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Original article

Fungal necrotizing fasciitis, an emerging infectious disease caused by *Apophysomyces* (Mucorales)

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ABSTRACT

Background: The mucoralean fungi are emerging causative agents of primary cutaneous infections presenting in the form of necrotizing fasciitis.
Aims: The aim of this study was to investigate a series of suspected necrotizing fasciitis cases by *Apophysomyces* species over one-year period in a northern Indian hospital.
Methods: The clinical details of those patients suspected to suffer from fungal necrotizing fasciitis were recorded. Skin biopsies from local wounds were microscopically examined and fungal culturing was carried out on standard media. The histopathology was evaluated using conventional methods and special stains. *Apophysomyces* isolates were identified by their morphology and by molecular sequencing of the internal transcribed spacer (ITS) region of the ribosomal genes. Antifungal susceptibility testing was carried out following EUCAST guidelines and treatment progress was monitored.
Results: Seven patients were found to be suffering from necrotizing fasciitis caused by *Apophysomyces* spp. Six isolates were identified as *Apophysomyces variabilis* and one as *Apophysomyces elegans*. Five patients had previously received intramuscular injections in the affected area. Three patients recovered, two died and the other two left treatment against medical advice and are presumed to have died due to their terminal illnesses. Posaconazole and terbinafine were found to be the most active compounds against *A. variabilis*, while the isolate of *A. elegans* was resistant to all antifungals tested.
Conclusions: *Apophysomyces* is confirmed as an aggressive fungus able to cause fatal infections. All clinicians, microbiologists and pathologists need to be aware of these emerging mycoses as well as of the risks involved in medical practices, which may provoke serious fungal infections such as those produced by *Apophysomyces*.

Fascitis necrotizante fúngica, una enfermedad infecciosa emergente causada por *Aphophysomyces* (Mucorales)

Palabras clave:
Apophysomyces
Hongos emergentes
Mucormicosis
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RESUMEN

Antecedentes: Los hongos mucorales son agentes emergentes causantes de infecciones cutáneas primarias presentes en forma de fascitis necrotizante.
Objetivos: La finalidad de este estudio fue la de investigar una serie de infecciones sugestivas de fascitis necrotizante causadas por alguna de las especies de *Apophysomyces* a lo largo de un año en un hospital del norte de la India.
Métodos: Se obtuvieron los datos de todos los pacientes con sospecha de fascistis necrotizante. Las biopsias de piel de la zona afectada fueron cultivadas en medios de cultivos estándar y se evaluaron

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histopatológicamente mediante tinciones convencionales y específicas para hongos. Los aislamientos de *Apophysomyces* fueron identificados morfológicamente y mediante la secuenciación del espaciador intergénico ribosomal (ITS). La sensibilidad antifúngica se determinó mediante el método EUCAST y la evolución de los pacientes fue monitorizada.

Resultados: Se encontraron siete pacientes con fascitis necrotizante causada por especies de *Apophysomyces*. Seis aislamientos fueron identificados como *Apophysomyces variabilis* y uno como *Apophysomyces elegans*. Cinco pacientes habían recibido previamente inyecciones intramusculares en el área afectada. Tres pacientes se recuperaron, dos fallecieron y de los dos restantes no se tiene seguimiento médico, aunque presumiblemente fallecieron debido a que padecían enfermedades terminales. El posaconazol y la terbinafina fueron los compuestos más activos frente a *A. variabilis*, mientras que el único aislamiento de *A. elegans* fue resistente a todos los antifúngicos ensayados.

Conclusiones: Se confirma que *Apophysomyces* es un hongo agresivo capaz de causar infecciones con desenlace fatal. Clínicos, microbiólogos y patólogos deben ser conscientes de los riesgos de estas micosis emergentes y de que determinadas prácticas médicas puedan provocar infecciones fúngicas graves como las producidas por *Apophysomyces*.

Necrotizing fasciitis, commonly known as flesh-eating disease, is one of the severe soft tissue infections of skin caused by bacterial as well as fungal pathogens. Some fungi of the Mucorales order can produce this rapidly progressive infection, which destroys the soft tissues that cover muscles, spreading along the fascial planes and invading deeper and deeper into the tissues, often being fatal even when treated in a timely manner.[16] Most commonly, the organism enters the body through a breach in continuity of skin, like a cut, burn, insect bite or puncture wound. The introduction of contaminated soil at the site of trauma may serve as the primary means of inoculation of this organism. The necrotizing infections are found mainly in immunocompetent patients, as well as in those with or without diabetes mellitus. The infection can also be associated to health-care risks such as post-surgery, intramuscular injections, skin tests and contamination of a plaster of Paris cast applied to reduce fractures, etc.[2,4,10,11]

The genus *Apophysomyces* (family Saksenaeaceae, order Mucorales) is a common cause of such devastating life-threatening infections.[3] The species in this genus are widely distributed in soil in tropical and subtropical climates, with clinical cases reported mainly from India, the United States and Australia. The type species of this genus, *Apophysomyces elegans*, was isolated for the first time in 1979 from two soil samples in a mango orchard in northern India.[12] The species of *Apophysomyces* are thermotolerant and can grow rapidly, even above the body temperature, up to 42 °C. The morphology of *Apophysomyces* in culture is quite similar to that of *Lichtheimia* species. However, *Apophysomyces* has dark brown champagne glass-shaped apophyses and a dark, thick-walled region in the sporangiophore just below the apophyses. In addition, *Apophysomyces* has a foot cell that resembles those produced by *Aspergillus* species. Recently, based on genetic, physiological and morphological features, three new species have been reported, i.e. *Apophysomyces ossiformis*, *Apophysomyces trapeziformis* and *Apophysomyces variabilis*, the latter being the most common in clinical infections.[1,9] The aim of this study was to investigate a series of cases suspected of necrotizing fasciitis caused by *Apophysomyces* species over one-year period in a northern Indian hospital.

Materials and methods

This prospective study was conducted over one-year period from September 2010 to August 2011 in the Departments of General Surgery, Microbiology and Pathology at the Government Medical College Hospital, Chandigarh (northern India) in association with the Mycology Unit, Rovira i Virgili University, Reus, and Mycology Department, Spanish National Center for Microbiology, Instituto de Salud Carlos III, Madrid (Spain). Tissue biopsy samples were handled delicately, avoiding fungal cell damage. The presence of mucoralean fungi was investigated, focusing on direct microscopic examination with potassium hydroxide and calcofluor white wet mounts. The histopathology exam was carried out by using hematoxylin and eosin, periodic acid-Schiff and Grocott's methenamine silver stains. Fungal cultures were made on blood agar, Sabouraud's dextrose agar (SDA; HiMedia, Mumbai, India), brain–heart infusion agar (BHIA; HiMedia, Mumbai, India) and oatmeal agar (OMA; HiMedia, Mumbai, India) in duplicate and incubated at 25 °C and 37 °C. The cultures were examined daily for the presence of Mucorales species, particularly, *Apophysomyces* spp., characterized by showing rapid growing (2–4 days), white cottony creamy-white colonies becoming pale-yellow on aging, filling almost the entire tube and/or petri dish. The isolates were identified by gross morphology along with microscopic examination on lactophenol-cotton blue (LCB) mounts. All isolates tentatively identified as *Apophysomyces* species in India were later confirmed phenotypically and molecularly in the reference laboratory at Reus (Spain). These isolates were grown on Czapek-Dox agar (BD Difco, USA) at 37 °C for one week, and microscopic features were determined on the sixth day in lactic acid mounts. The capacity to hydrolyse and to assimilate esculin was tested by growing the fungi on bile-esculin agar (BEA; Panreac, Barcelona, Spain) at 37 °C for one week.[1] The amplification and nucleotide sequencing of the internal transcribed spacer (ITS) region of the ribosomal genes were carried out, and the obtained sequences were compared with those of the type strains of *Apophysomyces* species.

The antifungal susceptibility testing of the *Apophysomyces* isolates was carried out using the EUCAST broth microdilution method.[15,17] The minimum inhibitory concentration (MIC) was calculated for amphotericin B (AMB), itraconazole (ITC), voriconazole (VRC), ravuconazole (RVC), posaconazole (PSC) and terbinafine (TRB), while minimum effective concentration (MEC) was calculated for caspofungin (CPF) and micafungin (MCF). Growth was examined visually after incubation at 35 °C for 24 h, and the MICs or MECs were determined. *Aspergillus fumigatus* ATCC 2004305 and *Aspergillus flavus* ATCC 2004304 were used as quality control strains.

Results

Seven cases of *Apophysomyces* infections were detected. The histopathology examination of the biopsy samples was positive in all cases, showing broad, ribbon-like sparsely septate hyphae,