CASE SERIES

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Acanthamoeba Keratitis- Camouflage Entity in Eastern Nepal: A Case Series

• Sanjay Kumar Singh[1] • Karthik Ambur[1] • Prija Poudyal[1] • Sagun Malla[1] • Amit Rajbanshi[1]

Submitted 12 May 2021 **Accepted** 12 June 2021 **Published** June 30 2021

Prija Poudyal
kgktssbh@gmail.com

https://orcid.org/0000-0003-4192-9791

[1] Biratnagar Eye Hospital, Biratnagar Morang, Nepal.

Citation

"*Singh SK, Ambur K, Poudyal P, Malla S, Rajbanshi A. Acanthamoeba keratitis-camouflage entity in eastern Nepal: A case series. JBPKIHS. 2021;4(1):59-63.*"

https://doi.org/10.3126/jbpkihs.v4i1.37071

Abstract

Acanthamoeba keratitis is a sight-threatening corneal infection and is a growing clinical problem in the world. Though Acanthamoeba keratitis is considered uncommon and rarely reported in Nepal, we encountered six cases in 2019. All patients had redness, photophobia, decreased vision, and pain with ring infiltrate. Ten percent potassium hydroxide mount revealed Acanthamoeba cyst in all cases. Non-nutrient agar overladen with Escherichia coli revealed feeding tracks and Polymerase Chain Reaction revealed T4 genotype Acanthamoeba in four cases. Amoebicidal treatment was started with chlorhexidine 0.02% eye drop half-hourly and supplementary treatment included moxifloxacin eye drop, a combination of polymyxin B sulfate, neomycin sulfate, and bacitracin eye ointment. After treatment, one patient had the best-corrected visual acuity of 6/9 while others had a visual outcome of hand movement. A high level of clinical suspicion and wet mount examination of specimen from infected corneal tissue are essential to aid in rapid diagnosis.

Keywords: *Acanthamoeba* keratitis, Cornea, Polymerase Chain Reaction, Nepal

Declarations

Ethics approval and consent to participate: Not applicable. However, Institutional Research Committee was informed about the case report.
Consent for publication: Informed consent obtained from patients for publication of identifying features.
Availability of data and materials: All data generated or analyzed during this study are included in this published article and the patients' files can be presented if required.
Competing interest: None
Funding: None
Authors' contributions: SKS: ophthalmic care, literature review, manuscript preparation and editing. KA: ophthalmic care, literature review, data collection. PP: laboratory investigations, literature review, manuscript preparation. SM: ophthalmic care, literature review, data collection. AR: laboratory investigation, literature review. All authors read and approved the final manuscript.
Acknowledgement: We would like to acknowledge Dr. Ibne Karim M. Ali, PhD, and Mr. Shantanu Roy, MSc. Microbiology, Division of Foodborne, Waterborne and Environmental Diseases, Center for Disease Control and Prevention (CDC), Atlanta for providing the real time PCR reports and for the immense support and cooperation.

Acanthamoeba species are free-living protozoans and have been isolated from air, water, and soil [1, 2]. It was first reported in 1973 [3]. Various risk factors are responsible for its causation including the use of contact lens in developed countries [4-6], and trauma or use of contaminated water in developing countries [3, 7-9]. *Acanthamoeba* adheres to the corneal epithelium with the help of a protein and then produce pathogenic proteases that degrade the basement membrane and cause cytolysis and apoptosis of cornea cellular elements and finally lead to the dissolution of the corneal stroma [10]. *Acanthamoeba* keratitis is often misdiagnosed as it shares similar symptoms and signs as that of herpetic, fungal, or bacterial keratitis. So, accompanying diagnostic tests with microscopy, culture, and Polymerase Chain Reaction (PCR) are highly supportive.

CASES

This retrospective case series included all the *Acanthamoeba* keratitis cases encountered in the year 2019 at Biratnagar Eye Hospital, Biratnagar, Nepal. All six *Acanthamoeba* patients were from agricultural background and none of the patients wore contact lenses. Four patients had a history of trauma with vegetative matters. Patients presented late to the eye hospital and the duration of onset of symptoms to presentation to our hospital (mean ± SD) was 19 ± 7.78 days. Five patients had best corrected visual acuity (BCVA) of hand movement to the perception of light vision and one had 2/60 in the affected eye at the time of presentation (**Table 1**).

All patients had redness, photophobia, and decreased vision. Severe excruciating pain was the main complaint in two patients (Case 1 and 3, Table 1). Ring infiltrate was the hallmark in all the patients (**Fig. 1**). The size of the corneal ulcer was > 6 mm in four patients (**Table 2**).

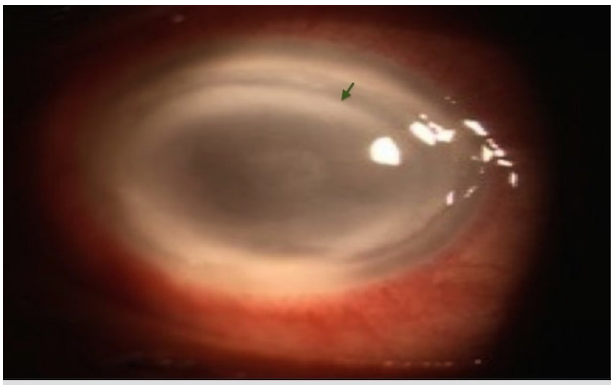

Figure 1: Ring infiltrate appreciated in an affected eye

MICROBIOLOGICAL LABORATORY WORK-UP

All patients underwent corneal scraping with a 15-degree Bard-Parker blade and scraped tissue was sent for gram stain and 10% potassium hydroxide (KOH) mount respectively. It was also inoculated in blood agar, chocolate agar, and Sabouraud dextrose agar. If the *Acanthamoeba* cysts were identified in either gram stain or KOH mount, then scraping was further inoculated in non-nutrient agar overladen with Escherichia coli.

Double-walled cysts of *Acanthamoeba* were noted on KOH mount (**Fig. 2a**) in the first scraping in four cases whereas only in the second scraping that was done after 3 days in two cases (**Table 3**). The second scrapings were done as there was high clinical suspicion suggesting *Acanthamoeba*. Gram stain revealed *Acanthamoeba* cyst (**Fig. 2b**) in one of the cases (Case 5). The culture of the first four cases in the non-nutrient agar with *Escherichia coli* showed clear/ feeding tracks along the lawn of *Escherichia coli* which signified the presence of migrating trophozoites that feed on the bacilli (**Fig. 2c**). Cysts isolated from culture showed inner polygonal and outer wrinkled wall (**Fig. 3a**) which was further highlighted with Giemsa stain (**Fig. 3b**). The

Table 1: Demographic and clinical profile of patients with *Acanthamoeba* keratitis

Case No	Age/ Sex	Involved eye	Vegetative trauma	Vision at presentation	Onset to presentation duration (days)
1	47/ F	Left	Yes	HMCF	15
2	28/ M	Right	No	POL*	30
3	32/ M	Right	Yes	2/60	10
4	32/ F	Left	No	POL*	25
5	33/ M	Right	Yes	HMCF	20
6	58/ M	Right	Yes	HMCF	12

HMCF: Hand movement close to face, POL: perception of light, projection of rays accurate in all quadrants*

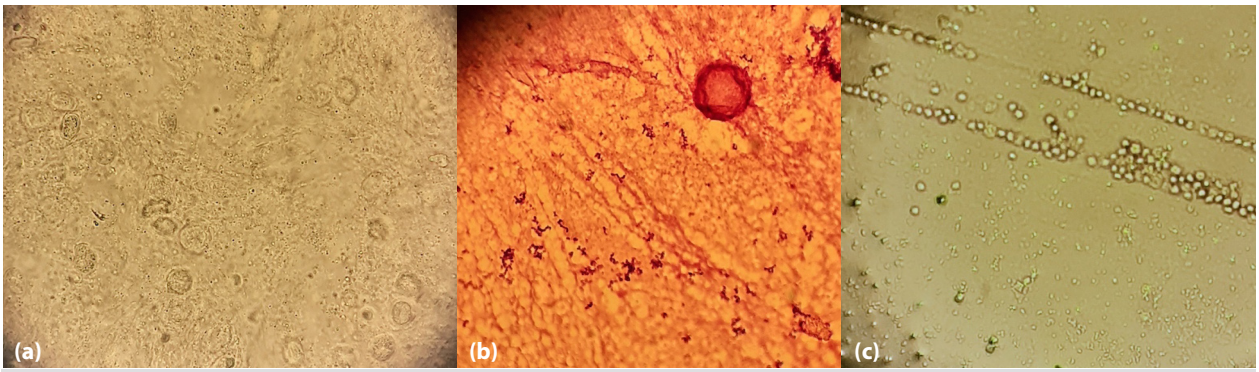

Figure 2: *Acanthamoeba* cyst (a) seen under 10% KOH mount (10X) (b) observed with Gram stain (100X) (c) amoebal feeding tracks appreciated along the lawn of E.coli (10X)

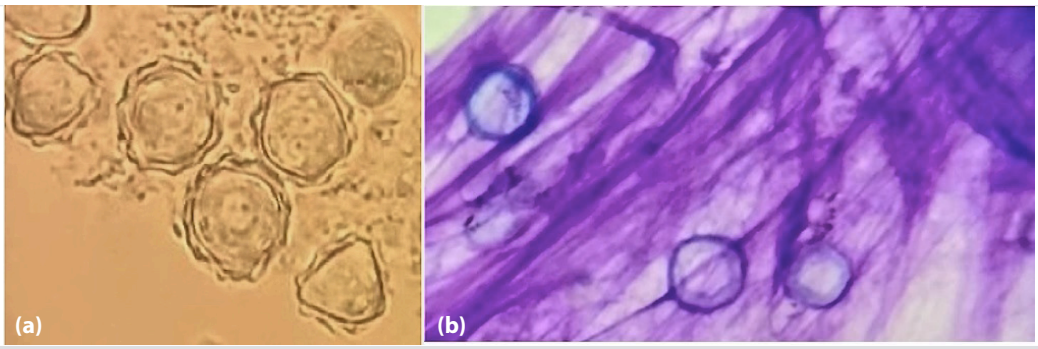

Figure 3: *Acanthamoeba* cyst (a) isolated from culture plates revealing inner polygonal and outer wrinkled wall (100X) (b) further highlighted with Giemsa stain(40X)

blood agar, chocolate agar, and Sabouraud dextrose agar cultures were negative in all cases.

PCR testing facility was not available anywhere in Nepal for *Acanthamoeba* at that time, so communication was made with the Centre for Disease Control and Prevention, USA and the samples of the initial four patients were sent in a special carrier media, as per their instructions. All first four samples were *Acanthamoeba* positive by a diagnostic real-time PCR (**Table 3**). These samples were genotyped successfully and belong to genotype T4. Two of the strains appeared identical in Sanger sequences to each other, while the other two were distinct. Three of these samples showed the most resemblance with the *Acanthamoeba* culbertsoni. Samples of the last two patients, who lost to follow-up, could not be sent.

TREATMENT

In this hospital, five patients except one (Case 3) received amoebicidal treatment after confirmation of clinical suspicion with direct microscopy reports. Out of these five patients, three were treated with antifun-

Table 2: Clinical findings on ophthalmic examination

Case No.	Ulcer location	Size of ulcer (mm)	Hypopyon
1	Central + paracentral	7.0	Present
2	Central + paracentral	7.0	Present
3	Paracentral	4.0	Absent
4	Central + paracentral	5.0	Absent
5	Central + paracentral	6.5	Absent
6	Central + paracentral	8.0	Present

Table 3: Microbiological and PCR findings from the scraped samples

Case No.	*Acanthamoeba* like cyst detected				Culture* positive on day	PCR
	1st scraping		2nd Scraping			
	Gram staining	KOH	Gram staining	KOH		
1	None	None	None	Yes	4	Positive
2	None	Yes	ND	ND	5	Positive
3	None	None	None	Yes	5	Positive
4	None	Yes	ND	ND	7	Positive
5	Yes	Yes	ND	ND	ND	ND
6	None	Yes	ND	ND	ND	ND

*ND: Not done, KOH: 10% potassium hydroxide, PCR: Polymerase Chain Reaction. *Non- nutrient agar with Escherichia coli.*

gal and antibiotics while two were treated with antiviral and antibiotics elsewhere before they approached us with a history of progression of infection and no alleviation of symptoms. Amoebicidal treatment was started with chlorhexidine 0.02% eye drop. Initially, the drug was started on a half-hourly basis and the dose was reduced after 3 days to an hourly basis, subsequently, it was tapered to 2 hourly after one week. Supplementary treatment included treatment with moxifloxacin eye drop 6 times a day, a combination of polymyxin B sulfate, neomycin sulfate, and bacitracin ointment at bedtime and atropine eye drop 3 times a day. Case no 3 was continued on antifungal (natamycin eye drop 5% half hourly and fluconazole eye drop 0.3% half hourly) and antibiotic (moxifloxacin eye drop 0.5% half hourly) as the patient was already receiving the medication before he was diagnosed in our hospital and the scarring had started around the ulcer despite thin cornea. He underwent cyanoacrylate glue application along with the bandaged contact lens. Gradually eye drops were tapered in all patients.

All patients showed early response to medication with a dramatic reduction in pain within one week as noted subjectively. Cases 1, 2 and 4 had healing with early corneal scarring within 2-3 weeks. One patient (Case 3) had gained BCVA of 6/9 whereas the remaining 3 patients had a final vision of hand movement. Case 5 and 6 visited another eye hospital to seek a second opinion and were lost to follow-up.

DISCUSSION

Unlike the reports of *Acanthamoeba* keratitis from the developed world [4-6], none of our patients wore contact lens, and all were farmers. Similar findings of higher association of *Acanthamoeba* keratitis with a history of trauma due to vegetative matter or other foreign body have been reported from other developing countries [3, 7-9].

In this series, excessive pain disproportionate to sign was complained by two patients (case no 1 and 3) while the other four patients complained of little pain. Ring infiltrate, a diagnostic sign for *Acanthamoeba* keratitis was noticed in all patients. Similar clinical features were described in earlier reports [3, 7].

In this case series, the sensitivity of KOH was higher than gram-stained smears in the detection of *Acanthamoeba*, as the cysts were easily identified in all six cases using KOH mount compared to one case using gram stain. Several other studies from India have reported superiority of KOH wet mount preparation for the diagnosis of *Acanthamoeba* keratitis [3, 7]. *Acanthamoeba* cysts with trophozoites were identified on non-nutrient agar overladen with *Escherichia coli* for initial four patients and PCR reporting was also reported to be positive in these patients.

All patients showed a clinical response to treatment. The pain was significantly reduced in all patients after starting medication and this could be due to the effect of amoebicidal and atropine eye drops. Two patients preferred to have a second opinion so they could not be followed up. Only one patient had BCVA of 6/9 and others had visual outcome of hand movement. Centrally located ulcer, size of the ulcer > 6 mm, and visual acuity hand movement/ perception of light at the time of presentation were associated with poor outcome.

Despite having one of the highest reported incidences of corneal ulcer in the world [11, 12], there are very few published reports of *Acanthamoeba* keratitis from Nepal [13]. It may be due to misdiagnosis relating to its simulation with bacterial, fungal, or viral infections. The diagnosis of *Acanthamoeba* keratitis should

be considered in patients with symptoms of photophobia and disproportionate pain. The pathognomic sign is a radial pattern of perineural infiltrates [14]. Also, in the presence of clinical signs of *Acanthamoeba* keratitis, diagnostic tests should always be performed [15]. To the best of our knowledge, this happens to be the first report of *Acanthamoeba* keratitis with PCR verified results in our country.

CONCLUSION

Acanthamoeba keratitis is a growing clinical problem in developed as well as developing countries. A sound clinical suspicion and accompanied microbiological investigation is needed for the early diagnosis of this condition.

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