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Phaeohyphomycosis Due to *Exophiala jeanselmei*: An Emerging Pathogen in India—Case Report and Review

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Abstract We present a rare case of a 30-year-old woman who presented with a swelling on the lateral aspect of her left forearm, present since 6 months, adjacent to a 16-year-old burn scar. X-ray of elbow joint and forearm revealed the subcutaneous nature of the swelling. Giemsa and periodic acid–Schiff-stained smears and potassium hydroxide mount of fine-needle aspirate of the swelling revealed dematiaceous, branching, and septate fungal hyphae. Fungal culture of the aspirated pus showed growth of *Exophiala jeanselmei*. Histopathological examination revealed brown-coloured hyphae with foreign body giant cell reaction and palisading granulomas in the surrounding tissue. The patient was successfully treated with surgical excision of the swelling. All the cases of phaeohyphomycosis due to *Exophiala* spp. in India are also reviewed.

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Introduction

Phaeohyphomycosis is a rare, distinct mycotic infection of the skin, or internal organs, caused by darkly pigmented, dematiaceous fungi, which are widely distributed in the environment. The term "phaeohyphomycosis" was first proposed by Ajello et al. [1] as, "infections caused by hyphomycetous fungi that develop in the host tissues in the form of dark-walled dematiaceous septate mycelial elements". McGinnis et al. [2] subsequently redefined the term to include infections caused by all agents appearing in tissues as dematiaceous yeast cells, pseudohyphae-like elements, septate hyphae, or combination of these.

Subcutaneous phaeohyphomycosis (phaeohyphomycotic cyst, previously known as phaeosporotrichosis) is an uncommon localised infection of the deep dermis and subcutaneous tissues caused by dematiaceous fungi [3]. In recent years, the incidence of phaeohyphomycosis as well as the diversity of causative organisms has been reported to be increasing. [4–6]. This is perhaps attributable to an increase in immunocompromised status owing to infections such as HIV, rising incidence of transplantation-associated immunosuppression, and increased incidence of diabetes.

Infection is thought to result from traumatic implantation of the causative fungal organism into the subcutaneous tissue. This form of infection is more common in warm climates and immunocompromised hosts [3]. It commonly presents as a single, well-encapsulated, subcutaneous mass or a nodule at the site of previous trauma, commonly on the extremities. The common causative organisms reported in the world include *Exophiala*, *Phialophora*, and *Cladophialophora* species. However, in India, *Exophiala* species is now an emerging pathogen and has a varied spectrum of disease presentation [7–22]. We present an unusual case of phaeohyphomycosis due to *Exophiala jeanselmei* adjacent to a burn scar in an immunocompetent patient.

Case Report

We present a case of a 30-year-old woman, resident of Delhi, a cook by profession, who presented with a single, well-defined swelling present on the lateral aspect of her left forearm since 6 months. The swelling was adjacent to a 16-year-old burn scar. There were no systemic or constitutional symptoms. There was no history of previous trauma, or use of topical or oral corticosteroids or other immunosuppressant drug intake. The patient was not a known diabetic. Her travel history was non-contributory.

Local examination revealed a soft, well-defined swelling of 3 × 3 cm (Fig. 1a). Skin over the swelling was pigmented, and chronic changes were present. The temperature over the swelling was normal. No discharge from the swelling was noted. It was non-tender.

Movement of the right elbow joint was not restricted, and there was no neurovascular deficit. The regional lymph nodes were not enlarged. Systemic examination was within normal limits. X-ray of the elbow joint revealed subcutaneous swelling, and extension to the bones was not seen. Fine-needle aspirate from the swelling yielded thick brown-coloured pus. It was sent for pyogenic, mycobacterial and fungal culture. The pyogenic and mycobacterial culture was sterile. On KOH mount, dematiaceous fungal hyphae with non-specific branching were seen. On Calcoflour white stain, septate fungal hyphae were seen. On Giemsa stain, branching septate hyphae were seen. Periodic acid–Schiff stain revealed PAS-positive fungal hyphae (Fig. 2).

Culture was inoculated on a duplicate set of Sabouraud's dextrose agar with and without antibiotics and was incubated at 25 and 37 °C, respectively. After 14th day of incubation, olive black colour colonies were seen, which were mucoid in the centre (Fig. 3). Lactophenol cotton blue mount of the colonies revealed brown septate hyphae with annelides giving rise to small, single-celled conidia arranged in groups (Fig. 4). The microslide culture on potato dextrose agar was done. The isolate morphologically resembled *Exophiala jeanselmei*. The isolate was confirmed as *E. jeanselmei* by ITS1, ITS2 and D1, D2 sequencing at NCCPF.

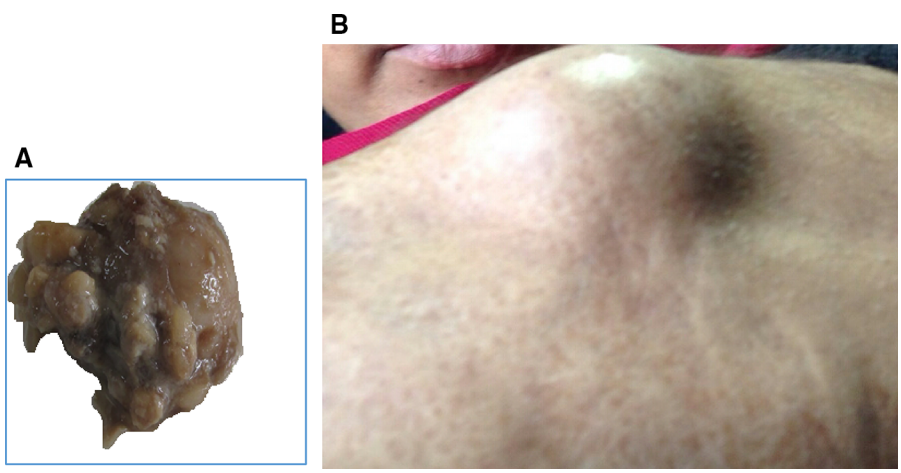

Fig. 1 **a** Gross appearance of excised phaeohyphomycotic cyst; **b** Subcutaneous cyst on right forearm near elbow with pigmentation

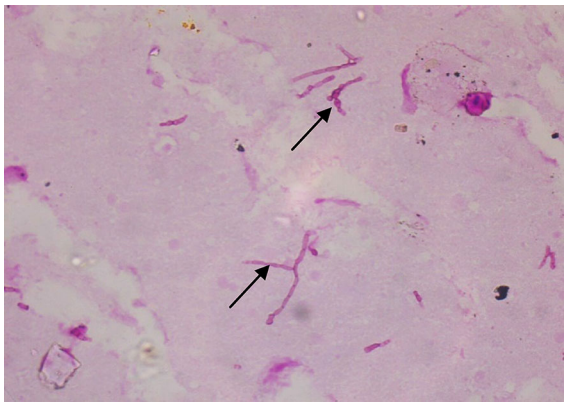

Fig. 2 Photomicrograph of the fine-needle aspirate showing PAS-positive fungal hyphae (*black arrows*) (PAS, ×200)

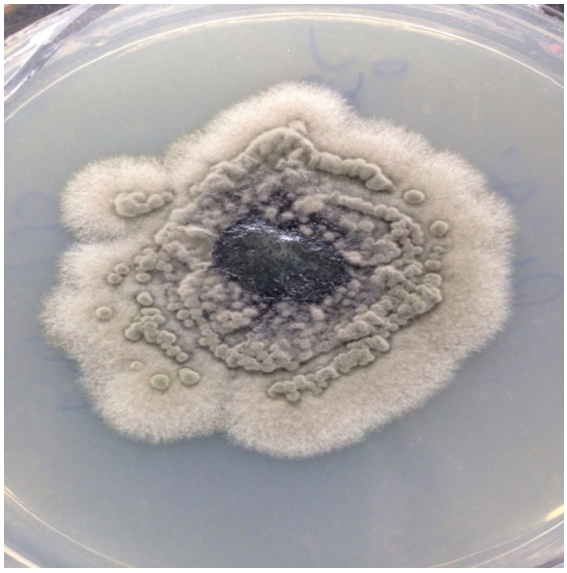

Fig. 3 On Sabouraud's dextrose agar, *olive black colour* colonies were seen, which were mucoid in centre. (Color figure online)

Following the cytological diagnosis, the swelling was completely excised and sent for histopathology. Grossly, it was well-encapsulated, grey brown cystic structure measuring 1.5 cm in diameter (Fig. 1b). On cut section, the cyst was filled with brown-coloured thick fluid. Histopathological examination revealed fibrocollagenous cyst wall, and the cavity showed necrotic material admixed with septate branching fungal hyphae eliciting tissue reaction comprising of palisading granulomas and foreign body giant cell reaction. The fungal hyphae were positive for PAS stain.

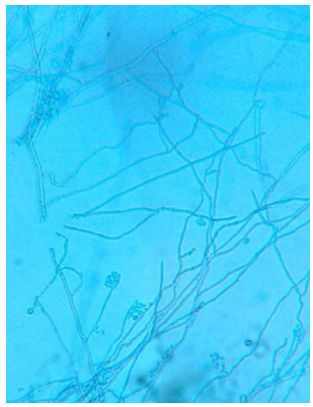

Fig. 4 Lactophenol cotton *blue* mount of the colonies revealed brown septate hyphae with annelides giving rise to small, single-celled conidia arranged in groups (×400). (Color figure online)

Further workup was done to find out the immune status of the patient. The patient was found to be non-diabetic, was not on any immunosuppressants, and HIV was ruled out. Total IgA, IgG, and IgE were within normal limits.

The excision was completely curative for the patient, and the patient did not need any additional antifungal course as the disease was localised and the patient was immunocompetent. After 7 months of follow-up, the patient was asymptomatic, and no recurrence was observed.

Discussion

Phaeohyphomycosis is an uncommon fungal infection, although its incidence has been reported to be on the rise globally including India [7–22]. To the best of our knowledge, only 16 cases of *Exophiala* spp. have been reported in India till date (Table 1). Most of these cases have been reported in immunocompromised patients. Also, in most cases of phaeohyphomycosis, there is a history of trauma in the past, which leads to inoculation of fungus in the subcutaneous tissues.

Table 1 shows the entire spectrum of *Exophiala* spp reported from India. Of the 16 reported cases of *Exophiala*, eight presented as cutaneous phaeohyphomycosis, one case of disseminated phaeohyphomycosis, one case of cerebral phaeohyphomycosis. Other varied presentations included onychomycosis, keratomycosis, endocarditis, and eumycetoma pedis. The

Table 1 Review of all *Exophiala* cases reported in India till date

S. no.	Study	No. of cases	Species	Clinical presentation	Diagnosis	Treatment	Outcome
1.	Rajam et al. [8], Punjab	1	*E. spinifera*	Chronic verrucous and crusted lesions on the face and skin of a 7-year-old boy (misdiagnosed as chromoblastomycosis)	Histopathology, KOH mount, and culture	–	Died
2.	Thammayya [9], Bengal	1	*E. jeanselmei*	65-year-old man with multiple nodules over right lower leg and foot with discharge with blackish granules (mycetoma pedis)	Histopathology, KOH mount, and culture	_	Cured
3.	Singh et al. [10], Gwalior	1	*E. jeanselmei varlecanii-cornii*	Cutaneous phaeohyphomycosis	Histopathology, KOH mount, and culture	Excision	Cured
4.	Rajendran et al. [11], Delhi	1	*E. spinifera*	Multiple, verrucous well-defined plaques over face, chest, arms, and thighs of a 12-year-old boy along with lymph node involvement	Histopathology, KOH mount, and culture	Itraconazole 100 mg BD	Cured
5.	Capoor et al. [12], Delhi	1	*E. jeanselmei*	8-year-old boy with swelling over dorsum of foot with purulent discharge and black granular deposits.(Eumycetoma pedis)	Histopathology, PAS stain,KOH mount, culture.	Excision, itraconazole	Cured
6.	Chander et al. [13], Delhi	1	*E. spinifera*	8-year-old boy with itchy, verrucous plaques all over body (Cutaneous Phaeohyphomycosis)	Histopathology, KOH mount, and culture	Itraconazole,Terbinafine	No clinical cure after 6 months of antifungals
7.	Gabhane et al. [14], Maharashtra	1	*E. jeanselmei*	30-year-old man with swelling left foot with a discharging sinus	FNACFungal culture		–
8.	Singal et al. [15], Delhi	1	*E. spinifera*	10–year-old immunocompetent boy with multiple verrucous disseminated phaeohyphomycotic lesions on legs	Histopathology, KOH mount, and culture	Itraconazole, Terbinafine, Fluconazole	Not cured
9.	Radhakrishnan et al. [16], Chennai	1	*E. spinifera*	20-year-old woman with non-healing skin ulcers with jaundice (phaeohyphomycosis)	Histopathology, KOH mount, and culture	Ketoconazole	Died due to hepatic failure
10.	Gill et al. [17], Rohtak	1	*Exophiala* (no subtyping done)	Corneal ulceration (keratomycosis)	KOH mount and culture	Natamycin eye drops	Cured
11.	Singh et al. [18], Chandigarh	1	*E. spinifera*On sequencing	26-year-old man with chronic, disfiguring facial lesions (phaeohyphomycosis)	Histopathology, KOH mount and culture	Itraconazole	Cured
12.	Sharma et al. [19], Shimla	1	*E. jeanselmei*	50-year-old man with black discolouration and hyperkeratosis of right great toe since 5 years (onychomycosis)	KOH mount and culture	Itraconazole	Cured

Table 1 continued

S. no.	Study	No. of cases	Species	Clinical presentation	Diagnosis	Treatment	Outcome
13.	Patel et al. [20], Gujarat	1	*E. dermatitidis*	Endocarditis on native cardiac valve in a post-renal transplant patient	Histopathology, KOH mount, and culture	Voriconazole	Cured
14.	Sood et al. [21], Rajasthan	1	*E. dermatitidis* on sequencing	21-year-old boy with weakness of right hand and slurring of speech for one and a half months (cerebral phaeohyphomycosis)	Histopathology, KOH mount, and culture	Amphotericin B, Voriconazole	Cured
15.	Karunakarreddy et al. [22], Karnataka	1	*E. dermatitidis*	52-year-old farmer with swelling over plantar aspect of foot	Histopathology, KOH mount, and culture	Surgical excision	Cured
16.	Venkateshwar et al. [23], Pondicherry	1	*E. oligosperma* on sequencing	Subcutaneous phaeohyphomycotic cyst in an immunocompetent host	Histopathology, KOH mount, and culture	Surgical excision	Cured
17.	Current case report	1	*E. jeanselmei* on sequencing	Subcutaneous phaeohyphomycotic cyst in an immunocompetent host	FNAC, Histopathology, KOH mount, culture, and sequencing	Surgical excision	Cured

E. Exophiala, *FNAC* fine-needle aspiration cytology

most common species isolated was *Exophiala spinifera* (six cases), followed by *E. jeanselmei* (five cases). There were three cases by *E. dermatitidis*. Other species reported was *E. oligosperma*. Amongst all the cases that were followed up, only two mortalities were reported, with rest of the patients having been cured completely. These cases were reported throughout India highlighting the isolations from varied geographical and climatic conditions [7–22]. In India, the isolations were in majority of cases from immunocompetent patients. Disseminated infection was observed in an immunocompromised patient post-renal transplant [19].

In this case, though the patient did not recall any inciting trauma, this may be attributed to the typically chronic disease course. The patient in this case was apparently immunocompetent. However, this form of phaeohyphomycosis is more common in warm climate, and immunocompromised patients are at an increased risk, but otherwise no particular group appears to be predisposed.

The usual clinical presentation in subcutaneous phaeohyphomycosis is the asymptomatic development of a single, well-encapsulated subcutaneous mass or nodule at the site of prior trauma [3].

The diagnosis in this case was confirmed by direct microscopy and culture of fine-needle aspirate. Slide culture on potato dextrose agar confirmed the aetiological agent at species level. The isolate was confirmed by DNA sequencing of the ITS region. In India, very few isolates of Exophiala spp. have been confirmed by molecular sequencing [11, 13, 14, 16].

Phaeohyphomycosis is caused by brown-pigmented fungi having melanin in their cell walls. Melanin acts as a virulence factor because of its scavenging effects over free radicals and hypochlorite produced by phagocytic cells, and moreover, it binds to hydrolytic enzymes [3]. This may be the explanation for infection in immunocompetent hosts.

Subcutaneous phaeohyphomycosis lesions are often surgically excised, allowing histopathological examination which may be needed to differentiate amongst clinically similar disease process. The differential diagnosis of subcutaneous phaeohyphomycosis includes fibromas, lipomas, ganglion cysts, chromoblastomycosis, mycetoma, and sporotrichosis [3].

In localised infection in immunocompetent patients, surgical excision of the entire lesion is usually curative and adjunctive antifungal therapy is often not necessary as was seen in this case.

In future, a large number of studies on black moulds are needed so that direct identification of fungal genera from tissue blocks using immunohistochemistry, in situ hybridisation, or DNA sequencing can be done for rapid diagnosis. In phaeohyphomycosis, the establishment of aetiological diagnosis by culture is very important as the aetiological agents are heterogenous group of emerging fungi, with no established CLSI break points for antifungals or serological tests or molecular assays available.

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