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Tropical medicine rounds

Chromoblastomycosis: a clinical and molecular study of 18 cases in Rio de Janeiro, Brazil

Marcelle de F. Mouchalouat[1], MD, MSc, Maria Clara Gutierrez Galhardo[1], MD, PhD, Rosely Maria Zancopé-Oliveira[2], PhD, Paulo Cezar Monteiro Fialho[2], MD, Janice Mery C. de Oliveira Coelho[3], MD MSc, Patrícia Morais Silva Tavares[2], DSc and Antonio Carlos Francesconi do Valle[1], MD, PhD

[1]Laboratório de Dermatologia, [2]Laboratório de Micologia, and [3]Laboratório de Anatomia Patológica, Instituto de Pesquisa Clínica Evandro Chagas (IPEC), Fundação Oswaldo Cruz, Brazil

Correspondence
Marcelle de F. Mouchalouat, MD, MSc
Rua Carlos Oswald 140, 1/105
Rio de Janeiro
Brazil
E-mail: marcelle.figueiredo@gmail.com

Abstract

Background Chromoblastomycosis (CBM) is a chronic subcutaneous mycosis caused by dematiaceous fungi.

Methods We described epidemiological data, clinical presentation, and treatment of 18 cases of CBM diagnosed in Rio de Janeiro, Brazil. Diagnosis was obtained by mycological, histopathological findings demonstrating typical muriform cells with confirmation of isolated by DNA sequencing of the ribosomal internal transcribed spacer.

Results The majority of patients were male (72.2%) ranging from 39 to 83 years old, farm laborers and construction workers. The duration of disease varied from four months to 32 years. The most common presentations were verrucous form in ten (55.6%) patients, followed by tumoral in three (16.7%) patients, primarily of moderate (55.6%) and severe (38.9%) intensity. Lower (44.4%) and upper limbs (33.3%) were the most affected sites. Fonsecaea pedrosoi isolated from 14 (77.8%), and Cladophialophora carrionii isolated from one case (5.6%). Fifteen patients (83.3%) were treated. Six patients (40%) received oral itraconazole 200–400 mg/day, five patients (33.3%) received oral itraconazole 200–400 mg/day combined with fluconazole 200 mg/day, and four (26.7%) patients were submitted to surgery. The duration of therapy varied from 12 to 48 months. Cure rate was 80% (12/15). No relapse was observed after two years of follow-up.

Conclusions Success was due to attending a center with specialized clinical care, laboratory support, and pharmaceutical care.

Introduction

Chromoblastomycosis (CBM) is a chronic subcutaneous mycosis caused by dematiaceous fungi with melanic-type pigment in their wall. These fungi are found in soil, plants, and plant debris. CBM has been reported on all continents, and most cases have been described in tropical and subtropical regions. Madagascar, Brazil, Japan, Australia, Mexico, and Venezuela are some important foci of this mycosis.[1–6] Worldwide, *Fonsecaea pedrosoi* is the most frequent agent, followed by *Cladophialophora carrionii* and *Phialophora verrucosa*. CBM mainly affects male individuals living in rural areas that work in contact with soil and suffer injuries. Diagnosis is often delayed for multiple reasons, including lack of basic health education and difficult access to outpatient clinics, both common to developing countries.[2] Clinical features vary, with the lesions classified as nodular, tumoral, verrucous, plaque, and cicatricial.[7] The verrucous form is the most common, with the lower limbs as the most frequent site, followed by upper limbs.[2,5,7,8] CBM is difficult to treat due to the long course of the disease and fibrosis of the lesions, resulting in poor response in most cases.

In Brazil, CBM is not a disease with mandatory reporting, and there are 520 cases of the mycosis reported in the literature. It has been described mainly in Pará, a State in Northern Brazil with agricultural activities as an important part of the economy and which accounts for 62.5% of all the country's cases. In this study, we report 18 cases of CBM from the State of Rio de Janeiro with confirmation of the dematiaceous fungal species isolated by DNA sequencing of the ribosomal internal transcribed spacer (ITS).

Materials and methods

This study was approved by the Research Ethics Committee of IPEC/FIOCRUZ.

The study included 18 patients with CBM treated from 1994 to 2008 at the Infectious Dermatology Outpatient Clinic of the Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil. All patients presented muriform cells, characteristic of CBM, in histological or mycological specimens. Lesions were classified according to location, clinical variety (nodular, tumoral, verrucous, plaque, and cicatricial)[7], and intensity (mild, moderate, or severe).[9] The mild form was defined as a single plaque or nodular lesion < 5 cm in diameter; moderate form as single or multiple nodular, verrucous, or plaque lesions < 15 cm on one or two adjacent skin areas; and the severe form as extensive lesions involving adjacent or non-adjacent skin areas and also including the tumoral and cicatricial types.

Biopsy of lesions was performed in all cases, and clinical specimens were analyzed through histological and mycological studies. Hematoxylin-eosin-stained histological sections and direct examination with 10% potassium hydroxide (KOH) were performed to detect the presence of dark brown muriform cells. Additional PAS and Grocott's methenamine silver stains were performed. Culture on Sabouraud 2% glucose agar isolated the agents, subsequently identified by slide culture microscopy (Riddel method). Before, during (every 2 months), and after antifungal treatment, the following laboratory tests were performed: complete blood count, blood glucose levels, and liver function tests for better control of possible adverse effects. Clinical, mycological, and histopathological evaluations were performed to demonstrate criteria for cure (sterile scars).

Ten isolates obtained in this study were also identified by molecular sequencing of the ITS1-ITS2 regions of the rDNA. DNA was prepared according to the method developed by de Andrade *et al.*[10] Two pairs of polymerase chain reaction (PCR) primers were used to amplify the rDNA, including ITS1, gene 5,8 S (rDNA), and ITS2 by PCR.[11] Primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were added to a final concentration of 0.2 mM each. Template DNA was added at a final concentration of 20 ng per 100 µl of the reaction mixture. Initial denaturation of template DNA was achieved by heating at 95 °C for 5 minutes, followed by 30 cycles of 30 seconds at 95 °C, 30 seconds at 58 °C, and 1 minutes at 72 °C. A final extension step was conducted for 10 minutes at 72 °C. Nucleotide sequencing of the PCR amplicons was performed with the Big Dye Terminator v.31 Cycle Sequencing kit using the ABI PRISM 3100 sequencer (Applied Biosystems, Foster City, CA, USA). DNA sequences were edited with BioEdit Sequence Alignment Editor (version 5.09; Tom Hall, Department of Microbiology, North Carolina State University, Raleigh, NC, USA) and aligned with those from other medically important dematiaceous fungi available at GenBank.

Table 1. Demographic and clinical features, time of evolution, treatment and follow-up of CBM cases enrolled in this study

Patient	Gender	Age (years)	Course	Clinical features/local	Intensity	Trial regimen (mg/day)	Length of treatment	Follow-up
1	Male	67	32 years	Verrucous + tumoral/extensive	Severe	ITZ 400 + FLC 200	48 months	Cure
2	Male	65	2 years	Tumoral/lower limb	Severe	[a]	–	–
3	Male	72	4 years	Verrucous/upper limb	Moderate	ITZ 400	19 months	Cure
4	Male	69	4 years	Verrucous + nodular/lower limb	Moderate	[b]	–	–
5	Female	42	20 years	Verrucous/lower limb	Moderate	ITZ 400 + FLC 200 TRB 500 + ITZ 400	[c]	In treatment
6	Male	47	11 years	Verrucous/extensive	Severe	ITZ 400 + FLC 200	44 months	Cure
7	Male	72	32 years	Verrucous/face	Severe	ITZ 400 + FLC 200	22 months	Cure
8	Male	73	15 years	Tumoral/lower limb	Severe	ITZ 400	12 months	Cure
9	Male	56	5 months	Plaque/upper limb	Mild	Surgery	–	Cure
10	Female	68	17 years	Tumoral/lower limb	Severe	ITZ 200 + FLC 200	36 months	Cure
11	Male	53	2 years	Verrucous/lower limb	Moderate	[a]	–	–
12	Male	39	5 years	Plaque/back	Moderate	Surgery	–	Cure
13	Female	55	8 months	Verrucous/lower limb	Moderate	Surgery	–	Cure
14	Female	77	12 years	Verrucous/upper limb	Severe	ITZ 200	4 months	Lost[b]
15	Male	45	9 months	Verrucous upper limb/	Moderate	ITZ 200	5 months	Cure
16	Female	36	4 months	Verrucous/lower limb	Moderate	Surgery	–	Cure
17	Male	61	11 months	Plaque upper limb/	Moderate	ITZ 200	11 months	Cure
18	Male	83	10 years	Verrucous upper limb/	Moderate	ITZ 200	10 months	In treatment

FLC, fluconazole; ITZ, itraconazole; TRB, terbinafine.
[a]Transferred to another service, treatment is not known.
[b]Died from another cause unrelated to CBM.
[c]Case 5: ITZ + FLC: 60 months; TRB + ITZ: 12 months.

Results

Epidemiological and clinical features

Demographic characteristics were: 13 males (72.2%) and five females (27.8%), with a mean age of 60 years (range: 36–83 years; Table 1). Regarding place of birth, 44.4% were natives of Rio de Janeiro, with the rest from other States of Brazil (Paraíba, Minas Gerais, and Piauí) and Portugal. As for place of residence, 16 patients (88.9%) lived in the State of Rio de Janeiro and two in other States (Piauí and Paraíba). Thirteen (72.2%) patients reported contact with soil: 10 (77%) during work activities (farm laborers, construction workers, gardeners) and three (23%) in recreational activities. Only five (27.8%) reported that the lesions had appeared after injuries with plants or wood. The course of the disease ranged from four months to 32 years. The most frequent location was in the lower limbs, in eight patients (44.4%), followed by upper limbs, in six patients (33.3%). Other sites were the back and face, in one patient each (5.6%). Two patients (11.1%) presented extensive lesions. The most frequent clinical variety was verrucous, in 10 (55.6%) patients (Fig. 1), followed by the tumoral and plaque forms, in three cases each (16.7%; Figs 2 and 3). A combination of two clinical forms was seen in two cases (11.1%). Regarding intensity, the moderate form was the most common, found in 10 (55.6%) patients, followed by severe in 7 (38.9%) cases and mild in only one case (5.6%). Symptoms included itching in five cases (27.8%) and pain in three cases (16.7%). Bacterial secondary infection was the most frequent complication (22.2%), followed by myiasis in 5.6%.

Associated diseases and immunosuppressive conditions

Arterial hypertension was seen in nine patients (50%), diabetes mellitus in four (22.2%), and cerebral cysticercosis in one (5.6%). Three patients presented CBM after beginning immunosuppressive drugs (16.7%): case 13 (tacrolimus 2.5 mg/day, mycophenolate mofetil 2 g/day, and prednisone 10 mg/day for two years) and case 16 (tacrolimus 5 mg/day and prednisone 10 mg/day for seven months) after kidney transplantation, and case 15 (prednisone 15 mg/day for one year) due to reactional leprosy.

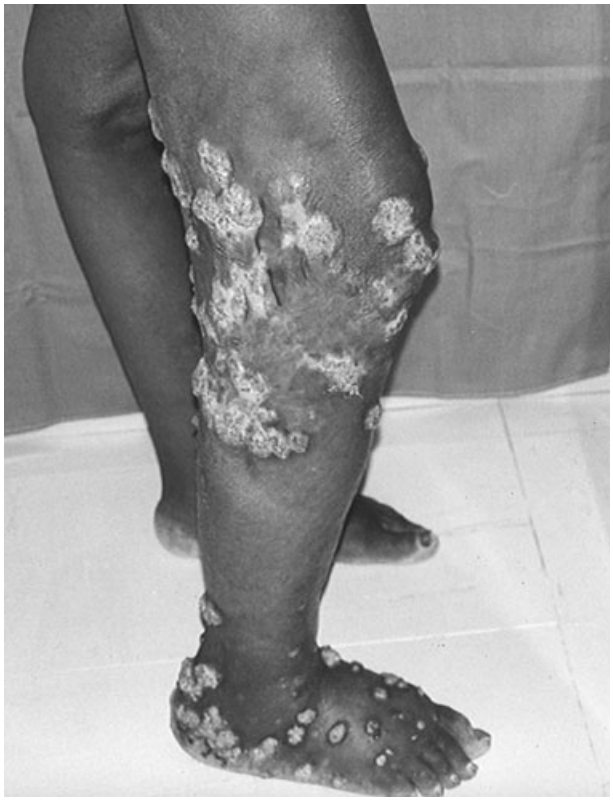

Figure 1 Case 1: verrucous and tumoral form of severe CBM with 32 years evolution located in right leg

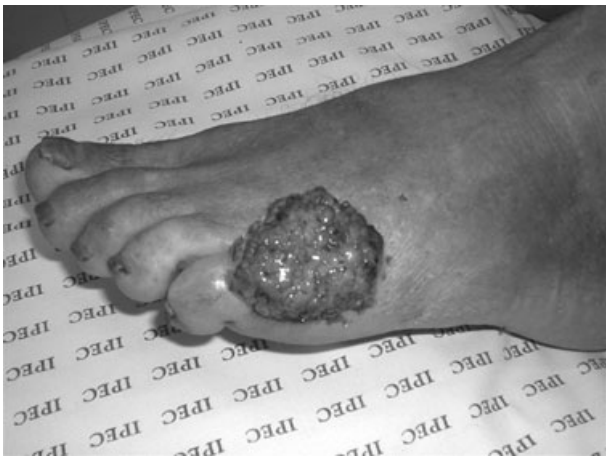

Figure 2 Case 2: tumoral form of CBM on the dorsum of the right foot

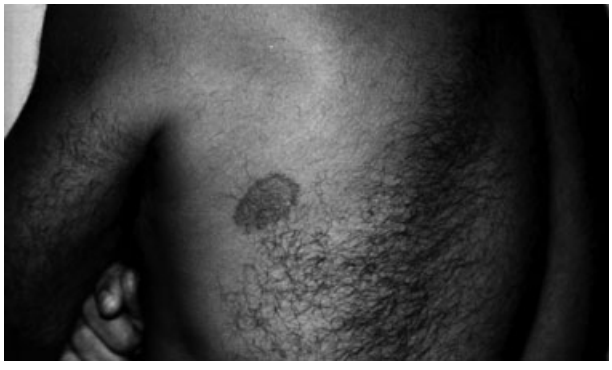

Figure 3 Case 12: plaque form of CBM on the back

Laboratory diagnosis

Direct mycological tests were positive in 12 of the 18 cases (66.7%). The primary causal agent was *F. pedrosoi* in 77.8% (14/18). *C. carrionii* was identified in only one patient (case 8). It was not possible to identify one strain by conventional methodologies, and two cases were culture-negative. Concerning histopathological findings, all patients presented chronic inflammatory infiltration and granulomatous process with microabscesses and inflammatory cells showing dark brown muriform cells.

Ten culture isolates were identified by molecular sequencing data from the rDNA ITS1-ITS2 and the 5.8S. The GenBank search demonstrated that the sequences from nine isolates (19571/184, 24687, 185, 19-112, 25811, 28656, 28479, 25543, and 28457) showed 98–100% similarity with *F. pedrosoi*, as previously described and deposited in the GenBank (accession number AB091205, AB117980, AB117978),[12] and one (28358) showed 91% homology with *C. carrionii* (accession number AB0109171.1),[13] thus supporting the morphological identification by mycological procedures.

Treatment and follow-up

Fifteen patients (83.3%) received clinical or surgical therapy (Table 1). In 11 patients (73.3%), clinical regimens were administrated as follows: monotherapy with oral itraconazole (ITZ) 200–400 mg/day in six cases (40%) and combined with fluconazole (FLC) 200 mg/day in five cases (33.3%). Case 5 was treated with ITZ 400 mg/day plus FLC 200 mg/day for five years. The patient was lost to follow-up and returned last year, when terbinafine (TRB) 500 mg/day plus ITZ 400 mg/day was introduced. The patient improved but has still not completed her treatment. Duration of antifungal therapy ranged from 5 to 48 months. Adjuvant cryotherapy was performed in two cases (20 s in two cycles/month for six months; cases 3 and 5).

Surgery was performed in four cases (26.7%) presenting small single lesions accessible to surgical excision (cases 9, 12, 13, and 16), including immunosuppressed patients.

Cure was observed in 12 (80%) of 15 cases that completed treatment, with no relapses after two years of follow-up. Two patients (13.3%) have still not completed treatment but have shown significant clinical improvement.

Three patients were not treated at IPEC/FIOCRUZ, and their treatment is unknown. Another patient died from causes unrelated to CBM.

Discussion

This study reports CBM in Rio de Janeiro, a State of Brazil, where this disease is rare. At IPEC/FIOCRUZ, a reference center for Infectious Diseases, CBM represented approximately 2% of all subcutaneous mycoses diagnosed during the study period, representing the second most common mycosis after sporotrichosis.

In this study, the cases were primarily males, older in age, and with extended disease progression and moderate and severe forms of CBM and comorbidities. Verrucous lesions were the most common clinical form. Most of the patients reported contact with soil.

CBM can usually course for many years with minimal discomfort, and medical attention is generally sought due to complications of the mycosis, including secondary infection, cosmetic issues, or elephantiasis. In this study, as expected, there was a predominance of males (72.3%) with CBM, probably because they are more involved in activities that expose them to the etiological agent. Even so, in Pará State, Brazil, where women work in contact with soil and have the same work-related risks as men, Silva *et al.* found only 6.7% of women among 325 cases of CBM. In Japan the rate difference according to gender is minimal.[3] In Brazil there are no reported cases of CBM in children and adolescents, contrary to Falcón State in Venezuela.[6] The differences are probably due to species specificities: while in Brazil the predominant etiological agent is *F. pedrosoi*, in the group reported by Pérez-Blanco *et al.* it was *C. carrioni*. Additionally, in Brazil mycological diagnosis is rarely performed in younger individuals.

The most common lesion sites were on the lower limbs, as in other case reports in Brazil[8] and elsewhere in the world.[5] Brazilian rural workers rarely wear appropriate personal protective equipment (boots and long pants) and often suffer injuries. In Venezuela patients commonly have a history of contact with *Cactaceae* plants and farm fences made of tree trunks, which could explain the location of lesions on the upper limbs.[6]

In Pará State, Brazil, *F. pedrosoi* was isolated from a patient's lesion and from the plant *Mimosa pudica* after an injury with the plant's thorns.[14] In Maranhão, Brazil, Marques *et al.*[15] reported isolation of a dematiaceous fungus from the babassu palm, a typical tree in this State and an important source of raw materials, thus acting as a probable risk factor for infection.

F. pedrosoi and surprisingly *C. carrionii*, an extremely rare agent in Brazil, were isolated in our study. This fact reinforces the importance of culture methods for the isolation of CBM agents.[16] Concerning histopathology, we found no characteristics that differed from other reports in the literature.[17]

Because CBM can be caused by different agents, molecular diagnosis based on PCR, a DNA-based methodology with high sensitivity and specificity, can be an important tool for confirming species identification of isolates, identifying strains when this is not possible by morphological methods, and probably also identifying new species.[10,13]

In this study, extended therapy with azoles (12–60 months) showed good tolerability. Combination therapy (ITZ and FLC) was used for severe forms and was suggested by our previous good experience in a case of entomophthoramycosis that had been treated unsuccessfully with other drugs.[18] Other authors have also reported successful treatment for other mycoses.[19,20] Both azoles act by inhibiting ergosterol synthesis, differing only in solubility and half-life. We have also used TRB and ITZ with good results in a patient presenting low compliance with antifungal therapy. This regimen was used by Gupta *et al.*[21] with good results in four patients with chronic CBM and poor response to several treatments. Cryotherapy with liquid nitrogen has been used in combination with azoles and proved beneficial in some patients, for example in the series by Castro *et al.*,[22] who used the technique in 22 patients with CBM with a longer freezing time (30 s–4 min in two cycles) than in our study and showed a 40.9% cure rate.

A remarkable aspect of the current study was that three patients (16.7%) were immunosuppressed but despite their condition presented moderate forms of CBM.[23] These patients had attended the outpatient clinic regularly due to their underlying diseases (kidney transplantation and leprosy), and because CBM shows a chronic course, the diagnosis was performed at earlier stages, and the single lesions allowed us to perform successful surgical excision. It is important to recall that ITZ is contraindicated in combination with tacrolimus. CBM-associated immunosuppressant conditions were reported in patients with systemic lupus erythematosus in use of prednisone and in renal transplant patients.[23,24] It would be interesting to study this type of association between immunosuppressant conditions and predisposition to CBM.

In this study, the cure rate among treated patients was high (80%), including all the severe forms, and no relapses were reported in two years of follow-up. Bonifaz *et al.*[5] observed cure in 31% of 51 patients, and Queiroz-Telles *et al.*[7] reported cure in four (44%) of nine patients with the severe form.

We believe that the successful results were due to the fact that patients were attending a center with specialized clinical care and laboratory support, with the capacity to diagnose and monitor infectious diseases, combined with pharmaceutical care providing the drugs required for the patients' complete therapy. Diagnosis in the early stages of the mycosis is also important, when the lesions are still small and can be treated promptly.

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