

Non-contact lens related *Acanthamoeba* keratitisPrashant Garg, Paavan Kalra, Joveeta Joseph<sup>1</sup>

The purpose of the study is to describe epidemiology, clinical features, diagnosis, and treatment of *Acanthamoeba* keratitis (AK) with special focus on the disease in nonusers of contact lenses (CLs). This study was a perspective based on authors' experience and review of published literature. AK accounts for 2% of microbiology-proven cases of keratitis. Trauma and exposure to contaminated water are the main predisposing factors for the disease. Association with CLs is seen only in small fraction of cases. Contrary to classical description experience in India suggests that out of proportion pain, ring infiltrate, and radial keratoneuritis are seen in less than a third of cases. Majority of cases present with diffuse infiltrate, mimicking herpes simplex or fungal keratitis. The diagnosis can be confirmed by microscopic examination of corneal scraping material and culture on nonnutrient agar with an overlay of *Escherichia coli*. Confocal microscopy can help diagnosis in patients with deep infiltrate; however, experience with technique and interpretation of images influences its true value. Primary treatment of the infection is biguanides with or without diamidines. Most patients respond to medical treatment. Corticosteroids play an important role in the management and can be used when indicated after due consideration to established protocols. Surgery is rarely needed in patients where definitive management is initiated within 3 weeks of onset of symptoms. Lamellar keratoplasty has been shown to have good outcome in cases needing surgery. Since the clinical features of AK in nonusers of CL are different, it will be important for ophthalmologists to be aware of the scenario wherein to suspect this infection. Medical treatment is successful if the disease is diagnosed early and management is initiated soon.

**Key words:** *Acanthamoeba* keratitis, deep anterior lamellar keratoplasty, *in vivo* confocal microscopy, nonusers of contact lens, radial keratoneuritis, ring infiltrate, sclerokeratitis

*Acanthamoeba* are ubiquitous microorganisms and are considered opportunistic pathogens in humans. These have been implicated in a variety of systemic infections including the eye. The first case of *Acanthamoeba* keratitis (AK) was described in the year 1974 from the UK.<sup>[1]</sup> From India, Sharma *et al.* published the first case.<sup>[2,3]</sup> Since then, many authors have published their experience with this disease and information has been compiled in review articles.<sup>[4-18]</sup> Thus, there has been substantial growth in the knowledge about the disease. However, nearly all review articles on this topic have been published from the Western world with primary focus on contact lens (CL)-associated keratitis. In India and most developing countries where CLs are not popular trauma is the most common predisposing factor and patients of AK suffer from prolonged and significant morbidity from delay in diagnosis due to lack of awareness and misunderstanding about clinical signs and symptoms among physicians and non-availability of anti-*Acanthamoeba* drugs. Therefore, we decided to write this review article with the following learning objectives:

- Summarize key features of AK and highlight differences between CL- and non-CL-associated keratitis
- Describe clinical situations under which to suspect infection by *Acanthamoeba*

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**Manuscript received:** 09.05.17; **Revision accepted:** 25.10.17

## Access this article online

Website:

www.ijo.in

DOI:

10.4103/ijo.IJO\_826\_17

## Quick Response Code:



- Describe ancillary clinical and laboratory tests for confirmation of the diagnosis including the role and limitations of *in vivo* confocal microscopy (IVCM)
- Discuss medical management including the indications and management of corticosteroid therapy
- Summarize indications of surgical treatment including the role and limitations of deep anterior lamellar keratoplasty (DALK)
- Discuss management of sclerokeratitis – a painful complication of the disease.

We recently analyzed 231 cases of AK managed at our center from 2012 to 2015 (unpublished data). In this article, we will present the data from this large series as well as review of the published literature to give practical perspective of the disease with primary focus on the disease in non-CL users.

## Epidemiology

Classically, AK is described to be associated with CLs. However, the experience in India and other developing countries as well as countries with high prevalence of CL-related keratitis clearly suggests that the disease occurs even without associated use of CLs.<sup>[11-18]</sup>

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**Cite this article as:** Garg P, Kalra P, Joseph J. Non-contact lens related *Acanthamoeba* keratitis. Indian J Ophthalmol 2017;65:1079-86.

In India, *Acanthamoeba* accounts for 2% of all cases of culture-positive corneal ulcers at tertiary eye care centers.<sup>[12-14]</sup> Trauma and exposure to contaminated water or soil are the important predisposing factors of AK not associated with CL use.<sup>[15-18]</sup> Additional risk factors are the use of contaminated water as home water supply,<sup>[19,20-22]</sup> warmer weather,<sup>[21,23]</sup> and poor socioeconomic conditions.<sup>[15]</sup> AK has also been reported after surgical trauma including penetrating keratoplasty (PK) and radial keratotomy and laser refractive surgery.<sup>[4,24,25]</sup>

#### *Acanthamoeba* keratitis in contact lens users

Although AK comprises of <5% of CL-related microbial keratitis, 80–85% cases of AK in the UK, the United States, and other developed Asian countries with high prevalence of CL uses are associated with CL uses.<sup>[9,26-28]</sup> The incidence is much higher in conventional soft CL users compared to those using daily wear rigid gas permeable lenses or planned replacement soft lenses. It is probably related to lens hygiene.<sup>[23,29]</sup> AK has also been reported with the use of orthokeratology lenses.<sup>[30]</sup>

AK is usually unilateral; however, there are reports of simultaneous affection of both eyes.<sup>[29,31]</sup> The cases occur all through the year, but there is some association with weather with increase in number of cases in summer and rainy season probably related to the increased contamination of water with the parasite. In India, the middle-aged individuals primarily engaged in outdoor activity and agriculture are most often affected by this disease.<sup>[14-16]</sup>

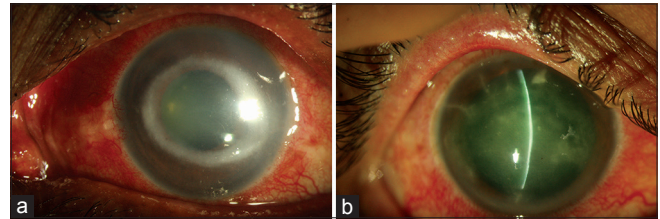
### Clinical Features

A textbook description of AK comprises of history of CL-wear, out of proportion pain, radial keratoneuritis, and ring infiltrate [Fig. 1]. However, one must remember that all patients with *Acanthamoeba* keratitis do not present with these classical symptoms and signs. *Acanthamoeba* infection of cornea is seen even in individuals with no history of CL wear as highlighted in epidemiology section. Reports from the Indian subcontinent and South America suggest that trauma and consequent exposure to contaminated water are the most important risk factors of AK in developing nations. Analysis of 231 cases (unpublished data) managed at our center between 2008 and 2013 showed that CL wear was the risk factor in only 3.5% cases. The population at risk of this infection, therefore, primarily comprised of agricultural workers and manual laborers. Publications from other centers in India also observed similar trends.<sup>[14,15,17]</sup>

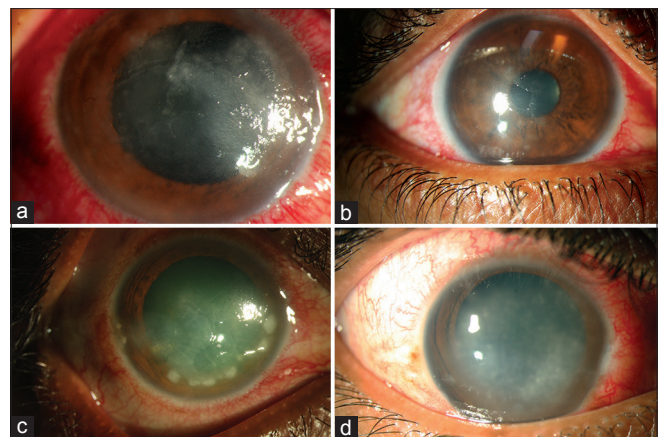
The clinical presentation of AK is also highly variable. Out of proportion pain was not a prominent feature in our series and was seen in only one-fourth ( $n = 52$ , 23%) of patients. Radial keratoneuritis was seen in 6 (2.7%) cases while ring infiltrate was seen in 74 (33%) cases. Our case series as well as cases published from other centers suggest that corneal signs vary depending partly on the duration of the disease. Early cases present with punctuate epithelial erosions, anterior stromal haze, nummular keratitis, and variable stromal edema associated with keratic precipitates on endothelium [Fig. 2]. It is not surprising that many early cases are misdiagnosed as herpes simplex viral keratitis and treated accordingly. The presence of inflamed corneal nerves (radial keratoneuritis) is a pathognomonic clinical sign and should prompt the diagnosis of *Acanthamoeba*. If undiagnosed early, the disease progresses to develop classical ring-shaped infiltrate, necrotizing keratitis

or diffuse infiltrative keratitis (features described in delayed presentation).

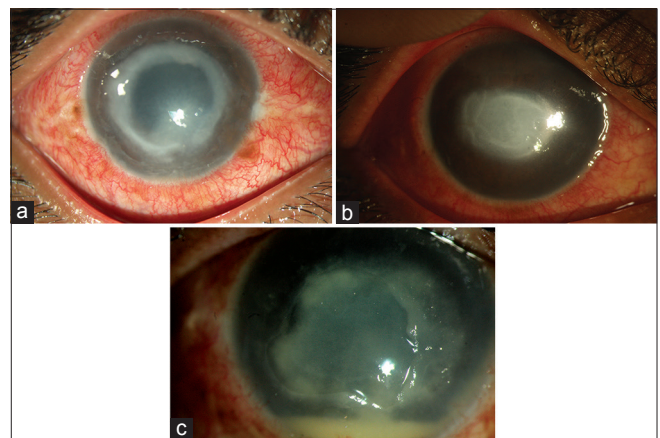
The clinical picture in patients presenting with advanced disease is characterized by ring infiltrate; dry-looking infiltrate mimicking fungal keratitis; diffuse infiltrate or ulcerative keratitis [Fig. 3]. In our case series, 74 (33%) patients presented with ring infiltrate while 141 (64%) cases presented with diffuse infiltrate. Two most common misdiagnosis made on the 1<sup>st</sup> day were herpes simplex virus (HSV) stromal keratitis and fungal keratitis. The series along with other published case series clearly highlight that absence of out of proportion pain, history



**Figure 1:** Classical clinical features of *Acanthamoeba* keratitis – radial keratoneuritis (a); ring infiltrate (b)



**Figure 2:** Nonclassical clinical features of *Acanthamoeba* keratitis – epitheliopathy with geographic or dendritic configuration (a and b); nummular keratitis (c); nonulcerative stromal keratitis (d)



**Figure 3:** Corneal picture of cases presenting late with advanced disease – ring infiltrate (a); necrotizing stromal keratitis (b); dry raised infiltrate mimicking fungal keratitis (c)



of CL wear, radial keratoneuritis, or ring infiltrate do not rule out *Acanthamoeba*. This knowledge is very important in making clinical diagnosis of this relatively rare disease.

### Clinical diagnosis

Since AK is often misdiagnosed, it will be important to be familiar with clinical scenario wherein to suspect *Acanthamoeba* etiology. They are as follows:

- Patient presenting with corneal infiltrates associated with inflamed corneal nerves – these are linear structures with inflammation of adjoining stroma continuous with limbus and may have branching at central end [Fig. 1a]
- Patients of chronic keratitis (history in weeks or months) associated with ring infiltrate [Fig. 1b]
- Patients with suspected HSV keratitis not responding to anti-HSV therapy
- Patients with ulcerative keratitis not responding to antibacterial or antifungal therapy.

All these patients must be subjected to laboratory tests for establishing the diagnosis.

## Laboratory Diagnosis

### Conventional laboratory procedures

The laboratory diagnosis of *Acanthamoeba* is performed using the same protocol as used for detection of bacteria and fungi with minor modifications. The protocol includes a combination of microscopic examination of smears and inoculation of scraped specimen on various culture media that allow growth of bacteria, fungi, and *Acanthamoeba* and is described in detail in our earlier publication.<sup>[32]</sup> The *Acanthamoeba* cysts are seen as double-walled structure with inner wall being hexagonal on microscopic examination of smears stained with calcofluor-white or Gram stain and provide immediate diagnosis [Fig. 4]. The culture confirmation on nonnutrient agar with *Escherichia coli* overlay may take 1–3 days [Fig. 5]. The medium, however, should be incubated for up to 2 weeks before concluding it as a negative culture.<sup>[4]</sup>

Patients with deep infiltrate and negative corneal scrapings might need corneal biopsy. The biopsy material is processed similar to corneal scrapings in microbiology laboratory.

For histopathology sections, a variety of staining protocols have been described in the literature. However, most staining procedures such as calcofluor-white, Gram, Giemsa, fluorescein-conjugated lectin, hematoxylin, and eosin delineate the cyst of *Acanthamoeba* very well showing the characteristic morphology of polygonal, double-walled structure with central nucleus.<sup>[33-35]</sup> Trophozoites, on the other hand, may be difficult to distinguish from inflammatory cells.<sup>[16,36]</sup> Immunostaining with either indirect fluorescent antibody<sup>[37,38]</sup> or immunoperoxidase technique<sup>[36,39]</sup> has also been described. These stains can be used on corneal scrapings as well as corneal tissue sections.

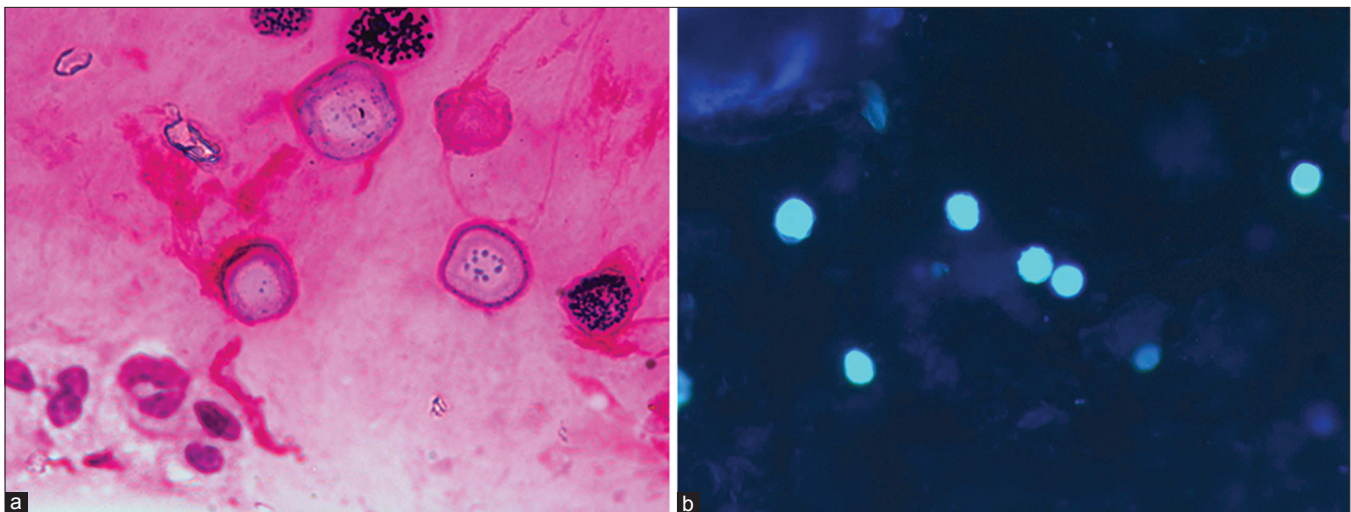
### Molecular methods of diagnosis

A number of molecular methods for the diagnosis of AK have been described. Most of these methods are based on amplification of 18S rDNA. Evaluation of molecular methods with conventional microbiology showed that the molecular methods are effective but have sensitivity similar to conventional microbiology.<sup>[40]</sup> Therefore, these tests have not been very popular in clinics. These are often used for identification of the parasite to subspecies level for understanding pathobiology and epidemiology.

Loop-mediated isothermal amplification, a newly developed highly specific, efficient, and less time-consuming DNA amplification technique which rapidly amplifies target DNA sequences under isothermal conditions, was evaluated, using 18S rDNA gene for specific detection of *Acanthamoeba* from corneal scraping samples, and was found particularly suitable for a rapid and accurate diagnosis of AK.<sup>[41]</sup> It takes 2–3 h lesser than polymerase chain reaction and thus offers a rapid, highly sensitive and specific, simple, and affordable diagnostic modality for patients suspected of AK, especially in resource-limited settings.

### Confocal microscopy for *Acanthamoeba* keratitis

In past decade, a lot of enthusiasm has been seen for the use of IVCN for etiological diagnosis of corneal infections.<sup>[42-47]</sup> IVCN is a noninvasive imaging technique that allows direct visualization of pathogens including *Acanthamoeba* cysts within the patient's corneal tissue. Two machines currently in clinical use are scanning slit IVCN (Confoscan, Nidek, Fremont,



**Figure 4:** Microscopic features of *Acanthamoeba* cyst in corneal scraping – Gram stain (a) and calcofluor-white (b). Note hexagonal inner wall of the cysts

USA) and laser scanning IVCN (HRT3/RCM, Heidelberg, Germany). Using microbiology as gold standard, the reported sensitivity and specificity of the technique for the diagnosis of *Acanthamoeba* varies between 80% and 100%.<sup>[42]</sup> American academy of ophthalmology published a technology assessment report on the value of confocal microscopy and concluded that confocal microscopy holds the potential to provide instantaneously a diagnosis, especially in cases of *Acanthamoeba* and fungal keratitis.<sup>[48]</sup> IVCN is particularly useful for detecting organisms in deep corneal infiltrates inaccessible to scraping. More recently, IVCN findings have also been correlated to the prognosis – the presence of clusters or chains of cysts has been found to be independently associated with a poor prognosis.

However, one needs to exercise caution – the diagnostic accuracy is strongly dependent on the experience of the observer.<sup>[49]</sup> Further, the technique requires cooperation of patients, which at times becomes difficult in patients suffering from painful condition such as keratitis.

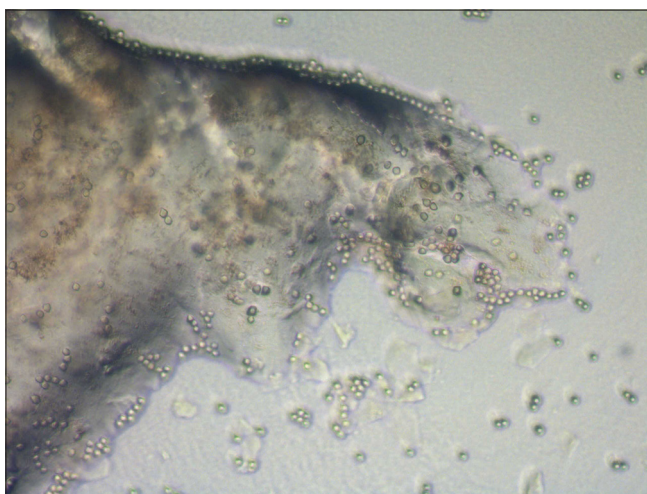
## Treatment

### Medical management

Table 1 lists drugs that have been used in the management of AK. However, none of the drugs effective in medical management are licensed for the use for AK treatment. The goals of medical therapy include the eradication of viable cysts and trophozoites and rapid resolution of the associated inflammatory response.

As regards *in vitro* activity, *Acanthamoeba* trophozoites are sensitive to most available chemotherapeutic agents (antibiotics, antiseptics, antifungals, antiprotozoals including metronidazole, antivirals, and antineoplastic agents). However, persistent infection is related to the presence of *Acanthamoeba* cysts, against which very few of these agents are effective.<sup>[50,51]</sup> Biguanides and diamidines have the best *in vitro* cysticidal activity and their use in clinical practice for the treatment of AK is supported by the vast *in vivo* experience in peer-reviewed literature.<sup>[4-8,52-57]</sup>

Therefore, topical biguanides with or without the addition of diamidines are the main stay of medical management of



**Figure 5:** Culture of *Acanthamoeba* on nonnutrient agar with an overlay of *Escherichia coli*. Clear tracks on the lawn of *Escherichia coli* suggests migrating trophozoites that feed on bacteria

this disease. At our center, a combination of PHMB 0.02% and chlorhexidine 0.02% is used as the primary therapy.<sup>[16]</sup> Concentration of chlorhexidine and PHMB can be varied from 0.02% to 0.2% and from 0.02% to 0.06%, respectively. However, one needs to be aware of toxicity. The drops are administered every hour, day, and night, for 48 h initially, followed by hourly drops by day till the clinical signs of resolution are observed. Intensive early treatment is given because organisms may be more susceptible before cysts have fully matured. The frequency is reduced subsequently based on the clinical response. However, the impression of no response should be made only after adequate time has been allowed for anti-*Acanthamoeba* therapy, as duration of medical treatment is usually prolonged. Mean duration of treatment among patients cured of infection in our series was 128 days. In a multicenter study from the United Kingdom, the average duration of medical therapy was 6 months (range, 0.5–29 months).<sup>[23]</sup>

One should also be cautious about the toxicity of drugs, especially if preparations not meant of ophthalmic use are prescribed to patients.

Other drugs including neomycin and antifungal drugs are not effective and should not be relied on as sole therapy in the management of this disorder. There are reports of beneficial effect of concomitant use of antibiotics. The rationale for the use of antibiotics is the parasites thrive on bacteria, and phagosomes-containing bacteria have been demonstrated in the cytoplasm of the parasitic cells.<sup>[58-60]</sup> Similarly, there are reports on successful treatment of infection with the addition of voriconazole in patients not responding to conventional anti-*Acanthamoeba* treatment.<sup>[61]</sup> One can consider these additional therapies in patients not responding to conventional medical management.

### Role of corticosteroids in the management of *Acanthamoeba* keratitis

The role of corticosteroid therapy in the management of AK is controversial.<sup>[62-64]</sup> While corticosteroids help control inflammation, in laboratory, they lead to transformation of cyst forms into trophozoites with consequent risk of worsening or recurrence of clinical disease.<sup>[65]</sup> On the other hand, trophozoites are more susceptible to anti-*Acanthamoeba* drugs. Despite this conflict, it is widely believed that corticosteroids have a definitive and beneficial role in the management of specific cases of AK provided these are used appropriately. The following are the indications of the use of corticosteroids in AK:

- Occurrence and progressive increase in deep vascularization of cornea

**Table 1: List of Anti-acanthamoeba drugs and doses**

Group	Drugs	Doses Topical/Oral
Biguanides	Chlorhexidine	0.02%-0.2%
	Polyhexamethylenebiguanide	0.02%-0.06%
Diamidines	Propamidine isethionate	0.1%
	Hexamidine	0.1%
Azoles	Voriconazole	Oral 100 mg per day
Aminoglycoside	Neomycin	1.0%



- Inflammatory complications of AK – scleritis, anterior chamber inflammation, persistent chronic keratitis
- Severe out of proportion pain.

Take the following precautions while using corticosteroids: (a) Corticosteroid therapy is not commenced until 2 weeks of biguanide treatment has been completed. (b) Antiamoebic therapy is maintained throughout and continued after the steroids have been withdrawn. (c) Watch for recurrence of infection from viable cysts in follow-up visits. (d) Continue corticosteroid therapy until good control of inflammation is achieved. (e) Continue biguanides in low doses (four times per day) after discontinuation of corticosteroid therapy. (f) When the eye has been free of inflammation for 4 weeks, while using a biguanide alone, the condition is labeled as cured and all therapies are discontinued.

### Surgical treatment

With achievement of high-cure rates with anti-*Acanthamoeba* therapy, the need for keratoplasty in active disease has reduced significantly.<sup>[52]</sup> However, a delay in diagnosis and initiation of specific therapy or its misdiagnosis as viral keratitis in the initial stages leads to more extensive disease. These cases may become too fulminant to be controlled medically or may develop descemetocoele/perforation (which is not amenable to gluing) and may have to undergo surgical treatment.

The current indications for surgical treatment are (a) large infiltrate extending or threatening to involve limbus; (b) worsening on medical treatment; and (c) gross thinning or actual perforation.

Full-thickness PK has become less popular and is preferred only in cases of pre- and intra-operative corneal perforation.<sup>[66-69]</sup> Deep lamellar keratoplasty has been shown to be associated with better prognosis both for eradicating infection as well as graft survival.<sup>[70,71]</sup> We analyzed 23 cases of AK that underwent DALK at our center (unpublished data). There were no cases of recurrence of infection. However, 4 cases had detached Descemet membrane and required rebubbling. One patient had primary graft failure. Fifteen (65%) individuals had clear graft at the last follow-up. Three patients developed graft infiltrate of bacterial etiology, and 4 patients were lost to follow-up. Anshu *et al.* achieved successful eradication of infection in 8 out of the 9 eyes with therapeutic DALK in the first attempt and with a repeat procedure in the remaining eye with

disease recurrence.<sup>[70]</sup> Sarnicola *et al.* performed deep lamellar keratoplasty in 11 cases early in the course of therapy with no episodes of failure or recurrence in follow-up.<sup>[71]</sup>

In view of success with medical therapy, keratoplasty should largely be avoided in active stages, and even if there is extensive corneal involvement, an attempt should be made to achieve control of infection medically as far as possible.<sup>[7]</sup> Further, all patients undergoing surgery should be started on antiamoebic therapy before surgery, which than is continued postoperatively to prevent recurrence of infection. We use PHMB and chlorhexidine eight time per day along with corticosteroids for 3 weeks after the surgery at which time anti-*Acanthamoeba* drugs can be discontinued if there is no recurrence of infection. Follow-up care and complications otherwise are similar to that for other cases of keratoplasty in inflamed eyes.

### Complications

AK can be associated with extracorneal complications. Important ones are as follows:

#### Cataract

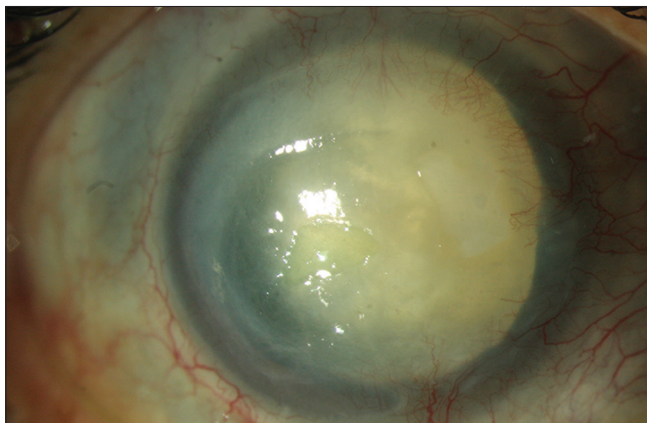
Cataract is seen in patients with severe and prolonged keratitis<sup>[72]</sup> [Fig. 6]. There are various theories of cataractogenesis in AK-toxicity from the use of topical anti-*Acanthamoeba* drugs, chronic inflammation, corticosteroids, and vascular thrombosis.

#### Iris atrophy and persistent dilated fixed pupil

This is seen in some patients and is again attributed to severe inflammation and vascular thrombosis<sup>[73]</sup> [Fig. 6].

#### Scleritis

Scleritis in patients with AK is an painful agonizing but an uncommon complication that generally occurs in otherwise immune-competent individuals.<sup>[74]</sup> It is unrelated to direct invasion of *Acanthamoeba* and attributable to an inflammatory response. Dying organisms and degenerating amoebic cyst walls have been shown to elicit an adaptive immune response in the animal model of keratitis and are proposed to cause persistent corneal and scleral inflammation. Other theories for scleritis and other posterior segment inflammatory reactions are AK induced autoimmunity through molecular mimicry and T-cell response from corneal antigen-presenting cells resulting in inflammatory response in vascularized ocular



**Figure 6:** Complication of *Acanthamoeba* keratitis: deep vascularization; cataract; dilated fixed pupil with peripheral anterior synechiae



**Figure 7:** Clinical picture of a case of *Acanthamoeba* sclerokeratitis

tissues.<sup>[75,76]</sup> Patients who receive topical steroids before the diagnosis of AK, usually following misdiagnosis as herpes simplex keratitis, tend to develop scleritis more commonly than those who do not.

The clinical picture of scleritis is characterized by severe deep pain, globe tenderness, engorgement of episcleral and scleral vessels, and in advanced cases by nodular or diffuse thickening of sclera [Fig. 7]. The pain of scleritis is usually very severe and was one of the indications of enucleation before the treatment with immunosuppressive therapy was in practice. Iovieno *et al.* described a step-ladder approach to the treatment of *Acanthamoeba* sclerokeratitis.<sup>[75]</sup> While milder form of scleritis gets controlled with topical steroids and oral NSAIDs, patients with severe disease require systemic immunosuppression in the form of oral steroids and steroid-sparing agents. The treatment is begun with intravenous methyl prednisolone 1-gram daily for 2–3 days along with oral prednisolone in the doses of 0.5–1 mg/kg/day. Unless there is rapid control of symptoms with oral prednisolone, steroid-sparing agents such as cyclosporine (3–7.5 mg/kg/day), mycophenolate (usually 1 g twice per day), or azathioprine (usually 100 mg oral daily) are added. In addition, all patients on systemic corticosteroid or immunosuppressive therapy are given oral antifungal therapy as prophylaxis against scleral invasion by trophozoites.<sup>[74,75]</sup> Treatment may last for months and is given in conjunction with anti-amoebic medications, which are continued after discontinuation of immunosuppressive treatment.

#### Intraocular spread

Although there are cases showing *Acanthamoeba* cysts in choroid and vitreous,<sup>[76,77]</sup> it is believed that in most cases, the parasite remains restricted to cornea and it is rare to see extracorneal spread. The mechanism of extracorneal spread in the absence of perforation is unknown.

#### Prognosis

The prognosis of AK depends on the severity of the disease and the time duration between onset of symptoms and initiation of definitive treatment with anti-*Acanthamoeba* drugs.<sup>[78–81]</sup> Cases diagnosed and treated early respond well to medical treatment. Two large series from the UK suggest that outcomes are good in patients where treatment was initiated within 3 weeks of onset of symptoms. We also had similar experience.

#### Conclusion

*Acanthamoeba* is an uncommon cause of keratitis. However, the outcome of medical management is good if the disease is diagnosed early and definitive treatment is initiated. The epidemiology and clinical features of AK in India are different than what is described in the Western world. Ophthalmologists are advised to be familiar with these differences. Routine laboratory tests are good enough for its diagnosis. Unfortunately, the drugs are not available and need to be prepared from chemical solutions that are not approved for ophthalmic use. However, these have good safety records, and enough experience suggests that these can be dispensed with reasonable confidence.

#### Financial support and sponsorship

Nil.

#### Conflicts of interest

There are no conflicts of interest.

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