**Case Report**

**Demodex** mite infestation of unknown significance in a patient with rhinocerebral mucormycosis due to *Apophysomyces elegans* species complex

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**Introduction**

Two members of the mite family, *Demodex folliculorum* and *Demodex brevis*, inhabit the pilosebaceous unit. These mites were previously thought to be harmless commensals occurring permanently on the human skin. However, results from recent studies claimed that the mites may be associated with pityriasis folliculorum, rosacea, perioral dermatitis, seborrhoic dermatitis, pustular eruption, blepharitis, seborrhoic alopecia and other skin lesions (Zhao et al., 2011). Data also exist on severe infestation and increased severity of demodicosis in immunosuppressed individuals (Seyhan et al., 2004). Herein, we report a case of a patient with rhinocerebral mucormycosis who had severe *Demodex* infestation of unknown significance.

**Case report**

A 45-year-old man, with a recurrent giant cell tumour of the left radius with lung metastasis, had received six courses of chemotherapy with the ifosfamide, carboplatin and etoposide regimen and palliative external radiotherapy (20 Gy for 5 fractions) to the lung mass. He received oral prednisolone (40 mg daily) for the past 8 months for breathlessness, and irregular treatment with insulin for 5 years on account of diabetes mellitus. He presented with progressive swelling and redness of the face, and had difficulty in breathing for 10 days. Swelling and redness were associated with itching. Dyspnoea was present throughout the day and was not associated with any positional variation. Upon general physical examination the patient was drowsy and had laboured breathing, bilateral eyelid swelling and periorbital oedema. He also had erythema, erosions and woody, hard induration of the cheeks and upper lip. A large palatal ulcer (1 × 2 cm) with a necrotic base in the anterior two-thirds of the palate and a small ulcer with a necrotic base on the nasal septum were observed. The patient had reduced air entry in the left lung fields. The rest of the physical examination was within normal limits. A provisional diagnosis of rhino-orbito-cerebral mucormycosis, angio-oedema, Cushing's syndrome and superior vena caval syndrome was made. Laboratory investigations revealed leukocytosis (count 29,900 µl⁻¹), raised levels of aspartate aminotransferase and alanine aminotransferase (244.2 U l⁻¹ and 942.2 U l⁻¹, respectively), hyperglycaemia (340 mg dl⁻¹), hypokalaemia (2.8 mEq l⁻¹), hypocalcaemia (7.29 mg dl⁻¹), hypochloro-aeemia (88.6 mEq l⁻¹) and hypoproteinaeima (4.68 g dl⁻¹). Other biochemical and haematological parameters were within normal limits. A potassium hydroxide wet mount (10 %) examination of the nasal scrapings from the base of the necrotic ulcer revealed the presence of 4–6 mites per low-power field (Fig. 1a) and broad aseptate hyaline hyphae (Fig. 1b). The mites were 0.3–0.4 mm long, with four legs and a long striated posterior segment. The detailed examination of the size and morphology of the mites identified them as *D. folliculorum*. Culture of nasal scrapings on Sabouraud dextrose agar (SDA) medium yielded white cottony, mycelial colonies after 4 days of incubation at 37 °C. As there was no sporulation on the SDA, water agar culture was carried out to induce sporulation. Microscopic examination of the scotch tape preparation of the water agar culture after 10 days of incubation at 30 °C showed pauciseptate hyphae, unbranched sporangiophores, pyriform sporangia with funnel-shaped apophyses. Sporangiospores were elongated and smooth walled. Based on these features, the isolate was identified as *Apophysomyces elegans* complex. The isolate was later subjected to molecular identification by amplifying the
Demodex folliculorum showing (a) characteristic features of Demodex folliculorum (magnification ×10) and (b) broad aseptate hyphae (magnification ×40).

DNA segments of the 18 S (partial), internal transcribed spacer (ITS), 5.8 S, ITS2 and 28 S (partial) ribosomal regions using primer pairs ITS1 (5’-GCATATCAATA-AGCGGAGGAAAAG-3’) and ITS4 (5’-GTTCCGTGTTC-AGACG-3’). Nucleotide sequences of the same region were analysed using the Big Dye Terminator Cycle Sequencing kit, version 3.1 (Applied Biosystems) for both strands. All the sequencing reactions were purified and analysed on an ABI 3130 Genetic Analyzer (Applied Biosystems). Consensus sequence was prepared from forward and reverse sequences using Bionumerics software version 6.6 (Applied Maths). A comparison of our sequence with those in the GenBank DNA database gave 98.6% identity with the ex-type strain of Apophysomyces variabilis UTHSC 06 (CBS 658.93). The nucleotide sequence data were submitted to the National Center for Biotechnology Information GenBank, accession number KC469686. The isolate is deposited at the National Culture Collection for Pathogenic Fungi (NCCPF), Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, accession number NCCPF-102057.

Amphotericin B deoxycholate (50 mg intravenously day⁻¹) and turpentine nasal wash three times a day were initiated to manage the patient’s condition. Surgical debridement was planned, but the patient refused further treatment after 2 days of antifungal therapy and left against medical advice. A follow-up did not occur.

Discussion

Rhinocerebral mucormycosis was suspected in this individual, as he was poorly compliant to diabetic therapy and had necrotic ulcers on the palate and nose. The diagnosis was confirmed by the presence of broad hyaline aseptate hyphae on microscopy and the isolation of the A. elegans species complex from the nasal scraping sample. Invasive mucormycosis is commonly caused by species of Rhizopus, Lichtheimia and Rhizomucor, and less commonly by species of the genera Mucor, Apophysomyces, Saksenaea, Cunninghamalla, Cokeromyces and Syncephalastrum (Chakrabarti et al., 2010). However, recent studies have shown that the genus Apophysomyces is a species complex comprising more than one species (Alvarez et al., 2010). Apophysomyces is an emerging pathogen in tropical countries such as India, as it is increasingly isolated from cases of mucormycosis (Chakrabarti & Singh, 2011). In India the A. elegans complex is the second most frequently isolated agent from mucormycosis patients (Diwakar et al., 2007). It commonly causes cutaneous and subcutaneous mucormycosis in immunocompetent hosts. Local wound contamination with soil or plant detritus after an accident is the single most common risk factor. Though it is not known how this patient became infected with this agent that caused rhinocerebral mucormycosis, inhalation of spores from contaminated air is expected. The patient was immunosuppressed, as he had undergone radiotherapy, was on long-term steroids and was poorly compliant to anti-diabetic therapy. Uncontrolled diabetes mellitus is the most predominant risk factor for mucormycosis in India and overshadows the other risk factors (Chakrabarti et al., 2010).

A high density of Demodex mites was found in the nasal scraping of the base of a necrotic ulcer in this patient. The significance of the mite infestation could not be evaluated as the patient left the hospital against medical advice 2 days after the initiation of therapy. Demodex mites belong to the family Demodicidae, of which two closely related species, Demodex folliculorum and Demodex brevis, infest man.

A new term demodicosis has been coined to denote patients with facial dermatosis who have a Demodex density of five mites cm⁻² from one surface biopsy or ten mites cm⁻² from two successive surface biopsies at the same site (Hay, 2010). Severe demodicosis has been reported in a number of patients with AIDS, haematological malignancies and allogenic bone marrow transplants, and those undergoing long-term corticosteroid, pimelolinus and erlotinib therapy. The etiopathogenesis of severe infestation has been attributed to suppression of cell-mediated immunity and secondary lymphocyte depletion, leading to an increased number of parasites in the skin (Seyhan et al., 2004). Our patient had erythema, erosions and induration on the face with pruritus. Though we did not attempt to detect the mites on the face, the mites were detected in the nasal ulcer only. The necrotic tissue at the site of infestation and immunosuppressed condition of the host might have allowed unhindered proliferation of these mites and the subsequent skin manifestation.

The treatment guideline for demodicosis in humans is not available. Several clinicians used systemic antiparasitic agents like ivermectin and metronidazole (Holzhuch et al., 2011; Román-Curto et al., 2012). The topical preparations like ointment permethrin, metronidazole, pilocarpine gel, tea tree oil, mercury oil and crotamiton have also been used (Bikowski & Del Rosso, 2009; Gao et al., 2005). In the present case study a turpentine wash was initiated as the mites were initially confused with maggots.

The assumption that Demodex mites were the cause of facial rash in this patient could not be confirmed as the patient left hospital against medical advice. To the best of
our knowledge, this is the first case of possible demodicosis associated with invasive mucormycosis. This case reflects the need for greater awareness about demodicosis amongst clinicians and laboratory physicians managing immuno-suppressed patients. Future studies are also needed to examine the possible role of diabetes mellitus in the predisposition of demodicosis.

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References


