifically, 30.04% of erythrocyte suspensions, 31.9% of thrombocyte suspensions, and 60.56% of fresh frozen plasma were used for patients with gastrointestinal bleeding. In trauma patients, 12.75% of requested erythrocyte suspensions, 0.083% of thrombocyte suspensions, and 13.89% of fresh frozen plasma were utilized.

Conclusion: Excessive requests for blood products in the emergency department can misuse resources. The transfusion committee should develop specialized strategies and increase physician awareness to reduce unnecessary requests and optimize resource utilization.

Keywords: Erythrocyte suspension, blood products, fresh frozen plasma, resource utilization, transfusion, thrombocyte suspension.

ÖZET

Amaç: Acil servis kan ürünü istemleri artıyor, ancak aşırı olup olmadığı belirsizdir. Acil hekimleri tarafından yapılan kan ürünü istemlerini ve kullanım oranlarını inceleyerek kan ürünleri istemlerinin aşırı olup olmadığını tespit etmeyi amaçladık.

Gereç ve Yöntem: Bu prospektif, gözlemsel ve tek merkezli çalışma, beş ay içinde üçüncü basamak bir yetişkin acil servisine başvuran 18 yaş ve üzeri hastaların demografik verilerini, kan transfüzyonu endikasyonlarını ve transfüzyon oranlarını incelemiştir.

Bulgular: Bin dört yüz yetmiş beş kan ürünü istemi (hasta başına ortalama 6,92 ünite) incelendi. Bu istemlerin %63,1'i kullanılmadı. Transfüzyon oranları eritrosit süspansiyonu için %40,89, trombosit süspansiyonu için %25,61 ve taze donmuş plazma için %44,34 idi. Kan ürünleri istemenin ana endikasyonları gastrointestinal kanama ve anemiydi. Gastrointestinal kanaması olan hastalarda spesifik olarak eritrosit süspansiyonlarının %30,04'ü, trombosit süspansiyonlarının %31,9'u ve taze donmuş plazmaların %60,56'sı kullanıldı. Travma hastalarında istenen eritrosit süspansiyonlarının %12,75'i, trombosit süspansiyonlarının %0,083'ü ve taze donmuş plazmaların %13,89'u kullanıldı.

Sonuç: Acil serviste kan ürünlerine yönelik aşırı istemler, kaynakların yanlış kullanılmasına neden olabilir. Transfüzyon komitesi, gereksiz istekleri azaltmak ve kaynak kullanımını optimize etmek için özel stratejiler geliştirmeli ve hekim farkındalığını arttırmalıdır.

Anahtar Sözcükler: Eritrosit süspansiyonu, kan ürünleri, kaynak kullanımı, taze donmuş plazma, transfüzyon, trombosit süspansiyonu.

Introduction

In recent years, blood transfusions in the emergency department (ED) have increased significantly compared to other clinical departments (1). This situation reveals the necessity of plans for blood transfusion management in EDs, which are the first responders to the preventable deaths due to bleeding.

The availability of blood products relies on continuous donations from the public. The blood donation rate required to meet basic blood needs is considered to be 1% of the country's population, but more than 70 countries worldwide cannot reach this rate (2). Globally, approximately 85 million units of erythrocyte suspensions (ESs) are transfused each year. Studies have indicated that 5-58% of blood products are transfused unnecessarily or inappropriately (1). Insufficient volunteer blood donors and inadequate storage conditions pose significant challenges. Furthermore, the tendency of physicians to routinely request large amounts of blood leads to a range of problems, including excess reserved blood after cross-matching, inventory issues, loss of shelf life, increased costs, and wastage of blood products (1,2).

There is limited data regarding the extent of blood product overuse in the ED. This study aims to determine the factors influencing the need for blood transfusion in ED patients and to identify usage rates of blood products requested from the ED, with the aim of reducing unnecessary requests.

Material and Methods

Study Design and Setting

This study was designed as a prospective-observational single-center study. The patients 18 or older admitted to a tertiary academic adult ED between July 1 and Dec 1, 2018, who were requested a blood product by an emergency doctor, were included in the study.

Blood product requests and transfusion decisions were made by emergency doctors, and the clinical decisions and patient management process were not interfered with. The demographic characteristics and comorbidities of the patients; the clinical indications of the blood products requests, requested blood product types, amounts, and transfusion numbers were recorded. The crossmatch/transfusion ratio (CTR) and transfusion ratio (TR) values were calculated. Hemoglobin (Hb, g/dL), hematocrit (Hct, %), mean erythrocyte volume (MCV, fL), red blood cell distribution width (RDW, %), mean platelet volume (MPV, fL), plateletcrit (PCT, %), platelet count (plt, 10^3 mm^3), activated partial thromboplastin time (aPTT in seconds), international normalized ratio (INR), fibrinogen (mg/dL), and Glasgow Blatchford Scores were also recorded.

This study was the doctoral thesis of one of the authors and was approved by the local non-interventional ethics committee with approval ID: GO 18/498-21, dated June 5, 2018.

Participants and Measurements

Within the scope of the study, a total of 213 patients who were requested blood products and provided consent to participate were examined. Patients (n=8) who were transferred to another center for blood product transfusion were excluded from the study. *Crossmatch-transfusion rate (CTR)* was used to evaluate the blood product request and utilization practices in the hospitals. A ratio of 1 indicates that all requested blood products were transfused, and no unnecessary requests were made. *Transfusion rate* (TR) was defined as the percentage of transfused blood products to crossmatched products (3,4).

Glasgow Blatchford Score is a scoring system that incorporates vital signs, physical examination findings, patient history, and laboratory parameters to assist clinicians in determining acute treatment needs for gastrointestinal bleeding (5).

The presence of any of the following symptoms along with anemia was considered symptomatic anemia: Bleeding, weakness, fatigue, tachycardia, tachypnea, palpitations, shortness of breath, especially on exertion, near syncope, syncope, chest pain and decreased exercise tolerance, hypotension, or pallor of the skin.

The first diagnosis the clinician thought of when the patient was admitted was the preliminary diagnosis. After the results were obtained and all procedures were completed, the patient's diagnosis was expressed as the final diagnosis.

Statistical Analyses

Statistical analyses were performed using IBM SPSS for Windows Version 22.0 package program.

Numerical variables were summarized as mean ± standard deviation and median [min - max] values, while categorical variables were presented as numbers and percentages. The normality of numerical variables was assessed using the Kolmogorov-Smirnov test, and the equality of variances among groups was examined using Levene's test. The Kruskal-Wallis test was used to compare more than two groups. The Spearman correlation coefficient was used to assess the relationship between numerical variables. The chi-square test was employed to determine the relationship between categorical variables. The cut-off points for distinguishing between the groups of used blood products was determined through ROC curve analysis. Sensitivity and specificity values for the optimal cut-off point were reported, and the area under the ROC curve was calculated. The significance level was accepted as $p<0.05$.

Results

The mean age of the total 213 patients was 57.68 ± 18.794 years, and 60.6% (n=129) of them were male. Comorbidities were present in 77% (n=164) of the patients. The most common comorbid disease was non-hematological malignancy with a ratio of 29.1% (n=62) (Table I). A total of 53.5% (n=114) of patients had signs of active bleeding.

According to the indications for blood product requests, the preliminary diagnoses of the patients and the percentages were as follows: Hemorrhagic shock 9.9% (n=21), cardiogenic shock 0.9% (n=2), septic shock 6.1% (n=13), symptomatic anemia of unknown cause 32.9% (n=70), intracranial hemorrhage 1.4% (n=3), trauma 18.3% (n=39), gastrointestinal bleeding 13.6% (n=29), thrombocytopenia 2.8% (n=6) and coagulopathies 5.6% (n=1). Blood products were prepared for urgent surgery in 22.5% (n=48) of the patients.

If examined in terms of their final diagnosis, 23% (n=49) had gastrointestinal system bleeding, 18.8% trauma (n=40), 7% shock (n=15), 2.3% a need secondary to a defined malignancy (n=5), 22.1% anemia (n=47), 6.6% bleeding from a mass (n=14), 4.7% hemoptysis (n=10), 2.3% thrombocytopenia (n=5), 1.4% intracranial hemorrhage (n=3), 3.8% elevated INR (n=8), 1.9% epistaxis (n=4), and 1.9% had aortic dissection (n=4).

In this study, it was found that a total of 1,475 blood product units were requested for a total of 213 patients: 719 units of ESs, 445 units of thrombocyte suspensions (TSs), 309 units of fresh frozen plasma (FFP), and 2 units of cryoprecipitates. Table II presents the data on blood product requests and the amounts transfused.

Table I. Baseline characteristics of patients.

Features	n (%)
Age, years[a]	57.68 ± 18.794
Gender, male	129 (60.6)
Comorbid disease[*]	164 (77.0)
Non-hematologic malignancy	62 (29.1)
Hypertension	58 (27.2)
Coronary artery disease	41 (19.2)
Diabetes mellitus	29 (13.2)
Hematologic malignancy	21 (9.9)
Chronic kidney disease	17 (8)
Chronic liver disease	11 (5.2)
Aplastic anemia	4 (1.8)
Factor VIII deficiency	3 (1.4)
Osler-Weber disease	1 (0.4)
Hemolytic anemia	1 (0.4)
Polycythemia vera	1 (0.4)
Thalassemia major	1 (0.4)
Platelet function disorder	1 (0.4)

[a]: Mean ± SD; *: Some patients had multiple comorbidities.

For patients with gastrointestinal bleeding, the usage rates of blood products were 30.04% for ESs, 31.9% for TSs, and 60.56% for FFP. In trauma patients, the usage rates were 12.75% for ESs, 0.083% for TSs, and 13.89% for FFP. ESs and TSs were used significantly higher in the case of symptomatic anemia without signs of active bleeding compared to other indications such as gastrointestinal bleeding and trauma ($p<0.001$; $p<0.002$, respectively). FFP was most commonly used for cases with high INR values ($p<0.010$). The rates of blood product usage according to the indications are presented in Table III.

Table II. Distribution of data on blood products requested and transfused

Blood products	Erythrocyte Suspension	Thrombocyte Suspension	Fresh Frozen Plasma	Total
Request, unit	719	445	309	1,475*
Usage, unit	294	114	137	545
Transfusion rate (%) (Number of transfused units / Number of crossmatches)	40.89	25.61	44.34	36.9
Crossmatch/transfusion ratio (Number of crossmatches/ Number of transfused units)	2.44	3.90	2.25	2.70
Average request per patient, units**	3.38 ± 2.451	2.09 ± 4.301	1.45 ± 3.13	6.92
Average usage per patient, units**	1.45± 2.036	0.54 ± 1.661	0.64 ± 2.045	2.56

*2 patients were requested for cryoprecipitate. **Mean ± SD.

The mean values of Hb, Htc, MCV, RDW, aPTT, fibrinogen, platelet, and platelet volume of the patients are presented in Table IV. There was a moderate negative correlation between the initial hemoglobin values of the patients and the usage ratios of ESs (*r=-0.645; p<0.001*).

Table III. Evaluation of the utilization rates of blood products based on indications for request

Blood Products And Request İndications	Usage Rate %	*p value**
Erythrocyte Suspension		*<0.001*
Symptomatic anemia (without bleeding)	64.73	
Shock	51.11	
Gastrointestinal bleeding	30.04	
Trauma	12.75	
Thrombocyte Suspension		*<0.002*
Symptomatic anemia (without bleeding)	51.30	
Gastrointestinal bleeding	31.9	
Trauma	0.083	
Fresh Frozen Plasma		*<0.010*
Elevated INR	64.58	
Gastrointestinal bleeding	60.56	
Trauma	13.89	

**p< 0.05* is significant. INR: International normalized ratio

Table IV. Pre-transfusion laboratory results of patients

	Mean value	Standard Deviation	Minimum	Maximum
Hemoglobin (gr/dL)	9.615	3.4572	1.9	19.9
Hematocrit (%)	29.287	10.2065	11.5	62.9
MCV (fL)	86.68	13.582	22	146
RDW (%)	17.45	4.552	10	43
aPTT (sn)	28.06	16.255	11	160
INR	1.4835	1.05359	0.70	9.30
Fibrinogen (mg/dL)	404.08	177.238	64	950
Platelet (/µL)	205805.16	180339.501	3000	1680000
MPV (fL)	8.514	1.4452	0.8	14.2
PCT (%)	0.17934	0.262964	0.003	3.600

MCV: Mean erythrocyte volume, RDW: Red blood cell distribution width, aPTT: Activated partial thromboplastin time, INR: International normalized ratio, MPV: Mean platelet volume, PCT: Plateletcrit

Discussion

Since emergency physician acts in the nature of making quick decisions and providing patient stabilization, they tend to request blood products excessively. This may cause unnecessary demand or improper consumption of blood product reserves. As a matter of fact, in our study, it was seen that 63% of the blood product requests were not used. The low transfusion rate and high CTR reveal the gravity of the issue.

According to our study that ESs and TSs were the most preferred blood products among blood transfusions by emergency physicians due to symptomatic anemia of unknown cause without signs of active bleeding. Gastrointestinal bleeding was the second-line indication with the highest number of ESs, TSs, and FFP requests; traumas were the third.

The blood transfusion rate in ED, the first visiting and intervention place for critically ill patients, have been increased compared to other clinical departments in recent years (1). It reveals the necessity of planning for blood transfusion management in EDs. Within the framework of these plans, it is important to properly evaluate each patient before administering a blood transfusion. This helps to ensure that the transfusion is necessary and that the correct type and amount of blood product is used. By doing so, we can avoid unnecessary transfusions and reduce the over-request of blood products. Today, blood products found to

be compatible are reserved on behalf of the patients after the compatibility tests between the patients requested for blood products, and the products are studied in blood centers. Afterward, it is kept in the inventory for specified periods and cannot be used for another patient before expiration. This makes it difficult for blood products to be used within their limited life and causes problems such as clot formation, segmentation, and hemolysis, especially in ESs. Emergency physicians' tendencies to request large volumes of blood products as part of their habits or routines; causes a series of problems, ranging from increased percentages of reserved blood products, inventory problems, deterioration of cells in these products due to inappropriate storage conditions, loss of shelf life, increased costs, and destruction of the blood product before it can be transported. Many studies have shown that transfusion rates are pretty low compared to the amount of blood requested (6-9).

Approximately 61% of the patients in our study were male. The mean age of all patients was 57.68 ± 16.45. In a study conducted by Kelly et al. in ED, the mean age of 255 transfused patients was 67, with 63% male (10). Cobain et al. examined the demographic data of patients who received a blood transfusion in the United States, England, Germany, and Australia and they reported that most of the transfused patients were male (range 51-52.7%), and transfusion rates increased with increasing age (11).

When the underlying diseases of the patients were examined, many comorbid diseases were detected, especially hematological and non-hematological malignancies. This depends on the population of our hospital patient profiles (our hospital has a large oncologic center). The final diagnoses of blood product requests were as follows, in request from most to least: gastrointestinal bleeding (23%), anemia (22.1%), trauma (18.8%), shock (7%), hemoptysis (4.7%), and high INR (3.8%). Other studies on this issue reported that the most common cause of transfusion was gastrointestinal bleeding (10-12). Although the rates varied in our study, blood product requests were mostly due to gastrointestinal bleeding and anemia, consistent with the literature.

The literature has reported that blood product usage rates vary between 39-66.4% (9, 10, 13). In our study, the blood product request/use rate was 36.9%. Our study's low demand/use rate was likely due to the high number of blood product requests made early in the admission of unstable patients to the ED.

In this study, in which a total of 719 units of ESs were requested (mean 3.38 ± 2.241 units) for 213 patients, we found that only 294 units (mean 1.45 ± 2.036 units) were transfused. The CTR of this tertiary ED was 2.44, and its TR was 40.89%. We revealed that only one out of every three units of ESs requests was transfused. In a study that included 1487 patients, blood requests and transfusion rates were examined over six months, and it was reported that the TR was 64.2% and the CTR was 1.6% throughout the hospital (9). In a study by Subramanian et al. examining 252 patients admitted to the trauma center, it was shown that the CTR was 2.5 (13). The results of our study show that the CTR is quite low, and the rate of unnecessary requests is high in blood transfusions in the ED.

The TR of ESs according to clinical indication in our study was as follows: symptomatic anemia 64.73%, shock 51.11%, gastrointestinal bleeding 30.04%, and trauma 12.75%. There was a significant difference between the rates of ESs use according to the diagnosis. The few rates of blood product use in trauma patients show that physicians habitually request inappropriate and excessive amounts of blood products at the time of first admission in these patients. It may be due to an urgency reflex, or it may be due to fear of malpractice. Health policies should take steps to prevent this misuse of resource utilization, and physicians should internalize clinical decision guides. The amount of unused blood product was observed to be quite high, especially in patients who started massive transfusion protocol. In a study by Dunbar et al. in which they examined the use and destruction of blood products due to massive transfusion protocols in three trauma centers, the rate of ESs use was 39-65%, the FFP usage rate was 43-66%, and thrombocyte use was found in patients who initiated massive transfusion protocols, unlike our center. It has been reported that the rate of use of cryoprecipitate varies between 67-93% (14).

It has been reported that using the Glasgow

Blatchford Scale would benefit studies conducted to evaluate the blood needs of gastrointestinal patients, the most frequently transfused patient group in EDs (15-17). In our study, the TR was found to be 30.04% for patients with gastrointestinal bleeding. A statistically significant moderate correlation was found between the Glasgow Blatchford Scale, which was examined to determine these patients' blood transfusion needs, and ESs use rates.

In our study, the mean Hb value for which transfusion was decided was 9.61±3.46 gr/dL, and the mean Htc value was 29.29±10.21%. In the study of Sadeghi et al., blood transfusions of 1000 patients were examined, and the mean Hb value before blood transfusions was found 7.4 ±2.3 gr/dL in ED; it was reported that it was 7.5 ±1.0 gr/dL in internal services, 10.4 ± 2.6 gr/dL in surgical services, and 9.1±2.3 gr/dL in intensive care units. It was determined that 22% of the patients (n=219) received ESs transfusions inappropriately and unnecessarily, even though the Hb value was greater than 10 g/dL (18). In the study of Diaz et al., the data of patients who received a total of 908 units of ESs transfusion in the ED were examined, and it was shown that 21.4% of blood transfusions were performed with inappropriate indications, according to pre-transfusion Hb values. 100% of the transfusion decision in patients with Hb value <7 g/dL, 95% in patients with Hb 7.0-7.9 g/dL, and Hb 8.0-8.9 gr/dL was reported to be 71% accurate (1). In a study by Kelly et al. in ED, the mean Hb value before transfusion was found to be 8.14 ±2.59 gr/dL (10). The Hb transfusion threshold value specified in the blood transfusion guidelines is 7-8 gr/dL on average (19-21). In our study, the mean Hb value before transfusion was higher in the ED compared to the literature, indicating that ESs transfusions were performed inconsistently with the guidelines' recommendations. Making incorrect decisions regarding ES transfusion can result in an increase in unnecessary requests.

In our study, the TSs transfusion rate was 25.61%. When evaluated according to the diagnoses, it was seen that this rate was 31.9% in gastrointestinal bleeding patients and 0.083% in trauma patients. In our study, there was a statistically significant moderate negative correlation between the rate of transfused TSs and PCT value *(r=0.407; p=0.001)*. Our literature review found no study on the correlation between PCT and blood transfusion rate.

The study of Reed et al., examining the use of blood products in the ED of their center in previous years, revealed that due to the strategy they utilized, the rate of blood product requests decreased by 64%, and the rate of transfusion by 39% (22). Within the scope of the results of our study, we believe that blood transfusion strategies should be developed by making continuous analyses to improve ED blood transfusion management. Sharing feedback on blood product requests and use with emergency physicians and involving clinical guidelines routinely at every stage of patient management will provide continuous improvement (20, 23, 24). It will reduce unnecessary requests and improper usage of blood products.

Conclusion

In the ED, blood product requests are mainly for gastrointestinal bleeding and anemia. We have revealed that the blood product requests for trauma patients are not used to a large extent. Hospitals must develop their transfusion strategies and protocols to optimize blood transfusion and demand quantities, and all hospital units have to follow this protocol consistently.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Brentuximab Vedotin Monotheraphy in Relapsed/Refractory T Cell Lymphoma Setting-Real Life Data

Relaps /Refrakter T Hücreli Lenfomada Brentuksimab Vedotin Monoterapisi-Gerçek Yaşam Verisi

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1320606
Geliş Tarihi | Received: 20.11.2023
Kabul Tarihi | Accepted: 23.02.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Darçın T, Serin İ, Ugur MC, et al. Brentuximab Vedotin Monotheraphy in Relapsed/Refractory T Cell Lymphoma Setting-Real Life Data. Hitit Medical Journal 2024;6(2):186-192 https://doi.org/10.52827/hititmedj.1320606

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Çalışma Abdurrahman Yurtaslan Onkoloji Araştırma ve Eğitim Hastanesi etik kurul onayı doğrultusunda gerçekleştirildi (etik onay no: 2023-02/03).
İntihal Kontrolleri: Evet - (ithenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: MA, MSD, BT, FA Tasarım: TD, TH, SD, İA Veri Toplama/Veri İşleme: TD, İS, MCU, ÖE, İYH, SA, EG, MHD, AD, MN Veri Analizi: TD, SN, SB Makalenin Hazırlanması: TD.
Hasta Onamı: Hasta onamı alınmasına gerek yoktur.
Finansal Destek: Finansal destek alınmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: The study was carried out in accordance with the approval of the ethics committee of Abdurrahman Yurtaslan Oncology Research and Training Hospital (ethical approval no: 2023-02/03)
Plagiarism Check: Yes - (ithenticate)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: MA, MSD, BT, FA Design: TD, TH, SD, İA Data Collection/Data Processing: TD, İS, MCU, ÖE, İYH, SA, EG, MHD, AD, MN Data Analysis: TD, SN, SB Article Preparation: TD.
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

Brentuximab Vedotin Monotheraphy in Relapsed/Refractory T Cell Lymphoma Setting-Real Life Data

ABSTRACT

Objective: We present data of patients with relapsed/ refractory T cell lymphomas treated with brentuximab vedotin (BV) in real-world practice.

Material and Method: This study is an observational, multi-center, retrospective study. The data of patients (n=17) treated with BV alone from January 2014 until July 2020 in thirteen centers from Turkey were collected.

Results: Bv was given as salvage chemotherapy to 17 patients with median age of 53. Nine (52.9%) patients had diagnosis of peripheral T cell lymphoma, not otherwise specified; 8 (47.1%) patients had anaplastic large T cell lymphoma. The median follow-up of the cohort was 20 months. Nine (52.9%) patients had complete response, 5 (29.5%) had partial response, 3 (17.6%) had progressive disease. The safety results aligned with the established profile of BV, included 2 pneumonia and 1 thrombocytopenia with grade 4. The median progression free survival of the cohort was 10 months. BV cycle and response to BV therapy were found to have an effect on the univariate analysis.

Conclusion: In patients with relapsed/ refractory T cell lymphomas, BV seems to have convincing antitumor activity with favorable safety profile.

Keywords: Brentuximab vedotin, relapse, T cell lymphoma.

ÖZET

Amaç: Brentuksimab vedotin (BV) ile tedavi edilen Relaps /Refrakter (R/R) THL'li hastaların gerçek yaşam verilerini sunmayı amaçladık.

Gereç ve Yöntem: Bu çalışma gözlemsel, çok merkezli, retrospektif bir çalışmadır. Ocak 2014'ten Temmuz 2020'ye kadar Türkiye'deki on üç merkezde yalnızca BV ile tedavi edilen tüm hastaların (n=17) verileri toplandı.

Bulgular: Ortanca yaşı 53 olan 17 hastaya kurtarma kemoterapisi olarak BV verildi. Dokuz (%52,9) hastaya periferik T hücreli lenfoma, diğer türlü sınıflandırılamayan tanısı konurken, 8 (%47,1) hastaya anaplastik büyük T hücreli lenfoma tanısı konuldu. Kohortun ortanca takip süresi 20 aydı. Dokuz (%52,9) hastada tam yanıt, 5 (%29,5) hastada kısmi yanıt, 3 (%17,6) hastada ilerleyici hastalık görüldü. Güvenlik verileri BV bilinen profiliyle tutarlıydı, 2 pnömoni ve 4. dereceli 1 trombositopeniyi içeriyordu. Grubun medyan progresyonsuz sağkalımı 10 aydı. Siklus sayısının BV tedavisine yanıtın üzerinde etkili olduğu değişkenli analiz ile bulundu.

Sonuç: R/R THL'leri olan hastalarda BV olumlu güvenlik profili ile tatmin edici antitümör aktivitesine sahip olduğu görülmektedir.

Anahtar Sözcükler: Brentuksimab vedotin, nüks, T hücreli lenfoma.

Introduction

T-cell lymphomas (TCLs) are a diverse category of non-Hodgkin lymphomas, encompassing many subclasses such as angioimmunoblastic T-cell lymphoma (AITL), anaplastic large-cell lymphoma (ALCL), mycosis fungoides (MF) and others (1). TCLs respond poorly to traditional chemotherapeutics, and their prognosis is dismal. Currently, first-line regimens with low response rates include CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) based regimens (2). Since most patients who receive first-line medications experience refractoriness or early recurrence, multiple trials have tested innovative medicines for these patients in recent years (3).

Anti-CD30 monoclonal antibody and cytotoxic antitubulin agent monomethyl auristatin E (MMAE) combine to form the antibody-drug combination product known as BV (4). After at least one previous multidrug chemotherapy regimen failed, the FDA authorized BV in August 2011 for the treatment of individuals with systemic ALCL. About malignant lymphoid tumors, BV has some advantages: a) has a stronger and longer duration of activity, b) has relatively stable concentrations, c) is less toxic to normal cells, d) may eradicate CD30+ non-malignant cells in the microenvironment that have protumor effects (5). In one study, 34 patients with at least two lines of treatment history and resistant CD30+ PTCL were treated with single-agent BV; 24% achieved complete remission (CR), and 14% achieved partial remission (PR). Among 13 patients diagnosed with AITL, five had a CR, and two had a PR. Of the twenty-one patients with PTCL, NOS, three achieved a CR and four a PR. In this trial, median progression-free survival (PFS) was 6.7 and 1.6 months, respectively (6). It is known that prospective randomized study results and real-life experiences could be different.

Here, we conducted a retrospective multicenter study on a cohort of relapsed or refractory TCLs patients treated with BV monotherapy. This study aimed to report real-life outcomes of BV monotherapy.

Material and Method

This is a retrospective, multi-center, observational research. We collected the data of patients (n: 17) treated with BV alone in thirteen centers from Türkiye between January 2014 to July 2020 using an automated database and a methodical chart review. Immunohistochemistry study was determined by each center. Refractory illness was defined as a recurrence that occurred within six months of the last therapy and less than a partial response (PR). Relapsed disease was defined as a relapse following at least a partial response.

Any drug-related side effects during the BV therapy were recorded. The clinical importance of adverse events was assessed. (For example, grade 3-4 hematologic toxicity or other severe effects that necessitated the termination or discontinuation of BV medication)

The response rate was assessed at the discretion of the individual physician using the 2007 updated response criteria for malignant lymphoma (7) and the International Working Group revised response criteria for malignant lymphoma (8).

The complete response rate (CRR) was defined as the proportion of CR found during BV therapy. The ORR was computed by summing the CR and PR rates obtained during BV treatment. Progression-free survival (PFS) is calculated from the start of BV therapy to progression, relapse, or death from any cause. Survival was calculated based on the final follow-up visits throughout the research period or if medication was changed for reasons other than progression or regression. Overall survival (OS) was estimated using survivors' most recent follow-up visit from the start of BV treatment to death from any cause.

The study was carried out in accordance with the Declaration of Helsinki and with the approval of the ethics committee of Abdurrahman Yurtaslan Oncology Research and Training Hospital (ethical approval no: 2023-02/03)

For analysis, IBM SPSS, Version 26.0 (IBM Corp., Armonk, N.Y., USA) was used. To demonstrate patient and illness characteristics, descriptive statistics were used. The median (minimum-maximum) value for continuous variables was used. Categorical variables were presented as numbers and percentages. PFS was estimated using Kaplan-Meier survival analysis. To assess the parameters impacting PFS, Cox regression analysis was used. Initially, univariate analysis was performed, and components with p-values less than

0.25 were included in multivariate analysis. $p < 0.05$ was considered statistically significant.

Results

Nine (52.9%) patients had a diagnosis of PCTL, NOS whereas, 8 (47.1%) patients had anaplastic large T cell lymphoma. The median age was 53 (21-78) and there was a male predominance (n=11(64.7%)). All patients were positive for CD30 by immunohistochemistry. The median follow-up of the cohort was 20 (4-87) months. Table I summarizes the patients' baseline demographic data and clinical characteristics.

Table I. Demographic Data and Patients' Clinical Features

Parameters	N=17 100%)
Gender (Male/Female)	11/6
Age (median)	53 (21-78)
Follow-up duration (months)	20 (4-87)
B symptom	8 (47.1%)
Bulky disease	3 (17.6%)
Performance (ECOG ≥2)	5 (29.4%)
Subtype	
Peripheral T cell NOS	9 (52.9%)
Anaplastic large T cell	8 (47.1%)
Advanced Stage (Ann Arbor 3-4)	13 (76.5%)
Extranodal involvement	8 (47.1%)
Bone marrow involvement	6 (35.3%)
Risk Scores	
International Prognostic Index (High-intermediate, High)	6 (35.3%)
AA- International Prognostic Index (High-intermediate, High)	7 (41.1%)
Prognostic Index for PTCL-U(PIT) (Group 3-4)	9 (52.9%)
International T cell lymphoma Project (Group 3-4)	9 (52.9%)

AA: age-adjusted, ECOG: Eastern Cooperative Oncology Group, NOS: not otherwise specified.

BV was given at a dose of 1,8 mg/kg every three weeks until progression or unacceptable toxicity for salvage chemotherapy to 17 patients.

After the evaluation of BV response 9 (52.9%) patients had a complete response, 5 (29.5%) had a partial response, and 3 (17.6%) had progressive disease. Patients had median 2 (1-6) line treatments, 16 (94.1%) received conventional chemotherapies and 1 (5.9%) received autologous stem cell transplant before BV initiation. The median age of BV initiation was 56 (21-78). BV was administered for median 6 (1-19) cycles. BV was utilized as salvage treatment before ASCT (bridge to SCT setting) in 7 (17.6%) patients and before allo-SCT in 1 (5.9%) patient. BV was discontinued in patients mostly for ASCT and progression. The BV data is demonstrated in Table II.

Table II. Brentuximab Therapy data

	N=17 (100%)
Age (median)	56 (21-78)
Previous CT lines (median)	2 (1-6)
BV cycle (median)	6 (1-19)
B symptom	8 (47.1)
Bulky disease	3 (17.6)
Performance (ECOG ≥2)	4 (23.5%)
Previous treatments	
CT	16 (94.1%)
ASCT	1 (5.9%)
Response Rate (ORR)	14 (82.3%)
CR	9 (52.9%)
PR	5 (29.5%)
PD	3 (17.6%)
Cause of Discontinuation	16 (94.1%)
Progression	6 (35.3%)
Bridging to Allo-SCT	1 (5.9%)
Bridging to ASCT	7 (17.6%)
Adverse Event	1 (5.9%)
Death	1 (5.9%)

ASCT: autologous stem cell transplant, BV: brentuximab vedotin, CT: chemotherapy, CR: complete response, ECOG: Eastern Cooperative Oncology Group, ORR: overall response rate, PR: partial response, PD: progressive disease.

Hematological toxicity was observed on 8 (47.1%) occasions. Neutropenia was observed in grades 1-2, thrombocytopenia was variable in grades 1-4. Pneumonia 4 (23.5%), neuropathy 3 (17.6%), renal failure 2 (11.7%), hyperkalemia 2 (11.7%), and infusion reactions 2 (11.7%) were the most seen non-hematological adverse events. BV was stopped in one patient due to adverse events. Other patients did not need dose reduction (Table III).

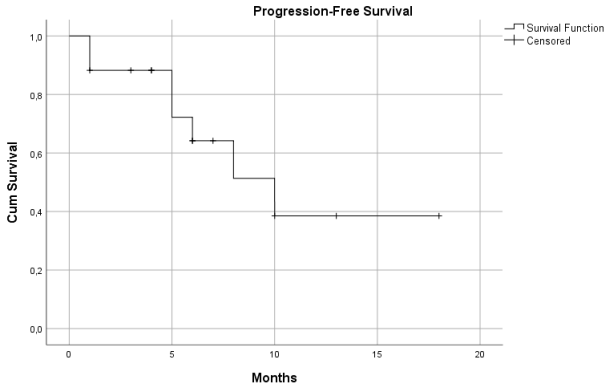
The median PFS of the cohort was 10 months (95% CI, 5.077-14.923). PFS curves are shown in Figure I. Median OS was not reached, and 4 patients deceased in the follow-up period.

Table III. Adverse Events

Adverse Events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Neutropenia	3 (17.6%)	1 (5.9%)			
Pneumonia	1 (5.9%)	1 (5.9%)		2 (10.8%)	
Neuropathy	2 (10.8%)		1 (5.9%)		
Hyperkalemia	1 (5.9%)		1 (5.9%)		
Acute renal failure	1 (5.9%)	1 (5.9%)			
Thrombocytopenia	1 (5.9%)	1 (5.9%)	1 (5.9%)	1 (5.9%)	
Infusion reactions			2 (10.8%)		

Common Terminology Criteria for Adverse Events (CTCAE) v5.0 were used for reporting adverse events.

Factors influencing PFS were analyzed, age at diagnosis, gender, clinical data-stage, extranodal disease, subtype, ECOG, received chemotherapy lines, and risk scores did not seem to affect outcome of BV therapy. BV cycle and response to BV therapy were found to affect the PFS; however, in multivariate analysis, no factor was identified to have a significant effect (Table IV).



Median PFS = 10 months (95% CI, 077-14.923)

Figure I: Progression-Free Survival

Table IV. Factors Predicting Progression-Free Survival in Brentuximab Therapy

	Univariate Analysis		Multivariate Analysis	
	Odds Ratio (95% CI)	***p value***	**Odds Ratio (95% CI)**	***p value***
Age at diagnosis	1.011 (0.973-1.050)	*0.586*		
Gender (based female)	1.222 (0.271-5.506)	*0.794*		
The stage at diagnosis (based on early stage)	2.595 (0.432-15.595)	*0.320*		
Extranodal disease at diagnosis (based on none)	0.635 (0.137-2.940)	*0.561*		
BM involvement at diagnosis (based on none)	3.378 (0.401-28.473)	*0.263*		
Subtype (based on PTCL NOS)	1.258 (0.279-5.665)	*0.765*		
Age at BV initiation	1.008 (0.968-1.049)	*0.697*		
Pre-BV CT lines (based ≤2)	0.976 (0.217-4.399)	*0.975*		
BV cycle (based ≤4)	14.401 (1.262-164.377)	*0.032**	4.414 (0.328-59.491)	*0.263*
Pre-BV ECOG (based ECOG 0-1)	0.968 (0.178-5.270)	*0.970*		
Pre-BV bulky disease (based none)	0.554 (0.106-2.980)	*0.484*		
Pre-BV B symptom	0.805 (0.166-3.903)	*0.787*		
Response (based on non-responders)	13.750 (1.430-132.184)	*0.023**	7.057 (0.541-92.053)	*0.136*
SCT (based none)	0.526 (0.101-2.731)	*0.445*		
IPI (based low-intermediate)	0.819 (0.182-3.689)	*0.794*		
AA_IPI (based low-intermediate)	1.379 (0.295-6.450)	*0.683*		
PIT (based group 1-2)	0.867 (0.188-3.991)	*0.854*		
ITLP (based groups 1-2)	1.421 (0.316-6.393)	*0.647*		

*Statically significant

AA_IPI: Age-adjusted International Prognostic Index, BM: bone marrow, BV: brentuximab vedotin, CT: chemotherapy IPI: International Prognostic Index, ECOG: Eastern Cooperative Oncology Group,ITLP: International T cell lymphoma Project, PIT: Prognostic Index for PTCL-U, PTCL-NOS: Peripheral T cell not otherwise specified. SCT: Stem cell transplantation

Discussion

With an observed 82.4% ORR and 52.9 percent CR, our results seem superior to those previously published in the context of patients with recurrent T cell lymphoma receiving chemotherapy. The OS was not reached and the median PFS was 10 months (2). It might not be realistic to draw this comparison

given the lower size of our cohort, but this could be explained by the fact that in our cohort, the patients were not exposed to BV or other targeted novel therapies. In a single-arm trial of 34 patients with recurrent T cell lymphoma treated with BV as a single drug, the response rate we saw seemed to be noticeably higher (ORR and CR within 41% to 82.4% and 24% to 52.9%, respectively) (6). The fact that our sample is younger and had less severe illness may help to explain our findings. After responding to BV treatment, 1 (5.8%) patient received allo-SCT, and 7 (41.1%) patients underwent ASCT, among the eligible patients of our 17 TCLs. Four patients (23.5%) had died during a follow-up of a median of 20 months (range 4-87 months) following the documentation of refractory/relapsed illness. The cohort's median PFS was 10 months (95% CI, 5.077-14.923). The median OS was not attained.

Age at diagnosis, gender, clinical stage, presence of extranodal disease, subtype of TCLs, ECOG performance status, previous chemotherapy history, and prognostic risk scores were not found to influence the outcome of BV therapy. The number of BV cycles and response to BV therapy were found to affect PFS, but no factor was identified as having a significant effect in multivariate analysis.

Although we were unable to define any causes that influence OS, our findings demonstrate the therapeutic importance of achieving CR. Our study had a limitation that our cohort was too small, and we were unable to statistically evaluate the parameters related to PFS or OS.

Neutropenia was observed slightly more than thrombocytopenia. There was no report of neutropenia of grade 3 or higher. One patient had grade 4 thrombocytopenia (5,9%). Pneumonia 4 (23.5%), neuropathy 3 (17.6%), renal failure 2 (11.7%), hyperkalemia 2 (11.7%), and infusion reactions 2 (11.7%) were the most seen non-hematological adverse events. BV was discontinued in one patient due to peripheral neuropathy. Other patients did not need dose reduction. Unlike the previous trials, we did not discover a greater level of toxicity in our entire cohort (6,9,10,11). In contrast, we have fewer peripheral neuropathies. This might be explained by underreporting of these episodes in patients' medical records, a well-known limitation of retrospective investigations. The low prevalence of neuropathies in our sample might be attributed to many of our patients' short-term BV exposure, as peripheral neuropathy is connected to cumulative BV exposure (12).

Conclusion

For orphan diseases due to rare incidence, sometimes it is not feasible to conduct randomized controlled studies, in these conditions real-life reports carry major significance. To the best of our knowledge, our study is the first study that presents real-life experience data for BV.

Our findings support the use of BV monotherapy as a therapeutic option for patients with R/R T-cell lymphomas. Its usage as a monotherapy has been related to an improvement in ORR, making it a feasible choice for young and SCT-naive patients as a bridge to high-dose treatment. The effectiveness of BV in T cell lymphomas, which is an unmet medical need, surely needs more research.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Malignant Melanomas Localized to the Parotid Gland

Parotis Bezi Yerleşimli Malign Melanomlar

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1429875
Geliş Tarihi | Received: 01.02.2024
Kabul Tarihi | Accepted: 08.04.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Düzköprü Y, Han Ü, Koçanoğlu A, Doğan Ö, Şahinli H, İmamoğlu Gİ. Malignant Melanomas Localized to the Parotid Gland. Hitit Medical Journal 2024;6(2): 193-199 https://doi.org/10.52827/hititmedj.1429875

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Çalışmanın onayı Ankara Etlik Şehir Hastanesi Klinik Araştırmalar etik kurulu tarafından verildi. (sayı: AEŞH-EK1-2023-783, tarih:10.01.2024)
İntihal Kontrolleri: Evet - (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: YD, AK Tasarım YD, AK Veri Toplama/Veri İşleme: OD, HŞ Veri Analizi: OD, HŞ Makalenin Hazırlanması: UH, Gİİ.
Hasta Onamı: Onam alınmasına gerek yoktur.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: The approval of the study was granted by the Clinical Research Ethics Committee of Ankara Etlik City Hospital. (number: AEŞH-EK1-2023-783, date:10.01.2024)
Plagiarism Check: Yes - (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: YD, AK Design: YD, AK Data Collection/Data Processing: OD, HŞ Data Analysis: OD, HŞ Article Preparation: UH, Gİİ.
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

Malignant Melanomas Localized to the Parotid Gland

ABSTRACT

Objective: Malignant melanoma situated in the parotid gland represents a rare clinical presentation, and the prognosis of these patients remains inadequately understood in comparison to other forms of malignant melanoma. This study aims to evaluate cases of parotid gland-located malignant melanoma under follow-up in our clinic.

Material and Method: Records of five patients aged 18 and above, diagnosed with melanoma localized within or adjacent to the parotid gland, were retrospectively reviewed. Relevant clinical information such as patients' demographic data including age and gender, medical histories, presenting symptoms, treatment modalities, and outcomes were evaluated. The overall survival of the patients was examined.

Results: None of the patients included in the study had primary parotid gland melanoma. Among all patients, 4 patients had primary lesions that were cutaneous melanomas originating from the head and neck region, while in one patient, the primary lesion was uveal melanoma of the eye. While 3 patients included in the study had died, 2 patients were still being followed up.

Conclusion: Primary melanomas localized to the parotid gland are extremely rare, and when encountered, a thorough medical history and careful physical examination can often reveal that the primary lesion is cutaneous melanoma, predominantly located in the head and neck region. It should be kept in mind that although rare, there may be primary intranodal melanoma cases whose primary is unknown or cannot be found in the parotid gland.

Keywords: Head and neck cancer, melanoma, neoplasms-unknown primary, parotid neoplasms.

ÖZET

Amaç: Parotis bezi içerisinde yer alan malign melanom, nadir bir klinik sunumu temsil eder ve bu hastaların prognozu, diğer malign melanom türleriyle karşılaştırıldığında yeterince anlaşılamamıştır. Bu çalışma, kliniğimizde takip edilen parotis bezi içerisinde bulunan malign melanom vakalarını değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: Parotis bezi içinde veya komşuluğunda lokalize melanom tanısı alan 18 yaş ve üstü 5 hastanın kayıtları geriye dönük olarak incelendi. Hastaların yaş ve cinsiyet gibi demografik bilgileri, tıbbi geçmişleri, başvuru semptomları, tedavi yöntemleri ve sonuçları gibi ilgili klinik bilgiler gözden geçirildi. Hastaların genel sağkalımları incelendi.

Bulgular: Çalışmaya dahil edilen hastaların hiçbirinde primer parotis bezi melanomu yoktu. Dört hastada primer lezyonlar baş ve boyun bölgesinden kaynaklanan kutanöz melanomlardı, bir hastada ise primer lezyon gözde yer alan üveal melanomdu. Çalışmaya alınan 3 hasta ölmüşken 2 hastanın takibi devam ediyordu.

Sonuç: Parotis bezinde lokalize olan primer melanomlar son derece nadir olup, parotis bezi melanomu ile karşılaşıldığında kapsamlı bir tıbbi öykü ve dikkatli bir fizik muayene ile primer lezyonun ağırlıklı olarak baş boyun bölgesinde yerleşen kutanöz melanom olduğu sıklıkla ortaya konulabilir. Ancak nadir de olsa parotis bezinde primeri bilinmeyen veya primer intranodal melanom vakaları olabileceği akılda tutulmalıdır.

Anahtar Sözcükler: Baş ve boyun kanseri, melanom, primeri bilinmeyen neoplaziler, parotis tümörleri.

Introduction

The most common cause of malignancies in the parotid gland is metastasis to the intra and periparotid lymph nodes (1). The most frequent cancers metastasizing to the parotid gland are squamous cell carcinoma and malignant melanoma (2). Melanoma of the parotid gland is a rare condition and usually develops due to metastasis from primary melanomas originating in the head and neck region (3,4). Although rare, careful examination of the head and neck may reveal regressed cutaneous melanomas when melanoma is observed in the parotid gland.

Due to the scarcity of cases, most studies in the literature have been presented as case reports. In this study, we aimed to discuss cases of parotid gland malignant melanoma treated in our clinic.

Material and Method

All patients diagnosed with malignant melanoma and followed in the oncology clinic of our hospital between January 2015 and January 2022 were retrospectively screened. Data were collected from medical records and the hospital database for patients with melanoma localized in the parotid gland, confirmed histopathologically. Patients' age, gender, primary or metastatic status, the reason for hospital admission, treatments received, last follow-up dates, and status at the last follow-up were recorded. In addition, histopathological examination and immunohistochemical findings were noted after re-evaluation.

Statistical analyses were performed using IBM SPSS Statistical Software (IBM SPSS Statistics version 22.0, IBM SPSS, USA). The clinical and demographic characteristics of the patients were analyzed by descriptive analysis. Categorical and numerical variables were presented as numbers and percentages (n, %). Continuous data were expressed as means ± standard deviation when the data follows normal distribution; otherwise, they were expressed as median and range. Survival outcomes were compared using the Kaplan-Meier method with the log-rank test. The time from the parotid gland metastasis to the last control or the date of death was accepted as overall survival (OS). The ethics committee approval of the study was given by the Ankara Etlik City Hospital ethics committee. (number: AEŞH-EK1- 2023-783, date:10.01.2024)

Results

A total of 5 patients were included in the study. Three (60%) of the patients were male. The median age was 64 (60-82) years. The clinicopathological characteristics of the patients are presented in Table I. No patient had primary parotid melanoma, and none of the patients' primary lesions had regressed spontaneously. In all patients, primary lesions and parotid metastases were pathologically confirmed. While 4 patients had cutaneous melanoma as the primary lesion, one patient had uveal melanoma as the primary lesion. Regarding the location of the primary lesions, one patient had the lesion in the left frontoparietal region, one patient in the left frontotemporal region, one patient on the left side of the neck, one patient under the left eye, and one patient had a primary lesion of the right eye, which was uveal melanoma (Figure I).

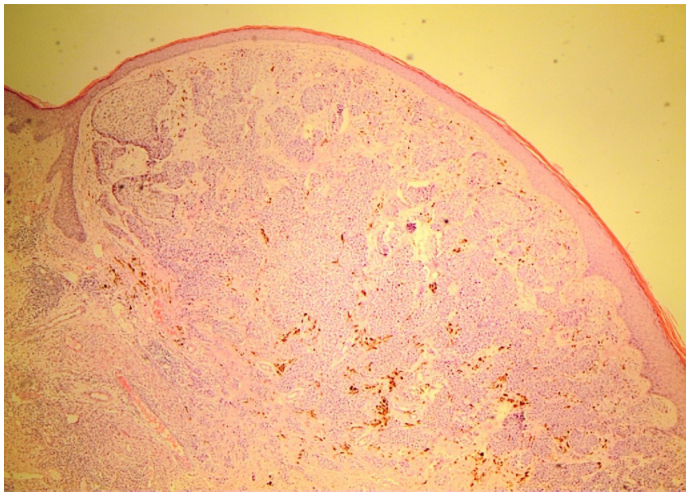

Figure I. Dermal nodular melanocyte nests in frontotemporal skin lesion (Hematoxylin and Eosinx40)

Two patients presented to the hospital due to swelling in the parotid region before the diagnosis, two patients due to lesions on the scalp, and one patient due to vision problems. In three patients, parotid metastasis was present at the time of diagnosis. In one patient, parotid metastasis developed 5 months after the initial diagnosis, and in another patient, it occurred 40 months later. Among the other metastatic sites, lung metastasis was observed in three patients, bone metastasis in three patients, liver metastasis in two patients, and lymph node metastasis in only one patient.

Table I. Clinicopathological characteristics of patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	Male	Male	Female	Female	Male
Age (at diagnosis, years)	60	82	63	77	64
Location of primary tumor	Left Frontal skin	Unknown	Right uveal melanoma	Left cheek skin	Left parietofrontal scalp
Time of parotid gland metastasis	At diagnosis	At diagnosis	40. month after diagnosis	5. month after diagnosis	At diagnosis
Other metastatik sites	Lymph node	Lung	Lung, liver, bone	Liver, bone	Bone
Involved parotid gland	Left	Left	Right	Left	Left
BRAF mutation	Mutant	Wild	Wild	Wild	Mutant
Treatment type	1. Dabrafenib +Trametinib	1. Temozolamid 2.Nivolumab	1. Temozolamid 2. Nivolumab 3. Paclitaxel	1. Temozolamid 2. Nivolumab	1. Temozolamid 2. Nivolumab
OS (months)	18 (Alive)	58 (Died)	37 (Died)	37 (Died)	13(Alive)

BRAF: B-Raf proto-oncogene, OS: Overall Survival

Diagnosis was confirmed through fine needle aspiration cytology (FNAC) of the relevant mass lesion and subsequent immunohistochemical staining of Human Melanoma Black-45 (HMB45), Melan A, S100 (pS100), and/or Sry-related HMg-Box gene 10 protein (SOX10) in the prepared cell blocks.

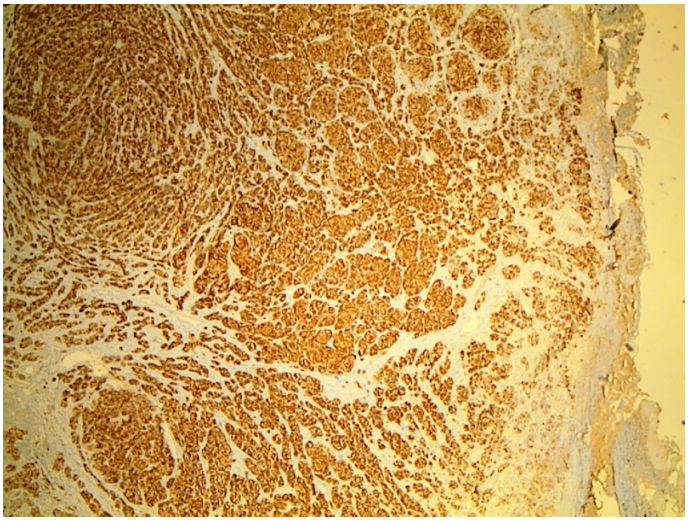

Figure II. Diffuse cytoplasmic HMB45 expression in melanocytes(Immunohistochemistry, HMB45x100)

Two patients with cutaneous primaries were found to have the B-Raf proto-oncogene (BRAF) V600E mutation, which is known to promote the growth and spread of melanoma cells. Treatment regimens were evaluated, and among the patients in the metastatic stage, three received temozolomide, one received Nivolumab, and one received dabrafenib+trametinib combination therapy.

In terms of OS, three patients died at the time of the last follow-up, while two patients were still under observation. The survival durations of the deceased patients were 37 months, 58 months, and 37 months, whereas the remaining patients were being followed up at 13 months and 18 months.

Discussion

Although primary malignant melanomas of the parotid glands are rare, melanomas are the second most common cause of parotid gland metastases (5). The most frequent origin of melanomas that metastasize to the parotid gland is known to be cutaneous melanomas in the head and neck region (4). Rarely, melanoma can be seen in the parotid parenchyma or parotid lymph nodes without a known primary, which may be attributed to regressed cutaneous melanomas or ectopic intranodal nevus cell inclusions (6,7).

Metastases from parotid melanomas are typically presented with swelling in the preauricular region. In these instances, ultrasonography is typically employed as the primary diagnostic tool, while positron emission tomography and computed tomography are often utilized for screening distant metastases. FNAC for diagnostic purposes and immunohistochemical examination of cell blocks serve as both non-invasive

and cost-effective methods.

In a series presented by Prayson et al. comprising 12 cases, the median age was 66 (range: 30-84) (8). Only one case had an unknown primary, and the remaining 11 patients had primaries in the head and neck region. Among these patients, 11 presented with a mass or nodule. The left parotid gland was involved in 5 patients, while the right parotid gland was involved in 6 patients (8). Some case reports have also described parotid metastasis in patients without a known primary, with two of those cases presenting with swelling in the right parotid gland (3,9). In our study, the median age resembled that of the aforementioned study, albeit with a higher incidence of left parotid gland involvement.

The identification and differentiation of lesions in the parotid gland pose a significant clinical challenge, particularly in cases where a malignant history is present. Although the most common tumors metastasizing to the parotid gland are cutaneous squamous cell carcinoma and melanoma (2), distinguishing between benign and malignant lesions, as well as determining whether they are primary or metastatic, remains complex. Given that benign tumors are predominant among parotid gland neoplasms (10), an essential consideration arises in isolating malignancies within parotid masses. While imaging techniques such as ultrasound, computed tomography, and magnetic resonance imaging are routinely employed for differential diagnosis, their efficacy may be limited. Consequently, FNAC, core needle biopsy, or excisional biopsy are invaluable for achieving a definitive pathological diagnosis. In our study, excisional biopsies were performed in one patient, while ultrasound-guided core needle biopsies were conducted in four patients.

The gold standard for diagnosing melanoma involves the demonstration of melanin pigment pathologically (11); however, in many instances, the diagnosis relies on the positivity of S100 and HMB45 immunohistochemical stains (12) (Figure II). Confirming the presence of melanin, along with the positive expression of S100 and HMB45 immunohistochemical stains in all our cases, unequivocally confirmed the pathological diagnosis of melanoma metastasis to the parotid gland. Also, the primary tumors in all patients originated from the head and neck region. Overall, patients with parotid metastasis tend to have shorter OS. In Prayson et al.'s series, half of the patients died within 2 years of parotid gland metastasis (8). In Wang et al.'s series of 17 patients, 9 patients died within an average of 2.6 years (13). In our series, after parotid metastasis, the survival durations were 37 months in 2 patients and 58 months in 1 patient, while the other 2 patients were still being followed up at 13 and 18 months after the parotid gland metastasis.

The main treatment for salivary gland malignancies, including melanomas, is generally surgery. Adjuvant radiotherapy may be added based on pathological evaluation after parotidectomy and neck dissection (14). In patients with widespread metastases and BRAF mutations, combination therapies targeting BRAF and Mitogen-activated protein kinase (MEK) inhibitors such as dabrafenib+trametinib, encorafenib+binimetinib, and vemurafenib+cobimetinib are used as targeted treatments (15-17). For patients without mutations, recently approved immunotherapy agents such as nivolumab, ipilimumab, and pembrolizumab are considered the first-line options (18,19). Additionally, alternative treatments such as temozolomide and historically significant interferon therapies are among the options. In our country, the lack of reimbursement for current treatments by the government and the challenges in accessing medications have resulted in none of our patients receiving the first-line standard treatments recommended in recent guidelines. The majority of patients underwent treatment with temozolomide, an older option that has fallen out of favor as a first-line therapy. The possible reason for our patients' overall shorter survival durations, as compared to current literature data, may be attributed to their inability to receive optimal treatment. The financial barriers and limited access to state-of-the-art therapies underscore the pressing need for improved healthcare policies and increased efforts to ensure that patients have equitable access to the latest and most effective treatments.

It is essential to acknowledge several limitations in our study. Firstly, the retrospective, single-center nature of the study introduces the potential for bias. Secondly, the study is constrained by a limited and heterogeneous patient population, thereby restricting the robustness of our findings. Additionally, the

notable limitation lies in the fact that the patients did not receive optimal treatment according to the evolving treatment algorithms in recent years. When interpreting the results of the study, these limitations should be taken into consideration, emphasizing the need for cautious interpretation and generalization of the findings. Future research endeavors should aim to overcome these limitations by incorporating larger, more diverse patient cohorts and adopting prospective study designs to enhance the reliability and applicability of the results.

In conclusion, primary melanomas of the parotid gland are extremely rare. When parotid melanoma is encountered, a thorough medical history and physical examination are essential. This allows for the identification of a primary lesion in many patients, with the most common origin being cutaneous lesions in the head and neck region. Sometimes, a previously excised skin lesion mentioned in the patient's history may serve as a primary lesion without any other lesions present.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Rare Pathogenic Lower Respiratory Tract Factors and Antibiotic Sensitivity Situations in Respiratory Intensive Care

Solunum Yoğun Bakımın Patojen Nadir Alt Solunum Yolu Etkenleri ve Antibiyotik Duyarlılık Durumları

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1400890
Geliş Tarihi | Received: 05.12.2023
Kabul Tarihi | Accepted: 20.03.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Özdemir L, Özdemir B, Gegin S, Aksu EA. Rare Pathogenic Lower Respiratory Tract Factors and Antibiotic Sensitivity Situations in Respiratory Intensive Care. Hitit Medical Journal 2024;6(2):200-207 https://doi.org/10.52827/hititmedj.1400890

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Samsun Üniversitesi Klinik Araştırmalar Etik Kurulundan 21/6/2023 tarihinden onaylanmıştır. (No: 2023/12/10)
İntihal Kontrolleri: Evet (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez:LÖ Tasarım: LÖ, BÖ Veri toplama/ Veri işleme: LÖ, BÖ, SG, EAA Veri analizi: LÖ Makalenin yazımı: LÖ Makalenin kontrolü: BÖ, SG, EAA.
Hasta Onamı: Hasta onamına gerek yoktur.
Finansal Destek: Finansal destek alınmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Ethical approval was received from Samsun University Clinical Research Ethics Committee on 21/6/2023 (No: 2023/12/10)
Plagiarism Check: Yes (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: LÖ Design: LÖ, BÖ Data Collection/Data Processing: LÖ, BÖ, SG, EAA Data Analysis: LÖ Manuscript Preparation: BÖ, SG, EAA.
Informed Consent: Not applicable.
Financial Disclosure: There are no financial funds for this article.

Rare Pathogenic Lower Respiratory Tract Factors and Antibiotic Sensitivity Situations in Respiratory Intensive Care

ABSTRACT

Objective: Determination of clinical features and antibiotic susceptibility of microorganisms rarely grown in lower respiratory tract (LRT) cultures of patients hospitalized in respiratory intensive care unit

Material and Method: Demographic characteristics, underlying diseases and risk factors, APHACHE-II scores, culture results and antibiotic resistance patterns, mortality status of 42 patients in whom rare microorganisms were detected as infectious agents in the LRT samples who were followed up and treated as inpatients in the respiratory intensive care unit between January 2019 and April 2023 were evaluated retrospectively.

Results: 42 patients, 30 males and 12 females, whose rare microorganisms were detected as the infectious agent in LRT samples, were included in the study. The average age was 73.7±10.9 years. The most common comorbidities in the patients were hypertension, chronic obstructive pulmonary disease (COPD), and heart failure. The most common rare pathogenic microorganisms were burkholderia cepaci (n=8), Stenotrophomonas maltophilia (n=6), proteus mirabilis (n=5), and entobacter aerogenes (n=5). The most common risk factors were urinary catheterization and the usage of antibiotics before the growth of rare pathogenic microorganisms. While 22 of the patients survived during their stay in intensive care, 20 of them died. The day when the first reproduction was detected in LRT samples *(p=0.012)* and APHACHE-II scores in the surviving group were significantly lower than those in the surviving group *(p=0.012).*

Conclusion: We think that the determination of antibiotic resistance patterns against microorganisms that rarely reproduce in intensive care units, such as microorganisms that reproduce frequently, will contribute to the use of antibiotics in our country and in our intensive care units.

Keywords: Antibiotic susceptibility, in-hospital mortality, intensive care, rare microorganisms.

ÖZET

Amaç: Solunum yoğun bakımında yatan hastaların alt solunum yolu (ASY) kültürlerinde enfeksiyon etkeni olarak nadir üreyen mikroorganizmaların klinik özelliklerinin ve antibiyotik duyarlılıklarının saptanması

Gereç ve Yöntem: Ocak 2019–Nisan 2023 tarihleri arasında solunum yoğun bakımında yatarak takip ve tedavi edilen ASY örneklerinde enfeksiyon etkeni olarak nadir üreyen mikroorganizmalar saptanan 42 hastanın demografik özellikleri, altta yatan hastalıkları ve risk faktörleri, APHACHE-II skorları ve kültür sonuçları ile antibiyotik direnç paternleri ile hastane içi mortalite durumları retrospektif olarak değerlendirildi.

Bulgular: Çalışmaya ASY örneklerinde enfeksiyon etkeni olarak nadir üreyen mikroorganizma saptanan 30'u erkek, 12'si kadın 42 hasta alındı. Yaş ortalaması 73,7±10,9 yıl idi. Hastalarda en sık eşlik eden hastalıklar hipertansiyon, kronik obstrüktif akciğer hastalığı (KOAH), kalp yetmezliği idi. Nadir patojen mikroorganizma olarak en sık burkholderia cepaci (n=8), Stenotrophomonas maltophilia (n=6), proteus mirabilis (n=5), entobacter aerogenes (n=5) saptandı. Risk faktörü olarak da en sık idrar sondası, nadir patojen mikroorganizma üremesi öncesinde antibiyotik kullanımı saptandı. Yoğun bakımda yatış sırasında hastalardan 22'si yaşarken, 20'si eksitus oldu. ASY örneklerinde ilk üremenin saptandığı gün *(p=0.012)* ve yaşayan gruptaki APHACHE-II skorları eksitus olan gruba göre anlamlı olarak düşüktü *(p=0.012).*

Sonuç: Yoğun bakımlarda sık üreyen mikroorganizmalar gibi nadir üreyen mikroorganizmalarda da hastane içi mortalitenin yüksek olduğu bu nedenle antibiyotik direnç paternlerinin daha yüksek sayıdaki olgularla değerlendirilmesinin ülkemizde ve yoğun bakımlarımızda antibiyotik kullanımına katkı sağlayacağını düşünmekteyiz.

Anahtar Sözcükler: Antibiyotik duyarlılığı, hastane içi mortalite, nadir patojen mikroorganizma, yoğun bakım.

Giriş

Yoğun bakım ünitelerinde (YBÜ) alt solunum yolu enfeksiyonları (ASYE) tüm yoğun bakım hastalarının %10-25'inde görülür ve %22-71 ölüm oranına sahiptir (1,2). Yoğun bakımlarda ASYE'ye yol açan etkenler süreç içinde değişmiştir. Etken olarak en sık pseudomonas, acinetobacter, klebsiella gibi gram negatif bakteriler saptanırken, son yıllardaki veriler koagülaz negatif staphylococ, staphylococcus aureus ve enterokok türleri gibi gram pozitif bakterin sıklığının arttığı bildirilmektedir (3).

YBÜ'lerinde takip edilen hastalarda antibiyotiklere doğal dirençli fırsatçı nadir mikroorganizmaların patojen olarak ortaya çıkmasında etkili olan çeşitli nedenler vardır. Bunlar içinde hastaların kliniğinin ağır olması, hastanede yatış süresinin uzun olması, komorbiditeler, uygulanan sık invaziv prosedürler, antimikrobiyal ajanların uygunsuz kullanımı sayılabilir. Tüm bunların sonucunda, çoklu ilaç direnci olan suşlar gelişmekte ve sağlıklı bireylerde nadiren enfeksiyona sebep olan citrobacter, stenotrophomonas maltophilia, burkholderia cepacia, proteus mirabilis ve serratia türleri gibi virulansı düşük mikroorganizmaların neden olduğu enfeksiyonlar hastaların hayatlarını tehdit etmektedir (3,4).

Bu çalışmada solunum YBÜ'de yatan hastaların balgam/trakeal aspirat kültürlerinde nadir olarak üreyen ve enfeksiyon etkeni olarak değerlendirilen mikroorganizmaların klinik özelliklerinin ve antibiyotik duyarlılıklarının saptanması planlandı.

Gereç ve Yöntemler

Çalışma için Samsun Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu'ndan onay alındı (Tarih:21.06.2023, Karar No:2023/12/10).

Çalışma retrospektif olarak, Samsun Üniversitesi Eğitim ve Araştırma Hastanesi Göğüs Hastalıkları solunum YBÜ'de Ocak 2019-Nisan 2023 tarihleri arasında yatarak takip ve tedavi edilen 1585 hastanın verileri değerlendirilerek yapıldı. Hastaların YBÜ'de yattığı süre boyunca gönderilen ASY örnekleri (balgam/trakeal aspirat kültürleri) hastane bilgi yönetim sisteminden tarandı. ASY örneklerinde enfeksiyon etkeni olarak saptanan nadir mikroorganizmaya (burkholderia, serratia, stenotrofomonas, proteus, enterobacter, citrobacter, corynebacterium, enterococcus, aspergillus, achromobacter, granulicatella, kocuria, pantoea) sahip 42 hasta çalışmaya dahil edildi. Çalışmamız 'Helsinki Deklerasyonu Prensipleri'ne uygun olarak yapılmıştır. Çalışmamız retrospektif olduğundan bilgilendirilmiş olur formu alınamamıştır. Kolonizasyon ve enfeksiyon etkeninin ayrımında kültürde üremenin olduğu zamanki beyaz küre ve C-reaktif protein (CRP) düzeyi değerlendirildi. ASY örneğinde patojen mikroorganizmanın üremesi saptandığında eş zamanlı beyaz küre ve CRP'si de yüksek olan hastalarda üreyen mikroorganizma etken olarak kabul edilirken, eş zamanlı beyaz küre ve CRP'si normal olan hastalarda üreme, kolonizasyon olarak değerlendirildi ve hasta çalışma dışı bırakıldı. Hastaların demografik özellikleri, altta yatan hastalıkları ve risk faktörleri, APHACHE-II skorları, hastane yatış süresi, ASY örneklerinde ilk üreme saptanma süresi ile nadir patojen mikroorganizma üreme süreleri, nadir patojen mikroorganizma üremesi öncesindeki ve sonrasındaki ASY kültürlerinde üreyen diğer mikroorganizmalar, üreyen mikroorganizmaların antibiyotik duyarlılıkları ile hastaların hastane içi mortalite durumları değerlendirildi.

İstatistiksel değerlendirme için SPSS 23.0 (SPSS Inc. Chicago, IL, ABD) programı kullanıldı. Elde edilen verilerin değerlendirilmesinde, normal dağılımlı değişkenler için ortalama ± standart sapma (SD), kategorik değişkenler için ki-kare, parametrik veriler için bağımsız örneklem t testi uygulandı.

Bulgular

Çalışmada 1585 hastanın 42'sinde ASY örneklerinde enfeksiyon etkeni olan nadir mikroorganizma saptandı. Hastaların 30'u (%71.4) erkek, 12'si (%28.6) kadındı. Yaş ortalaması 73.7±10.9 yıl idi. Cinsiyetler arasında yaş dağılımı (Kadın: 78.8±9, Erkek: 71.7±11.1) benzerdi (*p=0.386*).

Nadir enfeksiyon etkenleri için eşlik eden hastalık olarak en sık hipertansiyon, KOAH, kalp yetmezliği, alzheimer mevcut iken risk faktörü olarak da en sık idrar sondası, nadir enfeksiyon üreme öncesinde antibiyotik kullanımı ve mekanik ventilasyon (acil entübasyon n=6 / elektif entübasyon n=19) uygulanması saptandı. Hastaların 22'sinin yaşadığı, 20'sinin eksitus olduğu saptandı. APHACHE-II skorları yaşayan hastalarda 18.3±2.9 iken, eksitus olan hastalarda 22.5±1.5 idi (*p=0.012*). Hastaların ortalama hastanede yatış süreleri yaşayan grupta

Tablo I. Hastaların demografik özellikleri

	Yaşayan (n, %) **22 (52.4)**	**Eksitus (n, %)** **20 (47.6)**	**Total (n, %)** **42**	***p***
Yaş (Ort ± SD)	72.3 ± 11.5	75.3 ± 10.3	73.7 ± 10.9	*0.386*
Cinsiyet (n, %)				
Erkek	16	14	30 (71.4)	*0.845*
Kadın	6	6	12 (28.6)	
Komorbidite (n, %)				
Hipertansiyon	15	16	31 (73)	
KOAH	16	12	28 (66)	
Kalp Yetmezliği	10	11	21 (50)	
Alzheimer	9	9	18 (42.9)	
Postcovid	4	5	9 (21.4)	
Diyabet	3	5	8 (19)	
SVH	5	3	8 (19)	
Malignite	2	3	5 (11.9)	
Parkinson	2	0	2 (4.8)	
ALS	1	0	1 (2.4)	
Risk Faktörleri				
İdrar sondası	19	20	39 (92.9)	
Öncesinde antibiyotik kullanımı	18	17	35 (83.3)	
Mekanik ventilasyon	9	16	25 (59.5)	
Kan transfüzyonu	9	11	20 (47.6)	
Santral venözkateter	6	10	16 (38)	
Nazogastrıik sonda	6	8	14 (33.3)	
NIMV	6	4	10 (23.8)	
PEG	7	3	10 (23.8)	
Dekübit	5	5	10 (23.8)	
Trakeostomi	3	5	8 (19)	
İmmunsupresif tedavi	0	1	1 (2.4)	
APACHEII Skor	18.3 ± 2.9	22.5 ± 1.5		*0.012*
Hastane Yatış / gün	38 ± 29.6	53.4 ± 48.6	45.3 ± 40	*0.354*
Balgamda ilk üreme /gün	9.5 ± 8.3	18 ± 16	13.5±13.2	*0.012*
Nadir patojen üreme/günü	20.8 ± 18.9	33 ± 47.5	26.6±35.6	*0.161*

KOAH: Kronik obstrüktif akciğer hastalığı, SVH: Serebro vasküler hastalık, ALS: Amyotrofik Lateral Skleroz

38±29.6 gün, eksitus olan grupta 53.4±48.6 gün idi (*p=0.354*). Alt solunum yolu örneklerinin kültüründe yaşayan grupta (9.5±8.3/gün) eksitus olan gruba (18±16/gün) göre mikroorganizmaların ilk üreme günü daha önce idi (*p=0,012*). Alt solunum yolu örneklerinde nadir patojen mikroorganizmaların üreme günleri arasında anlamlı fark saptanmadı (*p=0.161*) (Tablo I).

Nadir patojen mikroorganizma olarak en sık burkholderia cepaci (n=8), stenotrophomonas maltophilia (n=6), proteus mirabilis (n=5), entobacter aerogenes (n=5) saptandı. Nadir patojen mikroorganizma üremeden önceki kültürlerde en sık pseudomonas aeruginosa (n=7), acinetobacter baumannii (n=6) saptanırken, nadir patojen mikroorganizma üremesi sonrasında en sık pseudomonas aeruginosa (n=8) ve klebsiella pneumoniae (n=3) saptandı (Tablo II).

Tablo II. Patojen mikroorganizma kültürleri

Nadir patojen mikroorganizma öncesi kültür (n=16)	Nadir patojen mikroorganizma kültür (n=42)	Nadir patojen mikroorganizma sonrası kültür (n=21)
Pseudomonas aeruginosa (n=7)	Burkholderia cepaci(n=8)	Pseudomonas aeruginosa (n=8)
Acinetobacter baumannii (n=6)	Stenotrofomonas maltophilia (n=6)	Klebsiella pneumoniae (n=3)
Klebsiella pneumoniae (n=2)	Proteus mirabilis(n=5)	Burkholderia cepaci (n=3)
Burkholderia cepaci (n=1)	Enterobacter Aerogenes(n=5)	Acinetobacter baumannii (n=2)
	Citrobacter koseri(n=3)	Entobacter Aerogenes(n=1)
	Corynebacterium spp. (n=2)	Enterobacter cloacae (n=1)
	Enterococcus faecium. (n=2)	Escherichia coli (n=1)
	Aspergillus spp (n=2)	Serratia marcescens (n=1)
	Achromobacter xylosoxidans (n=1)	Stenotrofomonas maltophilia (n=1)
	Granulicatella adiacens (n=1)	
	Kocuria kristinae (n=1)	
	Pantoea spp(n=1)	
	Pseudomonas fluorescens (n=1)	
	Serratia ficaria (n=1)	
	Serratia liquefaciens (n=1)	
	Serratia marcescens (n=1)	
	Serratia odorifera (n=1)	

Üreyen mikroorganizmaların cinsine göre antibiyotik duyarlılık durumları Tablo III'te gösterildi.

Tartışma

Biz çalışmamızda yoğun bakım ünitemizde uzun süre yatış öyküsü olan, yaşı ileri, komorbidite ve risk faktörlerine sahip hastalarda virülansı düşük fırsatçı nadir mikroorganizmaların patojen olarak ortaya çıktığını ve %42 oranında hastane içi mortaliteye sahip olduğunu gözlemledik.

Literatür taramamızda yoğun bakımlarda alt solunum yollarında nadir üreyen etkenler ve antibiyotik duyarlılıkları ile ilgili bir çalışma saptamadık. Yapılan çalışmaların daha çok sık üreyen etkenlerin risk faktörleri ve komorbiditeleri ile antibiyotik dirençlerinin değerlendirildiği çalışmalar şeklinde olduğu ve nadir üreyen etkenlerin sayısal veri olarak verildiğini gözlemlendik. Özer ve ark.'nın (5) yaptığı çalışmada yoğun bakımlardan gönderilen 908 alt solunum yolu (balgam/trakeal aspirat/bronkoalveolar lavaj) örneğinde nadir etken olarak en sık serratia marcescens (n=9), stenotrophomonas maltophilia (n=6), burkholderia cepacia (n=2), enterobacter aerogenes (n=2), pseudomonas putida (n=2), providencia stuartii (n=2), pseudomonas fluorescens (n=2), citrobacter koşeri (n=1), Gürbüz ve ark.'nın (6) yaptığı alt solunum yolu örneklerinin değerlendirildiği diğer bir çalışmada da nadir etken olarak proteus mirabilis (n=7), serratia spp. (n=4), enterococcus spp. (n=3), achromobacter xylosoxidans (n=2), citrobacter spp. (n=2), stenotrophomonas maltophilia (n=1) saptanmıştır. Çetin ve ark.'nın (3) yaptığı çalışmada serratia marcescens (n=12), proteus mirabilis (n=11), Küme ark.'nın (7) yaptığı çalışmada stenotrophomonas maltophilia (n=2) saptanmıştır. Bizde çalışmamızda benzer etkenleri saptadık.

Yoğun bakım hastalarında alt solunum yolu kültürlerinde sık üreyen pseudomonas, acinetobacter, klebsiella, staphylococcus aureus ve enterokok türleri için yaş, cinsiyet, hastanede yatış süreleri, altta yatan hastalıklar (kardiyovasküler, serebrovasküler hastalıklar, diyabet, KOAH, akut ve kronik böbrek yetmezliği, karaciğer hastalığı, malignite), invaziv prosedürler (idrar ve nazogastrik sonda, mekanik ventilasyon, diyaliz, santral venöz kateter, trakesotomi, PEG uygulaması), immunsüpresif tedaviler, parenteral beslenme, kan transfüzyonu, operasyon, yanık ya da travma, antibiyotik kullanımı gibi tanımlanmış risk faktörleri ve komorbiditeler mevcuttur (7,8). Sık üreyen bakterilerde tanımlanan risk faktörleri ve komorbiditelere ek olarak daha nadir üreyen bakterilerde de hastanede ≥28 gün yatış, APACHE-II>20, öncesinde dirençli mikroorganizmaların üremesi, huzurevinde veya uzun süreli bakım tesisinde yaşayanlar, kronik granülomatöz hastalıklar, primer immun yetmezlik durumları, maligniteler, kortikosteroid kullanımı, kistik fibrozis gibi risk faktörleri tanımlanmıştır (9-12). Bizde nadir üreyen patojen bakteriler için literatür ile benzer şeklide risk faktörleri ve komorbiditeler saptadık.

APACHE-II skoru, hastalığın ciddiyetini, prognozunu ve ölüm riskini değerlendirmek için en yaygın kullanılan kritik durum değerlendirme aracıdır (13). Alt solunum yolu örneklerinde S.maltophilia üremesi saptanan 18 çalışmanın değerlendirildiği bir meta-analizde

Tablo III. Antibiyotik duyarlılıkları

Nadir patojen mikroorganizma	Antibiyotik duyarlılıkları												
	Ampisilin (n)	Levofloksasin (n)	TMP/SMZ (n)	Pip/Tazo (n)	Colistin (n)	Tigecycline (n)	İmipenem (n)	Meropenem (n)	Cipro (n)	Amicasin (n)	Ceftazidim (n)	Cefepim (n)	Vanko (n)
B. cepaci (n=8)		5				7	4	3	5		8		
S. maltophilia (n=6)		2	6										
P. mirabilis (n=5)	1	1		3		1	2	3	5	5	1	2	
E. Aerogenes (n=5)			4	2	2		4	4	4	4	2	3	
C. koseri (n=3)			2	3	1	2		2	2	3	3	2	
Corynebacterium (n=2)													
E. faecium (n=2)						2							2
Aspergillus spp (n=2)													
A. xylosoxidans (n=1)					1							1	
Kocuria kristinae (n=1)													1
Pantoea spp (n=1)						1							
P. fluorescens(n=1)				1			1	1		1	1		
S. ficaria (n=1)													
S. liquefaciens (n=1)		1		1			1	1	1	1	1	1	
S. marcescens (n=1)				1			1	1	1	1			
S. odorifera (n=1)		1	1	1		1	1	1	1	1	1		
G. adiacens (n=1)													

APACHE-II skoru >20 olması ölüm oranı ile ilişkili bağımsız risk faktörü olarak saptanmıştır (9). Bizde eksitus olan grupta APHACHE-II skorunu 22.5±1.5 saptadık.

Yoğun bakımlarda üreyen etkenlere karşı kullanılan geniş spektrumlu antibiyotiklerin ampirik tedavide yaygın olarak kullanımı hastane florasında bulunan virülansı düşük antibiyotiklere doğal dirençli fırsatçı nadir mikroorganizmaların patojen olarak ortaya çıkmasına ve dirençli mikroorganizmaların baskın hale gelmesine sebep olmaktadır. Ülkemizde yapılan çalışmalar değerlendirildiğinde yoğun bakımlarda alt solunum yolunda nadir üreyen etkenlerin antibiyotik duyarlılıkları ile ilgili sınırlı sayıda veri olduğu ve yüksek dirence sahip oldukları gözlemlendi. Dizbay ve ark.'nın (14) nazokomiyal burkholderia cepaci üremelerini değerlendirdiği çalışmada %58.9'unun (23/39) alt solunum yolu örneklerinde ürediği ve ceftazidime 24 (%61.5), ciprofloksasin 21 (%53.8), amikasin 21 (%53.8), meropenem 19(%48.7), imipenem 18 (%46.1) dirençli olduğu saptanmıştır. Bizim de çalışmamızda 8 burkholderia cepaci'nın ceftazidime %100 duyarlı, meropeneme %72.5, imipeneme %50, ciprofloksasin ve levofloksasine %37.5 dirençli olduğu gözlendi.

S. maltophilia üremelerinin değerlendirildiği çalışmalarda karbapenem, β-laktam, kinolonlar, aminoglikositler gibi antibiyotik gruplarına yüksek seviyede doğal direnç gözlenmiştir (15). Şen ve ark.'nın (16) yaptığı alt solunum yolunda üreyen 69 (%34.1) hastanın verilerinin değerlendirildiği çalışmada TMP-SMZ'e %99 duyarlı iken, meropeneme %92.8, imipeneme 87.5 direnç saptanmıştır. Kayabaşı ve ark.'nın (2) yaptığı çalışmada 27 S. maltophilia üremesinde TMP-SMZ'e %96 duyarlı iken, seftazidime %52, levofloksasine %22 direnç saptanmıştı. Özdemir ve ark.'da(17) nazokomiyal pnömonili 7 olgunun TMP-SMZ ve amikasine duyarlı olduğunu, 1 olguda siprofloksasin, 6 olguda da imipenem dirençli olduğunu saptanmıştır. Bizim çalışmamızda da 6 S. maltophilia'nın TMP-SMZ %100 duyarlı, levofloksasine %66.6 dirençli olduğu saptandı.

Proteus mirabilis yapılan çalışmalarda daha çok idrar yolu ve yara kültürlerinde saptanmış olup alt solunum yollarında nadir olarak saptanmaktadır. Antibiyotik duyarlılıklarına bakıldığında kolistine ve tigesikline doğal direçlidir. Demirel tarafından yapılan bir çalışmada proteus suşlarının meropeneme %95.7, piperasilin/tazobaktama %95.7 ve fosfamisine %91.2

duyarlı olduğu saptanırken, tigesiklin duyarlılığı %15.4 olarak saptanmıştır (18). Bizim çalışmamızda da siprofloksasin ve amikasine duyarlılık yüksek iken, tigesikline, ampisiline duyarlılık düşük olarak saptandı.

Enterobacter aerogenes, hastane kaynaklı pnömoni, bakteriyemi ve idrar yolu enfeksiyonlarına neden olan önemli bir fırsatçı patojendir. Etken olarak en sık E. cloacae, E. aerogenes izole edilmektedir. Aydemir ve ark.'nın endotrakeal aspiratların değerlendirdiği bir çalışmada E. cloaca üreyen hastanın antibiyogramında seftriakson ve seftazidime %52.5 direnç saptanırken, piperasilin/tazobaktama %20.4 olarak saptanmıştır (1). Bizim de çalışmamızda E. Aerogenes'in imipenem, meropenem, siprofloksasin, amikasine duyarlılığın yüksek, seftazidime dirençli olduğunu gözlemledik. Serratia türleri yoğun bakım ünitelerinde nozokomiyal enfeksiyonlardan sorumlu nadir fırsatçı bir patojen olup solunum yolu, üriner sistem ve bakteriyemilerden sorumlu olabilmektedir. Tartar ve ark.'nın endotrakeal aspiratları değerlendirdiği çalışmada S. marcescens (n=23) Piperasilin-Tazobactama %91.3, amikasin ve TMP-SMZ'e %87, siprofloksasin, imipenem, seftazidime %78.3 yüksek direnç saptanmışken, colistine %4.3 direnç saptanmıştı (19). Bizim çalışmamızda serratia türlerinin piperasilin/tazobaktama, siprofloksasin, imipenem, amikasin duyalı olduğunu, sefepim ve TMP-SMZ'e direncin fazla olduğunu saptadık.

Citrobacter'lerde nadir izole edilen, solunum sistemi ve üriner sistemde infeksiyona neden olabilen fırsatçı patojenlerdir. Atmaca ve ark.'nın yaptığı çalışmada citrobacter'lere karşı en etkili antibiyotik amikasin saptanırken, en çok direncin seftazidime olduğu gözlenmiştir (20). Bizde çalışmamızda seftazidim, amikasin, piperasilin/tazobaktama duyarlılık saptarken, colistine duyarlılık daha az olarak saptandı.

Bu çalışmanın kısıtlılıkları vardı. Öncelikle tek merkezli, retrospektif bir çalışmaydı ve nadir üreyen mikroorganizma hasta sayımız azdı.

Sonuç olarak yoğun bakımlarda sık üreyen mikroorganizmalar gibi nadir üreyen mikroorganizmalarda da hastane içi mortalitenin yüksek olduğu bu nedenle antibiyotik direnç paternlerinin daha yüksek sayıdaki olgularla değerlendirilmesinin ülkemizde ve yoğun bakımlarımızda antibiyotik kullanımına katkı sağlayacağını düşünmekteyiz.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

The Impact of Nasal Septum Deviation on Paranasal Sinus Volumes

Nazal Septum Deviasyonunun Paranazal Sinüs Hacimlerine Etkisi

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1422398
Geliş Tarihi | Received: 19.01.2024
Kabul Tarihi | Accepted: 15.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Öztürk FY, Nahir M, Beyhan M. The Impact of Nasal Septum Deviation on Paranasal Sinus Volumes. Hitit Medical Journal 2024;6(2):208-215 https://doi.org/10.52827/hititmedj.1422398

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Çalışma Tokat Gaziosmanpaşa Üniversitesi Klinik Araştırmalar Etik Kurulu'na sunuldu ve 03.11.22 tarihinde 22-KAEK-240 kayıt numarasıyla onaylandı.
İntihal Kontrolleri: Evet (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: FYÖ, MN, MB Tasarım: FYÖ, MN, MB Veri toplama/Veri işleme: FYÖ, MN, MB Veri analizi: FYÖ, MN, MB Makalenin hazırlanması: FYÖ, MN, MB
Hasta Onamı: Hastalardan onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: The study was submitted to the Clinical Research Ethics Committee of Tokat Gaziosmanpaşa University and approved on 03.11.22 under registration number 22-KAEK-240
Plagiarism Check: Yes (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: FYÖ, MN, MB Design: FYÖ, MN, MB Data Collection/Data Processing: FYÖ, MN, MB Data Analysis: FYÖ, MN, MB Manuscript Preparation: FYÖ, MN, MB.
Informed Consent: Consent was obtained from the patients.
Financial Disclosure: There are no financial funds for this article.

The Impact of Nasal Septum Deviation on Paranasal Sinus Volumes

ABSTRACT

Objective: Researchers have extensively investigated the reasons behind variations in the volumes of paranasal sinuses, either among different individuals or between the right and left sides of the same individual. These differences in volumes have been associated with nasal septal deviation (NSD) and anatomical variations. In this study, we aimed to retrospectively analyzed the effect of NSD on frontal sinus, maxillary sinus, and sphenoidal sinus.

Material and Method: A total of 281 patients (151 females and 130 males) admitted to Gaziosmanpaşa University Hospital were included in the study. Paranasal sinus computed tomography (CT) images consisting of 0.625-mm-thick slices were obtained from the patients. The areas of the frontal sinus, maxillary sinus, and sphenoidal sinus were determined using ImageJ software. The volume of each sinus was calculated using Cavalieri's principle. We analyzed the relationship between the calculated volumes and nasal septal deviation.

Results: Our study found that the frontal sinus volume values were 4.67 cm^3 on the right side and 5.03 cm^3 on the left side in women. In men, the right-side volume value was 7.07 cm^3 and the left-side volume value was 7.77 cm^3. The sphenoidal sinus volume value was calculated as 6.35 cm^3 on the right side and 6.57 cm^3 on the left side in women. In males, the right-side volume value was 7.34 cm^3 and the left-side volume value was 7.62 cm^3. The maxillary sinus volume was calculated as 19.38 cm^3 on the right side and 19.37 cm^3 on the left side in women. In males, the right-side volume value was 22.80 cm^3 and the left-side volume value was 23.71 cm^3. The frontal sinus volume values in males were larger than those in females *(p=0.04)*. The maxillary sinus volume values in males were greater than the maxillary sinus volume values in females *(p=0.02)*. There was no significant relationship between the presence of septal deviation and sinus volumes *(p>0.05)*. The right, left, or s-shaped deviation direction did not cause a significant difference in sinus volumes *(p>0.05)*.

Conclusion: The findings show that there is no correlation between volume values and the presence and shape of nasal septal deviation. However, significant differences in volumes were observed between the genders. Because the severity of nasal septal deviation is related to the volume value, different results can be obtained by measuring the frontal sinus, maxillary sinus, and sphenoidal sinus volume values in individuals with more advanced deviation.

Keywords: Cavalieri principle, frontal sinus, ImageJ, maxillary sinus, sphenoidal sinus, volumes.

ÖZET

Amaç: Paranazal sinüslerin hacimlerinin farklı bireylerde ya da aynı bireyin sağ ve sol tarafı arasında farklılık gösterme sebepleri birçok araştırmacı tarafından incelenmiştir. Hacimlerdeki bu farklılıklar nazal septum deviasyonu, anatomik varyasyonlar gibi birçok sebeple ilişkilendirilmiştir. Bu çalışmada, NSD'nin sinus frontalis, sinus maxillaris ve sinus sphenoidalis hacimlerine etkisini retrospektif olarak incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmaya Tokat Gaziosmanpaşa Üniversite Hastanesi'ne başvuran 151 kadın, 130 erkek toplamda 281 hasta dahil edildi. Hastalardan, 0,625 mm kalınlığında kesitlerden oluşan paranazal sinüs bilgisayarlı tomografi görüntüleri alındı. ImageJ programı ile sinus frontalis, sinus maxillaris ve sinus sphenoidalis'in alanları tespit edildi. Her bir sinüsün hacmi Cavalieri Prensibi ile hesaplandı. Hesaplanan hacimlerin nazal septum deviasyonu ile ilişkisi incelendi.

Bulgular: Çalışmamızda, kadınlarda sinus frontalis hacim değerleri, sağ tarafta 4,67 cm^3, sol tarafta 5,03 cm^3 olarak hesaplandı. Erkeklerde ise sağ taraf hacim değeri 7,07 cm^3, sol taraf hacim değeri 7,77 cm^3 olarak hesaplandı. Sinus sphenoidalis hacim değeri, kadınlarda sağ tarafta 6,35 cm^3, sol tarafta ise 6,57 cm^3 olarak hesaplandı. Erkeklerde sağ taraf hacim değeri 7,34 cm^3 iken sol taraf hacim değeri 7,62 cm^3 olarak hesaplandı. Sinus maxillaris hacmi kadınlarda sağ tarafta 19,38 cm^3, sol tarafta ise 19,37 cm^3 olarak hesaplandı. Erkeklerde ise sağ taraf hacim değeri 22,80 cm^3, sol taraf hacim değeri ise 23,71 cm^3 olarak hesaplandı. Erkeklerde sinus frontalis hacim değerleri kadınların sinus frontalis hacimlerinden daha büyüktür *(p=0.04)*. Erkeklerde sinus maxillaris hacim değerleri kadınların sinus maxillaris hacim değerlerinden daha büyüktür *(p=0.02)*. Septum deviasyonun varlığı ile sinus hacimleri arasında anlamlı bir ilişki bulunamadı *(p>0.05)*. Deviasyon yönünün sağa, sola veya s şekilli olması sinüslerin hacimlerinde anlamlı bir fark oluşturmadı *(p>0.05)*.

Sonuç: Bulgularımıza göre hacim değerleri ile nazal septum deviasyonun varlığı ve şekli arasında ilişki bulunamazken, cinsiyetler arasında hacimlerde anlamlı farklılıklar görüldü. NSD'nin şiddeti ile hacim değerinin ilişkili olması nedeniyle daha ileri deviasyona sahip bireylerde sinus frontalis, sinus maxillaris ve sinus sphenoidalis hacim değerlerini ölçmek farklı sonuçlar elde edilebilir.

Anahtar Sözcükler: Cavalieri prensibi, hacim, ImageJ, sinus frontalis, sinus maxillaris, sinus sphenoidalis.

Introduction

The paranasal sinuses are four pairs of cavities found within the bones that make up the nasal cavity. These are the frontal sinus, sphenoidal sinus, maxillary sinus, and ethmoidal cells. They are formed by embedding the nasal mucosa into bones. The formation of paranasal sinuses significantly alters the size and shape of the face. They also contribute to changes related to growth and dental development and can be indicative of an individual's social context, such as gender and sexual maturity. Paranasal sinuses perform multiple functions, including contributing to voice resonance, lightening the weight of the skull, aiding facial growth and shaping, maintaining olfactory membrane moisture through mucus secretion, and balancing internal and external atmospheric pressure (1, 2). The location, shape, and size of nasal sinus openings vary greatly among individuals. This region, where the nasal cavity and paranasal sinuses are located, plays a crucial role in the pathogenesis of diseases and is also where anatomical variations are commonly observed (1, 3). The nasal septum is located in the middle part of the nasal cavity and is divided into the posterior section by the vomer and perpendicular plate of the ethmoidal bone and into the anterior section by the quadrilateral cartilage. NSD is the most common anatomic variation observed in adults, occurring in approximately 80% of cases. The volume of the nasal cavity decreases on the ipsilateral (convex) side of the NSD. Facial anomalies, birth traumas, other injuries, abnormalities in the growth of incisor and upper teeth and prolonged finger sucking are among the causes of NSD (4, 5).

To the best of our knowledge, studies have investigated the relationship between the volume of a single sinus and NSD. Nevertheless, a comprehensive study simultaneously examining all three sinuses, namely frontal sinus, sphenoidal sinus and maxillary sinus, has not been previously undertaken.

The volumes of the sinuses were measured using the Cavalieri principle. The Cavalieri principle provides the advantages of speed, reliability, and obtaining quantitative data compared to other measurement techniques. There are articles that measure the volumes of structures such as the spleen, liver and brain using the Cavalieri principle, but there are not many articles that measure the volumes of paranasal sinuses using this method (6, 7). It is crucial to accurately calculate the volumes of these cavities, which are adjacent to important anatomical structures such as the orbit, pituitary gland, and teeth, and to understand the effect of NSD. This will provide an advantage for the rapid and reliable implementation of surgical procedures in these regions in the future.

In this study, we aimed to evaluate the effect of NSD on the volume of the frontal sinus, sphenoidal sinus and maxillary sinus.

Material and Method

The study was submitted to the Clinical Research Ethics Committee of Gaziosmanpaşa University and approved on 03.11.22 under registration number 22-KAEK-240. Following ethical approval, we evaluated 281 participants who underwent paranasal sinus CT at the Gaziosmanpaşa University Faculty of Medicine Hospital between January 2018 and May 2023. The study included cranial CT images of 281 participants, comprising 151 females and 130 males, with ages ranging from 11 to 75 years (mean age: 36.6 ± 15.5 years). The mean ages for females and males were 36.3±15.9 and 36.01±16.1, respectively. The data were obtained from a GE-branded CT scanner, using images with a slice thickness of 0.625 mm (GE Optima CT660 -128 slice). The inclusion criteria encompass having brain CT images available and absence of any surgical history related to the head region. Patients with a history of head trauma, nasal polyposis, previous surgical interventions in the head region, chemotherapy and radiotherapy history targeting the head region, diagnosis of chronic sinusitis, and patients under the age of 14 years will be excluded.

The radiological images were downloaded from the SECTRA radiological image viewing software of Gaziosmanpaşa University Hospital's information technology system. They were initially transferred to a dedicated folder in DICOM (Digital Imaging and Communication in Medicine) format. Subsequently, the transferred images were opened using ImageJ software. Axial-oriented images with a slice thickness of 0.625 mm were selected from the opened image series for visual examination using the same program.

Images were checked for any intracranial pathologies. After this review, image series that were considered problem-free were sampled, ensuring at least 15 image slices for the frontal, sphenoidal, and maxillary sinuses. The paranasal sinuses were colored red using the 'Threshold' option under the Image, Adjust tab in ImageJ software. The program's polygon selection tool was used to establish the boundaries of the colored sinus. Using this method, the area covered by the sinus in each section was determined (Figure I). To calculate the volume of the sinus, these area values were multiplied by the section thickness in accordance with the Cavalieri Principle and then summed. In this way, the volume value of the respective sinus was calculated (Formula I).

Formula I: $V=t\times (a_1+a_2+.....+a_n)\ cm^3$

In the formula, t represents the average thickness of consecutive sections in centimeters for n number of sections, while $(a_1 + a_2 + ... + a_n)$ represents the cross-sectional areas in square centimeters.

Table I. Difference in sinus volumes according to gender

Group	Measure	Mean (cm^3)	SD	Z	*p*
Female	Right Frontal Sinus	4.67	3.77	-1.23	*0.22*
	Left Frontal Sinus	5.03	4.10		
Male	Right Frontal Sinus	7.07	4.52	-2.04	*0.04**
	Left Frontal Sinus	7.77	5.01		
Female	Right Sphenoidal Sinus	6.35	3.84	-0.55	*0.59*
	Left Sphenoidal Sinus	6.57	4.07		
Male	Right Sphenoidal Sinus	7.34	3.70	-0.62	*0.54*
	Left Sphenoidal Sinus	7.62	4.17		
Female	Right Maxillary Sinus	19.38	6.58	-0.57	*0.57*
	Left Maxillary Sinus	19.37	6.85		
Male	Right Maxillary Sinus	22.80	7.72	-2.29	*0.02**
	Left Maxillary Sinus	23.71	8.51		

*$p<0.05$, SD: Standard Deviation

The direction and angle of NSD were calculated using the approach defined by Gencer et al. Kapusuz Gencer et al. determined the direction of NSD by accepting the convex side of the nasal septum and classified the NSD angle accordingly. They categorized the deviation angle as follows: The deviation angle of the nasal septum is classified as mild (<9 degrees), moderate (angle between 9 and 15 degrees), or severe (>15 degrees) (18).

The Wilcoxon test was employed to compare the volumes of the paranasal sinuses between genders. In addition, the Wilcoxon test was used to assess the relationship between the volumes of sinuses and NSD. The obtained p-value was considered significant when it was <0.05.

Results

In our study, septal deviation was observed in 197 individuals. Among them, 99 had a deviation toward the right, 76 had a deviation toward the left, and 22 had an S-shape deviation. Table 2 provides the values between the volumes of the frontal sinus, sphenoidal sinus, and maxillary sinus and the direction of deviation. The most striking difference was the significantly larger sinus volumes in males (Table I). In male individuals, the volume of the left frontal sinus (7.77±5.01 cm^3) was greater than that of the right frontal sinus (7.07±4.52 cm^3) (*p=0.04*). There was no significant difference observed between the mean values of the right and left frontal sinus measurements in female participants (*p=0.22*). In both male and female participants, there was no statistically significant difference between the mean values of the right and left sphenoidal sinuses (*p=0.54, p=0.59*). Similarly, in female participants, no significant difference was observed in the mean values of the right and left maxillary sinuses (*p=0.57*). However, in male participants, a significant difference was observed (*p=0.02*), with the average values of the right maxillary sinus (22.80±7.72 cm^3) being lower than those of the left (23.71±8.51 cm^3). No significant difference was observed in the mean values of right and left frontal sinus measurements based on the variable of participants' deviation status (*p=0.16*). Similarly, there was no significant difference between the mean values of the right and left frontal sinus in participants without deviation (*p=0.052*). There was no significant difference between the mean values of the right and left sphenoidal sinus in participants with or without deviation (*p=0.20, p=0.55*). Similarly, there was no significant difference between the mean values of the right and left maxillary sinus in participants with deviation (*p=0.86*). In male individuals without deviation, the mean value of the right maxillary sinus (21.06±8.14 cm^3) was lower than that of the left maxillary sinus (22.17±7.90 cm^3) (*p=0.02*) (Table II).

Table II. Comparison of right and left sinus volumes according to deviation status

Group	Measure	Mean (cm^3)	SD	Z	*p*
Deviation	Right Frontal Sinus	5.83	3.90	-1.39	*0.16*
	Left Frontal Sinus	6.33	4.45		
Non-deviation	Right Frontal Sinus	5.67	5.10	-1.94	*0.052*
	Left Frontal Sinus	6.23	5.34		
Deviation	Right Sphenoidal Sinus	6.81	3.88	-1.27	*0.20*
	Left Sphenoidal Sinus	7.34	4.38		
Non-deviation	Right Sphenoidal Sinus	6.81	3.64	-0.60	*0.55*
	Left Sphenoidal Sinus	6.41	3.51		
Deviation	Right Maxillary Sinus	20.92	6.94	-0.17	*0.86*
	Left Maxillary Sinus	21.03	7.97		
Non-deviation	Right Maxillary Sinus	21.06	8.14	-2.32	*0.02**
	Left Maxillary Sinus	22.17	7.90		

*$p<0.05$, SD: Standard Deviation

There was no significant difference between the possible directions of septal deviation (right, left, and s-shaped) and the volumes of the right and left side sinuses (*$p>0.05$*) (Table III, Table IV, Table V).

Table III. Comparison of right and left frontal sinus volumes according to deviation direction

Group	Measure	N	Mean (cm^3)	SD	Z	p
De. Dir. Right*	Right Frontal Sinus	99	5.63	3.28	*-1.52*	*0.13*
	Left Frontal Sinus	99	6.33	4.26		
De. Dir. Left**	Right Frontal Sinus	76	6.23	4.52	*-0.23*	*0.82*
	Left Frontal Sinus	76	6.46	4.69		
De. Dir. S Shape***	Right Frontal Sinus	22	6.02	5.81	*-1.58*	*0.11*
	Left Frontal Sinus	22	6.69	6.45		
De. Dir Mid****	Right Frontal Sinus	84	5.48	4.72	*-1.85*	*0.06*
	Left Frontal Sinus	84	6.01	4.86		

*Deviation Direction Right, **Deviation Direction Left, ***Deviation Direction S Shape, ****Deviation Direction Middle, SD: Standard Deviation

Table IV. Comparison of right and left sinus sphenoidal sinus volumes according to deviation direction

Group	Measure	N	Mean (cm^3)	SD	Z	p
De. Dir. Right*	Right Sphenoidal Sinus	99	7.04	4.04	-0.16	0.87
	Left Sphenoidal Sinus	99	6.86	4.25		
De. Dir. Left**	Right Sphenoidal Sinus	76	6.77	3.80	-1.19	0.24
	Left Sphenoidal Sinus	76	7.73	4.70		
De. Dir. S shape***	Right Sphenoidal Sinus	22	6.00	3.08	-1.90	0.06
	Left Sphenoidal Sinus	22	8.11	3.79		
De. Dir. Mid****	Right Sphenoidal Sinus	84	6.79	3.71	-0.59	0.56
	Left Sphenoidal Sinus	84	6.38	3.43		

*Deviation Direction Right, **Deviation Direction Left, ***Deviation Direction S Shape, ****Deviation Direction Middle,
SD: Standard Deviation

Discussion

The paranasal sinuses are cavities located inside the bones and have the same name. They all open into the lateral wall of the nasal cavity and have small holes that allow for air balance and mucus clearance through a mucociliary pathway (3). The paranasal sinuses play pivotal roles, including modulating voice resonance during speech and diminishing the overall weight of the skull. Pathologies affecting the paranasal sinuses are of significance because of their proximity to anatomical structures such as the dentofacial region, orbit, nasus, and glandula hypophysialis. The mucosa of the sinuses is common with the nasal mucosa, which is critical for the spread of infection over a wide area (8, 9). The complex shape of certain anatomical structures in fields such as otorhinolaryngology, radiology, neurosurgery, and dentistry can make clinical tasks challenging. Our study found that men have larger paranasal sinus volumes than women. Emirzeoğlu et al. used the Cavalieri principle to measure paranasal sinus volumes on CT images. It was concluded that men have larger paranasal sinus volumes than women and that the volumes of the paranasal sinuses differ significantly between genders, except for the sphenoidal sinus similar to the study being referenced (10). Cavalieri principle produces precise and reliable results in a shorter time than other methods. Diverse methodologies have been employed in the literature

for quantifying paranasal sinuses volumes, with our study using the Cavalieri principle (23, 28).

Table V. Comparison of right and left maxillary sinus volumes according to deviation direction

Group	Measure	N	Mean (cm^3)	SD	Z	p
De. Dir. Right**	Right Maxillary Sinus	99	20.88	6.87	-0.08	*0.94*
	Left Maxillary Sinus	99	21.01	7.82		
De. Dir. Left***	Right Maxillary Sinus	76	21.24	7.15	-0.28	*0.78*
	Left Maxillary Sinus	76	21.37	8.60		
De. Dir. S Shape****	Right Maxillary Sinus	22	20.47	7.86	-0.23	*0.82*
	Left Maxillary Sinus	22	20.43	6.91		
De. Dir Mid*****	Right Maxillary Sinus	84	20.93	7.94	-2.37	*0.02**
	Left Maxillary Sinus	84	22.08	7.83		

**p<0.05,* **Deviation Direction Right, ***Deviation Direction Left, ****Deviation Direction S Shape,
*****Deviation Direction Middle, SD: Standard Deviation

In a retrospective study, Sahlstrand-Johnson et al. measured the volume of 120 maxillary sinuses (32 women, 28 men) on cranial CT images of individuals aged 18–65 years. The investigation revealed that the average volume of the maxillary sinus was 15.7±5.3 cm^3, as determined by morphometric measurements. Notably, gender-based analysis demonstrated a larger maxillary sinus volume in males than in females. Furthermore, when evaluating the effect of age on volume measurements, no statistically significant correlation was found in either the right or left side volume measurements, bilaterally, according to age groups (11). Pirner et al. measured the depth, height, and width of the maxillary sinus on CT images of 50 individuals (24 males, 23 females, and 3 cadavers) aged between 16 and 78 years. When evaluating the impact of the gender factor on volume values, it has been indicated that male individuals exhibit larger volume values than female individuals. In their study, Kawarai et al. noted a tendency for the sinuses of males to have larger volumes than those of females (12, 13). The growth rate of bones directly affects the size of these structures. Sinus volumes are larger in men because of physiological and physical differences between the genders. Bone mass peaks in the second decade of life and is influenced by dietary calcium and exercise during childhood and adolescence. In girls, the rate of increase begins to decline with menarche. Bone development continues until approximately 18 years of age in females and 21-22 years of age in males. Therefore, the male skeleton has larger bones (14, 15). In this study, we analyzed the effect of the direction and shape of NSD on the volumes of the paranasal sinuses. We found a significant difference between the mean volume of the right and left maxillary sinuses in participants without deviation. However, it was observed that the mean volume of the right maxillary sinus was lower than that of the left maxillary sinus in participants without deviation. The results showed that there was no significant difference in the volumes of the sinuses based on the deviation direction to the right, left, or S-shaped. The research undertaken by Sapmaz et al. examined coronal CT scans from a cohort of 1568 individuals aged between 18 and 60 years. The study specifically incorporated CT images from 402 participants, and the assessment involved the use of ImageJ software to compute parameters such as hard palate angulation, maxillary sinus volume, and nasal septal deviation angle. The study revealed a statistically significant difference in the volume of the maxillary sinus between the side with deviation and its contralateral counterpart (16). As per Kapusuz Gencer et al., there was an elevation in the volume of the maxillary sinuses on the contralateral side in cases of severe NSD. Likewise, Orhan et al. documented a statistically notable decrease in the volume of the maxillary sinus on the side corresponding to the septal deviation compared with that on the contralateral side (17, 18). Karataş et al. retrospectively analyzed the paranasal sinus volumes of 732 participants (410 males, 322 females) using CT images. Karatas et al. included 83 participants with deviated nasal septum, who were divided into three groups after excluding other concomitant sinonasal pathologies (19). The results showed that only moderate NSD affected maxillary sinus volume but not frontal sinus volume. Stallman et al. conducted a study and discovered that 65% of cases exhibited some degree of NSD. However, their findings did not establish a significant correlation between NSD and sinus conditions. Upon examination of these studies, it can be concluded that severe and

moderate septal deviation significantly affect sinus volume (20).

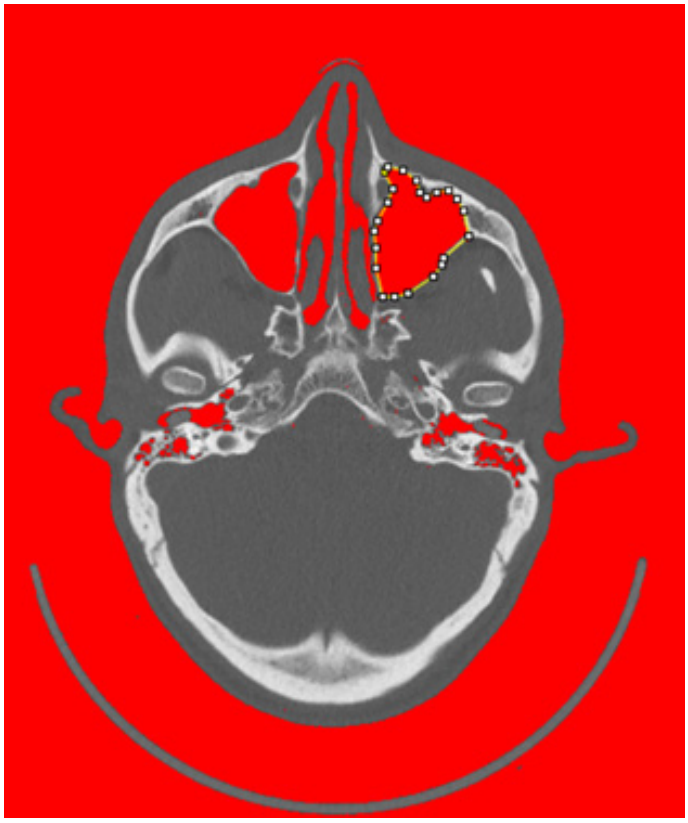

Figure I. Example of volumetric measuring of maxillary sinus. Fig. 1 displays a CT of the maxillary sinus based on the volumes measures by painting all plains of the scan.

In our study, we observed that NSD had no effect on the volume values of the frontal sinus and sphenoidal sinus. The direction of deviation, whether right, left, or s-shaped, does not significantly affect the volumes of the sinuses (21, 22).

Mild septum deviation may not have a significant effect on sinus volume. Consistent with findings in other studies, septum deviation demonstrated no impact on the volume of the frontal and sphenoidal sinuses. Additionally, the presence of mild NSD did not influence the volume of the maxillary sinuses.

Conclusion

The paranasal sinuses vary in shape, morphology, and size depending on gender. In our study, we concluded that the sinus volumes of male individuals were larger than those of female individuals. Additionally, it was determined that NSD had no effect on the volumes of the frontal sinus, sphenoidal sinus, and maxillary sinus. If the degree of NSD is mild, the presence and shape of the deviation have a very weak effect on the volumes of the sinuses. In our study, we observed that applying the Cavalieri principle for measuring volume values had advantages in terms of time, accuracy, and consistency compared to other methods. These conclusions can contribute to the perioperative assessment of craniofacial reconstruction, dental implant procedures, and sinus surgery and potentially facilitate the identification of sinus pathologies in subsequent evaluations. More data should be added to the literature on this subject, and comprehensive studies should be conducted.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Imidazopyridines in Overcoming Cancer Multidrug Resistance: New Hopes

Kanser Çoklu İlaç Dirençliliğinin Yenilmesinde İmidazopiridinler: Yeni Umutlar

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Derleme | Review Article
Doi: https://doi.org/10.52827/hititmedj.1425486
Geliş Tarihi | Received: 25.01.2024
Kabul Tarihi | Accepted: 06.04.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Yıldırım C, Yurtcu E. Imidazopyridines in Overcoming Cancer Multidrug Resistance: New Hopes. Hitit Medical Journal 2024;6(2)216-225 https://doi.org/10.52827/hititmedj.1425486

Hakem Değerlendirmesi: Hakem Değerlendirmesi: farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Etik beyana gerek yoktur.
İntihal Kontrolleri: Evet (intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: CY, EY Tasarım: CY, EY Veri toplama/Veri işleme: CY, EY Veri analizi: CY, EY Makalenin hazırlanması: CY, EY
Hasta Onamı: Hasta onamına gerek yoktur.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: No ethical declaration is required.
Plagiarism Check: Yes (intihal.net)
Conflict of Interest: The authors declared that there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: CY, EY Design: CY, EY Data Collection/Data Processing: CY, EY Data Analysis CY, EY Manuscript Preparation: CY, EY.
Informed Consent: Patient consent is not required.
Financial Disclosure: There are no financial funds for this article.

Imidazopyridines in Overcoming Cancer Multidrug Resistance: New Hopes

ABSTRACT

Pharmacotherapy protocols used in cancer treatment are far from curative for many patients due to reasons such as drug-related toxicity and decreased effectiveness of the drug. Multidrug resistance is a defense mechanism developed by the cancer cell against different drug groups or drug combinations. One of the most important reasons is the increase in the efficiency or number of ABC transporters that ensure drug removal from the cell. Imidazopyridines, which are incorporated into the structure of many biomolecules, have been produced under laboratory conditions for many years. Imidazopyridines are effective anticancer agents that can kill cancer cells in various ways. In this review, we presented a detailed summary of studies in which imidazopyridines were used to overcome multidrug resistance by targeting ABC transporters in cancer cells. For this purpose, we collectively evaluated the synthesis strategies and laboratory results of the agents synthesized and used as drugs and the compounds whose clinical trials have not yet started. The introduction of several imidazopyridine derivatives as prescription drugs reflects the potential of these molecules. We think that agents that can provide targeted therapy will be used more frequently in the clinic and will improve treatment success.

Keywords: ABC Transporters, cancer, imidazopyridine, multidrug resistance.

ÖZET

Kanser tedavisinde kullanılan farmakoterapi protokolleri ilaçlara bağlı toksisite ve ilacın etkinliğinin azalması gibi nedenlerden ötürü birçok hasta için tedavi edici olmaktan uzaktır. Çoklu ilaç direnci kanser hücresinin birbirinden farklı ilaç gruplarına ya da ilaç kombinasyonlarına karşı geliştirdiği bir savunma mekanizmasıdır. En önemli nedenlerinden biri hücreden ilaç atımını sağlayan ABC taşıyıcılarının etkinliğinin ya da sayısının artmasıdır. Birçok biyomolekülün yapısına katılan imidazopiridinler uzun yıllardır laboratuvar koşullarında üretilebilmektedirler. İmidazopiridinler kanser hücrelerini çeşitli yollarla öldürebilen etkin antikanser ajanlardır. Bu derlemede imidazopiridinlerin kanser hücrelerinde ABC taşıyıcılarını hedefleyerek çoklu ilaç direncinin üstesinden gelinmesinde kullanıldığı çalışmaların ayrıntılı bir özetini sunduk. Bu amaçla sentezlenen ve ilaç olarak kullanılan ajanlar ile henüz klinik denemelerine başlanmamış bileşiklerin sentezlenme stratejilerini ve laboratuvar sonuçlarını toplu halde değerlendirdik. Birkaç imidazopiridin türevinin reçete edilebilen ilaçlar olarak piyasaya sunulması bu moleküllerin potansiyelini yansıtmaktadır. Hedefe yönelik tedavi sağlayabilen ajanların klinikte daha sık kullanılacağını ve tedavi başarılarını iyileştireceğini düşünüyoruz.

Anahtar Sözcükler: ABC Taşıyıcılar, çoklu ilaç direnci, imidazopiridin, kanser.

Giriş

1. Bir Toplumsal Sağlık Problemi Olarak Kanser

Dünya Sağlık Örgütü'nün (DSÖ) 2020 Dünya Kanser Raporuna göre, 2018 yılında dünya genelinde 18 milyonun biraz üzerinde yeni kanser vakası ve yaklaşık 10 milyon kansere bağlı ölüm meydana gelmiştir (1). Kanser dünya çapında çoğu ülkede 30-69 yaş arası kişilerde erken ölümün birinci veya ikinci önde gelen nedenidir. Önleme çabaları kanser insidansını sınırlamak için kritik öneme sahip olsa da kanserin tedavisinde invazif cerrahi operasyonlardan radyasyon ile yok etme ve farklı kemoterapötik stratejilere kadar çeşitli yaklaşımlar uygulanmaktadır. Kanser tedavisinin etkinliğini tanı yaşı, tedavi süresi ve hastalığın yoğunluğu (kanser evresi) gibi çeşitli faktörler belirler. Ancak kanser tedavisi sıklıkla farmakolojik tedaviyi içerir (2). Sitotoksik kemoterapötik ajanlar, kanser farmakoterapisinde önemli bir rol oynamaya devam etmektedir, ancak keşif çabaları giderek daha fazla hedefe yönelik tedavilere (kanser hücrelerinin çoğalması ve yayılmasına özgü süreçlere müdahale eden ilaçlar) ve kanser için etkili ve daha az toksik farmakoterapi formları olarak immünoterapiye (bağışıklık sistemini güçlendirmek veya bağışıklık sisteminin işleyişini değiştirmek) yönelmiştir (3) Çok sayıda antikanser ilaç mevcut olmasına rağmen, birçok kanser türünün tedavisi halen zordur. Mevcut antikanser ajanlarla ilişkili toksisite, hızlı direnç gelişimi ve sınırlı etkinlik, mevcut ilaçların sınırlamalarının üstesinden gelebilecek yeni bileşikler keşfetmenin aciliyetini vurgulamaktadır (4).

a. Kanser Tedavi Modalitesi Olarak Kemoterapi

Kimyasal yapıları, doğaları, etki mekanizmaları ve diğer tedavilerle ilişkileri gibi çeşitli yönlere bağlı olarak farklılaşan farklı kemoterapi ilacı türleri vardır. Ancak kanseri tedavi etmek için kullanılan ilaçların tümü aynı etkiyi göstermez (5). Kemoterapi; içerisinde kilo kaybı, bitkinlik hissi, iştah azalması, yorgunluk hissi gibi hafif etkilerden bulantı, bağışıklık yetersizliği ve normal vücut hücrelerine zarar verilmesi gibi aşırı uçlara kadar değişen yan etkilere sahiptir. Kemoterapide kullanılan ilacın türüne bağlı olarak ortaya çıkan yan etkiler hastalarda farklılık gösterir. Hatta bu yan etkiler kemoterapi tedavi dönemleri arasında bile değişebilir (6).

Kanserlerin tedavisi için dünya çapında oldukça fazla çaba harcanmaktadır ancak kullanılan kemoterapi modalitelerinin yol açtığı yan etkiler ve zamanla gelişen ilaç direnci nedeniyle başarı halen sınırlıdır. Kanser hücrelerinde gelişen ilaç direnci, tümör hücrelerinin tam olarak ortadan kaldırılamaması nedeniyle genellikle tedavinin başarısız olması veya kanserin tekrarlaması ile sonuçlanır (7). İlaç direncinin gelişimi çeşitli faktörlerin etkisinde olan oldukça dinamik bir süreçtir (8).

Antikanser ajanların çoğunun etki gösterebilmesi için *in vivo* ortamda aktive edilesi gereklidir. İlaçların aktive edilmesi için gereken mekanizmalar oldukça karmaşıktır ve bu mekanizmalardaki değişiklikler ilacın etkinliğini olumsuz yönde etkilemekte hatta bazen ortadan kaldırmaktadır. Fizyolojik koşullarda sitokrom p450 enzimleri (CYP450), Glutatyon -S-transferazlar, gridin difosfo glukronozil transferazlar ilaçları aktive ya da inaktive ederler. Bu sistemleri değiştirebilen kanser hücreleri antikanser ilaçlara direnç kazanabilirler (9).

b. Çoklu İlaç Direnci

Kanser hücrelerinin ilaç direnci geliştirmesi tedavi başarısını olumsuz yönde etkileyen en önemli faktörlerden birisidir. Çoklu ilaç direnci (ÇİD) bir kanser hücresinin yapısal ve işlevsel olarak birbiriyle ilişkili olmayan ilaçlara ya da ajanlara aynı anda dirençli olması demektir ve en büyük endişe kaynaklarından birisidir. Direnç, spesifik bir ilaca veya ilaç kombinasyonuna karşı gelişebileceği gibi farklı moleküler hedeflere veya etki mekanizmalarına sahip ajanlara da karşı gelişebilir. Sonuç olarak bir birey daha önce hiç karşılaşmadığı ilaçlara karşı direnç kazanmış olabilir. İstatistiksel veriler, kanser hastalarının ölüm oranlarının %90'ından fazlasının ilaca karşı dirençten kaynaklandığını göstermektedir (10-12).

ÇİD genellikle çok faktörlüdür ve ilaç alımının azalması, ilaç akışının artması, lizozomlarda veya hücre içi keseciklerde ilaç tutulması, artan ilaç metabolizması, ilaca bağlı apoptozun bloke edilmesi, DNA hasarı onarım mekanizmalarının etkinleşmesi veya değişen hücre döngüsü dahil olmak üzere birçok farklı mekanizmadan kaynaklanabilir (11,13)

ÇİD'nin altında yatan en önemli moleküler mekanizmalardan birisi ATP bağlayıcı kaset (ABC) taşıyıcı süper ailesinden proteinlerin aşırı ifadelenmesidir. Dizi benzerliklerine göre 7 alt aile içinde (ABCA – ABCG) sınıflandırılan 49 insan ABC

taşıyıcısı vardır. ABC süper ailesindeki taşıyıcılar substratı olan endojen molekülleri ve ksenobiyotikleri ATP'den elde ettiği enerjiyi kullanarak zar boyunca konsantrasyon farkına rağmen taşıyabilir. ABC taşıyıcısından en az 17'sinin, kanser hücrelerinin kemoterapiye duyarlılığını azalttığı, hücre içi ilaç birikimini önlediği ve ilaç etkinliğini azalttığı gösterilmiştir. Bunlar arasında, *in vitro* ÇİD oluşturma yeteneği en iyi şekilde P-glikoprotein (P-gp:ABCB1; *ABCB1*), ABCG2 [meme kanseri direnç proteini: (BCRP);*ABCG2*] ve çoklu ilaç direnciyle ilişkili protein 1 (MRP1, [ABCC1;*ABCC1*]) için tanımlanmıştır (14-18). Paklitaksel, vinkristin ve doksorubisin gibi yaygın kullanılan kemoterapötik ajanlar bu taşıyıcıların substratları arasındadır ve bu taşıyıcıların inhibisyonu kemoterapiye cevabın artmasıyla ilişkili olabileceği uzun süreden beri bilinmektedir. Ancak yüksek afiniteli P-gp inhibitörlerinin geliştirilmesine rağmen klinikte beklenen sonuçlara ulaşılamamıştır (19,20) Bu yüzden araştırıcılar ÇİD'nin üstesinden gelebilecek yeni ajanları keşfetmeye yönelmişlerdir.

2. İmidazol Halkası İçeren Moleküllerin Biyolojik ve Tıbbi Önemi

İmidazol ilk olarak 1858'de Heinrich Debus tarafından sentezlenmiş olmasına rağmen çeşitli imidazol türevleri 1840'ların başlarından beri bilinmekteydi. İmidazol çekirdeği (Şekil I) içerisinde histidin, B12 vitamini, histamin, biyotin ve DNA baz yapısına katışan pürinler ve pirimidinler gibi insan organizmasında yer alan bazı iyi bilinen bileşenlerin ana yapısını oluşturur. Ayrıca birçok doğal veya sentetik ilaç molekülünün yapısında da mevcuttur. İmidazol içeren ilaçların çeşitli hastalıkların iyileştirilmesinde geniş bir uygulama alanı vardır. İmidazol, suda ve diğer polar çözücülerde çözünebilen 5 üyeli düzlemsel bir halkadır. Hidrojen atomu iki nitrojen atomundan herhangi birinde bulunabildiği için iki eşdeğer tautomerik formda bulunur. İmidazol amfoteriktir; yani hem asit hem de baz olarak işlev görebilir (21). Elektronca zengin nitrojen heterohalkası yalnızca protonu almak veya vermekle kalmaz, aynı zamanda çeşitli zayıf etkileşimleri de kolaylıkla oluşturabilir. İmidazol halkasının bu özel yapısal özellikleri, türevlerinin hidrojen bağları, koordinasyon, iyon-dipol, katyon-π, π-π etkileşimleri, hidrofobik etkiler, van der Waals kuvvetler vb., yoluyla biyolojik sistemlerdeki çeşitli enzimler ve reseptörlere kolayca bağlanması açısından faydalıdır. Bu yüzden biyoaktivite spektrumları oldukça geniştir (22,23).

a. İmidazopiridinler: Genel Bakış

Bir piridin halkasıyla kaynaşmış imidazolden oluşan imidazopiridin, biyolojik olarak aktif nitrojen içeren önemli bir heterosiklik moleküldür. İmidazopiridin halka sistemi, imidazol ve piridin halkalarının çeşitli halkaları tarafından üretilen ayrı nitrojen atomlarının konumlarına bağlı olarak dört sınıfa ayrılabilir (Şekil II). Çeşitli imidazopiridin türevleri arasında imidazo[1,2-a]piridin doğal ürünler ve farmasötikler alanında en önemli olanıdır. Bu türevler antifungal, antiinflamatuar, antitümör, antiviral, antibakteriyel, antiprotozoal, antipiretik, analjezik, antiapoptotik, hipnoselektif ve anksiyoselektif aktiviteler gibi geniş bir yelpazede biyolojik aktiviteler gösterirler. Ayrıca β-amiloid oluşum inhibitörleri, Gama Aminobütirik Asit (GABA) ve benzodiazepin reseptör agonistleri ve kardiyotonik ajanlar olarak da hareket ederler. İmidazo[1,2-a]piridin içeren ilaçlar arasında zolpidem (uykusuzluk tedavisinde kullanılır), olprinon (akut kalp yetmezliği tedavisinde kullanılır), zolimidin (peptik ülser tedavisinde kullanılır), alpidem, nekopidem ve saripidem (üçü de anksiyolitik ajan) piyasada mevcuttur (24).

İmidazo[4,5-b]piridin heterosiklik sistemi pürinin yapısal bir analoğudur ve türevleri DNA, RNA ve proteinler gibi büyük biyomoleküllerle *in vivo* olarak kolaylıkla etkileşime girer (25). İmidazo[4,5-b]piridin ve imidazo[4,5-c] piridin çekirdeği içeren bazı bileşiklerin çeşitli aktiviteleri kanıtlanmış olup bazıları klinik deney aşamalarındadır. Örneğin imidazo[4,5-c]piridin sistemine sahip Bamaluzol, bir antikonvülsandır ancak hiçbir zaman pazara çıkmamıştır (26). Benzer şekilde telcagepant imidazo[4,5-b] piridin kısmına sahip olan bir peptid reseptör antagonistidir. Başlangıçta migren için bir çare olarak klinik denemelerde bulunulmuşken 2009 yılında çalışmalara son verilmiştir (27). Tenatoprazol (TU-199) imidazo[4,5-b] piridin halkasına sahip olup mide proton pompa inhibitörü olarak kullanıma sunulmuştur. Bu ilaç ile ilgili halen aktif çalışmalar devam etmektedir (28).

ABD' de kullanılan FDA onaylı ilaçlar analiz edildiğinde, bu küçük moleküllü ajanların %59' unun nitrojen içeren heterosiklik bileşikler olduğu görülmektedir. İmidazol ilk on arasında yer alırken kaynaşmış imidazoller (benzimidazol ve

imidazopirimidin (pürin)) bu tür küçük moleküllü ilaçlarda en sık görülen ilk 25 içinde yer alır. Dakarbazin, bendamustin hidroklorür, fludarabin fosfat, nilotinib ve ponatinib gibi bir dizi antikanser ilaç, yapısal bileşenler olarak imidazol ve konjuge imidazol içerir (Şekil III) (29). Antikanser aktivite sergiledikleri düşünülen imidazol ve konjuge imidazol türevleri için birçok hedef arasında tubulin/mikrotübüller (30), kinazlar (31), histon deasetilazların (32) da yer aldığı biyolojik hedef araştırılmış ve tanımlanmıştır.

b. ÇİD'le Savaşta İmidazopiridin Türevleri

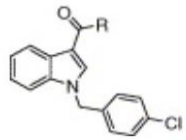
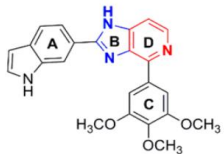
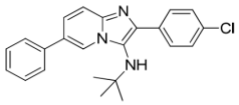
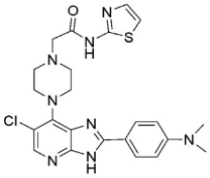
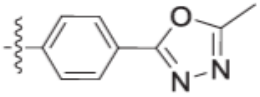
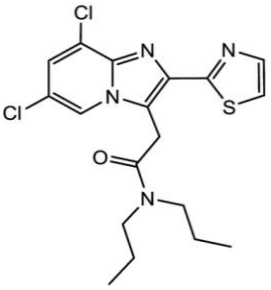
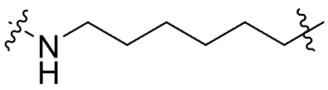
Uzun yıllardır imidazopiridin türevlerinin kanser hücreleri üzerindeki bu yöndeki potansiyeli araştırmaların konusu olmuş ancak ÇİD fenotipini doğrudan değerlendiren çok az sayıda çalışmaya ilişkin sonuçlar yayımlanmıştır. Bu amaçla araştırıcılar çoğu kez ilaç dirençli kanser hücre dizileri üzerinde çalışmalar yapmışlar ve birkaç çalışmada ajan etkinlikleri ksenograft modellerde de değerlendirilmiştir. Hücre dizileriyle yapılan çalışmalarda genellikle sentezlenen yeni bileşiklerin etkinliği kanser hücresinin dirençli olduğu ilaca kıyaslanarak belirlenmiştir. Bu amaçla hangi konsantrasyonda sitotoksik etki (IC50) gösterdiği hesaplanmış ayrıca, ABC taşıyıcılarının inhibisyonu ise substratlarının hücre içi birikiminin ölçülmesiyle gösterilmiştir. Bu derlemede sunulan ve araştırıcılar tarafından sentezlenen bileşiklerin kimyasal yapıları ve hangi hücre dizilerinde çalışıldığı Tablo I'de okuyucuların dikkatine sunulmuştur.

James ve arkadaşları (2006) sentezledikleri 13 bileşiğin etkilerini taksole dirençli lösemi hücre hattı HL60/TX1000 üzerinde denemişlerdir. Araştırıcılar çıkış molekülüne ekledikleri çeşitli yan grupların etkilerini değerlendirdikleri bu çalışmada imidazol halkası ile piridin arasına eklenen kimyasal grubun etkinliği azalttığını, ancak konjuge gruba göre etkinliğin dikkate değer şekilde arttığını göstermişlerdir. Bu kapsamda sentezlenen indol-piridoimidazol bileşiği 23 taksol dirençli MES-SA/DX5 ve HL60/TX1000 hücre hatlarında sırasıyla 0,05 ve 0,02 µM konsantrasyonda IC50 değerine sahipken bu oran taksol için her iki hücre hattında da 5 µM olarak bulunmuştur. Ancak ilaç dirençli olmayan MDA435, HL60, P388, DU145 ve MES-SA hücre hatlarında taksol daha etkilidir (33).

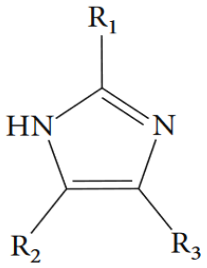
Hwang ve arkadaşları da (2015) sentezledikleri bir seri bileşik içinde indol konjuge imidazo[4,5-c] piridin bileşiği 43'ün melanoma ve prostat kanser hücre panelinde taksol direncinin üstesinden gelebileceğini ispatlamışlardır. Bileşik 43'ün A375 ve PC-3 hücreleri için çok güçlü inhibisyon aktivitesi (IC50 değerleri A375 için 3 nM, PC-3 için 8 nM) ve en iyi metabolik stabiliteyi sergilediği belirtilmiştir. Sentezlenen bileşik serisinin aynı zamanda tubulin polimerizasyonu üzerinde çok güçlü bir inhibitör etkiye de sahip olması klinikte kullanılabilecek ajan öncülleri açısından dikkat çekici bir özelliktir (34). Bileşik 43 bu çalışmadan sonra DJ95 olarak adlandırılmış ve preklinik çalışmaları daha detaylı gerçekleştirilmiştir. Benzer hücre dizileriyle ATPaz, koloni oluşumu, hücre göçü, endoteliyal hücre tüp oluşumu *in vitro* olarak belirlendikten sonra *in vivo* deneylere geçilmiş ksenograft model oluşturularak farmakokinetik çalışmaları takiben tolere edilebilen doz belirlenmiştir. DJ95'in *in vivo* sitotoksiteye neden olmadan damarlanmayı engellenmesini gösterilmesi ve kolşisinin bağlanma bölgesine bağlanması eşsiz özelliği olarak belirtilmiştir (35). Bu çalışmalar imidazol halkasına indol konjugasyonunun ABC proteinlerinin inhibisyonunda çok kuvvetli olduğunun gösterilmesi açısından dikkate değerdir.

Chan ve arkadaşları (2007) sentezledikleri CT129202 adlı bileşiğin *in vitro* ve *in vivo* koşullarda etkin bir Aurora kinaz inhibitörü olduğunu belirlemişlerdir. CCT129202 ksenograft modelde kanser hücrelerinin büyümesini durdurmuştur ve araştırıcılar bu bileşiğin gelecekte iyi bir antikanser ajan olacağını önermişlerdir (36). İlerleyen yıllarda bu küçük molekül çeşitli firmalar tarafından Aurora kinaz inhibitörü olarak satışa sunulmuştur. Cheng ve arkadaşları CCT129202'nin diğer kinazları etkilemediğini belirterek bu küçük molekülün etkin bir ABCB1 inhibitörü olabileceğini önermişlerdir. Araştırıcılar bazı ilaç dirençli hücre dizilerinde bu küçük molekülün ABCB1 ve ABCB2'ye bağlı ÇİD'i etkin bir şekilde geri çevirdiğini gösterdikten sonra *ex vivo* ve *in vivo* olarak da bu etkinin var olduğunu ispatlamışlardır. Ayrıca CCT129202'nin ABCB1 ve ABCB2 mRNA ve protein düzeylerini artırmaksızın sadece dirençli hücrelerde doksorubisin birikimini artırarak hücre ölümünde anlamlı bir artışa neden olduğunu göstermişlerdir (37).

Tablo I: Sentezlenen imidazopiridin türevleri, test edilen hücre dizileri

Bileşik adı/ Kimyasal formülü	Hücre Dizisi	Referans
23 	MES-SA/DX5 HL60/TX1000 MDA435 HL60 P388 DU145 MES-SA	(33)
43 	A375 PC-3/TxR	(34)
	L5178Y	(41)
6a 	HEK 293 Vero	(38)
CCT129202 	- KB V MCF-7/adr HL60/adr A549	(37)
6b 	MDAMB231 MDA435 HL60 MES-S MES-SA/DX5	(39)
TZ6 	A549 HCT-15 BxPC3 A431 MCF-7 A2780 A375 A498 C13* LoVo-OXP LoVo-MDR	(40)
10e 	A549/R	(42)

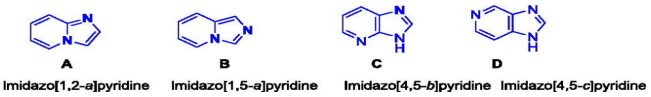
Çalışmalarda kullanılan kanser hücre dizileri: A2780:ovaryum, A375: melanoma, A431: servikal, A498: böbrek, A549/R: Taksol dirençli akciğer, A549: akciğer, BxPC3: pankreas, c13*: sisplatine dirençli ovaryum, DU145: Prostat, HCT-15: kolon, HEK 293: Embriyonik böbrek kanser, HL60/adr: doksorubisin dirençli akut promyelositik lösemi, HL60/TX1000: Taksol dirençli Akut promyelositik lösemi, HL60: Akut promyelositik lösemi, KB-V: vinkristin dirençli oral epidermoid karsinoma, L5178Y: Fare T lenfoma, LoVo-MDR: doksorubisine dirençli kolon, LoVo-OXP: oksaliplatine dirençli kolon, MCF-7/adr: doksorubisin dirençli meme, MES-SA/DX5: Doksorubisine dirençli uterin sarkoma, MES-SA: Uterin sarkoma, P388:Fare lenfoma, PC-3:Prostat, S1-M1-80 mitoksantron dirençli kolon karsinoma, VERO: maymun böbrek epitelyal



Şekil I: İmidazol çekirdeği (21)

Topoizomeraz II katalitik inhibitörleri, zehirlerin ve alkilleyici ajanların sitotoksik etkilerini modüle ederek ÇİD'nin üstesinden gelinmesinde yardımcı olmaktadır. Baviskar ve arkadaşları (2015), doğrudan ABCB1 proteinlerini hedeflemeseler de, 6a adını verdikleri 6-aril-imidazopiridin bileşiğinin topoizomeraz II'yi etkin bir şekilde inhibe ederek etoposide göre daha sitotoksik olduğunu belirlemişlerdir. Bileşik 6a HEK293 hücrelerinde 13 µM konsantrasyonda IC50 etkinliği sergilerken Vero hücrelerinde sitotoksik etki göstermemiştir. Bu özelliği seçici olarak kanser hücrelerini hedeflediğini göstermesi açısından önemlidir. Araştırıcılar ayrıca geliştirdikleri molekülün bakteriyel (DNA giraz) ve leishmanyal topoizomeraz II enzimi inhibisyonu için de faydalı olabileceğini önermişlerdir (38).

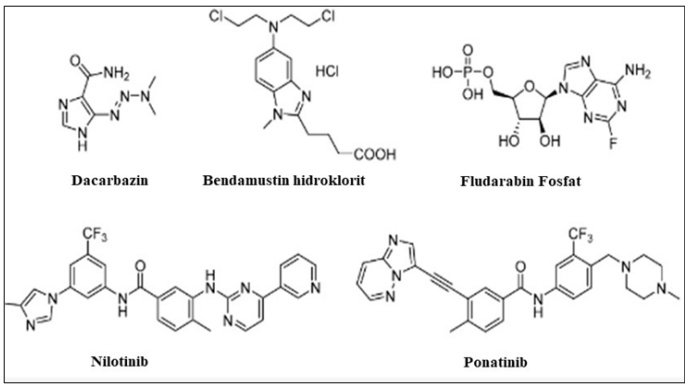
Sajith ve aarkadaşları (2015) sentezledikleri 40 adet imidazo[4,5-b] piridin türevinin yapı aktivite ilişkilerini değerlendirmişlerdir. Yapı aktivite çalışmaları esnasında heteroaril analoglarını 2-grubuna yerleştirdikleri zaman daha etkili bir molekül elde ettiklerini görmüşlerdir. Bu yolla sentezlenen bileşik 6b'nin MDAMB231, MDA435, HL60, MES-S MES-SA/DX5 hücreleri için sitotoksik değerleri sırasıyla 0,021, 0,028, 0,07, 0,025 ve 0,08 µM olarak belirlenmiştir. Bu oranlar paklitaksel için 0,457, 0,005, 0,007, 0,008, 5,1 µM'dır. Bileşik 6b üçlü negatif meme kanseri hücrelerinde paklitaksele göre çok daha etkin gibi görünmektedir (0,0021, 0,457). Ancak daha dikkat çekici bir diğer nokta ise bileşik 6d doksorubisine dirençli uterin sarkoma hücrelerinde 60 kattan daha az konsantrasyonda sitotoksik etki göstermiştir (39).



Şekil II: İmidazol halkası içeren piridin türevleri A: İmidazol[1,2-a] piridin, B: İmidazol[1,5-a]piridin, C: İmidazol[4,5-b]piridin, D:İmidazol[4,5-c]piridin (26)

Montagner ve arkadaşları (2017) mitokondrinin dış zarında bulunan, mitokondriyal redoks üzerine etki ederek çeşitli aracılarla hücre ölümünü uyaran translokatör proteini (TSPO) ligandı TZ6'nın Cu (II) ile konjuge edildiğinde oldukça etkili ve seçici yeni bir antikanser ajanı haline dönüşebileceğini raporlamışlardır. Bu amaçla 1 [$CuBr_2$(TZ6)] adını verdikleri bileşiğin antitümör etkinliğini çıkış molekülü olan TZ6, sisplatin ve oksaliplatine karşı, A549, HCT-15, BxPC3, A375, A431, MCF-7, A2780, ve A498 kanser hücrelerinde karşılaştırmıştır. TZ6 hiçbir hücre hattında ciddi bir sitotoksik etki göstermezken, bileşik 1 µM altı konsantrasyonlarda etki göstermiştir (sırasıyla 1,12, 3,16, 0,33, 2,22, 0,89, 0,21, 0,65, ve 1,16 µM). Dikkat çekici olarak MCF7 hücrelerindeki etkinliği sisplatin ve oksaliplatine karşı sırasıyla 36 ve 16 kat olarak gerçekleşmiştir. Ayrıca bileşik 1'in sisplatine dirençli ovaryum kanseri hücrelerinde 7 kat, oksaliplatin ve doksorubisine dirençli kolon kanseri hücre dizilerinde ise yaklaşık 3 kat düşük konsantrasyonlarda etkili olduğu ancak tümör olmayan hücrelerde sitotoksik etkili olmadığı gösterilmiştir. Araştırıcılar son aşama olarak *in vivo* fare modelinde bileşik 1'in tümör kütlesini %98 oranında azalttığını belirlemiştir. Bileşik 1'in hem *in vitro* hem de *in vivo* koşullarda tümör hücrelerini seçici olarak engellemesi bakır konjugasyonunun ne kadar etkili olduğunu göstermektedir (40).

Bourichi ve arkadaşları (2018) sentezledikleri 9 adet imidazo [4,5-b] piridin derivatifinin ABCB-1 aşırı ifade eden fare T lenfoma hücrelerinde etkinliğini belirlemeye çalışmışladır. Bir metoksi grubu taşımayan bileşik i en etkili bileşik olup rhodamine 123'ün hücre içi birikimini verapamile göre yaklaşık 5 kat artırmıştır. Metoksi grubu eklenmiş imidazopiridin bileşikleri de antikanser ajan olarak umut vadetmektedir (41).



Şekil III: İmidazol ve konjuge imidazol içeren ilaçlar (29)

Nikotinamid fosforibosiltransferaz (NAMPT) Nikotinamid Adenin Dinükleotid (NAD^+) oluşumunda rol oynayan hız sınırlayıcı enzimdir. NAD^+, kanser hücrelerinde biyoenerjetik, biyosentetik ve redoks homeostazisinin sürdürülmesi için gereklidir. IDO1 ise oldukça etkili bir immünosüpresif ajan olarak kanser tedavisinde umut verici bir hedeftir. Wang ve arkadaşları (2023) ilaca dirençli küçük hücreli dışı akciğer kanserinin tedavisi için nikotinamid fosforibosiltransferaz (NAMPT) ve indoleamin 2,3-dioksijenaz 1'i (IDO1) aynı anda inhibe eden bir ajan geliştirmeyi hedeflemişlerdir. Sentezlenen bir dizi molekül için 10e adlı bileşik taksol dirençli A549 hücrelerinde etkin bir şekilde her iki hedefi de inhibe ederek 5.35 µM konsantrasyonda sitotoksik etki sergilemiştir. 10e bileşiğinin epitelyal mezenşimal geçiş (EMT) sürecini ve lipit metabolizmasını engelleyerek A549/R hücrelerinin migrasyon yeteneklerini azalttığı da gösterilmiştir. Sonrasında A549/R ksenograft fare modelinde 10e'nin önemli bir sitotoksik etki olmadan tümör büyümesini büyük ölçüde inhibe ettiği belirlenmiştir (42). Tüm bu bulgular ikili hedefli moleküllerin terapötik potansiyelini göstermesi açısından dikkate değerdir.

Öte yandan ÇİD pompaları olan ABCB1 ve ABCG2 doğaları gereği hücre içinde moleküler hedefleri olan imidazopiridin türevlerini de substrat olarak kabul edebilir. Lee ve arkadaşları (2013) sentezledikleri bir bileşik olan HS173'ün, küçük hücreli dışı akciğer kanseri (NSCLC) hücrelerinin, Hep3B hücrelerinin ve SkBr3 hücrelerinin proliferasyonunu inhibe edebildiğini, G2/M basamağında hücre döngüsünü durdurduğunu ve apoptozu uyardığını göstermişlerdir (43,44). Wu ve arkadaşları ise (2023) HS173'ün ABCB1 ve ABCG2'nin bir substratı olduğunu bu yüzden etkinliğinin azaldığını ve bu durumun molekülün klinik kullanımını kısıtlayabilecek bir faktör olduğunu göstermişlerdir (45). Bu bulgular bize ÇİD'nin üstesinden gelmek için geliştirilen ajanların yine ABC taşıyıcıları tarafından hücre dışına taşındığını göstermesi açısından önemlidir. Aslında bu durum bir paradoksa da işaret etmektedir ve araştırıcılar için alınması gereken yolun büyüklüğünü göstermesi açısından önemlidir.

Sonuç

Tüm tedavi çabalarına rağmen kanser halen dünyada ölümlerin başta gelen sebepleri arasında yer almakta olup gelecekte daha fazla insanı etkileyeceği öngörülmektedir. Çok sayıda ilaç ile kanser tedavisi yapılmaya çalışılmasına rağmen yan etkiler, direnç gelişimi, ilaç etkinliğinin değişimi gibi sebepler yeni antikanser ajanların keşfi için itici bir güç oluşturmaktadır. Bu literatür özetinde gösterilmeye çalışıldığı gibi konjuge imidazol türevleri olan imidazopiridinlerin sentezlenmesi ve antikanser etkinliklerini ÇİD kapsamında değerlendirilmesi oldukça önemlidir. Bu derlemede, bu türevler kullanılarak gerçekleştirilen ve sayıları oldukça az olan *in vitro* ve *in vivo* çalışmalar gözden geçirilmiştir. Her ne kadar bu ajanların kanser tedavisi için etkinliklerini henüz kesin olarak belirlenememişse de aralarında umut verici olanları vardır.

Örneğin 2015 yılında rapor edilen bileşik 43 yıllar içerisinde DJ95 adını alarak preklinik aşamaya geçmiştir (34,35). Benzer şekilde 2007 yılında ilk kez aktivite çalışmaları gerçekleştirilen CCT129202 (36) daha sonra Aurora Kinaz inhibitörü olarak satışa sunulmuştur. Diğer ajanların da referans ilaçlara göre yüksek etkinlik sergilediği görülmektedir. Bu şekilde hedefe yönelik tedavi sağlayabilen ajanların klinik sonuçları iyileştireceği açıktır. Derlememizin bu alanda çalışan bilim insanları için yardımcı olacağını düşünüyoruz.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Therapeutic Effect of Thrombolytic Therapy on Motor Function Loss After Ischaemic Stroke: A Case Study

Trombolitik Tedavinin İskemik İnme Sonrası Motor Fonksiyon Kaybı Üzerindeki Terapötik Etkisi: Bir Vaka Çalışması

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Olgu Sunumu | Case Report
Doi: https://doi.org/10.52827/hititmedj.1418809
Geliş Tarihi | Received: 12.01.2024
Kabul Tarihi | Accepted: 11.03.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Karakoç E, Çamur S, Demirdöğen F, Akçay G. Therapeutic Effect of Thrombolytic Therapy on Motor Function Loss After Ischaemic Stroke: A Case Study. Hitit Medical Journal 2024;6(2)226-230 https://doi.org/10.52827/hititmedj.1418809

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Etik kurul onayına gerek yoktur.
İntihal Kontrolleri: Evet (iThenticate)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: EK, SÇ, FD Tasarım: EK, FD, GA Veri toplama/Veri işleme: SÇ, GA Veri analizi: FD, GA Makalenin hazırlanması: EK, SÇ, FD, GA.
Hasta Onamı: Çalışma için hasta ve/veya ailesinden onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Not applicable.
Plagiarism Check: Yes (iThenticate)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: EK, SÇ, FD Design: EK, FD, GA Data Collection/Data Processing: SÇ, GA Data Analysis: FD, GA Manuscript Preparation: EK, SÇ, FD, GA.
Informed Consent: Consent was obtained from the patient and/or family for the study.
Financial Disclosure: There are no financial funds for this article.

Therapeutic Effect of Thrombolytic Therapy on Motor Function Loss After Ischaemic Stroke: A Case Study

ABSTRACT

Stroke is a serious cerebrovascular disease that can cause disability and death if not treated with appropriate treatment methods at the right time. In ischemic stroke, recombinant tissue plasminogen activator (rt-PA) treatment is effective in motor deficit recovery, especially in the first 4.5 hours. In this case study, rt-PA thrombolytic treatment of patients with motor deficit after acute ischemic stroke aged 58 and 79 years was found to improve the loss of motor function.

Keywords: Hypertension, stroke, thrombolytic treatment.

ÖZET

İnme, doğru zamanda uygun tedavi yöntemleri ile tedavi edilmediği takdirde sakatlık ve ölüme neden olabilen ciddi bir serebrovasküler hastalıktır. İskemik inmede, rekombinant doku plazminojen aktivatörü (rt-PA) tedavisi, özellikle ilk 4,5 saatte motor fonksiyon bozukluğunun iyileşmesinde etkilidir. Bu vaka çalışmasında, akut iskemik inme sonrası motor fonksiyon kaybı olan 58 ve 79 yaşlarındaki hastaların rt-PA trombolitik tedavisinin motor fonksiyon kaybını iyileştirdiği bulunmuştur.

Anahtar Sözcükler: Hipertansiyon, inme, trombolitik tedavi.

Introduction

Stroke is a serious health problem that occurs as a result of occlusion of vessels by a thrombus or embolus called ischemic stroke; or rupture of the vessel named as hemorrhagic stroke. Approximately %80-85 strokes are ischemic (1). Stroke cases occur in approximately 20 million people worldwide annually and result in death or disability (1, 2). Although hypertension, diet, alcohol, age, diabetes and gender are factors affecting stroke, it has been observed in recent years that stroke mortality has decreased and quality of life has increased due to the identification of risk factors and early and effective treatment (3). Since stroke causes psychosocial and economic problems on the individual, family and society, the treatment of stroke is important for both the individual and the society (2). The main aim of stroke treatment is to restore perfusion in the ischemic area, to prevent secondary damages that may occur after ischemia and to put forward treatment approaches that will accelerate the recovery of cases and reduce mortality. Antiaggregant, anticoagulant, thrombolytic, antiedema and neuroprotective agents are widely used in the treatment of cerebral ischemia in the clinic. In these case reports, a 79-year-old female patient with hypertension and a 58-year-old male patient with no known disease presented to the clinic with complaints of weakness in arms and legs and difficulty in speaking. After thrombolytic treatment, motor deficit of the patients resulted in complete recovery.

Cases

Case 1: 79-year-old female patient. She had no history of systemic disease other than hypertension and complained of weakness in the left arm and leg 1 hour ago. Neurological Examination: Pupillary light reflex (PIR): ++/++, Pupils: isochoric. Left central facial paralysis (MFP): (+). Floor skin reflex: left (+). Motor deficit: left 4/5 hemiparetic. Cerebellar tests: Normal. MRI diffusion scan revealed an acute infarct area at the level of the right basal ganglion (globus pallidus and putamen) (Figure I.A). No pathology was found in hemogram, biochemistry and INR tests. Thrombolytic treatment was initiated with the consent of the patient's spouse (0.9 mg/kg, 10% of which was given as a puff and the rest as a 1-hour infusion). He was taken to intensive care unit for follow-up. After thrombolytic treatment, motor deficit resulted in complete recovery. Control computed tomography showed no hemorrhage, and the patient was transferred to the ward (Figure I.B). Electrocardiogram (ECG): Normal sinus rhythm. Ejection fraction on echo: 60%. Holter was recommended. Doppler ultrasound revealed no significant stenosis. She was discharged with the Holter result and neurology outpatient clinic was recommended. Written informed consent was obtained from the patient before thrombolytic treatment.

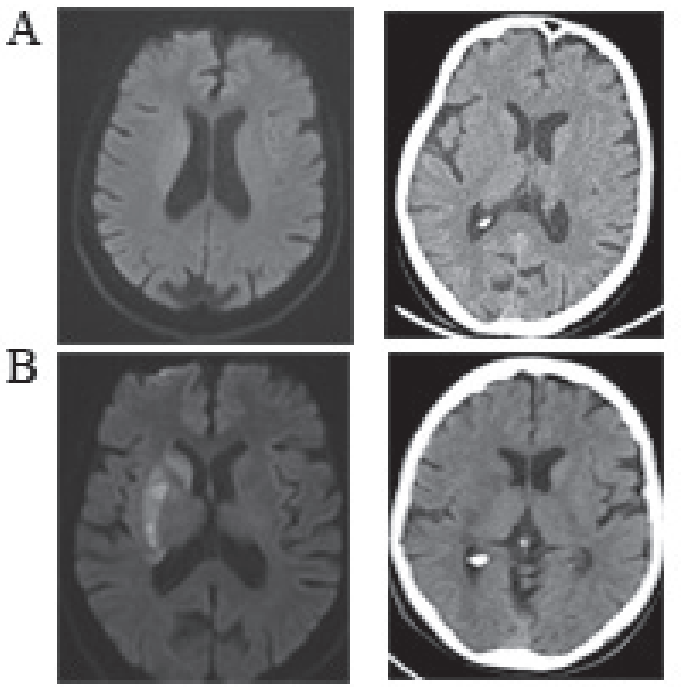

Figure I. A) Pre-operative MRI and CT B) Post-operative MRI and CT

Case 2: 58-year-old male patient. The patient had no known history of systemic disease and had sudden onset of weakness in the right arm and leg and difficulty in speaking 2 hours ago. Neurological Examination: pir:++/+++, pupils: isochoric. Left vulpian palsy (+). Right central facial paralysis (MFP): (+). Floor ciliary reflex: on the right (+). Motor deficit: right hemiplegia (+). Cerebellar tests: normal on the left, could not be evaluated on the right due to plegia. Diffusion MRI revealed diffusion limitation compatible with acute infarction in the cortex - subcortical grey matter in the anteromedial (parafalksian area) at the level of centrum semiovale in the frontal lobe in the

left hemisphere, in the frontal lobe in the anterior neighborhood of the central sulcus and in the cortex at the level of parietal lobe at the posterior level (Fig. II.A). No pathology was found in hemogram, biochemistry and INR tests. Thrombolytic treatment was initiated with the consent of the patient's spouse (0.9 mg/kg, 10% of which was given as a puff and the rest as a 1-hour infusion). He was taken to intensive care unit for follow-up. After thrombolytic treatment, motor deficit resulted in complete recovery. Right MFP persisted at a mild level. No hemorrhage was detected on control computed tomography and the patient was transferred to the ward (Fig. II.B). ECG: Normal sinus rhythm. Ejection fraction on echo: 60%. Holter was recommended. Doppler ultrasound revealed no significant stenosis. She was discharged with the Holter result and neurology outpatient clinic was recommended. Written informed consent was obtained from the patient before thrombolytic treatment.

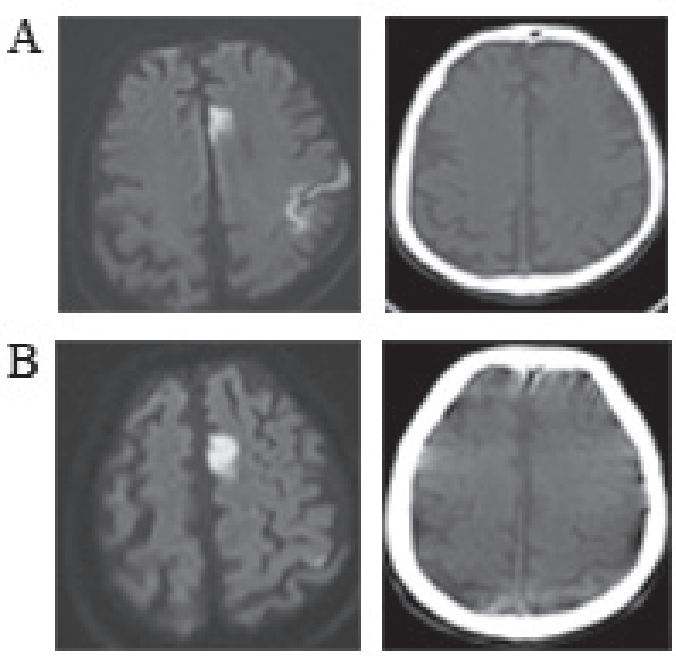

Figure II. A) Pre-operative MRI and CT B) Post-operative MRI and CT

Discussion

Stroke ranks third in terms of mortality and disability in the world after cardiovascular and cancer diseases (4). Approximately 87% of strokes are ischemic strokes (4). Ischemic stroke occurs as a result of occlusion of some or all of the vessels supplying the brain. If cerebral blood flow falls below 10 ml/min per 100 g of brain tissue, necrosis rapidly starts to develop within seconds. This area can maintain its viability for approximately 4-6 hours and permanent damage can be prevented by reperfusion (5). Therefore, the main aim in the treatment of Acute Ischemic Stroke (AIS) is to minimize cell death and damage by providing reperfusion (6). Interventional therapies including intravenous thrombolytic and endovascular treatment, which are the main treatment modalities applied for this purpose, are time-dependent therapies. Intravenous thrombolytic therapy is one of the most important strategies to recanalize the occluded vessel and provide timely reperfusion in the acute phase of stroke. The efficacy of recombinant tissue plasminogen activator (rt-PA) treatment has been proven in patients with AIS presenting in the first 4.5 hours (5). After treatment, 30% of patients may recover with minimal sequelae or without sequelae in the third month of the disease (7). In this study, thrombolytic treatment resulted in complete recovery of motor deficit in two patients with acute ischemic attack who presented to the hospital with weakness in the arm and leg.

In conclusion, Thrombolytic treatment administered within the first 4 hours after an acute ischemic attack improved the loss of motor function. thrombolytic treatment is an effective treatment method especially in the first hours of ischemia.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Isolated Percutaneous Endoscopic Gastrostomy Site Malignancy Due to Nasopharynx Cancer: A Case Report

Nazofarenks Kanserine Bağlı İzole Perkütan Endoskopik Gastrostomi Bölgesi Malignitesi: Olgu Sunumu

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Olgu Sunumu | Case Report
Doi: https://doi.org/10.52827/hititmedj.1393887
Geliş Tarihi | Received: 21.11.2023
Kabul Tarihi | Accepted: 20.03.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Karatepe YK, Bölük S, Bölük SE, Bilgiç Ç, Genç MS. Isolated Percutaneous Endoscopic Gastrostomy Site Malignancy Due to Nasopharynx Cancer: A Case Report. Hitit Medical Journal 2024;6(2) 231-235 https://doi.org/10.52827/hititmedj.1393887

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Etik kurul onayına gerek yoktur.
İntihal Kontrolleri: Evet (intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: YKK, SB, SEB Tasarım: ÇB, MSG Veri Toplama/Veri İşleme: YKK, SEB, MSG Veri Analizi: SB, ÇB Makalenin Hazırlanması: YKK, SB, SEB, ÇB, MSG.
Hasta Onamı: Çalışma için hasta ve/veya ailesinden onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Not applicable.
Plagiarism Check: Yes (intihal.net)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: YKK, SB, SEB Design: ÇB, MSG Data Collection/Data Processing: YKK, SEB, MSG Data Analysis: SB, ÇB Manuscript Preparation: YKK, SB, SEB, ÇB, MSG.
Informed Consent: Consent was obtained from the patient and/or family for the study.
Financial Disclosure: There are no financial funds for this article.

Isolated Percutaneous Endoscopic Gastrostomy Site Malignancy Due to Nasopharynx Cancer: A Case Report

ABSTRACT

PEG (Percutaneous Endoscopic Gastrostomy) procedure is a method used in patients with head and neck cancers whose oral intake is impaired. Although very rare, metastasis may occur due to the possible implantation of tumor cells compatible with primary malignancy at the PEG site. In our case report, we aimed to present a patient who was treated for nasopharyngeal cancer and was found to have a lesion compatible with metastasis at the old PEG site 7 years later.

Keywords: Metastasis, nasopharyngeal cancer, percutaneous endoscopic gastrostomy.

ÖZET

PEG (Perkütan Endoskopik Gastrostomi) işlemi, ağız yoluyla beslenme yeteneği bozulan baş ve boyun kanseri hastalarında kullanılan bir yöntemdir. Çok nadir olmakla birlikte, PEG bölgesindeki primer malign tümörle uyumlu hücrelerin implantasyonu nedeniyle metastaz meydana gelebilir. Vaka sunumumuzda, nazofarenks kanseri tedavisi gören ve eski PEG bölgesinde metastazla uyumlu bir lezyonun 7 yıl sonra görüldüğü bir hastayı sunmayı amaçladık.

Anahtar Sözcükler: Metastas, nazofarinks kanseri, perkütan endoskopik gastrostomi.

Introduction

Percutaneous endoscopic gastrostomy (PEG) is the process of endoscopically placing a feeding tube into the stomach to provide enteral nutrition to the patient in cases of impaired oral intake. The cause of oral intake disorder may be neurological diseases and masses that obstruct or narrow the passage in the nasopharyngeal region (1). It is a good alternative to surgical gastrostomy and long-term nasogastric feeding tube. It is also preferred in suitable cases because it does not generally require hospitalization after the procedure.

Nasopharyngeal cancers; It is an epithelial carcinoma arising from nasopharyngeal mucosal lining. In the nasopharynx, the tumor is often seen in the pharyngeal recess (Rosenmüller's fossa). Nasopharyngeal carcinoma and other epithelial head and neck tumors are distinctly different despite originating from similar cell or tissue lineages (2). In nasopharyngeal cancers, PEG placement is an option as the patient's oral intake may be impaired due to partial or complete obstruction. At the end of the treatment, the PEG is removed when it is no longer needed. Very rarely, in head and neck cancers, malignancy development may be observed due to tumor transplantation from the PEG site after its removal. In our case report, we aim to planned to present a patient diagnosed with nasopharynx cancer who developed malignancy at the PEG site, after its removal.

Case Report

A 71-year-old male patient was admitted to our hospital with a palpable mass and pain in the old PEG site. The patient was first diagnosed with nasopharyngeal cancer in 2015. The patient's histopathological diagnosis is squamous cell carcinoma. The percutaneous endoscopic gastrostomy (PEG), which was placed on the patient in 2016 due to malnutrition by 'pull' method, was removed in 2017 after remaining in place for 1 year. The primary treatment given to the patient for nasopharyngeal cancer is radiotherapy. No recurrence of the primary nasopharynx tumor was observed during the gastroscopy performed by us. The patient's current complaint has been present for 1 year. Granulation tissue was considered as the preliminary diagnosis in the patient and excision was performed (Figure I). The patient's pathology resulted in lymphoepithelial malignancy. In the pathologist's interpretation, it was stated that this type of malignancy was compatible with squamous cell nasopharynx cancer. Thoracoabdominal CT and PET CT were planned for the patient (Figure II). As a result of the examinations, it was observed that the lesion was compatible with a malignancy that also invaded the stomach (Figure I). No other pathology was detected.

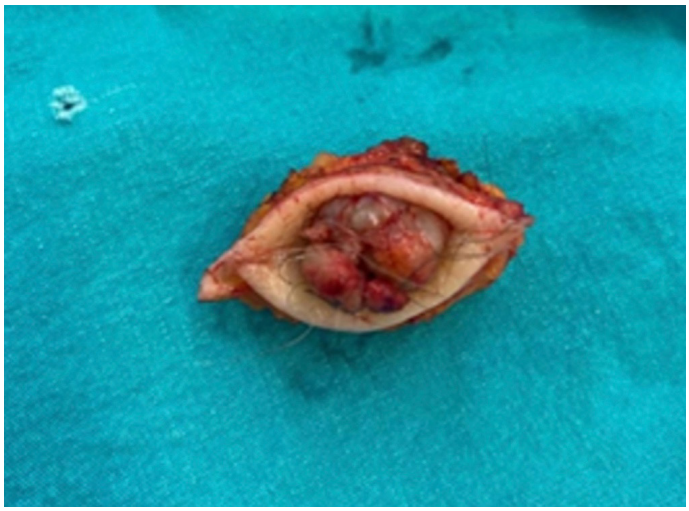

Figure I: Excision specimen of granulation tissue

Then, the patient underwent gastroscopy to determine the relationship between the mass with the stomach lumen. A 5 cm diameter mid-vegetative fragile mass lesion was seen in the same location in the distal part of the corpus (Figure III). The biopsy taken also confirmed malignant. The patient was evaluated multidisciplinary. A surgery decision was made.

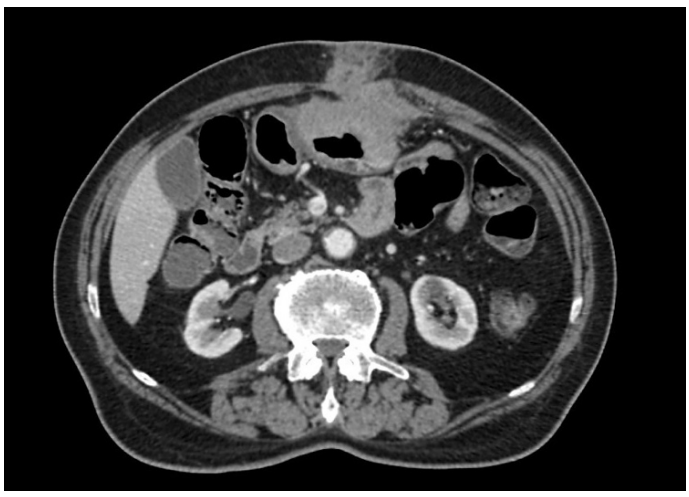

Figure II: Computed tomography image of the mass that extends into the corpus of the stomach

The patient underwent en bloc resection under general anesthesia (UGA), including the existing lesion and the relevant parts of the stomach. After the anterior wall of the stomach corpus was resected with a wedge resection, an additional resection was decided because the frozen pathology result showed microscopic continuity of the mass in the area close to the lesser curvature.

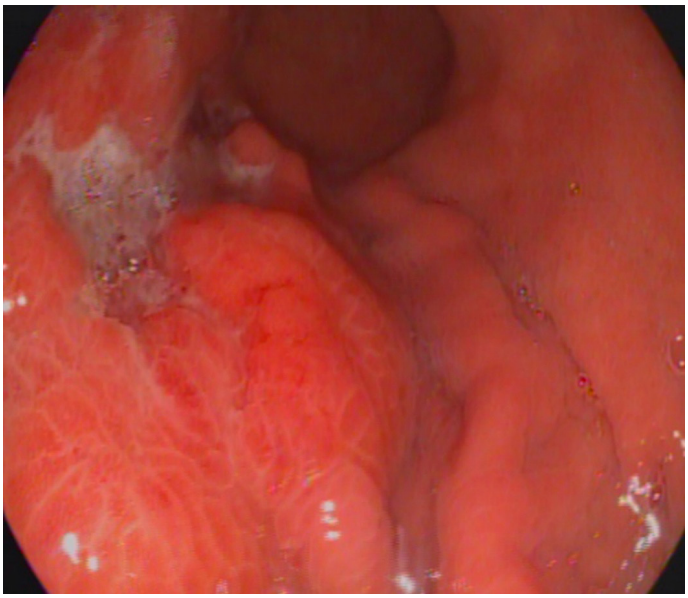

Figure III: View of malign lesion in retroflexion with the gastroscope

Considering that additional resection in the lesser curvature region could cause nutritional problems in the stomach, a partial gastrectomy operation was performed to include the stomach corpus. Then, the proximal and distal stomach segments were anastomosed to each other. Additionally, since there was a large fascia defect, the anterior abdominal wall was repaired by placing dual mesh on the fascia. The patient was discharged from hospital on the 6th day after surgery. No complications were observed during follow-up, except for slight fat necrosis in the incision line.

Discussion

Percutaneous endoscopic gastrostomy (PEG) is a procedure used when enteral nutrition cannot be provided by oral intake. PEG is indicated in patients who need long-term nutritional support, especially for more than 2 months, in obstructive neoplasms of the larynx, pharynx, or esophagus, in swallowing difficulties due to neurological disease or radiation therapy, and in facial trauma (3). Nasopharyngeal cancer is also a rare subtype among rare head and neck cancers. The female/male frequency is between 1/2 and 1/3. EBV can often play a role in the etiology (4). The patient we presented was a 70-year-old male patient. The patient's age at diagnosis and treatment of nasopharyngeal cancer was 62 years old. Nasopharyngeal cancer patients are often present in the hospital with symptoms such as lymph nodes in the neck, blood in saliva, bloody nasal discharge, and runny nose. After diagnosis, treatment methods include radiotherapy, chemotherapy, and surgery (5). During treatment, the patient may develop oral intake disorders. Even if there is no complete obstruction, tolerance to only liquid foods will cause the patient to develop malnutrition over time. For this reason, PEG insertion is a practice that can be used in head and neck cancers to prevent enteral nutrition from being interrupted during treatment. The endoscopy probe used in the endoscopic gastrostomy procedure is passed through the nasopharynx region where the neoplastic lesion is present and comes into contact with the malignant neoplastic area during its manipulation. During PEG insertion, the accessories of the set will also come into contact with the malignant area. This condition can cause metastases through direct implantation of neoplastic cells (6). With this theory of tumor transplantation in head and neck cancers, malignancy may develop at the old PEG site. It was observed that malignancy developed at the old PEG site in the patient presented in the article, who had a history of nasopharynx cancer.

The possibility of direct tumor implantation in patients to distant organs of the body is one of the controversial issues. There are examples in the literature of head and neck carcinoma metastasizing to the PEG region. However, the exact mechanism of this type of tumor spread remains unclear (7). Three possible mechanisms for metastasis at the PEG site are mentioned: 1) tumor cells are disrupted when the PEG tube is inserted from the upper digestive tract into a stoma in the abdominal wall and implant directly into the PEG site or into the esophagus 2) hematogenous dissemination of ruptured tumor cells and implant at the PEG site; 3) PEG site metastases may be random events

resulting from the hematogenous distribution of tumor cells (8,9).

The technique of inserting the gastrostomy tube may also play a role in implantation rate. Basically, a tube can be placed in the stomach using two types of methods under the guidance of endoscopy. These are the pull technique, in which the tube is inserted orally with the help of a guide wire, and the push technique, in which the tube is inserted percutaneously into the stomach directly. In the pull technique, the stomach is entered twice with an endoscope. In the push technique, it is sufficient to view the stomach with the endoscope once. Studies have shown that the implantation rate in the push technique is predictably lower (10). Since the PEG procedure report could not be obtained in the presented patient, it is not clear by which method it was inserted, but we believe that the pull technique was used, since there was a tumor implantation situation, and the pull technique has been used more frequently in recent years. It should also be noted that open surgical gastrostomy without using endoscopy is an alternative method to insert the feeding tube. However, it should not be forgotten that in this method, gastroscopy must be performed before the procedure to exclude a possible pathology in the stomach.

In conclusion; patients with head and neck tumors who have impaired oral intake, PEG insertion is a practice performed in selected patients. During PEG insertion, the endoscope is passed through the area where the tumor is present. This poses an implantation risk. Since it is a rare condition, it is not correct to conclude that open gastrostomy should be performed in all patients, but it should be kept in mind that if patients with a previous history of malignancy in the head and neck region also have a history of PEG, malignancy may develop at the old gastrostomy site.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Caudal Anesthesia for Bilateral Inguinal Hernia Repair in a Newborn with Laryngomalacia

Laringomalazili Bir Yenidoğanda Bilateral Kasık Fıtığı Onarımı için Kaudal Anestezi

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Olgu Sunumu | Case Report
Doi: https://doi.org/10.52827/hititmedj.1444951
Geliş Tarihi | Received: 29.02.2024
Kabul Tarihi | Accepted: 14.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Yeniay D, Değermenci M, Özdemir AY. Caudal Anesthesia for Bilateral Inguinal Hernia Repair in a Newborn with Laryngomalacia. Hitit Medical Journal 2024;6(2) 236-240 https://doi.org/10.52827/hititmedj.1444951

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Etik kurul onayına gerek yoktur.
İntihal Kontrolleri: Evet (intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
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Katkı Beyanı: Fikir/Hipotez: DY, AYÖ Tasarım: DY Veri toplama/ Veri işleme: MD Makalenin hazırlanması: DY.
Hasta Onamı: Çalışma için hasta ve/veya ailesinden onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Not applicable.
Plagiarism Check: Yes (intihal.net)
Conflict of Interest: The authors declared that there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: DY, AYÖ Design: DY Data Processing: MD Article Preparation: DY.
Informed Consent: Consent was obtained from the patient and/or family for the study.
Financial Disclosure: There are no financial funds for this article.

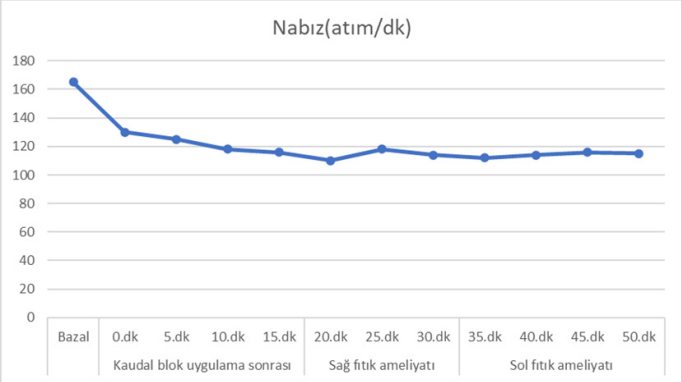
Caudal Anesthesia for Bilateral Inguinal Hernia Repair in a Newborn with Laryngomalacia

ABSTRACT
Laryngomalacia is the most common cause of stridor in newborns. Pediatric difficult airway is a major challenge for anesthetists and one of the main causes of perioperative respiratory complications. In order to prevent respiratory complications of general anesthesia, especially apnea, in newborns, regional anesthesia techniques such as caudal or spinal anesthesia may be preferred. Caudal anesthesia can be used as the sole anesthesia method, especially for subumbilical surgeries, or as an adjunct to general anesthesia and is an effective way to provide perioperative analgesia. In this article, we presented our experience with caudal anesthesia for bilateral inguinal hernia surgery in a newborn with laryngomalacia.
Keywords: Caudal Anesthesia, laryngomalacia, newborn.

ÖZET
Laringomalazi yenidoğanlarda stridorun en yaygın sebebidir. Pediatrik zor hava yolu anestezistler için büyük bir zorluktur ve perioperatif solunum komplikasyonlarının ana nedenlerinden biridir. Yenidoğanlarda genel anestezinin özellikle apne gibi solunumsal komplikasyonlarını önlemek amacıyla kaudal veya spinal anestezi gibi bölgesel anestezi teknikleri tercih edilebilir. Kaudal anestezi özellikle göbek altı cerrahiler için tek anestezi yöntemi olarak kullanılabileceği gibi genel anesteziye yardımcı olarak da kullanılabilir ve perioperatif analjezi sağlamanın etkili bir yoludur. Biz bu yazımızda, laringomalazili bir yenidoğanda bilateral herni ameliyatı için uyguladığımız kaudal anestezi deneyimimizi sunduk.
Anahtar Sözcükler: Kaudal anestezi, laringomalazi, yenidoğan.

Giriş

Laringomalazi yenidoğanlarda stridorun en yaygın sebebidir. Konjenital stridorlu tüm bebeklerin %45-75'i laringomalaziye sahiptir. Hastalığın ortaya çıkışı, ilerlemesi ve sonuçlarının spektrumu çeşitlidir. Laringomalazide supraglottik yapılar solunumun inspiratuar fazı sırasında hava yoluna çöker ve bu da inspiratuar stridor üretir. Laringomalazili bebeklerin çoğunda hafif semptomlar olur ve 12 ila 24 ay arasında düzelen iyi huylu bir hastalık seyri olur; ancak laringomalazi vakalarının tamamının iyi huylu bir seyir izlemediğinin bilinmesi önemlidir (1). Pediatrik zor hava yolu anestezistler için büyük bir zorluktur ve perioperatif solunum komplikasyonlarının ana nedenlerinden biridir (2).



Şekil I. Operasyon süresince nabız (atım/dk) değerleri

Kaudal anestezi perioperatif ve postoperatif analjezi sağlamada faydalı olabilen ve çocuklarda en sık uygulanan bölgesel anestezi tekniklerinden biridir. Tek anestezik olarak kullanılabileceği gibi genel anesteziye yardımcı olarak da kullanılabilir. Özellikle göbek altı cerrahiler için (bilateral herni onarımı, sistoskopi/transüretral manipülasyon, sünnet, anal atrezi, invajinasyon tedavisi veya kalça displazisi olan yenidoğanları hareketsiz hale getirmek için alçı uygulaması gibi) genel anestezi gereksinimini azaltmak ve perioperatif analjezi sağlamanın etkili bir yoludur. Sedasyonlu çocuklarda uygulandığında, yalnızca erken derlenmeyi sağlamakla kalmaz, aynı zamanda maksimum zor hava yolu riski olan hasta gruplarında işlem sırasında hemodinamik stabilite ve spontan solunum sağlar. Bunlar, özellikle erken doğmuş bebeklerde ve eşlik eden kardiyopulmoner hastalıkları olan çocuklarda genel anesteziye göre önemli avantajlardır (3).

Olgu Sunumu

Hastamız iki buçuk aylık 4 kg erkek bebek. Hasta tarafımıza çocuk cerrahi kliniği tarafından bilateral bilateral herni operasyonu için preoperatif değerlendirme amaçlı gönderildi. Hastamızın özgeçmişinde 39 hafta-2900 gr olarak doğması nedeniyle düşük doğum ağırlığı tanısı mevcut. Doğum sonrası kontrollerinde çocuk kardiyolojisi tarafından sekundum tip atriyal septal defekt tanısı konulmuş ve takip önerilmiş. Doğum sonrası geçmeyen inilti tarzı ses çıkarma şikâyeti olması üzerine kulak burun boğaz tarafından flexible endoskopla muayene edilen hastaya laringomalazi tanısı konulmuş. İstirahat sırasında morarma veya solunum güçlüğü olmayan, oral alımı normal olan hastaya hafif düzeyde semptom varlığı nedeniyle sadece yakın takip önerilmiş. Anne sütü alan hasta preoperatif 4 saatlik açlık ile ameliyathane masasına alındı. Nabız ve periferik oksijen saturasyonu için monitorize edildi. Oksijen maskesi ile 2 lt/dk'dan oksijen verildi. %100 saturasyonla operasyona alınan hastanın ameliyatı boyunca saturasyonunda düşme olmadı. Ameliyat süresince nabız değişikliği Şekil I'de gösterilmiştir. Başlangıçta 0,02 mg/kg atropin ve sedoanaljezi için 0,5 mg midazolam, 0,5 mcg/kg fentanil, 1,5 mg/kg ketamin intravenöz olarak uygulandı. Kaudal anestezi için lateral poziyona alındı. Sterilizasyon işlemi yapıldıktan sonra 25 gauge 30 mm kaudal iğne ile sakral hiatusa girildi. %0,25'lik 4 cc bupivakain ile kaudal anestezi sağlandı. Bloğun duyusal seviyesi pinprick testi ile değerlendirildi ve T5-6 düzeyinde belirlendi. Kaudal enjeksiyondan 20 dakika sonra hastadan yanıt alınmamasıyla (motor: bacak hareketi yok, duyusal: kalp atış hızında veya kan basıncında değişiklik yok) cerrahi başlatıldı. Ameliyat boyunca nabzında herhangi bir değişiklik olmayan hastanın ek analjezik ihtiyacı olmadı. Aktif stridoru olan hastanın omuz altı yükseltilerek operasyon boyunca daha rahat nefes alması sağlandı. Ameliyatın sonunda derlenme odasına alınan hasta yaklaşık 45 dk yakın takip edildi. Herhangi bir solunum sıkıntısı olmaması üzerine hemşire eşliğinde çocuk cerrahi servisine çıkarıldı. Postoperatif dönemde FLACC (yüz, ayaklar, aktivite, ağlama, teselli edilebilirlik) ağrı skoru ile takip edilen hastanın ilk analjezi ihtiyacı postoperatif 2.saatinde oldu. İntraoperatif ve postoperatif dönemde herhangi bir komplikasyon yaşanmadı. Başarılı ve sorunsuz geçen operasyonun

ardından hasta postoperatif 1. gününde taburcu edildi. Yazı için aileden onam alınmıştır.

Tartışma

Laringomalazi, inspirasyon sırasında supraglottik yapıların çökmesi olarak tanımlanır. Laringomalazi formlarının çoğu minördür (%70-90). Ağlama veya öksürmede herhangi bir değişiklik, dispne ve yutma bozukluğu olmaksızın izole ve aralıklı stridor şeklinde ortaya çıkar (4).

Laringospazm ve laringeal ödem, çocuklarda ekstübasyon sonrası üst hava yolu tıkanıklığının yaygın nedenleridir (5). Yenidoğanlarda bir saatten uzun süren endotrakeal entübasyon, özellikle travmatik entübasyondan sonra sık görülen subglottik ödeme yol açabilir (6). Konjenital veya edinsel hava yolu patolojisi olan çocukların zor ekstübasyon ve zor yeniden entübasyon yaşadıkları bilinmektedir (7). Düşük doğum ağırlıklı yenidoğanlarda doğru anestezi seçimi yaparken postoperatif komplikasyonları en aza indirmek dikkate alınmalıdır. Bu hasta grubunda temel sorun, genel anestezi sonrasında ortaya çıkma olasılığı daha yüksek olan apnedir. Yenidoğanlarda genel anestezinin özellikle apne gibi solunumsal komplikasyonlarını önlemek amacıyla kaudal veya spinal anestezi gibi bölgesel anestezi teknikleri tercih edilebilir. Kaudal anestezi, spinal anesteziye göre daha yaygın olarak kullanılan ve daha kolay bir tekniktir (8). Biz de bu hastada genel anestezi sonrası apneyi ortadan kaldırmak ve olası postoperatif komplikasyonları en aza indirmek için daha fazla deneyime sahip olduğumuz kaudal anestezi yöntemini uyguladık.

Birçok olgu raporunda tek başına kaudal anestezi kullanımı uygun bir anestezi tekniği olduğu gösterilmiştir. Geze ve ark. (9) inguinal herni tamirinde levobupivakainle kaudal anestezi uyguladıkları 15 düşük doğum ağırlıklı yenidoğan deneyimlerini paylaşmışlar. Spear ve ark. (10), alt vücut cerrahi prosedürleri için yedi uyanık veya sedasyonlu yüksek riskli yenidoğanda %0,25 1,0–1,3 ml/kg bupivakain ve 1:200,000 epinefrin kullanarak güvenli bir şekilde kaudal anestezi uygulamışlar ve yanlızca bir hastada ek ilioinguinal/iliohipogastrik blok gerekmiş. Bizim hastamızın ameliyatı başlangıçta yaptığımız sedasyon ile devam etti ve herhangi bir ek ilaç gerekmedi. Uyanık kaudal anestezide spinal blok, yetersiz analjezi ve anestezi gibi komplikasyonlar görülebilmektedir. Ameliyat süresi sınırlıdır. Ameliyat süresi uzadığında veya beklenmeyen cerrahi durumlar ortaya çıktığında genel anesteziye geçmek gerekebilir. Bilateral herni ameliyatlarında ortalama ameliyat süresinin kısa olması ve genel anestezi gerektirmemesi nedeniyle kaudal anestezi yeterli olmaktadır (8). Bizim olgumuzun ameliyat süresi için de kaudal anestezi yeterli olmuştur. Genel anestezi gerekmemiştir. Total spinal blok yaşanmamıştır.

Tek başına kaudal anestezi uygulanan ameliyatlarda yenidoğanlarda sedasyon amacıyla propofol kullanıldığında apne gelişebilmektedir ve ventilasyon gerekebilmektedir. Yenidoğanlarda spontan solunumu korumak amacıyla ameliyat tipi ve süresi değerlendirilerek minimal sedasyon tercih edilmelidir (9). Biz de kaudal blok öncesinde sedoanaljezi amacıyla solunumu durdurmayacak düzeyde midazolam, ketamin ve fentanil uyguladık. Ameliyat boyunca yeterli oldu ve genel anestezi uygulanmadı. İntraoperatif ve postoperatif dönemlerde apne ya da solunumsal komplikasyonla karşılaşılmadı. Daftary ve Jagtap (11) anorektal, inguinal ve abdominal cerrahi geçiren 43 preterm yenidoğan hastadan oluşan bir seride tek anestezik olarak kaudal epidural anestezi uygulamışlar. Bu uygulamanın hasta sonuçları üzerindeki etkisini değerlendirdiklerinde, bu hastalarda tek başına kaudal anestezi uygulamanın postoperatif açlık süresini ve hastanede kalış süresini kısalttığını bulmuşlar. Aynı zamanda postoperatif komplikasyon insidansının düşük olmasıyla ve yoğun bakım yönetiminin daha kolay olmasıyla da ilişkilendirmişlerdir. Bizim hastamızda da ameliyat sonrası herhangi bir komplikasyonla karşılaşılmadı ve postoperatif 1. gününde taburcu edildi.

Sonuç olarak, yenidoğanlarda bilateral herni onarımında sedasyonlu kaudal blok etkili ve güvenli bir anestezi tekniğidir. Düşük doğum ağırlıklı yenidoğanlarda postoperatif anestezi komplikasyonlarını önlemek için kaudal anestezi etkili bir teknik olarak önerilebilir. Laringomalazili bir yenidoğan için zor havayolu hazırlığı mutlaka yapılmış olmalıdır.

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HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

Kounis Syndrome Accompanying Diffuse Alveolar Hemorrhage due to Pinaverium Bromide: A Case Report

Pinaverium Bromide Bağlı Diffüz Alveoler Hemorajiye Eşlik Eden Kounis Sendromu: Olgu Sunumu

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Makale Bilgisi | Article Information
Makale Türü | Article Type: Olgu Sunumu | Case Report
Doi: https://doi.org/10.52827/hititmedj.1451459
Geliş Tarihi | Received: 12.03.2024
Kabul Tarihi | Accepted: 14.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
Güneş O, Öztürk A, Kuşdoğan M, Günay S, Erenler AK. Kounis Syndrome Accompanying Diffuse Alveolar Hemorrhage due to Pinaverium Bromide: A Case Report. Hitit Medical Journal 2024;6(2) 241-245 https://doi.org/10.52827/hititmedj.1451459.

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Etik kurul onayına gerek yoktur.
İntihal Kontrolleri: Evet (intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: AÖ, AKE Tasarım: AÖ, AKE Veri Toplama/Veri İşleme: SG Veri Analizi: MK, SG Makalenin Hazırlanması: OG, AÖ, MK, SG, AKE.
Hasta Onamı: Çalışma için hasta ve/veya ailesinden onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Not applicable.
Plagiarism Check: Yes (intihal.net)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: AÖ, AKE Design: AÖ, AKE Data Collection/Data Processing: SG Data Analysis: MK, SG Manuscript Preparation: OG, AÖ, MK, SG, AKE.
Informed Consent: Consent was obtained from the patient and/or family for the study.
Financial Disclosure: There are no financial funds for this article.

Kounis Syndrome Accompanying Diffuse Alveolar Hemorrhage due to Pinaverium Bromide: A Case Report

ABSTRACT

Kounis syndrome is defined as a coronary artery distortion due to hypersensitivity to various reasons such as drugs, environmental exposure, nutrients, coronary stents, etc. Diffuse alveolar hemorrhage is a life-threatening condition that clinically presents with hypoxemic respiratory distress, low hematocrit level, hemoptysis, and extensive pulmonary infiltration. Drugs involving pinaverium bromide as an active ingredient is frequently used for relieving gastrointestinal complaints. In this report, we present a case of a young female patient with Kounis syndrome accompanying diffuse alveolar hemorrhage admitted to our emergency department due to allergic reaction following Pinaverium Bromide intake.

Keywords: Anaphylaxis, diffuse alveolar hemorrhage, Kounis Syndrome, Pinaverium Bromide

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ÖZET

Kounis sendromu, çeşitli nedenlere bağlı olarak koroner arter bozulması olarak tanımlanır. Bu nedenler arasında ilaçlar, çevresel maruziyetler, besinler, koroner stentler vb. yer alabilir. Diffüz alveoler hemoraji hipoksemik solunum sıkıntısı, düşük hematokrit seviyesi, hemoptizi ve geniş pulmoner infiltrasyon ile klinik olarak kendini gösteren hayatı tehdit eden bir durumdur. Pinaverium bromid etken maddesi içeren ilaçlar sıklıkla gastrointestinal şikayetlerin hafifletilmesi için kullanılır. Bu raporda, Pinaverium bromid alımını takiben alerjik reaksiyon nedeniyle Acil Servisimize başvuran ve Kounis sendromu ile birlikte diffüz alveoler hemorajiye sahip genç bir kadın hastayı sunuyoruz.

Anahtar Sözcükler: Anafilaksi, diffüz alveolar hemoraji, Kounis Sendromu, Pinaverium Bromid

Introduction

Kounis syndrome (KS) is defined as a coronary artery distortion due to hypersensitivity to various reasons such as drugs, environmental exposure, nutrients, coronary stents, etc (1). The disease involves three variants as vasospastic allergic angina, allergic myocardial infarction, and stent thrombus consisting of obstructing thrombus formed by eosinophil and /or mast cell infiltration (1). The variant developing due to vasospasm after drug use is type 1, and there are many examples reported in the literature (2, 3). Diffuse alveolar hemorrhage (DAH) is a life-threatening condition that clinically presents with hypoxemic respiratory distress, low hematocrit level, hemoptysis, and extensive pulmonary infiltration (4, 5). Pulmonary-renal syndromes, connective tissue disorders, infections, and medication are the main reasons for DAH. Treatment of DAH mainly depends on treatment of the underlying cause and corticosteroid is the mainstay of the treatment in many cases. Cessation of medication is recommended in drug-related and other exposure-related DAH cases (4). Drugs involving Pinaverium bromide (PB) as an active ingredient is frequently used for relieving gastrointestinal complaints (6). In this report, we present a case of a young female patient with KS accompanying DAH admitted to our emergency department (ED) due to allergic reaction following PB intake.

Case report

A 26-year-old female patient with abdominal pain was admitted to family physician and a medication involving PB as an active ingredient was administered. After medication, the patient developed tongue swelling, itching on the body, chest pain, and shortness of breath. In her medical history, any chronic disease or smoking was not determined. Her vital signs were as follows: Blood pressure: 89/60 mmHg, heart rate: 86 beats/minute, temperature: 36,1°C, oxygen saturation: 98%. On physical examination, an uvula edema was determined.

On electrocardiogram (ECG); ST elevations on DI, aVL leads and ST depression on V1, V2, V3, V4, V5, and V6 leads were observed, and the patient was diagnosed with KS (Figure I). The chest radiograph showed a reticulonodular pattern that could be consistent with diffuse alveolar hemorrhage (Figure II). On thorax computed tomography (CT), findings of DAH were determined in lung parenchyma without any findings of pulmonary embolus (Figure III). On blood analysis White blood cell (WBC) count was 20.3 10^9/L with neutrophil dominance, troponin was 585 ng/L, pH was 7.29 and lactate was 3.16 mmol/L (Table I).

Table I. Laboratory findings of the patient on admission

Parameter	On Admission	Second Day of Hospitalization	Reference Range
Leukocyte (10^9/L)	20.30	14.69	4.49-12.68
Hemoglobin (g/dL)	16	15.8	11.9-14.6
Hematocrit (%)	46.9	46.1	36.6-44
Platelet (10^9/L)	244	282	154-400
RDW (%)	38.5	39.4	38.2-49.2
INR	1.15	1.17	0.8-1.2
PT (sec)	13.4	13.7	10-14
Troponin (ng/L)	585	424	0-300
D-dimer (mg/dL)	0.12	0.19	0-0.55
Creatinine (mg/dL)	0.6	0.7	0.5-1.1
LDH (U/L)	225	263	5-248
Total bilirubin (mg/dL)	0.52	0.42	0.3-1.2
Direct bilirubin (mg/dL)	0.11	0.08	0-0.2
CRP (mg/dL)	3.34	6.1	0-5
pH	7.29	7.37	7.35-7.45
pCO_2 (mmHg)	43	44	41-51
pO_2 (mmHg)	34.5	36.2	35-45
Lactate (mmol/L)	3.16	1.31	0.5-2
Bicarbonate	20.4	24.4	20-24

Based on these findings, supportive care was initiated for the diagnoses of KS and DAH, and to address the underlying anaphylaxis, 0.5 mg of intramuscular adrenaline was administered. Additionally, 4 mg dexamethasone intravenous was administered.

The patient was consulted with a cardiologist, chest diseases specialist, anesthesiologist, and dermatologist. The cardiologist recommended observation with medical support. The patient was hospitalized in the intensive care unit (ICU) by the anesthesiologist. The patient left with written consent on the second day of hospitalization.

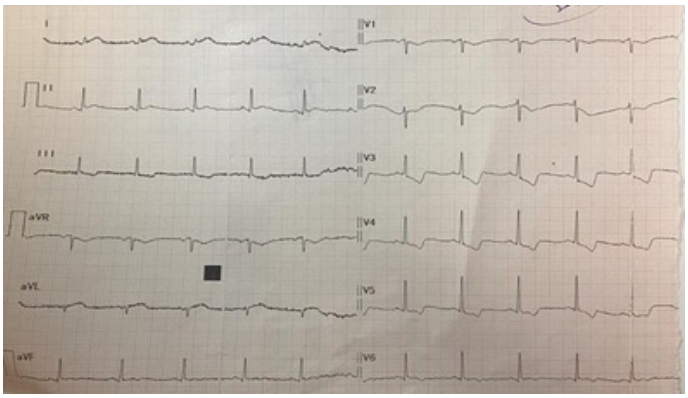

Figure I. The patient's initial ECG shows ST depressions on leads V1, V2, V3, V4, V5, and V6, with ST elevations on leads DI and aVL.

Informed consent was obtained from the patient and their relatives for the use of medical data and images related to this study.

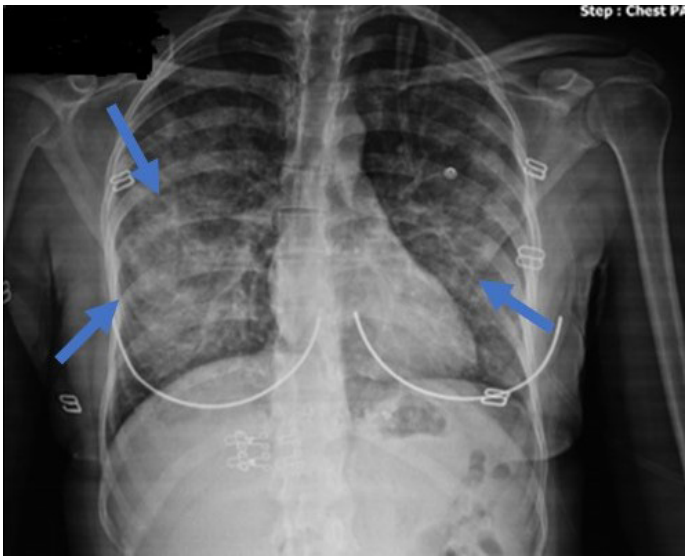

Figure II. Chest X-ray: Diffuse reticulonodular pattern

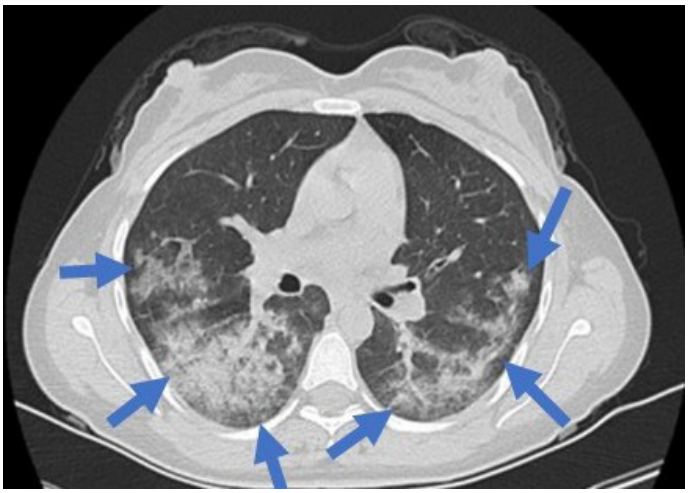

Figure III. Thorax Computed Tomography: Diffuse alveolar hemorrhage in lung parenchyma

Discussion

The main clinical signs and symptoms of KS are associated with chronic allergic reactions accompanied by cardiac symptoms. Cardiac symptoms and findings are related to ECG changes such as ST segment elevation/depression, heart block in any grade, and cardiac arrhythmias mimicking digitalis intoxication (1). KS has 3 types. Type I represents coronary spasm due to endothelial dysfunction and microvascular angina. Type II involves patients with a history of occult atheromatous disease. Type III represents patients with aspired coronary artery stent thrombus with eosinophil and mast cell involvement observed with hematoxylin eosin and Giemsa dye. KS is a complex form of acute coronary syndrome needs to be diagnosed and treated promptly. After surviving the acute event, a 12-derivation ECG, echocardiogram, and a complete cardiac assessment involving risk factor modifications must be performed (7). Although it is not a rare disease it is rarely diagnosed and may easily be misdiagnosed (1). Thus, it is important for EM physicians to obtain a 12-lead ECG and a detailed cardiac anamnesis. DAH should be kept in mind when a patient admits with hypoxemia, new-onset anemia, and alveolar infiltrations on chest X-ray. Nevertheless, in approximately 1/3 of the patients, hemoptysis cannot be determined (4). A detailed history involving exposure to drugs, physical examination, and laboratory findings should be evaluated in combination to establish a diagnosis (5). DAH is a medical emergency and immediate cessation of medication is recommended in DAH cases (4). PB is a calcium channel blocker agent generally used for irritable bowel syndrome (6). In the literature, we could not coincide a case of DAH due to PB. To our knowledge, this is the first case of KS accompanying DAH due to PB. In conclusion, PB is an easily available and frequently used medicine. Clinicians should be aware of lethal complications of PB such as KS and DAH.

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