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Brief report

First case of chromoblastomycosis due to *Phoma insulana*

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ABSTRACT

Chromoblastomycosis is a chronic infection, caused by pigmented fungi affecting skin and subcutaneous tissues characterized by verrucous nodules or plaques. *Fonsecaea pedrosoi* and *Cladophialophora carrionii* are the prevalent agents in the endemic areas. *Phoma* is an uncommon agent of human infection and involved mainly with phaeohyphomycosis cases. The case of a patient with a history of laceration in foot followed by verrucous aspect and scaly lesions, which had evolved for 27 years is presented. On physical examination disease was clinically compatible with chromoblastomycosis and the microscopic examination of scales showed fumagoid cells. On culture a dematiaceous fungus was grown. The agent was confirmed to be *Phoma insulana* based on its morphology and PCR-sequencing. This fungal agent has not been previously reported in association with this pathology.

Primer caso de cromoblastomicosis causado por *Phoma insulana*

Palabras clave:
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Phoma insulana
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RESUMEN

La cromoblastomicosis es una infección crónica causada por hongos pigmentados que afecta la piel y el tejido subcutáneo y que se caracteriza por nódulos o placas verrugosas. *Fonsecaea pedrosoi* y *Cladophialophora carrionii* son los agentes prevalentes en las áreas endémicas. *Phoma* es un agente raro de infección humana y está involucrado principalmente en casos de feohifomicosis. Se presenta el caso de un paciente con antecedente de laceración en el pie, seguida de lesiones de aspecto verrugoso y descamativas, que evolucionaron durante 27 años. En el examen físico la enfermedad fue clínicamente compatible con cromoblastomicosis y el examen microscópico de escamas mostró células fumagoides. En el cultivo creció un hongo dematiáceo. El agente fue confirmado como *Phoma insulana* en base a su morfología y PCR seguida de secuenciación. Este agente fúngico no ha sido reportado previamente en asociación con esta patología.

Introduction

Chromoblastomycosis is an infection caused by the traumatic implantation of species of dematiaceous fungi, primarily in the skin and subcutaneous tissues of the lower limbs. The disease generally starts as a cutaneous nodule or papule which gradually increases in the adjacent areas and develops a scaly, greyish surface. After the lesion may evolve into one of the following described clinical forms: the nodular, tumoral, verrucous, cicatricial and plaque types. Histologically, a granulomatous reaction associated with acanthosis and pseudoepitheliomatous hyperplasia in the stratum corneum and epidermis is observed. The fungal structure in infected scales or tissues appears as rounded, dark-brown yeast-like bodies (5–15 μm in diameter) with thick, planate-dividing walls that are known as sclerotic cells (also referred as "copper pennies," "fumagoid cells," "Medlar bodies," or "muriform cells").[1]

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Chromoblastomycosis is a frequent disease in countries with tropical and subtropical climates, particularly in Latin America, Africa and Asia. The most frequent causative agents are *Fonsecaea pedrosoi*, *F. compacta*, *Phialophora verrucosa*, *Cladophialophora carrionii*, and *Rhinocladiella aquaspersa*. These fungi inhabit the soil and vegetal matter; therefore, the disease is more frequent in rural populations.[2]

This work describes the case of a patient living in deficient hygienic and socioeconomic conditions who presented a chronic subcutaneous infection compatible with chromoblastomycosis. The identified etiological agent has not been previously associated with this pathology.

Case

During a health campaign in rural areas organized by the Faculty of Chemical Sciences at the Autonomous University of Oaxaca the patient of this case was addressed. He was an indigent 79-year-old man, resident in Miahuatlan, Oaxaca (Mexico), and was a peasant with a history of chronic alcoholism and smoking. He began his current dermatological illness 27 years ago after the use of tight footwear that caused a laceration on his right heel. From that time, his lesion changed, with progressive thickening, pigmentation and peeling of the local skin. Several small nodules appeared and extended to the foot. The patient applied several empirical and aggressive treatments without improvement. He also sought medical assistance, but the treatment yielded unsatisfactory results. Five years ago, the presence of a progressive ulcerative lesion was detected.

On physical examination, pigmented verrucous plaques were observed, predominantly on the anterior side, heel and internal side of the right foot. Moderate oedema and thick, yellow and adherent squamae were noteworthy on the leg. The patient had an ulcer located on the internal side of the lower third of the leg (10 cm × 5 cm), with a well-defined border and a haematopurulent and foetid exudate. Additionally, larvae (miasis) were observed in the ulcer. The patient did not report symptoms localized in the affected zone but manifested asthenia, adynamia and general discomfort. He presented pallor, hearing loss, uncontrolled salivation, involuntary movements of both hands, and urinary incontinence. Authorization to take an image of his lesion was obtained (Fig. 1a). Additionally, scales and blood samples were collected for mycological study and analysis, respectively.

Laboratory studies

Haematological analysis revealed values within normal parameters.

Mycological study

Microscopic examination of the skin scales with 20% potassium hydroxide showed individual or grouped, brown, thick-walled, planate-dividing round cells, 10 μm × 13 μm in diameter, compatible with fumagoid cells (Fig. 1b). The scales were cultivated on Sabouraud dextrose agar (SDA) with and without antibiotics at 28 °C, with periodic revision. After two weeks, several small, pigmented, downy colonies were observed on SDA without antibiotics. No growth was present on SDA with antibiotics, and these tubes were discarded after three weeks of incubation. Microscopic examination of the primary culture revealed pigmented and irregular hyphae, and swollen cells, insufficient features to identify the fungus (Fig. 1c).

After observation of fumagoid cells in scales and a dematiaceous fungus in culture, the chromoblastomycosis diagnosis was established. The patient was visited at home and informed about his disease. However, he refused any topical or systemic treatment, and a follow-up visit was not possible. After the final identification of the fungal isolate, a colleague again went to visit the patient around three months later. The neighbours informed about the patient's death due to "cardiac failure".

Morphological study

The fungus was grown on SDA, on potato dextrose agar (PDA) and on lactrimel agar (LA) for 6 days at 28 °C. The microscopic morphology on SDA and PDA was similar to that observed for the primary isolate, but it revealed numerous chlamydoconidia on LA. After four weeks on PDA, many irregular and round pycnidia were observed. The fungus was later grown on malt extract agar (MEA) and on oat agar (OA) for 8 days at 28 °C (Fig. 2a and b). The size of colony was 7.0 and 6.7 cm of diameter, respectively. Microscopic morphology on two media was similar, but only description on OA is done. Abundant globose or piriformis, intercalary, terminal or in chains, of variable size (5–14 μm diameter) chlamydospores, were observed (Fig. 2c). Numerous globose (200 μm), piriformis (200 μm × 360 μm) or irregular pycnidia, with one to three ostioles or pores (Fig. 2d) were observed. Polyhedral cells formed the pycnidial wall (Fig. 2e). Abundant ellipsoidal, hyaline conidia with one or two polar guttules emerging from picnidia were present (Fig. 2f). Conidial matrix whitish was evident. The morphological characteristics were integrated according to Boerema et al.[3]

Molecular identification

DNA was extracted from a monosporic culture in Sabouraud dextrose broth using the GeneAll Exgene Plant SV mini kit (GeneAll Biotechnology Co., Ltd., Seoul, Korea). Three PCR reactions were

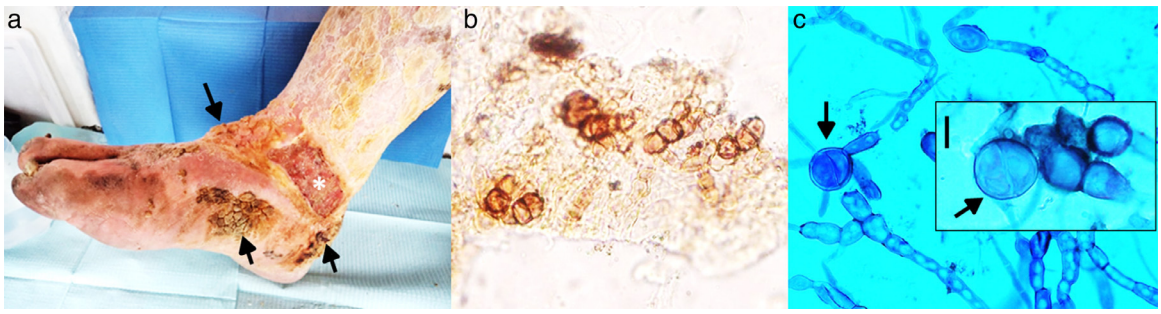

Fig. 1. (a) Pigmented verrucous lesions on the foot (arrows), an ulcerative lesion (*) on the lower part of the leg, and yellowish, thick, crusty lesions on the leg are observed. (b) Microscopic examination of scales, showing thick walled, round-to-ovoid brown cells with septa characteristic of fumagoid cells (40×). (c) Microscopic examination with lactophenol blue from primary culture: irregular and pigmented hyphae with internal guttules, chlamydoconidia-like cells and some septate globose structures similar to fumagoid cells (arrows) are observed (scale bar: 10 μm).