Original Article

Acanthamoeba keratitis: A 4-year review from a tertiary care hospital in North India

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Abstract

Introduction: *Acanthamoeba keratitis* (AK) is a blinding condition reported from both developed and developing countries. Limited knowledge on the clinical characteristics of AK and scarce laboratory diagnostic facilities in such countries poses difficulties in the accurate diagnosis.

Objective: To describe the epidemiological and clinical characteristics as well as management of *Acanthamoeba* keratitis in a tertiary care hospital in North India.

Methods: All clinically suspicious cases of Acanthamoeba keratitis (AK) presenting to our centre were screened for *Acanthamoeba*. All patients diagnosed as *Acanthamoeba* on microscopic examination, culture and polymerase chain reaction (PCR) were given Polyhexamethylene biguanide (PHMB) eye drops 0.02% half hourly for 1 week, then hourly for 1 week and then gradually tapered according to the response. Out of 300 consecutive patients evaluated, Acanthamoeba was detected in 11(3.6%) patients. A history of trauma was elicited in majority of the patients, 6 (55%). The most common complaints were eye pain, redness and watering in all of the patients, diminution of vision (8, 72.7%), photophobia (7, 63.6%) and foreign body sensation (2, 18.2%). Complete healing with vascularization and scarring was observed in 7 patients (63.6%) patients whereas progression to perforation of corneal ulcer and corneal melt was seen in 3 (27.3%) cases and these patients underwent therapeutic keratoplasty later. 1 patient did not come for follow up examination.

Conclusion: The most common risk factor for the occurrence of Acanthamoeba Keratitis is trauma followed by contact lens use.

Key words: *Acanthamoeba,* Keratitis, PHMB, Non nutrient agar, PCR.

Key message: Trauma and contact lens use are the most common risk factors for the development of Acanthamoeba Keratitis in North India.

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Introduction

In recent years Acanthamoeba Keratitis (AK) has been increasingly reported from various parts of the world. While this clinical entity was initially recognized in developed countries among contact lens wearers, its reports from developing countries in both contact lens wearers and non-wearers are on the rise. Limited knowledge of the clinical

characteristics of AK and scarcity of diagnostic facilities in such countries poses difficulties in the accurate diagnosis of AK. Trauma followed by exposure to dirty water are the most common risk factors implicated in cases of AK from developing countries (Bharathi JM et al, 2007; Radford CF et al, 2002). In recent years, few reports from South India describe an increasing incidence of AK from rural areas (Lalitha P et al, 2012). It is very common for AK to be initially diagnosed as viral (herpes simplex virus) keratitis or even fungal keratitis and the conflicting clinical manifestations often result in misdiagnosis of this condition (Mascarenhas J et al, 2014). Patients have a prolonged course of disease and are commonly left with debilitating corneal scarring, visual morbidity and often require penetrating keratoplasty. The resistant cyst stage of *Acanthamoeba* further complicates treatment. Early diagnosis of this condition before the *Acanthamoeba* have penetrated deep into the corneal stroma ensures successful therapy and better outcome (Morales J L et al, 2015). Thus, the early and accurate diagnosis of this devastating infection is important particularly in developing nations as limited resources may prevent many patients from obtaining sufficient treatment. This study describes clinical characteristics and management of patients with AK presenting at our centre.

Material and methods

A prospective analysis of all clinically suspicious cases of AK (total of 300 patients) was performed over 4-year study duration. (2014-2018). Clinically suspicious cases were defined as patients with history of contact with dirty water, history of contact lens use as well as signs and symptoms suggestive of AK; such as corneal ulcers with typical ring infiltrates, perineural infiltrates and limbitis etc. Patients with corneal ulcers who fail to respond promptly to antiviral or antibacterial therapy were also included.

The demographic data, detailed clinical history, and ophthalmological findings were recorded. A written informed consent was obtained from all participants and Institute ethical committee clearance vide NK/953/PH.D/8656 prior to the commencement of the study. All patients were examined using slit-lamp bio-microscope. The size of the epithelial defect, size, depth of the stromal infiltrate and the size of the hypopyon if any was measured. Predisposing factors for developing corneal ulcers such as trauma, use of contact lenses, use of topical steroids and the presence of various comorbid diseases were noted.

All suspected patients of AK were subjected to corneal scraping and contact lenses; lens solutions and lens case (in case of lens wearer) were collected for microscopic examination (giemsa, calcofluor white and gram stain). The material was scraped under magnification from the edge and the base of the ulcer and was directly inoculated on non-nutrient agar plate (NNA) plate overlaid with Escherichia coli. Molecular diagnosis of *Acanthamoeba* infection was also done by conventional PCR and Real time PCR targeting 18S rDNA gene of *Acanthamoeba.*

All patients diagnosed as AK were given Polyhexamethylene Biguanide (PHMB 0.02%) half hourly for 1 week, then hourly for 1 week and then gradually tapered according to the response. The local pharmacy prepared PHMB was used in our study. All patients were continued with PHMB (0.02%) drops 2 weeks after complete healing. All the patients were given topical antibiotics (gatifloxacin 0.05%) to prevent secondary bacterial super infection. Cycloplegics (atropine 1% eye drops) and oral analgesics were given in all cases as an adjunctive. None of the patients received any treatment with steroids before presentation. Treatment success was defined as total resolution of the corneal infiltrates with scarring, disappearance of hypopyon,

and healing of the epithelial defect. Treatment failure was defined as i) increase in size of the infiltrate and/or epithelial defect or ii) increase in the size of hypopyon or endothelial plaque, or iii) if there was a corneal perforation.

In addition, the anatomical and functional outcome in these eyes was studied as follows:

a. Anatomical outcome- i.e., the type of corneal opacity (maculo-leucomatous corneal opacity, leucomatous corneal opacity or adherent leucoma).
b. Functional outcome- Final visual acuity.

Results

During this study period, 300 clinically suspicious *Acanthamoeba* patients underwent corneal scrapings. A diagnosis of AK was established in 11 patients by microscopic examination on calcofluor white staining/ giemsa, culture on NNA (Figure 1) as well by molecular diagnosis by PCR/Real Time PCR. While seven patients were positive on microscopic examination by calcoflour or giemsa stain; nine patients were positive for *Acanthamoeba* after 3 days of culture on NNA and all patients were positive on PCR and Real time PCR. One patient had co-existing bacterial infection with serratia marcescens.

Demographic details and predisposing factors

The median age of patients with AK was 33 years (range 21 -73 years) and a male: female ratio of 8:3 was observed. The majority of patients (7, 63.6%) belonged to a rural background. The most common occupation amongst all cases was farming (4, 36.6%) followed by office-based professional (3, 27.3%), students (2, 18.2%), driver (1, 9%) and housemaid (1, 9%). A history of trauma was present in 6 (55%) cases with exposure to vegetative matter in 4 and to dust and unknown foreign body in one patient each. Three (27.3%) patients were contact lens users. Two of the patients were using commercial lens cleaning solutions and one was using tap water to clean the lenses. History of overnight contact lenses use was present in one patient and he presented with bilateral infiltrates. A history of previous ocular surgical procedures was noted in 2 (18%) cases. Amongst them one patient underwent a corneal collagen cross-linking with postoperative bandage contact lens while one patient had undergone a scleral buckle surgery for exposed buckle. One patient had hypertension, however no co morbidity was identified in the other patients.

Clinical details

The most common complaints described by all patients (100%) were eye pain, redness and watering followed by diminution of vision (8, 72.7%), photophobia (7, 63.6%) and foreign body sensation (2, 18.2%). Bilateral eye involvement was seen in only one patient; majority had right eye involvement (7, 63.6%) and 3 (27.3%) had left eye involvement. The ophthalmological findings of the patients with AK are described in Table 1.

The interval between onset of symptoms and diagnosis ranged from 3 days to 84 days. Medical treatment was initiated in all cases in the form of PHMB 0.02% eye drops.

Complete healing with corneal vascularization and scarring was observed in 7 patients (63.6%) patients. Visual acuity (VA) in the range of 6/6-6/60 was seen in 4 (36.3%) patients; whereas 3 patients (64.5%) had VA in the range of perception of light-HMCF. Progression to perforation of corneal ulcer and corneal melt was seen in 3(27.3%) cases and these patients underwent therapeutic keratoplasty while one patient had persistent infection even after keratoplasty (Figure 2) and the eye went into phthisis bulbi. One patient did not come for follow up examination. Table 2 describes case description and clinical outcome of AK patients.

Table 1: Ophthalmological findings of patients with Acanthamoeba keratitis

Eye involvement B/E (Bilateral) R/E (Right eye) L/E (Left eye)	 1/11 (9.09%) 7/11 (63.63%) 3/11 (27.27%)
Corneal infiltrate (Location) Central Peripheral Scleritis (suppurative)	10/11 (90.9%) 5 /11 (44.4%) 5/11 (44.4%) 1/11(9.09%)
Size of ulcer: 1. Less than 5 mm and or less than 2/3rd stromal thickness involvement. (Mild-moderate) 2. More than 5 mm and or more than 2/3rd stromal thickness involvement. (Severe)	 5/11(44.4%) 5/11(44.44%)
Multifocal lesions	4(36.36%)
Ring like infiltrate	1(9.09%)
Dendritic ulcer/ Geographic ulcer	2(18.18%)
Dry looking with convex hypopyon	3(27.27%)

Table 2: Brief case description and clinical outcome of Acanthamoeba keratitis patients

Cases	Time of presentation/ duration of symptoms	Size of the ulcer	Visual acuity at presentation	Duration of treatment	Clinical outcome
1.	2 weeks	4.5x 6 mm central	PL+ve	4weeks	Perforated-underwent TPK
2.	8 weeks	1.6x 5mm	HMCF	6 weeks	Healed corneal opacity
3.	2 weeks	9x7.5mm	CFCF	10 weeks	Healed corneal opacity
4.	1 week	1x1mm (Multiple)	6/6	5 weeks	Healed corneal opacity
5.	5 days	2x2 mm (Multiple)	6/24	8 weeks	Healed corneal opacities
6	3 days	2mmx2mm (Multiple)	6/9	5 weeks	Healed corneal opacities
7.	1 week	0.5x0.5mm (Multiple)	6/6	6 weeks	Healed opacities
8	4 weeks	7x8mm	CFCF	4weeks	Perforated-underwent TPK
9.	12weeks	6x5mm	CFCF	12 weeks	Healed corneal opacity
10.	20 days	6x5mm	PL+ve	3 days	Perforated-underwent TPK
11.	6 days	Scleral buckle suppuration	NA	1week	Lost to follow up

PL - Perception of light; HMCF-Hand movement close to face; CF-Counting Finger; CFCF-Counting Finger Close to Face; TPK-Therapeutic Penetrating Keratoplasty; NA-Not Available

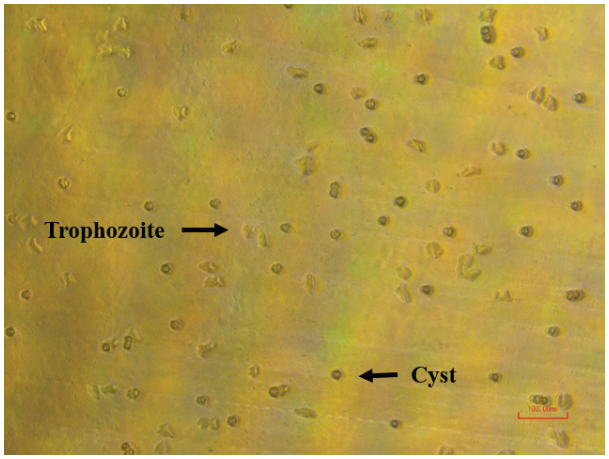

Figure 1: Microscopic image (10 x magnification) of Acanthamoeba trophozoites and cysts seen in the nutrient agar.

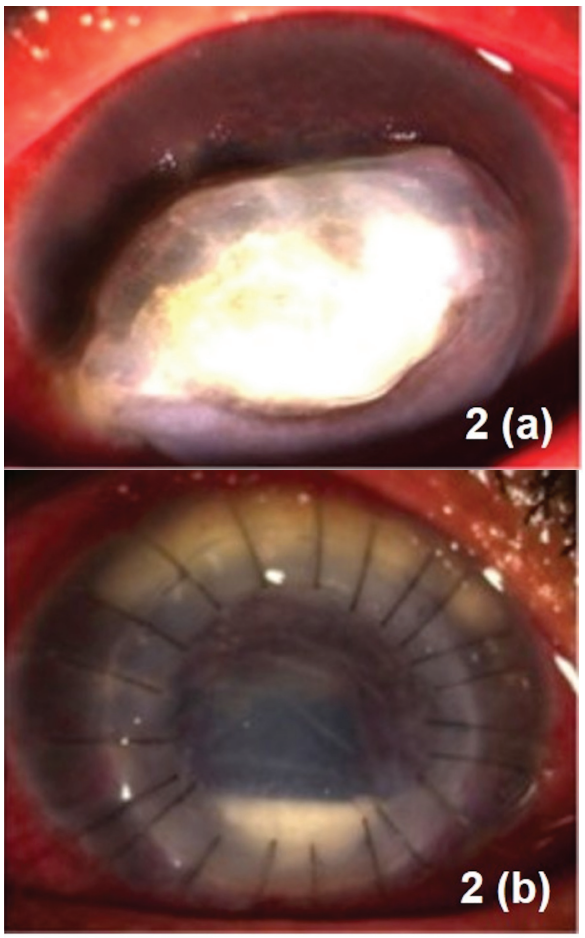

Figure 2: Slit lamp photograph of the right eye of a patient with Acanthamoeba keratitis a) Perforated corneal ulcer with slough b) Day 11 post therapeutic penetrating keratoplasty showing persistent infiltrates along with 3 mm hypopyon.

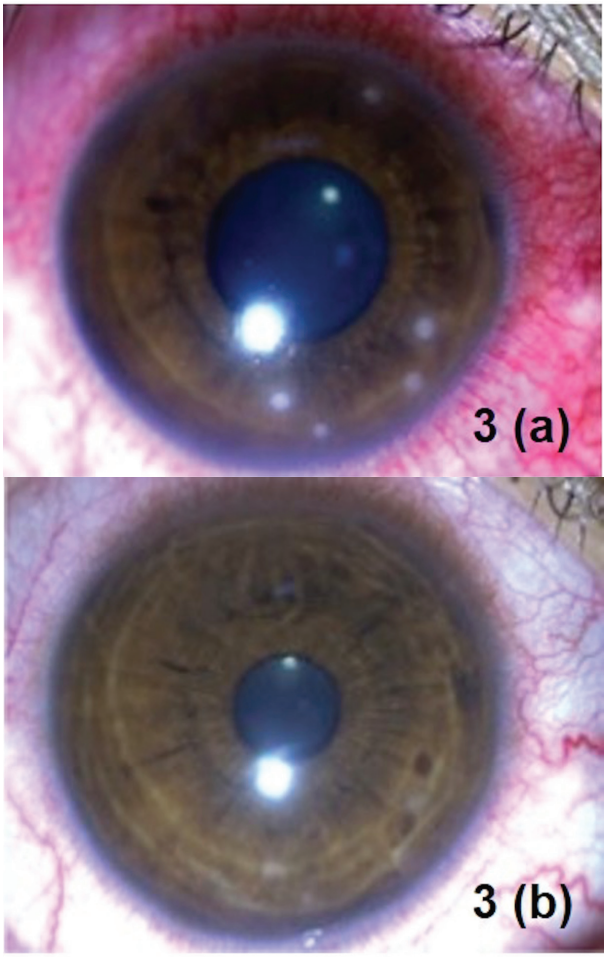

Figure 3: Slit lamp examination of the left eye of a patient with Acanthamoeba keratitis. a) Multifocal infiltrates involving the peripheral cornea b) Healed peripheral corneal opacities.

Discussion

In our study we have analyzed cases of AK diagnosed at our centre over 4-year study duration. On studying the predisposing factors for infection it was noted that most patients (55%) had history of trauma to the eye while 27% were soft contact lens wearers. Trauma has been seen to contribute to the onset and development of AK. Initially the adhesion of trophozoite to the corneal epithelium is necessary for causing infection (Panjwani N, 2010). It has been shown that subsequent to corneal trauma, mannose-containing glycoproteins at the surface of the cornea are

exposed to which mannose binding proteins of *Acanthamoeba* get attached (Garate M et al, 2006; Garate M et al, 2004; Garate M et al, 2005).

In our study, we found a subset of patients who were users of daily wear soft contact lenses. The use of tap water to clean the lenses allows pathogenic *Acanthamoeba* species to bind to lens material and subsequently get transferred to eye. Acanthamoebae have been shown to survive in contact lens cases as well as solutions (Morlet N et al, 1997; Radford C.F, 2002; Kilvington S et al, 2004). In a recent epidemiological study from India, only 0.9% of reported cases of AK were found to be linked with contact lens wear (Lalitha P, 2012). Thus, contact lens wear is also a risk factor for the development of AK and also supported by our study. In a study by Jiang C et al (2015) the most common risk factor was ocular trauma (53.1%), followed by contact lens wear (29.8%) which is in concordance with our findings. Most of the patients in their study were farmers (50.8%), which are similar to our study as the majority of patients described here, were agriculturists (36%) (Jiang C et al, 2015). Amongst all cases of AK described in our study, 18% had a history of ocular surgery. Microbial keratitis has been reported following laser refractive surgeries (Sharma DP et al, 2011). A combination of excessive pain, radial kerato-neuritis and a stromal ring infiltrate is said to be diagnostic of AK (Dart JKG et al, 2009). Initial ocular findings are punctate epithelial keratitis or scattered sub-epithelial infiltrates with radial perineural infiltrates, often with associated limbitis. Advanced stages show central epithelial loss, stromal thinning, corneal melt, uveitis and hypopyon. (Clarke B et al, 2012). In our study only 1 patient presented with a classical ring infiltrate suggestive of AK. In our study almost 36% patients presented with multifocal peripheral infiltrates and all these patients were contact lens users (Figure 3). Three patients (27.27%) presented with typical fungal keratitis and 2 patients (18.18%) presented with dendritic ulcers and were on presumptive antivirals and antifungals from 2 weeks before presenting to our centre. In a study almost 50% patients were initially suspected to be viral or fungal infections. (Tanheco T et al, 2010). We also observed a rare case where *Acanthamoeba* was identified from the infected scleral wound. That patient had undergone scleral buckling surgery 3 years back for retinal detachment and had undergone scleral buckle explant 1week back. This rare presentation has never been reported in the literature.

In our study we found that about 44 % of the ulcers were severe ulcers (Size was >5mm and or more than 2/3rd of the stromal involvement). This manifestation is obvious in Indian scenario, as most of the patients are started empirical antibiotics, antifungal and antivirals without microbiological diagnosis in most of the peripheral hospitals. It is only when they do not respond to the conventional treatment such patients are referred to a tertiary eye hospital and then the diagnosis of AK is made. The delay in diagnosis leads to complications and poor visual outcome. In our study, 3 patients worsened on treatment and progressed to corneal perforation. These patients later underwent therapeutic keratoplasty.

Clinical suspicion is the most important step in diagnosis of AK. First is the presence of specific history of contact lens wear, prior trauma and exposure to dirty water. Second is the failure to respond to antiviral or antibacterial therapy. These factors along with specific signs should raise the suspicion of AK and must be investigated by appropriate microbiological techniques.

In a study, Mathers et al (1996) reported a striking rate of co-infections (46%) in 43 patients with AK. Also, Sharma R et al (2013) in their study reported 23% cases with bacterial co-infections. In the present study, a bacterial

co-infection serratia marascens was found in one eye (9%).

The combined use of antiacanthamoeba agents such as chlorhexidine, neomycin, metronidazole along with debridement, and cauterization may be a best choice of treatment for AK (Sun X et al, 2006). Polyhexamethylene biguanide (PHMB) is a polymeric biguanide that has been used in the treatment of AK. PHMB kills both the cystic as well as the trophozoite stage of *Acanthamoeba* and appears to be relatively nontoxic (Larkin DF et al, 1992). Although the precise mode of action of PHMB against Acanthamoeba is uncertain, it is likely to be similar if not the same as its disruptive action on the phospholipid bilayers on the bacterial cell wall (Berry M et al, 1993). All the patients in the present study were managed with topical PHMB (0.02%) 1 hourly and then tapered according to the response to treatment. 7 patients (63.6%) had a favorable anatomical outcome as the ulcer in these patients healed with the formation of a vascularized corneal opacity. Three patients (27.27%) in our study did not respond to treatment and progressed to corneal perforation. These patients underwent therapeutic keratoplasty. In our study we observed a higher rate of corneal perforation and the requirement for therapeutic penetrating keratoplasty.

From our study we can state that trauma followed by contact lens use are the most important risk factor for developing AK in North India. Timely diagnosis is essential for the effective management of AK. Therefore, careful and hygienic use of contact lenses is of utmost importance in decreasing the incidence of this disease. In our study, although the clinical resolution and improvement in subjective symptoms is achieved in some cases with timely management, the visual outcome in most of the patients was not favorable due to a healed scar.

Conclusion

Our study suggests that trauma is the most common risk factor followed by contact lens use for the development of *Acanthamoeba Keratitis* (AK).

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