

## Case Reports

## Successful treatment of chromoblastomycosis of 36 years duration caused by *Fonsecaea monophora*

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We report a case of chromoblastomycosis in a 67-year-old female farmer, which involved a large (20 × 30 cm) cicatricial erythematous plaque on the inner side of her right thigh. The lesion was initially a small nodule which gradually extended over 36 years. Direct microscopic examination revealed a granulomatous lesion with muriform cells surrounded by giant cells. The mould recovered in cultures was dark olivaceous and identified as *Fonsecaea monophora* by ribosomal internal transcribe spacer (ITS) sequence data. The lesion was successfully cured after 4 months treatment with itraconazole, but there was a relapse.

**Keywords** Chromoblastomycosis, *Fonsecaea monophora*, itraconazole

### Introduction

Chromoblastomycosis is a chronic, cutaneous and subcutaneous infection characterized by slowly expanding skin lesions with muriform cells in tissue, provoking a granulomatous immune response. The disease occurs worldwide, but most cases have been reported from tropical and subtropical climates. It is caused by members of the ascomycete order *Chaetothyriales*, comprising the black yeasts and relatives. To date, six species have been proven as a causative agent of the disease, i.e., *Fonsecaea pedrosoi* [1,2], *F. monophora* [3,4], *Cladophialophora carrionii* [5], *C. samoënsis* [5], *Phialophora verrucosa* [6,7] and *Rhinocladiella aquaspersa* [8,9]. Several *Exophiala* species (*E. jeanselmei* and *E. spinifera*) have been confirmed as occasional agents of the disease [10,11]. The etiologic agents are supposed to gain entrance through the skin by traumatic implantation of contaminated material. The majority of lesions are observed on extremities of outdoor

workers [1,12–14]. Carrión [15] and Queiroz-Telles *et al.* [1] described five types of lesions; nodular, tumorous, verrucose, cicatricial and plaque types. As yet it is unknown whether these types are associated with specific etiologic agents or are dependent on host responses.

The present case reports a chronic infection caused by a *Fonsecaea* species. The genus *Fonsecaea* presently comprises two species, *F. pedrosoi* and *F. monophora* [3,16]. While morphologic differentiation is difficult, their separation was recently confirmed by Najafzadeh *et al.* [16] using multilocus analysis. The pathology of the two species may be somewhat different, i.e., *F. pedrosoi* thus far is strictly associated with chromoblastomycosis, while *F. monophora* seems to be a more general opportunist [3], including, for example, cases of brain infection [17].

### Case report

While the patient was a 67-year-old female farmer living in Spain, she was born in Equatorial Guinea. She presented with a cicatricial erythematous plaque (20 × 30 cm in diameter) with verrucous margins in the internal face of the right thigh (Fig. 1). The lesion started 36 years earlier in Guinea as a small subcutaneous nodule which was pruritic

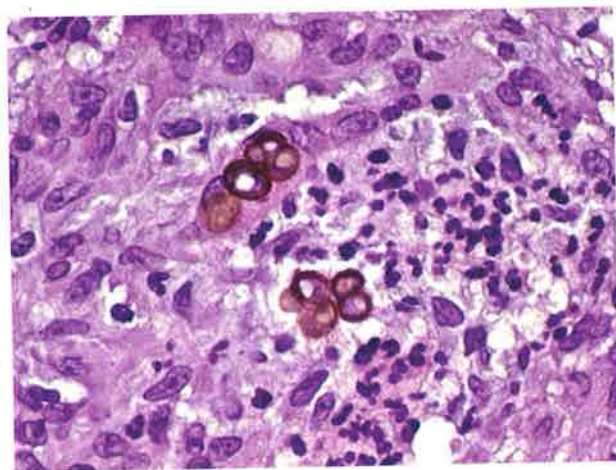
Received 4 March 2009; Received in final revised form 23 April 2009; Accepted 30 April 2009

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**Fig. 1** Cicatricial erythematous plaque in the internal face of the right thigh with verrucous zones.

and painless. A trauma or inoculation was not recalled. No satellite lesions or lymphadenopathies were observed and she had not received any antifungal therapy. Although a partial resection was made 12 years earlier, there had been a relapse in the lesion. Results of blood tests were within normal limits except for a slight neutropenia and glycaemia (7.5 mM/L). Thyroid function and urine biochemistry were normal. Examination of potassium hydroxide mounts from the lesion revealed brown muriform cells. Histopathology with haematoxylin and eosin staining of the epidermis showed hyperkeratosis and parakeratosis, and a granulomatous response with histiocytes, plasma



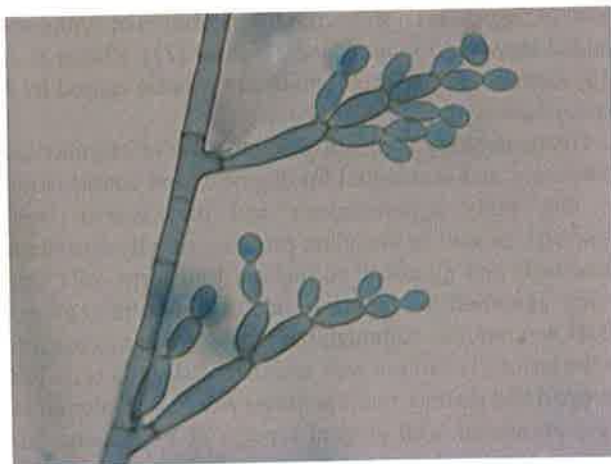
**Fig. 2** Muriform cells in histopathological examination of biopsy (hematoxylin-eosin stain).



**Fig. 3** Macroscopic appearance of *Fonsecaea monophora*.

cells, polymorphonuclear cells and giant cells including muriform cells (Fig. 2). These results confirmed the clinical diagnosis of chromoblastomycosis.

Fungal culture of skin scales on malt extract agar (MEA) yielded velvety to cottony, dark olivaceous colonies after 14 days at 25°C (Fig. 3). Microscopic appearance was indistinguishable from that of *F. pedrosoi* (Fig. 4). Partial sequences of the rDNA Internal Transcribed Spacer (ITS), actin (*ACT1*) and  $\beta$ -tubulin (*TUB1*) domains were compared with GenBank and aligned with voucher strains maintained at CBS including ex-types of *Fonsecaea* species. The isolate showed close sequence similarity with CBS 269.37, the ex-type strain of *F. monophora*. The sequence data for the isolate were deposited in GenBank with accession numbers FJ785471, FJ785472 and FJ785473



**Fig. 4** Microscopic appearance of *Fonsecaea monophora* (CBS 123849).

for ITS, *ACT1* and *TUB1*, respectively. The isolate was preserved in the reference collection of the CBS-KNAW Fungal Biodiversity Centre with accession number CBS 123849. The patient showed good response to treatment with 400 mg/day for the first month and 200 mg/day for the next three months of systemic itraconazole. However, discontinuation of treatment resulted in a relapse.

## Discussion

*Fonsecaea monophora* is presently recognized as one of the agents of human chromoblastomycosis. Xi *et al.* [4] described 20 cases of chromoblastomycosis caused by this species, which proved to be the predominant etiologic agent of the disease in southern China. Zhang *et al.* [18] reported three further cases caused by *F. monophora* in southern China. Yaguchi *et al.* [19] discussed 27 cases by *F. monophora* from Japan and showed that Chinese populations of the fungus are very similar in sequence of ITS region to those from Japan and they located in Subgroup B-2. The present report is the first proven case of chromoblastomycosis caused by *F. monophora* originating from the African continent. An earlier African case was concerned with a brain infection [17]. Clinically our case could be classified [1] as the plaque type of chromoblastomycosis, with slight elevation of the lesion and having a sharp reddish margin. There is no definite incubation period and most cases have a slow, chronic course. In the present case, the initial lesion gradually extended over 36 years and represents the longest course of the disease reported. During the long duration of the infection, the lesion had enlarged considerably, and could be classified as 'severe' according to Queiroz-Telles *et al.* [1].

Bonifaz *et al.* [20] studied 51 cases involving *F. pedrosoi* and *Phialophora verrucosa* in Mexico and found that the longest course of disease was 24 years, with a minimum of 2 months. In another study from Malaysia, where the etiologic agent was not specified, the duration of symptoms ranged between 5 months and 13 years [21]. Zhang *et al.* [18] reported a case of chromoblastomycosis caused by *F. monophora* with a 24 year history.

Histopathology is identical in all types of chromoblastomycosis, and is essential for diagnosis and confirmation. In this study hyperkeratosis and parakeratosis were observed, as well as abundant presence of polymorphonuclear cells and giant cell formation. Muriform cells were easily identified in routine haematoxylin-eosin stain and KOH wet mounts. Although the host defence mechanisms in the lesions is still not well understood, Davila *et al.* [22] reported that distinct immune-histopathological alterations were correlated with clinical aspects of the lesions. The authors suggested that patients with lesions presenting as verrucous plaques had a type Th2 immunological response,

while patients with lesions presenting as erythematous atrophic plaque had a type Th1 response.

Patients are primarily male rural workers who are supposed to acquire the infection after having been pricked by contaminated thorns or wood splinters. Our patient did not recall any trauma but a microtrauma may have occurred. The infection process in chromoblastomycosis is still not well understood. Salgado *et al.* [12] isolated a fungus morphologically identified as *Fonsecaea pedrosoi* from thorns of the plant *Mimosa pudica* at the site of infection specified by one of their patients. However, Vicente *et al.* [23] demonstrated that such environmental strains may differ at the molecular level from clinical strains. The involvement of environmental *F. pedrosoi* isolates has thus far not been proven by direct isolation methods. Possibly this is related to differential virulence and predilection of the species concerned. *Fonsecaea monophora* seems to be less strictly associated with chromoblastomycosis than *F. pedrosoi*, and can be directly isolated from the environment, whereas for *F. pedrosoi* enrichment via a mouse or another mammal is required [23].

Treatment of chromoblastomycosis may be difficult because of the presence of therapy-refractory muriform cells and differential susceptibilities between taxonomically closely related groups [20]. There is no drug of choice for treatment of the disorder and results may depend on the size and severity of the lesions [1], etiologic agent, patient status and clinical localization [20]. In the present case the patient was initially cured after 4 month treatment with systemic itraconazole 200–400 mg/day. The result of the present study confirms earlier reports which indicated that itraconazole provides an effective therapy of chromoblastomycosis caused by *Fonsecaea*, *Phialophora* and *Cladophialophora carrionii* [20,24–27], but the period of treatment should probably be extended. Some authors have reported that a combination of itraconazole treatment with cryosurgery using liquid nitrogen has a synergistic effect and appears to be the best treatment for extended lesions [20,28,29]. Alternative, combination therapy of itraconazole with terbinafin might be a treatment option. Voriconazole and posaconazole also have *in vitro* activity against these fungi [24,30,31].

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on Early Online on 01 February 2010.