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

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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
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).

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

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

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

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1st scraping</th>
<th>2nd scraping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram staining</td>
<td>KOH</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>4</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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

**References**