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Recalcitrant Primary Subcutaneous Phaeohyphomycosis Due to *Phialophora verrucosa*

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Abstract *Phialophora verrucosa* has rarely been reported for causing phaeohyphomycosis, which tends to occur in immunocompromised individuals. The case of primary subcutaneous phaeohyphomycosis due to *P. verrucosa* in an otherwise healthy Chinese female is presented. The girl presented with asymptomatic skin lesions when she was only 16 year old. Histological examinations revealed multiple dematiceous hyphael elements in the dermis and subcutaneous tissues. Fungal cultures were identified as *P. verrucosa* repeatedly based on the morphological features and confirmed by the internal transcribed spacer region nucleotide sequencing. The infection was so extremely recalcitrant that prolonged systemic antifungal regimens for 12 years revealed limited effect. The skin lesions slowly progressed and caused marked disfigurement despite the encouraging results of in vitro susceptibility. However, no relevant side effects have been reported in the course, and the patient gave birth to a healthy baby while under the maintenance treatment of itraconazole and terbinafine. This case is special in terms of the early onset, the rare clinical aspect of the pathogen, the discrepancy between in vitro and in vivo antifungal activities and especially the prolonged and recalcitrant course in such an otherwise healthy host.

Keywords *Phialophora verrucosa* · Phaeohyphomycosis · Subcutaneous · Recalcitrant

Introduction

Phialophora verrucosa is a well-known cause of chromoblastomycosis, but an uncommon one for phaeohyphomycosis. It is occasionally observed in immunosuppressed individuals, and subcutaneous phaeohyphomycosis is the most common presentation that also includes cases with dermal involvement [1].

In this study, we report an unusual case of primary subcutaneous phaeohyphomycosis caused by *P. verrucosa* in an immunocompetent Chinese female with the onset age as early as 16. The recalcitrant course lasting for 12 years demonstrated the disagreement between in vitro and in vivo antifungal activities. To further clarify the disease, 6 other cases that share the same causative agent, clinical and pathological features were reviewed.

Case Report

A 28-year-old otherwise healthy female from Hebei Province, People's Republic of China, presented with extensive skin lesions. The skin lesions developed

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since she was only 16 year old. Over the years, the skin eruptions gradually progressed onto her left face and generalized to the right side, back, and left upper limb.

Twelve years earlier, a skin-colored painless subcutaneous nodule with a diameter of about 1.5 cm appeared in her retroauricular area without evident history of trauma. After surgical excision and treatment under the diagnosis as tuberculosis, the skin lesions exacerbated with slight weeping and multiple red-to-violet nodules developed. She discontinued the anti-tuberculosis therapy and was referred to another hospital, where she was treated with itraconazole 200 mg/day under the diagnosis of cutaneous cryptococcosis. The lesions gradually improved in the first 3 weeks, but new lesions developed later. She came to our hospital in November 1998.

Physical Examination

Cutaneous examination revealed multiple sharply demarcated red-to-violet papules and palpable nodules, ranging in size from 2 mm to 1 cm with slight desquamation involving the left retroauricular area (Fig. 1). No obvious discharge or crust was found. Regional lymph nodes were not palpable. Except for these lesions, the girl was generally in good health.

Histopathological Findings

Histological slides of the biopsy tissues were stained with hematoxylin and eosin and Periodic acid–Schiff (PAS). No specific change was found in the epidermis. Intense inflammatory infiltrates composed of neutrophils, eosinophils, lymphocytes, plasma cells, histiocytes, Langhans and foreign body giant cells were noted in the dermis and subcutaneous tissues. Within the infiltrate were short, slender, strongly septate, dematiaceous hyphae and occasional large yeast-like cells extracellularly and intracellularly within the giant cells (Fig. 2). Sclerotic cells were not observed. Hence, a histopathological diagnosis of subcutaneous phaeohyphomycosis was made.

Mycological Findings

Direct microscopic examinations of the lesions revealed numerous dematiaceous, septate and branching hyphae (Fig. 3). Fungal culture of the lesions was performed. Brown to black colonies grew slowly in Sabouraud's dextrose agar, attained a diameter of 2 cm in 14 days at 27 °C with abundant short gray aerial hyphae. Slide culture revealed subhyaline to pale brown, smooth-walled, septate, branched hyphae bearing vase-shaped phialides laterally or terminally with a flared cup-like collarette and hyaline-to-brown, round-to-oval conidia accumulating at the apex of the phialides as cohesive clusters, giving the appearance of a vase of flowers: the typical *Phialophora* type of sporulation (Fig. 4). The isolate was morphologically identified as *P. verrucosa* and was confirmed by the internal transcribed spacer region nucleotide sequencing and sequence similarity searching using BLAST in the CBS fungi database, which demonstrated 99 % identity with the *P. verrucosa* sequence (CDC-B2152).

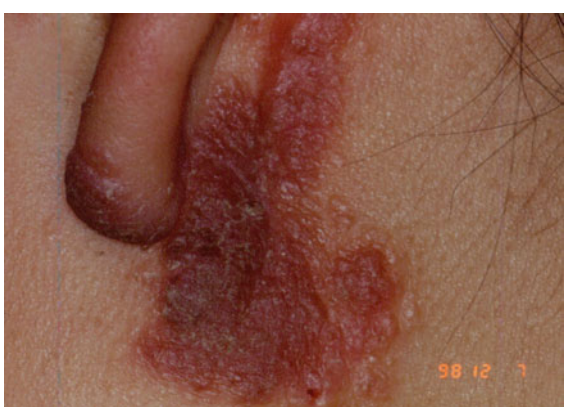

Fig. 1 *Red to violet* papules and palpable nodules some of which had coalesced involving her *left* retroauricular area and auricular lobule in Dec. 1998

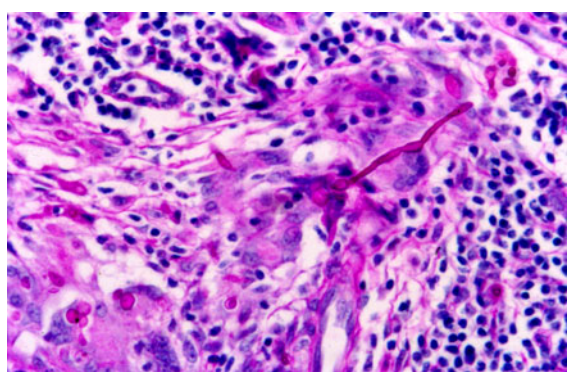

Fig. 2 Histopathology stained with PAS revealed multiple dematiaceous hypha and large yeast-like cells extracellularly and intracellularly within the giant cells

Laboratory Examination

Full blood counts were normal in 1998. However, after years of antifungal therapies, the blood routine tests revealed that RBC counts, HGB and HCT decreased to around the lower limits. WBC counts also were near the lower limits with the lowest at 2.74×10^9/l. The proportion of lymphocytes was slightly elevated ranging from 44.6 to 55.5 %. However, total T cell count, total B cell count, CD4/CD8 ratio, NK cell count, serum immunoglobulin (IgG, IgM and IgA) levels and complement levels were all within normal limits. Liver and renal function tests were all normal during the 12 years.

In Vitro Antifungal Susceptibility

In vitro susceptibility of those isolates against 5-Flucytonine (5-FC), fluconazole (FCZ), amphotericin B (AMB), terbinafine (TRB), itraconazole (ITR), voriconazole (VOR), caspofungin (CAS) and micafungin (MICA) was determined using Clinical Laboratory Standard Institute recommended broth microdilution method of M38-A2. In vitro susceptibility of those isolates against posaconazole (POSA) was determined using *E* test. The minimal inhibitory concentration (MIC) endpoints were determined (visually) as the lowest drug concentration that prevented any discernible growth (i.e., optically clear). For the echinocandins, the minimal effective concentration (MEC) was used for endpoint determination. The MEC was defined as the minimal antifungal concentration that produced morphological alterations of hyphal growth. The MICs and MECs results were not read until the hyphae grew well in the growth control. The MICs and MECs were determined in duplicate for all isolates. The results showed that the MIC values of those isolates against 5-FC, FCZ, AMB, TRB, ITR, VOR and POSA were $\geq$32, $\geq$64, 4, 0.006, 1.00, 1.00, and 0.125 μg/ml, respectively, while MEC values against CAS and MICA were 2.00 and 1.00 μg/ml, respectively.

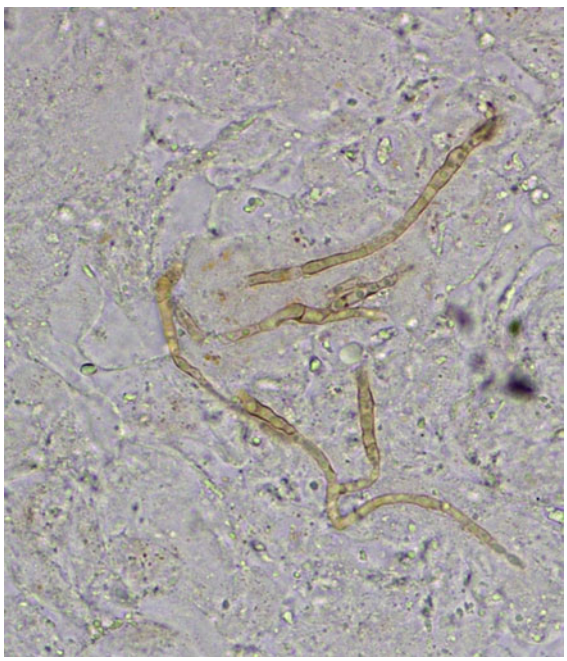

Fig. 3 Smear of the scrapings showed numerous dematiaceous, septate and branching hyphal elements

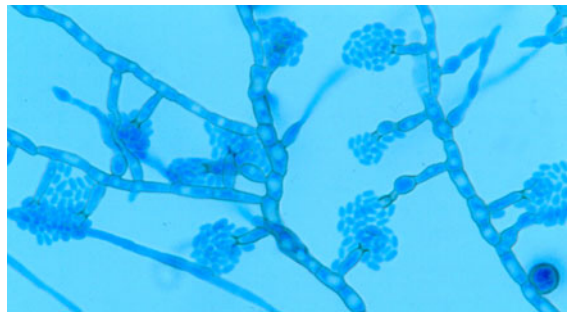

Fig. 4 Slide culture revealed subhyaline to *pale brown*, *smooth-walled*, septate, branched hyphae bearing vase-shaped phialides with a flared cup-like collarette characteristic of *P. verrucosa*

Disease Course

After the diagnosis of subcutaneous phaeohyphomycosis was confirmed by mycological and histological examinations, this patient has been under systemic antifungal therapy for almost 13 years. Different therapy strategies were applied during her admissions (Table 1). Local heat was also applied to the skin lesions since 1999 and that seemed to be quite useful as the skin lesions showed tendency of subsidence. However, when the patient discontinued the heat apply, the lesions exacerbated. A combination of oral ITR and TRB has been applied as maintenance treatment since 2002. However, despite all these, the lesions still slowly progressed in this lengthy course and significantly aggravated during her pregnancy

although she did not halt the therapy. As shown in Table 1, new lesions developed on her back and left upper limb in 2009. Direct microscopy, skin culture and biopsy of the new lesions revealed the same findings as found in the lesions on the faces. In vitro susceptibilities of these new isolates to various antifungal agents were exactly the same. Now, those lesions have already caused marked disfigurement (Fig. 5).

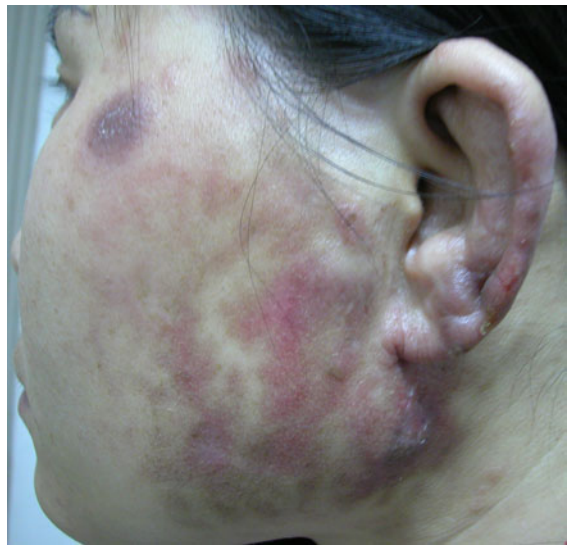

Fig. 5 Progressed skin lesions involved her *left* ear and face causing disfiguration in Dec. 2009

Discussion

Although *P. verrucosa* has long been known to be a classic agent causing chromoblastomycosis, it has rarely been isolated from cases of phaeohyphomycosis [1]. The first case of subcutaneous phaeohyphomycosis due to *P. verrucosa* was reported by Iwatsu et al. in 1978 [2]. And since then, sporadic cases have been reported [3–7]. Apart from the case presented above, we found 6 other cases reported in the English-language literature in the PubMed database (Table 2) [3–7].

It was noteworthy that 6/7 of the *P. verrucosa*-associated subcutaneous phaeohyphomycosis cases were observed in female, and 5 of the 7 had underlying immunosuppressed factors. This suggests its predilection for females with associated underlying diseases and locally or systemic immunological deficiencies (Table 2), whereas chromoblastomycosis occurs predominantly with male patients who work outside.

Table 1 Antifungal therapies and important events of the disease course

Years	Antifungals	Duration	Dosage	Treatment efficacy
1998.6	ITR (po)	5 months	200 mg Qd	Improved at first 3 weeks but progressed again
1998.11	AMB (iv)	1 month	~1 g	Limited
1999.1	TRB (po); AMB (intra-lesion)	2 months	250 mg Qd	Limited
1999.3	TRB (po); local heat	Continually	250 mg Qd	Significantly relieved
2002.4	ITR (po)	Continually	200 mg Qd	Limited
	New lesions on the right face after withdrawal of local heat			
2002.8	ITR (iv)	14 days	200 mg Bid	Limited
2006.4	ITR (iv)	14 days	200 mg Bid	Limited
2006.5	VOR (iv)	14 days	200 mg Bid	Limited
2007.8–2008.4	Significantly exacerbated during pregnant (therapies continued)			
2009.2	New lesion on her back after withdrawal of local heat			
2009.11	New lesion on her left upper limb after skin breakdown			
2009.12	AMB (iv)	1 month	~0.3 g	Limited
2011.5	TRB	Continually	500 mg Qd	Lesions improved but not entirely fade away
	ITR	Continually	400 mg Bid	

A combination of oral ITR (capsules, 200 mg Qd) and TRB (250 mg Qd) has been applied as maintenance treatment since 2002. The dosage of ITR and TRB was doubled since April 2011

KCZ ketoconazole, *ITR* itraconazole, *FCZ* fluconazole, *VOR* voriconazole, *TRB* terbinafine, *AMB* amphotericin B

Table 2 Clinical characteristics of patients with primary subcutaneous phaeohyphomycosis due to *P. verrucosa*

Year (publication)	Gender	Age	Underlying disease		Therapy	Prognosis
			Disease	Associated treatment		
1978	F	21	SLE	Betamethasone[b] * 5 yrs	–	–
1986	F	34	sarcoidosis	Prednisone[b]	KCZ	–
1995	M	39	AIDS	Anti-virus drugs	ITR 200 mg/day	Died of pneumococcal pneumonia with bacteraemia and meningitis
1997	F	45	Asthma	Steroids[b] * 9 yrs	ITR * 3 months	Cure without recurrence
2002	F	53	Evans' syndrome	Prednisolone[b] * 3 yrs	FCZ * 200 mg/day Surgical removement	Died from sepsis from a traumatic wound
2003	F	85	Immunocompetent	ITR 100 mg/day	Lost contact	
2010[a]	F	16	Immunocompetent	ITR/VOR/TRB/AMB	Recalcitrant but alive.	

[a] Present case; [b] Systemic

– not mentioned, *yrs* years, *F* female, *M* male, *SLE* systemic lupus erythematosous, *AIDS* acquired immunodeficiency syndrome, *KCZ* ketoconazole, *ITR* itraconazole, *FCZ* fluconazole, *VOR* voriconazole, *TRB* terbinafine, *AMB* amphotericin B

Of the 7 cases, 5 were in Asian countries (3 in Japan, 1 in India, and 1 in China), and 2 were in the western hemisphere. In addition to these 7 patients listed above, another 3 patients of *P. verrucosa*-associated subcutaneous phaeohyphomycosis have been reported in Japanese language. These show a prominent geographical feature that is in concordance with the geographical distribution of *Phialophora* species [8]. In China, *P. verrucosa* has never been implicated as a causative agent of phaeohyphomycosis. And this case, to the best of our knowledge, is the first documented subcutaneous phaeohyphomycosis due to *P. verrucosa* in China.

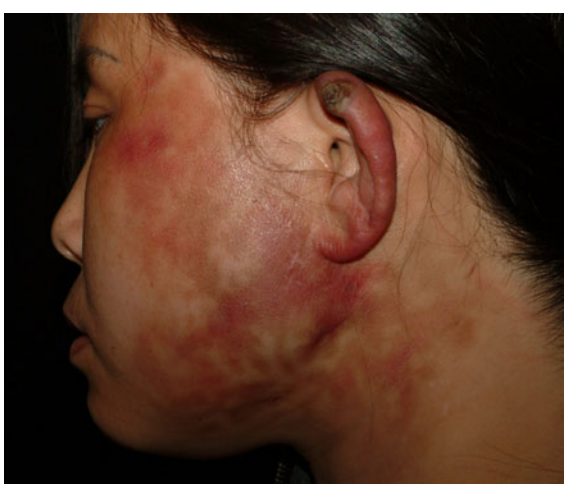

Fig. 6 Lesions 6 months after we doubled the dosage of ITR and TRB. Nodules exist

Relatively limited information was available on the treatment of subcutaneous phaeohyphomycosis. Many physical and pharmacological antifungal therapies for *Phialophora* spp. infection have generally been disappointing [9, 10]. None of the other 6 reports has provided information on in vitro antifungal susceptibility and only 1 provided information of definite cure [5].

Our case is special in terms of the early onset age (16 years old) when compared to the average age (41.86 years) and in terms of the prolonged and recalcitrant course in such an otherwise healthy host. In our case, however, in vitro antifungal susceptibility of strains isolated in different times and different lesions has already excluded the possibility of drug-induced resistance. Nevertheless, our case turned out to be a therapeutic dilemma as various antifungal regimens failed to stop the lesions from progressing and spreading. Because invasive *P. verrucosa* infection had previously been reported [11], hematogenous dissemination of *P. verrucosa* should be suspected in this patient with multiple independent lesions. However, the patient was otherwise well through these years. No nephrotoxicity or hepatotoxicity was reported, and she successfully gave birth to a healthy baby. In addition, there was no detectable (1,3)-β-D-glucan in her plasma, which suggested hematogenous

dissemination least possible in this patient. Although the patient did not recall evident history of trauma prior to the initial lesion, there might have been some minute skin break down that introduced the pathogen into local skin. Based on the fact that a minute trauma preceded the nodule on her left arm, it was likely that autoinoculation might have played an important role in the spread of skin lesions.

The patient has undergone remittent aggravation from time to time these years. However, the dosage of ITR and TRB used may be likely inadequate for this infection. The patient did get better after we doubled the dosage of ITR and TRB these 6 months (Fig. 6). However, there is still far away to reach the goal of complete cure. After all, the fact that this disease being so recalcitrant and persistent in such a well-appearing person and the discrepancy between in vitro antifungal susceptibility and in vivo activities reflects the difficulties in the treatment of phaeohyphomycosis. Endeavors should be made to elucidate the pathogenesis and to seek effective therapy strategies to improve the treatment outcome.

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Conflict of interest None.

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