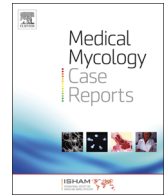




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Subcutaneous mycoses caused by *Rhytidhysterion* species in an immunocompetent patient

K. Mishra^a, S. Das^b, S. Goyal^a, C. Gupta^b, G. Rai^b, M.A. Ansari^b, R. Saha^b, A. Singal^c^a Department of Pathology, University College of Medical Sciences, Delhi 110095, India^b Department of Microbiology, University College of Medical Sciences, Delhi 110095, India^c Department of Dermatology, University College of Medical Sciences, Delhi 110095, India

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ABSTRACT

Subcutaneous mycoses are chronic fungal infections of the skin and subcutaneous tissues caused by variety of fungal agents and usually occur following trauma with vegetative matter. We report a case of subcutaneous mycoses caused by rare fungus belonging to the genus *Rhytidhysterion*, in an immunocompetent male who presented with a subcutaneous nodule on left foot. This unusual species was identified and confirmed by molecular methods.

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1. Introduction

Subcutaneous mycoses include a heterogeneous group of fungal infections that develop at the site of transcutaneous trauma and can occur in both immunocompetent and immunocompromised patients. Infection evolves slowly as the etiological agent survives and adapts to the adverse host tissue environment. The main subcutaneous fungal infections include sporotrichosis, chromoblastomycosis, mycetoma, lobomycosis, rhinosporidiosis, subcutaneous zygomycosis, and subcutaneous phaeohyphomycosis [1]. The common fungal etiologic agents like *Sporothrix schenckii*, *Cladophialophora carrionii*, *Fonsecaea pedrosoi*, *Phialophora verrucosa*, *Rhinochrysiella aquaspersa*, *Exophiala jeanselmei*, *Exophiala spinifera*, *Wangiella dermatitidis*, *Acremonium* spp., *Conidiobolus coronatus* and *Basidiobolus ranarum* have been associated with subcutaneous mycotic infections [2–5]. The subcutaneous mycoses caused by rare fungi such as *Colletotrichum* species, *Diaporthe*, *Fusarium subglutinans*, *Chaetomium funicola* etc. have been described in the literature so far [6–9]. We report a case of subcutaneous mycoses in an immunocompetent male caused by a dematiaceous fungus belonging to genus *Rhytidhysterion*.

2. Case

A 65-year old, hawker by profession, resident of Delhi, presented with a painless subcutaneous nodule on tendoachilles

region in the left foot with duration of 10 years. The nodule was about $4 \times 4 \text{ cm}^2$ in size, well circumscribed, indurated, nontender, and the overlying skin was hard and blackish in color (Fig. 1). There was no discharging sinus, grains or any ulceration. Patient was healthy otherwise, with no evidence of immunosuppression or diabetes. There was no history of travel or treatment. However, history of trauma could not be excluded owing to bare feet walking in his profession. All investigations were done on the day 0 when patient first presented to the hospital. The routine laboratory investigations which included complete blood counts, random blood glucose, urine analysis, liver and renal function test and X-ray chest were normal. Patient was seronegative for HIV.

Fine needle aspiration cytology (FNAC) was done from the nodule and aspirate was sent for analysis. Direct microscopic examination of aspirate after digestion with 10 percent potassium hydroxide (KOH) demonstrated thick brown hyphae. No spherical or sclerotic bodies were seen, ruling out the diagnosis of chromoblastomycosis. FNAC smears revealed septate and branching pigmented fungal hyphae which were PAS positive and surrounded by intense neutrophilic reaction (Fig. 2). Acid fast stains for aspirate was negative. Fungal cultures were performed on Sabouraud's Dextrose Agar with and without cycloheximide and incubated at 25 °C. After 2 weeks grayish black, floccose colonies with black reverse were observed on culture tubes (Fig. 3). Standard Tease Mounts using Lacto Phenol Cotton Blue and slide cultures using Potato Dextrose Agar (PDA) and Malt Extract Agar

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Fig. 1. Swelling measuring $4 \times 4 \text{ cm}^2$ on tendoachilles in left foot in a 65-year-old male.

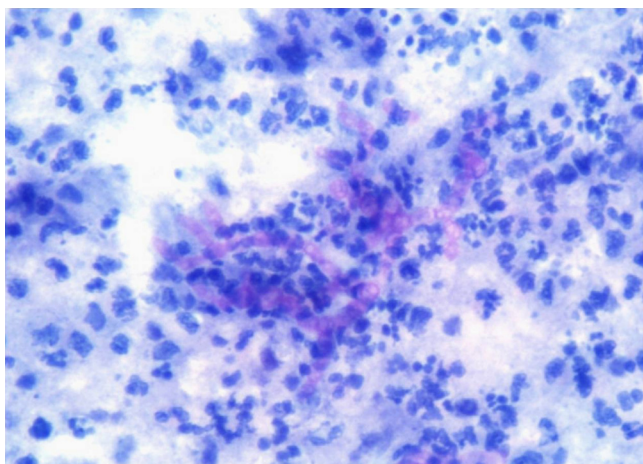


Fig. 2. Fine needle aspirate from the foot swelling shows branching septate hyphae, which are Periodic Acid Schiff (PAS) positive and surrounded by intense neutrophilic reaction ($400 \times$).



Fig. 3. Culture shows fluffy, black colonies Sabouraud's dextrose Agar at 25°C .

(MEA) were prepared to observe the microscopic morphology and sporulation respectively. Microscopically, it showed thick, brown septate hyphae but the isolate was non-sporulating (Fig. 4).

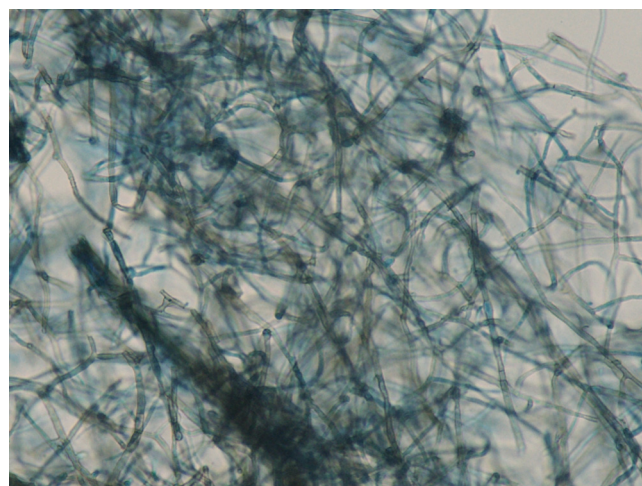


Fig. 4. Lacto phenol Cotton Blue mount shows branching hyphae having black cell walls with tiny conidia sticking to them ($400 \times$).

Based on the macroscopic and microscopic observations, the isolate could not be identified, hence, was subjected to molecular methods for identification. The culture grown on SDA slant was subjected to PCR amplification and sequencing using the panfungal primers ITS1 (5' TCCGTAGGTGAACCTGCCG 3') and ITS4 (3' TCCTCCGCTTATTGATATGC 5') to amplify the internal transcribed spacer (ITS) region of 18S rRNA in an outsourced laboratory (CromDx Solutions, India). A homology search of all the sequences was carried out using BLAST (National Center for Biotechnology Information, Washington, DC). The isolate was identified as *Rhytidhysterion* species, with the closest link being *Rhytidhysterion rufulum* with 97% identity to the GenBank accession number AM711974.1. However, due to considerable uncertainty of species classification in the genus and lack of sufficient molecular data, species classification cannot be achieved unequivocally. The GenBank accession number of the small subunit sequence is KM052345. The isolate was deposited to the National Reference and Research Laboratory, Mycology division; Post Graduate Institute of Medical Education Research (PGIMER), Chandigarh, India for further confirmation.

The patient was treated with oral terbinafine (250 mg/day) and itraconazole (100 mg/day) and the swelling partially regressed after 3 months. He has been advised to continue treatment for 1 year and is on regular follow-up.

3. Discussion

Rhytidhysterion genus was described initially in the poorly known family of Ascomycetes, Patellariaceae [10]. It is characterized by hysteriform ascomata that become discoidal at maturity and paraphyses covered by a gelatinous layer (pseudoeipithecium). The ascospores are thick walled and deeply pigmented. The genus comprised two species: *R. rufulum* and *R. hysterinum*. Recently on the basis of amino acid and sequence data provided by Boehm et al. in 2009, the *Rhytidhysterion* genus, previously placed in the Patellariaceae, has been transferred to the Hysteriaceae [11].

Rhytidhysterion spp. are common in the tropics and subtropics where it grows as a saprobe or weak parasite on wood of a variety of dicotyledonous plants. These are rare human pathogens. Very few reports of mycoses caused by *Rhytidhysterion* sp. have been described in literature [12,13]. In our report, the patient was immunocompetent and we hypothesize that walking bare footed might have led to a penetrating injury from plants resulting in

entry of this fungus as it is a saprobe with a diverse geographical distribution in tropical countries. This suggests that *Rhytidhysterion* sp. is not an opportunistic pathogens and can cause infection in both immunocompetent and immunocompromised patients with different clinical presentations. As described by Chowdhary et al. [12], our isolate also failed to sporulate on laboratory media and was identified by ITS sequencing, highlighting the necessity of molecular techniques for identification of non-sporulating fungus. As the previous cases of human infections caused by this fungus have been reported only from India, presence of this fungus in Indian environment is speculated. The fungus in the genus *Rhytidhysterion* are not known pathogens in humans and currently no specific management protocols exist, so the patient was treated with antifungal combination of itraconazole and terbinafine considered appropriate for management of subcutaneous mycoses caused by dematiaceous fungus. Since experience with this fungus has been limited so far, further case reports and studies may be helpful in establishing the epidemiology, morphological features and management protocol for this group of mycoses.

In conclusion, subcutaneous mycoses can be caused by both common and rare fungal species. Apart from routine fine needle aspirate cytology and fungal cultures, molecular methods for identification of non-sporulating fungus should be included in the diagnostic workup of such cases. Further characterization of an unusual fungal species and its antifungal susceptibility profile should be performed to guide the clinicians so that they can optimize the accurate treatment.

Conflict of interest

None.

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None.

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