HAMIDIYE MEDICAL JOURNAL
The Official Journal of University of Health Sciences Türkiye, Hamidiye Faculty of Medicine
E-ISSN: 2718-0956
December - 2023
Volume - 4
Issue - 3

## ORIGINAL ARTICLES



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# HAMIDIYE MEDICAL JOURNAL

2023
Volume 4

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**Web:** www.galenos.com.tr **Publisher Certificate Number:** 14521
**Online Publication Date:** December 2023 **E-ISSN:** 2718-0956
International periodical journal published three times in a year.

hamidiye med j

2023/Volume 4

HAMIDIYE
MEDICAL JOURNAL

# Aims and Scope

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**English Title:** Hamidiye Medical Journal

**Official abbreviation:** Hamidiye Med J

**E-ISSN:** 2718-0956

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HAMIDIYE
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### CASE REPORTS

ORIGINAL ARTICLE

**DOI:** 10.4274/hamidiyemedj.galenos.2023.86158

Hamidiye Med J 2023;4(3):161-167

# Long-term Cosmetic and Functional Outcomes of Feminizing Genital Surgery

## Feminizan Genitoplastinin Uzun Dönem Kozmetik ve Fonksiyonel Sonuçları

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ABSTRACT

**Background:** In this study, long-term cosmetic and functional results, quality of life, emotions, and feelings of patients who underwent feminizing genitoplasty were evaluated.

**Materials and Methods:** Twenty-two disorders of sex development cases who underwent feminizing genitoplasty were retrospectively evaluated. The primary pathology of the cases, the age of the operation, and the operations performed were recorded from the patient files. Cosmetic evaluation was performed. The sensitivity of the clitoris and vagina was evaluated. A questionnaire was administered to the patients to evaluate gender role and quality of life.

**Results:** The age of 22 cases included in the present study ranged from 12.33 to 26.02 years, and the mean age was found to be 18.50±4.23 years. Except for three cases, all cases perceived a heat difference of 2 °C below and above 37 °C. All patients stated that they were cosmetically satisfied. In the present study, it supports the behavior similar to male subjects. It was found that the quality of life was not affected in patients with disorders of sex development.

**Conclusion:** In most studies, it remains a subjective assessment because of the small number of cases and the absence of standardized scales. The fact that the culture of each country is different and the living standards are different can also change the treatment and prognosis of the patients. These patients face multiple life-long problems, from urinary tract infections to psychiatric problems. As the treating team, we should carefully evaluate the needs of these cases, keeping this in mind.

**Keywords:** Feminizing genitoplasty, outcome, cosmetic, longterm, disorder of sex development

ÖZ

**Amaç:** Çalışmamızda feminizan genitoplasti yapılan olguların uzun dönem kozmetik, fonksiyonel sonuçları ve yaşam kalitesi değerlendirildi.

**Gereç ve Yöntemler:** Feminizan genitoplasti yapılan 22 cinsiyet farklılaşma sorunu olan olgu retrospektif olarak değerlendirildi. Olguların birincil patolojisi, ameliyat yaşı ve yapılan ameliyat hasta dosyalarından kaydedildi. Kozmetik değerlendirme hasta ve cerrah tarafından ayrı ayrı yapıldı. Klitoris ve vajina hissi değerlendirildi. Cinsiyet rolü ve yaşam kalitesi anketler ile değerlendirildi.

**Bulgular:** Çalışmaya katılan 22 olgunun yaş aralığı 12,33 ila 26,02 yıl arasında değişmekteydi. Ortalama yaş 18,50±4,23 yıl olarak bulundu. Üç olgu dışında diğerlerinde 37 °C'nin 2 °C altı ve üstü ısı farkını algıladı. Tüm olgular kozmetik olarak memnundular. Olguların davranış değerlendirmesi erkek yönü desteklemekteydi. Cinsiyet farklılaşma sorunu olan olgularda yaşam kalitesinin bozulmadığı görüldü.

**Sonuç:** Cinsiyet farklılaşma sorunu olan olgular çok yönlü değerlendirme gerektirmektedir. Çoğu çalışmada hem olgu sayısı azlığı hemde standart ölçeklerin olmayışı nedeniyle subjektif değerlendirme olarak kalmaktadır. Her ülkenin kültürünün farklı oluşu hayat standartlarının farklı oluşu da olguların tedavi ve prognozunu değiştirebilmektedir. Bu olgular idrar yolu enfeksiyonundan, psikiyatrik sorunlara kadar yaşam boyu çoklu sorunlarla karşılaşmaktadır. Tedavi veren ekip olarak bunu akılda tutarak bu olguların gereksinimlerini iyi değerlendirmeliyiz.

**Anahtar Kelimeler:** Feminizan genitoplasti, sonuç, kozmetik, uzun dönem, cinsiyet farklılaşma sorunu

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**Received:** 15.01.2023 **Accepted:** 05.06.2023

## Introduction

Disorders of sex development (DSD) are a broad definition that includes hormonal, metabolic, and chromosomal abnormalities that result in abnormal genital development (1). Its incidence has been reported as 1:4,500 in the literature (2).

A case with a sex development problem should be diagnosed by a multidisciplinary team as quickly as possible, but without making a hasty decision. Gender should be determined, and treatment should be planned (2-4).

In infants of female sex, gonadectomy may be required in addition to hormone replacement therapy, genitoplasty, and genital surgery. Genital surgery varies from vaginal reconstruction or dilatation of the vaginal opening to clitoral reduction and labioplasty. The primary purpose of surgery is to make the masculine external genitalia look female and provide sexual intercourse in adult life (5). Research in the 1950s showed that sex development was based on environmental factors after birth. It is accepted that babies are psychosocially neutral at birth and that sex develops with the behavior of families and the environment, based on whether they are male or female genitalia (6). According to this, as the most appropriate sex policy, early genital cosmetic surgery was recommended based on male or female external genitalia appearance that is suitable for sexual function. Today, sexual identity differentiation is a multifactorial and complex process that includes genetic, prenatal and postnatal hormonal, psychological, and sociological effects. The importance of external genitalia appearance in this process is unknown, but it is argued that it may be minimal. Feminizing genitoplasty is also believed to have a positive effect on psychological outcomes (7). In a study conducted on cases with congenital adrenal hyperplasia, it was found that good surgical results and satisfaction with genital appearance reduced behavioral problems (8).

Although signs of progress have been observed in long-term results in genital repair and establishing a diagnosis in recent studies, the timing of sex determination and genital surgery remains controversial (9,10). Some authors recommend delaying genital surgery and sexual decisions until the child is old enough to make his own decision (10,11). Despite improvements, some patients still face difficulties and traumatic experiences for treating DSD (12).

In the present study, long-term cosmetic and functional results, quality of life, emotions, and feelings of patients who underwent feminizing genitoplasty were evaluated. This study aimed to evaluate the long-term results of the operation and especially the clitoris sensation in patients who underwent nerve-sparing clitoroplasty.

## Material and Methods

After institutional review board approval (2012-023) and obtaining informed consent from the patients and their parents, 22 DSD patients aged 12 years and over were evaluated. The patients were those who had previously undergone feminizing genitoplasty in our clinic or had feminizing genitoplasty in another center and were followed up in our clinic. The primary pathology of the cases, the age of the operation, and the operations performed were recorded from the patient files.

A cosmetic evaluation was performed by genital examination, by the categories clitoral length (normal/none-long), glans-clitoris length (normal/none-long), the distance between the clitoris and urethra (normal/none-long), labia majora normal/none-long), labia minora (normal/none-long), whether the labia majora covers the clitoris (normal/none-long), perineal distance (normal/none-long), whether there are two separate orifices (yes/no), whether the urethra vaginal entrance is in its normal place (yes/no), and whether the vaginal width is appropriate (yes/no). This scale was scored separately not only by the surgeon but also by the third person (nurse) who was not present during the operation and by the case, giving 1 point when normal. It was evaluated whether there was a difference between the scores.

The sensitivity of the clitoris and vagina was evaluated. As in the "Genitosensory Analyzer", 2 instruments with adjustable temperatures were made. One of them was between 24 °C and 50 °C , and the other was between 24 °C and 10 °C, which could be adjusted with buttons and had metal plugs at the ends. Starting at 37 °C, the patient's clitoris and anterior vaginal wall were touched, and the patient's sensation of heat and cold was questioned. The sensation of the patient was examined by increasing or decreasing 1 degree each time. A maximum of 40 °C and a minimum of 20 °C were set as the confidence limits. The sensation was evaluated by touching the clitoris and vagina of the patients with cotton. The correlation between the type of operation performed and clitoral sensation was evaluated.

In this study, a questionnaire including 19 health-related and 27 gender-role-related questions was administered to the patients to evaluate gender roles and quality of life. The answers of the patients were evaluated in five parameters and were scored from 1 to 5 (1: never, 5: always). For this purpose, the questionnaire that Julka et al. (13) used to evaluate the quality of life and gender role was translated into Turkish and used.

### Statistical Analysis

In this study, statistical analyses were performed using (the Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. Alpha Cronbach values were calculated to determine the reliability of the scales used. In the evaluation of the data, in addition to descriptive statistical methods (mean, standard deviation, frequency, percentage distributions), a one-way analysis of variance test was used in comparisons between groups, Pearson's correlation test was used to determine the relationships of variables with each other, and the weighted Kappa test was used to determine doctor and nurse evaluation concordance. The results were evaluated at a significance level of $p<0.05$.

## Results

Twenty-one of our cases were followed up with a diagnosis of congenital adrenal hyperplasia (CAH) and one with a diagnosis of mixed gonad dysgenesis. One of the CAH cases had a 46XY genetic structure and 17 β-Hydoxysteroid Dehydrogenase deficiency. Others had a 46XX genetic structure and 21 hydroxylase deficiency.

The age of 22 cases included in the present study, ranged from 12.33 to 26.02 years, and the mean age was found to be 18.50±4.23 years. Eleven cases were older than 18 years. The body weight of our patients ranged from 38.5 to 80 kg, and the mean was 56.25±11.02 kg. Height ranged from 140 to 162 cm, with an average height of 150.75±6.33 cm.

Of the cases, 4 (18.18%) were university graduates, 13 (59.09%) were high school graduates, and all (100%) were secondary school graduates or still continuingtheir studies. Twenty- of 22 cases (95.45%) in the study group were receiving hormone replacement therapy.

Clitoroplasty was performed in 21 patients. Although the age range of these cases varied between 1 and 16 years, the mean age of clitoroplasty was found to be 4.48±4.83. The age range of 21 patients who underwent vaginoplasty ranged from 1 to 16 years. The mean age of vaginoplasty was found to be 5.76±5.34 (Figure 1).

Of 21 patients who underwent clitoroplasty, 3 underwent clitoroplasty without preserving the neurovascular structure, and 18 underwent clitoroplasty with preservation of the neurovascular structure. None of the patients had a history of sexual experience. In the evaluation of the sensation of the cases, 2 of 22 cases (9.09%) had no clitoris sensation. It was found that in 1 (4.55%) of the cases, there was no vaginal or tactile sensation. The temperature difference in the clitoris was not found in 3 (13.63%) cases (Table 1).

The normal evaluation rates of the surgeon and nurse in the cosmetic evaluation are given in Table 2. The cases evaluated the cosmetic appearance as normal at a rate of 100%. The width of the vagina and the distance between the clitoris and the urethra were found to be the most inadequate by the surgeon and nurse in cosmetic evaluation. Clitoral length, glans- clitoris width, labia majora, labia minora, glans clitoris covering of labia majora, perineal distance, having two separate orifices, evaluation of the urethra, and urethra entrance were evaluated as normal at rates ranging from 60% to 100%. The evaluation of the operating doctor and the non-operating person (nurse) was found to be statistically significant (p=0.0001).

Nocturnal and/or daytime urinary incontinence was in 5 of 22 (22.72%) cases. Four cases (8.18 %) describe urgency. Postvoid dribbling was found in 2 cases (9.09%). Of these, only 3 cases (13.63%) were found to affect life. Urinary tract infection history was found in 4 (18.18%) of the cases.

The total number of answers of 22 cases to 18 questions about quality of life ranged from 25 to 43. The mean was found to be 35.27±4.77. The total number of answers of 22 cases to 27 questions about gender roles varies between 41 and 79. The mean was found to be 59.13±11.28. The general assessment of quality of life was answered between 5 and 10 out of 10. The mean value was 8±1.92.

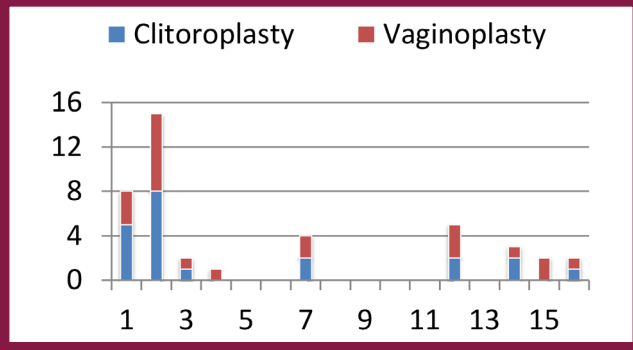


**Figure 1.** Age distribution of patients undergoing clitoroplasty and vaginoplasty

**Table 1. Distribution of postoperative sensation assessment measurements in the study group**

| | | n | % |
|---|---|---|---|
| **Sexual experience** | **No** | 22 | 100.00 |
| **Clitoris sense** | **No** | 2 | 9.09 |
| | **There is** | 20 | 90.91 |
| **Vaginal sensation** | **No** | One | 4.55 |
| | **There is** | 21 | 95.45 |
| **Sense of touch** | **No** | One | 4.55 |
| | **There is** | 21 | 95.45 |
| **Temperature difference in the climate** | **No** | 3 | 13.63 |
| | **There is** | 19 | 86.36 |

**Table 2. Comparison of physician and nurse postoperative cosmetic evaluation measurements**

| | | Doctor | | Nurse | | p |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Clitoral length | None-long | 5 | 22.73 | 6 | 27.27 | $K_w$=0.879 p=0.0001 |
| | Normal | 17 | 77.27 | 16 | 72.73 | |
| Glans clitoris width | None-long | 8 | 36.36 | 10 | 45.45 | $K_w$=0.814 p=0.0001 |
| | Normal | 14 | 63.64 | 12 | 54.55 | |
| The distance between the clitoris and urethra is sufficient | None-long | 12 | 54.55 | 8 | 36.36 | $K_w$=0.645 p=0.001 |
| | Normal | 10 | 45.45 | 14 | 63.64 | |
| Labia majora | None-long | 0 | 0.00 | 0 | 0 | $K_w$=1 p=0.0001 |
| | Normal | 22 | 100.00 | 22 | 100.00 | |
| Labia minor | None-long | 4 | 18.18 | 4 | 18.18 | $K_w$=1 p=0.0001 |
| | Normal | 18 | 81.82 | 18 | 81.82 | |
| Does labia majora cover clitoris? | None-long | 5 | 22.73 | 5 | 22.73 | $K_w$=1 p=0.0001 |
| | Normal | 17 | 77.27 | 17 | 77.27 | |
| Perineal distance | None-long | 5 | 22.73 | 4 | 18.18 | $K_w$=0.861 p=0.0001 |
| | Normal | 17 | 77.27 | 18 | 81.82 | |
| Are there two separate orifices? | No | 6 | 27.27 | 6 | 27.27 | $K_w$=1 p=0.0001 |
| | Yes | 16 | 72.73 | 16 | 72.73 | |
| Urethra vagen entry | No | 6 | 27.27 | 6 | 27.27 | $K_w$=1 p=0.0001 |
| | Yes | 16 | 72.73 | 16 | 72.73 | |
| Vagen width suitable | No | 12 | 54.55 | 12 | 54.55 | $K_w$=1 p=0.0001 |
| | Yes | 10 | 45.45 | 10 | 45.45 | |

## Discussion

Cases that reached puberty were included in the study to evaluate sexual development and sexual experience. However, although there were 11 patients older than 18 years in our study, no patient had sexual experience.

According to the 2012 data from the Turkish Statistical Institute, the average height of girls in Turkey is 162.8 cm. Accordingly, DSD cases are 12 cm shorter than the average. In an article in which the results of 35 different studies conducted with CAH cases were evaluated, it was found to be 3.5 cm shorter than their peers (14). In this study, it was argued that the short stature was related to the drug treatment that the patients received. The higher rate of short stature in our study may be due to poor planning of the treatments or other factors (quality of life, surgery, depression) besides the treatment. When the quality of life and stature of the patients were compared, no statistically significant correlation was observed. In Turkey, cases with sexual development disorder hide their diagnoses because of cultural reasons, and they hesitate to talk about it. Therefore, there may be a predisposition to depression, as well as life-long treatment. Surgery may affect the quality of life and may be a predisposition to depression.

According to the 2012 data from the Turkish Statistical Institute, the average weight of girls in Turkey is 58.1 kg. According to this, the weights of the cases are below the average. Although there is no study directly related to weight average in the literature, obesity is expected in CAH cases due to insulin resistance (15). The low mean weight in our study may be because the weights of the patients ranged widely from 38.5 to 80 kg.

Recent publications have recommended clitoral surgery in infancy (16,17). In our clinic, it is preferred to perform clitoroplasty in patients under the age of 2 years. However, the average age of clitoroplasty was found to be high because of late admissions. There is still no consensus on the age of vaginoplasty. However, it is advocated that it should be performed under the age of 1 or starting from adolescence (18,19). In our clinic, vaginoplasty is also preferred for patients under the age of 2, but the cases performed in the past years and the late admission age increase the average vaginoplasty age.

There is no standard scale to evaluate the cosmetic results of patients who underwent feminizing genitoplasty.

Most of the evaluations were made by the surgeon's examination as a good or bad cosmetic result. This causes evaluations to be subjective most of the time. In an article published in 2007, the cosmetic results of 82 patients who underwent feminizing genitoplasty were evaluated by the patient herself or her family and by the surgeon. The cosmetic results were excellent. The mothers of all cases stated that they were satisfied with the cosmetic results, but the surgeon did not use any objective criteria in the evaluation (20).

In our study, all patients stated that they were cosmetically satisfied. The evaluations of the surgeon and the third person were compatible with each other, but they were not as satisfied with the cosmetic results as the cases. This may be because the cases could not be compared. In our study, the evaluation of the third person who did not undergo surgery allowed us to obtain a more objective result. In another study performed with different techniques and at different ages, the clitoris was evaluated as 59%, vaginal opening 18%, vaginal length 73%, and labia 61% normal. The evaluation was made only by the surgeon, but the evaluation was made using a scale they created (21). The Fortunoff vaginoplasty technique was used in our study, and we could say that our cosmetic results are better than these results.

There are very few publications that evaluate the sensitivity of genitoplasty. In a study by Crouch et al., they evaluated 28 cases of genital sensitivity with the Genitosensory Analyzer (GSA) in cases who underwent feminizing genitoplasty compared with the control group.

The threshold of clitoral warmth perception of the cases to whom feminizing genitoplasty was applied was found higher (42.8 °C, the control group: 39.2 °C) and the threshold of coldness perception was found to be lower (28 °C, the control group: 33 °C) than the control group (8). CAH cases participated in the vaginal sensitivity test, and no difference was observed compared with the control group of 4 people. There are 2 separate probes giving heat and vibration in this device. When the patient felt the heat, she stopped the test by pressing the button in her hand. Thus, the threshold values for hotness and coldness were determined. Different techniques were used in the surgery of the patients, but no comparison was made (22). Again, in a similar study, which Lesma et al. conducted with 12 cases and 12 control groups, it was found that sensitivity in the clitoris was lower than in the controls. He found no difference in vaginal sensitivity compared with controls. Only the Passerini Glazel technique was used in this study (23). In this study, we used 2 devices, similar to the GSA device, with which we can adjust the desired temperature. When the patients felt the heat difference, they gave verbal feedback. Except for three cases, all cases perceived a heat difference of 2 °C below and above 37 °C. In our study, there was no control group, and we did not have the opportunity to use decimal degrees while increasing the temperature. In 3 of our cases, clitoroplasty was performed without preserving the neurovascular structure. It was found that 3 patients without a clitoris temperature difference did not undergo nerve-sparing surgery.

In a study conducted by Nabhan et al. (24), urinary infection history was found to be 8.5% in patients operated on before 36 months and 3% in patients operated on after 36 months. It was stated that this was consistent with the same age group without health problems (24). In the study by Lesma et al. (20), no urinary infection was found. In the study of Canalichio et al. (25), urinary infection was found to be 10% and urinary incontinence 19%. The incidence of urinary tract infection and urinary incontinence in our patients was higher than that in other studies.

The evaluation criteria of the publications evaluating the quality of life in patients with DSD are different from each other. Some have made qualitative evaluations and some have made quantitative evaluations. Qualitative evaluations show that sexual functions and sexual satisfaction of DSD cases decrease (26,27). In the quantitative evaluation, the cases were evaluated using scored questionnaires. Outcomes ranged from a better quality of life than controls to severely affected (28-31). In the evaluation of Julka et al. (13), the mean score of quality of life was found to be 39.5±6.8 in DSD cases, 34.3±7.6 in healthy controls; in the present study, the score of DSD cases was found to be 35.27±4.77. As in the study by Julka et al. (13), it was found in this study that the quality of life was not affected in DSD cases.

Studies have argued that the rate of male-type behaviors is high in female patients with CAH (32-34). In only one of our cases, the mother complained of male-type behavior. Julka et al. (13) found the mean score of gender role to be 84±5.4 in female DSD cases, 51.2±6.0 in male DSD cases, 82.5±8.7 in healthy female cases, and 53.2±7.1 in healthy male cases. In this study, all cases were raised as females, and the mean score was 59.13 59.13±11.28. The study by Julka et al. (13), supports that female DSD cases behave like healthy females. On the other hand, in this study, it supports behavior similar to male subjects. Julka et al. (13) included 8 female and 5 male DSD cases. This reduces the reliability of the study. Having 22 cases in this study may provide more reliable results.

**Study Limitations**

However, because of the lack of a control group in the present study, it could not be evaluated whether the results obtained were different from those of healthy individuals

in our society. The lack of a standard scale to evaluate the cases and the cultural, structural, and religious understanding of each society may lead to different results in each study. In addition, because of the high number of cases and the use of a quantitative scale in the present study, its reliability is higher than that in other studies.

## Conclusion

In the present study, as in other studies, it was found that patients who did not undergo nerve-sparing clitoroplasty did not perceive the temperature difference and had no sense of touch. This is an indication that neurovascular structures must be protected while performing clitoroplasty.

In addition, DSD cases require multidisciplinary evaluation. In most studies, it remains a subjective assessment because of the small number of cases and the absence of standardized scales. The fact that the culture of each country is different and the living standards are different can also change the treatment and prognosis of the patients. These patients face multiple life-long problems, from urinary tract infections to psychiatric problems. As the treating team, we should carefully evaluate the needs of these cases, keeping this in mind. There is a need to use standard scales and studies with control groups to evaluate DSD cases.

### Ethics

**Ethics Committee Approval:** Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital Non-Pharmaceutical Clinical Research Ethics Committee (decision date: 20.06.2021/decision no: 2012-023).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally and internally peer reviewed.

### Authorship Contributions

Surgical and Medical Practices: Z.A.K., T.T., Concept: T.T., Design: T.T., Data Collection or Processing: T.T., Analysis or Interpretation: T.T., Literature Search: Z.A.K., T.T., Writing: Z.A.K.

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

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

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ORIGINAL ARTICLE

**DOI:** 10.4274/hamidiyemedj.galenos.2023.21033

Hamidiye Med J 2023;4(3):168-176

# The Comparison of Apoptosis Anti-apoptosis and Autophagy Markers in Epicardial Tissue in Patients with Coronary Artery Disease and in Patients have Valvular Disease, HOCM, Ascending Aortic Aneurysm without Coronary Artery Disease

## Koroner Arter Hastalığı Olan ve Koroner Arter Hastalığı Olmayan Kapak Hastalığı, HOKM, Asendan Aort Anevrizması Olan Hastalarda Epikardiyal Dokuda Apoptoz Anti-apoptoz ve Otofaji Belirteçlerinin Karşılaştırılması

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ABSTRACT

**Background:** Apoptosis is a highly programed mechanism that occurs as a part of cell life. Anti-apoptotic and pro-apoptotic proteins regulate this process. Autophagy is a catabolic process that involves the degradation and recycling of aged cytoplasmic components. In this study, we evaluated the expression rates of bax (apoptosis), Bcl-2 (anti-apoptosis), and beclin (autophagy) proteins in epitadial tissue in cardiovascular diseases.

**Materials and Methods:** Patients with coronary artery by-pass graft (CABG) due to coronary artery disease constituted the patient group. Patients who underwent mitral valve repair (MVR) for severe mitral regurgitation, or who underwent aortic valve replacement (AVR) for severe aortic regurgitation, or who underwent myectomy for hypertrophic obstructive cardiomyopathy (HOCM) or Benthall procedure for ascending aortic aneurysm were included in the study as a control group. Biopsy samples were taken from the epicardial tissues during surgery. Sections taken from the samples were stained with hematoxylin and eosin and examined histopathologically under a light microscope to evaluate inflammation intensity. Samples found suitable were stained immunohistochemically with bronchioloalveolar carcinoma, Bcl-, and Beclin antibodies.

**Results:** Inflammation in patients with CABG was considerably higher than that in the control group. In patients with CABG with inflammation, Bax expression increased in parallel with inflammation. Bcl-2 expression was significantly increased in patients with MVR, AVR, or myectomy. The highest rate of Beclin expression was observed in HOCM patients. Similar results were obtained in the patient who underwent the Benthall procedure as in the HOCM patient. Beclin expression was also increased in patients who underwent AVR or MVR and showed high Bcl-2 expression.

**Conclusion:** All of the participants had Bax, Bcl-2, and Beclin expression, albeit at different levels. Inflammation level is determinative for Bax and Beclin expression. In patients with inflammatory CABG, Bax expression increases in parallel with inflammation. In patients with MVR or AVR or myectomy without ischemic heart disease, Bcl-2 expression increased much more than in patients with CABG.

**Keywords:** Apoptosis, cardiovascular diseases, Bcl-2, Bax, Beclin

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**Received:** 03.05.2023 **Accepted:** 16.08.2023

ÖZ

**Amaç:** Apoptoz, hücre yaşamının bir parçası olarak ortaya çıkan yüksek düzeyde programlanmış bir mekanizmadır. Anti-apoptotik ve pro-apoptotik proteinler bu süreci düzenler. Otofaji, yaşlanmış sitoplazmik bileşenlerin parçalanması ve geri dönüşümünü içeren katabolik bir süreçtir. Bu çalışmada kardiyovasküler hastalıklarda epikadiyal dokuda Bax (apoptoz), Bcl-2 (anti-apoptoz), Beclin (otofaji) protein ekspresyon oranlarını değerlendirdik.

**Gereç ve Yöntemler:** Koroner arter hastalığı nedeniyle koroner arter by-pass grefti (KABG) yapılan hastalar hasta grubunu oluşturmuştur. Şiddetli mitral yetersizliği nedeniyle MVR yapılan veya şiddetli aort yetersizliği nedeniyle AVR yapılan veya hipertrofik obstrüktif kardiyomiyopati (HOCM) nedeniyle miyektomi yapılan veya çıkan aort anevrizması nedeniyle Benthall prosedürü uygulanan hastalar kontrol grubu olarak çalışmaya dahil edildi. Ameliyat sırasında epikardiyal dokulardan biyopsi örnekleri alındı. Örneklerden alınan kesitler hematoksilen ve eozin ile boyandı ve inflamasyon yoğunluklarını değerlendirmek için ışık mikroskobu altında histopatolojik olarak incelendi. Uygun bulunan örnekler immünohistokimyasal olarak BAC, Bcl- ve Beclin antikorları ile boyandı.

**Bulgular:** KABG hastalarında enflamasyon kontrol grubuna göre oldukça yüksekti. Enflamasyonlu KABG hastalarında Bax ekspresyonu enflamasyona paralel olarak artmıştır. MVR veya AVR veya miyektomi yapılan hastalarda Bcl-2 ekspresyonu anlamlı derecede artmıştır. En yüksek Beclin ekspresyonu oranı HOCM hastasında gözlendi. Benthall prosedürü uygulanan hastada da HOCM hastasında olduğu gibi benzer sonuçlar elde edildi. Beclin ekspresyonu AVR veya MVR yapılan ve yüksek Bcl-2 ekspresyonu gösteren hastalarda da artmıştır.

**Sonuç:** Katılımcıların hepsinde farklı düzeylerde de olsa Bax, Bcl-2 ve Beclin ekspresyonu vardı, enflamasyon düzeyi Bax ve Beclin ekspresyonu için belirleyicidir, enflamatuvar KABG'li hastalarda özellikle Bax ekspresyonu inflamasyona paralel olarak artar, İskemik kalp hastalığı olmayan MVR veya AVR veya miyektomili hastalarda Bcl-2 ekspresyonları KABG'li hastalara göre çok daha fazla artmıştır.

**Anahtar Kelimeler:** Apoptoz, kardiyovasküler hastalıklar, Bcl-2, Bax, Beclin

## Introduction

Apoptosis is a highly programed, energy-dependent mechanism that occurs as a part of cell death and development, tissue turnover, and the immune system without damaging the surrounding tissue (1). Apoptosis is the bridge between pro-death and survival signals, and its outcome is key to cell fate (2). It is characterized by its specific morphology, which includes cell shrinkage, chromatin condensation, DNA fragmentation, membrane blebbing, and formation of apoptotic bodies (1). Data obtained from the examination of human heart tissue demonstrated increased apoptosis in idiopathic dilated cardiomyopathy, ischemic cardiomyopathy, and hypertrophic cardiomyopathy (1). The degree of apoptosis associated with such conditions is usually quite low; however, it is thought that the gradual loss of heart cells over time may contribute to eventual heart failure (1). In addition, the majority of tissues examined were obtained in the last stage of the disease, and it has been suggested that apoptosis may play a role in the transition from mild hypertrophy to end-stage heart failure (2). Apoptosis is regulated by the complex interplay of numerous prosurvival and prodeath signals. In particular, the Bcl-2 protein family consists of both anti-apoptotic (Bcl-2, Bcl-xl) and pro-apoptotic (Bax, Bid) proteins and exerts its effects by altering the integrity of the mitochondrial membrane and by releasing apoptotic intermembrane proteins (2).

Autophagy is a catabolic process involving the degradation and recycling of aged cytoplasmic components, such as long-lived proteins and organelles, with the aid of lysosomes (3). Under normal conditions, it is responsible for maintaining homeostasis. In nutrient-deprived cells, autophagy is a cell-survival mechanism but also mediates cell death under certain conditions (3). It has various physiological and pathophysiological roles such as adaptation to nutrient deprivation, protein intracellular clearance, growth, anti-aging, elimination of microorganisms, cell death, tumor suppression, and antigen presentation (4). Autophagy in the heart appears to be upregulated in conditions such as ischemia -reperfusion and heart failure (3). It is unclear whether autophagy is beneficial or dangerous in the heart because it appears to modulate cell viability and death. The presence of autophagic vacuoles in dying cells can be assessed in one of two ways: cells activate autophagy for survival or as part of cell death (3). In a cell with insufficient autophagic activity, aged proteins and defective organelles accumulate and eventually apoptotic cell death occurs (4). Conversely, if autophagy degrades proteins and organelles beyond a certain threshold, autophagic cell death will occur, especially in cells with an inadequate apoptotic response (4). It can be concluded that the crossover mechanism occurs between the two mechanisms and that the balance between autophagy and apoptosis maintains homeostasis. Autophagy is mainly controlled by autophagy-related genes that regulate autophagosome formation (3). Beclin-1 is required for the vesicle nucleation stage of autophagy (3).

In this study, we evaluated the expression rates of bax (apoptosis), Bcl-2 (anti-apoptosis), and beclin (autophagy) protein in epitadial tissue in patients with coronary artery

by-pass graft (CABG) or mitral valve replacement (MVR) or aortic valve replacement (AVR) or Benthall procedure or myectomy.

## Materials and Methods

### Study Design and Study Population

The study was conducted between 2016 and 2018 at the University of Health Sciences Türkiye Kartal Koşuyolu High Specialization Training and Research Hospital. In the study, patients who underwent CABG because of obstructive coronary artery disease constituted the patient group. The patients were evaluated in the cardiology outpatient clinic because of chest pain, and coronary angiography was performed according to the results of the effort test or myocardial perfusion test. All patients had normal troponin levels. Patients with normal coronary arteries detected on preoperative coronary angiography who underwent MVR for severe mitral regurgitation, or who underwent AVR for severe aortic regurgitation, or who underwent myectomy for hypertrophic obstructive cardiomyopathy (HOCM) or Benthall procedure for ascending aortic aneurysm were included in the study as a control group. The left ventricular systolic functions of the patients were preserved both preoperatively and postoperatively (ejection fraction >50%). One of the patients with severe mitral regurgitation had mitral valve prolapse, whereas the others had rheumatic valve disease. All patients with advanced aortic regurgitation have degenerative valve disease. The study was carried out with the approval of the local ethics committee (2023/06/683). All patients were informed in detail about biopsy procedures, surgical procedures to be performed, all complications that may occur during and after surgical procedures, the follow-up period, and patient consent forms.

### Histopathological Examination

Biopsy samples were taken from the epicardial tissues of the patients during surgery. Formalin-fixed paraffin blocks were prepared from the biopsy samples. Sections of 4-5 microns taken from the samples were stained with hematoxylin and eosin (H&E) and examined histopathologically under a Nikon Eclipse E600 light microscope. Inflammation intensities in the patients were assessed by considering the number of inflammatory cells entering the high magnification field in light microscopic examination and were classified as none (score 0), mild (score 1), moderate (score 2) and severe (score 3) (Figure 1). Samples found suitable because of histopathological examination were stained immunohistochemically with BAC, Bcl- and Beclin antibodies.

### Immunohistochemical Examination

Samples were stained immunohistochemically using the ABC method for Bax, Bcl- and Beclin antibodies. Sections of 4 µm thickness taken on adhesive slides (Surgipath, X-tra Adhesive Microslides, Illinois, USA) and kept in an oven at 56 °C for 12 h. The sections were dried in xylene for 30 min and kept in 100% (absolute), 96%, 90%, and 80% ethyl alcohol for 15 min for dehydration. The sections were washed with water and distilled water. To block endogenous peroxidase activity, 3% $H_2O_2$ prepared in methanol was added for 10 min. Sections were washed

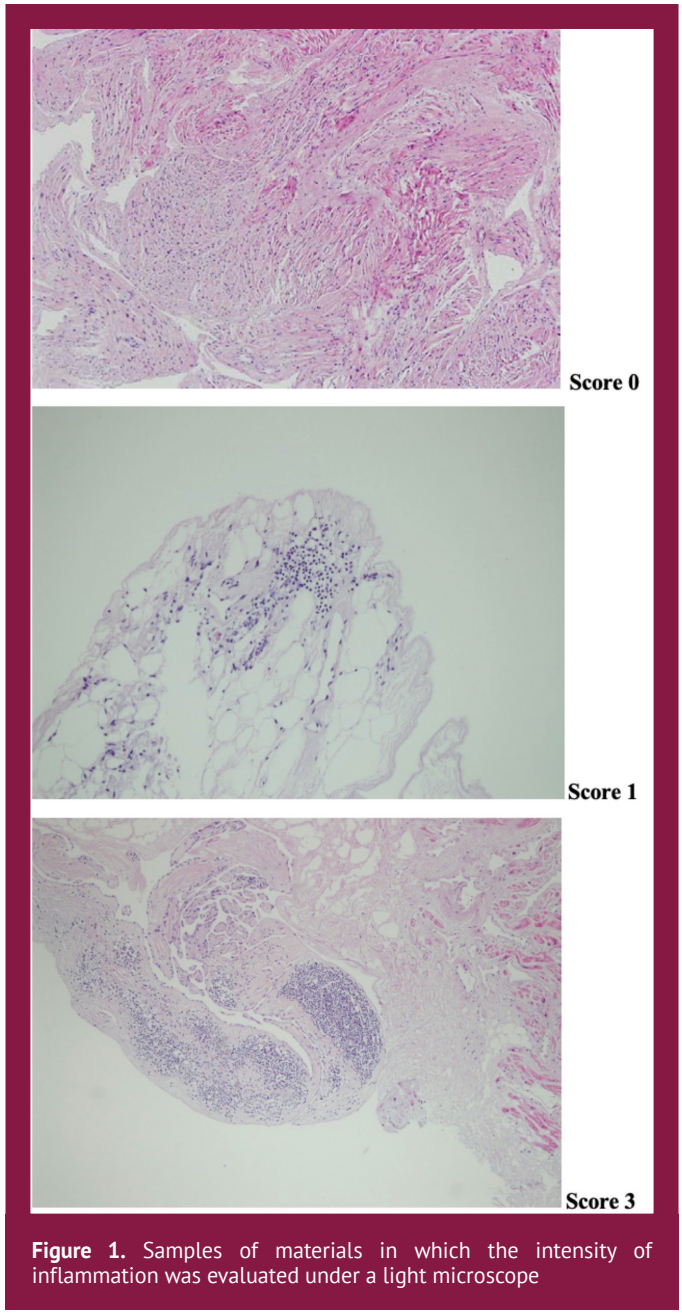


**Figure 1.** Samples of materials in which the intensity of inflammation was evaluated under a light microscope

with phosphate-buffered saline (PBS) (pH =7.60) using the spray method. To reveal the antigenic structures masked in the tissue due to formalin fixation and paraffin blocking, the sections to which both antibodies will be applied are in antigen retrieval solution (0.01M sodium citrate buffer, pH =6.00), medium for the first 10 min, and high for the last 5 min. It was processed in a microwave oven for 15 min. Subsequently, the sections were incubated at room temperature for 30 min and washed three times with distilled water followed by PBS. The Zymed Histostain Plus Broad Spectrum kit (Lot 1385070, Zymed laboratories Inc., Carlsbad, CA, USA) was used for all antibodies. Sections were kept in non-immune blocking serum for 5-10 min. Then, iNOS (polyclonal antibody RB -1605-P1; Neo Marker, Fremont, CA, USA), HSP27 (monoclonal antibody clone G3.1, MS-101- R7, Neo Marker, Fremont, CA, USA), and HSP 60 (monoclonal antibody clone LK1, 101-R7, Neo Marker, Fremont, CA, USA) at +4 $^{0}$C for 18-20 hours. After reaching room temperature, the sections were washed with PBS for 5 min by spraying and dried, and then biotin-bound (secondary) antibody was applied for 20-30 min. After the sections were washed again with PBS and dried, alkaline phosphatase conjugated with streptavidin was applied and left for 20-30 min. Subsequently, the sections were washed with PBS and dried. The mixture was incubated with AEC chromogen solution (Zymed Laboratories, San. Francisco, Calif, USA, Lot: 00-0027) for approximately 10-15 min to provide color rendering. Sections were run thrice in distilled water. It was kept in Mayer's hematoxylin for 30 s for background staining. Sections were washed first in tap water and then in distilled water. The slide was then closed using a water-based sealant (Large volume vision mount, Lot:UG14124, labvision Fremont, CA, USA).

### Immunohistochemical Evaluation

Cytoplasmic and nuclear staining of myocyte cells in epicardial fat samples were considered positive for Bax, Bcl-2, and Beclin expression. Each sample considered positive was graded according to the expression intensity. In addition, other cells in the connective tissue that showed positivity were not recorded.

### Statistical Analysis

Descriptive statistics were used to summarize the case data by diagnostic group. Continuous variables; were evaluated using mean, standard deviation, minimum and maximum values, and ratio and percentage values for categorical variables. One-way analysis of variance was used to compare the numerical variables with more than two groups that were normally distributed, and the Kruskal-Wallis test was used for the variables that were not normally distributed. Comparisons between the two groups were made using Student's t-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. Categorical variables were analyzed using crosstalk statistics (chi-square test, Fisher test). In crosstalk statistics, when the hypothesis of the test to be used in the analysis was not met, the necessary transformations (group aggregation) were made and the data were reanalyzed. Statistical significance was defined as a p value of 0.05. IBM SPSS Statistics version 12.0 (SPSS Inc, Chicago USA) was used in the analysis.

## Results

The study included 28-CABG, 4 MVR, 3 AVR, 1 myectomy, and 1 Benthall procedure patients. There were 5 women and 23 men aged between 44 and 75 years in the patient group, whereas the control group included 3 women and 7 men aged between 24 and 68 years. The comorbid diseases of the patients are shown in Table 1.

In the examination of sections obtained from biopsy samples by H&E staining, it is noteworthy that the inflammation in patients diagnosed with ischemic heart disease and therefore undergoing CABG was considerably higher than that in the control group (Table 2).

Bax, Bcl-2, and Beclin expressions in the patient and control groups are shown in Table 3.

It was observed that Bax, Bcl-2, and Beclin expression was observed in all the patient and control groups, albeit at different levels. In patients with CABG with inflammation, it was observed that Bax expression increased in parallel with inflammation. Bcl-2 expression was significantly increased in patients with MVR, AVR, or myectomy without ischemic heart disease compared with patients with CABG (Figure 2). Bax expression, which was detected at a high rate in patients with CABG, suggested that ischemia also triggers apoptosis. In addition to high Bax expression in CABG patients, Bcl-2 expression was increased in these patients. The highest rate of Beclin expression was observed in HOCM patients (Figure 3). Similar results were obtained in the patient who underwent the Benthall procedure as in the HOCM patient (Figure 4). Beclin expression was also increased in patients who underwent AVR or MVR and showed high Bcl-2 expression.

No myocardial infarction, stroke, or death occurred during the in-hospital follow-up of the patients.

## Discussion

In the patient group, there were patients with pre-operative and post-operative LVEF >50% who underwent diagnostic angiography for suspected coronary artery

**Table 1. The intensity of inflammation in the study population. The first 28 patients consisted of CABG patients. Biopsy samples were obtained from the right atrium (RA) appendix and the periventricular region adjacent to the left anterior descending artery (LAD). Control group patients are shown in red**

| Patient | Sex | RA appendix | LAD periventricular |
|---|---|---|---|
| 1 | M | 0 | 0 |
| 2 | F | 0 | 1 |
| 3 | M | 0 | 0 |
| 4 | M | 0 | 0 |
| 5 | M | 1 | 0 |
| 6 | M | 3 | 0 |
| 7 | M | 0 | 0 |
| 8 | M | 0 | 1 |
| 9 | M | 0 | 0 |
| 10 | M | 0 | 0 |
| 11 | M | 0 | 0 |
| 12 | M | 1 | 0 |
| 13 | M | 0 | 0 |
| 14 | M | 3 | 0 |
| 15 | M | 0 | 0 |
| 16 | M | 3 | 0 |
| 17 | M | 3 | 0 |
| 18 | M | 1 | 0 |
| 19 | M | 0 | 0 |
| 20 | M | 0 | 0 |
| 21 | M | 0 | 0 |
| 22 | M | 0 | 0 |
| 23 | F | 0 | 0 |
| 24 | M | 3 | 1 |
| 25 | M | 0 | 0 |
| 26 | M | 0 | 0 |
| 27 | M | 3 | 0 |
| 28 | F | 3 | 0 |
| 29 | F | 0 | 0 |
| 30 | F | 0 | |
| 31 | F | 0 | |
| 32 | M | 0 | |
| 33 | M | 0 | |
| 34 | F | 0 | |
| 35 | M | 0 | |
| 36 | M | 0 | |
| 37 | F | 0 | |
| 38 | M | 0 | |

F: Female, M: Male, LAD: Left anterior descending artery, RA: Right atrium

disease and whose coronary artery disease was confirmed; therefore, CABG was performed. The control group included patients with MVR, AVR, Benthall procedure, or myectomy with normal or plaque coronary arteries on preoperative coronary angiography and pre-operative and post-operative LVEF >50%. The expression rates of Bax (apoptosis), Bcl-2 (antiapoptosis), and Beclin (autophagy) proteins were measured in epicardial tissue samples taken from the patients during surgery.

Several proteins can initiate apoptosis in mitochondria. These proteins neutralize cytochrome c and endogenous apoptosis inhibitors. The choice between cell death or survival is determined by mitochondrial permeability, which is controlled by more than 20 proteins, the prototype of which is Bcl-2. Multiple sensors are activated when cells are deprived of growth factors and other life signals or when they encounter DNA-damaging factors or accumulate large amounts of misfolded proteins. These sensors are members of the Bcl-2 family called "BH3 proteins". BH3 proteins activate two proapoptotic members of the family, Bax and Bak. These dimerize and enter the mitochondrial membrane and form channels through which cytochrome c and other mitochondrial proteins can pass into the cytosol. Again, the same sensors inhibit anti-apoptotic molecules called Bcl-2 and Bcl-xL, making it easier for mitochondrial proteins to pass into the cytosol. Cytochrome c with other cofactors activates caspase-9. Other proteins that pass from mitochondria to the cytosol block caspase antagonists, which act as physiological inhibitors of apoptosis. The net result of all these interactions is the activation of the caspase cascade, which results in fragmentation of the cell nucleus. However, when cells continue to receive growth factors and other life signals, they synthesize anti-apoptotic members of the Bcl-2 family. One of the two main types is Bcl-2 itself, and the other is Bcl-xL. Because these proteins antagonize Bax and Bak proteins, they limit the exit of pro-apoptotic proteins from mitochondria. Cells deprived of growth factors not only activate the Bax and Bak proteins. They also decreased the levels of Bcl-2 and BclxL, causing the balance to shift more toward cell death. The mitochondrial pathway appears to be responsible for apoptosis in most cases, as described later (5-9).

Detection of Bax > Beclin in patients undergoing CABG for myocardial ischemia shows that ischemia causes severe death in myocytes not only by necrosis but also by apoptosis and autophagy (even if LVEF is preserved). In the process of necrosis, which starts with cell membrane damage after the prolongation of the ischemia period and the increase in the severity of the existing ischemia and therefore the decrease in intracellular energy, the calcium

entering the cell can directly stimulate the caspases and initiate apoptosis via the intrinsic way through the mitochondria.

Only 30% of the cells in the heart wall are myocytes, but because oxygen consumption is high in these cells, ischemia and apoptotic processes triggered by ischemia should be expected to develop predominantly in these cells. It was thought that the increase in apoptosis determined by the increase in Bax in the heart wall was observed in ischemic myocytes. In this process, it can be thought that the general lack of nutrients in the cells of the heart wall stimulates autophagy through Beclin.

The high expression of Bcl-2 in the control group patients with normal coronary angiography and preserved LVEF indicates that the cells escaped death and that the expression of claims was significantly increased in these patients, the adaptation capacity of the cells to the physical stresses causing the surgery was exceeded, myocyte death started via autophagy, and the heart began to dilate may be an indicator.

In the patient group without ischemic findings in the heart wall, heart wall cells showed Bcl-2 expression and Bax expression was low. This suggested that there was no intense stress that could cause cell death in the heart wall cells. However, as in heart valve diseases, the strain of heart

**Table 2. Bax, Bcl-2, and Beclin expressions in the patient group**

| | Expressio | Standard deviation | | Expressio | Standard deviation | | Expressio | Standard deviation |
|---|---|---|---|---|---|---|---|---|
| 1A BAX | 28.787 | 6.785 | 11A-BAX | 32.662 | 9.003 | 21A BAX | 23.631 | 5.452 |
| 1A BCL2 | 34.196 | 5.84 | 11A BCL2 | 28.611 | 8.473 | 21 ABCL2 | 29.875 | 4.267 |
| 1A BECLIN | 30.026 | 8.78 | 11A-BECLIN | 37.07 | 10.612 | 21A-BECLIN | 22.345 | 4.342 |
| 2A BAX | 28.033 | 6.819 | 12A-BAX | 34.718 | 6.894 | 22A-BAX | 23.761 | 5.592 |
| 2A BCL2 | 24.262 | 5.317 | 12A BCL2 | 36.136 | 6.319 | 22A BCL2 | 30.067 | 4.342 |
| 2A BECLIN | 29 | 8.025 | 12A-BECLIN | 24.289 | 4.784 | 22A-BECLIN | 23.267 | 4.769 |
| 3A BAX | 33.25 | 9.101 | 13A BAX | 32.382 | 7.526 | 23A BAX | 24.346 | 6.415 |
| 3A BCL2 | 25 | 7.841 | 13A BCL2 | 30.487 | 4.658 | 23A BCL2 | 33.917 | 4.353 |
| 3A BECLIN | 41.687 | 13.905 | 13A-BECLIN | 31.585 | 9.005 | 23A BECLIN | 25.296 | 4.741 |
| 4A BAX | 33 | 8.613 | 14A BAX | 27.113 | 7.276 | 24A-BAX | 39.462 | 9.995 |
| 4A BCL2 | 36.899 | 7.229 | 14A BCL2 | 27.833 | 7.297 | 24A BCL2 | 56.898 | 9.421 |
| 4A BECLIN | 28 | 7.563 | 14A-BECLIN | 26.976 | 8.273 | 24A-BECLIN | 31.193 | 8.564 |
| 5A BAX | 27.816 | 5.341 | 15A-BAX | 25.352 | 5.591 | 25A BAX | 61.252 | 18.715 |
| 5A BCL2 | 22 | 7 | 15A BCL2 | 41.466 | 9.609 | 25A BCL2 | 73.388 | 13.326 |
| 5A BECLIN | 37.099 | 9.29 | 15A-BECLIN | 25.698 | 6.042 | 25A BECLIN | 47.255 | 13.88 |
| 6A BAX | 21 | 4.823 | 17A-BAX | 43.059 | 17.01 | 27A BAX | 42.72 | 12.13 |
| 6A BCL2 | 22.895 | 6.59 | 17A BCL2 | 28.641 | 9.743 | 27A BCL2 | 58.174 | 14.496 |
| 6A BECLIN | 28 | 7.92 | 17A-BECLIN | 29.474 | 6.654 | 27A BECLIN | 41.762 | 12.86 |
| 7A BAX | 35.745 | 9.885 | 18A BAX | 18.314 | 7.338 | 28A BAX | 37.403 | 10.939 |
| 7A BCL2 | 37 | 7.431 | 18A BCL2 | 15.471 | 6.621 | 28A BCL2 | 30.212 | 9.103 |
| 7A BECLIN | 41.249 | 13.402 | 18A-BECLIN | 11.306 | 4.977 | 28A-BECLIN | 29.498 | 8.084 |
| 8A BAX | 24 | 6.696 | 19A BAX | 23.061 | 8.274 | 29A BAX | 26.079 | 10.861 |
| 8A BCL2 | 22.531 | 6.094 | 19A BCL2 | 21.733 | 4.431 | 29A BCL2 | 17.725 | 8.95 |
| 8A BECLIN | 23 | 5.702 | 19A-BECLIN | 15.087 | 4.033 | 29A BECLIN | 18.822 | 6.105 |
| 9A BAX | 33.934 | 5.529 | 20A BAX | 29.546 | 6.177 | 30A-BAX | 11.206 | 4.033 |
| 9A BCL2 | 22 | 6.732 | 20A BCL2 | 19.132 | 6.139 | 30A BCL2 | 14.614 | 4.469 |
| 9A BECLIN | 27.259 | 8.221 | 20A-BECLIN | 17.13 | 6.445 | 30A-BECLIN | 13.68 | 4.522 |
| 10A-BAX | 26 | 7.738 | | | | | | |
| 10A BCL2 | 37.693 | 6.33 | | | | | | |
| 10A-BECLIN | 25 | 6.189 | | | | | | |

**Table 3. Bax, Bcl-2, and Beclin expressions in the control group**

| | Expressio | Standard deviation | | Expressio | Standard deviation |
|---|---|---|---|---|---|
| 16A BAX | 34.945 | 9.599 | 34A BAX | 38.095 | 11.374 |
| 16A BCL2 | 32.942 | 9.551 | 34A BCL2 | 46.135 | 16.556 |
| 16A BECLIN | 28.902 | 9.883 | 34A BECLIN | 32.812 | 9.026 |
| 26 A BAX | 33.197 | 7.556 | 35A BAX | 35.915 | 8.186 |
| 26A BCL2 | 28.629 | 7.789 | 35A BCL2 | 54.032 | 14.249 |
| 26A-BECLIN | 29.521 | 6.189 | 35A BECLIN | 44.192 | 13.468 |
| 31A BAX | 13.504 | 4.69 | 36A-BAX | 36.423 | 8.448 |
| 31A BCL2 | 13.574 | 4.773 | 36A BCL2 | 57.451 | 11.506 |
| 31A-BECLIN | 13.008 | 6.278 | 36A-BECLIN | 42.602 | 18.452 |
| 32A-BAX | 24.954 | 7.894 | 37A BAX | 32.286 | 7.868 |
| 32A BCL2 | 50.942 | 16.65 | 37A BCL2 | 33.153 | 8.31 |
| 32A-BECLIN | 41.231 | 9.87 | 37A-BECLIN | 30.909 | 7.004 |
| 33A BAX | 27.555 | 4.171 | 38A-BAX | 28.84 | 6.221 |
| 33A BCL2 | 34.987 | 6.758 | 38A BCL2 | 34.221 | 7.776 |
| 33A-BECLIN | 33.374 | 14.932 | 38A-BECLIN | 26.788 | 5.002 |

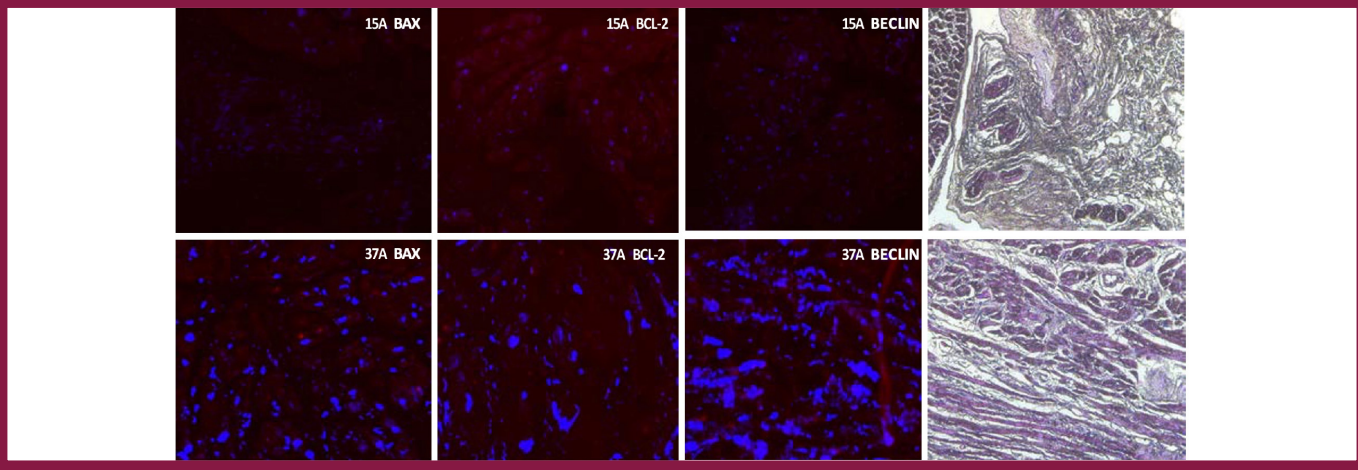


**Figure 2.** Outcomes of AVR patients (anti-apoptosis > apoptosis ⩾ autophagy)
*AVR: Aortic valve replacement*

wall cells and high energy needs lead to the depletion of energy sources in the environment, suggesting that autophagy is stimulated through nutrient deficiency. Here, the energy source includes oxygen in the short term, but glucose and other substances can be converted into energy in the long term. It can be thought that the heart wall cells use substances that they can convert into energy in the cell after oxygen deficiency and go to autophagy in the final stage. Similarly, it can be thought that autophagy develops after the use of oxygen and then substances that can be converted into energy in the ischemic heart muscle.

### Study Limitations

This study was conducted with a small group of patients. Not all valve diseases were evaluated in this study. Mixed serious valve diseases were not evaluated together. There is no long-term follow-up of patients after discharge.

## Conclusion

All of the participants had Bax, Bcl-2, and Beclin expression, albeit at different levels. Inflammation level is determinative for Bax and Beclin expression. In patients with inflammatory CABG, Bax expression increases in

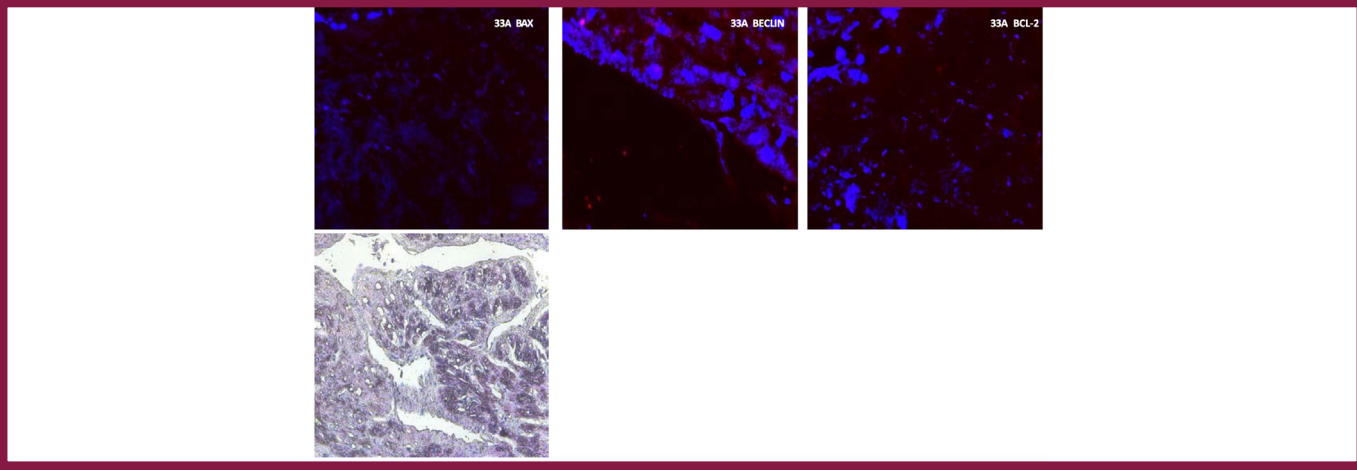


**Figure 3.** HOCM (anti-apoptosis > autophagy > apoptosis)
*HOCM: Hypertrophic obstructive cardiomyopathy*

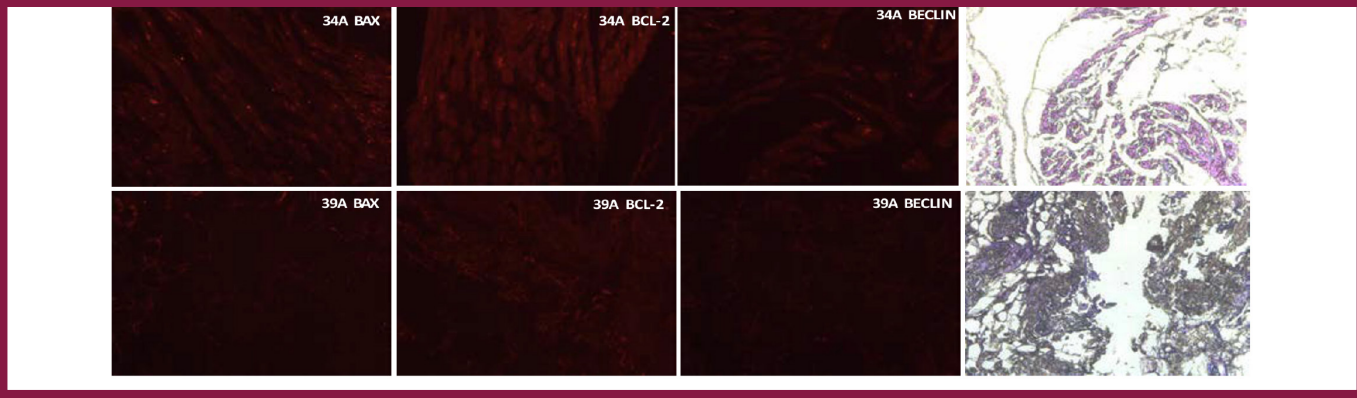


**Figure 4.** Benthall procedure (anti-apoptosis > apoptosis ⩾ autophagy)

parallel with inflammation. In patients with MVR or AVR or myectomy without ischemic heart disease, Bcl-2 expression increased much more than in patients with CABG. The increased rate of inflammation in patients with CABG possibly developed secondary to ischemic necrosis, and the high rate of Bax in the same patient group suggested that ischemia triggered not only coagulation necrosis but also severe apoptosis in coronary artery patients who underwent CABG. Because coronary blood flow will be restored with bypass grafts in the patient group undergoing CABG, free radical damage and apoptosis will continue in myocytes that are in ischemia and encounter oxygenated blood again, which will cause high Bax expression. Considering the increased Bcl-2 expression in patients with CABG in addition to increased Bax expression, Bcl-2 expression represents myocytes that resist death, it was understood that high Bax expression in patients with CABG was directly caused by ischemia, considering the high Bcl-2 levels in the control groups without ischemia. The increase in Beclin expression in patients who had AVR or MVR showed high Bcl-2 expression, indicating that the adaptation capacity of myocytes against increased stress was reached and that myocytes died by autophagy without ischemia. In patients with HOCM, the highest rate of Beclin expression in genetically thickened myocytes leads to the death of myocytes via autophagy when increased protein synthesis in the cell exceeds the storage capacity of the cells, even in the absence of ischemia and physical stress. Patients who underwent ascending aortic grafting for an ascending aortic aneurysm had similar results to those of HOCM patients. In line with these results, it was understood that ischemia is the strongest death herald for myocytes, causing coagulation necrosis by hypoxia, as well as triggering apoptosis at a high rate. We have shown that it is not caused by necrosis or

apoptosis, but rather by autophagy. This can be used in the timing of surgery, especially in patients with moderate-to-severe valve disease without coronary artery disease.

### Ethics

**Ethics Committee Approval:** The study was carried out with the approval of the local ethics committee (2023/06/683).

**Informed Consent:** All patients were informed in detail about biopsy procedures, surgical procedures to be performed, all complications that may occur during and after surgical procedures, the follow-up period, and patient consent forms.

**Peer-review:** Externally and internally peer reviewed.

### Authorship Contributions

Concept: M.K., G.A., Design: M.K., G.A., Data Collection or Processing: M.K., T.Ö., G.A., Analysis or Interpretation: M.K., N.K., T.Ö., G.A., Literature Search: M.K., K.E.P., N.K., Writing: M.K., K.E.P., N.K.

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

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

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ORIGINAL ARTICLE

**DOI:** 10.4274/hamidiyemedj.galenos.2023.80775

Hamidiye Med J 2023;4(3):177-183

# Repeatability Assessment of Optical Coherence Tomography Angiography in the Overall and Different Age Groups of the Turkish Population

## Türk Popülasyonunun Genel ve Farklı Yaş Gruplarında Optik Koherens Tomografi Anjiyografinin Tekrarlanabilirlik Değerlendirmesi

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ABSTRACT

**Background:** To measure the repeatability of foveal avascular zone (FAZ) area, vessel density (VD), and flow area using AngioVue Analytics optical coherence tomography angiography (OCTA) in the overall and different age groups of the Turkish population.

**Materials and Methods:** This study included 100 eyes of 100 healthy subjects (54 females; mean age: 43.2±15.2 years). They were divided into five age groups- 20-29, 30-39, 40-49, 50-59, and >60 years- with 20 subjects each. The optical disk region and macula were imaged thrice using a 4.5x4.5 mm and 3x3 mm scan, respectively. Intraclass correlation coefficient (ICC) and coefficient of variation (CV) analyses were used to evaluate repeatability.

**Results:** CV and ICC for the FAZ area were 2.96% and 0.990, respectively. ICC values of entire image VD of superficial capillary plexus (SCP) and deep capillary plexus (DCP) were 0.828 and 0.842, respectively. CV and ICV for peripapillary VD were 2.32% and 0.990, respectively. ICC was 0.865 for the choriocapillaris flow area. CV values of different age groups showed no statistical differences, except for the FAZ area.

**Conclusion:** OCTA measurements of the FAZ area, VDs of the SCP, DCP, and optic nerve, and the choriocapillaris flow area showed relatively good overall repeatability. Repeatability results of different age groups should guide the optimal use of OCTA.

**Keywords:** Flow area, foveal avascular zone, OCTA, repeatability, vessel density

ÖZ

**Amaç:** AngioVue Analytics optik koherens tomografi anjiyografi (OKTA) kullanarak foveal avasküler bölge (FAB) alanı, damar yoğunluğu (DY) ve akış alanının tekrarlanabilirliğini Türk popülasyonunun genel ve farklı yaş gruplarında ölçmek.

**Gereç ve Yöntemler:** Bu çalışmaya 100 sağlıklı kişinin (54 kadın; ortalama yaş: 43,2±15,2 yıl) 100 gözü dahil edildi. Her biri 20 denek olmak üzere beş yaş grubuna (20–29, 30–39, 40–49, 50-59 ve >60 yaş) ayrıldılar. Optik disk bölgesi ve maküla sırasıyla 4,5x4,5 mm ve 3x3 mm tarama kullanılarak üç kez görüntülendi. Tekrarlanabilirliği değerlendirmek için sınıf içi korelasyon katsayısı (SKK) ve varyasyon katsayısı (VK) analizleri kullanıldı.

**Bulgular:** FAZ alanı için VK ve SKK sırasıyla %2,96 ve 0,990 idi. Yüzeyel kapiller pleksus (YKP) ve derin kapiller pleksus (DKP) tüm görüntü DY'sinin SKK değerleri sırasıyla 0,828 ve 0,842 idi. Peripapiller DY için VK ve SKK sırasıyla %2,32 ve 0,990 idi. Koryokapillaris akış alanı için ICC 0,865 idi. Farklı yaş gruplarının VK değerleri, FAZ alanı dışında istatistiksel olarak farklılık göstermedi.

**Sonuç:** FAZ bölgesinin OKTA ölçümleri; YKP, DKP ve optik sinirin DY'leri; ve koryokapillaris akış alanı genel olarak nispeten iyi tekrarlanabilirlik gösterdi. Farklı yaş gruplarının tekrarlanabilirlik sonuçları, OKTA'nın optimal kullanımına rehberlik etmelidir.

**Anahtar Kelimeler:** Akım alanı, foveol avosküler bölge, OKTA, tekrarlanabilirlik, damar yoğunluğu

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**Received:** 25.07.2023 **Accepted:** 20.10.2023

## Introduction

Imaging of the vascular structure of the retina is necessary for the diagnosis, follow-up, and treatment of most ocular diseases. To date, retinal vasculature has been studied using various instruments and methods, such as histological techniques, fundus fluorescent angiography (FA), and laser ophthalmoscopy (1,2). However, these techniques have limited use in clinical practice and research because they are non-quantitative methods. FA is the gold standard method for evaluating retinal vascular diseases. However, it requires a dye injection to observe the retinal microvasculature, making it invasive and requiring a certain amount of time.

Optical coherence tomography angiography (OCTA) was first reported by Makita et al. (3) using Doppler OCT (3). OCTA is a highly up-to-date, non-invasive, and non-contact imaging method that allows detailed imaging of the retinal capillary network (4). This method relies on motion contrast to distinguish structures with blood flow from those without blood flow. The AngioVue Imaging System (RTVue XR Avanti; Optovue, Inc., Fremont, CA) uses its automated algorithm, split-spectrum amplitude decorrelation angiography (SSADA). This algorithm enables simultaneous three-dimensional structural visualization of the retina and the creation of en face maps of blood flow (5). It is widely used in the evaluation of retinal diseases and glaucoma. OCTA also enables quantitative measurements of the retinal microvasculature (6,7). Many studies have reported the repeatability of OCTA measurements in the eyes of healthy individuals; however, the repeatability in the normal eye across different age groups has not been sufficiently studied (6,8-12).

Repeatability is important for assessing the change in normal and the diagnosis of diseased eyes and their change over time. The repeatability of microvasculature measurements at the same retinal region of the same patient is critical during the follow-up and treatment periods. Many previous studies have evaluated OCTA in terms of repeatability in a specific retinal vascular structure region. In the present study, repeatability was evaluated comprehensively in terms of the foveal avascular zone (FAZ) area, peripapillary area, choriocapillaris flow area, and vascular density (VD) of the superficial capillary plexus (SCP) and deep capillary plexus (DCP).

In this study, we aimed to evaluate the repeatability success of OCTA measurements and obtain OCTA data across different age groups to provide an additional contribution to database creation for a Turkish population.

## Materials and Methods

This retrospective interventional comparative study was approved by the Local Ethics Committee (2023.06.236) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before registration.

Subjects with healthy eyes without any ocular diseases were included in this study and divided into five age groups-20-29, 30-39, 40-49, 50-59, and >60 years old-with 20 subjects each. Patients with any ocular diseases causing corneal opacities, cataract, vitreous opacities, retinal diseases, glaucoma, or optic nerve pathologies were excluded. In addition, smokers, pregnant women, patients with a history of any ocular surgeries, axial length (AL) of <21 mm_or >24 mm and with spherical refraction of more than ±3.0 D, cylinder refraction of more than ±2.0 D, and best-corrected visual acuity (BCVA) of less than 20/25 were excluded from this study. All subjects who met the inclusion criteria underwent a complete ophthalmologic examination (external ocular examination, auto-refractometry, BCVA, slit-lamp and fundoscopy examination, Goldmann applanation tonometry, and AL measurement using an AL-Scan (Nidek Co., Gamagori, Japan).

### OCTA Imaging Protocol

OCTA images were acquired through previously dilated pupils by the same experienced technician, and similar mesopic conditions were adopted throughout the study. OCTA imaging was performed using an SSADA algorithm to detect flow area and vascular density. The SSADA algorithm has been described in detail in previous studies (7,13). Before imaging, each subject's right pupil was dilated with a combination of tropicamide (0.5%) twice and phenylephrine (2.5%) once, 5 min apart. Study participants underwent imaging consisting of the macula (3 mm x3 mm) and optic disk (4.5 mm x4.5 mm) with the eye tracking system activated. Imaging was repeated three times for only the right eye with 5 min between scanning sessions. Manual adjustments were not conducted because it was planned to analyze the repeatability of the FAZ area, VD, and flow area measured using AngioVue software automatically. Imaging with a signal strength <70 were considered poor quality and excluded, and repeated acquisitions were performed until the signal strength index was ⩾70.

### Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 26.0 (SPSS, Inc., Chicago, IL, USA). The coefficient of variation (CV) and intraclass correlation

coefficient (ICC) were calculated to analyze the repeatability of the OCTA measurements in healthy subjects.

Repeatability was examined by CV, coefficient of variation, and ICC. CV was accepted as 20% or less. Comparisons of numerical variables in independent groups were made using the Kruskal-Wallis test. Subgroup analyses were performed using the Mann-Whitney U test and interpreted with Bonferroni correction. The statistical alpha significance level was set as $p<0.05$.

## Results

A total of 100 eyes of 100 healthy subjects [54 females (54%) and 46 males (46%)] were included in this study. The mean age was 43.2±15.2 years. The mean intraocular pressure was 15.5±3.0 mmHg, and the mean AL was 23.4±1.0 mm.

The mean FAZ area was 0.28±0.10 $mm^2$; further, the CV and ICC values for the FAZ area were 2.96% and 0.990, respectively. Table 1 shows the repeatability assessment of the FAZ area for the overall age groups. When different age groups were evaluated separately, the CV value was below 10% in all age groups (Table 2). Specifically, the CV values for the 20-29, 30-39, 40-49, 50-59, and >60 years age groups were 1.52%, 1.41%, 4.38%, 4.16%, and 3.61%, respectively; this indicates that the measurement repeatability between the groups differed significantly ($p<0.05$). Further, as shown in Table 3, the corresponding ICC values were 0.994, 0.998, 0.987, 0.981, and 0.975 for the age groups, respectively; this indicates that the ICC values evaluated separately for all groups showed excellent reliability.

The ICC and CV values were assessed for VDs of five subregions of the SCP and DCP: whole area, superior hemi, inferior hemi, fovea, and parafovea. The whole image VDs showed good reliability for both SCP and DCP. The VDs of the SCP and DCP for different age groups showed CV values below 10% for all age groups (Table 2). CV values did not show significant differences between the different age groups ($p>0.05$, for all). The ICC values showed moderate or better reliability for all age groups (Table 3).

ICC and CV values were assessed for VDs of three subregions of the optic disk: entire image, inside disk and peripapillary. The CV and ICC values for the peripapillary were 2.32% and 0.740, respectively. The VD of the optic disk entire image showed moderate reliability overall. VDs of the optic disk for different age groups showed CV values below 10% for all age groups (Table 2). The ICC values showed moderate or better reliability for all age groups (Table 3).

The mean choriocapillaris flow area for all volunteers was 2.08±0.12 $mm^2$. The ICC value of the choriocapillaris flow area showed good overall reliability. The ICC values of

**Table 1. CV and ICC values of OCTA parameters for overall age groups**

| | | Mean ± SD | CV (%) | ICC |
|---|---|---|---|---|
| **FAZ** | | 0.28±0.10 | **2.96** | **0.990** |
| **Vessel density of the SCP** | Whole image | 47.2±3.5 | **2.69** | **0.828** |
| | Superior hemi | 47.1±3.7 | **2.73** | **0.837** |
| | Inferior hemi | 47.3±3.4 | **2.86** | **0.794** |
| | Fovea | 17.3±5.7 | **6.43** | **0.954** |
| | Parafovea | 50.2±3.8 | **2.62** | **0.842** |
| **Vessel density of the DCP** | Whole image | 52.0±3.7 | **2.19** | **0.876** |
| | Superior hemi | 52.0±4.2 | **2.96** | **0.658** |
| | Inferior hemi | 52.0±3.7 | **3.04** | **0.510** |
| | Fovea | 33.6±6.8 | **2.97** | **0.972** |
| | Parafovea | 54.0±4.4 | **3.32** | **0.562** |
| **Vessel density of the optical disk** | Whole image | 50.0±2.1 | **2.02** | **0.736** |
| | Inside disk | 49.7±4.9 | **4.66** | **0.745** |
| | Peripapillary | 52.7±2.5 | **2.32** | **0.740** |
| | Superior | 53.1±2.4 | **2.53** | **0.670** |
| | Inferior | 52.3±3.0 | **2.48** | **0.793** |
| **Flow area** | Choriocapillaries | 2.08±0.12 | **1.86** | **0.865** |

CV: Coefficient of variation, ICC: Intraclass correlation coefficient, FAZ: Foveal avascular zone, SCP: Superficial capillary plexus, DCP: Deep capillary plexus, SD: Standard deviation
Interpretation for ICC agreement measures: 0.50, poor; between 0.50 and 0.75, moderate; between 0.75 and 0.9, good; greater than 0.9, excellent reliability

**Table 2. Average and CV values of the OCTA parameters for different age groups**

| | | 20-29 years | | 30-39 years | | 40-49 years | | 50-59 years | | ⩾ 60 years | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SD | CV (%) | Mean ± SD | CV (%) | Mean ± SD | CV (%) | Mean ± SD | CV (%) | Mean ± SD | CV (%) | p |
| **FAZ** | | 0.27±0.08 | 1.52 | 0.33±0.13 | 1.41 | 0.26±0.11 | 4.38 | 0.27±0.08 | 4.16 | 0.29±0.10 | 3.61 | 0.012[a*] |
| **Vessel density of the SCP** | Whole image | 48.7±2.9 | 2.58 | 47.6±3.7 | 3.05 | 47.2±2.2 | 2.58 | 47.8±3.5 | 2.34 | 44.7±3.8 | 2.84 | 0.931[a] |
| | Superior hemi | 48.7±3.0 | 2.60 | 47.7±3.6 | 3.15 | 47.1±2.3 | 2.42 | 47.6±3.4 | 2.42 | 44.3±4.4 | 2.99 | 0.436[a] |
| | Inferior hemi | 48.7±3.0 | 2.66 | 47.5±3.9 | 3.06 | 47.4±2.2 | 2.88 | 47.9±3.7 | 2.55 | 45.1±3.3 | 3.09 | 0.956[a] |
| | Fovea | 19.1±5.4 | 5.21 | 15.1±6.6 | 7.44 | 19.0±5.5 | 5.23 | 18.6±4.2 | 5.46 | 15.0±5.4 | 8.63 | 0.369[a] |
| | Parafovea | 51.8±3.0 | 2.56 | 50.9±4.2 | 2.83 | 50.0±2.4 | 2.76 | 50.8±3.8 | 2.14 | 47.7±4.4 | 2.71 | 0.854[a] |
| **Vessel density of the DCP** | Whole image | 53.5±4.1 | 2.02 | 52.5±3.5 | 2.32 | 53.4±2.5 | 2.05 | 51.4±4.5 | 2.40 | 49.5±2.0 | 2.18 | 0.866[a] |
| | Superior hemi | 53.6±4.2 | 2.11 | 52.3±3.6 | 2.43 | 52.8±5.0 | 5.38 | 51.7±4.7 | 2.48 | 49.3±2.4 | 2.26 | 0.894[a] |
| | Inferior hemi | 53.4±3.9 | 2.34 | 52.7±3.6 | 2.23 | 53.3±2.5 | 2.27 | 50.2±5.0 | 6.36 | 49.9±2.1 | 2.61 | 0.980[a] |
| | Fovea | 36.0±5.0 | 2.37 | 31.7±8.3 | 2.65 | 35.1±7.2 | 2.59 | 35.3±5.2 | 3.29 | 30.5±5.8 | 4.03 | 0.208[a] |
| | Parafovea | 55.6±4.0 | 1.72 | 54.2±5.2 | 5.07 | 55.4±2.2 | 2.18 | 53.6±4.7 | 2.03 | 50.9±4.3 | 5.28 | 0.525[a] |
| **Vessel density of the optical disk** | Whole image | 49.8±1.6 | 2.30 | 50.0±2.8 | 2.20 | 50.8±1.8 | 1.35 | 49.8±2.2 | 2.20 | 49.4±2.0 | 2.09 | 0.088[a] |
| | Inside disc | 51.3±4.2 | 5.30 | 50.6±5.6 | 5.12 | 49.8±5.3 | 4.08 | 48.7±3.9 | 4.41 | 48.1±4.7 | 4.35 | 0.751[a] |
| | Peripapillary | 52.5±1.9 | 2.62 | 52.9±2.9 | 2.57 | 53.3±2.4 | 1.71 | 52.6±2.7 | 2.49 | 52.2±2.7 | 2.25 | 0.089[a] |
| **Flow** | Choriocapillaries | 2.20±0.11 | 1.57 | 2.10±0.11 | 1.83 | 2.05±0.08 | 1.92 | 2.04±0.11 | 1.71 | 2.04±0.13 | 2.25 | 0.764[a] |

[a]One-way ANOVA with *post-hoc test (Tukey-Kramer test)
In the post-hoc test, while there was no difference between other age groups in terms of FAZ, the 30-39-year age group was statistically different from the others (p<0.001).
CV: Coefficient of variation, FAZ: Foveal avascular zone, SCP: Superficial capillary plexus, DCP: Deep capillary plexus

the choriocapillaris flow area for the different age groups were all below 10%. As shown in Table 2, the CV values for the 20-29, 30-39, 40-49, 50-59, and >60 years age groups were 1.57%, 1.83%, 1.92%, 1.71%, and 2.25%, respectively; there was no statistically significant difference between the different age groups (p>0.05). The ICC values showed good reliability for all age groups (Table 3).

## Discussion

The accuracy of repeatability measurement is important when considering the implementation of OCTA in clinical use. We demonstrate that the mean FAZ area, vessel density, and flow measurements are reliable with higher repeatability by OCTA. We believe that repeatability results of different age groups are needed to determine strict standard values of OCTA measurements for diagnosis, follow-up, and treatment.

FAZ represents a non-capillary region in the center of the fovea and may vary significantly in dimension even among healthy individuals. The vascular layers in the retina end as they approach the center of the fovea. The FAZ area could be a useful parameter in the detection and follow-up of retinal vascular pathologies. FAZ area quantification has a significant positive correlation with the severity of capillary non-perfusion in various retinovascular diseases (14). Many devices used in clinical practice for ophthalmic evaluation have been discontinued because of low reliability. Studies that used FA to analyze the FAZ area showed that the FAZ area in healthy subjects varied greatly, ranging from 0.205 to 0.405 mm² (15-17). The high variability of FA measurements casts doubt on the reliability of this invasive technique used to measure the FAZ area (18). This method is also limited by overlaps in the capillary plexus and leaks during FA imaging. OCTA is non-invasive, can simultaneously image the retinal vasculature and microanatomy, and provides better FAZ measurement than FA imaging (19). This study investigates the repeatability of OCTA for measuring the FAZ area. Using automatic software, we found that the FAZ area had excellent reliability for ICC values for different age groups. For CV values, the FAZ area can be measured at a younger age with higher repeatability. OCTA repeatability studies have shown the greatest success for the FAZ area (7-9). FAZ is useful for detecting and monitoring the progression of retinovascular diseases, making OCTA useful for follow-ups of these diseases. The mean FAZ area was 0.28±0.10 mm²; this result will guide us in determining the mean FAZ area of healthy individuals in the Turkish population. The FAZ area in the 30-39-year age group was significantly higher than in the other groups (p<0.001).

OCTA enables the observation of SCP and DCP of the retina with high resolution and three-dimensional

**Table 3. ICC values of the OCTA parameters for different age groups**

| | | 20-29 years | | | 30-39 years | | | 40-49 years | | | 50-59 years | | | ≥60 years | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ICC | 95% CI | | ICC | 95% CI | | ICC | 95% CI | | ICC | 95% CI | | ICC | 95% CI | |
| | | | Min | Max | | Min | Max | | Min | Max | | Min | Max | | Min | Max |
| **FAZ** | | **0.994** | 0.988 | 0.998 | **0.998** | 0.997 | 0.999 | **0.987** | 0.973 | 0.994 | **0.981** | 0.959 | 0.993 | **0.975** | 0.949 | 0.989 |
| **VD of the SCP** | Whole image | **0.789** | 0.615 | 0.902 | **0.809** | 0.658 | 0.908 | **0.600** | 0.358 | 0.794 | **0.867** | 0.731 | 0.945 | **0.859** | 0.733 | 0.936 |
| | Superior hemi | **0.782** | 0.604 | 0.899 | **0.795** | 0.638 | 0.901 | **0.591** | 0.346 | 0.788 | **0.881** | 0.758 | 0.951 | **0.885** | 0.778 | 0.948 |
| | Inferior hemi | **0.795** | 0.624 | 0.905 | **0.820** | 0.675 | 0.913 | **0.580** | 0.335 | 0.781 | **0.814** | 0.639 | 0.921 | **0.768** | 0.586 | 0.891 |
| | Fovea | **0.968** | 0.935 | 0.986 | **0.966** | 0.931 | 0.985 | **0.942** | 0.885 | 0.974 | **0.943** | 0.878 | 0.977 | **0.917** | 0.836 | 0.964 |
| | Parafovea | **0.775** | 0.592 | 0.895 | **0.843** | 0.713 | 0.925 | **0.584** | 0.340 | 0.784 | **0.892** | 0.778 | 0.955 | **0.893** | 0.792 | 0.952 |
| **VD of the DCP** | Whole image | **0.871** | 0.761 | 0.945 | **0.864** | 0.749 | 0.936 | **0.920** | 0.843 | 0.964 | **0.772** | 0.572 | 0.901 | **0.645** | 0.412 | 0.824 |
| | Superior hemi | **0.921** | 0.843 | 0.965 | **0.852** | 0.729 | 0.930 | **0.851** | 0.689 | 0.941 | **0.758** | 0.552 | 0.894 | **0.703** | 0.491 | 0.856 |
| | Inferior hemi | **0.876** | 0.762 | 0.944 | **0.858** | 0.738 | 0.933 | **0.881** | 0.772 | 0.946 | **0.818** | 0.648 | 0.957 | **0.560** | 0.305 | 0.773 |
| | Fovea | **0.961** | 0.921 | 0.983 | **0.989** | 0.977 | 0.995 | **0.979** | 0.956 | 0.991 | **0.946** | 0.885 | 0.978 | **0.939** | 0.877 | 0.973 |
| | Parafovea | **0.933** | 0.866 | 0.971 | **0.874** | 0.781 | 0.957 | **0.903** | 0.812 | 0.956 | **0.817** | 0.643 | 0.923 | **0.815** | 0.612 | 0.917 |
| **VD of the optic disk** | Whole image | **0.521** | 0.262 | 0.747 | **0.788** | 0.625 | 0.897 | **0.855** | 0.729 | 0.933 | **0.741** | 0.519 | 0.887 | **0.703** | 0.491 | 0.856 |
| | Inside disc | **0.548** | 0.283 | 0.768 | **0.768** | 0.596 | 0.886 | **0.850** | 0.722 | 0.930 | **0.693** | 0.449 | 0.863 | **0.786** | 0.611 | 0.901 |
| | Peripapillary | **0.535** | 0.275 | 0.757 | **0.730** | 0.536 | 0.866 | **0.846** | 0.713 | 0.929 | **0.747** | 0.528 | 0.890 | **0.810** | 0.652 | 0.912 |
| **Flow area** | Choriocapillaries | **0.864** | 0.741 | 0.939 | **0.856** | 0.732 | 0.932 | **0.857** | 0.734 | 0.927 | **0.860** | 0.718 | 0.942 | **0.840** | 0.699 | 0.927 |

ICC: Intraclass correlation coefficient, VD: Vascular density, FAZ: Foveal avascular zone, SCP: Superficial capillary plexus, DCP: Deep capillary plexus, CI: Confidence interval, min: Minimum, max: Maximum
Interpretation for ICC agreement measures: 0.50, poor; between 0.50 and 0.75, moderate; between 0.75 and 0.9, good; greater than 0.9, excellent reliability

microcirculation imaging of the retina (20). Two-dimensional FA imaging methods can be used to explore the retina in just one plane. In contrast, OCTA provides a non-invasive approach for evaluating three-dimensional retinal microcirculation imaging (20,21). OCTA can also obtain individual images of the two vascular networks of the retina and indicate their morphology and characteristics, which is not possible using FA (22). The repeatability of VD measurements has been previously reported for the same and other OCTA devices. For the RS-3000 Advance OCTA device (Nidek) and its included measurement software, the CV and ICC for the SCP were 5.2% and 0.90, respectively (6). In a study of healthy participants using the RTVue device and an automated measurement tool, the agreement was reported as 0.78-0.99 (23). In this study, VD of the SCP and DCP as obtained using the AngioVue Imaging System for image analysis showed good repeatability. However, the inferior hemi and parafoveal VD of the DCP showed moderate reliability with ICC values of 0.510 and 0.562, respectively. This study showed a higher vessel density in the DCP than in the SCP. These findings are consistent with those of previous studies using other techniques (24). The different age groups did not show any differences in repeatability. Owing to the interest in using OCTA-derived quantitative measurements in clinical trials and practice, it is important to determine its repeatability limits. The repeatability of SCP and DCP did not differ statistically for different age groups.

The VD of the optic disk was divided into different sectors; several studies have evaluated the repeatability of OCTA measurements of the peripapillary region (25-27). Wang et al. (27) evaluated the repeatability of the peripapillary vessel density of 15 healthy individuals and found that CV was 1.21%. Liu et al. (25) evaluated the repeatability of the mean peripapillary vessel density in two groups-12 normal subjects and 12 glaucoma patients- and found that CV was 1.9% and 4%, respectively. This study showed that VD measurements using OCTA are reliable and have higher repeatability. Peripapillary VD showed moderate reliability with CV <2.32% and ICC of 0.661-0.808. CV values of the peripapillary region for different age groups showed no differences in terms of repeatability.

Perfusion of retinal vessels within natural limits is vital for retinal function to be within normal limits (28). OCTA, first described in 2012, enables examination and measurement of macular. Studies are increasingly investigating new techniques for studying changes in macular perfusion. In the study with healthy participants, the evaluation of the choriocapillaris flow area with the RTVue XR device showed high repeatability (20). In this study, the choriocapillaris flow area in the different age groups showed good repeatability with no statistically significant difference. Yu et al. (29) reported that the RTVue XR device had a mean ICC of 0.925 for the macular perfusion flow index in healthy participants. Wei et al. (30) reported that OCTA could be used with high repeatability to measure macular perfusion in healthy individuals. Al-Sheikh et al. (31) reported that the flow index of the choriocapillaris had medium to high repeatability in the subfoveal region.

### Study Limitations

One limitation of this study is that the population included only healthy subjects. Therefore, it may not be possible to obtain these reliability rates in patients with poor fixation owing to maculopathy. In algorithms using automatic segmentation, problems may occur in the detection of FAZ and vascular structures secondary to pathologies such as macular edema that cause changes in retinal thickness. Furthermore, the sample size was relatively small. To test the repeatability of OCTA measurements, a rigorously designed prospective study representing subjects of all ages is required.

## Conclusion

Preliminary results suggest that RTVue XR with OCTA may be appropriate and reliable for evaluating changes in the follow-up and treatment of ocular diseases. It can be used as a non-invasive, reliable method to quantitatively determine changes in FAZ shape and size. Therefore, the measurement of retinal VD by OCTA is reliable; the flow area could be a valuable way to detect and follow up choroidal disease in the future.

### Ethics

**Ethics Committee Approval:** This retrospective interventional comparative study was approved by the Local Ethics Committee (2023.06.236) and conducted in accordance with the Declaration of Helsinki.

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

**Peer-review:** Externally peer reviewed.

### Authorship Contributions

Surgical and Medical Practices: M.K., Y.C.Y., Concept: M.K., Y.C.Y., Design: M.K., Y.C.Y., Data Collection or Processing: M.K., Analysis or Interpretation: M.K., Y.C.Y., Literature Search: M.K., Y.C.Y., Writing: M.K., Y.C.Y., D.Ö.

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

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

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ORIGINAL ARTICLE

**DOI:** 10.4274/hamidiyemedj.galenos.2023.77487

Hamidiye Med J 2023;4(3):184-190

# Epidemiological Characteristics and Microbiological Profile of Infectious Keratitis in the Last Decade at a Tertiary Care Center in İstanbul: A Retrospective Study

## Enfeksiyöz Keratitlerin Epidemiyolojik Özellikleri ve Mikrobiyolojik Profili: İstanbul'da Bir Üçüncü Basamak Merkezde On Yıllık Retrospektif Analiz

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ABSTRACT

**Background:** Regional epidemiological studies are needed for the management of microbial keratitis. The aim of this study was to analyze the epidemiological features, risk factors, causative microorganisms, and antibiotic susceptibility patterns in microbial keratitis in the last decade.

**Materials and Methods:** Medical and laboratory records of patients with microbial keratitis who underwent corneal scrapings between 2013 and 2023 were reviewed. Risk factors, culture results, and antibiotic sensitivity of the microorganisms were evaluated. **Results:** We obtained a 45.0% culture-positive rate (90/200). The mean age of the patients was 64±19 years (range: 18-94) (45 female, 45 male). The most common risk factor was corneal transplantation (42.2%). Of all positive cultures, 75 (83.3%) were bacterial and 15 (16.7%) were fungal keratitis. Polymicrobial growth was detected in 13 cultures. In total, 87 bacteria and 16 fungi were isolated. The 103 isolated microorganisms consisted of 46 gram-positive bacteria (44.7%), 41 gram-negative bacteria (39.8%), and 16 fungi. The most frequent microorganisms in bacterial keratitis were *Pseudomonas aeruginosa* (16.5%), *Staphylococcus aureus* (13.6%), and *Candida* species (8.7%) in fungal keratitis. The gentamicin and vancomycin susceptibilities of gram-positive bacteria were 100%. The susceptibility of gram-negative bacteria to various aminoglycosides ranged from 76.5% to 87.0%, which was comparable to that of ceftazidime (81.8%). The susceptibility of all bacterial species to various fluoroquinolones ranged from 77.8% to 100% **Conclusion:** Bacteria are the most common causative agent of microbial keratitis. The most frequent microorganisms were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. According to our results, empirical treatment of bacterial keratitis may be initiated with a combination of vancomycin and cephalosporin or aminoglycoside. Early treatment modification may be considered when a clinical response is not achieved.

**Keywords:** Microbial keratitis, epidemiology, etiology, antibiotic resistance

ÖZ

**Amaç:** Mikrobiyal keratitlerin yönetimi için bölgesel epidemiyolojik çalışmalara ihtiyaç vardır. Çalışmamızda, kliniğimizde son on yılda görülen mikrobiyal keratit hastalarının epidemiyolojik özelliklerini, predispozan risk faktörlerini, kornea kültürlerinden izole edilen mikroorganizmaları ve antibiyotik duyarlılığını analiz etmek amaçlanmıştır. **Gereç ve Yöntemler:** Kliniğimizde 2013-2023 yılları arasında keratit tanısı ile kültür örneği alınmış hastaların dosyaları retrospektif olarak tarandı. Hastalar risk faktörlerinin varlığı, kültür sonuçları ve antibiyotik duyarlılığı açısından değerlendirildi. **Bulgular:** Mikrobiyal keratit tanısı ile kültür alınan 200 hastanın, 90'ında kültür pozitifliği elde edildi (%45,0). Hastaların yaş ortalaması 64±19 (18-94) olup 45'i kadın 45'i erkekti. En yaygın görülen risk faktörü korneal transplant (%42,2) idi. Pozitif kültürlerin 75'inden (%83,3) bakteriyel, 15'inden fungal keratit sorumlu idi (%16,7). On üç kültürde polimikrobiyal üreme oldu. Toplamda 87 bakteri ve 16 mantar izole edildi. İzole edilen 103 mikroorganizmanın 46'sı gram-pozitif bakteri (%44,7), 41'i gram-negatif bakteri (%39,8) ve 16'sı mantar (%15,5) idi. Bakteriyel keratitlerde en yaygın mikroorganizmalar *Pseudomonas aeruginosa* (%16,5), *Staphylococcus*

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**Received:** 26.02.2023 **Accepted:** 31.10.2023

ÖZ

*aureus* (%13,6), iken fungal keratitlerde *Candida* türleri (%8,7) idi. Gram pozitif bakterilerin Gentamisin ve Vankomisin duyarlılığı %100 idi. Gram-negatif bakterilerin çeşitli aminoglokozidlere duyarlıkları %76,5 ile %87,0 arasında olup Seftazidim (%81,8) ile karşılaştırılabilir düzeydeydi. Tüm bakteri türleri için çeşitli florokinolonlara duyarlılık %77,8 ile %100 arasında değişmekteydi. **Sonuç:** Kliniğimizdeki mikrobiyal keratitlerin en yaygın etkeni bakterilerdi. En yaygın mikroorganizmalar *Pseudomonas aeruginosa* ve *Staphylococcus aureus* idi. Sonuçlarımıza göre bakteriyel keratit ampirik tedavisine vankomisin ile bir sefalosporin veya aminoglikozid kombinasyonu ile başlanabilir. Yakın takip ile kültür sonuçlanana kadar ya da kültür negatif olgularda yanıt alınamaması durumunda erken tedavi modifikasyonunu düşünülebilir.

**Anahtar Kelimeler:** Mikrobiyal keratit, epidemiyoloji, etiyoloji, antibiyotik direnci

## Introduction

Microbial keratitis is a corneal infection caused by bacteria, fungi, parasites, or viruses. Keratit is an important cause of ocular morbidity, which can cause corneal scarring, corneal perforation, and endophthalmitis and is associated with a risk of severe vision loss. It is one of the most important causes of visual impairment worldwide (1,2).

Keratitis is an ophthalmic emergency that can progress rapidly and requires effective treatment. Successful treatment of patients depends on early diagnosis, appropriate antibiotic selection, and close follow-up (2). In the management of microbial keratitis, culturing with corneal swab or scraping is necessary to identify the organism responsible for the infection and to determine the appropriate antimicrobial therapy (3). Broad-spectrum antibiotics are used as empirical therapy until the corneal culture results are obtained. Empirical treatment selection is based on epidemiological data. The etiology of infectious keratitis varies depending on demographic characteristics, risk factors, geography, and climate (4,5). For empirical treatment selection, careful evaluation of the patient's history and clinical signs and knowledge of regional microbial profiles and antibiotic susceptibility patterns are important. Therefore, current local epidemiological studies are required.

The aim of this study was to analyze the epidemiological features and predisposing risk factors of patients with microbial keratitis, microorganisms isolated from corneal cultures, and antibiotic susceptibility patterns of the pathogens in our tertiary care center in the last decade.

## Materials and Methods

This cross-sectional descriptive study was conducted by examining the clinical records and microbiological reports of patients diagnosed with microbial keratitis in the ophthalmology clinic of our hospital, which is a tertiary care institution. The study was approved by the local ethics committee (approval number: HNEAH-KAEK 2022/218), and the results were consistent with the tenets of the Declaration of Helsinki. Informed consent was not required as the design of the study was retrospective.

The medical records of all inpatients diagnosed with keratitis between 2013 and 2023 were retrospectively reviewed. Eyes without culture samples or negative cultures were excluded. Patients with typical viral keratitis findings were excluded because the protocols required to analyze these microorganisms were not available in our laboratory. Interstitial keratitis, marginal keratitis, peripheral ulcerative keratitis associated with autoimmune diseases, Mooren's ulcer, and neurotrophic keratopathy were also excluded from the study.

### Microbiological Examination

Samples for culture were obtained from patients with central, large, deep, chronic, antibiotic-resistant, or atypical infiltrates. All samples were collected under topical anesthesia. Samples from the infiltrate were obtained using a sterile cotton-tipped applicator or a sterile scalpel. The corneal samples were sent to the Microbiology Laboratory for culture and antibiotic susceptibility testing with transport medium within 2 h. Samples were inoculated on MacConkey, blood agar, chocolate agar, and Sabouraud dextrose agar and incubated for 48 h at the appropriate temperature. Selective media for the anaerobic bacteria Mycobacterium and Acanthamoeba were used in clinically suspicious cases. The causative microorganisms were identified using VITEX® 2 (BioMérieux, France) and MALDI-TOF MS (bioMerieux, France), and antibiotic susceptibility was determined using an automated antimicrobial identification and sensitivity system (VITEX® 2). Antibiotic susceptibility test results were evaluated according to the Clinical and Laboratory Standards Institute) criteria until 2018 and according to theEuropean Committee on Antimicrobial Susceptibility Testing) criteria after 2018.

Patients were treated with hourly empirical topical fortified ceftazidime (50 mg/mL) and vancomycin (50 mg/mL) or moxifloxacin hydrochloride 0.5% monotherapy until culture results were available. Topical fortified antifungals were added to the treatment regimen if there was a suspected fungal etiology (soil contamination or

vegetative trauma) or if clinical features of fungal keratitis were present. The treatment was then adjusted according to the culture and antibiotic susceptibility results.

The patients were evaluated in terms of demographic characteristics, presence of risk factors (ocular surface diseases, previous ocular surgery, ocular trauma, using contact lenses, systemic diseases, corticosteroid use), culture results, and antibiotic susceptibility of pathogens.

### Statistical Analysis

Data were analyzed using Microsoft Excel and SPSS version 22 (Package for the Social Sciences). Descriptive statistical methods (mean, standard deviation, frequency, ratio, minimum, maximum) were used to evaluate the study data. The chi-square test was used for the comparison of the two proportions. P<0.05 was considered statistically significant

## Results

Corneal samples were taken from 200 patients diagnosed with microbial keratitis between 2013 and 2023, and pathogens were recovered in 90 of these patients (45.0%). Of the patients with a positive culture, 45 were female and 45 were male. The mean age of the patients was 64±19 years and ranged from 18 to 94 years.

### Risk Factors

Eighty-one patients (90%) had identifiable risk factors, whereas nine (10%s) did not have a predisposing factor. The most common risk factor was corneal transplantation (42.2%). Among the corneal transplant patients, 16 had graft failure, two had suture keratitis, five had topical antiglaucomatous use and ocular surface disease, and one had herpetic keratitis superinfection. Ocular surface disease (13.3%) and topical medication (13.3%) were other common risk factors. The other risk factors are shown in Table 1.

### Microbiology Spectrum

Of 90 patients with positive cultures, 75 (83.3%) had bacterial and 15 (16.7%) had fungal keratitis. Thirteen cultures showed polymicrobial growth (12 bacterial, one fungal). In total, 87 bacteria and 16 fungi were isolated. Of the 103 isolated microorganisms, 46 were gram-positive bacteria (44.7%), 41 were gram-negative bacteria (39.8%), and 16 were fungi (15.5%). The most common microorganisms in bacterial keratitis are *Pseudomonas aeruginosa* (16.5%), *Staphylococcus aureus* (13.6%), coagulase-negative *staphylococci* (10.7%), and *Streptococcus pneumoniae* (10.7%), whereas *Candida species* (8.7%) are the most common in fungal keratitis. Table 2 shows all the isolated microorganisms.

Patients who underwent corneal transplantation were compared with patients with other risk factors. Bacteria were the causative agent in 86.8% of patients with corneal transplantation and in 80.8% of patients with other risk factors (p=0.445). Of the bacteria isolated in patients with corneal transplant, 57.6% were gram-positive bacteria, compared with 45.2% in patients with other risk factors (p=0.289). The two groups differed in terms of bacterial spectrum (p=0.026).The most common bacteria recovered in patients with corneal transplantation was *Staphylococcus aureus* (33.3%), whereas in other patients it was *Pseudomonas aeruginosa* (25.6%).

### Antibiotic Susceptibility

Antibiotic susceptibility patterns of gram-positive and gram-negative microorganisms are shown in Table 3. The susceptibilities to some antibiotics commonly used for treating bacterial keratitis were as follows:

The susceptibility of gram-positive bacteria to both gentamicin and vancomycin was 100%. Ciprofloxacin, moxifloxacin, and levofloxacin susceptibilities were 77.8%, 83.3%, and 86.4%, respectively. Three of the *Staphylococcus aureus* species (21.4%) were methicillin-resistant *Staphylococcus aureus*, and one of the coagulase-negative *staphylococci* was methicillin-resistant coagulase-negative *Staphylococcus aureus* (9.1%).

**Table 1. Predisposing risk factors**

| Risk factors | n | % |
|---|---|---|
| Trauma | 5 | 5.6 |
| Ocular surface disease | 12 | 13.3 |
| • Bullous keratopathy | 6 | 6.7 |
| • Meibomian gland dysfunction | 1 | 1.1 |
| • Dry eye | 3 | 3.3 |
| • Eyelid disorder | 2 | 2.2 |
| Corneal transplant | 38 | 42.2 |
| • Graft failure | 16 | 17.8 |
| • Suture keratitis | 2 | 2.2 |
| • Medications (anti-glaucomatous) | 5 | 5.6 |
| • Herpetic keratitis (superinfection) | 1 | 1.1 |
| Contact lens wear | 6 | 6.7 |
| Medications (systemic/topical steroid, antiglaucomatous) | 12 | 13.3 |
| Ocular surgeries | 3 | 3.3 |
| Herpetic keratitis (superinfection) | 2 | 2.2 |
| Systemic diseases (diabetes mellitus, autoimmune disease under immunosuppressant) | 24 | 26.7 |
| Unknown | 9 | 10 |

The susceptibilities of gram-negative bacteria to amikacin, netilmicin, tobramycin, and gentamicin were 87.0%, 76.5%, 81.3%, and 84.6%, respectively. Ceftazidime and ceftriaxone sensitivity was 81.8% and 100%, respectively. The sensitivity to ciprofloxacin, moxifloxacin and levofloxacin was 81.8%, 100% and 85.7%, respectively.

Antifungal susceptibility was evaluated in seven cultures of *Candida* species. Of these, sensitivity was reported to amphotericin B in seven cultures, voriconazole in five cultures, fluconazole in six cultures, caspofungin in six cultures, and micafungin in six cultures. Antifungal susceptibility could not be evaluated for filamentous fungi.

**Table 2. Microorganisms isolated from microbial keratitis**

| | n | % |
|---|---|---|
| **G (+) cocci** | 40 | 38.8 |
| *Coagulase-negative staphylococci* (*CNS*) | 11 | 10.7 |
| • *Staphylococcus epidermidis* | 5 | 4.9 |
| • *Staphylococcus capitis* | 1 | 1 |
| • *Staphylococcus hominis* | 3 | 2.9 |
| • *Staphylococcus saprophyticus* | 2 | 1,9 |
| • *Methicillin-resistant coagulase-negative staphylococci* (*MRCNS*) | 1 | 1 |
| *Staphylococcus aureus* | 14 | 13.6 |
| *Streptococcus pneumoniae* | 11 | 10.7 |
| *Streptococcus parasanguinis* | 1 | 1 |
| *Streptococcus mitis/oralis* | 1 | 1 |
| **G (+) bacilli** | 6 | 5.8 |
| *Corynobacterium* turneri | 5 | 4.9 |
| *Bacillus cereus* | 1 | 1 |
| **G (-) cocci** | 9 | 8.7 |
| *Moraxella* turneri | 8 | 7.8 |
| E. coli | 1 | 1 |
| **G (-) bacilli** | 32 | 31.1 |
| *Pseudomonas aeruginosa* | 17 | 16.5 |
| *Serratia marcescens* | 7 | 6.8 |
| *Klebsiella* turneri | 4 | 3.9 |
| *Acinetobacter lwoffii* | 1 | 1 |
| *Burkhelderia* | 1 | 1 |
| *Eikenella corrodens* | 1 | 1 |
| *Sphingomonas paucimobilis* | 1 | 1 |
| **Yeast-like fungi** | 9 | 8.7 |
| *Candida parapsilosis* | 4 | 3.9 |
| *Candida albicans* | 4 | 3.9 |
| *Candida ferri* | 1 | 1 |
| **Filamentous fungi** | 7 | 6.8 |
| *Fusarium solani* | 2 | 1.9 |
| *Aspergillus terreus* | 1 | 1 |
| *Aspergillus niger* | 1 | 1 |
| *Paecilomyces* spp. | 1 | 1 |
| *Penicillium* | 1 | 1 |
| *Acrenomium* | 1 | 1 |

**Table 3. Antibiotic susceptibility patterns of microorganisms isolated from microbial keratitis**

| Antibiotic | Gram-positive | | Gram-negative | |
|---|---|---|---|---|
| Ampicillin sulbactam | 100% | 5/5 | 0% | 0/12 |
| Amoxicillin clavulanate | 100% | 1/1 | 41.7% | 5/12 |
| Penicillin | 40.9% | 9/22 | 100% | 3/3 |
| Oxacillin | 60% | 3/5 | | |
| Ceftazidime | | | 81.8% | 18/22 |
| Sefepim | | | 82.6% | 19/23 |
| Cefoxitin | 80% | 4/5 | 0 % | 0/5 |
| Cefoxitin | 100% | 6/6 | 80% | 4/5 |
| Cefazolin | | | 11.1% | 1/9 |
| Cefuroxime | | | 45.5% | 5/11 |
| Ceftriaxone | 100% | 9/9 | 100% | 7/7 |
| Meropenem | | | 84.2% | 16/19 |
| Imipenem | | | 69.2% | 9/13 |
| Piperacillin | | | 72.2% | 13/18 |
| Piperacillin tazobactam | | | 85.7% | 24/28 |
| Ciprofloxacin | 77.8% | 14/18 | 81.8% | 27/33 |
| Moxifloxacin | 83.3% | 5/6 | 100% | 2/2 |
| Levofloxacin | 86.4% | 19/22 | 85.7% | 18/21 |
| Amikacin | | | 87.0% | 20/23 |
| Netilmicin | | | 76.5% | 13/17 |
| Tobramicin | | | 81.3% | 13/16 |
| Gentamicin | 100% | 15/15 | 84.6% | 22/26 |
| Eritromicin | 65.5% | 19/29 | 100% | 7/7 |
| Clindamycin | 73.3% | 22/30 | | |
| Trimethoprim-sulfamethoxazole | 84% | 21/25 | 76.2% | 16/21 |
| Fusidic acid | 76.5% | 13/17 | | |
| Tetracycline | 59.1% | 13/22 | 57.1% | 4/7 |
| Colistin | | | 45.5% | 6/11 |
| Chloramphenicol | 100% | 3/3 | 100% | 1/1 |
| Tigecycline | 100% | 6/6 | 50% | 3/6 |
| Vancomycin | 100% | 10/10 | | |
| Teicoplanin | 100% | 4/4 | | |
| Daptomycin | 100% | 7/7 | | |
| Linezolid | 100% | 4/4 | | |

## Discussion

The distribution and resistance patterns of isolates from microbial keratitis vary with geography and change over time. Therefore, it is important to analyze regional microbial profiles and antibiotic susceptibility of pathogens for evidence-based selection of empirical treatment regimens. In our study, the demographic characteristics and microbiological profile of patients with microbial keratitis and antibiotic susceptibility of the responsible pathogens are presented. To the best of our knowledge, this is the first study in Turkey in which antibiotic susceptibility of keratitis cases has been reported.

In our study, positive culture was observed in 90 of 200 patients who underwent corneal cultures with the diagnosis of microbial keratitis. We obtained a 45.0% culture-positive rate. In current reports in the literature, culture positivity rates range from 35.1% to 71.6% (6-14). These rates are affected by antibiotic therapy before culture, inadequate sampling, limited culture media, or sensitive microorganisms. Most of our cases were consulted in our clinic, and culture results may have been affected by antibiotic treatment that began in the center they were first referred to.

The cornea has a natural resistance to infections, with its healthy epithelium acting as a protective barrier against pathogens. Microbial keratitis rarely affects healthy eyes (3). Predisposing factors such as trauma, wearing of contact lenses, previous corneal surgery, and long-term use of corticosteroids weaken the defense mechanisms of the ocular surface and facilitate the invasion of the cornea by microorganisms (3). While the leading risk factors are using contact lenses and ocular surface disease in developed countries (10,12,15), trauma is more prevalent in developing countries (6,8,14). The most common local risk factors in our study were corneal graft and ocular surface disease. The high rate of corneal grafts in our study may be related to frequent keratoplasty surgery and follow-up of patients with keratoplasty because of the presence of an eye bank in our clinic.

Consistent with the literature, bacterial keratitis (83.3%) was the most common type of infectious keratitis among culture-positive patients in our study, followed by fungal keratitis (16.7%) (6-15). Although there were cases that were cultured for suspected Acanthamoeba keratitis, the pathogen could not be isolated in our laboratory. While 44.7% of the isolated microorganisms were Gram-positive bacteria, 38.8% were gram-negative bacteria. Gram-positive bacteria dominance has been demonstrated in most of the studies in the literature (7-15). In a recent study reported in our country, *Pseudomonas aeruginosa* was reported as the most common pathogen with gram-negative bacteria dominance (16). In studies reported in different countries, coagulase-negative *Staphylococci* (10-12,15), *Staphylococcus aureus*, and *Streptococcus pneumoniae* (9,13) are the most frequently reported gram-positive agents. *Pseudomonas aeruginosa* is the most frequently isolated agent among gram-negative bacteria (9,12-15,17). The most common pathogens in our study were *Pseudomonas aeruginosa* (16.5%), *Staphylococcus aureus* (13.6%), coagulase-negative *Staphylococci* (10.7%), and *Streptococcus pneumoniae* (10.7%). *Pseudomonas aeruginosa* keratitis is more severe than keratitis caused by gram-positive bacteria (3). The frequency of need for treatments such as corneal transplantation and evisceration is higher in patients with a progressive clinical course. Therefore, the prevalence of *Pseudomonas aeruginosa* may have been high in patients referred to our clinic.

In this study, the rate of fungal keratitis was 16.7%, and *Candida* species (8.7%) were the most common agents. The rate of fungal keratitis varies between 2% and 46.6% in the current literature (6-14). Higher rates have been reported in studies conducted in developing countries with larger rural populations (6,7,9,16). Fungal keratitis is more common in tropical and subtropical regions than in temperate climates (6,9). In addition, while *Candida* species are the most frequently isolated fungi in temperate climates, filamentous fungi, particularly *Fusarium* species, are more frequently encountered in tropical regions (14). Because of the small number of patients referred to our clinic from rural areas, the rate of fungal keratitis may be small.

The World Health Organization has defined antimicrobial resistance as a growing public health threat (18,19). This is due to the prolonged and inappropriate use of antibiotics. To maintain the efficacy of empirical therapy, it is necessary to provide low rates of resistance to selected antibiotics. Here, the importance of antibiotic susceptibility reports is highlighted. Some studies in the literature have shown an increase in antibiotic resistance against fluoroquinolones, including the fourth generation (3,11,13,14,20). In the present study, the sensitivity of gram-positive bacteria to various fluoroquinolones was between 77.8% and 86.4%, and that of gram-negative bacteria was between 81.8% and 100%. The reason for the resistance to fluoroquinolones may be the widespread use of fluoroquinolones for the treatment of infections such as conjunctivitis and for postoperative prophylaxis. Considering the widespread use of fluoroquinolones in our country, their use for empirical monotherapy may not be a good option.

In our clinic, a combination of vancomycin and ceftazidime is often preferred for the empirical treatment of bacterial keratitis. In our antibiogram reports, vancomycin susceptibility was observed in cases after 2019, and all

of the gram-positive bacteria evaluated were susceptible to vancomycin. Vancomycin continues to be used as the first choice in the empirical treatment of gram-positive agents in our clinic. Our results, which are consistent with studies reporting high sensitivity to vancomycin, support this hypothesis (13,15,20-23). In our study, the sensitivity of gram-negative bacteria to ceftazidime was 81.8%. This rate was comparable to the sensitivity to aminoglycosides (76.5% to 87.0%). According to the antibiogram results, ceftazidime, which is frequently preferred in empirical treatment, was not superior to other agents. If there is no clinical response until culture results are obtained or in cases where culture positivity cannot be obtained, it may be beneficial to act early to choose an alternative treatment to empirical therapy.

### Study Limitations

The main limitation was the retrospective design of the study. All the follow-up data of the patients could not be reached and their progression could not be determined. Antifungal susceptibility was not detected in filamentous fungal species. Our results may not reflect the microbial profile and antibiotic resistance pattern of the general population because of the referral of more complex cases caused by resistant pathogens to our clinic.

## Conclusion

In our clinic, the most common etiology of microbial keratitis was bacteria (83.3%). *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most prevalent pathogens. *Candida* species were responsible for most fungal keratitis. Based on our antibiogram results, a combination of vancomycin with cephalosporin or aminoglycoside can be selected as the initial therapy in the empirical treatment of bacterial keratitis. Patients should be closely followed, and early treatment modification may be considered if there is no clinical response. We presented the data of our clinic, which is a tertiary center in Istanbul, the most populated city in Türkiye. We believe that our study may contribute to the literature on microbiological profiles and antibiotic susceptibility of keratitis.

### Ethics

**Ethics Committee Approval:** The study was approved by the local ethics committee (approval number: HNEAH-KAEK 2022/218), and the results were consistent with the tenets of the Declaration of Helsinki.

**Informed Consent:** Informed consent was not required as the design of the study was retrospective.

**Peer-review:** Externally and internally peer reviewed.

### Authorship Contributions

Surgical and Medical Practices: M.B.Y., E.Y., E.T.U., R.B., N.K., Concept: M.B.Y., E.Y., E.T.U., R.B., N.K., S.A., Design: M.B.Y., E.Y., E.T.U., R.B., N.K., S.A., Data Collection or Processing: M.B.Y., E.Y., E.T.U., R.B., N.K., S.A., Analysis or Interpretation: M.B.Y., E.Y., E.T.U., R.B., N.K., S.A., Literature Search: M.B.Y., E.Y., E.T.U., Writing: M.B.Y., E.Y., E.T.U.

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

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

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CASE REPORT

**DOI:** 10.4274/hamidiyemedj.galenos.2023.70298

Hamidiye Med J 2023;4(3):191-193

# Inferior Myocardial Infarction Recanalized by Multiple Embolization to Distal Branches

## Distal Dallara Multiple Embolizasyon ile Rekanalize Olan İnferior Miyokard Enfarktüsü

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**ABSTRACT**

Coronary artery embolization resulting in STEMI is a rare condition that is a challenging issue in the field of interventional cardiology in terms of determining the etiology and management. In this manuscript, we present a case characterized by distal multiple thrombus embolization from an infarct-related lesion located in the mid-segment of right coronary artery and discuss the management strategies.

**Keywords:** STEMI, distal embolization, PTCA

**ÖZ**

Koroner embolizasyon ve buna bağlı görülen STYMI tablosu nadiren görülen ve gerek etiyoloji araştırması gerekse tedavi stratejileri bakımından girişimsel kardiyoloji alanının zorlu konularından biridir. Biz bu yazımızla RCA mid bölgede infarktan sorumlu lezyondan distal dallara multiple trombüs embolizasyonu ile karakterize bir olgu sunuyor ve tedavi yöntemlerini tartışıyoruz.

**Anahtar Kelimeler:** STYMI, distal embolizasyon, PTKA

## Introduction

Coronary embolization is a condition reported to be responsible for approximately 3% of ST-segment elevation myocardial infarctions (1). The most common causes of coronary embolization are atrial fibrillation, dilated cardiomyopathy, endocarditis, and systemic diseases such as malignancy, autoimmune diseases, and antiphospholipid syndrome. However, the etiology cannot be determined in approximately 25% of patients (2).

In this article, we present a case that underwent emergency coronary angiography because of acute inferior + posterior + right myocardial infarction (MI). We found total stenosis in the proximal regions of both the posterior descending artery (PDA) and posterolateral artery (PLA) due to distal thrombus embolization originating from an infarct-related lesion in the mid-region of the right coronary artery (RCA).

## Case Report

A 65-year-old male patient was referred to the emergency department of our hospital with a diagnosis of 2-h chest pain and acute inferior + posterior + right MI. After the first evaluation in the emergency department, 300 mg of acetylsalicylic acid was orally administered, and 180 mg of ticagrelor was loaded. In the RCA angiography during the primary procedure (Figure 1), we determined a hazy plaque that did not cause significant stenosis in the mid-region of the RCA and total stenoses in the proximal PDA and PLA, thought to be due to embolism (Figure 2).

In addition, 80% stenosis was detected in the left anterior descending artery during angiography, and an elective procedure was decided. To treat both total stenoses in the distal segments of the RCA, we engaged the RCA ostium with a right Judkins guide catheter. We passed the total stenosis in the PDA ostial and then the PLA ostial stenosis, respectively, using Choose Floppy guidewire (Boston Scientific/Scimed,

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**Received:** 26.05.2023 **Accepted:** 28.09.2023

Inc., Maple Grove, Minnesota). Balloon dilatations were performed using a 2.0x12 mm Shunmei semi-compliant balloon at a pressure of 12-14 atm. Complete patency and TIMI 3 flow were obtained in both arteries. There was no plaque imaging in the areas with total stenosis after successful ballooning of these segments; thus, embolism from the lesion in the mid-RCA was confirmed. In addition, there was a significant regression in the blurry appearance and stenosis rate in the mid-region during the distal ballooning process (Figure 3).

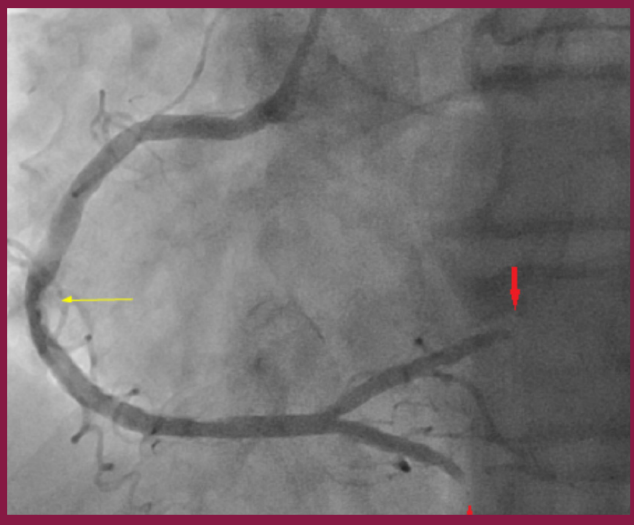

**Figure 1.** Multiple distal embolizations in the distal branches of the right coronary artery (red arrows) and a non-critical hazy lesion in the mid-region (yellow arrow) associated with the infarct

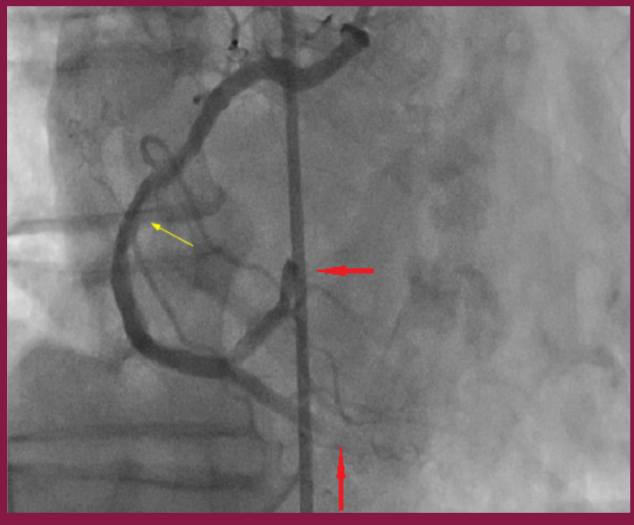

**Figure 2.** Multiple distal embolizations in the distal branches of the right coronary artery (red arrows) and a hazy lesion in the mid-region (yellow arrow)

Therefore, we did not consider stenting in the mid-region of the RCA. After the procedure, there was no angina, hemodynamic, or electrical abnormality during the clinical follow-up in the hospital. Thus, we did not plan any further intervention. Early echocardiographic imaging revealed that the inferior and inferior septum of the left ventricular wall was hypokinetic, the ejection fraction was 45%, and there was no valve pathology or source of intracardiac thrombus. In addition, there was no pathology to explain the hematological cause of thromboembolism.

## Discussion

Although coronary embolization is detected in approximately 3% of all STEMI patients, the source of embolism may not be detected in some patients (1). Coronary embolism is a controversial issue in terms of both its causes and treatment methods, which the field of interventional cardiology cannot find clear answers to (3). For treating distal embolization, methods such as thrombus aspiration, glycoprotein 2b/3a antagonists, and thrombolytic administration are used according to the characteristic features of the thrombus (4,5). It should be noted that the source of distal embolization may be an acute responsible lesion in the proximal segments in patients with multiple distal total lesions presented with STEMI. In addition, in this case, it should be considered that the antegrade flow successfully provided by a low-pressure balloon dilatation with a diameter that will not cause dissection in the coronary can dissolve the thrombus.

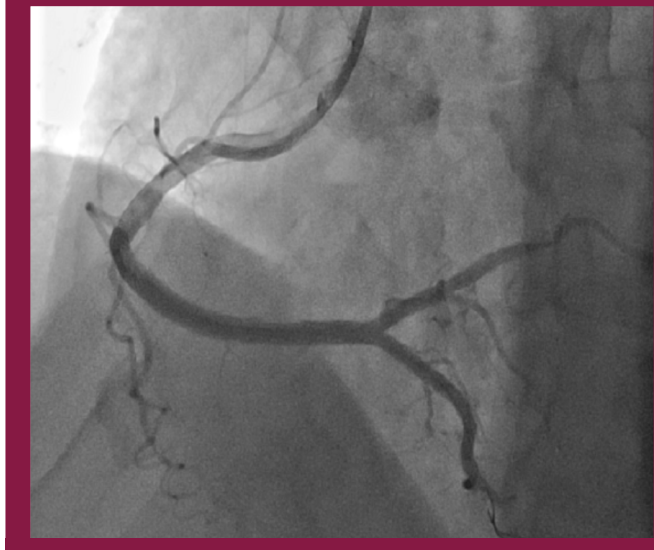

**Figure 3.** Distal TIMI 3 flow and non-critical plaque in the mid-region after balloon angioplasty

**Informed Consent:** An informed consent form was obtained from the patient.

**Peer-review:** Externally peer reviewed.

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

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CASE REPORT

**DOI:** 10.4274/hamidiyemedj.galenos.50251

Hamidiye Med J 2023;4(3):194-197

# Wide Resection Treatment of Angiomatoid Fibrous Histiocytoma in a 42-Year- Old Female

## Anjiyomatoid Fibröz Histiositomaası Bulunan 42 Yaşındaki Hastanın Geniş Rezeksiyon ile Tedavisi

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**ABSTRACT**

Angiomatoid fibrous histiocytoma is a rare neoplasm with malignant potential that primarily occurs in the subcutaneous tissues or dermis of the extremities in children or young adults. Its characteristic histological appearance involves the nodular growth of histiocytic, epithelioid, or spindle cells surrounded by a fibrous pseudocapsule containing a lymphocytic cuff. It may also feature a prominent myxoid stroma. Unresectable or metastatic tumors may have limited treatment options. Immunotherapy using PD-1/PD-L1 inhibitors could play a role in the future, although it is not yet widely adopted. The standard surgical approach for angiomatoid fibrous histiocytoma involves wide or radical resection of the lesion. In this article, we discuss the case of a 42-year-old female patient who presented to our clinic with shoulder pain and was subsequently diagnosed with angiomatoid fibrous histiocytoma after biopsy.

**Keywords:** Angiomatoid fibrous histiocytoma, Pd-1/pd-l inhibitors, wide resection

**ÖZ**

Anjiyomatoid fibröz histiyositoma, çocuklarda veya genç erişkinlerde çoğunlukla deri altı dokularda veya ekstremitelerin dermisinde ortaya çıkan nadir bir malign potansiyelli neoplazmadır. Lenfositik bir manşon içeren fibröz bir psödo kapsül ile çevrili histiyositik, epitelioid veya iğsi hücrelerin nodüler büyümesiyle karakterize edilen histolojik bir görünümü vardır. Belirgin miksoid stroma içerebilir. Rezeke edilemeyen veya metastatik tümörler sınırlı tedavi seçeneklerine sahip olabilir. PD-1/PD-L1 inhibitörleri ile immünoterapi planlaması gelecekte yer alabilir ancak henüz yaygın olarak kullanılmamaktadır. Anjiyomatoid fibröz histiyositomanın yaygın cerrahi tedavisi, lezyonun geniş veya radikal rezeksiyonunu içermektedir. Bu yazıda, kliniğimize omuz ağrısı şikayetiyle başvuran ve biyopsi sonrası anjiomatoid fibröz histiyositoma tanısı konulan 42 yaşındaki kadın hastayı tartıştık.

**Anahtar Kelimeler:** Anjiyomatoid fibröz histiyositoma, Pd-1/pd-l inhibitörleri, geniş rezeksiyon

## Introduction

Angiomatoid fibrous histiocytoma is a rare neoplasm with malignant potential that predominantly occurs in the subcutaneous tissues or dermis of the extremities in children or young adults. most reported cases involve individuals aged between 10 and 30 years (1).

Angiomatoid fibrous histiocytoma belongs to the group of soft tissue tumors with unknown differentiation. Local recurrence has been reported in 15% of the cases, and metastases have been reported in 2-5%. There is no correlation between clinicopathological factors and clinical course (2).

It is most commonly found in the upper and lower extremities. The neck and scalp are also not uncommon locations in reported cases (3). The pelvis is not a common site for angiomatoid fibrous histiocytoma. Although it is not very common, some individuals with pelvic infiltration are treated with wide resection of the lesion (4).

Chemotherapy and radiotherapy can be used in patients with metastasis or widespread dissemination of the disease; however, there is no strict consensus on these treatments. Some centers use PD-1/PD-L1 inhibitors for treatment, but long-term results have not yet been reported for cases of angiomatoid fibrous histiocytoma. The most common surgical treatment is wide resection of the lesion. The

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**Received:** 29.04.2023 **Accepted:** 28.09.2023

prognosis heavily depends on the presence of metastasis, with the lungs being the most common site of metastasis reported (5).

Its histological appearance is characterized by histiocytic, epithelioid, or spindle cells surrounded by a fibrous pseudocapsule containing a lymphocytic cuff (6).

## Case Report

A 42-year-old female patient was admitted to our clinic with left shoulder pain. Her primary complaint was pain during exertion while trying to perform household tasks. The patient was diagnosed with breast cancer in 2014 and had a history of chemotherapy, radiotherapy, and 1 surgery. Before treatment, consent was obtained from both the patient and the doctors.

On physical examination, there was no asymmetrical view of the shoulders. There was no swelling or redness in the left shoulder. There was a small amount of pain with palpation on the posterior superior edge of the humerus. The patient had a full range of motion in both shoulders, and there was no pain during shoulder joint movement. In her radiological images, a soft tissue lesion around 5x5 cm was identified on the superior posterior aspect of the humerus extending into the deltoid muscle. Magnetic resonance imaging was reported as a soft tissue lesion of a potentially benign nature. A full-body metastasis scan was performed on the patient using positron emission tomography-computed tomography before surgery, and no metastasis was detected.

Following the radiological evaluations (Figures 1, 2), the patient was discussed in the tumor board, and it was deemed appropriate to perform a fine needle aspiration biopsy by the interventional radiology department. The results of the biopsy revealed a low-grade/benign mesenchymal tumor with spindle cells and myxoid stroma. The report also stated that the tumor's myxoid component was predominant. There was no necrosis, and the atypia was mild.

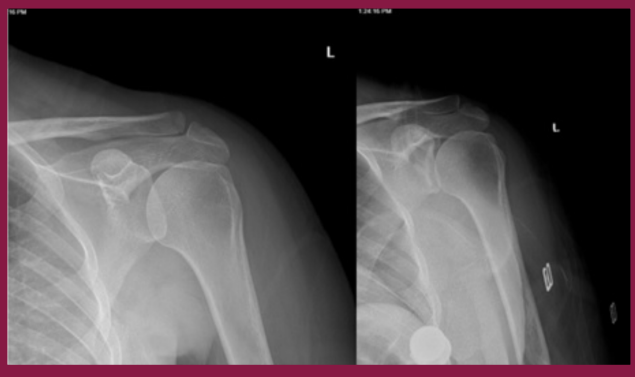

**Figure 1.** Direct radiological views of the shoulder joint

A few weeks after the fine needle biopsy, a wide resection of the lesion was performed (Figures 3-5) on the patient, and the specimens were sent for histopathological

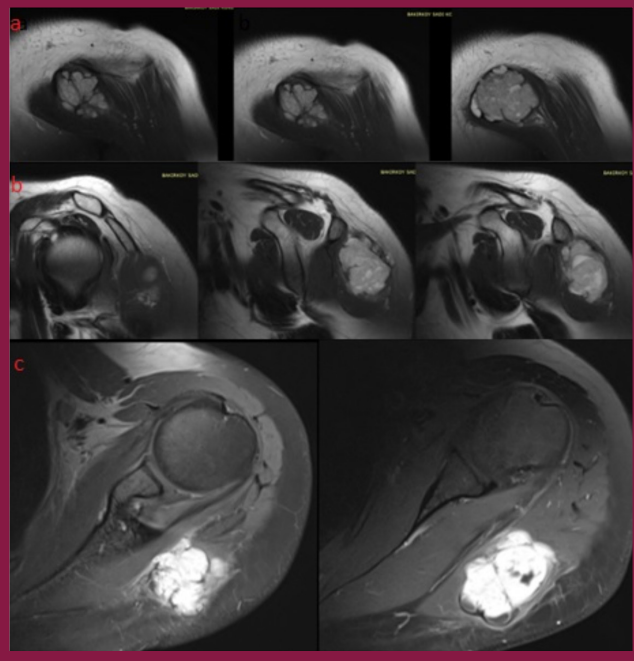

**Figure 2.** a-c. Magnetic resonance images of the lesion from different views

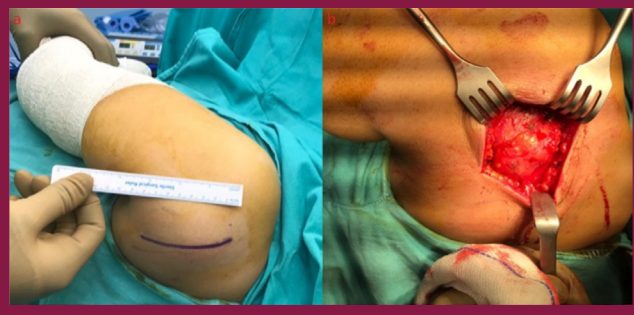

**Figure 3.** a, b. Peroperative images of the lesion. a) Before surgery, b) During surgery

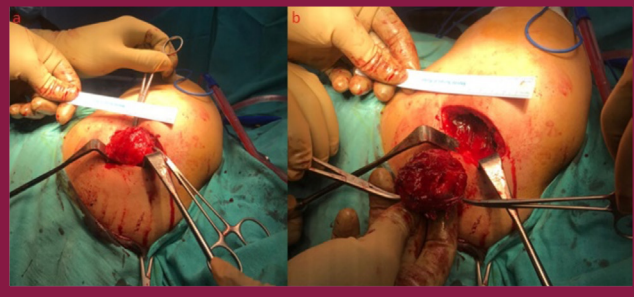

**Figure 4.** Peroperative images of the lesion during removal

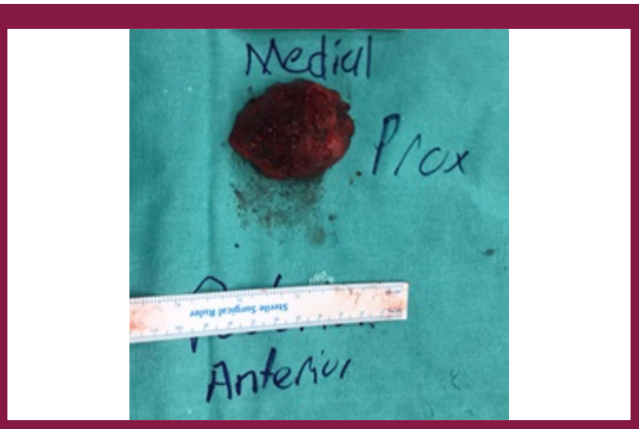


**Figure 5.** Peroperative pictures of the lesion after removal

investigations. The result of the wide resection material was "angiomatoid fibrous histiocytoma, myxoid form". During surgery, pathological examination revealed a negative surgical margin. The immunohistochemical markers were as follows:

- CD99: Cytoplasmic positive
- Desmin: Generalized diffuse positive
- EMA: Positive
- SMA: Focal positive
- S100: Negative
- BCOR: Negative
- STAT-6: Cytoplasmic positive
- CD34: Negative
- Ki67: 3-4%

The patient was discharged from the hospital on the day of surgery. Before discharge, the patient was taught pendulum and passive elbow exercises. The wound site was clear, and there was no leakage. The patient was then asked to visit for weekly check-ups in the polyclinics. During the check-ups, the patient was content and relieved of pain symptoms. The patient's shoulder range of motion was complete and without limitation.

After the pathological results were released, further molecular rearrangement of EWSR1 was studied and found to be positive. The report stated that no mutations, copy number increases, or rearrangements have been detected in the studied genes (7).

During the 2 years of postoperative checkups, there were no signs of recurrence or metastasis of the lesion in the control magnetic resonance images. No PD-1/PD-L1 inhibitors were used before or after treatment. The patient was recommended to undergo annual contrast-enhanced MRI follow-up.

## Discussion

Most of the reported cases comprise female individuals between the ages of 10 and 30. However, our case was a 42-year-old female patient with a lesion occurring in a relatively common site. Some studies have shown that age has no prognostic value in angiomatoid fibrous histiocytoma (8,9).

Angiomatoid fibrous histiocytoma is classified as a soft tissue tumor with unknown differentiation. The patient's pathology report states, "the tumor's myxoid component is predominant. There is no necrosis, and the atypia is mild". In cases of angiomatoid fibrous histiocytoma with low atypia and either no or minimal necrosis, metastasis is uncommon, and the tumor does not exhibit aggressive behavior. These findings are consistent with the clinical presentation of our patient (9).

Among the options of fine-needle aspiration biopsy, core biopsy, and surgical biopsy, surgical biopsy is the most effective and definitive method for making a diagnosis. To assess the possibility of medical treatment, a fine-needle aspiration biopsy was initially performed. Surgical excision was considered appropriate following the diagnosis of angiomatoid fibrous histiocytoma.

The most notable treatment for angiomatoid fibrous histiocytoma is surgical-wide resection of the lesion. We also performed wide resection surgery, and there were no complications during or after the surgery. The lesion was encapsulated and limited to the soft tissue, with no spread to bone or neurovascular bundles. Resection of the lesion was easily accomplished during surgery (7).

The time between diagnosis and surgery was approximately 1 month. During the surgery, the size of the lesion was approximately the same as that in the magnetic resonance image, and there was no growth between the two periods [the magnetic resonance imaging (MRI) was taken about 2 and a half months before the surgery].

We did not use any biological drugs or PD-1/PD-L1 inhibitors during the patient's treatment. There may be beneficial results in postoperative follow-ups, but the long-term effects are still uncertain (7). The most commonly used PD-1/PD-L1 inhibitors are pembrolizumab, nivolumab, and cemiplimab, and atezolizumab, avelumab, and durvalumab. They have been predominantly employed in diseases such as melanoma, lung cancer, and urothelial cancer. (10). Reviews conducted to date indicate that in most cases, wide resection of the lesion is sufficient as treatment, and further chemo/radiotherapy or biological agents are not required. Similar to the literature, our patient has been successfully treated with only-wide excision without receiving chemotherapy or radiotherapy.

## Conclusion

Angiomatoid fibrous histiocytoma is a rare neoplasm that predominantly occurs in subcutaneous tissues of the extremities in children or young adults and can be successfully treated with surgical removal. Chemotherapy and radiotherapy are generally not recommended for treatment. Wide resection is the preferred approach. The use of PD-1/PD-L1 inhibitors is still under discussion and may play a primary role in future treatment.

**Informed Consent:** Before treatment, consent was obtained from both the patient and the doctors.

**Peer-review:** Externally and internally peer reviewed.

### Authorship Contributions

Surgical and Medical Practices: A.C., M.K.Ö., H.B., Concept: A.C., M.K.Ö., H.B., Design: A.C., Data Collection or Processing: A.C., M.K.Ö., Analysis or Interpretation: A.C., M.K.Ö., H.B., Literature Search: A.C., M.K.Ö., Writing: A.C.

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

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

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