Acanthamoeba keratitis - Camouflage entity in Nepal

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Brief report

Keywords:

Posted Date: June 22nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-37382/v1>

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Abstract

Background Acanthamoeba keratitis is a sight threatening corneal infection and is a growing clinical problem in developing countries as well as developed countries. Acanthamoeba keratitis is a local infection of the eye and does not produce systemic illness. Proper diagnostic techniques and appropriate medical management are necessary for good prognosis. Findings We report a series of six cases of acanthamoeba keratitis diagnosed and managed at Biratnagar Eye Hospital in the year 2019. The patients age ranges from 28-58 years, with four males and two females. The mean duration of presentation to hospital was 19 days. Four patients had trauma with vegetative matter. All patients had redness, photophobia, decreased vision, pain among which only two had pain out of proportion. One patient had visual acuity of 2/60 whereas the rest had hand movement to perception of light at time of presentation. Ring infiltrate was noted in all the cases. Five patients had ulcer in central and paracentral regions, four had ulcer larger than 6mm and three showed presence of hypopyon. KOH stained smears revealed acanthamoeba cyst in all cases while Gram stain was positive in only one case. Non-nutrient agar overlaid with *Escherichia coli* revealed feeding tracks in four cases and polymerase chain reaction revealed T4 genotype acanthamoeba in four cases. Amoebicidal treatment was started with Chlorhexidine 0.02% eye drop half hourly. Other drugs Natamycin 5% eye drop half hourly, Fluconazole 0.3% eye drop half hourly and antibiotic Moxifloxacin 0.5% eye drop half hourly were also used simultaneously. Pain was reduced in all patients. After treatment, only one patient had BCVA of 6/9 while others had visual outcome of hand movement. Conclusions Acanthamoeba keratitis can be severe and vision-threatening. Centrally located ulcer, size of the ulcer > 6mm and visual acuity Hand Movement/Perception of light at the time of presentation were associated with poor outcome. Clinically suspected lesions associated with microbiological assessment help in early diagnosis and prompt treatment.

Introduction

Acanthamoeba species are free-living protozoan parasite [1,2]. Ocular infection by Acanthamoeba is rare (3). It was first reported in 1973 in both USA and UK [4]. Acanthamoeba likely invades the cornea through a physical opening in its protective barriers, such as a minor abrasion in the corneal epithelium [5]. Various risk factors are responsible for its causation including use of contact lens in developed countries [3,7,13,16,17] and trauma or use of contaminated tap water in developing countries [4,6,8,10,20,21]. A high level of clinical suspicion and wet mount examination of specimen from infected tissue are essential to aid in rapid diagnosis of Acanthamoeba Keratitis [6].

Case Description

All six acanthamoeba patients were in between 28 to 58 years age group, 4 were males and 2 were females. All were from agricultural background and none of the patients wore contact lens. Four patients had history of trauma with vegetative matters. Patients presented late to the eye hospital and mean duration of having symptoms to presentation to Biratnagar Eye Hospital was 19 days (Standard

deviation 7.78). Five patients had best corrected visual acuity (BCVA) of hand movement to perception of light vision at the time of presentation and one had 2/60 in the affected eye. The details of all the patients including demographic characteristics, history of trauma, duration and vision at presentation have been summarized in Table 1.

Table 1 Demographic and clinical profile of patients with Acanthamoeba keratitis

Case No	Month	Age/Sex	Occupation	Predisposing factors/traumatic agents	Vision at presentation	Duration of presentation
1	April 2019	47/F	House wife	H/o vegetative trauma 15 days back followed by use of tube well water in LE after trauma	LE: Hand Movement close to face	15 days
2	June 2019	28/M	Farmer	None	RE: PL, PR accurate in all quadrants	30 days
3	July 2019	32/M	Farmer	H/O vegetative trauma	RE-2/60	10 days
4	September 2019	32/F	House wife	None	LE- PL, PR accurate in all quadrants	25 days
5	November 2019	33/M	Farmer	H/O vegetative trauma	RE- HM close to face	20 days
6	December 2019	58/M	Farmer	H/O vegetative trauma	RE- HM close to face	12 days

Severe excruciating pain was the main complaint in two patients. However, ring infiltrate was the hallmark in all the patients [Fig. 1]. Based on the history and clinical findings, provisional diagnosis of Acanthamoeba keratitis was made in four patients. One patient was clinically suspected as fungal keratitis and the other as viral keratitis.

The details of the corneal ulcer, their size and the presence of hypopyon have been described in Table 2.

Table 2 Corneal findings of the patients

Case No	Details of ulcer		Size of the ulcer		Presence of hypopyon	
	Central+ Paracentral	Paracentral	2to 6 mm	More than 6 mm	Yes	No
1	✓	-	-	✓	✓	-
2	✓	-	-	✓	✓	-
3	-	✓	✓	-	-	✓
4	✓	-	✓	-	-	✓
5	✓	-	-	✓	-	✓
6	✓	-	-	✓	✓	-

Microbiological laboratory work-up

All patients underwent corneal scraping with 15-degree bard parker blade and scraped tissue was sent for Gram and KOH stain. It was also inoculated in Blood agar, Chocolate agar and Sabouraud Dextrose Agar (SDA). If the acanthamoeba cysts were identified in either Gram and KOH stain, then scraping was further inoculated in non-nutrient agar overladen with *Escherichia coli*.

Results of microbiological assessment-

Double walled cysts of *Acanthamoeba* were noted on 10% KOH mount [Fig. 2] in first scraping in four cases (Case 2,4,5 and 6) whereas only in second scraping in two cases. Gram stain revealed acanthamoeba cyst [Fig. 3] in one of the cases (Case 5). Culture of first four cases in the non-nutrient agar with *E.coli* showed clear/ feeding tracks along the lawn of *E.coli* which signifies the presence of migrating trophozoites that feed on the bacilli [Fig. 4]. Cysts isolated from culture showed inner polygonal and outer wrinkled wall [Fig. 5a] which was further highlighted with Giemsa stain [Fig. 5b]. The Blood agar, Chocolate agar and SDA cultures were negative in all cases.

Polymerase Chain Reaction (PCR) report: PCR testing facility is not available at our hospital and in Nepal for *Acanthamoeba* so a communication was made with Centre for Disease Control and Prevention (CDC), USA and samples of initial four patients were sent in a special carrier media, as per their instructions. All four samples were *Acanthamoeba* positive by a diagnostic real-time PCR. All 4 samples have been genotyped successfully and belong to genotype T4. Two of the strains appeared identical in Sanger sequences to each other, while the other two were unique. Sample of last two patients could not be sent, as they were lost for the follow-up. The laboratory findings are illustrated in Table 3.

Table 3 Microbiological and Polymerase chain reaction findings from the scrapped samples

Case No	1 st Scraping		2 nd Scraping		Culture (Non Nutrient Agar with <i>E.coli</i>)	PCR
	Gram	KOH	Gram	KOH		
1	No organisms isolated	No organisms isolated	No organisms isolated	Cyst like structure resembling Acanthamoeba	In 2 nd scrapping, Amoebal feeding tracks along the lawn of E.Coli noted after 4 days.	Positive
2	No organisms isolated	Cyst like structure resembling Acanthamoeba			Amoebal feeding tracks along the lawn of E.coli noted after 5 days.	Positive
3	No organisms isolated	No organisms isolated	No organisms isolated	Cyst like structure resembling Acanthamoeba	In 2 nd scrapping, Amoebal feeding tracks along the lawn of E.coli noted after 5 days.	Positive
4	No organisms isolated	Cyst like structure resembling Acanthamoeba			Amoebal feeding tracks along the lawn of E.coli noted after 7 days.	Positive
5	Cyst like structure resembling Acanthamoeba	Cyst like structure resembling Acanthamoeba			Not done	Not done

6	No organisms isolated	Cyst like structure resembling Acanthamoeba	Not done	Not done
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Treatment

All patients except case number 3 received amoebicidal treatment for the first time at Biratnagar Eye Hospital. Amoebicidal treatment was started with Chlorhexidine 0.02% eye drop prepared at the pharmacy of Sagarmatha Choudhary Eye Hospital. Initially the drug was started on half hourly basis and dose was reduced after 3 days to hourly basis, subsequently it was tapered to 2 hourly after one week. Supplementary treatment included treatment with Moxifloxacin eye drop, a combination of polymyxin B sulfate, neomycin sulfate and bacitracin. Case no 3 was continued on antifungal (Natamycin eye drop 5 % half hourly + 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 had the scarring started around the ulcer despite having thin cornea. This patient underwent cyanoacrylate glue application along with BCL. Gradually eye drops were tapered in all patients.

All patients showed early response to medication with dramatic reduction in pain. First four patients came for regular follow-up and showed a healing with corneal scarring. Case number 3 who received antifungal treatment only had gained BCVA of 6/9 whereas remaining 3 patients had vision of hand movement. Case 5 and 6 went to other eye hospitals to seek further opinion and were lost for the follow-up.

Discussion

Unlike the reports of acanthamoeba keratitis from developed world, none of our patients wore contact lens users [7,13,16,17,18]. All of them were from agricultural background and four patients had history of trauma with vegetative matter. Similar findings of higher association of acanthamoeba keratitis among agricultural worker with history of trauma due to vegetative matter have been reported from other developing countries [4,6,19,20,21].

In our series, pain disproportionate to sign was found in two patients while other four patients complained of little pain. Ring infiltrate, a diagnostic sign for acanthamoeba keratitis was noticed in all patients and was associated with perineuritis in two patients and severe limbitis in one patient. Based on the classical sign of ring-shaped infiltrate, acanthamoeba keratitis was suspected on all except two patients at the time of presentation. Similar clinical feature was described in earlier reported cases [4,6]. One patient was suspected as viral keratitis in the beginning and other patient was suspected as fungal keratitis as he had history of trauma of vegetative origin and anterior stromal infiltrate. These two patients developed ring shaped infiltrate after 1–2 weeks of treatment at the hospital. In most of the

reported cases, a primary diagnosis of Acanthamoeba Keratitis was not made and a delay in correct diagnosis ranged from 7 weeks to 12 months [6].

In this case series, the sensitivity of KOH was higher than Gram stained smears in detection of Acanthamoeba, as the cysts were easily identified in all six cases using KOH stain compared to one case using Gram stain. Several other studies from South India have reported superiority of KOH wet mount preparation for the diagnosis of acanthamoeba keratitis [4,6]. Various studies have shown increasing prevalence of Acanthamoeba keratitis due to increased awareness of the clinical features and easy diagnostic technique [7, 25] Acanthamoeba cysts with trophozoites were identified on non-nutrient agar overlaid with Escherichia coli among all patients identified with KOH mount. PCR reporting with the help of CDC from USA was received for initial four patients and all were reported to be positive.

Mean duration of symptom to presentation to hospital was 19 days. All patients showed clinical response to treatment. Pain was significantly reduced in all patients after starting medication and this could be due to the effect of amoebicidal and Atropine eye drop. Last two patients preferred to have second opinion from other eye hospitals in India 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 > 6mm and visual acuity Hand Movement/Perception of light at the time of presentation were associated with poor outcome. Various other studies have reported similar findings [8].

The frequency and distribution of microbial keratitis varies from one place to another based on climate, geographic, socio-economic and habits of inhabitants [7]. Despite having one of highest reported incidence of corneal ulcer in the world till now, there is no published report of acanthamoeba keratitis from Nepal [22,23,24]. A high degree of clinical suspicion, a good support from Microbiology department and mutual interaction between clinical ophthalmologist and microbiologist for the diagnosis of uncommon causes of microbial keratitis especially in non-responding cases is a must. Upgradation of microbiology laboratory facility and availability of a qualified full-time microbiologist at Biratnagar Eye Hospital could be the important factor for the diagnosis of acanthamoeba keratitis in our set-up.

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.

Abbreviations

BCVA: Best corrected visual acuity; BCL: Bandaged contact lens

KOH: Potassium hydroxide; SDA: Sabouraud dextrose agar; PCR: Polymerase chain reaction

HM: Hand movement; PL: Perception to light; PR: Projection of rays

CDC: Center for disease control and prevention

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Written informed consents were obtained from the patients for publication of their individual details and accompanying images in the manuscript

Availability of data and material

All data generated or analysed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests

Funding

None to disclose

Authors contribution

SKS,KA,SM involved in ophthalmic care. PP, AR carried out laboratory investigations. SKS, KA, PPSM, AR participated in literature review. SKS, PP did the manuscript writing. KA, SM collected the data. SKS corrected the final manuscript. All authors read and approved the final manuscript.

Acknowledgements

We the authors 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.

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References

1. Stothard DR, Schroeder-Diedrich JM, Awwad MH, Gast RJ, Ledee DR, Rodriguez-Zaragoza S, Dean CL, Fuerst PA, Byers TJ (1998) The evolutionary history of the genus *Acanthamoeba* and the identification of eight new 18S rRNA gene sequence types. *J Eukaryot Microbiol* 45(1):45-54
2. Marciano-Cabral F, Cabral G (2003) *Acanthamoeba* spp. as agents of disease in humans. *Clinical Microbiology Reviews* 16(2):273-307
3. Moore MB, McCulley JP, Luckenbach M, Gelender H, Newton C, McDonald MB, Visvesvara GS (1985) *Acanthamoeba* keratitis associated with soft contact lenses. *Am. J. Ophthalmol* 100(3):396-403
4. Bharathi JM, Srinivasan M, Ramakrishnan R, Meenakshi R, Padmavathy S, Lalitha PN (2007) A study of spectrum of *Acanthamoeba* keratitis: A three year study at a tertiary eye care referral center at South India. *Indian Journal of Ophthalmology* 55(1):37-42
5. Simitzis-Le Flohic AM, Hasle DP, Paniagua-Crespo E, Colin J, Lagoutte F, Donval A, Bellon C (1989) *Acanthamoeba* keratitis. Epidemiologic and parasitologic study. *J Fr Ophtalmol* 12:361
6. Sharma S, Srinivasan M, George C (1990) *Acanthamoeba* keratitis in non-contact lens wearers. *Archives of Ophthalmology* 108(5):676-678
7. Stehr-Green JK, Bailey TM, Visvesvara GS (1989) The epidemiology of *Acanthamoeba* keratitis in the United States. *The American Journal of Ophthalmology* 107(4): 331-336
8. Davamani F, Gananaselvan J, Anandakannan K, Sridhar N, Sundararaj T (1998) Studies on the prevalence of *Acanthamoeba* keratitis in and around Chennai. *Indian Journal of Medical Microbiology* 16(4):152-153
9. Sharma S, Srinivasan M, George C (1990) Diagnosis of *acanthamoeba* keratitis- A report of four cases and review of literature. *Indian J Ophthalmol* 38:50-6
10. Parmar DN, Awwad ST, Petroll WM, Bowman RW, McCulley JP, Cavanagh HD (2006) Tandem scanning confocal corneal microscopy in the diagnosis of suspected *Acanthamoeba* keratitis. *Ophthalmology* 113(4):538-47
11. Theodore FH, Jakobiec FA, Juechter KB, Ma P, Troutman RC, Pang PM, Iwamoto T (1985) The diagnostic value of a ring infiltrate in *acanthamoebic* keratitis. *Ophthalmology* 92(11):1471-1479
12. Yera H, Zamfir O, Bourcier T, Ancelle T, Batellier L, Dupouy-Camet J, Chaumeil C (2007) Comparison of PCR, microscopic examination and culture for the early diagnosis and characterization of *Acanthamoeba* isolates from ocular infections. *European Journal of Clinical Microbiology and Infectious Diseases* 26(3):221-224
13. Chin J, Young AL, Hui M, Jhanji V (2015) *Acanthamoeba* keratitis: 10-year study at a tertiary eye care center in Hong Kong. *Cont Lens Anterior Eye* 38(2):99-103
14. Maycock NJ, Jayaswal R (2016) Update on *Acanthamoeba* Keratitis: Diagnosis, Treatment, and Outcomes. *Cornea* 35(5):713-20
15. Fu L, Gomaa A (2019) *Acanthamoeba* keratitis. *N Engl J Med* 381(3):274
16. Lindsay RG, Watters G, Johnson R, Ormonde SE, Snibson GR (2007) *Acanthamoeba* keratitis and contact lens wear. *Clinical and Experimental Optometry* 90(5):351–60

17. Stehr-Green JK, Bailey TM, Brandt FH, Carr JH, Bond WW, Visvesvara GS (1987) Acanthamoeba keratitis in soft contact lens wearers. A case-control study. JAMA 258 (1) :57-60
18. Moore MB, McCulley JP, Newton C, Cobo LM, Foulks GN, O'Day DM, Johns KJ, Driebe WT, Wilson LA, Epstein RJ (1987) Acanthamoeba keratitis. A growing problem in soft and hard contact lens wearers. Ophthalmology 94(12): 1654–1661
19. Garg P, Kalra P, Joseph J (2017) Non-contact lens related Acanthamoeba keratitis. Indian J Ophthalmol 65(11):1079-1086
20. Imam AM, Mahgoub E (2008) Blindness due to Acanthamoeba: first case report from Sudan. International Journal of Health Sciences 2(2): 163–166
21. Sharma S, Garg P, Rao GN (2000) Patients characteristics, diagnosis and treatment of non-contact lens related Acanthamoeba The British journal of ophthalmology 84(10): 1103-8
22. Upadhyay MP, Karmacharya PC, Koirala S, Shah DN, Shakya S, Shrestha JK, Bajracharya H, Gurung CK, Whitcher, JP (2001) The Bhaktapur eye study: ocular trauma and antibiotic prophylaxis for the prevention of corneal ulceration in Nepal. The British journal of ophthalmology 85(4): 388–392
23. Puri LR, Shrestha, GS (2017) Microbial keratitis: A five years retrospective clinical study in tertiary eye hospital of eastern region of Nepal. Journal of Kathmandu Medical College 4(4): 118-125
24. Sitoula RP, Singh SK, Mahaseth V, Sharma A, Labh RK (2015) Epidemiology and etiological diagnosis of infective keratitis in eastern region of Nepal. Nepalese journal of ophthalmology 7(1):10–15
25. Bacon AS, Dart JK, Ficker LA, Matheson MM, Wright P (1993) Acanthamoeba keratitis. The value of early diagnosis. Ophthalmology 100(8): 1238–1243

Figures

Figure 1

Ring infiltrate appreciated in the patient eye

Figure 2

Acanthamoeba cyst seen under 10%KOH mount (100X)

Figure 3

Acanthaamoeba cyst observed with Gram stain (100 X)

Figure 4

Amoebal feeding tracks appreciated along the lawn of E.coli (10X)

Figure 5

a Acanthamoeba cyst isolated from culture plates reveal inner polygonal and outer wrinkled wall (100X) b further highlighted with Giemsa stain(100X)