Case Report

Facial subcutaneous phaeohyphomycosis caused by *Phialophora verrucosa*: successful treatment with itraconazole and local resection

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Introduction: The fungal disease phaeohyphomycosis tends to occur in immunosuppressed individuals and has rarely been reported to be caused by *Phialophora verrucosa*.

Case presentation: Here, we report a primary subcutaneous phaeohyphomycosis case caused by *P. verrucosa* in an immunocompromised Chinese female with a *CARD9* mutation that was cured with local resection and further treated with itraconazole. The current case is placed in perspective with a review of the relevant literature. The patient presented with painless dark erythema and a plaque on the right part of her face that had been present for the past 20 years. Histological examinations revealed multiple brown hyphae, bead-like pseudohyphae and yeast-like cells either within the giant cell or distributed in the dermis and subcutaneous tissues. The fungal cultures were morphologically identified as *P. verrucosa* and were confirmed by internal transcribed spacer region nucleotide sequencing. A partial surgical focal excision was performed, and the patient was treated with oral itraconazole 200 mg daily for 1 year as maintenance therapy, resulting in complete resolution of the lesions.

Conclusion: This case is notable due to the prolonged course before a definitive diagnosis was made, the rarity of *P. verrucosa* as the cause of subcutaneous phaeohyphomycosis and the dramatic improvement after the focal lesion was excised and treated with itraconazole.

Keywords: itraconazole; phaeohyphomycosis; *Phialophora verrucosa*; subcutaneous.

Introduction

Dematiaceous fungal infections are classified into the groups of chromoblastomycosis and phaeohyphomycosis, depending on the characteristic appearance of the culprit in the infected tissue (McGinnis, 1983). Subcutaneous phaeohyphomycosis is a distinct clinical form characterized by dematiaceous fungal infection localized to the skin. Unlike chromoblastomycosis, sclerotic bodies are absent or rare. Instead, the causative fungus is observed in affected tissue as combinations of dematiaceous yeast-like cells with budding, septate hyphae and pseudohyphae. *Phialophora verrucosa* is a well-known cause of chromoblastomycosis, while *Exophiala* spp. are the most common causative agents in phaeohyphomycosis (McGinnis, 1983; Hoffmann et al., 2011). *P. verrucosa* rarely causes phaeohyphomycosis and tends to occur in immunocompromised individuals (McGinnis, 1983; Duggan et al., 1995; Lundstrom et al., 1997; Ohira et al., 2002; Tong et al., 2013). Subcutaneous phaeohyphomycosis caused by *P. verrucosa* with *CARD9* mutations and TH17 cell deficiencies were recently reported and further proved that genetic and immune deficiencies predispose patients to a chronic subcutaneous *P. verrucosa* infection.

Here, we report an uncommon case of primary subcutaneous phaeohyphomycosis caused by *P. verrucosa* in an immunocompromised Chinese female with a *CARD9* gene mutation. This case is placed in perspective with 15 other phaeohyphomycosis cases that were reported to be caused by *P. verrucosa*.
Case report

A 43-year-old female Chinese farmer presented at the Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital with painless dark brown erythema and a plaque on her right face that had been present for the past 20 years. The lesions gradually progressed but were still limited to the right part of her face. A red-coloured and bean-sized asymptomatic nodule appeared and progressed slowly on her right face 20 years ago. She denied any evident history of trauma and had no underlying disease or similar family history. Fifteen years ago, the lesions developed into a plaque with a diameter of 3 cm. The lesions did not improve after treatment of streptomycin 1.0 g day\(^{-1}\), isoniazide 300 mg day\(^{-1}\) and rifampicin 600 mg day\(^{-1}\) under the diagnosis of ‘lupus vulgaris’. The anti-tuberculosis therapy was discontinued and she was referred to another hospital where a skin biopsy showed infectious granuloma that was found to be positive by Periodic acid–Schiff (PAS) stain but did not yield a fungal culture. Subsequently, the patient was treated with oral itraconazole 400 mg day\(^{-1}\) and 250 mg day\(^{-1}\) terbinafine over a period of 2 months, but new lesions presented with verrucous granuloma that developed with exudate and crust. The patient was then referred to the Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital in March 2012.

Physical examination

Cutaneous examination revealed a dark brown, diffuse and infiltrated erythema and plaque involving the right face area with dirty brown-coloured crusts that had a well-defined but irregular border with slight hyperplasia. A verrucous granuloma with discharge close to the nose with a diameter of 1×1 cm was also present (Fig. 1a). The regional lymph node was not palpable; otherwise, the patient was generally in good health.

Mycological findings

Scrapings collected from the crusted lesions were examined by direct microscopy and revealed numerous dematiaceous, septate and branching hyphae (Fig. 1b). The fungal culture was positive and showed a slow radially growing grey/black colony when incubated at 28 °C for 2 weeks on potato dextrose agar (PDA) (Fig. 1c). The reverse of the colony was black in colour and abundant spores were observed. The slide culture of the PDA demonstrated round to oval conidia and flask-shaped conidiogenesis from the mouth of the phialides with a flared cup-like collarette, suggestive of a Phialophora-type of sporulation (Fig. 1d). Based on these morphological characteristics, the isolate was identified as \(P.\ verrucosa\).

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Fig. 1. (a) Dark brown, diffuse and infiltrated erythema and plaque on the right side of the face. (b) Smears of the scrapings from lesions showing multiple brown-coloured, septate and branching hyphae. (c) A compact and wooly grey/black colony grew on PDA after culture at 28 °C for 2 weeks. (d) Slide culture demonstrating conidiogenesis from the mouth of the vaseshaped phialides with a flared cup-like collarette. (e–g) PAS staining of biopsied material revealing multiple hyphae (e), ‘string-of-beads’-like pseudohyphae (f) and large yeast-like cells (g) intracellularly and extracellularly within the giant cells.
To confirm the identity of the fungus, molecular identification was performed by amplification and sequencing of the internal transcribed spacer (ITS) region (Caligiore et al., 1999). We performed similarity searching using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), which demonstrated a 100 % match with the type strain of P. verrucosa (BMU07163) in GenBank (accession no. KF360975.1).

In vitro antifungal susceptibility was performed using the Clinical and Laboratory Standards Institute-recommended microdilution method (CLSI, 2008) for the compounds fluconazole (FCZ), 5-fluorocytosine (5FC), amphotericin B (AMB), itraconazole (ITR), voriconazole (VOR), posaconazole (POS), terbinafine (TRB), micafungin (MICA) and caspofungin (CAS) (CLSI, 2008; Yu et al., 2008). For MICA and CAS, the minimal effective concentration (MEC) was defined as the minimal antifungal concentration; for the other compounds, the MIC endpoints were determined as the lowest drug concentration that yielded no fungal growth. The MIC and MEC results were determined when hyphal growth was detected in the control. The experiment was carried out in duplicate. The MIC values were: FCZ, 16 μg ml⁻¹; 5FC, 4 μg ml⁻¹; AMB, 4 μg ml⁻¹; ITR, 1 μg ml⁻¹; VOR, 0.25 μg ml⁻¹; POS, 1 μg ml⁻¹ and TRB, 0.06 μg ml⁻¹, while the MEC values against both CAS and MICA were 8 μg ml⁻¹.

Histopathological findings

The skin biopsy from the granuloma that was taken from close to the nose was stained with haematoxylin and eosin and with PAS and showed parakeratosis, stratum spinosum hypertrophy and false epithelioma hyperplasia in the epidermis. Numerous inflammatory infiltrates composed of neutrophils, plasma cells, lymphocytes, histiocytes and foreign-body-type giant cells were noted in the deep dermis to the subcutaneous tissues. In addition, multiple brown hyphae, bead-like pseudohyphae and yeast-like cells (Fig. 1e–g) were distributed intra- and extracellularly within the giant cells.

Laboratory examination

No abnormalities were found on blood and urine routine tests, chest X-ray, electrocardiogram or abdominal ultrasound examination. Serum immunoglobulin (IgA, IgG, and IgM) and complement (C3 and C4) levels were within the normal levels. Antibodies to HIV, hepatitis B and hepatitis C were detected to be negative. The liver and renal function tests were all normal during the therapeutic course.

Diagnosis

The diagnosis of subcutaneous phaeohyphomycosis caused by P. verrucosa was confirmed by mycological and histological examinations.

Clinical course and treatment

After the granuloma was removed by surgery, the patient was administrated itraconazole capsules (200 mg, twice a
<table>
<thead>
<tr>
<th>Case no.</th>
<th>Year</th>
<th>Country</th>
<th>Age</th>
<th>Sex</th>
<th>Lesions form</th>
<th>Anatomic sites</th>
<th>Fungal elements in the tissue</th>
<th>Underlying disease(s)</th>
<th>Effective therapy/prognosis</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1</td>
<td>1978</td>
<td>Japan</td>
<td>14</td>
<td>F</td>
<td>Papillomatous</td>
<td>ND</td>
<td>SC, SB, HY</td>
<td>Nodular metastasis</td>
<td>ND</td>
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<tr>
<td>2</td>
<td>1978*</td>
<td>Japan</td>
<td>21</td>
<td>F</td>
<td>Abscess/cystic granuloma</td>
<td>Buttock</td>
<td>SC, SB, HY</td>
<td>Nodular and skin metastasis</td>
<td>Surgical excision/ND</td>
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<tr>
<td>3</td>
<td>1981</td>
<td>Japan</td>
<td>21</td>
<td>F</td>
<td>Nodules</td>
<td>ND</td>
<td>SB, HY</td>
<td>Trauma</td>
<td>ND</td>
<td>Kagawa (1981)</td>
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<tr>
<td>4</td>
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<td>Japan</td>
<td>73</td>
<td>F</td>
<td>Abscess</td>
<td>ND</td>
<td>SB, HY, SC</td>
<td>Immune deficiency, Evan’s syndrome, steroids</td>
<td>ND</td>
<td>Ito and Iwatsu (1985)</td>
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<td>5</td>
<td>1985</td>
<td>Japan</td>
<td>39</td>
<td>F</td>
<td>Nodules</td>
<td>ND</td>
<td>SB, HY</td>
<td>Immunocompetent</td>
<td>FCZ and surgical excision/died</td>
<td>Ohira et al. (2002)</td>
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<tr>
<td>6</td>
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<td>53</td>
<td>F</td>
<td>Nodules</td>
<td>Buttock</td>
<td>SB, HY</td>
<td>ITR/diminished and lost contact</td>
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<td>7</td>
<td>2003</td>
<td>Japan</td>
<td>85</td>
<td>F</td>
<td>Nodules/pustules</td>
<td>Upper limb</td>
<td>SB, HY</td>
<td>Immune deficiency</td>
<td>KCZ/ND</td>
<td>Schnadig et al. (1986)</td>
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<tr>
<td>8</td>
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<td>Western Hemisphere</td>
<td>34</td>
<td>F</td>
<td>Cystic lesion</td>
<td>Foot</td>
<td>SB, HY</td>
<td>Sarcoidosis, immunosuppressive, steroids</td>
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<td>Duggan et al. (1995)</td>
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<td>1995</td>
<td>USA</td>
<td>39</td>
<td>M</td>
<td>Ulcerated lesion</td>
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<td>USA</td>
<td>42</td>
<td>F</td>
<td>Invasive</td>
<td>Tracheal</td>
<td>HY</td>
<td>Leukaemia bone-marrow transplant</td>
<td>AMB/died</td>
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<td>11</td>
<td>1998*</td>
<td>India</td>
<td>45</td>
<td>F</td>
<td>Ulcerated lesion</td>
<td>Lower limb</td>
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<td>Asthma, steroids</td>
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<tr>
<td>12</td>
<td>2011</td>
<td>China‡</td>
<td>19</td>
<td>M</td>
<td>Erythema and nodules</td>
<td>Face and ear</td>
<td>SB, HY</td>
<td>Pulmonary tuberculosis, anti-tuberculosis</td>
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<tr>
<td>13</td>
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<td>China‡</td>
<td>16</td>
<td>M</td>
<td>Erythema and crusting</td>
<td>Face</td>
<td>SB, HY</td>
<td>Immunocompromised</td>
<td>AMBand ITR/relapsing</td>
<td>Xu et al. (2011)</td>
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<tr>
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<td>2013</td>
<td>China‡</td>
<td>64</td>
<td>M</td>
<td>Purulent plaque</td>
<td>Face/neck/</td>
<td>SB, HY</td>
<td>HIV-negative, CD4 lymphopenia</td>
<td>ITR and TRB/ND</td>
<td>Tong et al. (2013)</td>
</tr>
<tr>
<td>15</td>
<td>2013</td>
<td>China</td>
<td>16</td>
<td>F</td>
<td>Erythema and nodules</td>
<td>Face</td>
<td>SB, HY</td>
<td>Immunocompromised</td>
<td>ITR, VOR, TRB and AMB/</td>
<td>Gao et al. (2013)</td>
</tr>
<tr>
<td>16</td>
<td>2014†</td>
<td>China‡</td>
<td>43</td>
<td>F</td>
<td>Erythema and nodules</td>
<td>Face</td>
<td>SP, SB, HY</td>
<td>Immunocompromised</td>
<td>ITR and local resection/cured</td>
<td></td>
</tr>
</tbody>
</table>

*Resection.
†Present case.
‡With CARD9 gene mutation (Wang et al., 2014).
F, female; M, male; SB, string-of-beads-like pseudohyphae; HY, hyphae; SC, sclerotic cell; SP, spore; ND, not determined; SLE, systemic lupus erythematosus.
day), with a good clinical response (Fig. 2a, b). At the 1-month follow-up, the hyperplasia on the edges of the lesions, exudates and crust on the face had improved significantly (Fig. 2c). Oral itraconazole was used as a maintenance treatment over a 1-year period and finally resulted in complete resolution of the lesion (Fig. 2d). The patient remains under medical supervision and has so far not presented with a relapse.

**Discussion**

Since the first reported case of subcutaneous phaeohyphomycosis caused by *P. verrucosa* by Iwatsu & Miyagi in 1978, a total of 16 sporadic cases, including the present case, have been reported (Table 1), comprising 10 cases in the English-language literature in the PubMed database, three cases published in Japanese and two cases published in Chinese. Thirteen of the 16 cases were in Asian countries (seven in Japan, one in India and five in China), and three were in western countries; three-quarters of the cases were observed in females, and 13 of the 16 occurred in young adults or middle-aged people; 14 of the 16 had underlying immunosuppressive factors. Other than our case, only one report provided a prognosis of a complete cure (Tendolkar *et al*., 1998). Some cases were recurrent and refractory (Xu *et al*., 2011; Gao *et al*., 2013), and some patients even died (Duggan *et al*., 1995; Lundstrom *et al*., 1997; Ohira *et al*., 2002). These outcomes suggest that *P. verrucosa*-associated subcutaneous phaeohyphomycosis is predominant in Asian, female, young or middle-aged and immunocompromised individuals, with a poor prognosis (Table 1).

Subcutaneous phaeohyphomycotic infection is usually caused by traumatic skin inoculation of contaminated material carrying inocula of ubiquitous environmental fungi, which are usually located in decaying wood, living plants, plant debris and soil (Iwatsu *et al*., 1981). Although all Chinese cases so far, including the patient in the current report, had no evident history of traumatic wounds or of foreign body inoculation, very tiny traumas like mosquito bites might be overlooked by patients. Our patient was a farmer, with more likelihood of contact with plants and exposure to soil, and it is likely that she had acquired material contaminated with *P. verrucosa* from a very small injury. The predominant geographical characteristic is consistent with the geographical distribution of *Phialophora* spp., as *P. verrucosa* has been recovered from pine bark, soil and lumbar woods in Japan (Geuzele *et al*., 1972; Iwatsu *et al*., 1981). However, it is unknown if similar ecological niches exist in China, and thus further environmental studies need to be carried out.

Surgical excision and antifungal therapy remain the standard treatments for cutaneous and subcutaneous phaeohyphomycosis (Silveira & Nucci, 2001). Simple surgical excision can eradicate the localized cutaneous and subcutaneous phaeohyphomycosis (Bogle *et al*., 2004; Nulens *et al*., 2006). Recently, itraconazole has been reported to be highly effective in some phaeohyphomycotic infections, even in some refractory cases, but it is still unable to achieve a complete cure in some recalcitrant cases (Gao *et al*., 2013). It has been reported that a relapsing and invasive case in a lung-transplant patient was cured by a local resection combined with antifungal therapy (Chua *et al*., 2001). The development of new antifungal drugs and combination therapies may be helpful in treatment. Chronic, refractory and cerebral phaeohyphomycosis treated successfully with voriconazole and caspofungin have also been reported previously (Trinh *et al*., 2003; Proia & Trenholme, 2006; Koo *et al*., 2010).

*P. verrucosa* has historically been reported as a rare causative agent of phaeohyphomycosis in China (Hu *et al*., 2011; Xu *et al*., 2011; Gao *et al*., 2013; Tong *et al*., 2013). We were able to establish some similarities among the five published cases occurring in China: all were immunocompromised individuals (one had CD4<sup>+</sup> lymphopenia and one had a history of tuberculosis and received anti-tuberculosis treatment); they had a relatively early onset; they had no trauma history; they originated in the countryside and occurred on the face with subcutaneous infections, except for one case that developed into a generalized form involving the neck and dorsal area; no systemic infection has been reported in China to date; and three of the five cases were students. Wang *et al.* (2014) investigated underlying genetic and immune deficiencies in four Chinese patients with subcutaneous phaeohyphomycosis caused by *P. verrucosa* that was found to be partly related to the CARD9 genetic deficiencies.

In our patient, complete surgical excision was deemed to be impractical, and the local resection appeared to be quite effective, as the lesions improved significantly after the surgery and the administration of itraconazole. Therefore, local excision of the lesions followed by itraconazole is recommended in the treatment of facial cutaneous and subcutaneous phaeohyphomycosis. Compared with the previous cases in China, our patient demonstrated the best treatment effect using itraconazole combined with surgical excision, thereby achieving a complete cure.

The treatment of phaeohyphomycosis is always a serious challenge. Early diagnosis, surgical excision and high doses of itraconazole and other new antifungal medications provide promising and highly effective methods for treating this emerging disease. Further study is needed on what lies behind the CARD9 deficiencies and the susceptibility to recalcitrant opportunistic subcutaneous *P. verrucosa* infections.

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**References**


