Case report

Phaeohyphomycosis caused by *Exophiala spinifera* in India

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The second case of phaeohyphomycosis due to *Exophiala spinifera* in India has been diagnosed 46 years after the initial case. The present case involved a 12-year-old female patient with no known immunocompromising conditions. She presented with multiple verrucous, well-defined plaques encompassing phaeohyphomycotic lesions of varying sizes on her face, chest, arms and thighs. Lymph node involvement in dissemination was confirmed by demonstrating pigmented fungal elements in histopathology of the left axillary node. The infection responded positively to prolonged administration of itraconazole. The original case involved a young boy and was similarly disseminated but was more severe, with bone involvement, and had a fatal outcome. It is likely that other such cases have occurred in the intervening time but have not been reported.

**Keywords** Exophiala spinifera, India, Phaeohyphomycosis, Uttar Pradesh

Introduction

It was in 1954 that the first known human case of mycosis due to *Exophiala spinifera* was observed. The patient was a 7-year-old boy from a village near Amritsar, Punjab, India and the case was reported as chromoblastomycosis due to *Hormodendrum dermatidis* (Kano) Conant [1]. When later studies showed that the fungus produced annellides on spine-like conidiophores, the organism was reclassified as *E. spinifera* [2,3]. Recently, after a gap of 46 years, we encountered another human case of *E. spinifera* infection in India. We report this case not just to record a second Indian occurrence, deriving from an area of the country where no such case has been seen previously, but also to highlight the salient features of the current case in comparison with those of the previous case.

Case report

In July 2000, a 12-year-old female student from a village near Allahabad, Uttar Pradesh, presented with multiple verrucous, well-defined plaques encompassing lesions of varying sizes on her face (Fig. 1), mainly on the left side, as well as her chest, arms and thighs. The lesions were of about 1.5 years’ duration. History revealed that the infection first manifested as mildly itchy, small, erythematous papular lesion on the forehead near the left eyebrow. There was no history of apparent trauma. Gradually, the lesion increased in size and, after 2–3 months, similar lesions developed on the left cheek and on the side of the nose; these lesions also gradually increased in size. Over the next 3 months, new lesions continued to develop on the upper chest, arms and thighs. The last lesion to develop was on the right cheek. Initially, the case was diagnosed as lupus vulgaris and was treated for 7–8 months with antitubercular therapy; no relief was obtained. Subse-

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We dedicate this paper to the late K. C. Kandhari, who is popularly known as the father of Indian dermatology.
Fig. 1  Phaeohyphomycosis due to Exophiala spinifera on the face of a 12-year-old female patient showing multiple verrucous lesions.

Fig. 2  Primary isolation of Exophiala spinifera from a piece of the excised lesion from a 12-year-old female patient with phaeohyphomycosis on SGA at 27°C incubated for 22 days.

sequently, the patient was treated presumptively for sarcoidosis with daily prednisolone, 15–20 mg for 4 weeks, but again with no improvement.

At the time of presentation to us, the lesional areas contained erythematous plaques and papules of varying sizes (0.5–12 cm diameter) with well-defined irregular margins. While the larger plaques showed signs of healing and some scarring, with verrucosity at the active border, the smaller plaques had smooth surfaces. There was no history of fever, cough with expectoration, loss of appetite or loss of weight. Systemic examination revealed no abnormality except non-tender, small, discrete lymph nodes in the left submandibular, axillary and bilateral inguinal areas.

**Laboratory investigations**

Complete blood count, urinalysis and blood urea were in the normal range. X-rays of the chest and the affected portions showed no internal involvement. A portion of the lesional material was excised and was processed for direct microscopy and histopathology, as well as mycobacterial and fungal culture. Ziehl–Neelsen smears and culture in Lowenstein–Jensen medium were negative for mycobacteria. Direct microscopy in 10% KOH, however, revealed globose to subglobose, brown-coloured fungal elements occurring either singly or in small groups.

For the isolation of any fungal pathogen, Sabouraud glucose agar (SGA) medium containing chloramphenicol (0.05 mg/ml) was used. Plates inoculated with the materials were incubated at 27 and 37°C. Within 7–10 days, moist, shiny, chocolate-brown to blackish, yeast-like colonies appeared on plates incubated at both temperatures. Gradually, the colonies developed mycelium (Fig. 2).

Histopathological sections stained with haematoxylin and eosin revealed hyperkeratotic and acanthotic epidermis. In the dermis, a granulomatous reaction was observed consisting of histiocytes, lymphocytes, few plasma cells, neutrophils, eosinophils and giant cells. Several unicellular, hyaline to pale brown yeast cells mostly 4–8 µm in diameter were seen within and outside histiocytes and giant cells. Some of them showed budding and the occasional formation of pseudohyphae was also seen clearly in sections stained with periodic acid-Schiff stain (Fig. 6). Biopsy of the lymph node from the left axilla showed an epithelioid cell granuloma with giant cells. Several giant cells showed the presence of pigmented fungal elements. Fine-needle aspiration cytology from the right inguinal lymph node showed features of reactive lymphadenitis.

**Description of the fungus**

Colonies on SGA at 27°C attaining a diameter of 2 cm in 20 days, initially moist, shiny and black, growing gradually with a smooth margin and slowly developing a short nap of mycelium over the surface, ultimately becoming pulvinate to velvety and olivaceous grey to black with a grey to black reverse. At 37°C growth was slower than that at 27°C; colonies attained a diameter of 1.5 cm in 20 days. Colonies were initially yeast-like
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but becoming mycelial and folded, cushion-like and centrally raised, greyish black to dark brown with shiny and moist margins. In microscopy, the moist colonies consisted predominantly of yeast cells, but accompanying hyphae were hyaline to lightly brown, septate and branched. Conidiophores arose from these hyphae as lateral branches, and were spine-like and generally darker than the vegetative hyphae. Conidiogenous cells were pale brown annellides, cylindrical to lageniform, with distinctive, snout-like, closely annellated apices (Fig. 3). The annelloconidia were hyaline to subhyaline, unicellular, subglobose to cylindrical, smooth, 1.6–2.4 × 3.2–4.8 μm. The fungus prominently produced a Phaeoannellomyces synanamorph (i.e. the yeast cells present could be seen to be producing daughter cells from annellidic apertures rather than via ordinary blastoconidial budding). These yeast cells were hyaline to pale brown, one celled, globose to ovoid, encapsulated and 1.6–3.2 × 3.2–6.4 μm in size (Figs. 4 and 5).

The organism was identified as Exophiala spinifera and a subculture of the same was preserved under No. Myco/NICD 204-2000 in the culture collection of the Medical Mycology Laboratory, National Institute of Communicable Diseases, Delhi, India. A dried voucher culture was deposited at the culture collection of the Center for Advanced Study in Botany, University of Madras, Guindy Campus, Madras, India, under the number UBL-452.

Fig. 3 A slide culture mount showing conidiophores bearing snout-like, closely annellated conidiogenous cell and conidia of Exophiala spinifera (× 2800).

Fig. 4 Exophiala spinifera producing annellidic yeast cells (× 2800).

Fig. 5 India ink mount of yeast cells of Exophiala spinifera showing encapsulation (× 2300).

Treatment

The patient was treated with itraconazole 100 mg twice daily orally and within 1.5 months there was significant flattening of all the lesions and almost complete clearance of the smaller papules. After 3 months, the lesions had almost completely cleared except minimal induration and mild post-inflammatory hyperpigmentation. The patient is still under follow-up.
Discussion

Although phaeohyphomycosis caused by E. spinifera is a disease of rare occurrence, the reported cases are from different geographical regions of the world, indicating that this species is widely distributed [4-10]. In addition to human infections, two cases in which cats were infected were reported by Kettlewell [11]. E. spinifera has also been reported as having been isolated from organic matter in nature by Mackinnon et al., from Uruguay, as early as 1973 [12].

It is interesting that a second case of E. spinifera phaeohyphomycosis in India has been observed only after a gap of 46 years. It is unlikely that infections due to E. spinifera did not occur in India in the intervening time. In all probability, a number of cases might have remained undiagnosed, or have been misdiagnosed, or have been diagnosed as chromoblastomycosis, because the concept of phaeohyphomycosis has been part of common medical knowledge only recently [13-16]. The recent finding by Barba-Gomez et al. [17] that ‘chromoblastomycosis and phaeohyphomycosis represent extremes of a continuum of infections’ may strengthen this interpretation of the historical record.

The initial E. spinifera case report by Rajam et al. was from Punjab state [1], while the present case is from another northern Indian state, Uttar Pradesh. In both cases, the victims were children, a 7-year-old boy in the first case and a 12-year-old girl in the present case. Both patients were from a rural background. Clinically, the two patients share some similarities, but in the earlier case the lesions were extensive and involved certain bones, indicating the potentially highly virulent nature of the pathogen in some patients. In the present case, major involvement was seen on the face, but though the infection then started to spread all over the body, no bone involvement was detected.

With regards to treatment, while the earlier Indian case did not respond to any kind of therapy available at the time and ultimately ended fatally, our case responded well to regular but prolonged itraconazole therapy. Probably our case also would have ended fatally if the diagnosis had not been made relatively early and appropriate therapy had not been implemented in time. Itraconazole has previously been shown to be effective against E. spinifera infection by Kotylo et al. [9] and Barba-Gomez et al. [17].

It is noteworthy that our isolate of E. spinifera produced in culture prominently a Phaeoannelomyces synanamorph consisting of annelidic yeast cells as demonstrated by Kettlewell et al. [11]. Encapsulation of the yeast cells could easily be demonstrated in India ink preparations in our isolate as was originally observed by Mackinnon et al. [12]. Another notable feature of our isolate was that it could grow at 40°C. However, despite growing the organism on cornmeal agar, we could not detect the Phialophora synanamorph reported by Nielsen and Conant [4] and Barba-Gomez et al. [17].

The identity of E. spinifera cannot be absolutely ascertained without sequencing [18], but this could not be done by us due to the limitations of our expertise in this specific area. Also, our attempts to have the organism sequenced overseas were unsuccessful due to difficulty in sending the cultures outside India, as the Government’s new regulations on transfer of biological materials, including microorganisms, out of India is highly restrictive.

References


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