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A Rare Case Report of Subcutaneous Phaeohyphomycotic Cyst Caused by *Exophiala oligosperma* in an Immunocompetent Host with Literature Review

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Abstract We report a rare case of phaeohyphomycotic cyst in an immunocompetent patient caused by *Exophiala oligosperma*. This fungus is earlier known to cause infections in the immunocompromised. Identification of black fungi at species level is more challenging by conventional methods, and hence final identification of the fungi was based on sequencing of rDNA. The patient was managed with surgical excision. To the best of our knowledge, this is the first case report of *E. oligosperma* human infection from India.

Keywords Exophiala · Oligosperma · Immunocompetent · Phaeohyphomycosis · Fungus · Sequencing

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Introduction

Exophiala species are dematiaceous (dark pigmented) fungi acquired by man through accidental penetrating injury with material contaminated with the fungal elements. The species most commonly causing human infection are *Exophiala jeanselmei*, *Exophiala spinifera* and *Exophiala dermatitidis* and less frequently other species [1]. These fungi generally produce a heterogenous group of chronic infection known as phaeohyphomycosis which exhibit either yeast-like or hyphal forms on histopathology. These infections can be superficial, subcutaneous or systemic in location [1–3]. Superficial and subcutaneous infections often start as innocuous swellings which progress insidiously to a well-defined nodule. Infections caused by *Exophiala* species usually occur in the immunocompromised host. We report a phaeohyphomycotic cyst caused by a recently described fungus *Exophiala oligosperma* in an immunocompetent individual.

Case Report

A 38-year-old male, construction worker, presented with a single, non-tender, nodule on the dorsum of the right elbow (Fig. 1). The nodule was soft, diffuse, mobile, 5 × 5 cm in diameter. Initially, he noticed a small swelling, which progressively increased in size over the last 2 years. He did not recollect any history of trauma to the affected part. He was moderately built and nourished, and his general condition was good. He

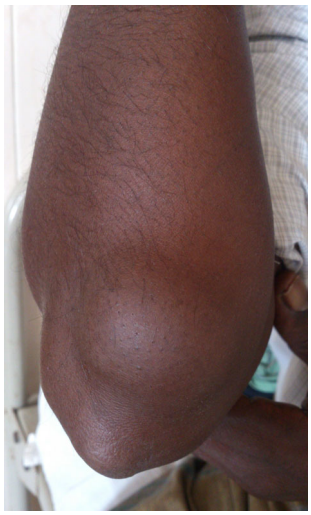

Fig. 1 Solitary, non-tender, nodule on the dorsum of the right elbow

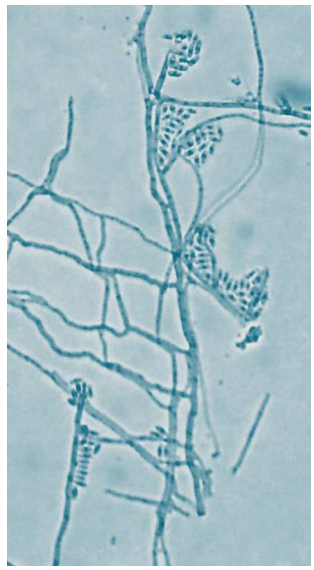

Fig. 2 Lactophenol cotton blue from slide culture revealed branching, septate hyphae, with subglobose annelloconidium arranged in clusters at the tip of the annellides (×400)

was afebrile. The patient was non-reactive for HIV antibodies. He was not known to have diabetes mellitus, nor was immunocompromised due to other causes.

Fine needle aspiration of the nodule yielded thick purulent material. Smears were stained with May-Grunwald Giemsa, Papanicolaou, periodic-acid Schiff (PAS) and Zeihl-Neelsen stains. The smears revealed an inflammatory exudate rich in polymorphs, lymphocytes and histiocytes and numerous branching septate fungal hyphae with a faint brownish pigmentation. The hyphae also stained positive with PAS stain. Stain for acid-fast bacillus was negative. A preliminary diagnosis of phaeohyphomycosis was made, and the aspirate was subjected to culture for final identification.

The purulent material was cultured for multiple pathogens, including fungi and bacteria. Gram stain and culture of the material for aerobic and anaerobic bacterial isolates were negative. Examination of the aspirate with KOH (10 %) showed septate, branching, phacoid hyphae. The specimen was inoculated onto Sabourauds dextrose agar (SDA) and incubated at 25 and 37 °C. After 1 week of incubation velvety, olivaceous green colonies appeared which turned black on prolonged incubation. A black pigment was noted on the reverse. Microscopic examination revealed phacoid branching, septate hyphae with subglobose annelloconidium arranged in clusters at the apex of the annellides (Fig. 2). The isolate failed to grow at 42 °C, and it was presumptively identified as *Exophiala* species. The isolate was submitted for further species identification to the Center for Advanced Research in Medical Mycology and WHO Collaborating Center, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Molecular identification of the isolate was done by sequencing ITS1-5.8S-ITS2 region of ribosomal deoxyribonucleic acid (DNA) gene. Sequencing was performed using primer pairs ITS1 (GCATATCAATAAGCGGAGGAAAAG) and ITS4 (GGTCCGTGTTTCAAGACGG) with Big Dye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems, CA, USA) for both strands as per the manufacturer's instructions. Sequencing reactions were purified and analysed on ABI 3130 genetic analyser (Applied Biosystems). For each gene, the consensus sequences were prepared from the sequence obtained by forward primer and reverse primers with the help of BioNumerics software version 7.1 (Applied Maths, Ghent, Belgium). The consensus sequences were compared with the sequences of the GenBank DNA database. The ITS1-5.8S-ITS2 sequence showed 99 % identity with the *E. oligosperma* (98 % identity with the sequence of *E. oligosperma* strain CBS 658.76, GenBank accession–JN625230). Sequence has been deposited in the GenBank database with the accession number KF780537. The isolate has been deposited at

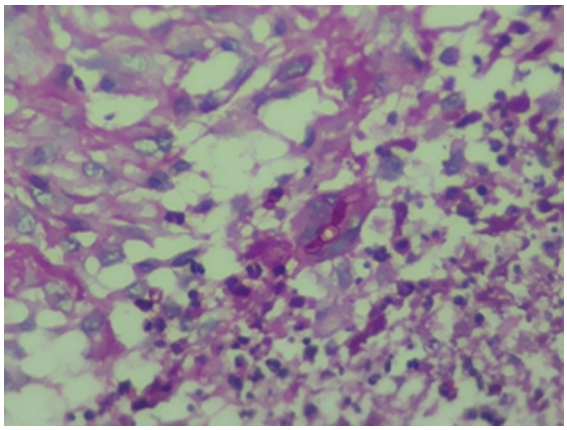

Fig. 3 PAS stain (×400) a giant cell showing fungal hyphae

NCCPF, PGIMER, Chandigarh as NCCPF-106017. Based on morphology and sequencing results, the isolate was identified as *E. oligosperma*.

Surgical excision of the nodule was performed and sent for histopathological examination. The cyst contained purulent material in the centre. Microscopy revealed a subcutaneous granuloma encapsulated by fibrocollagenous tissue with a central abscess cavity filled with necrotic debris and mixed inflammatory infiltrate. The granulomatous infiltrate was composed of histiocytic aggregates and foreign-body-type giant cells. Faint brown branching septate fungal hyphae were noted in the granuloma. Some of the fungal hyphae were phagocytosed by multinucleated giant cells (Fig. 3).

Post-operatively the wound healed well, and the patient was advised to come regularly for follow-up to monitor the progress and early detection of subsequent lesions if any. He did not have any relapse during 15-month follow-up.

Discussion

Studies have shown that overall accuracy of fungal identification in histological/cytological specimen is 79 % [4]. Various pitfalls in diagnosing fungi in histological and cytological specimens do occur such as morphological overlap and difficulty in identifying yeast forms [4]. However, a careful scrutiny of cytological features is helpful in the recognition and appropriate classification of rare fungal species also. Presence of brown pigmentation of the fungal hyphae in our case helped us to suspect phaeohyphomycosis and submit aspirated material for culture and PCR. FNAC helps in the rapid and preliminary identification of these rare fungal infections which often mimic soft tissue neoplasms clinically.

Differentiation of phacoid genera from clinical specimens can be difficult by culture while using non-selective media like SDA [5]. Moreover, *Exophiala* species often share common morphological characteristics despite molecular diversity. Rapid molecular diagnostic techniques like sequencing of the internal transcribed spacer region (ITS) of the rDNA genes are more definitive in identification at the species level. These methods have revealed evidence of novel species and helped to re-identify previously described entities.

Literature review reveals cases where it was difficult to identify *Exophiala* to the species level. Lau et al. [6] have described a case of CAPD peritonitis caused by *Exophiala* (isolated in 2000) which could best be identified to the genus level. Recently Woo et al. [7] identified this isolate as *Exophiala xenobiotica* with the aid of ITS and RpB1 sequencing . Woo et al. [7] have also described a case of onychomycosis (2010) caused by a novel fungus which exhibited phenotypic characteristics that did not fit into the pattern of any known *Exophiala* species. ITS, Rpb1, β-tubulin and β-actin gene sequencing unambiguously showed that it is closely related to but distinct from other *Exophiala* species. They have proposed to name this novel species as *E. hongkongensis* [7]. In the present case, the fungus was identified as *E. oligosperma* on the basis of microscopic morphology and molecular identification by sequencing ITS1-5.8 S-ITS2 region of DNA gene.

It is of paramount importance to differentiate various species of *Exophiala* because of the clinical, therapeutic and epidemiological importance. *Exophiala* species have preferred sites of infection and are associated with distinct clinical syndromes. *E. jeanselmei* has been reported to cause eumycetoma also [5, 8, 9]. *Exophiala dermatitis* has been associated with neurotropic infections in young immunocompetent individuals, but such rare cases are restricted to Asia only [5, 10]. *E. xenobiotica* reported as a causative agent of subcutaneous phaeohyphomycosis has the ability to grow in high concentrations of xenobiotics like xylene and toluene [5, 11]. Some species like the recently described *Exophiala asiatica* (China) can

Table 1 Summary of previously reported cases of human infections with *E. oligosperma*

S. no.	Case reports (references)	Age/ gender	Predisposing factors	Management	
				Medical	Surgical
1	Bossler et al. [2]	62/M[a]	Wegner's granulomatosis	Antifungal (amphotericin B)	_
2	Al-Obaid et al. [15]	3/F[a]	Leukaemia	Antifungal (amphotericin B)	_
3	Gonzalez-Lopez et al. [16]	72/F	Renal transplant	Antifungal (itraconazole)	_
4	Badali et al. [17]	20/F	Refractory chronic rhinosinusitis	Corticocosteroids	Yes (FESS)
5	Tokuhisa et al. [18]	57/F	None	Antifungal (terbinafine)	_
6	Kan et al. [19]	71/F	Wegner's granulomatosis	Antifungal (voriconazole, itraconazole)	Yes (surgical excision)
7	Rimawi et al. [20]	50/F	SLE	Antifungal (voriconazole)	_
8	Fukai et al. [21]	58/F	Sjogren's syndrome diabetes mellitus	Antifungal (itraconazole)	_
9	Present case report	38/M	None	None	Yes (surgical excision)

[a] *M* male, *F* female, *FESS* functional endoscopic sinus surgery

cause fatal disseminated cerebral phaeohyphomycosis [12]. Though the available data are relatively sparse, literature review shows that *Exophiala* species have varied antifungal susceptibility [13, 14]. Appropriate therapy should also be based on the immune status and extent of lesions, as discussed later. The knowledge of the prevalence of rarer species increases the awareness of the role of these fungi in human infections and moreover will further improve the fungal taxonomy.

Clinically significant infections caused by *E. oligosperma* are rare, and only eight cases have been reported so far. Interestingly, the current case and the very first case report caused by this fungus in the literature were male and the rest were female. The majority of *E. oligosperma* cases have been reported in immunosuppressed individuals and were treated with antifungal therapy (Table 1). However, one case was managed with surgical excision along with antifungal [19]. Badali et al. [17] have reported a case of fungal ball of the paranasal sinus which was effectively managed with functional endoscopic sinus surgery (FESS) followed by corticosteroid spray therapy Recently, Fukai et al. [21] have used local hyperthermia to treat *E. oligosperma* presenting with multiple subcutaneous nodules and abscesses resistant to antifungal therapy.

The optimal therapy for subcutaneous phaeohyphomycosis infections is still not standardized. Therapy of subcutaneous lesions caused by dematiaceous fungi is usually based on the immune status and extent of the lesions [5]. Antifungal drugs are frequently used in conjunction with surgical excision to prevent possible disseminated infections, especially in immunocompromised individuals [5]. However, surgical excision alone has proved to be successful in solitary, well-defined lesions [3]. Even subcutaneous infections in organ-transplant individuals have been managed effectively with surgical excision alone [5, 22–25]. Antifungal agents are quite expensive and some antifungal agents are difficult to obtain in developing nations [5]. Moreover, prolonged antifungal therapy has several adverse effects. Our case presented with a solitary nodular lesion and was managed effectively with surgical excision. Post-operatively, the wound healed well and no relapse was evident during 15-month follow-up. Hence, surgical excision alone was sufficient for this patient and a need for starting antifungal medications was not felt.

In summary, we describe a rare case of *E. oligosperma* presenting as a single subcutaneous cystic nodule in an immunocompetent individual. This is the first case reported from India. DNA sequencing helped in the identification of this rare species of *Exophiala*. We report this case to raise an awareness that *E. oligosperma* can manifest in immunocompetent individuals also. Our case also emphasizes that surgical excision is effective in the management of solitary phaeohyphomycosis lesions caused by *E. oligosperma.*

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