Case Report: Corneal Coinfection with Fungus and *Amoeba*: Report of Two Patients and Literature Review

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Abstract. We report two cases of corneal coinfection with *Acanthamoeba* and *Fusarium* sp. along with the review of published literature. A 35-year-old woman and 65-year-old man presented to the institute with corneal ulcer refractory for treatment with topical antibiotics. Microbiological examination revealed the presence of *Acanthamoeba* cysts along with septate, hyaline fungal filaments. After emergency therapeutic penetrating keratoplasty (TPK) in both, the corneal tissue was sent for histopathologic examination, which confirmed the presence of *Acanthamoeba* and fungal coinfection. One patient had a recurrence of fungal infection after TPK. In subjects with a rapid progression of mycotic ulcer, coinfection with other microorganisms including *Acanthamoeba* should be suspected. The two cases presented here emphasize the importance of microbiology in making prompt diagnosis and appropriate management of these cases at an early stage.

INTRODUCTION

Microbial keratitis has been reported to be due to infection with a wide range of organisms. There are regional variations in the predominance of different microbes, reflecting different patient population and climatic effects.¹ Although fungal keratitis is more common in tropical and subtropical areas, *Acanthamoeba* keratitis rarely presents with a positive history of contact lens use in India and South Asia, and is more often associated with a previous history of trauma or exposure to contaminated water.² Compared with bacterial keratitis, isolated infection with organisms such as *Acanthamoeba* and fungi tend to follow a more insidious course and may need a therapeutic surgical intervention. A complicating factor in the treatment of keratitis is the coinfection with microorganisms which may result in significant changes for both and increased virulence in the mammalian hosts.³ So far, 13 cases of coinfection of cornea with *Acanthamoeba* and fungus have been reported, and the use of contact lenses was involved in five of them.^{4–7} Treatment regimens have varied from the biguanides, polyhexamethylene biguanide (PHMB) and chlorhexidine, to the diamide propamidine, the aminoglycoside neomycin, and the antifungal clotrimazole. The drugs have been used in different combinations, with varying degrees of success.⁸ The standard medical treatment of fungal keratitis is the frequent use of topical natamycin 5%, but any delay in diagnosis usually leads to therapeutic penetrating keratoplasty (TPK).⁹ Mixed infections can be difficult to manage because *Acanthamoeba* and fungus are resistant to initial empirical antimicrobial agents.³ The purpose of this study was to report two patients of keratitis where microbiological examination of the corneal scrapings showed both *Acanthamoeba* and *Fusarium* sp., and the diagnosis was confirmed by histopathology of the corneal tissue after TPK. We also present the current literature that is available on this dual infection of the cornea.

METHODS

This study was conducted at the L. V. Prasad Eye Institute, Hyderabad, India, a tertiary eye care facility in South India. A retrospective, consecutive review of medical and microbiology records of all patients seen between January 2012 and December 2016 identified two cases having infection with fungus and *Acanthamoeba*. Demographic details, cause and duration of symptoms, presenting and final visual acuity, surgical interventions, and laboratory data of these patients were collected. Both patients had undergone complete eye examination under slit lamp biomicroscopy and indirect ophthalmoscopy following collection of history and demographic details.

The patients had undergone complete microbiological investigations as described by us earlier.¹⁰ In brief, corneal scrapings were obtained from the patients under topical anesthesia (0.5% proparacaine hydrochloride) using a sterile surgical blade no. 15 on a Bard Parker handle. The corneal scrapings were examined by direct microscopic examination after staining with calcofluor white and Gram stain. Culture included a variety of media such as blood agar (5% sheep blood), chocolate agar (5% sheep blood), brain heart infusion broth, Sabouraud dextrose agar, and non-nutrient agar with an overlay of *Escherichia coli* (not taken for case 2) for the possible growth of bacteria and/or fungus and/or *Acanthamoeba*. Incubation of the media and their interpretation were as per our protocol published earlier.¹⁰

Post-penetrating keratoplasty in both cases, half corneal button (fixed in 10% buffered formalin) was sent to the pathology laboratory for histopathology examination. Paraffin embedded tissue sections were stained with hematoxylin and eosin, periodic acid-Schiff, and Gomori's methenamine silver stain (GMS) following standard procedures.¹¹ The other half of the corneal buttons were sent in sterile containers to the microbiology laboratory for culture. Minced tissue was inoculated on blood agar, brain heart infusion broth, Sabouraud dextrose agar, and non-nutrient agar with *E. coli*.

Case 1. A 35-year-old woman presented to the cornea clinic with pain and redness in the left eye since 7 days. She had no history of trauma or use of contact lenses. Visual acuity was 20/40. Slit-lamp examination revealed anterior- to midstromal corneal infiltrate measuring 7 × 5.5 mm (Figure 1A).

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FIGURE 1. (A) Slit-lamp photograph of the left eye at initial presentation-needs some description, (B) Gram stain from the first scrapings showing filamentous structures with chlamydospores ($\times 100$), (C) photograph from the culture specimens showing *Fusarium* spp. ($\times 40$), (D) slit-lamp photograph of the left eye at day 10 showing rapid worsening characterized by diffuse corneal involvement up to the limbus, (E) calcofluor white mount from repeat scraping showing coinfection of *Fusarium* and *Acanthamoeba* ($\times 40$), and (F) Histopathology of the corneal button showing fungal filaments and *Acanthamoeba* on special staining (Gomori's methamine silver staining) at $\times 20$. This figure appears in color at www.ajtmh.org.

Microbiological examination of the corneal scrapings showed fungal filaments with chlamydospores on calcofluor white stain (CFW) and Gram stain (Figure 1B) which later grew *Fusarium* sp. in culture (Figure 1C). She was treated with 5% Natamycin eye drops hourly and oral ketoconazole 200 mg twice a day. A rapid worsening of the symptoms and progression of the ulcer was noted over the next few days. Vision was reduced to light perception and slit-lamp examination revealed a full thickness infiltrate involving the whole of the cornea with melt and ectasia inferiorly (Figure 1D). In view of rapid worsening, the patient was taken for TPK and corneal scrapings were repeated. Microbiological smears of the second scrapings revealed both fungal filaments (Figure 1E, arrowhead) and *Acanthamoeba* cysts (Figure 1E, arrow) on CFW, and both grew in culture. Histopathological examination of the corneal specimen revealed plenty of fungal hyphae and double walled *Acanthamoeba* cysts in Gomori's methenamine silver staining, suggestive of coinfection with fungus and *Acanthamoeba* (Figure 1F, arrow). The culture of the corneal button grew only *Fusarium* spp. Postoperatively, the patient received topical natamycin (5%) and 0.02% chlorhexidine along with oral ketoconazole (200 mg, twice daily). The postoperative course was uneventful with no recurrences until 6 months of follow up.

Case 2. A 65-year-old farmer presented with pain and redness in the left eye since 3 months, following injury with stick. He had consulted at several places and his records revealed a nonresponse to therapeutic trial with topical antibacterials. In one of the records, he was diagnosed to have viral keratitis with secondary glaucoma. At presentation, visual acuity was light perception with inaccurate projection of light rays. On slit-lamp examination, the lids were edematous, conjunctiva was congested, cornea showed a full thickness total corneal infiltrate with hypopyon. The intraocular details of

the eye could not be appreciated because of the hazy cornea. Therefore, B scan ultrasonography of the posterior segment of the eye was performed to rule out any intraocular extension of the infection. The test was unremarkable with no echos seen in the vitreous cavity of the eye, indicating no involvement of the vitreous or retina. Microbiological examination of the corneal scrapings showed septate hyaline fungal filaments along with *Acanthamoeba* cysts on a CFW (Figure 2A) and Gram stain (Figure 2B). The extensive corneal involvement necessitated immediate TPK, following which the patient was advised hourly topical 5% Natamycin, 0.02% PHMB, and 0.02% Chlorhexidine. Histopathological examination of the corneal specimen revealed extensive lamellar stromal necrosis with the co-existence of both empty cysts and branching hyphae on Gomori's methenamine silver staining (Figure 2C, arrow). The culture of the corneal button grew *Fusarium* species. A month later, the patient developed a graft infiltrate with anterior chamber exudates. A clinical impression of recurrence of infection was made. Corneal scrapings from the infiltrate site revealed the presence of Gram-positive cocci and septate filamentous fungus on microbiological smear examination. Cultures grew *Staphylococcus epidermidis* and *Fusarium* sp. Treatment with natamycin was continued, however, he was lost to follow up.

DISCUSSION

In the setting of recalcitrant corneal ulcers unresponsive to medical treatment, the differential diagnoses include polymicrobial infections, resistant organisms, atypical etiologic agents, coexisting ocular surface disorders, and systemic ailments such as diabetes mellitus.¹² Combined fungal and *Acanthamoeba* keratitis is a rare entity and can be difficult to

FIGURE 2. (A) Calcofluor white stained smear of corneal scraping ($\times 40$) showing presence of both fungus (arrowhead) and *Acanthamoeba* (arrow), (B) Gram stained smear of corneal scraping ($\times 100$) showing both fungus (arrowhead) and *Acanthamoeba* (arrow), (C) Histopathology of the corneal button ($\times 20$) showing Fungal filaments (arrowhead) and *Acanthamoeba* (arrow) on special staining (Gomori's methamine silver staining). This figure appears in color at www.ajtmh.org.

diagnose and manage.⁶ Of the 3,733 fungal corneal infections treated at our institute during the study period, coinfection with fungus and bacteria was observed in 267 cases, and two cases were identified to have a coinfection with fungus and *Acanthamoeba*. In the first case presented here (Case 1), the initial microbiology reports showed the presence of fungus alone with chlamydo spores; however, microbiological evaluation of the second scrapings identified both fungus and *Acanthamoeba* cysts. It is possible that at the first instance the cysts were missed as they resemble the fungal chlamydo spores and one may tend to identify the predominant organism. In the second case, however, in the initial microbiological investigation itself both fungus and *Acanthamoeba* infection was revealed. Both the patients here required a therapeutic keratoplasty, and one had a recurrence of fungal infection after keratoplasty. Several factors are implicated in poor response to medical management in coinfection with *Acanthamoeba* and fungus. Treatment failures and limitations of medical

treatment even in isolated fungus and *Acanthamoeba* infections is well known. In our first case, there was a rapid worsening of the infiltrate over a few days, but the diagnosis of *Acanthamoeba* along with fungus was made only on repeat scrapings after worsening of symptoms. In the second case, the infection was long standing and had involved the entire cornea at the time of initial presentation which could account for the poor prognosis and the recurrence in the second case.

Literature search revealed 13 cases with concurrent *Acanthamoeba* and *Fusarium* keratitis (Table 1); all were initially misdiagnosed as bacterial or viral keratitis.^{4-8,13-16} Of these cases, five had a history of use of contact lens⁴⁻⁸ whereas injury by foreign body was seen in two cases,⁵ including one from our series. Corneal trauma allows the microorganisms access to the stroma and deep epithelial layers. In most reported cases, the scrapings showed the presence of *Acanthamoeba*, and only after corneal perforation followed by subsequent TPK has the diagnosis of fungal corneal infection

TABLE 1
Details of Previous Reports on mixed infection of *Acanthamoeba* and fungal keratitis in the Literature (PubMed)

S. no	Reference	Organism	No of patients	Risk factor	Treatment	Outcome (VA)
1	Gussler et al. (1995) ¹⁵	<i>Fusarium</i> sp.	1	Radial keratotomy (tap water)	PHMB	TPK (20/20)
2	Tien and Sheu (1999) ⁵	Mould	3	DM, mud-related trauma, and SCL	Neomycin, metronidazole (case 1), amikacin, piperacillin (cases 2, 3), PHMB, fluconazole, TPK	TPK (HM, 20/100, 20/20)
3	Froumis et al. (2001) ⁴	<i>Scenedosporium apiospermum</i>	1 (two eyes)	Contact lens	Cyclosporin A, Pred-Forte, propamidine, neomycin, natamycin, PHMB	TPK, lensectomy, vitrectomy (CF) 20/30
4	Babu and Murthy (2007) ¹⁶	<i>Fusarium</i> sp.	1	Agriculturist	Cycloplegics	TPK
5	Slade et al. (2008) ⁶	Fungus (HP section)	1	Intacs corneal implants	PHMB, propamidine, clotrimazole, natamycin	20/20, 20/200
6	Lin et al. (2009) ⁸	<i>Fusarium</i> sp.	2	Contact lens and FB (case 2)	Propamidine isothionate, natamycin, fluconazole, neosporin cycloplegic eye drops	Improved
7	Gupta et al. (2010) ¹³	<i>Curvularia</i> sp.	1	Pond water	Propamidine, chlorhexidine, neomycin/polymyxin B	TPK twice (20/400)
8	Lee et al. (2010) ⁷	<i>Fusarium solani</i>	1	Silicone hydrogel contact lens	PHMB, chlorhexidine, natamycin	TPK (CF)
10	Current case 1	<i>F. solani</i>	1	Unknown	PHMB, chlorhexidine, natamycin	TPK (LFU)
11	Current case 2	<i>Fusarium</i> sp.	1	Injury-stick	PHMB, chlorhexidine, natamycin	TPK (LFU)

CF = counting fingers; DM = diabetes mellitus; FB = foreign body; HM = hand movements; HP = histopathology; LFU = lost to follow up; PHMB = polyhexamethylene biguanide; SCL = soft contact lens; TPK = therapeutic penetrating keratoplasty.

been discovered through corneal histopathology.^{4–7} Only one case established a diagnosis with the help of confocal microscopy.¹⁶ In both our patients, the direct examination of the corneal scrapings and histopathological examination of the corneal tissue were confirmatory of the diagnosis of *Fusarium* and *Acanthamoeba* infection. Molecular tests such as polymerase chain reaction would have been performed if required. However, they were not considered necessary in our patients. In addition, Lin et al.⁸ suggested that *Acanthamoeba* may progress faster because the fungi may serve as nutrient for the amoeba in mixed infections, whereas Hu et al.,¹⁷ suggested that as the amoebae and human macrophage share similar features, the accidental selection of fungal virulence traits by *Acanthamoeba*, may help *Fusarium solani* to escape or to survive in human macrophages, which may lead to worsening of keratitis. The delay in fungal diagnosis may represent the faster progression of *Acanthamoeba* keratitis in dual infections leading to corneal perforation.

Regardless, coexistent atypical infectious keratitis with these two organisms, although extremely rare, resulted in severe keratolysis and necessitated TPK with poor visual outcomes like most of the cases described previously. Better visual outcomes are most often reported in cases where TPK could be avoided.^{8,13,16} It is probable that the infectious keratitis in our patients would have responded favorably if combined antifungal and anti-amoebic treatments were instituted simultaneously in the early stages of the disease before complete corneal involvement. Specific diagnosis was not made and both our cases had not received specific therapy. Meticulous microbiological investigation of all cases with clinical diagnosis of microbial keratitis is recommended.

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