Mycopathologia (2019) 184:115–119
https://doi.org/10.1007/s11046-018-0284-3

ORIGINAL PAPER

Case Report: A Case of Chromoblastomycosis Caused by *Fonsecaea pedrosoi* in Vietnam

Tran-Anh Le · Khac-Luc Nguyen · My-Hang Pham · Thuat-Thang Vi · Ngoc-Anh Do

Received: 31 October 2017 / Accepted: 27 June 2018 / Published online: 4 July 2018

Abstract

Background Chromoblastomycosis is a chronic fungal infection of the skin and subcutaneous tissues caused by different melanized fungi. The disease occurs worldwide, particularly in tropical and subtropical regions but not reported in Vietnam.

Case Summary A 47-year-old women was admitted to hospital 103, Hanoi, Vietnam, with a 10-year lasting lesion on backside of her right shank. Diagnosis of chromoblastomycosis was made after discovery of a muriform cell in histopathological examination. A black, slow-growth fungus was isolated and identified as *Fonsecaea pedrosoi* after molecular analysis. After 1-month treatment with itraconazole, the lesion has significant improvement.

Conclusion This is the first case of chromoblastomycosis caused by *Fonsecaea pedrosoi* reported in Vietnam.

Keywords Case report · *Fonsecaea pedrosoi* · Chromoblastomycosis · Human · Vietnam · Molecular analysis

Abbreviations

CBM	Chromoblastomycosis, chromomycosis
KOH	Potassium hydroxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
PCR	Polymerase chain reaction

Introduction

Chromoblastomycosis (chromomycosis, CBM) is a chronic fungal infection of the skin and subcutaneous tissues caused by different melanized fungi. The disease occurs worldwide, particularly in tropical and subtropical regions with higher prevalence in Madagascar, South Africa, Brazil and Costa Rica [1]. Cases have been reported in Asia including India [2], Sri Lanka [3], Bangladesh [4], Korea [5], Japan [6], China [7], [8], Taiwan [9], Thailand [10], Philippine [11]. Vietnam is an agricultural country with tropical climate, but no case of CBM has been reported [12].

Handling Editor: Yuping Ran.

T.-A. Le (✉) · K.-L. Nguyen · N.-A. Do
Department of Parasitology, Vietnam Military Medical University (VMMU), Phung Hung Street, Ha Dong Town, Hanoi, Vietnam
e-mail: anh_lt@vmmu.edu.vn

M.-H. Pham
Department of Dermatological Disease, Hospital 103, Vietnam Military Medical University (VMMU), Hanoi, Vietnam
e-mail: myhang3003@gmail.com

T.-T. Vi
Department of Pathology, Hospital 103, Vietnam Military Medical University (VMMU), Hanoi, Vietnam

Most pathogen of CBM are members of a single order in the fungal kingdom, the *Chaetothyrials,* and the species most frequently associated with CBM belong to the genera *Fonsecaea* and *Cladophialophora* [12]. *Fonsecaea pedrosoi* is among the most common agent. In Madagascar, Esterre et al. (1996) reported 1343 cases of CBM, and 61.8% of the isolated strains were *F. pedrosoi* [13]. In a retrospective study of 325 cases in Amazonic Region (Brazil), *F. pedrosoi* was present in 77/78 CBM cases that the etiological agent was isolated and identified [14]. In Sri Lanka, the agents were isolated from 69 cases of CBM and 64 cases were *F. pedrosoi* [3]. The speciation of pathogenic fungus is based on molecular approach because the identification using conventional mycological methods, like morphology and physiology, may be inadequate [15].

Here, we report a case of CBM caused by *F. pedrosoi* in Vietnam.

Case Report

A 47-year-old women was admitted in hospital 103, Hadong, Hanoi, Vietnam, on February 27, 2017, with a lesion on backside of her right shank. The lesion lasted for about 10 years. At first, the lesion appeared as an erosion of about 1 cm with itching and pain and continued spreading peripherally. She had been treated with antibiotics and corticosteroids without improvement. Clinical examination on admission revealed a purple, approximately 12 × 20 cm, non-elevated, irregular-shaped ulcer on backside of her right shank. Brown crust with black dots covered edges of the lesion, and under the crust was a pink flesh with increasing papilla. The center of the lesion tended to healing with pink skin and hard background (Fig. 1).

Complete blood count and liver function tests were done, and all were in normal range. Direct microscopic with wet mount examination (KOH 20%) of skin scrapings, crusts, aspirated debris did not reveal any fungal structures. Histological examination (hematoxylin and eosin stain) showed pseudoepitheliomatous hyperplasia and a mixed granulomatous inflammatory infiltrate in the dermis. There was a single muriform cell about 5 μm in diameter, polyhedral shaped, thick walled, dark pigmented and having both transverse and longitudinal cross-walls in the dermis (Fig. 2).

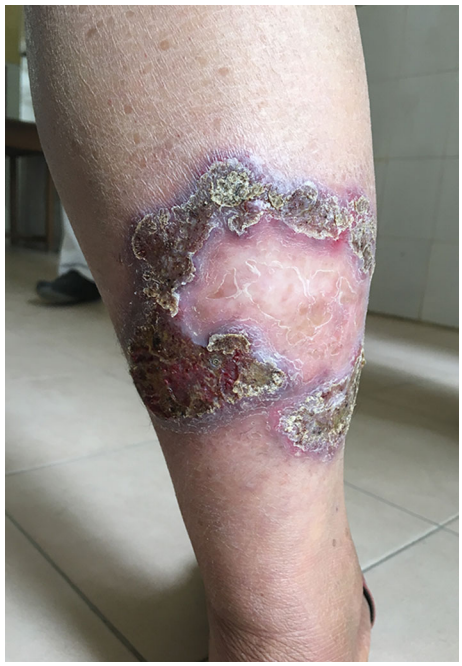

Fig. 1 The lesion on backside of her right shank. A purple, approximately 12 × 20 cm, non-elevated, irregular-shaped ulcer and brown crust with black dots covered edges of the lesion. Under the crust was a pink flesh with increasing papilla. The center of the lesion tended to healing with pink skin and hard background. (Color figure online)

Culture of biopsy material was performed on Sabouraud dextrose agar (Bio-Rad, France), and a dark colony with a velvety surface and a raised center

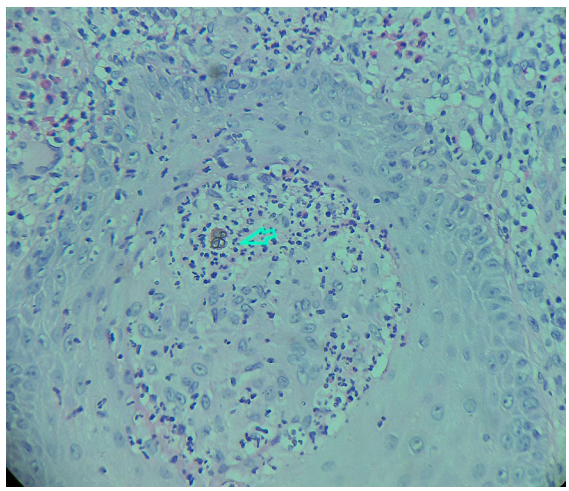

Fig. 2 A single muriform cell in the dermis (arrow)

developed after 4 weeks of incubation at room temperature (Fig. 3).

When viewed under the microscope, colony sample on Sabouraud agar showed only treelike branched, septate hyphae without any conidia. After being transferred to Malt extract agar (Merck, Germany) many oval, one-celled conidia on conidiogenous cells that grew in dense clusters were seen (Fig. 4).

To determine the isolated fungi, deoxyribonucleic acid (DNA) from the isolate was extracted by Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corp., Canada) according to manufacturer's instructions. 18S-ITS1-5.8S-ITS2-28S rDNA region was amplified using primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) [16] and NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′) [17]. The PCR reagents were obtained from Thermo Fisher Scientific (Germany). All PCR steps were carried out in a 50 µL reaction volume with 0.2 µM concentration of each dNTP, 0.1 µM of each primer, 10 ng of template DNA and 1.25 U of Taq polymerase. The PCR profile consisted of denaturation for 3 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 55 °C for 45 s and 72 °C for 60 s and a final extension at 72 °C for 10 min. The PCR products were purified using GeneJET PCR Purification Kit (Thermo Fisher Scientific (Germany)) and underwent sequencing. The sequence was compared with those in GenBank by Blast program, and the fungus was identified as *F. pedrosoi*. The sequence was deposited in the GenBank under accession number MF173064.

A diagnosis of CBM was made, and the patient received treatment with oral itraconazole 200 mg twice daily. After 4 weeks, she returned for re-examination, and the lesion was in healing process (Fig. 4). For unknown reason, she has not come back to be consulted again, so we could not do any further examination (Fig. 5).

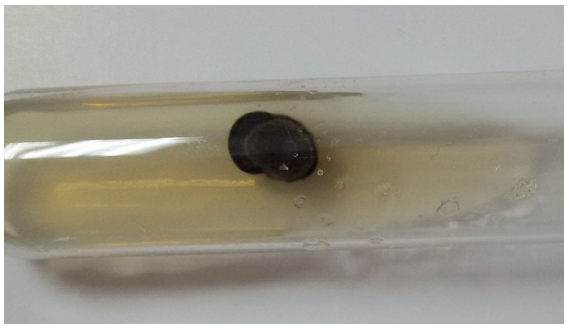

Fig. 3 A black, velvety with raised-center colony after 4 weeks of incubation of biopsy material on Sabouraud agar

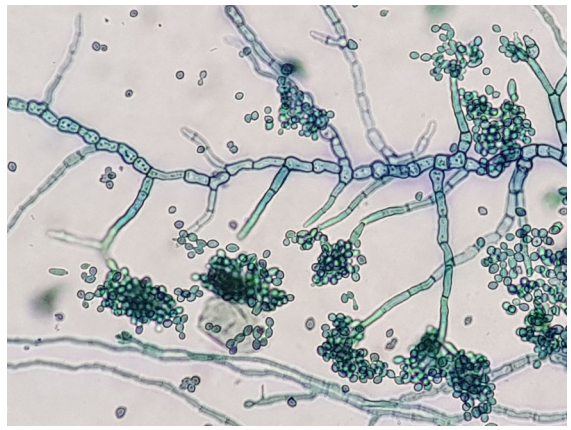

Fig. 4 Hyphae, conidiophores and conidia of the isolate on Malt extract agar

Discussion

With clinical, histopathological and mycological results, as well as molecular biological findings, we

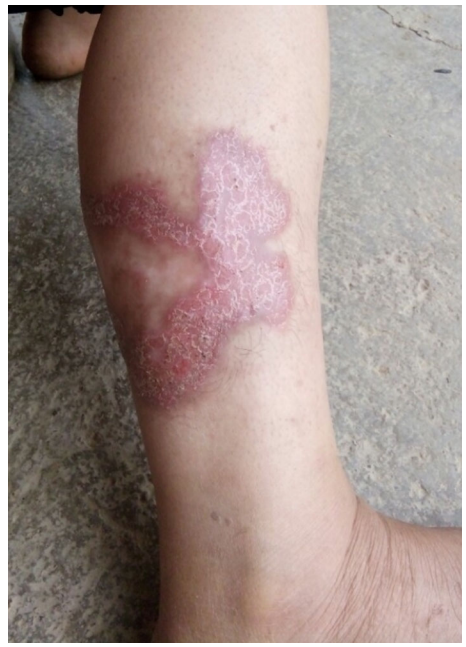

Fig. 5 The lesion with healing trend after 4 weeks of treatment with itraconazole

were able to diagnose the patient as having CBM caused by *F. pedrosoi*.

Our patient is a farmer, 47-year-old and lives in a tropical country, so she is among a group at high risk of infection as CBM is generally found in tropical and subtropical areas [18]. The responsible agents for CBM are usually saprophytic fungi found in decaying vegetation and soil and can cause human infection by transcutaneous implantation [19], so that the disease is more prevalent in rural worker [15, 20]. Our patient did not remember or realized the traumatic cutaneous injury that made her infected by the fungus, a phenomenon noticed by some other reports [21, 22].

The location and slow progression of her lesion were also typical for the disease. The time from the appearing of the lesion to diagnosis is 10 years. In a review of 27 cases by Correia et al. [20], an average length of time between appearing of the lesion to diagnosis is 109.33 months in rural workers and 69.18 months of all other professions. The lesion was graded as moderate and cicatricial type according to classification of Queiroz-Telles et al. [18].

The discovery of a muriform cell on histopathology and isolation of a black, slow-growth fungus on Sabouraud agar determined the diagnosis of CBM. Histopathology is identical in all types of CBM, and the presence of the muriform cells (dark-walled polyhedral structures which were easily recognized in routine hematoxylin–eosin stain) is the hallmark of the disease [15], [23]. Muriform cells can be also visible in KOH wet mounts but not found in our case which is agreed with other authors that biopsy is preferred material for diagnosis of CMB [15]. The slow-growing dark-pigmented colony on Sabouraud media was also characteristic of agent responsible for the disease.

Systemic antifungal agent is needed considering the size of the lesion (12–20 cm) as suggested by some authors [24]. Itraconazole is prescribed because it is considered the standard therapy for CBM and it is also the most commonly used antifungal drug [12]. After one month using itraconazole (400 mg daily), significant clinical improvement was observed (Fig. 4). We cannot access more about the result of treatment because the patient was lost to follow-up after 1 month of treatment. Result of treatment is promising, but CBM is a chronic mycosis that is resistant to most treatments and prone to recurrence, and long-time follow-up is necessary [12].

Conclusion

Our report determines the existence of CBM and *F. pedrosoi* in Vietnam. Regarding the high risk and lack of epidemiological profile of CBM in Vietnam, detailed national surveys involving epidemiological and etiological information are needed for early control of the infections. The disease should be suspected when patients having slow-progress lesion in lower limbs and clinical samples such as skin scrapings, crusts and biopsy should be collected for laboratorial analysis. Itraconazole may be an effective drug to treat CBM caused by *F. pedrosoi*.

Acknowledgements The authors are grateful to Mr. Robert Mayrhofer and Ms. Linh Khanh Le for revising the English text.

Authors' Contributions KLN and NAD conducted the study, as well as morphological and molecular analyses of the isolated strain. MHP collected clinical data. TTV made the histopathological examination. TAL designed the study and created the final draft of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Availability of Data and Material The sequence generated and analyzed during the current study is available in the GenBank under the code MF MF173064.

Consent for Publication The authors agreed to publish this article in *Mycopathologia*.

Ethics Approval and Consent to Participate Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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