Brazilian Journal of Development

Chromoblastomycosis and Chagas' disease: a case study in the Brazilian Northeast

Cromoblastomicose e doença de Chagas: um estudo de caso no nordeste brasileiro

DOI:10.34117/bjdv5n10-274

Recebimento dos originais: 10/09/2019
Aceitação para publicação: 22/10/2019

Yankee Costa Magalhães Diniz
Pós-graduanda no Programa de Ciências da Saúde / PPGCS pela Universidade Federal Maranhão.
Instituição: Universidade Federal Maranhão - UFMA
Endereço: Av. dos Portugueses, 1966 - Vila Bacanga, São Luís - MA, Brasil.
E-mail: yankeecm@gmail.com

Eudes Alves Simões Neto
Médico Infectologista pelo Instituto de Infectologia Emílio Ribas - São Paulo.
Instituição: Universidade Federal Maranhão - UFMA
Endereço: Estrada Pinheiro/Pacas, Km 10, s/n, Enseada. Pinheiro - MA, Brasil.
E-mail: eudesimoes@gmail.com

Maria Rosa Quaresma Bomfim
Pós-Doutora em Ciências Biológicas pela Universidade Federal de Minas Gerais.
Instituição: Universidade CEUMA
Endereço: Rua Josué Montello, 01, Renascença II, São Luis - MA, Brasil.
E-mail: mrqbomfim@gmail.com

Patrícia Cristina Ribeiro Conceição
Mestre em Biologia Parasitária pela Universidade CEUMA
Instituição: Hospital Universitário da Universidade Federal do Maranhão, HUUFMA
Endereço: R. Barão de Itapari, 227 - Centro, São Luís – MA, Brasil.
E-mail: patriciacsribeiro@hotmail.com

Raimunda Ribeiro da Silva
Doutora em Fisiopatologia pelo DINTER/FISCLINEX (UFMA/UERJ)
Instituição: Universidade Federal Maranhão – UFMA
Endereço: Av. dos Portugueses, 1966 - Vila Bacanga, São Luís - MA, Brasil.
E-mail: rr.silva@ufma.br

Sirlei Garcia Marques
Doutora em Ciências Biológicas (Microbiologia) pela Universidade Federal de Minas Gerais UFMG
Instituição: Hospital Universitário da Universidade Federal do Maranhão, HUUFMA
Endereço: R. Barão de Itapari, 227 - Centro, São Luís – MA, Brasil.
E-mail: sirleigmarques@gmail.com

Brazilian Journal of Development

Vânia Aparecida Vicente
Pós-doutora em Taxonomia Molecular de Fungos pelo Instituto CBS (Centraalbureau voor Schimmelcultures- Fungal Biodiversity Centre) na Holanda.
Instituição: Universidade Federal do Paraná, UFPR, Curitiba, Brasil. Jardim das Américas, Curitiba, PR – Brasil.
E-mail: vicente@ufpr.br

Conceição de Maria Pedrozo e Silva de Azevedo
Doutora em Ciências Biológicas (Microbiologia) pela UFMG.
Instituição: Universidade Federal Maranhão – UFMA
Endereço: Av. dos Portugueses, 1966 - Vila Bacanga, São Luís - MA, Brasil.
E-mail: conceicaopedrozo@gmail.com

ABSTRACT

Background: Chromoblastomycosis is a disease caused by melanized fungi that have a slow evolution, and the disease may be chronic due to a lack of treatment at the onset of the disease. Besides chromoblastomycosis, other neglected diseases primarily affect people of low-income living in rural areas. The objective of this study was to analyze a case of coinfection with *Trypanosoma cruzi* and *Fonsecaea pedrosoi* in a patient with these agents. Methods: For the diagnosis of chromoblastomycosis, a biopsy of the lesion site was performed, and fungal tests were performed with KOH, cultured on Sabouraud Dextrose and Mycobiotic Agar. A microculture on the Potato Agar Dextrose was performed, followed by histopathology stained with hematoxylin-eosin. The diagnosis of Chagas disease, with the examination of gout on a slide, and staining using the Walker method. Results: The thick drop blood test was positive for Trypanosoma cruzi. Additionally, muriform brown corpuscles were visualized in the direct mycological biopsy, and also Fonsecaea pedrosoi was identified through the culture and microcultivation and sequencing, being thus diagnosed the chromoblastomycosis. **Conclusions:** Chromoblastomycosis and Chagas disease are neglected diseases in Brazil, especially in the states of the Northeast and North regions, where the prevalence of cases is still high.

Keywords: Chromoblastomycosis; Fonsecaea pedrosoi; Chagas Disease; Trypanosoma cruzi.

RESUMO

Antecedentes: A cromoblastomicose é uma doença causada por fungos melanizados que têm uma evolução lenta, e a doença pode ser crônica devido à falta de tratamento no início da doença. Além da cromoblastomicose, outras doenças negligenciadas afetam principalmente pessoas de baixa renda que vivem em áreas rurais. O objetivo deste estudo foi analisar um caso de coinfecção com Trypanosoma cruzi e Fonsecaea pedrosoi em um paciente com esses agentes. Métodos: Para o diagnóstico de cromoblastomicose, foi realizada uma biópsia do local da lesão e foram realizados testes fúngicos com KOH, cultivado em Sabouraud Dextrose e Agar Micobótico. Foi realizada uma microcultura no Dextrose de Agar de Batata, seguida de histopatologia corada com hematoxilina-eosina. Diagnóstico da doença de Chagas, com exame de gota em lâmina e coloração pelo método Walker. Resultados: O exame de sangue com gota espessa foi positivo para Trypanosoma cruzi. Além disso, os corpúsculos marrons muriformes foram visualizados na biópsia micológica direta, e também Fonsecaea pedrosoi

Brazilian Journal of Development

foi identificado através da cultura e microcultivação e sequenciamento, sendo assim diagnosticada a cromoblastomicose. Conclusões: A cromoblastomicose e a doença de Chagas são doenças negligenciadas no Brasil, principalmente nos estados das regiões Nordeste e Norte, onde a prevalência de casos ainda é alta.

Palavras-Chaves: Cromoblastomicose; Fonsecaea pedrosoi; Doença de Chagas; Trypanosoma cruzi.

1 INTRODUÇÃO

Chromoblastomycosis is a disease caused by melanized fungi that damage the epidermis and dermis and have a polymorphic appearance [1]. The disease affects mainly the lower limbs, because the inoculation of the fungus occurs through contaminated vegetation, primarily infecting people who perform agricultural activities [2]. It is a disease with a slow evolution. It gradually increases from a pink papule at the site of fungal inoculation, causing many delays in treatment and leading to chronic disease [3]. The lesions evolve slowly and, if not treated, can reach the lymphatic pathway, leading to loss or functional disability of the affected limb, with tissue fibrosis that evolves to carcinomas and lymphedema [4].

The main agent of chromoblastomycosis is a fungus of the genus *Fonsecaea*, although other agents include *Cladosporium Rhinocladiella*, *Phialophora Cladophialophora*, *Rhinocladiella, Exophiala, Veronaea* and *Cyphellophora* [3,5].

Chromoblastomycosis is a cosmopolitan disease with a higher incidence in tropical and subtropical regions. Several areas are endemic, including Japan, Southeast Asia, Australia, Madagascar, South America and Central America. Brazil is an endemic area, with the disease being observed in all states and with an estimated prevalence of 1/196 thousand inhabitants [3]. In Maranhão State, the disease is of epidemiological importance. There are endemic areas where the population goes without diagnosis and without treatment. The population includes low-income inhabitants of the rural area who survive on the subsistence economy [6].

Cases of Chagas disease occur mainly in populations of rural and low-income areas. It is caused by the protozoan *Trypanosoma cruzi*, which is transmitted by approximately 130 species of triatomids (hematophagous) of the family *Reduviidae*, which are popularly known in Brazil as "barbers". Among the endemic parasitic diseases, it is the third most common in the world after malaria and schistosomiasis [7,8].

According to the World Health Organization (WHO), Chagas disease is one of the greatest public health problems in Latin America, because it has disabling effects and its

mortality rate can range from <10% to > 80% [9]. It mainly affects people living in rural areas and in precarious accommodations [8].

Cases of infection by the protozoan occur worldwide, and it is estimated that between 6 and 7 million people are affected. Among these, 5 million are in Latin America, and 12,000 people die annually. In Brazil, it is currently estimated that from 1 to 4.6 million people are infected, and approximately 6,000 deaths occur per year [10,11,12].

Maranhão is not considered an endemic state for Chagas disease, but there has been a significant increase in the number of cases, although not all are adequately reported to institutions. Currently, the institutions responsible for the registration of cases are the Information System of Notifiable Diseases (SINAN) and the National Health Foundation (FUNASA) [13]. There were few reports on the prevalence data of Chagas disease in Maranhão in the National Survey from 1978 to 1980 SUCAM (National Health Foundation). In the population of the Northeast, the highest prevalence was in Bahia (7.40%) and the lowest was in Maranhão (0. 20%) [14].

This study reports the first case of coinfection by *Trypanosoma cruzi* and *Fonsecaea pedrosoi* in Brazil. It is important to emphasize the need for further studies on these two pathogens that cause neglected diseases in Brazil. Researchers should obtain more knowledge about the care, prevention and treatment of such diseases, which may enable the planning of strategic actions within social and health programs for rural communities. This could reduce the incidence of these diseases and result in treatments being performed systematically and continuously.

2 METHODS

2.1 CLINICAL DATA

Case study of chromoblastomycosis and Chagas disease in a patient attended at the CREDIP (Reference Center of Infectious-Parasitic Illnesses) of the Universidade Federal do Maranhão (UFMA) from February 2017 to August 2018. This is a Reference Center in Maranhão for chromoblastomycosis. The patient is a 56-year-old farmer residing in the municipality of Pedro do Rosário, Maranhão State. The clinical picture started with a low, daily, evening fever associated with dyspnea and a dry cough. Besides, after 15 years of the infection, the patient presented on the lower limb D, a large plaque vegetative and verrucous acquired due trauma in the field.

We sought to understand the clinical evolution of a patient with chromoblastomycosis and Chagas disease and to follow the clinical response to medications used for chromoblastomycosis infection and Chagas disease.

2.2 MICROBIOLOGICAL AND HISTOLOGICAL EXAMS

Mycological and histopathological examinations were performed at the time of the initial clinical diagnosis and repeated with 12 months of treatment, including another biopsy to evaluate the local inflammatory response. Data were recorded by the outpatient service and included sociodemographic, clinical and therapeutic variables, as well as the description of histopathological findings.

The clinical samples were collected by punch tissue biopsy using local anesthesia (5% lidocaine). Samples were packed in a sterile glass tube containing 2 ml of saline solution at 0.85% and transported to the microbiology laboratory in a thermal box, as recommended by [15]. The choice of the biopsy site was determined by the presence of black spots in the lesions. The fragments were used to perform direct mycological examination and culture for fungi. The direct examination was performed with potassium hydroxide (KOH) at 20%, and Sabouraud Dextrose agar Difco™ medium (SDA) and Mycobiotic agar BBL™ were used for culture. The cultures were incubated at 28°C to 37°C in an incubator. The fungus was identified based on the macromorphology of the colonies and the micromorphology of conidiogenesis based on the microcultivation technique [16]. For the micromorphological examination, potato Dextrose Difco™ agar was used (PDA). Two PDA cubes were cut with a sterile number 15 scalpel blade and placed on the surface of a glass blade wrapped inside a Petri dish and packed at room temperature for 12 days [16]. During this period, they are assembled on slides with lactophenol-blue cotton.

In the preparation of the biopsy material for histopathological examination, the samples were embedded in paraffin, followed by staining with hematoxylin-eosin (HE).

2.3 Examination for Chagas disease.

For the thick drop test, two drops of blood of approximately 25 µL were used, arranged side by side on the blade to form a square (1 cm ² in diameter) using the tip of another blade. Then, the blade was dried at room temperature and stained.

The thick drop was stained with the Walker method. First, 0.1 ml of methylene blue solution was added to the drop for 2 seconds. The blade was then washed with buffered water, and on

the inverted blade, Giemsa dye was used, with staining for 10 minutes. After staining, the blade was washed with buffered water and left to dry. To visualize the protozoan, an optical microscope was used with a 100x objective under immersion oil.

2.4 MOLECULAR IDENTIFICATION OF THE FONSECAEA PEDROSOI ISOLATES BY THE POLYMERASE CHAIN REACTION AND SEQUENCING

To confirm the phenotypic identification of the *Fonsecaea pedrosoi* isolate the genetic material was extracted with the MagaZorb® DNA Kit (Promega, USA) according to the manufacturer's protocol. PCR amplification was performed in Mycycler Biorad model 580BR3578 thermocycler in a final volume of 25 µl containing 10 picomoles of each primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) previously described [17], plus 12.5 µL PCR Master Mix - Promega® [Taq DNA polymerase (dNTPs, MgCl 2, PCR buffer [pH 8.5]), 1.5 µl nuclease-free water and 100 ng of template DNA. The amplification occurred under the following conditions: 94ºC for 5 minutes (initial denaturation) followed by 30 cycles of 94ºC for 1 minute, 58ºC for 1 minute and 72ºC for 1 minute and a final extension step at 72ºC for 7 minutes.

The PCR product was purified with the Wizard® SV Gel and Clean-Up System (PROMEGA) following the guidelines of the manufacturer.

The sequencing of the purified product was done by the dideoxynucleotide chain termination method [18], in both directions of the double-strand (positive and negative sense) with the ABI Prism BigDye Kit in the sequencer. 3130 Genetic Analyzer (Applied Biosystems). The sample was sequenced at least twice in each direction of the tape, making a total of four sequences from the same sample.

3 CASE REPORT

The patient is a 56-year-old farmer born in Pedro do Rosário, located in the Baixada Maranhense. The initial clinical signs were a low fever, daily, in the evening, with dyspnea and a dry cough. He also had mild abdominal pain. The symptoms persisted for two weeks, and he sought medical care and was hospitalized. He lives in a Taipa house in a Quilombola community that is difficult to access. He ingested bacaba juice (*Oenocarpus bacaba* Mart.) (Figure 1) 10 days before the onset of symptoms during a home party. Physical examination showed bilateral cervical and axillary lymphadenopathy, approximately 1.5 cm, mobile, elastic and discretely painful. Cardiac and respiratory auscultation were normal. The

examination of the abdomen showed the liver at the height of the costal rim with smooth edges and no pain on palpation. Traube's space was occupied, with a palpable spleen only after Schuster's maneuver. The skin had vegetative lesions (verrucous) measuring more than 10 cm. The lesions had an erythematous base with areas of whitish scaling and blackened hyperpigmentation.

Two thick-drop exams were performed to diagnose Chagas disease with a positive result for trypanosomiasis (Figure 2). Electrocardiogram (ECG) was performed and showed a right bundle branch block, and the chest x-ray showed a normal heart with no signs of congestion. Treatment with benznidazole at 100 mg was initiated, and the patient took 2 tablets in the morning and 1 tablet at night. There was a complete regression of symptoms in one week, and the treatment lasted 60 days.

The patient had a vegetative plaque lesion and extensive verrucous in the right lower limb (Figure 3). The disease was acquired after trauma working in the field and had been chronic for 15 years. The patient had more than 10 lesions of 12.5 cm, and had moderate disease. The fungus was studied with KOH and muriform brown corpuscles were visualized (Figure 4A).The fungus was identified as *Fonsecaea pedrosoi* in microculture on a blade with cotton blue and the patient was diagnosed with chromoblastomycosis (Figure 4B). This was confirmed with histopathology using (HE) staining. Histopathological included intraepithelial microabscesses with polymorphonuclear leukocytes, lymphocytes, histiocytes and muriform corpuscles (Figure 5A). The patient started treatment for chromoblastomycosis with 2 tablets of itraconazole (200 mg/day) after lunch and dinner. After 6 months of treatment, the dose was increased to 400 mg/day after lunch and dinner.

3.1 ETHICS STATEMENT

The present study was submitted to the Research Ethics Committee of the Universidade Federal do Maranhão (UFMA), and it was approved under a consubstantiated opinion number: 1.276.342. All presentations of the case report were consented to by the patient, having been authorized through a Free and Informed Consent Term (TCLE).

4 RESULTS

4.1 CLINICAL DATA

In the present study, the patient presented clinical signs and symptoms characteristic of Chagas' disease, such as fever, dyspnea and dry cough, after 10 days of consumption of

bacaba juice contaminated with the protozoan. The thick drop blood test was positive for *Trypanosoma cruzi*, and the patient was treated for Chagas disease in the acute phase with benznidazole and progressed well.

4.2 HISTOPATHOLOGICAL FINDINGS

Histopathological findings of the biopsy of the lesion showed a marked number of microabscesses, of fungus located in the corneal layer of the skin, and polymorphonuclear and pseudoepitheliomatous hyperplasia (Figure 5A)

After 6 months of treatment for chromoblastomycosis with 2 tablets of itraconazole (200 mg/day) was prescribed an increased dose of itraconazole (400 mg/day) after lunch and dinner. A new biopsy of the lesion to evaluate the clinical findings showed a slight reduction of the load scores of the fungus, granulomas with lymphocytes, histiocytes, multinucleated giant cells and muriform corpuscles (Figure 5B). The patient continues to be treated for chromoblastomycosis, and a new clinical evaluation was performed, and the reduction of the lesion was observed with the formation of scar tissue around the plaque (Figure 6).

4.3 PCR AND SEQUENCING

The sequencing result of the purified PCR product identified the isolate as *Fonsecaea pedrosoi*. The obtained sequence showed 99% similarity with this fungus, and the sequence was deposited in Genbank under number MN461171.

5 DISCUSSION

Chagas disease may be one of the main causes of sudden death, arrhythmias and heart failure [8]. Different species of triatomines are considered the vectors of *Trypanosoma cruzi*. The triatomines make their nest in the açaí palm and are transported together during collection, allowing contamination by protozoa due to lack of sanitary care during the preparation of the juice, where the triatomines are crushed during preparation [19]. The bacaba fruit (*Oenocarpus bacaba* Mart.) is very similar to açaí (Euterpe oleracea), which has been involved in several outbreaks of Chagas disease in the states of Pará and Amapá [7]. This was corroborated by [13] in a study in Maranhão, where the data obtained from FUNASA showed that the main form of transmission in this period was oral (n = 23/62. 16%).

After infection, Chagas disease has two phases: acute, lasting 2 months [20], and chronic, which lasts throughout life [19]. Notably [8], benznidazole is the first-choice

treatment in cases of Chagas disease, as it results in better treatment efficacy and fewer adverse effects. This is because it acts by interrupting protein synthesis and damaging the formation of the parasite. According to [13], in Maranhão the SINAN and FUNASA demonstrated that most people survive Chagas disease when treated in the acute phase. Most patients were cured even if the response to treatment was not immediate.

Regarding the distribution of Chagas disease among the regions of Maranhão, it was found in the FUNASA database that the northern Mesoregion had the highest number of cases of the disease, 16 cases (43.24%), distributed among six municipalities. The municipality of Pinheiro, located in Baixada Maranhense, was the municipality of the North Mesoregion with the highest number of cases of infection, 9 cases (56.25%) [13]. Similar data were obtained for chromoblastomycosis, where most of the reported cases occurred in Baixada Maranhense [21]. It was verified that the state of Maranhão is an endemic region for chromoblastomycosis, especially the area of Baixada Maranhense [22]. Regarding the primary lesion of chromoblastomycosis, [23] the lesion develops at the site of inoculation, usually in one of the extremities, and remains localized for years. which may represent the attempt of the immune system to perform transepithelial elimination [24]. However, elimination did not occur, because the lesion was 15 years old and the disease was already in a moderate stage with characteristics of a mixed granuloma (suppurative and epithelioid). Therefore, it was necessary to treat with itraconazole and follow-up the clinical evolution.

The treatment of choice for chromoblastomycosis is itraconazole, because it has better results at high doses over 6 to 12 months. However, severe disease is often refractory to treatment [1].

6 CONCLUSIONS

Chromoblastomycosis and Chagas disease are neglected diseases in Brazil, especially in the states of the Northeast and North regions, where the prevalence of cases is still high. Environmental and socioeconomic conditions favor the occurrence of new outbreaks annually. A large part of the populations of states located in the Amazon region, as in the case of Maranhão, still have plant extracts (wood, leaves and fruits) as a main source of income. People generally live in "pau-a-pique" houses that are covered with straw, and they have poor nutrition, providing favorable conditions for infection by Chagas disease and chromoblastomycosis. However, in this scenario, the lack of adequate conditions for the collection and handling of these natural products also led to the contamination of consumers,

not only in the field but also in large urban centers. Health education programs and standardized basic hygiene procedures in the handling of these foods should be provided to those that deal with the preparation of the açaí and bacaba pulps, which have been presented as an important vehicle for contamination by the protozoan that causes Chagas disease.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

Funding

No financial support.

Authors' contributions

YCMD preparation, isolation in culture media, microculture preparation, reading of mycological slides, preparation of histopathology, recording of images and collection of epidemiological data. EASN performed the care, diagnosis and treatment of Chagas Disease, performed the follow-up and obtained the medical history of the patient and collected data in the municipality of Pedro do Rosário. PCRC performed direct mycological reading on the slide with KOH. MRQB analysis and interpretation of data, drafted the manuscript. RRdaS performed a biopsy on a slide with HE staining and a histopathological description. SGM identified the fungus based on the macromorphology of the colony and the micromorphology of the conidiogenesis in microculture and stained in lactophenol-blue cotton. VAV confirmed the identification of the isolated fungus.

CMPSA performed patient care, biopsy collection, diagnosis, treatment for chromoblastomycosis and patient follow-up.

ACKNOWLEDGEMENTS

The authors thank technical staff from Labóratório Cedro, São Luis-MA.

REFERENCES

Antonello VS, Silva MCA da Cambruzzi E, Kliemann DA, Santos BR, Queiroz-Telles F. Treatment of severe chromoblastomycosis with itraconazole and 5-flucytosine association. Rev. Inst. Med. Trop. São Paulo. 2010; 52: 329-331.

Correia RTM, Valente NTYS, Criado PR, Martins JEC. Cromoblastomicose: relato de 27 casos e revisão da literatura. An Bras Dermatol. 2010; 85: 448-54.

Gomes RR, Vicente VAV, Azevedo CMPS de, Salgado CG, Silva MB da S, Queiroz-Telles F, Marques SG, Santos DWCLS, Andrade TS de, Takagi EH, Cruz KSC, Fornari G, Hahn RC, Scroferneker ML, Caligine RB, Ramirez-Castrillon M, JO DP de A, Heidrich D, Colombo A L, Hoog G S de. Molecular Epidemiology of Agents of Human Chromoblastomycosis in Brazil with the Description of Two Novel Species. PLOS Neglected Tropical Diseases. 2016; 10: 1-20.

Minotto R, Bernardi C D, Mallmann L F, Edelweiss MI, Scroferneker M L. Cromoblastomycosis: A review of 100 cases in the state of Rio Grande do Sul, Brasil. J. Am. Acad. Dermatol. 2001; 44: 585.

Queiroz-Telles F, Santos DWC, Azevedo C de MP. Fungal infections of implantation (chromoblastomycosis, mycetoma, entomophthoramycosis, and lacaziosis). In Hospenthal D, Rinaldi MG (ed), Diagnosis and treatment of fungal infections, 2nd ed. Springer International Publishing, Basel, Switzerland. 2015. p 271–276.

Queiroz-Telles, F, Hoog S de, Santos, DWCL, Salgado CG, Vicente VA, Bonifaz A, Roilides E, Xi L, Azevedo CM, Silva da MB. Chromoblastomycosis. Clin. Microbiol. Rev. 2017; 30: 233–276.

Xavier S C das C, Roque ALR, Bilac D, Araujo VAL de, Neto SF da C, Lorosa ES, Silva LFCF da S, Jansen AM. Transmissão *Distantia* de *Trypanosoma cruzi*. 2014. Um Novo Aspecto Epidemiológico da Doença de Chagas Aguda no Brasil. 2014; 8: 1-9.

Pavan TBS, Silva JW da, Martins L C, Costa SCB, Almeida EA de. Hepatic changes by benznidazole in a specific treatment for Chagas disease Plos One. 2018; 13: 1-11.

World health organization (WHO). Chagas disease *(American trypanosomiasis)*. Geneva: WHO. 2015; 90:33-44. https://www.who.int/wer/2015/en/. Accessed 05 May 2019.

Nóbrega AA da, Araújo WN, Vasconcelos AMN. Mortality due to Chagas disease in Brazil according to a specific cause. Am J Trop Med Hyg. 2014; 3:528-533.

Martins-Melo FR, Ramos AN-JR, Alencar CH, Heukelbach J. Mortality from neglected tropical diseases in Brazil, 2000–2011. Bull World Health Organ 2016; 94:103–110.

Simões TC, Borges LF, Assis ACP de, Silva MV, Santos J dos, Meira KC. Chagas disease mortality in brazil: a bayesian analysis of age-period-cohort effects and forecasts for two decades. PLOS Neglected Tropical Diseases, 2018; 12: 3-20

Rodrigues JRA., Silva JR JLR da, Paredes A de O, Reis A S., Silva LA de C. Doença de Chagas aguda no Estado do Maranhão, Brasil: uma comparação entre os bancos de dados do SINAN e da FUNASA. 2013. Manag Prim Health Care. 2013; 4: 3-9.

Dias JCP, Machado EMM, Fernandes AL, Vinhaes MC. Esboço geral e perspectivas da doença de Chagas no Nordeste do Brasil. Cad. Saúde Pública, Rio de Janeiro. 2000; 16 Suppl. 2:13-34.

Agência Nacional de Vigilância Sanitária (ANVISA). RDC nº 302, 13 de outubro de 2005. Available in:
<http://portal.anvisa.gov.brdocuments/10181/2718376/RDC_302_2005_COMP.pdf>.
Accessed 9 May.

Riddell, RW. Permanent stained mycological preparations obtained by slide culture. Mycology. 1950; 42: 265-270.

Abliz P, Fukushima K, Takizawa K, Nieda N, Miyaji M, Nishimura K. Rapid identification of the genus *fonsecaea* by PCR with specific oligonucleotide primers. J Clin Microbiol. 2003; 2:873-876.

Sanger F, Nickelen, S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S. v. 74, p. 5463-5467, 1977.

Bern C, Martin DL, Gilman RH. Acute and congenital Chagas disease. Advances in Parasitology. 2011; 75:19–47.

Maclean LM, Thomas J, Lewis MD, Cotillo I, DAVID W. GRAY, Rycker M de. Development of *Trypanosoma cruzi* in vitro assays to identify compounds suitable for progression in Chagas' disease drug discovery. PLOS Neglected Tropical Diseases. 2018; 12: 1-22.

Mello e Silva ACC, Neto AS, Galvão CES, Marques SG, Saldanha ACR, Silva CMP, Fischman O, Silva RR, Costa MRSR.; Costa JML. Cromoblastomicose produzida por *Fonsecaea pedrosoi* no Estado do Maranhão. I – Aspectos clínicos, epidemiológicos e evolutivos. Rev. Soc. Bras. Med. Trop. 1992; 25:1, 37-44

Marques SG, Silva CM, Saldanha PC, Resende MA, Vicente VA, Queiroz-Telles F, Costa JM. Isolation of *Fonsecaea pedrosoi* from the shell of the babassu coconut (*Orbignya phalerata* Martius) in the Amazon region of Maranhão Brazil. Nihon Ishinkin Gakkai Zasshi 2006; 47:305-11.

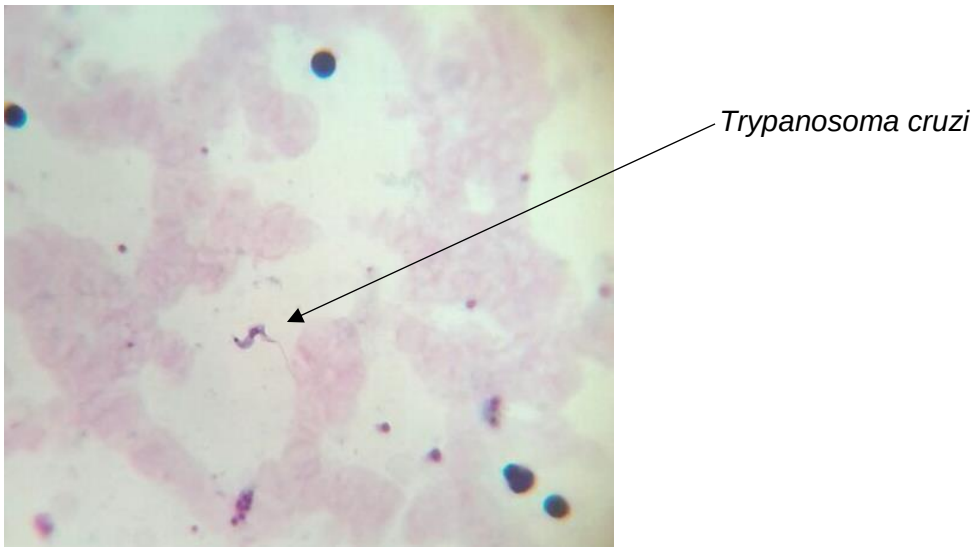
Sarkar M, Das D, Sinhababu A. Chromoblastomycosis as a mimicker of neoplastic lesion: A case report International Journal of Medical Science and Public Health. 2017; 6: 659-651.

Uribe JF, Zuluaga AI, Leon W, Restrepo A. Histopathology of chromoblastomycosis. Mycopathologia. 1989; 105: 1-6.

Attachments

Figure 1 Bacaba fruit (*Oenocarpus bacaba* Mart.)

Figure 2 Visualization of Trypanosoma cruzi in Giemsa, 100x.



Brazilian Journal of Development

Figure 3 Injury with a verrucous plaque and vegetative lesion of chromoblastomycosis in the lower limb D (1st collection before treatment).

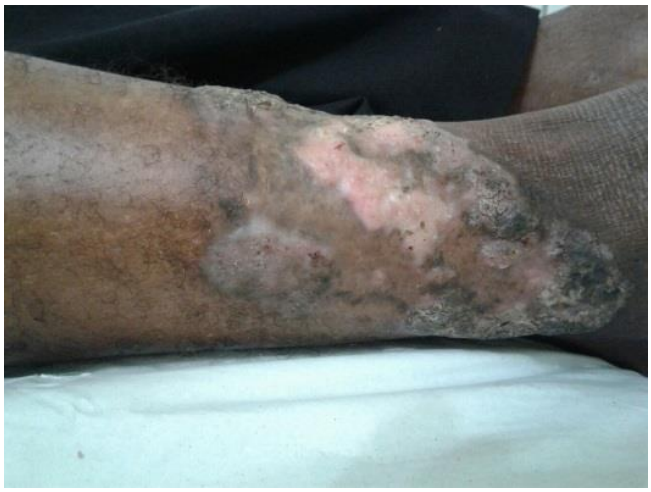
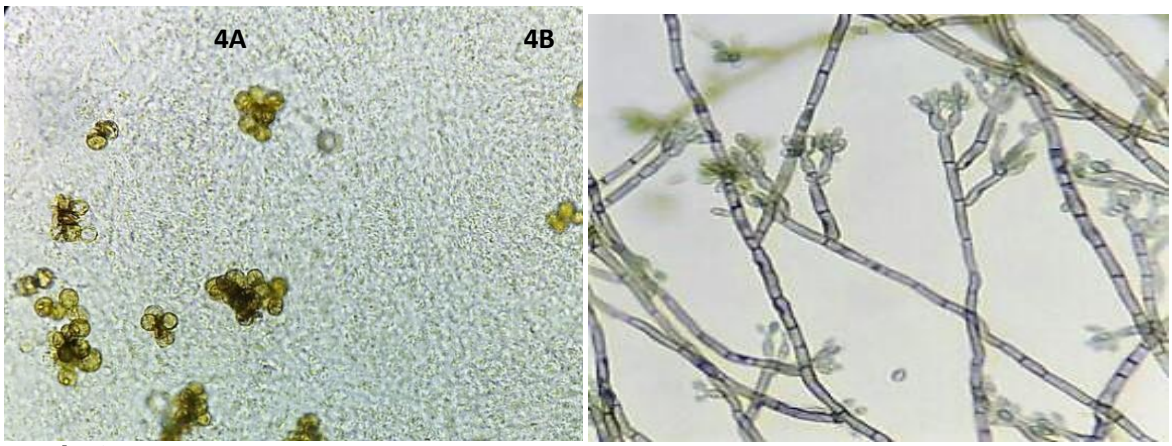
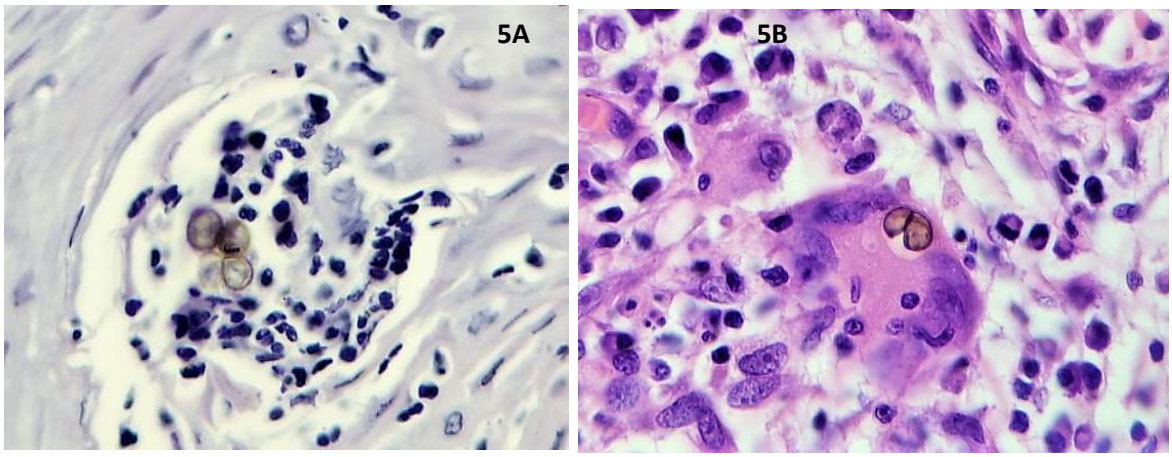


Figure 4A. Fungus Fonsecaea pedrosoi search in KOH. Presence of muriform brown corpuscles. Figure 4B: Identification of fungus in microculture on a blade with cotton blue.



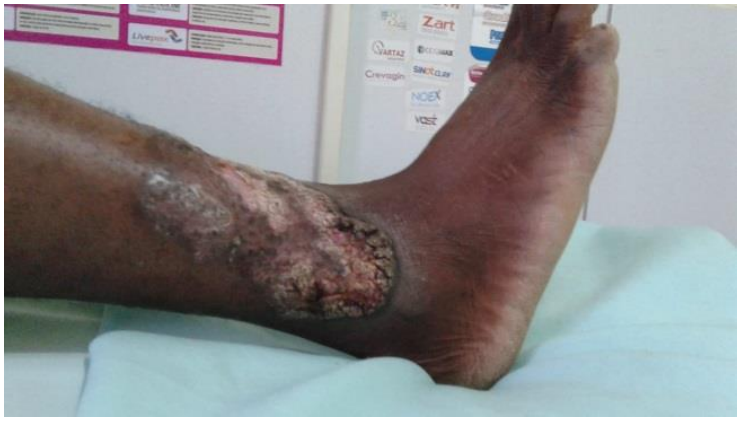
Legend: 40x.

Figure 5A. Histopathological section of the first collection before treatment for chromoblastomycosis. 5B. Histopathological section of the second collection during the treatment of chromoblastomycosis.



Legend: 5A - Intraepithelial microabscesses with polymorphonuclear leukocytes, lymphocytes, histiocytes and murifórmes corpuscles. HE, 100x. 5B-Detail of the inflammatory mono and polymorphonuclear infiltrate with a predominance of multinucleated giant cells phagocytizing the muriformes corpuscles. HE, 100x.

Figure 6 Clinical aspects of the chromoblastomycosis lesion six months of the treatment with itraconazole



Brazilian Journal of Development