Contact Lens and Anterior Eye xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Review article

Acanthamoeba in the eye, can the parasite hide even more? Latest developments on the disease

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A R T I C L E I N F O

Keywords:
Acanthamoeba
Keratitis
Re-emerging pathology
Diagnosis
Therapy

A B S T R A C T

Acanthamoeba spp. is a free living protozoan in the environment, but can cause serious diseases. *Acanthamoeba* keratitis (AK), a severe and painful eye infection, must be treated as soon as possible to prevent ulceration of the cornea, loss of visual acuity, and eventually blindness or enucleation. Although the disease affects principally contact lens (CLs) wearers, it is recognized nowadays as a cause of keratitis also in non-CLs wearers. Although the number of infections caused by these amoebae is low, AK is an emerging disease presenting an extended number of cases each year worldwide mostly due to the increasing use of CLs, but also to better diagnostic methods and awareness.

There are two principal causes that lead to severe outcomes: misdiagnosis or late diagnosis of the causal agent, and lack of a fully effective therapy due to the existence of a highly resistant cyst stage of *Acanthamoeba*.

Recent studies have reported different genotypes that have not been previously associated with this disease. In addition, *Acanthamoeba* can act as a reservoir for phylogenetically diverse microorganisms. In this regard, recently giant viruses called Pandoravirus have been found within genotypes producing keratitis. What potential risk this poses is not yet known.

This review focuses on an overview of the present status and future prospects of this re-emerging pathology, including features of the parasite, epidemiology, clinical aspects, diagnosis, and treatment.

1. Introduction

Numerous free-living amoebae cause opportunistic infection in humans. *Acanthamoeba* genus is found in the air, soil, and fresh or brackish waters. Some strains of *Acanthamoeba* are responsible for causing human infections [1].

AK is an infiltrative corneal ulceration caused by some *Acanthamoeba* strains. It has been recognized as a potentially blinding disease, often only diagnosed at a late stage. The clinical presentation is sometimes confused with other infectious keratitis, particularly those of herpetic and fungal origin [2].

The causal agent exists in both active (trophozoite) and dormant (cyst) forms. The cysts are able to survive for long periods of time in hostile environments, including chlorinated swimming pools, hot tubs, and subfreezing temperatures in fresh water lakes, turning into trophozoites when environmental conditions are appropriate. The trophozoites produce a variety of enzymes that aid in tissue penetration and destruction [3]. Both trophozoites and cysts can adhere to the surface of unworn soft or rigid CLs [4], and then a break in the corneal epithelium may allow them to invade the eye tissues.

Most of *Acanthamoeba* infections are associated with CLs wear [5], and the expected incidence in developed countries is one to 33 cases per million CLs wearers [6]. Since CLs users numbers are growing every year worldwide and awareness and better diagnostics are available, the disease will become increasingly important over time [7,8].

AK is known to be difficult to diagnose and treat, despite advances in pharmacotherapy. Most patients are initially wrongly treated for viral, fungal, or bacterial keratitis before the diagnosis of *Acanthamoeba*

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https://doi.org/10.1016/j.clae.2017.12.017
Received 15 August 2017; Received in revised form 12 December 2017; Accepted 13 December 2017

is made. The need therefore, is to make a quick and accurate diagnosis to ensure effective treatment, and a good prognosis. When AK is suspected, a provisional diagnosis can be made through clinical features and confocal microscopy. However, a definitive diagnosis is only possible by culture, histology, or identification by polymerase chain reaction (PCR) [9].

The aim of this review is to highlight current information about the disease including general characteristics, epidemiology, clinical aspects, diagnosis, and treatment, focusing in recent discoveries in the biology of the parasite that involve changes in the prognosis and treatment of the AK.

2. *Acanthamoeba*'s free life in the environment

The life cycle of *Acanthamoeba* consists of two stages: a vegetative active trophozoite stage (25–40 μm) and a dormant protective cyst stage (13–20 μm) [10]. During the trophozoite stage *Acanthamoeba* actively feeds on bacteria, algae, yeasts or small organic particles and divides mitotically under optimal environmental conditions. Exposure to harsh conditions, such as lack of food, hyper- or hypo-osmolarity and extreme pH or temperature, results in cellular differentiation into a double-walled cyst form [11], in which *Acanthamoeba* can survive *in vitro* for more than 20 years [12].

Acanthamoeba is remarkably tolerant to a wide range of environmental conditions, being probably the most common protozoon found in soil, water, and in air samples [13,14], in a wide variety of habitats, from tropical to arctic regions. Recently, pathogenic genotypes were isolated from soils and water resources in Pakistan [15,16], from public thermal baths in Hungary [17], and from reservoirs of drinking water in Taiwan [18].

The major incidence factor in the distribution of *Acanthamoeba* in the environment is the presence of an available food supply. *Acanthamoeba* can take in bacteria via phagocytosis, after which it lyses them in the phagolysosomes [19]. However, some bacteria have established a stable symbiotic relationship with amoebae, a situation which may serve as reservoir for maintaining and dispersing pathogenic bacteria in the environment. It is puzzling that *Acanthamoeba* can host bacteria protecting them from the adverse environment and, at the same time, feeds on them to cover its nutritional requirements [19].

3. *Acanthamoeba* genotypes in AK

Species of *Acanthamoeba* were originally classified into three groups (I–III) based in differences in cyst morphology [20]. However, as culture conditions affect morphology, identifications are nowadays based on rRNA gene sequences and the genus is divided into 20 different evolutionary lines or clades (T1-T20) [21,22]. In the world, most of the clinical isolates from both keratitis and non-keratitis samples, have been typed as T4 genotype [23]. Other genotypes that have also been found associated to AK include T2a, T3, T5, T6, T10, T11, and T15 [[23],24]. Grun et al. [25] have recently reported the genotype T13 as an etiological agent of keratitis. Although T4 is the most frequently isolated genotype [26], non-T4 genotypes produce worse symptoms and have poorer response to medical therapy [27].

4. Pathogens' reservoir

Acanthamoeba trophozoites and cysts have the ability to harbor a variety of microorganisms [19]. Several pathogenic bacterial species were isolated from the inside of *Acanthamoeba* species, including *Salmonella enterica* [28], *Pseudomonas* spp. [29–31], *Mycobacterium* spp. [32,33], *Legionella pneumophila* [30], *Helicobacter pylori* [34], *Campylobacter jejuni* [35], *Listeria* spp., and *Vibrio* spp. [36]. In this regard, the presence of *P. aeruginosa* enhances the attachment of *Acanthamoeba* trophozoite to hydrogel CLs [37], but not to silicone ones [38], and Dini et al., 2000 [39] reported a case of AK where both agents were present. More work is needed to understand this relationship as inhibition of *Acanthamoeba* growth by *Pseudomonas* has also been observed [40].

Other protozoa, including *Toxoplasma gondii* [41]; fungi, as *Cryptococcus*, *Blastomyces*, *Sporothrix*, *Histoplasma*, *Exophiala*; and virus, as mimivirus, coxsackieviruses, adenoviruses [42], poliovirus, echovirus, enterovirus, vesicular stomatitis virus [19] have also been found as *Acanthamoeba* endosymbionts. Recently, a giant virus found inside *Acanthamoeba* strains producing keratitis was identified as a member of the emerging Pandoravirus family [43] and later its whole genome was sequenced [44]. On the other hand, studies on *Acanthamoeba polyphaga* mimivirus showed an inhibition of the amoebal encystment [45], which may represent an advantage in AK treatment.

5. AK epidemiology

Despite its wide distribution, diseases caused by *Acanthamoeba* are relatively uncommon. Among the infections, AK is the most frequent, although other types can be produced in immunocompromised hosts [46,47].

The epidemiological features of infectious keratitis may vary among different geographic regions, climate conditions, and living environments. Traditionally, predisposing factors of AK included corneal trauma associated with exposure to contaminated water, soil or vegetation [48,49]. Nevertheless most cases of AK were reported in association with CLs wear [50–55]. However, in Asian countries the majority of the AK occurred in non-CLs wearers [56–59], being secondary to corneal injury. This scenario has been identified as the major risk factor for infectious keratitis in rural areas [60–62].

A recent review of case-control studies showed that the use of CLs increased by 10 times the risk of suffering AK [5]. It was found that *A. castellanii* trophozoites and cysts adhere not only to soft, but also to rigid CLs [[64],63]. Infections related to CLs are often associated with improper wear such as overuse, poor cleaning and sleeping or swimming with them. Exposure of CLs to water seems to be a significant risk factor for AK [64]. According to research conducted by the Center for Disease Control (CDC) in the US (Table 1), topping off and storing lenses in water were associated with more than four-fold increases in risk, closely followed by shorter duration of use, handling lenses with wet hands and rinsing cases with water before storage [65]. The fact that the risk is higher in patients with fewer years of use would indicate that the experience in handling could play a role in prevention of AK.

Some researchers suggested that municipal water supply and its treatment may play a role in the development of AK. In the UK the AK incidence was 0–42 cases per million inhabitants, this variability being associated with the distribution of hard and soft water around the country. It has been hypothesized that hard water, that leads to limescale deposits in house water tanks, provides a favorable environment for amoebae and reduces the efficiency of chorine disinfection systems [66]. The presence of *Acanthamoeba* spp. in swimming pools may

Table 1
Hygiene risk factors in CLs-related *Acanthamoeba* keratitis.
Source: USA CDC 2014 Multivariable analysis (Cope JR; 2014).

	Cases n/N (%)	Control n/N (%)	mOR[a]	(95% CI)
Topping off	69/101 (68)	48/144 (33)	4.46	(2.19–9.81)
CLW ≤ 5 yrs	35/109 (32)	26/157 (17)	2.98	(1.47–6.35)
Storing lenses in water	25/102 (25)	5/145 (3)	4.38	(1.47–15.88)
Handling with wet hands	66/109 (61)	82/155 (53)	2.19	(1.10–4.59)
Rinsing case before store	55/97 (57)	51/138 (37)	2.10	(1.06–4.32)

CLW = CLs wearing.
[a] mOR: m Odds ratio adjusted for age, sex, and CLs type using exact conditional logistic regression.