Case Report

A rare case of fungal keratitis: diagnosis and management

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Introduction: We report a case of keratitis caused by a member of the *Phoma* species of fungi.

Case presentation: A 59-year-old contact lens wearer developed a non-healing corneal ulcer. Microbiological culture and subsequent PCR analysis were performed using samples obtained from corneal scraping. A fungus, phenotypically identified as a member of the *Phoma* species of fungi, was cultured. PCR from infected tissue confirmed the diagnosis.

Conclusion: Although rarely pathogenic to humans, members of the ubiquitous *Phoma* species of fungi can occasionally cause keratitis. Corneal microtrauma associated with contact lens wear is probably important in precipitating such infections.

Keywords: corneal graft; keratitis; *Phoma* fungi.

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Introduction

Fungi of the genus *Phoma* or the synonymous genus *Peyronellaea* are part of a complex species of fungi designated *Pleurophoma* (Gordon *et al.*, 1975). These fungi belong to class Coelomycetes, order Sphaeropsidales and family Sphaeropsidaceae (Oh *et al.*, 1999).

Phoma species can be distinguished from other dematiaceous or 'dark-coloured' fungi by the formation of brown pycnidia (asexual fruiting bodies) without setae or bristles, which are lined with conidiophores. The pycnidia also possess protruding ostioles or openings from which masses of unicellular conidia (also termed chlamydospores, pycnidiospores or chlamydoconidia), i.e. asexual, non-motile spores, emerge (Young *et al.*, 1973).

More than 2000 *Phoma* species of fungi have been described. They are ubiquitous. They are established soil saprophytes, obtaining nutrients from dead organic matter and plant pathogens (Oh *et al.*, 1999; Zaitz *et al.*, 1997). Only nine species are considered pathogenic to humans and animals; *Phoma hibernica*, *Phoma glomerata*, *Phoma cava*, *Phoma cruris-hominis*, *Phoma euphratica*, *Phoma nunmella*, *Phoma minutispora*, *Phoma oculis-hominis* and *Phoma sorghina* (Montel *et al.*, 1991; Zaitz *et al.*, 1997).

Infection of a lesion on the leg by *P. hibernica* was the first reported human *Phoma* infection (Bakerspigel, 1970). Indeed, most human infections with *Phoma* species involve the skin and subcutaneous tissues (Rosen *et al.*, 1996; Zaitz *et al.*, 1997). They usually occur on exposed areas – the hands, feet and face – suggesting that such infections are probably the result of small local inoculations to the skin with, or at least at the sites of, trauma (Stone *et al.*, 1988). They often affect immunocompromised hosts (Rosen *et al.*, 1996; Zaitz *et al.*, 1997).

Medical treatment has been successful in nearly all cases of infection caused by *Phoma* species (Rosen *et al.*, 1996). *In vitro* studies have suggested that itraconazole followed by ketoconazole, fluconazole and griseofulvin are the most effective drugs in treating infections caused by these fungi (Rosen *et al.*, 1996).

To the best of our knowledge, there has been only one previously well-documented case of *Phoma* keratitis (Rishi & Font 2003). Keratitis caused by *P. oculo-hominis* has also been reported but the associated short communication detailed only the mycology of the organism and not the clinical or histopathological findings (Punithalingam, 1976).

We wish to increase the awareness of the *Phoma* species of fungi as a potential cause of keratitis and to provide clinical, morphological, microbiological and histopathological

Abbreviation: VA, visual acuity.

features that might permit its future earlier identification in a clinical setting.

Case report

A 59-year-old daily disposable soft contact lens wearer attended eye casualty with a painful right eye. Her visual acuity (VA) was 6/24 unaided and 6/7.5 with use of a pin-hole. A small central corneal stromal infiltrate with an overlying epithelial defect was noted. Guttate ofloxacin hourly and guttate cyclopentolate 1 % three times daily were prescribed.

Two weeks later, despite persistence of the patient's ocular pain, her VA had improved to 6/12 unaided and 6/7.5 with use of a pin-hole. Three small pinpoint corneal stromal opacities that did not stain with fluorescein were notable. Guttate Maxitrol (dexamethasone 0.1 %, w/v, polymyxin B sulfate 6000 IU ml⁻¹ and neomycin sulfate 3500 IU ml⁻¹; Alcon Laboratories) three times daily was prescribed.

The patient's condition subsequently deteriorated rapidly, with a reduction in VA to 6/60 and the development of a 5 mm central corneal abscess. Corneal scrapings were performed. Guttate gentamicin and chloramphenicol six times daily were commenced. Topical acyclovir was also admi-

nistered on the suspicion that the patient had herpes simplex virus disciform keratitis.

Investigations

Corneal tissue obtained by a corneal scrape was plated on Sabouraud's agar and incubated at 37 °C in CO₂.

DNA was extracted from a similar specimen. PCR was performed using the pan-fungal primers F-forward and DF-reverse, which amplify a broad spectrum of different fungal DNA without cross-amplification of prokaryotic, viral or other eukaryotic genomes.

A portion of each PCR was resolved electrophoretically on a 1.8 % agarose Tris/borate/EDTA gel and visualized using ethidium bromide and UV excitation.

Diagnosis

A sample of corneal tissue taken by corneal scraping grew dark brown, broadly spreading colonies on Sabouraud's agar after 3 days. One such agar plate is shown in Fig. 1(a). Sclerotia admixed with septate hyphae were seen at microscopy. The pycnidia typical of the *Phoma* species of fungi were also noted (Fig. 1b).

Growth from the first of these plates was sent to the Mycology Reference Laboratory, Public Health Laboratory,

Fig. 1. (a) Fungal growth on Sabouraud dextrose agar after 14 days incubation in CO₂ at 37 °C. The colony morphology is typical of *Phoma* species, growing in spreading, velvety grey-brown colonies. (b) Microscopy was performed using lactophenol cotton blue staining. Sclerotia and septate hyphae can be seen as well as pycnidia. (c) Denudation of the epithelium and disruption of the stromal architecture was apparent when the keratectomy specimen was stained with haematoxylin and eosin. Neutrophils and fungal organisms are visible throughout the tissue. (d) When a similar sample was stained with periodic acid-Schiff stain, sclerotia at the edges of corneal perforations and large fungal hyphae were seen. Magnification $\times 10$ (c, d).

Bristol, UK, where the isolate was phenotypically identified as a member of the *Phoma* species of fungi.

The specimen containing the *Phoma* species yielded a single product with an approximate size of 360 bp on PCR analysis. Further analysis revealed this species to be genetically similar but not identical to *Phoma opuntiae*.

Treatment

Treatment with guttate amphotericin 0.5 mg ml^{-1} hourly was commenced and maintained for 1 week following which there was no further enlargement of the patients' corneal infiltrate. This topical therapy was then reduced in frequency to six times daily for a further 3 weeks. Over this time, the VA from this patient's right eye stabilized at 6/36 with use of a pin-hole. However, a plaque-like stromal opacity with an overlying epithelial defect and conjunctival injection, all of which can be seen in Fig. 2(a), persisted. Amniotic graft placement with the aim of promoting corneal re-epithelialization was performed on two occasions.

Five months after her initial diagnosis, her right eye having again become inflamed despite ongoing topical amphotericin 0.5 mg ml^{-1} therapy four times daily, the patient underwent right penetrating keratoplasty in the hope of removing the infected corneal tissue and potentially improving her vision. Following this procedure, the patient was treated with guttate amphotericin 0.5 mg ml^{-1} , dexamethasone 0.1 % w/v and chloramphenicol 0.5% w/v four times daily.

Outcome and follow-up

Three months later, the patient had discontinued all antimicrobial medications. Her VA was 6/36 unaided and 6/15 with use of a pin-hole. As can be seen from Fig. 2(b), there

was no evidence of recurrent fungal infection in her corneal graft.

Histopathological analysis of the keratectomy specimen revealed corneal tissue with a focally denuded surface epithelium and a disordered stromal collagen fibre arrangement, as can be seen in Fig. 1(c). Vascularization of the cornea was also apparent. The stroma was moderately infiltrated by neutrophils. Periodic acid–Schiff staining showed large fungal hyphae. Spherules of variable diameter were observed at sites of corneal perforation, as shown in Fig. 1(d).

Discussion

In the other case of *Phoma* keratitis described in the literature, the fungus was thought to have entered the cornea secondary to trauma (Rishi and Font, 2003). Corneal microtrauma secondary to contact lens wear may have precipitated the case described here.

The history of antecedent trauma and the indolent course of this patient's infection is characteristic of fungal keratitis. Furthermore, fungal keratitis is chronic and difficult to treat, as was the case here. Also as here, penetrating keratoplasty was required in the previously described case of *Phoma* keratitis as corneal perforation had occurred. Consequently, keratitis caused by *Phoma* species must be considered to be serious.

The clinical presentation of the patient in our report bears some resemblance to that of *Acanthamoeba* keratitis. Initially, the pain she described seemed disproportionate to the findings at clinical examination. The patient's condition ultimately deteriorated following the use of topical steroid. At one point, she was suspected of having herpes simplex virus disciform keratitis – a classic stumbling block in the diagnosis of *Acanthamoeba* keratitis.

Fig. 2. (a) Clinical photograph showing a large central corneal infiltrate with fluffy white borders, the presence of conjunctival injection and peripheral corneal vascularization. (b) Clinical photograph showing the same eye following penetrating keratoplasty.

Of note, samples taken by corneal scrape were both culture and PCR negative for *Acanthamoeba*.

Amniotic membrane grafting may promote corneal epithelial healing, augment the corneal stroma and prevent perforation in ocular surface disorders that have caused persistent epithelial defects and/or underlying corneal stromal melting. However, in cases of corneal fungal infection, when the penetration of appropriate topical antimicrobials is often not good in the first instance, it must be acknowledged that amniotic membrane graft placement may further reduce drug penetration. Consequently, in such cases, amniotic graft placement may perhaps be best reserved only for those in which there is stromal thinning and risk of perforation.

Skin and subcutaneous infections with members of the *Phoma* species often affect immunocompromised hosts (Rosen *et al.*, 1996; Zaitz *et al.*, 1997). Our patient had no known systemic immunological defects, but the use of topical dexamethasone may have been influential in the progression of her keratitis.

PCR technology has allowed the rapid detection of DNA in clinical samples, even in situations where copy numbers are low. The pan-fungal specific primers used, which are designed based on the nucleotide sequence of the multiple-copy highly conserved 5.8S rRNA internal transcribed sequence 2 and 28S rRNA regions, amplify a wide variety of different fungal DNA templates (Kercher *et al.*, 2001). Thus, it is both sensitive and specific. The predicted and empirical product sizes of several common ocular fungal pathogens have been described previously. A single product of approximately 360 bp was amplified from the specimen in the case reported here.

The sensitivity and specificity of the pan-fungal-specific PCR assay is extremely advantageous because it makes it possible to rapidly screen ocular specimens, even for rare fungal infections. Further analysis of the PCR products by Southern blotting, restriction fragment digestion or sequencing techniques can then be used for genus-specific identification (Rishi and Font, 2003).

In summary, we identify *Phoma* species as a rare causative organism for keratitis in humans for which contact lens

wear is probably the main risk factor. We also emphasized the potential of PCR assays to aid the laboratory confirmation of such a fungal keratitis.

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