Failure of chemotherapy in the first reported cases of *Acanthamoeba* keratitis in Pakistan

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Acanthamoeba keratitis is a painful and progressive infection of the cornea that can result in loss of vision. Here, for the first time in Pakistan, we report two cases of *Acanthamoeba* keratitis. The first patient was a 37-year-old female who presented with severe itching, redness, pain, along with loss of vision. The patient was a regular soft contact lens wearer. The second patient was a 25-year-old female who had been using soft contact lenses for the past two years. She presented with a burning sensation and extreme pain, along with loss of vision. Both patients were treated for a possible microbial keratitis with topical moxifloxacin hydrochloride drops, vancomycin drops, propamidine isethionate ointment, amphotericin B drops, and amikacin drops. However, the response was inadequate and both patients were referred for corneal transplant. *Acanthamoeba castellanii* was isolated by placing contact lenses and contact lens cases on non-nutrient agar plates containing a lawn of non-invasive *Escherichia coli* K-12 HB101 bacteria. The polymerase chain reaction (PCR) using genus-specific probes confirmed the identity of *Acanthamoeba* spp., whereas the morphological characteristics of trophozoites and cysts were suggestive of *A. castellanii* in both cases. With growing use of contact lenses for vision correction/cosmetic use coupled with sub-standard lens care in this region and the possibility of non-contact lens-associated *Acanthamoeba* keratitis, a need for increased awareness of this sight-threatening infection is discussed further.

Keywords: *Acanthamoeba*, Keratitis, Contact lens, Polymerase chain reaction, Treatment

Introduction

Acanthamoeba castellanii is a unicellular protist pathogen that is extensively dispersed in nature.^{1–3} During the last few decades, *Acanthamoeba* species have become progressively acknowledged as important microbes. They are known to cause *Acanthamoeba* keratitis, a sight-threatening ulceration of the cornea that is associated with excruciating pain.⁴ Furthermore, *Acanthamoeba* species are able to cause a rare form of encephalitis known as granulomatous amoebic encephalitis (GAE), mostly limited to individuals with a compromised immune system.² The genus *Acanthamoeba* consists of 17 genotypes (T1–T17);^{5–8} however, the T4 genotype has been most frequently associated with blinding keratitis.⁹ The sequence of events in *Acanthamoeba* keratitis involves the breakdown of the epithelial barrier, stromal invasion by *Acanthamoeba*, keratocyte depletion, induction of an intense inflammatory response, and finally stromal

necrosis.^{4,10} Treatment is problematic and consists of hourly topical application of a mixture of drugs including polyhexamethylene biguanide or chlorhexidine digluconate together with propamidine isethionate or hexamidine. Moreover, chloramphenicol or neomycin is also given to prevent mixed bacterial infection.⁴ The treatment lasts for several months and even then, recurrence can occur.¹¹

Ever since *Acanthamoeba* keratitis was first diagnosed in 1973,¹² the incidence has risen, most likely due to the increased use of contact lenses, and also due to improved diagnostic methods leading to increased awareness. In addition over the last few decades, it has become apparent that contact lens users are at increased risk of corneal infections. Studies estimate that 90% of *Acanthamoeba* keratitis patients are the users of contact lenses.^{13,14}

The burden of *Acanthamoeba* keratitis in the region of Pakistan is unknown, despite the rise of contact lens wear, both for vision correction as well as their cosmetic use. For the first time in Pakistan, here we report two cases of *Acanthamoeba* keratitis in female patients who were contact lens wearers.

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Case Report

Case 1

A 37-year-old female was referred to the Ophthalmology Department, Aga Khan University Hospital, Karachi, Pakistan, with a 5-day history of severe pain, redness, and watering in her left eye. She was a regular soft contact lens wearer. At presentation, the patient had a visual acuity of light perception in the affected eye and 20/25 in the right eye with the best correction. The initial diagnosis in this case was corneal ulceration associated with contact lens wear. The patient was treated for a possible microbial/viral keratitis and included anti-*Acanthamoeba* (propamidine isethionate), anti-fungal, and anti-bacterial agents. The combination of drugs was given topically, every hour for up to 5 days. The drug combination included moxifloxacin hydrochloride drops, vancomycin drops, propamidine isethionate ointment, amphotericin B drops, and amikacin drops. However, the response was inadequate and *Acanthamoeba* was suspected to be the etiological agent of her illness. The contact lens cases and contact lenses were consequently examined. Samples were spread onto non-nutrient agar plates seeded with non-invasive *Escherichia coli* K-12 laboratory strain, HB101.¹⁵ Culture plates were Parafilm-sealed and incubated at 37°C. The plates were examined daily under an inverted microscope for *Acanthamoeba* using morphological features of trophozoites as well as cysts. The identity of *Acanthamoeba* was confirmed with polymerase chain reaction (PCR) using genus-specific primers AcantF900 (5'-CCC AGA TCG TTT ACC GTG AA-3) and AcantR1100 (5'-TAA ATA TTA ATG CCC CCA ACT ATC C-3) to amplify fragments of approximately 180 base pairs as previously described.¹⁶ A clinical isolate of *A. castellanii* belonging to T4 genotype, originally isolated from a keratitis patient (American Type Culture Collection, ATCC 50492), was used in the present study as a positive control. The PCR analysis revealed that the infecting agent was *Acanthamoeba* species (Fig. 1), whereas the morphological features of trophozoites and cysts were suggestive of *A. castellanii*. As the treatment was ineffective, consequently the patient was referred for keratoplasty surgery and has recovered well with no recurrence reported in the past 3 months.

Case 2

A 25-year-old female was referred to the Ophthalmology Department, Aga Khan University Hospital, Karachi, Pakistan, with a 3-week history of severe pain, redness, watering, and itching in her right eye. At presentation, the patient had a visual acuity of hand movement in the right eye and 20/25 in the left eye with the best correction. She was a regular soft contact lens wearer. The initial diagnosis was corneal perforation/melt due to contact lens-associated severe corneal

Figure 1 Polymerase chain reaction (PCR) assays confirmed the identity of *Acanthamoeba* spp. To determine the identity of *Acanthamoeba*, DNA was isolated and used for PCR analysis using genus-specific primers. Note that using *Acanthamoeba*-specific primers, PCR products were found at ~180 bp confirming the identity of *Acanthamoeba*. A clinical isolate of *Acanthamoeba castellanii* belonging to T4 genotype, originally isolated from a keratitis patient (American Type Culture Collection, ATCC 50492), was used as a positive control. Lane 1 is 100 bp DNA ladder; lane 2 is positive control; lane 3 is *Acanthamoeba* from contact lens of patient 1; lane 4 is *Acanthamoeba* from contact lens case of patient 1; lane 5 is *Acanthamoeba* from contact lens of patient 2; lane 6 is *Acanthamoeba* from contact lens case of patient 2; lane 7 is no DNA; and lane 8 is 100 bp DNA ladder.

infection. The patient had visited another eye clinic, a week before, and was receiving hourly topical moxifloxacin hydrochloride drops, vancomycin drops, propamidine isethionate ointment, amphotericin B drops, and amikacin drops. However, the response was inadequate and *Acanthamoeba* was suspected to be the etiological agent of her illness. The contact lens cases and contact lenses were spread onto non-nutrient agar plates seeded with non-invasive *E. coli* K-12 laboratory strain, HB101¹⁵ and plates examined for *Acanthamoeba* as described above. The identity of *Acanthamoeba* was confirmed using PCR (Fig. 1), whereas the morphological features of trophozoites and cysts were suggestive of *A. castellanii*. As the treatment was ineffective and there was a corneal perforation at presentation, the patient was advised an urgent right corneal transplant and has recovered well with no recurrence reported in the past 3 months.

Discussion

To our knowledge, not a single case of *Acanthamoeba* keratitis has been reported from Pakistan prior to this report and the burden of this sight-threatening infection is unknown. Our personal communication with the consultant ophthalmologist at the Aga Khan University Hospital, Karachi (a leading private hospital in Pakistan) has revealed that keratitis cases are prevalent, suggesting there is an urgent need for awareness of this difficult to treat infection. The question arises that if we are witnessing such

infections in a small, private hospital in a cosmopolitan and progressive city with the state-of-the-art facilities, the situation must be extremely dire in poor communities and the remaining country, particularly small cities and villages where there is little or no access to hospitals. While *Acanthamoeba* keratitis is generally reported in contact lens wearers¹⁷ and there has been a rise in contact lens use, both for corrective and cosmetic purposes, it is frequently observed in non-contact lens wearers, in particularly from developing countries.^{18–20} In non-contact lens wearers, predisposing factors reported are corneal trauma by items from the environment such as dirty water splash, insects, vegetation, traditional eye medicine, surma (kohl), and other particulate matters. In neighboring India, such cases have been often observed in patients from a low socioeconomic background,¹⁸ suggesting that *Acanthamoeba* keratitis may actually be rampant in Pakistan also, but is probably missed or mistreated because clinicians unduly associate *Acanthamoeba* keratitis with contact lens wear. Thus, the reported cases from Karachi may only represent the tip of the iceberg and the actual numbers of *Acanthamoeba* keratitis cases are most likely far higher. Contrary to what occurs in *Acanthamoeba* keratitis in contact lens wearers, complaint of severe pain in non-contact lens wearers may not be a significant feature, while radial keratoneuritis has been reported in 2.5% patients and a ring infiltrate is observed in 41.1% patients.¹⁸ As *Acanthamoeba* keratitis in non-contact lens wearers is often presented at the advanced stage with clinical features that may be atypical of contact lens-associated *Acanthamoeba* keratitis and there is a poor visual outcome, these findings suggest the need for increased awareness by the public as well as health professionals of *Acanthamoeba* keratitis in non-contact lens wearers. Patients who present with keratitis with no history of contact lens wear but the aforementioned predisposing factors should also be routinely suspected of *Acanthamoeba* keratitis. This coupled with the availability of molecular-based diagnostic tools in this part of the world warrants an increase in the reported cases of *Acanthamoeba* keratitis from Pakistan.

For contact lens wearers, the development of *Acanthamoeba* keratitis is a multifaceted process, comprising of several factors such as contact lens wear for extended periods of time, lack of personal hygiene, inappropriate cleaning of contact lenses, biofilm formation on contact lenses, and exposure to contaminated water.² This was consistent with our observations as both patients were regular users of contact lenses. It is distressing to note that none of the marketed contact lens disinfecting solutions in Karachi, Pakistan were effective in killing

Acanthamoeba including ReNu MultiPlus, DuraPlus, Ultimate Plus, OptiFree Replenish, OptiFree Express, Kontex Clean, Kontex Normal, Kontex Multisol extra+, and Kontex Soak (unpublished findings). Cysts treated with aforementioned contact lens disinfecting solutions tested re-emerged as viable amoeba upon inoculation in the growth medium (unpublished findings). Worryingly, contact lenses as well as their contact lens cleaning solutions can be bought without any instructions given to the consumer, majority of whom are unaware of the associated risks or they are teenagers in the case of cosmetic contact lens users. Additionally, contact lenses for cosmetic as well as corrective use are routinely available over the counter in developing countries such as Pakistan without any instructions of proper hygiene to handle contact lenses given to the users. These findings are of great concern for contact lens users in this region. Overall, the reported cases serve as an illustration that *Acanthamoeba* is an emerging cause of severe keratitis in Pakistan affecting healthy people and suggest the need for increased awareness among ophthalmologists as well as public to address this serious issue.

Conclusion

For the first time in Pakistan, we report two cases of *Acanthamoeba* keratitis. Both patients were treated with the recommended anti-*Acanthamoeba* chemotherapy. However, the response was inadequate and both patients were referred for corneal transplant. There is a need for increased awareness by the public and the health professionals of this sight-threatening infection.

Competing Interests

The authors declare no competing interests.

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