Acanthamoeba Keratitis

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ABSTRACT: Acanthamoeba keratitis (AK), contact lens wear, confocal microscopy, diamidines, biguanides

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First described in the early 1970s, Acanthamoeba keratitis is a rare but potentially devastating corneal infection [1]. A notable increase in cases of AK was observed in the early to mid-1980s due to the increasing popularity of soft contact lens wear. Up to 70% of reported cases have been associated with contact lens use [2]. Soft lenses probably carry a higher risk for AK than daily-wear rigid lenses [3], and planned-replacement soft contact lenses, which require daily cleaning and overnight storage, probably carry a higher risk than daily disposable lenses, possibly due to inadequate hygiene. More recently, orthokeratology lenses, which are rigid and worn overnight, have been associated with AK [4].

Acanthamoeba keratitis in non-contact lens wearers is often overlooked as a cause of keratitis and is diagnosed late, as evidenced by the 3–15% incidence in Britain and the United States [5-7]. In non-lens users AK is usually associated with trauma and exposure to contaminated water or soil, often in agricultural workers. Additional potential risk factors are the use of contaminated tank-fed water in the home, warm weather, and poor socioeconomic conditions. AK has also been reported after surgical trauma, including penetrating keratoplasty and radial keratotomy.

One should suspect Acanthamoeba keratitis in eyes with recent traumatic corneal erosion, corneal abscess with poor response to wide-spectrum antibiotic treatment, and in all contact lens wearers

There are variations in the reported incidence of the disease among coutnries, from as few as 0.15 per million in the U.S. to as high as 1.4 per million in Britain, with other countries for which figures are available, such as Sweden and New Zealand, having an intermediate incidence [8]. In India where contact lens use is much less widespread, AK has been less reported as well [9,10].

BIOLOGY

Acanthamoebae species are free-living cyst-forming ubiquitous protozoa found in air, dust, soil and fresh water. They exist as normal flora in the upper respiratory tract of humans of whom 50%–100% have antibodies to Acanthamoeba. There are eight species of Acanthamoeba that can be distinguished by their cyst morphology and mitochondrial DNA analysis. *Acanthamoeba castellani* and *A. polyphaga* are the most common reported subspecies to cause keratitis [8]. The Acanthamoeba life cycle includes the motile protozoan (15–45 µm in diameter) and the dormant cyst (10–25 µm diameter) forms. Both forms are found in infected tissue, but only the trophozoite form is infectious. Cysts are double walled and resistant to environmental stressors or intentional killing by freezing, desiccation, and chlorine in water supplies, swimming pools and hot tubs. The trophozoite has an amoeboid shape with pseudopodia, and feeds on small algae, bacteria and other protozoans. In the cornea, they are thought to feed on keratocytes. Reproduction is asexual by binary fission.

Over the disease course, parasite adhesion to the host cell is a primary step and is mediated by a 130 kDa mannose-binding protein and a 28.2 kDa laminin-binding protein expressed on the surface of Acanthamoeba [11]. The initial binding leads to secondary events such as phagocytosis and toxin production, which result in the formation of phosphotidylinositol 3-kinase. The latter initiates a cascade that culminates in host cell apoptosis [12].

Corneal infection alone does not induce protective immunity against the parasite antigen. However, the role of secretory immunoglobulin A in the tear film was questioned in a recent study in which neither normal tears nor AK tears had any protective effects on Acanthamoeba-mediated corneal epithelial cell cytotoxicity. Tear factors, in addition to secretory IgA such as lysozyme, lactoferrin, beta-lysins, prostoglandins, and other compounds with antimicrobial and immunological properties, were also shown to have no significant effects on Acanthamoeba-mediated binding to and cytotoxicity of human corneal epithelial cells [13].

Tears also contain complement that is composed of serumborne molecules in a cascade-like manner. Acanthamoeba directly activates the complement system via the alternative pathway; however, pathogenic amoebae are resistant to

AK = Acanthamoeba keratitis

IgA = immunoglobulin A

complement-mediated lysis due to expression of complement-regulatory proteins including a decay-accelerating factor [14]. The presence of macrophages in corneas exposed to the parasite-laden contact lenses was found to prevent the development of full-blown AK in vivo by inducing an inflammatory response, in particular secretion of macrophage inflammatory protein-2 [15].

CLINICAL PRESENTATION

Diagnosing AK is difficult and is occasionally mistaken for other corneal infections. A high level of suspicion is crucial. AK should be considered in cases of corneal trauma associated with soil or contaminated water as well as in all contact lens wearers, especially if improper contact lens hygiene is suspected. It should also be suspected in cases of persistent corneal infection that does not respond properly to treatment. In some cases AK is co-infected with other pathogens such as herpes simplex virus, various bacteria or fungi. Delayed diagnosis of more than a few weeks is associated with worse visual outcome [16].

For AK diagnosis, deep corneal scraping should be obtained and cultured on non-nutrient agar with *E. coli*

Patients with amoebic keratitis may present with various complaints: severe ocular pain usually disproportionate to the clinical findings and probably secondary to perineural inflammation, blurred vision, photophobia, blepharospasm, foreign body sensation and tearing usually in one eye. In the early stages, within the first month, AK presents with a unilateral epitheliopathy including a punctate keratopathy, pseudodendrites, epithelial or subepithelial infiltrates, and perineural infiltrates. Limbitis may also be present. At a later stage, clinical signs may include ring infiltrates or anterior uveitis; in advanced stages corneal abscess may progress to corneal melting and perforation. Other possible signs include decreased corneal sensation, iritis, hyphema, hypopyon, elevated intraocular pressure and scleritis.

LABORATORY EVALUATION

A definitive diagnosis of AK is made by visualizing amoebae in stained smears or by culturing organisms obtained from corneal scraping. Another modality is to identify amoebic DNA with polymerase chain reaction; this method appears to have a sensitivity of 84% and specificity of 100% [8]. When scraping, the highest diagnostic yield is accomplished in early disease, hence the pathogen is located in the superficial epithelium and penetrates deeper into stroma only later on where superficial scraping may not be sufficient. In such circumstances, biopsy is needed to confirm the diagnosis. In contact lens-associated infections, the contact lenses case should be examined.

Corneal scraping material is well stained by acridine orange or calcoflour white. Non-nutrient agar with *Escerichia coli* or *E. aerogenes* overlay is the preferred medium for culturing amoebae. Acanthamoeba can also grow on blood agar, chocolate agar or buffered charcoal-yeast extract agar.

Acanthamoeba can also be visualized by confocal microscopy. Confocal microscopy is fast emerging as an important method in the early diagnosis of various corneal conditions. It offers magnifications of up to x 200 to x 500 with increased image contrast and allows details to be visualized even in hazy corneas. Its non-invasive nature could make it an important modality for the rapid diagnosis of AK. It can be used repeatedly, which is valuable not only for diagnosis but for management and follow-up as well. The cystic form of Acanthamoeba is more distinct and appears as a highly reflective round-to-oval structure measuring from 11 to 100 μm in different studies and has a bilayered, target-shaped or coffee-bean appearance. Trophozoites are pear- or irregularly wedge-shaped configurations measuring 23 to > 100 μm. Foci of keratoneuritis appear as high contrast beaded nerves and subepithelial or anterior stromal honeycomb [17]. This technology, which has been prominently displayed at numerous research meetings over recent years, produces impressive high quality images. Limitations to confocal microscopy remain its cost and lack of standardized interpretation. Further normalization of the measurements estimated from the in vivo laser scanning confocal microscopy to those of the Confoscan 3.0 is needed.

MANAGEMENT

Cases diagnosed during the early epithelial stage of the disease respond well to epithelial debridement, followed by a short (3–4 month) course of anti-amoebic therapy. The prognosis for visual recovery with only mild residual stromal involvement is very good. Once stromal infiltrates appear, eradication of organisms is more difficult and treatment may require 6–12 months.

Acanthamoeba trophozoites are sensitive to most available chemotherapeutic agents (antibiotics, antiseptics, antifungals, antiprotozoals including metronidazole, antivirals and antineoplastic agents). However, when infection related to the presence of Acanthamoeba cysts is persistent few of these agents have any effect [18,19], and only agents that are cysticidal in vitro against cysts can be expected to be efficacious. Although neomycin has been used widely, it is ineffective against cysts in vitro. In addition, like all aminoglycosides it is toxic to the corneal epithelium and can cause further damage. The mainstay agents that are used as a treatment for AK are diamidines and biguanides, which were found to be cysticidal anti-amoebics in vitro. Polyhexamethylene biguanide 0.02–0.06% (200–600 g/ml) and chlorhexidine 0.02–0.2% (200–2000 g/ml) are two biguanides

that are in use. The biguanides interact with the cytoplasmic membrane, resulting in loss of cellular components and inhibition of respiratory enzymes. Both drugs have been effective as primary therapy. Clinically, corneal epithelial toxicity has been minimal for both chlorhexidine 0.02% and PHMB 0.02%. Therefore, biguanides are considered a first-line treatment for AK either alone or in combination with diamidines [8].

Available diamidines include propamidine isethionate 0.1% (1000 g/ml) and hexamidine 0.1% (1000 g/ml). The antimicrobial effects of the diamidines result from the cationic surface-active properties inducing structural membrane changes that affect cell permeability. When these molecules penetrate into the amoebic cytoplasm, denaturation of cytoplasmic proteins and enzymes occurs. Propamidine and hexamidine have been clinically effective against both the trophozoite and cyst forms of Acanthamoeba. However, clinically resistant isolates have been reported with a concentration of 125–500 g/ml for both propamidine and hexamidine [20,21]. Hence, diamidines should not be used as monotherapy for AK. Clinically, the diamidines are well tolerated by ocular tissues, although prolonged treatment with propamidine may lead to toxic keratopathy [22].

When AK is diagnosed or highly suspected, topical chlorhexidine 0.02% should be started as soon as possible since early diagnosis and treatment improve prognosis significantly

Topical steroids are unnecessary in most cases diagnosed early but can be considered in patients with persistent inflammation (anterior scleritis, severe pain, indolent ulcers, corneal inflammation, anterior chamber inflammation). Topical steroids can be prescribed if at least 2 weeks of biguanide treatment have been completed.

Limbitis is common in both the early and late course of the disease. Limbitis might cause significant pain that can be well controlled by an oral non-steroidal anti-inflammatory. Scleritis is a severe complication that is considered to be an autoimmune phenomenon, fortunately not common. It can progress to scleral necrosis, causing uncontrollable pain that does not respond to NSAIDs. High dose systemic corticosteroids with systemic cyclosporine has proven helpful [23].

Persistent epithelial defect is common in severe cases of AK. If bacterial superinfection and/or persistent Acanthamoeba are ruled out then most topical therapies should be discontinued for several days. Preservative-free wide-spectrum antibiotic eye drops should be administered. Anti-amoebic therapy may be reintroduced after re-epithelialization. Epithelial debridement over the affected area should be performed early, when the disease is still intraepithelial for obtaining cultures and histology material. This improves corneal drug permeability and the therapeutic effect.

For persistent culture-positive AK unresponsive to the above therapies cryotherapy can be performed. In vitro experiments have shown that cryotherapy eradicates trophozoites but not cysts, unless combined with topical therapy [24,25].

Penetrating keratoplasty in eyes with active infection usually portends a poor outcome [26-28]. It should therefore be reserved only for cases with a poor response to medical treatment. Since the introduction of the biguanides as medical therapy, penetrating keratoplasty has not been recommended as a treatment for the elimination of organisms. Lamellar keratoplasty with a conjunctival flap has been successful and showed better refractive outcome in some patients [8]. Amniotic membrane transplantation for progressive corneal lesions with persistent epithelial defects may also be effective in controlling inflammation and delaying penetrating keratoplasty [16]. In cases where penetrating keratoplasty is inevitable, systemic steroids should be prescribed prior to surgery if concomitant limbitis or scleritis is present. Later these are tapered as inflammation is controlled in the post-graft period. Unlike grafts for other corneal infections, which should be large enough to remove all contaminated tissue, the graft for AK should be kept to the minimum size required to excise all ulcerated and necrotic tissue, retaining clinically healthy (but usually subclinically infected) tissue. This is because of the risk of rejection with large grafts and because repeat grafting may be needed in the event of recurrence; a further graft represents a new food source for the organism and can be used to attract residual amoebae. Anti-amoebic therapy should be used pre- and postoperatively since residual Acanthamoeba may host the fresh corneal graft.

PROGNOSIS

The prognosis of AK is correlated with severity of the disease at presentation and initiation of treatment. Patients who present with severe disease have a poorer prognosis. Late diagnosis and treatment initiation more than 3 weeks after appearance of the first symptom holds a poorer prognosis.

CONCLUSIONS

Acanthamoeba keratitis is not a common disease. The patient's history, careful clinical examination, appropriate laboratory testing and a high level of suspicion are necessary to reach an early diagnosis and achieve a better outcome.

PHMB = polyhexamethylene biguanide
NSAID = non-steroidal anti-inflammatory drug

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Capsule

Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX3CR1hi cells

The intestinal microbiota has a critical role in immune system and metabolic homeostasis, but it must be tolerated by the host to avoid inflammatory responses that can damage the epithelial barrier separating the host from the luminal contents. Breakdown of this regulation and the resulting inappropriate immune response to commensals are thought to lead to the development of inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. Diehl and co-workers propose that the intestinal immune system is instructed by the microbiota to limit responses to luminal antigens. They demonstrated in mice that, at steady state, the microbiota inhibits the transport of both commensal and pathogenic bacteria from the lumen to a key immune inductive site, the mesenteric lymph nodes (MLNs). However, in the absence of Myd88 or under conditions of antibiotic-induced dysbiosis, non-invasive bacteria were trafficked to the MLNs in a CCR7-dependent manner, and induced both T cell responses and immunoglobulin A production. Trafficking was carried out by CX3CR1hi mononuclear phagocytes, an intestinal cell population previously reported to be non-migratory. These findings define a central role for commensals in regulating the migration to the MLNs of CX3CR1hi mononuclear phagocytes endowed with the ability to capture luminal bacteria, thereby compartmentalizing the intestinal immune response to avoid inflammation.

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Eitan Israeli

"A clear conscience is usually the sign of a bad memory"

Anonymous

"A superior man is modest in his speech, but exceeds in his actions"

Confucius (c. 551-478 BCE), Chinese philosopher and teacher. His philosophy emphasized personal and governmental morality, correctness of social relationships, justice and sincerity