Acanthamoeba keratitis for the microbiologist: An eye opener

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Acanthamoeba is a microscopic, cyst-forming, free-living amoeba (single-celled living organism), best known for its offensive habit of invading the cornea. The amoeba is found worldwide in the environment – in air, water and soil. Although free-living, they are also opportunistic pathogens, causing:

1. Acanthamoeba keratitis (AK), an infection of the eye that can result in permanent visual impairment or blindness;
2. Granulomatous amoebic encephalitis, a serious infection of the brain and spinal cord; and
3. Disseminated infection. Groups at high risk for systemic infections include immunocompromised and immunosuppressed individuals, while keratitis occurs in otherwise healthy individuals.[1]

Acanthamoeba keratitis is a rare yet increasingly diagnosed, severe, potentially sight-threatening, devastating ocular parasitic infection prevalent in developing countries. In India, the reported incidence of AK is about 1% among culture-positive infective keratitis.[2] Contact lens usage is rarely associated with it (0.9%). In non-contact lens users, it is often overlooked as a cause of keratitis and diagnosed late.[3] The protracted, painful clinical course, and frequently encountered treatment failures especially due to the resistance of *Acanthamoeba* cysts make it difficult to treat.

Acanthamoeba keratitis may occur in patients of any age, sex or race. The risk factors include corneal foreign bodies or any micro trauma, contact with non-sterile water, contact lens wear, concurrent eye disease, and microbial keratitis.[4] Sometimes, there need be no pre-disposing factor at all.[5] AK is sometimes co-infected with other pathogens such as herpes simplex virus, various bacteria or fungi.

Access this article online

Quick Response Code:

Website: www.jacmjournal.org

DOI: 10.4103/0972-1282.158814

Diagnosing AK is difficult, and it is occasionally mistaken for ocular herpes or fungal keratitis. It should be considered in cases of corneal trauma associated with soil or contaminated water, contact lens users, and persistent corneal infection not responding to proper treatment. Delayed diagnosis only leads to poor visual outcome.

The genus Acanthamoeba is a group of nearly 25 named species of which 11 are pathogenic.[3,6] *A. castellani* and *A. polyphaga* are the most common species to cause keratitis.[7]

Acanthamoeba has two stages in its life cycle – a vegetative, feeding, dividing, active, and infective trophozoite (which is 14-40 μm in diameter) and a resting, metabolically dormant, environmentally sturdy cyst (which has a double-layered wall with a diameter of 12-16 μm).[5] The trophozoite feeds on organic particles as well as other microbes.[2,3] They are minute oval or triangular unicellular organisms that can change their size and shape at any moment with their acanthopodia – a specific form of pseudopodia which are usually short and fine. They are constantly formed and reabsorbed to induce locomotion. The advancing acanthopodia are 'wide and tongue-shaped'. The distinguishing features of the trophozoite are a prominent contractile vacuole in the cytoplasm and a nucleus with a large central nucleolus. Reproduction occurs by binary fission.[7] Exposure to harsh, unfavourable conditions, such as extreme temperature, high or low pH, dryness or starvation results in cellular differentiation into a double-walled cyst form. Both walls are normally separated by a space, except at certain exit points for the excysting trophozoites.[2] Cysts are resistant to disinfectants.

I wish to describe my encounter with this organism as a microbiologist at a Tertiary Eye Care Centre in Trivandrum, Kerala, South India – The Regional Institute of Ophthalmology. Here, AK was mostly associated with fungal keratitis. Almost 20 different fungi could be isolated and reported from here. Aspergillus species was the predominant one followed by Penicillium, Fusarium, Curvularia, Mucor, Alternaria, Helminthosporium, Bipolaris, Phoma,

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Acremonium, Nigrospora and Aureobasidium to name a few. The patients ranged from manual labourers to housewives, professionals, and students. The source could probably be from water, especially from wells, overhead tanks, ponds, lakes, seawater, air conditioning units, and swimming pools. Among the cases that we encountered, the majority (99.5%) were using well water. The usual presentation was pain, photophobia, redness, diminution of vision, foreign body sensation, watering, and irritation in the affected eye. Pain was the pre-dominant presenting symptom. Sometimes, there was no history at all of any sort of trauma to the eye.

A corneal scraping is taken from the base and edge of the ulcer after instilling proparacaine hydrochloride local anaesthetic drops, ideally with a flame sterilised Kimura's spatula or a sterile Bard–Parker blade (no. 15) or by a 26G needle by the ophthalmologist. The procedure is performed under the magnification of a slit lamp or operating microscope.

The material obtained is initially smeared onto clean sterile labelled glass slides for the wet saline mount, 10% KOH wet mount and Gram-stain. The material obtained by the next scrape is inoculated directly onto the surface of a solid media such as sheep blood agar, chocolate agar, Mac Conkeys agar, Sabarauds's dextrose agar or non-nutrient agar in rows of C-shaped streaks, and also inoculated into the depth of liquid media such as brain heart infusion broth, thioglycollate medium, and glucose broth.

Acanthamoeba are seen as tiny specks and spots on the specimen surface in direct microscopy with low power objective. Under high power, the trophozoites in the specimen the specimen may be seen moving slowly. Any such movement from within or outside the specimen will catch your attention. Rarely, the specimen itself becomes so shaky due to the struggle of the Acanthamoeba to come out into the free space that it is even difficult to focus it under the microscope. The squirting movement is typical if ever you could observe it. The cytoplasm has the property to contract and isolated pools are seen to writhe, producing squirting motions. Sometimes, the amoebae are so hidden within the specimen that on continued exposure to the uncomfortable and disturbing heat and light of the examining microscope, they push their way out, forming channels in the specimen. This escape to the outside appears much like a march or a parade. In the free space outside the specimen, they typically move around very slowly, but sometimes, they remain completely still, making the smear appear lifeless, and the diagnosis, confusing. Very few of them may continue changing their shape when observed carefully. Sometimes, only one or two may be seen in the whole specimen and that too with great difficulty when the parasitic load is low or infection is less severe.

The cysts are single celled, with a nucleus, and they appear clearly with a lactophenol cotton blue stain. They have clear double-walls, a wrinkled outer wall, and a round, star-shaped, hexagonal or polygonal inner wall. Sometimes the cysts seem swollen up in the saline wet mount!

SOME CHALLENGES ENCOUNTERED WHILE PURSUING THIS PARASITE

1. An immediate second or even third scraping has helped many a time to reach the diagnosis. When the initial scraping is inconclusive, ask for a repeat sample, but this may not reveal anything at all. It is then the third scraping that reveals the culprit. Even if you notice some movement of the parasite in a few smears, it can stop in no time.
2. Sometimes, the whole specimen is so shaky that it is difficult even to focus the specimen. The *Acanthamoeba* are using all of their strength to come out of the specimen. Sometimes they march out of the specimen, one after the other in a row!
3. Cysts are seen more frequently during the rainy season (monsoons) in India. They appear packed in the specimen. With cysts alone, confirmatory diagnosis is difficult by direct microscopy. When the surface teams with cysts, the deeper layers have few trophozoites. A repeat scraping within 2 days has always given a positive result for *Acanthamoeba*. Cysts produce resistant, non-healing corneal ulcers that are difficult to treat.
4. *Acanthamoeba* may be easily mistaken for pus cells. A Gram stain can, with difficulty but with experience, help distinguish the trophozoites but not the cyst. In Gram-stained smears, the structure of the cyst was not as remarkable and resembled tissue macrophages, mononuclear cells or degenerative epithelium.

A history of exposure of eyes to well water and of trauma/injury to the eye, typical presenting symptoms, occupation, and duration of illness along with tiny spots on the surface of the corneal scraping from infective keratitis under low power of the microscope should be noted by the microbiologist as a clue to AK. In my experience, simple microscopic examination as wet mount preparation with normal saline is all that was required to diagnose and confirm Acanthamoeba. We could also culture and grow Acanthamoebae successfully; both from corneal scraping as well as from the well water from patients' homes and establish the well water as the major source of Acanthamoeba.

Treatment is with topical antiparasitic drugs like reconstituted Chlorhexidine eye drops, Neomycin ointment and/or poly hexamethylene biguanide drops along with other supportive measures. Therapeutic response is adequate in most cases, though healing is delayed with a chronic indolent course

in mixed infections, in elderly and in those with associated ocular surface diseases. Prompt diagnosis and early treatment is the most important factor deciding treatment success.

There is indeed a very powerful role here for the dedicated microbiologist. All clinical microbiologists attached to eye hospitals should be aware of this organism and try their best to get trained in microscopy and isolation of this sight-threatening parasite.

MY PERSONAL EXPERIENCE IN A FEW WORDS

'This parasite, if present, can play hide and seek with you, sometimes, a shake, a twist or a turn is all that is visible. Thus, it is all a mysterious game. They play or they dance or they hide and stay still and silent. You watch and watch, and they most often are caught red-handed! Sometimes, you have to wait for its acrobatics to end to focus the smear. This is then a real treat to our eyes – the dance is very graceful and peaceful – till it is tired. I have observed this dance for 35 min at a stretch; took a video of this, and its performance was on-going, when my eyes and neck felt strained, and I gave up! What if you are short on time? No way; it demands all your time, concentration, attention, and persistent viewing'.

ACKNOWLEDGEMENTS

Dr. Girija Devi P.S., Director and Head of Regional Institute of Ophthalmology (RIO), Trivandrum, Kerala, India, who was sending cases with a strong clinical suspicion of AK to the microbiology lab, expecting a confirmatory diagnosis through direct microscopy and culture. Her constant encouragement and patience made me spend hours and hours on the microscopic examination of the corneal scrapings in search of *Acanthamoeba*, because till then, I had never encountered this sight-threatening, blinding parasite except in literature.

It was owing to the continuous and daily interaction with a few ophthalmologists, in particular, Dr. Reena A. and Dr. Anitha Balachandran, that this dream of catching *Acanthamoeba* live became a reality. I also thank my lab staff Beena, Suma, Seeja, and Anjana.

My gratitude always to Dr. Mabel Legori and Dr. Saramma T.I. (my mentors), and Dr. Ramani Bai JT, Professor, and Head of Department of Microbiology, Medical College, Trivandrum and Dr. Kavita Raja, Professor of Microbiology, SCTIMST, Thiruvananthapuram for their encouragement in all my ventures.

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How to cite this article: Cherian A. Acanthamoeba keratitis for the microbiologist: An eye opener. J Acad Clin Microbiol 2015;17:60-2.
Source of Support: Nil. **Conflict of Interest:** None declared.