Exophiala spinifera as a cause of cutaneous phaeohyphomycosis: Case study and review of the literature

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Introduction

Over one hundred species of dematiaceous fungi have been reported to cause infections in humans [1]. These etiologic agents contain melanin in their cell walls and are widely distributed throughout the environment. Members of the order Chaetothyriales are of particular significance as they are recurrent agents of infection. These fungi are oligotrophic, particularly occurring at low frequency in low-nutrient or hydrocarbon-polluted environments [2]. Black moulds are etiologic agents of both chromoblastomycosis and phaeohyphomycosis. Chromoblastomycosis occurs most commonly in tropical environments and is a slow, progressive disease, often developing over several years. It is characterized clinically by verrucous lesions, usually on the lower extremities, and appears histopathologically as sclerotic bodies in tissue. Phaeohyphomycosis occurs worldwide, usually develops over a shorter duration, and presents in variable forms in tissue, including phaeoid yeastlike cells, hyphal elements, swollen cells, pseudohyphal elements, and/or moniliform hyphae.

The clinical syndromes of phaeohyphomycosis include allergic conditions, such as fungal allergic sinusitis [3,4], cutaneous disease (superficial, dermal or subcutaneous), as well as life-threatening disseminated

Exophiala spinifera has been reported as an agent of cutaneous disease 18 times in the literature. Clinical presentations of cutaneous lesions vary widely, including erythematous papules, verrucous plaques, and deep subcutaneous abscesses. The clinical distribution and course of disease are also variable, depending on the age and immune competency of the patient. Histologic appearance occurs in one of two patterns – phaeohyphomycosis or chromoblastomycosis. While E. spinifera appears to be susceptible to multiple antimicrobial agents in vitro, clinical experience with treatment modalities has been variable. Prior to the availability of sequencing methods, species identification was based on the histopathologic presentation in tissue and morphologic features of the fungus in culture. It is likely that E. spinifera cutaneous infections have been underreported due to its incorrect identification based on earlier methods. We report an additional case of E. spinifera phaeohyphomycosis, the first to be definitively identified by sequencing. In addition, we summarize the variable clinical, histopathologic, and morphologic features, as well as treatment responses described in previously reported cutaneous infections caused by E. spinifera.

Keywords Phaeohyphomycosis, Exophiala spinifera, itraconazole, chromoblastomycosis

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infection [5], often characterized by brain abscess formation [6]. Acquisition of the organism varies among syndromes. Cutaneous disease presumably occurs through traumatic implantation of colonized material, while allergic sinusitis and disseminated infections including central nervous system involvement are likely acquired through inhalation of fungal structures.

Until recently, species identification of various genera has been based upon recognition of macroscopic and microscopic features of the fungus in culture, temperature studies, and a limited number of physiologic assays. These methods, however, have proved unreliable in differentiating very similar species, particularly in the genus Exophiala [7]. Definitive molecular techniques employing ribosomal DNA sequencing are now available for species identification [2]. This will become increasingly important, as treatment modalities have variable effectiveness across species [8]. E. spinifera has previously been implicated, primarily based on its morphology, in eleven cases of phaeohyphomycosis and three of chromoblastomycosis in the English literature. We report an additional case of phaeohyphomycosis caused by E. spinifera and review the literature to summarize patterns in clinical presentation, histologic appearance, morphologic features, molecular characterization, genetic variation, and treatment strategies.

Case report

A 49-year-old Caucasian man presented to the outpatient dermatology clinic of the University of Pennsylvania School of Medicine for evaluation of a non-healing ulcer on his right shin that had enlarged over the previous four months. Eleven years prior to presentation, he underwent a bilateral lung transplant for α1-antitrypsin deficiency, and his transplant was maintained with prednisone (10 mg/day), tacrolimus (2.5 mg twice/day) and mycophenolate mofetil (500 mg twice/day). He denied any systemic signs or symptoms, including other skin lesions, difficulty breathing, headache, or neurologic symptoms. The patient is a building contractor who works outdoors doing physical labor.

Physical exam revealed a cushingoid man with an edematous right lower leg and a large ulcer with an indurated border on the pretibial aspect. Two erythematous nodules were present in close proximity to the ulceration (Fig. 1). There was no palpable lymphadenopathy.

Two separate punch biopsies, one from the indurated ulcer border and another from a nearby nodule, each demonstrated pseudopitheliomatous hyperplasia associated with a dense neutrophilic infiltrate. Pigmented yeast-like and pseudoahyphal elements were noted on routine hematoxylin and eosin staining and were highlighted with GMS, PAS and Fontana-Masson stains (Fig. 2).

A portion of the biopsied tissue and a separate wound swab were inoculated onto blood agar. Within one week, identical pigmented moulds were observed growing in cultures prepared from both specimens. The isolate was referred to the Fungus Testing Laboratory in the Department of Pathology at the University of Texas Health Science Center for further identification and accessioned into their stock collection as UTHSC 06-3355. Colonies were identified on potato flakes agar (PFA) [9] in ambient air with an on and off light cycle at 25°C. They measured 26 mm after 14 days and were moist initially but...
became more filamentous at maturity (Fig. 3). Microscopic features included long, brown, septate, annellophores with ovoidal annellocondia (2–2.5 × 3–4 μm) produced at the apices of the conidiogenous cells as well as at intercalary loci (Fig. 4). A capsular black yeast synanamorph was also present. The isolate assimilated nitrate [10] and grew at 35°C but failed to grow at 40°C. Based upon the features noted, the isolate was identified as an *Exophiala* species, not *E. dermatitidis*.

The isolate was subsequently sequenced. After incubation overnight on potato dextrose agar at 30°C, a small amount of material was scraped off the plate and inoculated into 50 μl of Prepmn Ultra reagent (Applied Biosystems, Inc., Foster City, CA). The cells were lysed according to the manufacturer’s instructions and the suspension pelleted in a microfuge at 14,000 g for 10 min. PCR was performed on 5 μl of the supernatant in a 0.5 ml microfuge tube in a 50 μl reaction volume using Triple Master Taq DNA polymerase (Eppendorf/Brinkmann Instruments, Inc., Westbury, NY) according to the manufacturer’s instructions. Two primer pairs were used to amplify two regions of the ribosomal repeat from the genomic DNA template. The ITS region (ITS1-5.8S-ITS2) was amplified using ITS1 (5’-TCCGTAGGTTACCTGCGG-3’) and ITS4 (5’-TCCGCGCATATGAGATGC-3’) as described [11]. The D1/D2 region of the large ribosomal DNA subunit was amplified with primers NL-1 (5’-GCATATCAACCTGCGG-3’) and NL-4 (5’-GGTCCGTGTTTCAGGCAGG-3’) as described previously [12]. All PCR reactions were performed in a PTC-100 thermocycler (MJ Research, Watertown, MA) using the preprogrammed three-step protocol as the standard program for all reactions. PCR reactions were electrophoresed through a 0.7% agarose gel to confirm amplification. The remaining template DNA was then cleaned using a Qiagquick PCR purification kit (Qiagen, Inc., Valencia, CA). Sequencing was performed on both strands at the UTHS CSA Advanced Nucleic Acids Core Facility using all four PCR primers. Both ITS and D1/D2 sequences were used to perform nucleotide-nucleotide searches using the BLASTn algorithm at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). Identifications were made when BLAST searches yielded >98% identity. A definitive identification of *Exophiala spinifera* was made based upon a 100% and 99% identity with D1/D2 and ITS sequences, respectively, when compared with GenBank nucleotide deposits for this species and the Centraalbureau voor Schimmelcultures (CBS) *Exophiala* database. Both the ITS and D1/D2 sequences were submitted to Genbank under accession numbers EU257701 (ITS), and EU257702 (D1/D2).

The patient received oral itraconazole at an initial dose of 200 mg twice/day that was later tapered to 100 mg twice/day. Importantly, itraconazole inhibits the cytochrome P450 enzyme CYP3A4 and thus typically results in increased plasma levels of tacrolimus, part of the immunosuppressive regimen used with our patient. Previous reports have shown that a
reduction in tacrolimus dose of 50-66% was necessary in transplant patients started on itraconazole [13]. In anticipation of this interaction, our patient’s tacrolimus dose was initially decreased by approximately 50%, from 2.5 mg (twice/day) to 1.5 mg (twice/day). Elevated plasma levels of tacrolimus on follow-up testing required temporary discontinuation, restarting at a low dose (0.5 mg twice/day), and close monitoring in follow-up while on itraconazole. The patient experienced rapid improvement and resolution of the lesions. He completed a total of six months antifungal therapy and to date has not experienced recurrence or new lesions.

Discussion and review of the literature

Summary of cases

Until recently, species identification of dematiaceous fungi has been based on appearance in tissue and in culture. By these criteria, Exophiala spinifera has been implicated in a total of 18 cases of clinical infections from ten different countries, with 14 cases appearing in the English literature. The initial case was reported in 1954 in a boy from India who succumbed to the infection. The pathogen was misclassified as Hormo

dendrum dermatitidis but was later reclassified based on modern morphologic criteria [14].

Of the 14 reported cases of E. spinifera infection in the English literature, 11 had histologic characteristics of phaeohyphomycosis and three of chromoblastomycosis (Table 1). Six cases were reported in children 13 years of age or younger—all had disseminated infection and two resulted in death. While both deaths occurred in healthy children, their clinical courses were marked by delayed diagnosis and ineffective treatment for a prolonged period prior to appropriate therapy. The remaining nine cases (including our report) were localized infections in adults and were easily treated in all but one. Clinical presentations were variable, including erythematous papules, verrucous plaques, deep subcutaneous abscesses, and an ulcer in our case. The site of initial infection (by history or exam) was on the face in four children but on an extremity in seven of nine adults. Various distant sites including skin, lymph nodes, bone, lung, and possibly the eye have been reported in cases involving dissemination. Unlike disseminated phaeohyphomycosis caused by other species [6], none involved the central nervous system.

Five of the nine adults were immunosuppressed, and all suppressive regimens included prednisone or prednisolone in doses from 2.5–16 mg/day. One adult patient who was not on an immunosuppressive regimen during infection had a remote history of oral corticosteroids for asthma and her disease was exacerbated during pregnancy, a physiologically immunosuppressed state [14–27]. Our case is the 15th E. spinifera clinical infection reported in the English literature, and the first in which identification was definitively established by molecular characterization.

Histology

The histologic features of E. spinifera cutaneous infection are typical for the cutaneous manifestations of a deep fungal infection, including epidermal hyperkeratosis, acanthosis, pseudopilomatous hyperplasia, and intraepidermal pustule formation. Dermal findings have included a dense mixed infiltrate, as well as supplicative and granulomatous infiltrates. Multinucleated giant cells are a common feature. Pigmented fungal elements can be detected most often in areas of inflammation, within or adjacent to multinucleate giant cells. GMS, PAS, and Fontana stains can be employed to highlight the fungal elements in instances involving lightly pigmented organisms. The fungal elements display one of two distinct histologic morphologies: a phaeohyphomycotic form or a chromoblastomycotic form. Three of the fourteen previously reported cases were classified as chromoblastomycosis and the remaining cases, including ours, as phaeohyphomycosis.

Specifically, phaeohyphomycosis is defined by the presence of dematiaceous fungi in tissue with a yeast-like, hyphal, or pseudohyphal morphology. These morphologies can be present uniformly or mixed. In culture-proven E. spinifera phaeohyphomycosis, the fungal elements have been described either as solitary forms or clusters of budding cells, thick-walled cells, or branched or septated hyphae. Other histologic descriptions have included budding yeast-like cells, thick walled chlamydospores, branched hyphae that were constricted at prominent septations, toruloid hyphal elements, and hyphae without prominent swellings. Some have observed hyaline to pale brown yeast cells with variable budding and the formation of pseudohyphae. In a case where the phaeohyphomycosis infection spread to an axillary lymph node, epithelioid cell granulomas with giant cells containing pigmented fungal elements were seen [14,15,20].

Chromoblastomycosis is defined by the presence of pigmented fungal elements in the form of muriform cells, thought to be an intermediate fungal form in the transition between the yeast and hyphal morphology. Other common terms for these elements include sclerotic bodies, fumagoid cells, copper pennies, Medlar

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bodies, and chlamydospores. In cases of *E. spinifera*, these elements have been described as round, chestnut brown or brownish in color, having a central pale area, and thick walls. Padhye et al. described the muriform cells as round-to-oval and ranging from 5 to 12 micrometers in diameter. They also described the presence of dematiaceous sporangium-like cells, which measured 12–20 micrometers in diameter and contained endocnidia. Propagation of the muriform cells in tissue was demonstrated by planate division [24–26].

Cases of *E. spinifera* cutaneous infection with concurrent chromoblastomycosis and phaeohyphomycosis forms have not been reported to date. It is likely, therefore, that these diagnoses represent two poles of a spectrum of disease and that the development of either form in a particular patient may depend on a specific strain-individual-environmental interaction [24].

Mycology

The black yeast genus *Exophiala* contains several species capable of causing human disease but the identification and differentiation of these species remains problematic for routine clinical laboratories. Factors contributing to this difficulty include the pleomorphic nature of the genus, i.e., the ability of several species to form yeast and filamentous phases [28], limited physiologic differences among them, and their very similar macroscopic and microscopic morphologies. Prior to the development of molecular techniques to characterize these agents, the most common species reported in clinical cases were *E. jeanselmei* and *E. dermatidis*. Our understanding of the genus greatly expanded with the advent of sequencing methods and the analysis of sequence data of the ribosomal DNA (rDNA) Internal Transcribed Spacer (ITS) regions. This information permitted the differentiation of the genus into specific ‘clades’ or clusters of taxa, including the *E. spinifera* clade [8,29,30]. The common clinical species *Exophiala jeanselmei*, long known to be a heterogeneous collection of yeast-like fungi, was also further defined and shown to consist of several species including *E. heteromorpha*, *E. lecanii-corni*, *E. oligosperma* and *E. xenobiotica* [31], as well as *E. jeanselmei* in the restricted sense. In a recent study of the distribution of *Exophiala* species recovered from clinical specimens in the United States using ITS sequence data, the order of frequency for the most common isolates was *E. dermatidis* (29.3%), *E. xenobiotica* (19.7%), *E. oligosperma* (18.6%), *E. lecanii-corni* (6.9%), and *E. phaeomuriformis* (6.4%). The recovery of *E. jeanselmei* and *E. spinifera* were at 3.7% and 2.7%, respectively [7]. Although *E. spinifera* is infrequently recovered and/or reported from clinical cases as a result of misidentification or lack of species identification, it is a cause of significant cutaneous disease.

Treatment

Definitive identification of the causative agent of phaeohyphomycosis is clinically important, as general species may vary in their susceptibility to antimicrobial agents [8]. *In vitro* studies suggest that *E. spinifera* is
most highly susceptible to itraconazole but poorly responsive to amphotericin B [8,32]. In a recent study evaluating the in vitro susceptibility patterns of clinically relevant Exophiala species in the US to the currently available antifungal agents, no significant differences were noted between species, except for amphotericin B resistance noted with three E. attemata isolates [7]. A summary of the in vitro susceptibilities of E. spinifera to various drugs is listed in Table 2. One study showed that the addition of quinolones to itraconazole or amphotericin B improved their antifungal activity against E. spinifera; however, this has not yet been tested clinically [33]. Importantly, it has been noted that in vitro susceptibility data seem to correlate poorly with in vivo efficacy [32].

Multiple antifungal therapeutic protocols have been utilized to treat infection with E. spinifera, including amphotericin B, ketoconazole, fluconazole, itraconazole, voriconazole, 5-fluorocytosine, terbinafine, griseofulvin, posaconazole, streptomycin, as well as physical treatment methods such as heat, cryosurgery and excision [8,14-26]. However, as detailed below, few of these treatments were ultimately found to be effective. Seven of the 12 patients who recovered from their infections, including our patient, were receiving itraconazole as part of their treatment regimens, and five were taking this medication as monotherapy. Four patients received amphotericin B in their initial treatment regimens of whom two required additional antifungal agents to obtain adequate clinical response, one died, and the last showed early improvement in short-term follow-up study [15-17,22]. In two cases there was increased resistance to itraconazole over the course of treatment that was verified by in vitro testing [15,25], and one report noted decreasing efficacy of multiple antifungal treatments over a prolonged course of treatment [22]. These investigations suggest that E. spinifera possesses the ability to develop resistance to antifungal therapy. A recent report described that a panel of treatments that included itraconazole, terbinafine, fluconazole, and cryosurgery was ineffective in treating a case of E. spinifera infection [18]. Other treatments that were used in the remaining patients who recovered without itraconazole treatment included posaconazole, ketoconazole, and 5-fluorocytosine antifungal therapies, liquid nitrogen cryotherapy, and surgical excision without additional therapy.

Conclusions

In conclusion, phaeohyphomycosis caused by E. spinifera: (1) typically presents in children as a lesion on the face, usually from an unidentified exposure, whereas in adults it primarily occurs on an extremity and is secondary to traumatic implantation; (2) may develop into disseminated disease, which is more common in children than in adults and has a higher rate of mortality; (3) usually occurs in healthy children but more than half of reported adult patients were on an immunosuppressive regimen which included daily prednisone; (4) may present histologically with either a phaeohyphomycosis or chromoblastomycosis morphology; and (5) has been most successfully eradicated when treatment protocols included itraconazole.

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