The first case represented in Turkey; onychomycosis caused by Chaetomium globosum in an immunocompetent patient

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ABSTRACT

It was reported as a case of distal subungual onychomycosis of the thumb of the right foot of a 25-year-old female patient in this article. Nail was examined in our laboratory and it was detected distal subungual onychomycosis. In direct microbiological examination septate hyphae was observed by using 20% KOH. Scraping of nail taken from patient was cultured on two Sabouraud’s Dextrose Agar with cycloheximide and without cycloheximide and it was kept at 25 °C for one week. After growth of fungal was detected slide cultures were prepared and brown-colored septated hyphae, perithecia, lemon-shaped ascospores were observed by light microscopy. The causative agent was identified as Chaetomium globosum. It was determined by using M38-A2 microdilution method, minimum inhibitory concentration values of, amphotericin B, fluconazole, itraconazole, miconazole, ketoconazole, flucytosine voriconazole were determined as 4->64, 1-0, 125-0,5 μg/mL, respectively. Fluorocytosine and fluconazole were determined as resistant for Chaetomium globosum while miconazole and ketoconazole MIC values was determined as the best effective antifungal. The patient was treated with daily (250 mg/gün) oral itraconazole and amorolfin %5 tırnak cilası and 12 haftada iyileşme kayıt edildi.

Anahtar Kelimeler: chaetomium, onikomikoz,
Onychomycosis is a chronic fungal infections of toenail and fingernail. The most causative agents are observed as dermatophyte, yeast and nondermatophyte species. Several types of nondermatophyte mould such as *Aspergillus* spp., *Fusarium* spp., *Chaetomium* spp. may cause these infection (1, 2). The genus *Chaetomium*, which belongs to (family *Chaetomiaceae*, class *Sordariomycetes*, phylum *Ascomycota*), is dematiaceous nondermatophyte fungus that are commonly found in deteriorating wood products, soil and cellulosic substrates (3). Up to date, different authors reported variable taxonomic data about *Chaetomium* (4-6). *Chaetomium globosum* is the most observed species and these species produced the toxic chaetoglobosins A and C (7). The genus *Chaetomium* is the possible causative agents of fungal infections and selection of effective antifungal therapy is important for infected patients. Antifungal susceptibility test and minimal inhibitory concentration (MIC) values of these species have not been created yet. They reproduce with ascospores instead of conidia and therefore inoculum solution prepared by ascospores is more concentrated than the one prepared by conidia. Thus few modifications were made from reference microdilution method (8, 9).

**CASE REPORT**

A 25-year-old female presented with brownish-yellow discoloration on the right toenails. She was working in the textile company. Patient's history revealed that clinical symptoms began two years ago. Nail examination was examined in our laboratory. Distal subungual onychomycosis was detected (Figure 1). Complete blood count, protein electrophoresis, metabolic test (glucose, cholesterol, triglycerides) urinalysis, hepatic and renal function tests were within normal limits for patients and systemic diseases were not found. She had been not applied antifungal treatment for onychomycosis previously.

**Mycological Examination**

In direct microbiological examination, septate hyphae was observed by using %20 KOH. Scraping of nail was cultured on two Sabouraud’s Dextrose Agar (SDA) with cycloheximide and without cycloheximide slants at 25 °C for one week. Growth of colony was observed in without cycloheximide slants. Initially, colour of colony appeared as velvety white but turned to dark gray and then brown (Figure 2). After growth of colony, slide cultures were prepared and stained with lactophenol cotton blue and brown-colored septated hyphae, lemon-shape shaped ascospores were observed by light microscopy (Figure 3). Perithecia appears as dark-brown to black, globose to ovoid (egg-shaped) opaque structures covered with thick hair-like hyphal filaments (Figure 4).
Figure 1. Distal subungal onycomycosis and brownish-yellow discoloration on the right toenails.

Figure 2. Dark gray to brown colony on Sabourauds Dextrose Agar.
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Figure 3. Brown-colored septated hyphae and, lemon shaped ascospores.

Figure 4. Dark brown to black, flask-shaped perithecia.
**DNA Extraction, PCR Amplification, and Analysis of the ITS Region**

Fungal DNA is amplified by using universal fungal primers (ITS1 and ITS4) by PCR described elsewhere (10). Positive PCR band is purified and sequenced bidirectionally. Obtained DNA sequences are edited, aligned and a consensus sequence is constructed. BLAST search is performed with obtained consensus sequence and maximum similarity is founded with *Chaetomium globosum*.

**Antifungal Susceptibility Testing**

Reference broth microdilution method performed according to M38-A2 document (11). Pure antifungal powders of known potency were supplied by the respective manufacturing companies (fluconazole, voriconazole (Pfizer, Istanbul, Turkey); amphotericin B, miconazole, flucytosine, ketoconazole, itraconazole (Sigma-Aldrich, St. Louis, Missouri, USA)). RPMI 1640 Medium (Sigma) with 0.2% glucose L-glutamine and without bicarbonate was employed. The inoculum was prepared by overlaying mature slants with sterile distilled water and gently scraping the surface with a wooden applicator stick. The suspension was permitted to sit for five minutes to allow large particles to settle out. Inocula of *C. globosum* were prepared with a hemocytometer. The minimum inhibitory concentrations (MICs) were read at 72 h. The final inocula were adjusted as $0.34 \times 10^4$ to $6.5 \times 10^4$ spores/mL in the microtiter plates. MIC values were determined for amphotericin B (4 μg/mL), fluconazole (≥64 μg/mL), itraconazole (1 μg/mL), miconazole (0.125 μg/L), ketoconazole (0.125 μg/mL), flucytosine (≥64 μg/mL), voriconazole (0.5 μg/mL). The most effective antifungal agents were found as miconazole (0.125 μg/mL) and ketoconazole (0.125 μg/mL). The patient was treated by using oral itraconazole (250 mg /a day) and local application of amorolfine 5% nail lacquer was used and it was seen to heal in 12 weeks.

**DISCUSSION**

In this report we discussed mycological examination and antifungal treatment of onycomycosis stemming from *C. globosum*. These species produce chaetoglobosins A and C and mycotoxin exposure may be associated with cutaneous, subcutaneous, and opportunistic fungal infection (7). Until today, author reported a small number of onycomycosis caused by *C. globosum* in adult patients (12-16). Among these cases only one mixed toenail infection caused by *C. Globosum* and *Trichophyton mentagrophytes* was reported (17). In all cases, male patients were found to be more affected than female patients. In this case, onycomycosis occurred in a female patient's toenail.

Clinical presentation of onychomycosis resulting from *C. globosum* is often nonspecific. Since this fungi is believed as common laboratory contaminants, it is difficult to recognize between the pathogen and the contaminant. If their characteristic images are seen in microscopic examination and the same strain is identified on repeated cultures, they can be considered as a pathogen of onychomycosis. In addition to phenotypic identification, DNA sequence analysis is useful in corroborating the diagnosis in difficult cases of onychomycosis (13). In our case, more or less flask-shaped, mostly ostiolate (perithecia) fruit body, ascospores and hyphae were seen in microscopic examination and fungal growth was observed in three different cultures. Mycological examination and DNA sequence analysis were used for *C. globosum* identification. For molecular biologic analysis, it was compared to the base sequence of *C. globosum* strain KM579606, which was stored in GenBank, using the Blast program and the result was 100% matched (Figure 5).
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ITS1-4

ATTGTGACGTTACCTATACCATTGCTTCCGGCAGCGCCGCCCGGGTTTACCCTCCCGGCGCCTGCGGCCCGGCAGGCTACCAAACTTGGATAATTATGCGCTCTCTGAATACGATAAAGTCACAACACTTCAACACCGAATCTCTGGTTCTGAGATCGATGAAGAAACGCAGCAAATGCCTAAGTAAATGTGATATGCGAACTCAGCTCACTCGAATCTTGAACGCACATTTGCGCCCGCAGCATCCTGGCCGGCTGCTGCTGCTGAGCTGACGCAACGCCAGGGCGGCTTGTGTTGGGAACCTGCCCGCTGCGAGCCGGCTGACGAGGACAGCTGCTACATCTCGCTTGGTGCAGCGCGCCGGGTTCCCGCGCTTAAACCACCTTTT

Figure 5. Sequences producing significant alignments.

Onycomycosis is classified as different clinical types for etiological agent and treatment (18). In this case, clinical form was detected as distal subungual onycomycosis. We observed brownish discoloration without periungual inflammation in dermatological examination.

Eradication of onycomycosis caused by non dermatophyte mold is difficult and time consuming. Because these molds don’t respond well to antifungal treatment and antifungal susceptibility of these species is not well established. Our information about the antifungal susceptibility of Chaetomium is limited. Serena et al., (9) investigated antifungal susceptibilities of Chaetomium and they reported that in vitro activities of ravuconazole, voriconazole, albaconazole were obtained as good, but micafungin was not. Guarro et al., (19) tested the activities of six antifungal agents against clinical and environmental strains of Chaetomium spp. fluocytosine and fluconazole were determined as resistant for all strains and the best effective antifungal was determined as itraconazole. Similarly, in our study MIC values of fluocytosine and fluconazole were found as resistant. The best fungal activities were determined as miconazole (0.125 μg/mL) and ketoconazole (0.125 μg/mL).

In literature, successful treatment was reported by using itraconazole and terbinafine (13,16). The Food and Drug administration (FDA) approve treatment regimen for toenails is itraconazole 200 mg per day for 3 month (20). Itraconazole MIC value was determined as (1 μg/mL) in our antifungal susceptibility study. Clinician administered oral itraconazole (250 mg /a day) and local application of amorolfine 5% nail lacquer. As a result, the patient was completely cured as clinically and mycologically.

In conclusion, we report the onychomycosis caused by C. globosum in an immunocompetent patient which was confirmed by mycological examination and molecular analysis. Antifungal susceptibility was performed and the most effective agent was determined as ketoconazole and miconazole, but clinical recovery was provided by using itraconazole.
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