Unusual Case of *Acanthamoeba polyphaga* and *Pseudomonas aeruginosa* Keratitis in a Contact Lens Wearer from Gauteng, South Africa

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*Acanthamoeba* species can cause a chronic, progressive ulcerative keratitis of the eye which is not responsive to the usual antimicrobial therapy and is frequently mistaken for stromal herpes keratitis. An unusual case of coinfection with *Acanthamoeba polyphaga* and *Pseudomonas aeruginosa* as causes of corneal keratitis in a contact lens wearer from Gauteng, South Africa, is reported. These two pathogens have previously been assumed to be selectively exclusive. Cysts of the isolated *acanthameba* tolerated an incubation temperature of 46°C, indicating it as a pathogenic species. This case highlights the importance of culture methods in the diagnosis of corneal infection and the choice of treatment regimen. The patient's history of careless contact lens disinfecting habits emphasizes the need to adhere strictly to recommended methods of contact lens care.

Free-living amebae belonging to the genus *Acanthamoeba* are found worldwide in air, dust, and water and are relatively resistant to normal levels of chlorine in tap water. These amebae occur in two forms in humans: as an active, invasive trophozoite stage and as a dormant, cystic stage (2). Several species of *Acanthamoeba* can cause a chronic, progressive ulcerative keratitis of the eye, which is a painful and potentially sight-threatening condition. Many such ocular infections are associated with minor corneal trauma, and it is thought that contact lenses (usually soft lenses) may predispose the wearer to corneal infection. Acanthamoeba keratitis is being recognized with increasing frequency in both the developed and the developing world (16, 20). This is probably due to a greater understanding of the disease process and the development of sophisticated, noninvasive diagnostic techniques, such as use of the confocal microscope. This microscope enables direct visualization of the *acanthamebae* in vivo upon slit lamp examination (24). It has been postulated that *Acanthamoeba* species may have been the cause of many cases of clinically presumed herpes simplex virus keratitis, bacterial keratitis, and other corneal diseases. In one study, 84% of the *acanthameba*-positive patients were found to have had a clinical diagnosis other than *acanthameba* keratitis made upon initial examination (19, 27).

The earliest sign of *acanthameba* infection, patchy edema, occurs at the epithelial level. This causes irregular dendritic ulceration, which often leads to misdiagnosis as keratitis due to herpes simplex virus. A clue to the correct diagnosis is that the patient is young and wears contact lenses; herpetic ulceration is rare in this group. Certain clinical features facilitate identification of the therapeutically resistant stromal keratitis in which an *Acanthamoeba* species should be suspected as the etiologic agent. Anterior stromal infiltration in the shape of a complete or partial ring is a consistent finding, with recurrent breakdown and healing of overlying epithelium. The ring is paracentral in early disease, while the central cornea is clear. Pain is unusually severe for the degree of keratitis and may be related to perineural infiltration by the amebae. If the condition is left untreated, the amebae penetrate the full depth of the cornea, forming a ring abscess, which may eventually lead to corneal perforation. Most patients have antibodies to *acanthamebae*, but the immune system alone seems unable to halt progressive corneal infection (2, 28).

CASE REPORT

A 31-year-old woman was referred to the Johannesburg Hospital eye clinic by a private ophthalmologist. She had been hospitalized 2 weeks previously for a *Pseudomonas aeruginosa* corneal ulcer and had received subconjunctival injections of gentamicin, amikacin, and ceftazolin, to which the organism had shown sensitivity in the laboratory. She had also received topical ciprofloxacin therapy and was subsequently discharged on this treatment. Within a week, the corneal ulcer had relapsed.

The patient had a history of disposable soft contact lens wear and was careless with the disinfecting routine. She regularly rinsed her contact lenses and case in tap water instead of sterile saline solution. Her corrected visual acuity was counting fingers in her right eye and 6/6 in the left. The conjunctiva of the right eye was injected. There was marked blanphoriasms and photophobia, and the patient complained of severe pain. She had a fluorescein-staining corneal ulcer measuring 4.8 by 3.6 mm, with an underlying grayish-white, paracentral, ring-shaped stromal infiltrate (Fig. 1A). The anterior chamber had a 20% hypopyon. The left eye was normal.

Treatment was initiated on the assumption that the causative organism was *P. aeruginosa*. The patient received topical ciprofloxacin, atropine, and chloramphenicol, oral paracetamol, and subconjunctival injections of amikacin (20 mg daily) and cefazolin (125 mg daily). Over the following 10 days, the ulcer reepithelialized, but the stromal infiltrate worsened, forming a complete ring. The hypopyon remained at 20%.
MATERIALS AND METHODS

The corneal surface was sampled under local anesthesia with calcium alginate swabs. Microscopy and culture for amebic, bacterial, mycobacterial, and fungal organisms were performed. Swabs from the cornea were inoculated onto two Escherichia coli-seeded, 1% nonnutrient agar (ECNNA) plates (Difco [Detroit, Mich.] agar) (13). In addition, the patient’s disposable contact lenses were placed onto two ECNNA plates. Ten milliliters of the patient’s contact lens-disinfecting solution was centrifuged at 3,000 rpm (Universal 16 centrifuge; Hettich) for 10 min, and the sediment was inoculated onto two ECNNA plates. All of the ECNNA plates were incubated at 25 and 37°C for 20 days. The disinfecting solution was cultured for bacteria.

The acanthameba isolate was cloned by diluting a suspension of cysts in sterile ameba saline, spreading them on agar under a microscope, and selecting individual cysts by using low magnification (7). A piece of agar bearing the selected cyst was cut out and transferred face down to a fresh ECNNA plate. Several plates were prepared in this manner from sequential cultures, and each time the
RESULTs

After 7 days of incubation at 37°C, the ECNNA plate that had been inoculated with the contact lens case swab yielded cysts and trophozoites of Acanthamoeba species (Fig. 1B and C). These trophozoites and cysts proved to be viable at 37°C. Trophozoites incubated at 40°C encysted or died after 15 days, whereas cysts were viable after 15 days at 40°C, i.e., they encysted and the trophozoites multiplied at 37°C after cysts were exposed to 40°C for 15 days. These temperature tolerance results indicate that this strain of acanthameba is pathogenic (8).

The mean cyst diameter was 14.1 μm. The squarish endocyst was attached to the ectocyst at the corners, with the folded ectocyst widely separated from the endocyst in places. Based on morphological characteristics, the species was identified as Acanthamoeba polyphaga

When microbiological culture confirmed the presence of an Acanthamoeba species, a treatment regimen with topical neomycin and 0.02% polyhexamethylene biguanide drops given hourly was begun. Within 48 h, the hypopyon had resolved completely, and over the next 3 weeks, the stromal infiltrate showed slight improvement. The treatment was continued for a prolonged period, tapering off over 12 months. At present, the patient’s best corrected vision is counting fingers. The eye shows no signs of inflammation but does have a few superficial blood vessels in the superior temporal quadrant. There is a large, central corneal scar obscuring the visual axis. The anterior chamber is deep and quiet, and there are posterior synechiae and a cataract present. The intraocular appearance is normal, and ultrasonography indicates a normal posterior segment. The patient is currently awaiting a corneal graft.

DISCUSSION

Amebae were not cultured from the superficial corneal swab, probably because sampling was inadequate. However, the clinical history and presentation, the recovery of acanthameba from the contact lens case, and the response to treatment, all strongly support the diagnosis of acanthameba keratitis. This case emphasizes the need to suspect acanthameba infection in soft contact lens wearers who present with keratitis. Inquiry should be made into the patient’s contact lens cleaning habits. Acanthameba infection should be considered in the differential diagnosis of any chronically progressive ulcerative keratitis and in progressively worsening corneal ulcers that are not responsive to the usual antimicrobial therapy (6). Most cases are mistaken for stromal herpes keratitis, since both disorders exhibit stromal necrosis and because cultures for bacteria and fungi are negative in both conditions (18). However, the clinical appearance of acanthameba keratitis is often characteristic: there is usually a central stromal opacity with an epithelial defect and a pronounced surrounding ring of inflammatory infiltration (10). Severe ocular pain, the corneal ring infiltrate, and recurrent epithelial breakdown are all important diagnostic features of acanthameba keratitis (33).

It is also important to consider the possibility of a coexisting bacterial (P. aeruginosa in this case) and Acanthamoeba species infection. It has been suggested that contamination of contact lens care systems with Acanthamoeba species and a bacterial species capable of supporting amebic growth may be the first step in the pathogenesis of ameba-induced keratitis by the provision of large inocula of amebae (4). Three other studies have shown that almost 50% of acanthameba-positive eyes had cultures that were positive for bacteria as well. In light of these observations, it is advisable to culture for bacteria and to use the appropriate antibacterial treatment in acanthameba infections (19, 23, 27). Aerobic gram-negative bacilli, including P. aeruginosa, are the predominant causative agents of acute necrotizing keratitis in the contact lens wearer (34). Previously, it was assumed that Acanthamoeba species and P. aeruginosa acted as selectively exclusive eye pathogens, because the latter induces amebicidal activity when cocultivated with Acanthamoeba species in vitro (25). In the present case, however, both organisms were isolated from a patient presenting with a corneal ulcer. It is important to bear in mind that acanthamebae may temporarily respond to antibacterial treatment, because the organism may become dormant upon exposure to antibiotics or to the preservatives contained in the antibiotic preparations (31). If acanthameba keratitis is suspected in a patient, smears should be made from corneal scrapings and culture for acanthamebae should be performed. The patient’s contact lenses and the lens case should also be obtained for culturing.

The treatment of acanthameba keratitis is controversial. Early diagnosis means a better prognosis for the patient. However, most affected eyes eventually require corneal graft surgery to restore vision. There is also a risk of toxicity to the corneal epithelium associated with the use of topical therapy (14, 17). Recently, it has been suggested that the most successful method of treatment for acanthameba keratitis is a combination of either 0.02% polyhexamethylene biguanide or 0.02% chlorhexidine with 0.1% propamidine isethionate. This regimen lowers the risk of drug resistance developing as a result of low-dose, single-drug therapy (17, 22, 30). In addition, a recent study has shown that a 0.5 to 2.5% povidone-iodine solution (PVP-I [Betadine]) has better in vitro antiamebic activity on both trophic and cystic stages of acanthamebae than chlorhexidine (11).

A breach of the normal barrier function of the corneal epithelium predisposes to acanthameba keratitis (5). Consequently, a number of risk factors have been identified: the wearing of soft contact lenses (especially extended-wear lenses); the use of nonsterile, homemade saline solutions; the use of chlorine-based disinfection rather than alternative chemical systems; the rinsing of contact lenses and cases in domestic tap water; the wearing of lenses while swimming; trauma to the eye; and preexisting corneal epithelial disease. Solutions used for soft contact lenses are not as effective against acanthamebae as those for hard and gas-permeable lenses (21). Previous corneal disease alters the normal epithelium and exposes the cornea to infection by opportunistic organisms (31). If acanthameba keratitis is suspected in a patient, smears should be made from corneal scrapings and culture for acanthamebae and antibiotic prepa-
the use of lens disinfection systems that are effective against the organism (26, 32).

The contact lens storage case is the usual source of microbial contamination, originating from tap water or household dust. Colonization is also facilitated by the ability of the acanthamebae to adhere to the polymers used to produce contact lenses. One study found that at least one contaminating organism was detected in 54% of the contact lens cases studied, and at least one well-recognized corneal pathogen was isolated from 34% of the cases. Four percent of storage cases were contaminated by amebae (5). Contact lenses should be stored in a clean, uncontaminated case that has been washed with boiled and cooled water (at 70°C). The case should be kept dry when not in use in order to prevent the multiplication of gram-negative rods and amebae (12). Some disinfectant-containing contact lens solutions can induce inflammation of the ocular tissues, unless the contact lenses have been rinsed before insertion (9). In addition to omitting postdisinfection rinsing, our patient often used nonsterile tap water to wash the lens case and the contact lenses. These factors contributed to the development of keratitis.

Acanthamoeba keratitis is too often a late diagnosis, and this may be attributed to a lack of awareness of the prevalence and pathogenicity of the organism. If the condition is diagnosed early in disease as a result of successful isolation, medical treatment may result in a cure and obviate the need for penetrating keratoplasty. In this regard, clinicians must suspect acanthameba infection in at-risk patients with suggestive clinical signs (1, 15). Furthermore, close collaboration with a microbiologist familiar with the culture requirements and appearance of the organism is imperative in order to allow for earlier treatment and improved prognosis (2). For all contact lens wearers, fastidious lens hygiene and strict attention to disinfector routines are the most important aspects of prophylaxis against microbial keratitis (29).

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REFERENCES