Cutaneous infection by *Phaeoacremonium parasiticum*

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Infección Cutánea por *Phaeoacremonium parasiticum*

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Abstract:

Background

Phaeoacremonium parasiticum is considered a rare infectious agent that is part of a heterogeneous group of fungi causing phaeohyphomycosis. This organism is capable of producing subcutaneous infections, eumycetomas, osteomyelitis, arthritis, myositis and also disseminated diseases, such as fungemia and endocarditis.

Aims

The aim of this study is to report a case of cutaneous infection by *Phaeoacremonium parasiticum* diagnosed in our hospital.

Materials and Methods

We described a case of cutaneous infection by *Phaeoacremonium parasiticum* in a kidney transplant patient diagnosed in our hospital. The identification of this microorganism was performed by microbiological and histopathological studies and confirmed with the sequence of gene encoding β-tubulin and a real time panfungal PCR targeting 18S ribosomal RNA gene.

Results

The microorganism was correctly identified by phenotypic and molecular methods. The patient was treated with oral antifungal therapy and a debulking surgery; evolving without any complication.

Conclusions

The diagnosis of this infection is difficult and usually affects a kidney transplant patients, but this association is still unknown.

Resumen:

Antecedentes

Phaeoacremonium parasiticum es considerado un agente infeccioso poco común, que forma parte de un grupo heterogéneo de hongos causantes de feohifomicosis. Este

microorganismo es capaz de producir infección cutánea, eumicetoma, osteomielitis, artritis, miositis e incluso enfermedad diseminada como fungemia y endocarditis.

Objetivo

El objetivo del estudio es describir un caso de infección cutánea por *Phaeoacremonium parasiticum* diagnosticado en nuestro hospital.

Materiales y Métodos

Se describe un caso de infección cutánea por *Phaeoacremonium parasiticum* en un paciente trasplantado renal diagnosticado en nuestro hospital. Para la identificación del microorganismo se realizaron pruebas microbiológicas y histopatológicas y se confirmó la identificación con la secuenciación del gen de la β-tubulina y una PCR a tiempo real para la detección del gen 18S rRNA.

Resultados

El microorganismo fue identificado correctamente por métodos fenotípicos y moleculares. El paciente recibió tratamiento con antifúngicos orales y citorreducción quirúrgica, evolucionando sin ninguna complicación.

Conclusiones

El diagnostico de esta infección usualmente aparece en pacientes trasplantados renales y su diagnóstico puede ser complicado. Sin embargo, la asociación de esta infección con este tipo de pacientes aun es desconocida.

Phaeoacremonium species have a wide distribution in the environment, It was thought that it only produced disease in plants (El-Herte et al., 2014)·(Aroca, Raposo, & Lunello, 2008), but in 1974 the first case of cutaneous infection in a kidney transplant patient was described. *P. parasiticum,* previously known as *Phialopora parasitica* is considered a rare infectious agent although in the last years new cases have been described (Aroca et al., 2008)·(Mostert et al., 2005)·(Mulcahy & Chew, 2011)(Crous PW, Gams W, Wingfield MJ, 1996)). *Phaeoacremonium parasiticum* is part of an heterogeneous group of fungi causing phaeohyphomycosis, a disease that includes a broad spectrum of infections caused by fungi that produce septate hyphae with melanin in the tissue (Alayeto Ortega et al., 2015)·(Marques et al., 2006). This organism has been reported to produce subcutaneous infections, eumycetomas, osteomyelitis, arthritis, myositis and also disseminated diseases, such as fungemia and endocarditis (VP Baradkar, M Mathur, S Kumar.Department of Microbiology & Medical College and General Hospital, Sion, Mumbai- 400 022, 1974). We describe a case of cutaneous infection caused by *P. parasiticum* in an immunosupressed kidney transplant patient.

Case report

A 77-year-old man went to a local hospital with an injury in his right hand caused by plants of his garden. The wound was cleaned and a daily antiseptic cleaning was prescribed. However, after several weeks the wound didn't improve. He came back to the same center and at the time of admission the patient confirmed that he was under immunosuppressive treatment due to a kidney transplant in 2015.

In the sample taken from the lesion grows filamentous fungi identified as *Exophiala dermatitidis* by microscopic identification. He started treatment with

voriconazole but after four days the patient stopped the treatment because of intolerance symptoms.

The patient continued with the lesion and 10 months later went to the dermatology department of our hospital. Physical examination revealed a nodular lesion in the right hand without any systemic symptoms. Oral treatment with itraconazole was started and a debulking surgery was performed. During the surgery samples for culture and histopathological study were taken. The histopathological examination of the tissue from the debridement showed aggregations of neutrophils forming micro abscesses, a moderate lymphoid infiltrate and numerous granulomas. In the center of one granuloma a material of vegetal appearance whith fungal structure compatible whit non-septate hyphae was observed Fig.1

Samples were plated on potato dextrose agar and incubated at 30ºC. After 7 days of incubation; colonies of spongy appearance with irregular borders and olivaceus- gray color were observed. Culture direct microscopy with lactophenol cotton blue staining showed hyaline hyphae, thin-walled phialides, tapering towards the tip often proliferating, with small funnel-shaped collarettes and hyaline conidia in balls. This appearance is consistent with *Phaeoacremonium* genus.

Identification of the isolate was confirmed by the analysis of the sequence of gene encoding β-tubulin and a real time panfungal PCR targeting 18S ribosomal RNA gene. DNA extraction was performed as described by Turenne (Turenne, Sanche, Hoban, Karlowsky, & Kabani, 1999)with some changes(Villanueva et al., 2017). For the extraction we used a vortexing with glass beads to improve the lysis of fugal cells wall. Amplification was performed in an automated PCR-System Smartcycler (Cepheid,USA) with cycles of 95ºC for 120 seconds, 45 cycles at 95ºC for 10 seconds, 52ºC for 30

seconds and 72ºC for 10 seconds, using Sybergreen to detect the amplification products (Sensifast SYBR Hi-Rox Kit, Bioline, UK). Melting curve analysis was performed in both methods.

The PCR products of both targets were sequenced (BigDye Applied Biosystem) and the sequences obtained were compared with those available from GenBank using a BLAST search. A *Phaeoacremonium parasiticum* was identified; the sequence ID of both results matched two sequences with accession number KU375504 and KX268647 with 100% and 99% of similarity respectively.

Antifungal susceptibility testing was performed on Sensititre YeastOne panel (Thermo scientific diagnostic systems, UK) following EUCAST rules for inoculated the sample(Chairman1 et al., n.d.). The following MIC values were obtained: voriconazole 0.06 µg/ml, itraconazole 0.12 µg/ml, posaconazole 0.06 µg/ml and amphotericin B 8 µg/ml. The isolate was considered as susceptible to azoles and reduced susceptibility to amphotericin B. The patient continued with oral itraconazole and progressed favorably.

P. parasiticum is an infrequent microorganism that causes infection especially in transplant patients receiving immunosuppressive treatment (Colombier et al., 2015),(El-Herte et al., 2014),(Mazzurco, Ramirez, & Fivenson, 2012),(Mulcahy & Chew, 2011). There are few reports of infections caused by this microorganism, but some reviews suggest an association with kidney transplantation. Up to 36% of infections occurred in kidney transplanted patients (Baddley, Mostert, Summerbell, & Moser, 2006),(Alayeto Ortega et al., 2015),(Marques et al., 2006), as in our patient and in some cases an invasive infection was observed (Colombier et al., 2015),(Alayeto Ortega et al., 2015). However, the cause of this association is not yet clear.

Patients with several immunocompromising hematological diseases, stem cell transplantation, rheumatoid arthritis treated with infliximab have also been recognized as a risk group for infections by *P. parasiticum*(Mazzurco et al., 2012),(El-Herte et al., 2014)[1,2]. Disseminated infection is rare and in some cases can be fatal. No death related to localized infection has been reported(Mazzurco et al., 2012).

Traumatic implantation is the main infective route, taking into account that species of *Phaeoacremonium* are ubiquitous in nature (Mazzurco et al., 2012), once the lesion occurs the dermis is mainly affected and in some cases, can be recurrent (Mazzurco et al., 2012). Injuries caused by plants could be the origin of the infection.

The etiology of phaeohyphomycosis involves a broad spectrum of microorganisms such as *Acrophialophora, Alternaria, Cladosporium, Exophiala,* among other species that include *Phaeoacremonium,* being this last one a very rare cause (Alayeto Ortega et al., 2015),(Baddley et al., 2006). Due to the similar morphology of these microorganisms it is challenging to obtain a reliable identification only by microscopic methods (Aroca et al., 2008),(Ellis., 2017),(Colombier et al., 2015),(Mostert et al., 2005). Therefore, sequenciation of selected regions of the β-tubulin gene and 18S ribosomal RNA gene are recommended to reach definitive diagnosis(Aroca et al., 2008),(Colombier et al., 2015). The performance of only microscopic identification may lead to mistakes in diagnosis as happened in this case which was firstly identified as *Exophiala dermatitidis.* The combination of molecular and morphological diagnosis is the most appropriate way for the identification (Colombier et al., 2015).

Antifungal susceptibility testing for *Phaeoacremonium* is not standardized yet, thus caution must be taken when interpreting the results. Although a specific treatment

against this microorganism is still unknown, it is believed that azoles can give optimum results. A study of in *vitro* activity against *P. parasiticum* showed a MIC range of voriconazole 0.125– 2 μg/ml, mean 0.55 μg/ml, amphotericin B range 1–16 μg/ml, mean 3.08 μg/ml and itraconazole range 0.25–32 μg/ml, mean 6.17 μg/ml (El-Herte et al., 2014). Some reports indicate a lower activity of itraconazole(El-Herte et al., 2014)(Baddley et al., 2006),(Alayeto Ortega et al., 2015)(Chowdhary et al., 2014)(Chowdhary et al., 2014). However, in our susceptibility study the result was interpreted as susceptible and this azole was used as part of the patient treatment.

In conclusion the diagnosis of this infection is difficult. However, there is a need to continue investigating, especially on kidney transplanted patients. The combined treatment of surgery and antifungal therapy seems to be the best option to treat this infection.

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Fig.1. Hematoxylin eosin stain at 40x showed a material of vegetal appearance whit fungal structure compatible whit non-septate hyphae in the center of one the granulomas.

Fig.2. Lactophenol cotton blue stain at 40x following growth in culture at 7 days reveals hyaline hyphae, thin-walled phialides with small funnel-shaped collarettes and hyaline conidia in balls.