Invasive fungal infection in tissue biopsy sample: *Candida albicans*

Doku biyopsisi örneğinde invaziv fungal enfeksiyon: *Candida albicans*

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ABSTRACT

The incidence of invasive fungal infections has increased significantly in recent years. There are hundreds of *Candida* species that cause invasive candidiasis. The most common species causing infections include *C. albicans*, *Nakaseomyces glabrata* (*C. glabrata*), *C. parapsilosis*, *C. tropicalis* and *Pichia kudriavzevii* (*C. krusei*). *Candida albicans* is the most frequently detected microorganism that causes candidiasis. Detection of the microorganism that poses a risk for invasive fungal infections and application of antifungal sensitivity tests are of great importance for the treatment of patients. The study demonstrates the effective use of the clinical microbiology laboratory in terms of laboratory diagnosis of invasive fungal infection and directing treatment. A 49-year-old male patient, who underwent femoral popliteal bypass surgery at an external center due to peripheral vascular artery disease, was admitted to Ağrı Training and Research Hospital due to discharge and increased temperature in the left popliteal region. The tissue biopsy sample of the patient, who was re-operated

ÖZET

İnvaziv mantar enfeksiyonlarının insidansı son yıllarda ciddi artış göstermektedir. İnvaziv kandidoz etkeni yüzlerce *Candida* türü bulunmaktadır. Enfeksiyonlara neden olan en yaygın türler arasında *C. albicans*, *Nakaseomyces glabrata* (*C. glabrata*), *C. parapsilosis*, *C. tropicalis* ve *Pichia kudriavzevii* (*C. krusei*) bulunmaktadır. *Candida albicans* en sık saptanan kandidoz etkeni mikroorganizmadır. İnvaziv fungal enfeksiyonlar için risk oluşturan mikroorganizmanın tespiti ve antifungal duyarlılık testlerinin uygulanması hastaların tedavisi için büyük önem taşımaktadır. Çalışma, invaziv fungal enfeksiyon etkeninin laboratuvar tanısı ve tedavinin yönlendirilmesi açısından klinik mikrobiyoloji laboratuvarının etkin kullanımını ortaya koymaktadır. Periferik vasküler arter hastalığı nedeniyle dış merkezde femoral popliteal bypass operasyonu geçiren 49 yaşındaki erkek hastanın sol popliteal bölgesinde akıntı ve ısı artışı olması nedeniyle Ağrı Eğitim ve Araştırma Hastanesi'ne başvurmuştur. Kardiyovasküler Cerrahi tarafından tekrar opere

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by Cardiovascular Surgery, was sent to the Medical Microbiology Laboratory. Gram-stained microscopic examination was performed on the tissue biopsy sample. For culture, cultivation was done on %5 sheep blood agar, EMB agar, chocolate agar, Saboraud dextrose agar and chromogenic Candida agar. Gram staining and germ tube testing were performed on pure colonies. Species level identification and antifungal susceptibility testing were performed with the VITEK-2 Compact (Biomerioux, France) device. Gram-stained microscopic examination of the tissue biopsy sample revealed pseudohyphae and budding yeast fungi. Green and well-circumscribed colonies were detected on chromogenic Candida agar and the germ tube test was positive. It was identified as *Candida albicans* at species level with the VITEK-2 Compact (Biomerioux, France) device. Fluconazole was added to the patient's treatment. He was discharged after two weeks of treatment. In invasive fungal infections identification of the infective pathogen and performing antifungal susceptibility tests guide the clinician in applying effective antifungal therapy in treatment. As a result, microbiological diagnostic methods were used effectively in the study and the treatment was implemented as quickly as possible.

Anahtar Kelimeler: *Candida albicans*, tissue biopsy, fluconazole, germ tube

edilen hastanın doku biyopsi örneği Tıbbi Mikrobiyoloji Laboratuvarına gönderilmiştir. Doku biyopsi örneğine Gram boyalı mikroskobik inceleme yapılmıştır. Kültür için %5 koyun kanlı agar, EMB agar, çikolata, agar, Saboraud dextroz agar ve kromojenik Candida agara ekimi yapılmıştır. Saf kolonilerden Gram boyama ve germ tüp testi gerçekleştirilmiştir. Tür düzeyinde tanımlama ve antifungal duyarlılık testi ise VITEK-2 Compact (Biomerioux, Fransa) cihazı ile çalışılmıştır Doku biyopsi örneğinde Gram boyalı mikroskobik incelemesinde psödohif, tomurcuklanan maya mantarı görülmüştür. Kromojenik Candida agarda yeşil ve düzgün sınırlı koloniler ve germ tüp testi ise pozitif saptanmıştır. VITEK-2 Compact (Biomerioux, Fransa) cihazı ile tür düzeyinde *Candida albicans* olarak tanımlanmıştır. Hastanın tedavisine flukonazol eklenerek düzenlenmiştir. İki hafta tedaviden sonra taburcu edilmiştir. İnvaziv fungal enfeksiyonlarda enfektif patojenin tanımlanması ve antifungal duyarlılık testlerinin yapılması tedavide etkin antifungal terapinin uygulanması açısından klinisyene yol gösterici olmaktadır. Sonuç olarak, çalışmada mikrobiyolojik tanı yöntemleri etkin olarak kullanılmış ve tedavinin en hızlı şekilde uygulanması sağlanmıştır.

Key Words: *Candida albicans*, doku biyopsi, flukonazol, germ tüp

INTRODUCTION

The incidence of invasive fungal infections has increased significantly in recent years (1). There are hundreds of Candida species that cause invasive candidiasis. The most common species causing infections include *C. albicans*, *Nakaseomyces glabrata* (formerly *C. glabrata*), *C. parapsilosis*, *C. tropicalis* and *Pichia kudriavzevii* (formerly *C. krusei*).

Candida albicans is the most frequently detected microorganism that causes invasive candidiasis. Hydrolytic enzymes secreted by *C. albicans* cause tissue destruction in the host tissue and allow the fungus to invade the host tissue. It has been reported that especially the enzymes aspartyl proteinase and phospholipase are significantly responsible for the virulence of *C. albicans* (2). Due to its biofilm-forming feature, its adhesion on external substance is an important virulence factor.

European Committee on Antimicrobial Susceptibility Tests (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines are used in antifungal susceptibility testing of yeasts in the clinical microbiology laboratory. In antifungal susceptibility tests of yeasts, standardization has been achieved for broth microdilution methods with the CLSI M27 guideline, disk diffusion method with the CLSI M44-A2 guideline, and broth microdilution methods with the EUCAST E.Dis 7.4 guideline (3-5). Technical difficulties in applying standard methods and visual evaluation of broth microdilution results require experience. For this reason, alternative methods that can be more easily applied have been used for antifungal susceptibility tests in routine laboratories. Gradient testing, flow cytometry, agar dilution, ergosterol amount determination, colorimetric microdilution methods and various automated systems are used as alternatives. The VITEK-2 Compact (Biomerieux, France) device is a fully automated commercial system. With VITEK-2 Compact system, yeasts are evaluated spectrophotometrically and identification and antifungal susceptibility tests can be performed simultaneously (6).

Identification of yeasts at the species level and susceptibility testing are necessary to accurately guide antifungal therapy and predict clinical response. The availability of sensitive and specific diagnostic tests in the laboratory diagnosis of patients allows antifungal treatment to be started earlier. The study demonstrates the effective use of the clinical microbiology laboratory in directing the laboratory diagnosis and treatment of invasive fungal infection agents.

CASE REPORT

A 49-year-old male patient with peripheral vascular artery disease underwent femoral popliteal bypass surgery at an external center. On November 16, 2023, he was admitted to Ağrı Training and Research Hospital due to discharge and increased temperature in the left popliteal region. The patient was re-operated by Cardiovascular Surgery. The graft was removed from the infected area. The operation area was washed with sterile saline. Tissue biopsy samples were taken from the infected area with scalpel. Tissue biopsy specimens were sent to Medical Microbiology Laboratory at Ağrı Training and Research Hospital.

The tissue biopsy samples were divided into small pieces on a slide with a sterile scalpel, then samples were crushed by adding 1 mL of sterile saline. Homogenized tissue biopsy samples were plated on 5% sheep blood agar (Oxoid, UK), EMB agar (Oxoid, UK), chocolate agar (Merck, Germany), chromogenic Candida agar (AEM Medikal, Türkiye) and Sabouraud dextrose agar (SDA, Merck, Germany). It was incubated at 37 ^{0}C under aerobic conditions for 24 hours. Gram-stained microscopic examination of the tissue biopsy sample was performed. Structures were seen in the yeast morphology in Gram staining. At the end of incubation, germ tube test was applied to identification. For this purpose, pure colonies taken from the sample were added to the 500 µL serum in tube and incubated at 37 ^{0}C for 4 hours (Figure 1). Then the results were evaluated.

Species level identification and antifungal susceptibility tests were performed with the VITEK-2 Compact (Biomerieux, France) device in accordance with the manufacturer's recommendations. YST card was used for identification of yeast isolate and AST-YS08 card was used for antifungal susceptibility. Pure yeast colonies were adjusted to a 2.0 McFarland (1.8-2.2; DensiCheck Plus, BioMérieux, France) standard in 0.45% sterile saline suspension for identification and antifungal susceptibility. Antifungal susceptibility test results were evaluated using species-specific breakpoints specified in the CLSI M27-A3 guideline.

Since it is a case report, ethics committee approval is not required; Written consent of the patient was obtained for the presentation of the case.

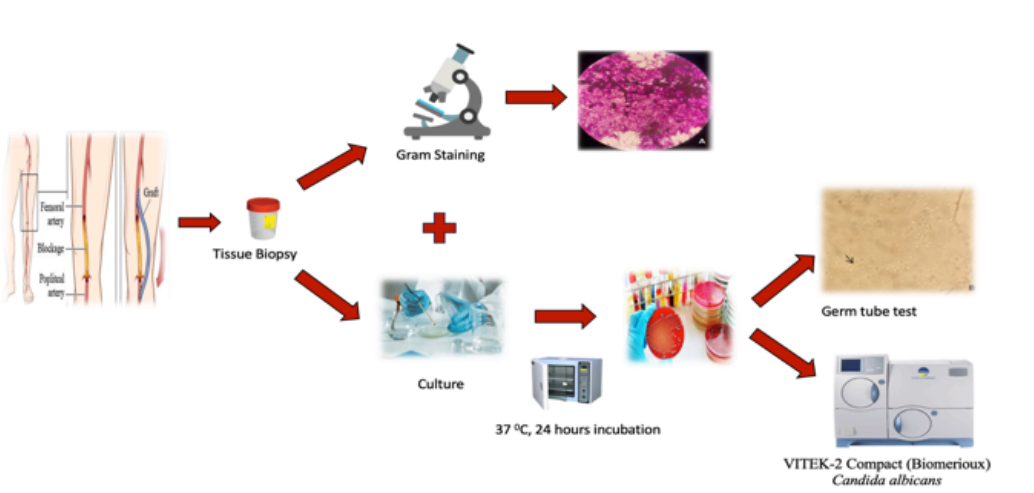

Figure 1. Graphical summary of tissue biopsy sample collection, transportation and processing stages

RESULTS

When the patient's biochemical parameters were examined, leukocyte 8.42.109/L (neutrophil 80.4%, lymphocyte 12.9%), hemoglobin 9.4 g/dL, hematocrit 31.2%, platelet 139.109/L and CRP 133.12 mg/L was detected.

In the Gram-stained microscopic examination of the tissue biopsy sample, pseudohyphal structure and budding yeast were observed (Figure 2-A). Cream-colored, well-circumscribed colonies were detected on Sabouraud dextrose agar. Green colored, well-circumscribed colonies were observed on chromogenic Candida agar (Figure 3). The germ tube test, one of the mycological phenotypic methods, of the yeast colonies isolated at the end of incubation was found positive (Figure 2-B). It was identified as *C. albicans* with the VITEK-2 Compact device. According to the antifungal susceptibility test results, MIC values were determined as fluconazole ≤0.5 µg/mL sensitive, voriconazole ≤0.12 µg/mL sensitive, micafungin ≤0.06 µg/mL sensitive, and amphotericin B 1 µg/mL sensitive. Fluconazole was added to the patient's treatment. He was discharged after two weeks of treatment.

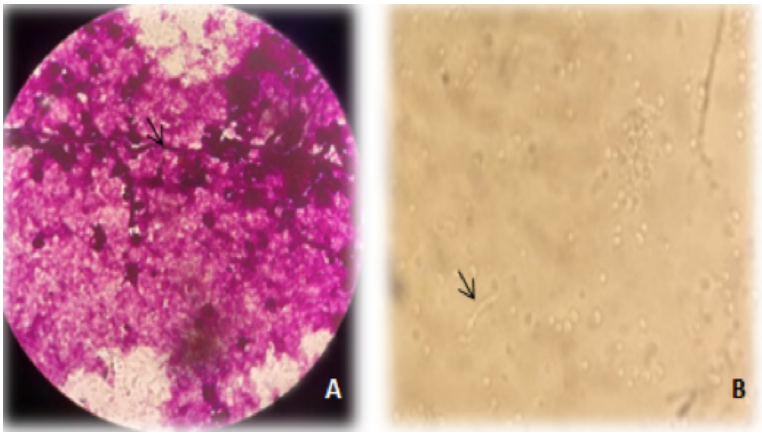

Figure 2. A) Pseudohyphae and budding yeast cells in gram-stained microscopic examination (x1000)
B) Germ tube positive yeast (x400)

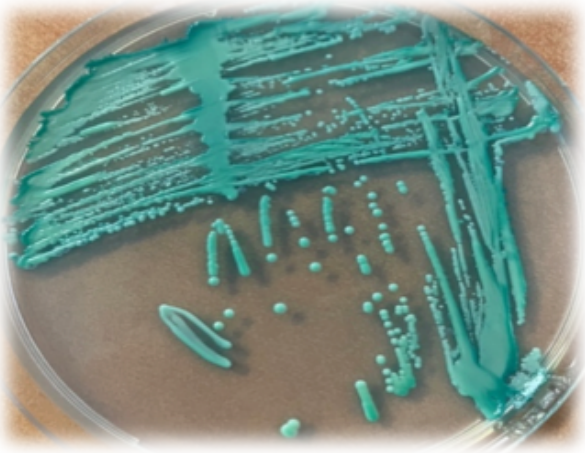

Figure 3. Colony morphology of *Candida albicans* on chromogenic Candida agar

DISCUSSION

The incidence of invasive fungal infections has increased significantly in recent years. Among the yeast, *Candida albicans* is the most common pathogen. There are hundreds of Candida species that cause invasive candidiasis. The most common species causing infections include *C. albicans*, *Nakaseomyces glabrata* (formerly *C. glabrata*), *C. parapsilosis*, *C. tropicalis* and *Pichia kudriavzevii* (formerly *C. krusei*) (14). Apart from these factors, an important pathogen *Candidozyma auris* (formerly *C. auris*) emerges as a cause of invasive candidiasis worldwide and in certain regions of the United States (7, 8). Due to the increase in microorganisms resistant to antifungal treatments, identification of the causative organism at the genus and species level is important in order to provide appropriate and adequate treatment (9).

Candida albicans, which causes invasive candidiasis, is found as a commensal in areas such as the mouth, throat, intestine, vagina, and mucosal surfaces. However, in some people who are at high risk for *Candida albicans* infection. It causes opportunistic infections due to reasons such as human immunodeficiency virus infection, diabetes mellitus, immunosuppressed patients, use of steroids, broad-spectrum antibiotics or cytotoxic drugs (10). Healthcare workers can also carry Candida species through their hands. For this reason, several outbreaks of candidemia have been linked to the hands of healthcare workers.

The ability of *C. albicans* to infect such a variety of host niches is supported by a wide range of virulence factors. In-vivo polymorphism, expression of adhesins and invasins on the cell surface, biofilm formation, phenotypic change and secretion of hydrolases enzymes are important virulence factors. Additionally, their traits include rapid adaptation to fluctuations in environmental pH, metabolic flexibility, robust nutrient acquisition systems, and stress response mechanisms (11). The pathogenicity of *C. albicans* is related to virulence factors and the host's immune response to these factors (12). Hydrolytic enzymes secreted by *C. albicans* cause tissue destruction in the host tissue and lead to invasion of the fungus into the host tissue. It is reported that especially the enzymes aspartyl proteinase and phospholipase are significantly responsible for the virulence of *C. albicans*. Issue invasion by *C. albicans*, which has strong virulence enzymes, therefore poses a risk (2).

Identification of fungal pathogens to the species level is necessary due to differences in virulence and susceptibility of Candida species and increasing rates of antifungal resistance. The availability of

sensitive and specific diagnostic tests in the clinical microbiology laboratory diagnosis allows antifungal treatment to be started earlier (13). In invasive fungal infections, identifying the infective pathogen and performing antifungal susceptibility tests guide the clinician in applying effective antifungal therapy in the treatment.

ETHICS COMMITTEE APPROVAL

* Since it is a case report, ethics committee approval is not required; Written consent of the patient was obtained for the presentation of the case.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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