

Case Report

Chromoblastomycosis due to *Fonsecaea pedrosoi*: an old wine in a rare bottle

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Abstract

Chromoblastomycosis is a chronic subcutaneous mycosis commonly caused by *Fonsecaea*, *Phialophora*, and *Cladophialophora* spp. Out of these, *Fonsecaea pedrosoi* is the most common etiological agent, implicated in 70%–90% of the cases reported worldwide. The histopathological diagnosis of chromoblastomycosis is based on visualization of medlar or sclerotic bodies in the tissue. These sclerotic bodies divide by planar division. Rarely, budding is seen in these sclerotic bodies. As this entity can be confused with phaeohyphomycosis, it is important to be aware of such a presentation also. We report two cases of chromoblastomycosis that showed budding sclerotic bodies.

Key words: chromoblastomycosis; *Fonsecaea pedrosoi*; sclerotic bodies.

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Introduction

Phaeohyphomycosis is a group of heterogeneous fungal infections caused by dematiaceous fungi. The infections in this group include superficial phaeohyphomycosis, subcutaneous phaeohyphomycosis, chromomycetoma, and chromoblastomycosis. The term chromoblastomycosis is exclusively used for a slow-growing chronic fungal infection involving the skin and subcutaneous tissue, which produces pigmented cells called sclerotic bodies or muriform bodies in the tissue [1].

The etiological agents of chromoblastomycosis are dematiaceous fungi which belong to the *Fonsecaea*, *Phialophora*, *Rhinochrysiella*, and *Cladosporium* genera [2]. These agents are common saprophytes found in the soil and vegetation. Infection is acquired by traumatic inoculation of the fungus into the exposed skin, usually by a thorn or a splinter. Although this condition is frequently reported from tropical and sub-tropical regions, it has a worldwide distribution. *Fonsecaea pedrosoi* is the most common species associated with chromoblastomycosis [3]. The histopathological diagnosis of chromoblastomycosis is based on visualization of medlar or sclerotic bodies in the tissue. These sclerotic bodies divide by planar division. Rarely, budding is seen in these sclerotic

bodies [4]. The presence of budding forms of sclerotic bodies can be confused with phaeohyphomycosis, which is defined as the presence of yeast-like cells, hyphal forms, or pseudohyphae-like elements in the tissue, without the presence of sclerotic bodies. We report two cases of chromoblastomycosis that showed budding sclerotic bodies in otherwise typical cases of chromoblastomycosis.

Case report 1

A 57-year-old male agriculturist presented to the outpatient department with an asymptomatic raised lesion on the anterior aspect of the right lower limb. The lesion had started as a small plaque near the ankle joint and progressed to the current size over two months. The patient did not give any history of traumatic injury in the region with the lesion. On examination, a 5 x 4 cm, single, oval, flesh-colored hypertrophic plaque was seen on the right lower limb (Figure 1). The surface of the lesion was cerebriform and was studded with black dots. A skin biopsy was taken from the lesion and sent for histopathological examination and fungal and mycobacterial culture.

The histopathological examination showed focal pseudo-epitheliomatous epidermal hyperplasia. Extensive dermal infiltrates of epithelioid histiocytes,

dense clusters of neutrophils, lymphocytes, occasional plasma cells, and eosinophils were seen. Brown pigmented structures resembling copper pennies, or medlar bodies, were seen surrounded by neutrophilic infiltrate (Figure 2).

Microscopic examination of the biopsy specimen using 10% KOH showed black, yeast-like structures 7–8 μm in size, with budding (Figure 3). The specimen was subcultured on Sabouraud dextrose agar (SDA) and incubated at 25°C and 37°C. A slowly growing black velvety colony was observed in the SDA slant incubated at 25°C after two weeks. Growth appeared in the slant incubated at 37°C after three weeks. The colonies were jet black, velvety, and embedded in the medium with an olivaceous reverse color. Microscopic examination of the culture showed

septate, dark brown hyphae with sub-erect conidiophores that were highly branched at the apices. Three types of conidiogenesis were seen: *Fonsecaea* type, showing primary and secondary conidia; *Cladosporium* type, showing branching chains of dematiaceous conidia; and *Rhinocladiella* type, which were sympodial with denticles. Based on the patterns of conidiogenesis, macroscopic features and the presence of barrel-shaped conidia, the fungus was identified as *Fonsecaea pedrosoi* (Figure 4).

The patient was started on oral itraconazole (tablet, 200 mg daily) and weekly cryotherapy for three months. The lesion regressed completely by the end of three months with post-inflammatory hyperpigmentation.

Figure 1. Hypertrophic plaque with cerebriform surface studded with black dots



Figure 3. Budding sclerotic bodies seen on 10% KOH mount resembling black yeasts

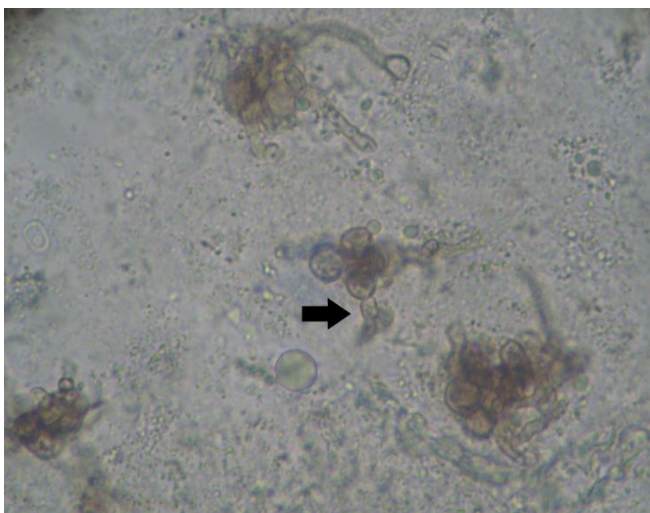


Figure 2. Hematoxylin- and eosin-stained section showing medlar bodies surrounded by neutrophilic infiltrate

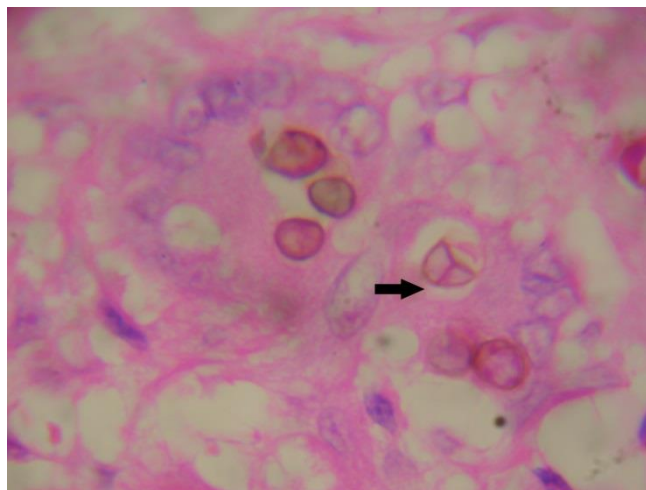
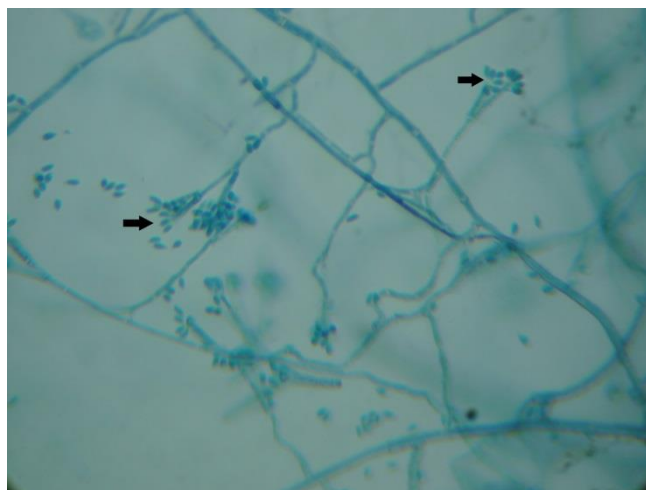


Figure 4. *Rhinocladiella* pattern of conidiation in *Fonsecaea* culture on lactophenol cotton blue mounts



Case report 2

A 58-year-old male patient presented with an asymptomatic raised scaly lesion on the dorsum of the right foot, of six months' duration. The patient gave a history of seeking treatment from multiple practitioners and application of multiple topical medications, none of which showed any effect on the lesion. On examination, a large hyperkeratotic plaque with areas of atrophy and depigmentation was seen. Histopathology of the skin biopsy showed broad bulbous acanthosis and hyperkeratosis with the dermis showing multiple thick-walled, rounded, "copper penny" bodies along with inflammatory cells.

Microscopy of the tissue sample in 10% KOH showed thick double-walled brown sclerotic bodies along with budding sclerotic bodies, which showed long pseudohyphal forms also. The fungal culture of the tissue grew a dematiaceous fungus, which was identified as *Fonsecaea pedrosoi* (Figure 6).

The patient was treated with oral itraconazole (tablet, 200 mg daily) and weekly cryotherapy for three months. The lesion had reduced in size after three months (Figure 5). The patient required three weeks of additional cryotherapy and itraconazole for complete regression of the lesion.

Discussion

Chromoblastomycosis is a rare, hard-to-diagnose disease commonly seen in the tropics, affecting males and rural workers, and predominantly involving the limb extremities [5]. Cutaneous chromoblastomycosis ensues following minor abrasions or trauma of the extremities, as the etiological agents are found as saprophytes in the soil and vegetation. One of our patients was an agriculturist who gave a history of a minor abrasion at the site where the lesion developed.

Clinically, the disease is usually asymptomatic and progresses slowly. Therefore, patients very often seek medical care years or decades after acquiring the infection or developing the skin lesion [6]. Early lesions start as an ulcer, nodule, or papule, and slowly progress to form plaques with verrucous appearance over years [7]. The disease is usually localized, but satellite lesions may develop as a result of autoinoculation during scratching or lymphatic dissemination.

The pathology of chromoblastomycosis is characterized by transepithelial elimination, a spontaneously occurring dermo-epidermal process. The black dots seen on the surface of the lesions are a consequence of damaged connective tissue, foreign matter, and the fungal etiologic agents in the dermis

Figure 5. Healed lesion with post-inflammatory hyperpigmentation (case 2)



Figure 6. *Fonsecaea*-type of conidiation in slide culture (case 2)



being expelled through the epidermis [8,9]. Both patients described here had cerebriform lesions studded with black dots, which was suggestive of chromoblastomycosis.

While the most common etiological agent is *Fonsecaea pedrosoi*, which was seen in our cases, other agents reported less frequently include *Cladosporium carrioni*, *Phialophora verrucosa*, *Rhinochrysiella aquaspersa*, *Fonsecaea compacta*, *Exophiala dermatitidis*, *Exophiala jeanselmei*, and *Exophiala spinifera* [10-14]. The diagnosis of *Fonsecaea pedrosoi* on microscopy is based on the four different patterns of conidiogenesis seen in microcultures. The *Fonsecaea* type of conidiogenesis shows primary sympodial conidiogenous cells giving rise to secondary and tertiary conidia, forming a complex conidial head. The *Cladosporium* type of

conidiogenesis shows primary shield-shaped conidia giving rise to long, branching oval chains of dematiaceous conidia. The *Rhinocladiella* type of conidiogenesis shows sympodial conidia with denticles, and the *Phialophora* type of conidiogenesis shows vase-shaped phialids with conidia at their apices. Isolates from both the patients described here showed the *Fonsecaea* type, *Cladosporium* type, and *Rhinocladiella* type of conidiogenesis. The *Phialophora* type of conidiogenesis, which is rarely seen, was not observed in these isolates [1].

Histopathologically, the diagnosis can be confirmed by the visualization of sclerotic or medlar bodies, which are aggregates of brownish-yellow cells with thick pigmented walls that divide by septation rather than budding in the parasitic phase [1]. These sclerotic cells are seen as the tissue stage of chromoblastomycosis agents, irrespective of the etiologic agent involved. They are highly resistant forms whose morphology is well known, but their physiology is obscure, as it is difficult to induce these tissue forms in traditional culture media [15]. Several studies have effectively demonstrated that a low pH, deprivation of Mn^{2+} or a supply of calcium are important in inducing sclerotic cell formation from chromoblastomycotic fungi [16-19]. In addition to this, sclerotic cells also result when polar growth (bud or hyphal development) is retarded, but isotropic cell enlargement continues unabated [15,20].

These sclerotic bodies usually divide by septation. Rarely, budding might be seen in these sclerotic bodies. Although a few reports of budding have been mentioned in *in vitro* studies where primary cultures containing hyphae and conidia have been converted into sclerotic cells in the presence of propranolol, case reports describing this characteristic are rarely seen in medical literature [15]. While we were able to observe septations in the sclerotic bodies in the histopathology sections, the 10% KOH mounts showed active budding of these sclerotic bodies. Production of budding (as well as hyphae) might be seen either from superficial samples, including scales and crusts in contact with air, or when the unfixed sample has been left for some time before processing. Some cells may have the appearance of budding because of the adherence of two globose cells, but growth by budding rarely occurs [10]. Correlation with histopathology and growth of *Fonsecaea pedrosoi* in the culture aided in the diagnosis of chromoblastomycosis. While sclerotic bodies are the hallmark of chromoblastomycosis, phaeohyphomycosis, caused by a diverse group of > 100 dematiaceous fungal species,

may present itself in the invasive form in tissue as phaeoid yeast-like cells, swollen cells, hyphal elements, pseudohyphal elements, moniliforme hyphae, or combinations of these [21]. The diagnosis of chromoblastomycosis may be missed if only budding sclerotic bodies are seen. Therefore, it is important to look for the planar forms of sclerotic bodies when budding black yeast-like cells are seen in tissues in order to differentiate phaeohyphomycosis and chromoblastomycosis.

Treatment of chromoblastomycosis is associated with variable cure rates of 15% to 80%, with *Fonsecaea pedrosoi* being less sensitive to antifungal therapy [7]. Non-comparative open clinical trials from Brazil have shown clinical and biological cure in 42% of patients having mild to moderate chromoblastomycosis who were given itraconazole for a mean duration of 7.2 months. Drug therapy involves the use of high doses of antifungal agents for a long duration. While early lesions respond well to surgical resection, extensive lesions are more responsive to antifungal therapy. However, in patients with dermal fibrosis and edema, the tissue antifungal levels may be reduced, leading to therapeutic failures or long courses of therapy [23]. Itraconazole and terbinafine have shown good *in vitro* activity against the causative agents of chromoblastomycosis [24-25]. Excision of small, localized lesions with a wide surgical margin can be successful [7]. Cryotherapy, involving the use of liquid nitrogen on lesions, freezes and destroys the diseased tissue. As the lesions were small in both the cases presented here, a combination of drug therapy with cryotherapy cured the patients at the end of four months. The second patient required three additional weeks of cryotherapy, as his lesion was more extensive and longer in duration compared to the first patient.

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