

# Successful culture of *Acanthamoeba* remains a key towards diagnosis of unusual clinical presentation of keratitis: A first case report from chhattisgarh

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### ABSTRACT

A fifty-nine-year-old female with corneal ulcer with a history of trauma in the past having clinical presentation of fungal keratitis visited the Ophthalmology outpatient department with a history of redness, watering, pain and white discoloration of the right eye cornea for two months. Sample was cultured on non-nutrient agar medium and revealed *Acanthamoeba*. Patient was managed with Chlorhexidine eye drop and keratoplasty.

**Keywords:** Free living amoebae, granulomatous amebic encephalitis (GAE), keratitis, keratoconjunctivitis, NN medium

### Introduction

Keratitis is the most common cause of monocular corneal blindness worldwide. The ocular trauma and corneal ulceration results in 1.5–2 million new cases of corneal blindness annually in the developing countries. Corneal blindness due to keratitis is a major public health problem in India.<sup>[1]</sup> Microbial keratitis is a spectrum of ocular infectious diseases, affecting the cornea resulting from bacterial, fungal, and protozoa organisms which may be responsible for ocular morbidity and disability. Most predominantly it is due to bacteria then fungi. *Acanthamoeba* keratitis is a rare but sight-threatening corneal infection.<sup>[1]</sup>

Herewith, reported an unusual case of keratitis due to *Acanthamoeba* in the contact lens non-user with clinical presentation resembling to that of fungal keratitis.

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### Case

A 59-year-old female patient referred to the ophthalmology out-patient department of our institute in August, 2019 with a history of redness, watering, pain and white discoloration of the right eye cornea for two months. She had a history of an accidental injury to the right eye with stone. She had been treated at a local hospital but did not improve. While presenting to us she was on eye-drops Chlorhexidine and Atropine and had been advised therapeutic keratoplasty. Patient did not suffer from any systemic illnesses and had undergone cataract surgery in both eyes a year back. Patient did not suffer from any systemic illnesses.

On examination, visual acuity was found to be with hand movement in the right eye and 6/12 (p) in the left eye, vitals were stable, general examination and systemic examination were within normal limits. Local examination of the right eye revealed both upper and lower lid edema, circumcorneal congestion. Limbal infiltrates at 2 'O' clock and 4 'O' clock positions, corneal haze, central corneal ulcer approximately 9 × 8 mm in size with feathery margins, ring shaped infiltrates,

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anterior chamber was well formed with hypopyon which was immobile with upward concavity, pupils were 7 mm dilated not reacting to light and corneal sensations were intact [Figure 1]. Anterior segment of left eye was within normal limits. Syringing was patent on both sides. Rest of the ocular examination was normal. A provisional diagnosis of fungal keratitis of right eye was made.

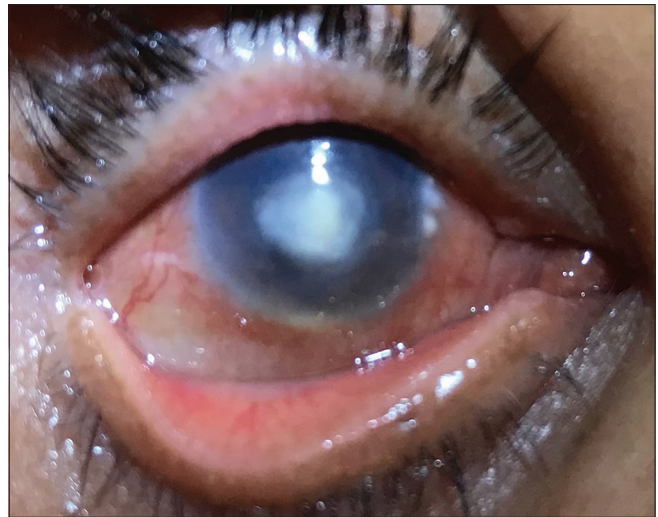
She was admitted and corneal scraping was done, specimen sent for Gram's staining, KOH, bacterial culture and fungal culture. She was started on oral Itraconazole. Topically, eye drops Moxifloxacin, Natamycin and eye ointment Atropine were also started. As the KOH revealed no fungal elements, Itraconazole was stopped on the second day and moxifloxacin dosing was increased to hourly and Natamycin was reduced. Neosporin was added.

On sixth day, the peripheral cornea became clear and margins of the ulcer was noted to be more defined. Bacterial culture was negative by this time and repeat corneal scraping specimen was sent on day seven. The patient was symptomatically and clinically better, so she was discharged on the same treatment and was advised to follow-up in the OPD after one week.

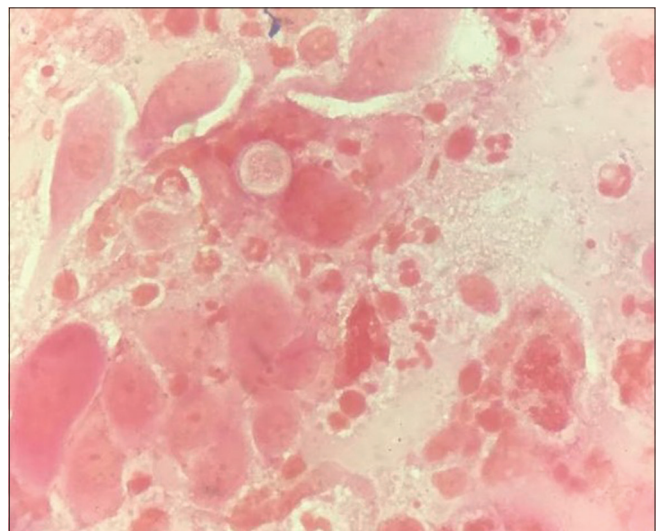
The follow-up showed clinical improvement in the form of decrease in size of the ulcer and margins becoming more defined. Fungal cultures subsequently came out to be negative. She was followed up regularly every one week but as the hypopyon was still persistent so a repeat corneal scraping was done. Gram stain of the corneal scrapping revealed moderate pus cells and many rounded to irregular double walled structures of approximate size of 10 – 50 micrometers suspicious of cysts [Figure 2] were seen. Suspecting it to be Acanthamoeba cyst, the ophthalmologist was requested for a repeat corneal scrapping and was again sent for microbiological investigation. KOH mount of the corneal scrapping was negative for any fungal elements. Calcofluor white stain showed fluorescent spherical structures of variable size [Figure 3].

Corneal scrapping was again taken using all aseptic precautions and inoculated on non-nutrient agar (NN agar) and SDA with a C-shaped inoculation. The NN agar plates, were incubated at 30°C after overlaying with *Escherichia coli* broth culture. Gram stain of the corneal scrapping showed spherical, well defined double-wall structures with irregular inner wall. Giemsa stain showed purple spherical cyst like structure with two walls containing internal multi angular layer. The incubated Non-nutrient agar plates were screened daily for signs of Acanthamoeba growth under the low-power (10X) objective using a light microscope.

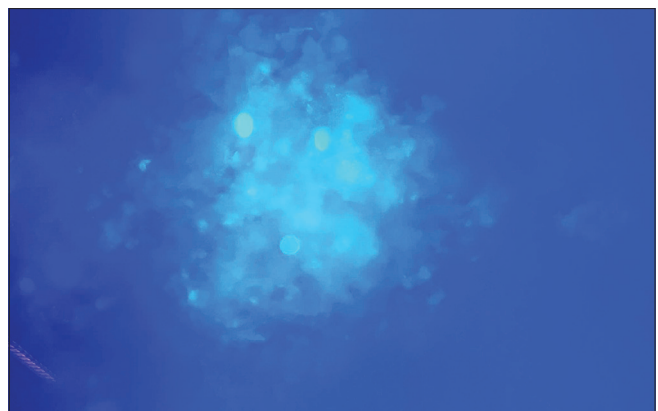
After 3 days of incubation the NN agar showed various shapes of trophozoites with acanthopodia, a prominent contractile vacuole and many hyper refractile cysts with multiple shapes (round, triangular, square) [Figure 4]. Amoebae migration tract was visible after 2 days. Giemsa stain was done from the smear made from the culture scrapping of NN agar revealed many double walled



**Figure 1:** Clinical presentation



**Figure 2:** Gram stain of Direct Smear of corneal scraping



**Figure 3:** Calcofluor stain of Direct clinical specimen

polyhedral cysts. Patient was managed with chlorhexidine eye drop. She was perusing regular follow-up till one week after that she missed the follow-up.



**Figure 4:** Microscopic pictures showing Trophozoites of Acanthamoeba from culture

## Discussion

Acanthamoeba is a free-living amoeba known to cause granulomatous amebic encephalitis (GAE) or an eye infection referred to as Acanthamoeba keratitis.<sup>[2]</sup> Acanthamoeba keratitis is a chronic infection of the cornea caused by several species of Acanthamoeba including *A. castellanii*, *A. culbertsoni*, *A. hatchetti*, *A. polyphaga*, and *A. rhysodes* and infections are being diagnosed with increasing frequency.<sup>[3,4]</sup> The exposure to Acanthamoeba species appears to be common due to its saprophytic nature still the incidence of Acanthamoeba keratitis is less common. In India, Acanthamoeba accounts for 2% of all cases of corneal ulcers.<sup>[5]</sup>

The common risk factors for Acanthamoeba keratitis are wearing contact lens for prolonged periods, corneal trauma, and wrong practices of contact lens rinsing, swimming with contact lenses, and biofilm formation on contact lens.<sup>[5]</sup> In the present study, the patient was contact lens non-user. The patient did not give any history of swimming in fresh water but gave history of trauma due to stone two-months before, so the possibility of contamination of wound with water could not be denied as fresh water is the source of free-living amoebae.

Studies from India and South America reported that trauma and consequent exposure to contaminated water are the most important risk factors of AK in developing nations. More risk population are primarily comprised of field workers.<sup>[6-8]</sup>

Clinical suspicion is required to diagnose a rare pathogen. Differential diagnosis of AK is bacterial, fungal, herpetic keratitis. In the present study clinical presentation was likely to be fungal keratitis as the corneal ulcer was with feathery margin. Anterior chamber of right eye had formed hypopyon. One must remember that all patients with Acanthamoeba keratitis is variable and do not present with classical symptoms and signs of AK like radial keratitis, ring infiltrates, stromal edema.<sup>[9]</sup> These were absent when patient had visited and in the follow-up. So complete investigations to rule out bacterial, fungal and protozoal agents must be undergone. In the present case study, KOH could not reveal anything, Gram stain findings were significant and revealed some circular empty structures and proved to be key to make provisional or differential diagnosis as protozoal keratitis and after cultivation the diagnosis was made confirmed. Newer techniques are also there like PCR and LAMP are better diagnostic technique to diagnose and speciate Acanthamoeba.

Early diagnosis may prove the key of successful treatment as management can be initiated soon. Rarely encountered infection so, standardized therapy or specific drugs are not available but still drugs like Brolene and Poly hexamethylene biguanide or combination of Neosporin drops, Clotrimazole drops and Ketoconazole tablet were also tried. Larkin *et al.*,<sup>[10]</sup> study experience had demonstrated good results starting with biguanide (Chlorhexidine 0.02%) in combination with oral itraconazole or ketoconazole. PHMB 0.02% may be used in combination, as PHMB has been shown to kill both the trophozoite and cyst without toxic effect to the healing epithelium.<sup>[11]</sup> Alizadeh *et al.*,<sup>[11]</sup> also suggest that pentamidine may be the diamidine of choice for Acanthamoeba keratitis. Murakawa GJ *et al.*,<sup>[12]</sup> had tried Pentamidine intravenously in disseminated AK.

The distinctiveness of this Acanthamoeba keratitis case was that, the patient was non-contact lens user; presentation was like fungal keratitis, it was associated with hypopyon of anterior chamber of affected eye, not typical of AK. So Natamycin with Moxifloxacin were given. Symptomatically patient was better and was discharged. But in the weekly follow-up, it was observed that the lesion had not recovered and there was persistent hypopyon. When scrapping was recollected the Acanthamoeba was detected. After appropriate therapy she responded well. Detection of Acanthamoeba by culture remains the key for appropriate diagnosis and management of this vision-threatening infection. For the same, this case also proves that complete microbiological investigations protocol for detection of bacteria, fungi and protozoa should follow for its successful management. Reporting of rarely encountered infections with its fate after management is needed to be address. It will help us for the management of future cases and will also help for the further research.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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## Conflicts of interest

There are no conflicts of interest.

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