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A review of Acanthamoeba keratitis in the Middle East and Iran

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ABSTRACT

Acanthamoeba keratitis, a vision-threatening disease, is caused by a free-living amoeba of genus Acanthamoeba. Amoeba is widely distributed all around the world. Several cases of Acanthamoeba keratitis have been reported from the Middle East and Iran as well. The infection is mainly caused by inappropriate use of contact lens; therefore, it is more common among lens wearers. The number of reported cases worldwide is increasing annually due to the increasing number of contact lens wearers for medical or cosmetic reasons. It is known that early diagnosis and treatment can reduce consequent damages, while the delay in these processes will deteriorate the vision. Nowadays contact lens wearing, surgery and use of corticosteroids are known to predispose to Acanthamoeba keratitis, and it was also believed that trauma was the main cause. Increasing public knowledge about Acanthamoeba infection, inquiring the history of patients, clinical signs and laboratory findings can be helpful to early diagnosis and better treatment. Regardless of the increasing knowledge of diagnosis and treatment, this disease is still a challenge. Considering the long curative time, and insufficient efficacy of available treatments, it seems that the prevention is more important than the treatment. This review aimed to explain Acanthamoeba keratitis in the Middle East and Iran from the aspects of epidemiology, diagnosis, and therapeutic treatment.

1. Introduction

Eye diseases affecting the cornea are a major causes of blindness worldwide. *Acanthamoeba* keratitis (AK), a rare sight-threatening corneal infection, is caused by the ubiquitous free-living amoebae of *Acanthamoeba* species[1]. The first case of AK was reported in 1974 by Naginton *et al.*, in the United Kingdom, and shortly later the second case in 1975 by Jones *et al.*, in the United States[2,3]. The widespread distribution of *Acanthamoeba* in the environmental

E-mail: h.habibpour59@gmail.com Fax: +98-61-33332036; sources, make it important[4]. There are several known risk factors including: wearing contact lens (CL) which may lead to corneal trauma, contamination of water or lens solutions with *Acanthamoeba*, and cornal trauma[5,6]. It is estimated that 90% of AK have occured among the contact lens wearers[7]. In recent years, the cases of AK have been increasing in the Middle East and Iran due to the wide use of CLs for vision correction and cosmetic reasons[8-10]. Corneal trauma even in very small scale can

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lead to AK, as injured cornea expresses mannose on surface two fold more than healthy one, and Acanthamoeba has more affinity for binding to this surface[10]. Symptoms of AK including severe pain, photophobia, ring-like stroma infiltration, epithelial defect and lid edema. Chorioretinitis, glaucoma hyphema, angiogenesis and blindness are other complications in the severe cases of AK[11]. Infectious keratitis is considered an ocular emergency and it needs special and immediate care to ensure the best visual outcome for the patient. Diagnosis of AK is challenging and mainly based on and the clinical and laboratory examination. Culture and microscopic examination are common laboratory methods to diagnose Acanthamoeba. Misdiagnosis of AK, results to penetration of amoeba to deeper layers of the infected eye and into the corneal stroma, which can destroy the tissue. Symbiosis between free-living amoeba and bacteria or fungi makes the diagnosis of AK very difficult. Although microscopy detection of amoeba is a reliable method, but it is hard to differentiate Acanthamoeba with other free-living amoeba, therefore, both culture and molecular assays are needed for definitive diagnosis of AK[10].

Despite of the increasing knowledge in the diagnosis and treatment of AK, it is still hard to diminish the threat of this disease. Eye injuries due to corneal scarring and vascularization usually lead to blindness through the life. Surgical intervention after corneal blindness depends on well-trained surgeon and staff with availability of high quality equipment, reliable eye bank facilities, and adequate clinical services for a long period after operation for monitoring and prevention of graft rejection[12]. Considering the severe consequences, prevention of sight-threatening consequences, increasing public knowledge about infection, inquiring the symptoms and signs of the disease and using the appropriate diagnostic methods can lead to the early and sufficient treatment[13]. The present review aimed to briefly described the epidemiological aspects, the diagnostic methods and available treatments of AK in the Middle East and Iran.

2. Epidemiology of AK

Acanthamoeba spp usually lives in the surface of water sources and is frequently found in different environments where human can be exposed to amoeba[14]. The presence of Acanthamoeba in agricultural channels/rivers[15] and places like hospitals[16], pools[17], hot springs[18,19] has a great effect on human health. Exposure with contaminated water, wearing of contact lens during the night, insufficient cleaning the contact lens, swimming with lens and other kinds of poor lens hygiene are main reasons for AK. The incidence in people using rigid contact lens is 9.5 times lower than for soft contact lense wearers because they have to be removed at night and be cleaned daily. AK is a rare disease in developing countries in compare with bacterial and fungal keratitis[20,21]. In a study Orti et al. examined 143 eyes of 129 patients and found that the left eyes of 55 patients (38.4%) and the right eyes of 60 patients (41.9%) and both eyes of 14 cases (9.7%) were suffering from keratitis and in

17 cases were infected with Acanthamoeba[22]. In 2017, Badawi et al. found that 245 of 247 cases were infected with Acanthamoeba. Also 243 patients suffered from infection in one eye and only two cases in both eyes. Another factor was age, and 70 cases (28.6%) were under 40 years and 118 cases (48.2%) were 40-59 years while 57 cases (23.3%) were 60 years old. The most common causes of keratitis were trauma (51.4%), and systemic factors included instance diabetes (15.1%), external beats to eye (5.7%), topical steroid usage (5.5%), local injuries after surgery (4.5%) and ulcers related to using of CLs (2.4%) and 17 cases (9.9%) showed no sign of the disease[23]. A case report study performed by Rezaei et al. found two cases of AK in one man and one woman who were using CL diagnosed by confocal microscopy method[24]. Demirci et al. reported one AK case in 5 years old boy with history of swimming and playing with soil[25]. Bower et al. found AK in one of the soldiers who was using CL[26]. In a study carried out by Ertabaklar et al. in a 22-year-old man in Turkey, direct eye contact with amoeba was found as the main cause of amoebic keratitis and T9 genotype was reported for the first time from Turkey[27]. A case report carried out by Alfawaz et al. reported AK in two women wearing CL. First one was a 28 years old woman who was using soft lens and had pain, reddish and blurred vision in left eye, and the other one was a 24 years old CL wearer woman who was complained of photophobia, reddish and pain in her left eye[28]. Abedkhojasteh et al reported one case of AK in CL wearer[29]. Patel et al. reported a case of AK with confocal microscopy in a 28-year-old woman who was using CL and was complaining of pain in his left eye and photophobia[30]. Sengor et al. reported a 41 old man with an AK who was also a contact lens wearer[31].

In 2007, a ten-year survey regarding AK reported by Rezaeian *et al.* showed that among 142 patients, 49 (34.5%) presented with AK. The most common age was between 15-25 year (75.5%). Interestingly, 44 patients (89.79%) were contact lens wearers for cosmetic purposes or visual corrections. Among them 41 patients (93.18%) wore soft contact lenses and three patients were hard contact lens wearers[32].

Another study by Niyyati et al. in Iran revealed Acanthamoeba as a causal agent in 15 (30%) of 50 keratitis samples. Among these clinical isolates, 13 (86.7%) were female patients and 2 (13.3%) were male. All positive infection occurred in soft contact lens wearers and only one in a patient with a history of hard contact lens usage. Regarding genotype identification, 13 (86.7%) of these isolates belonged to T4 genotype. However, it is important to mention that there was a mixed genotype of Acanthamoebae T4 and T11 genotypes in one patient. Other genotypes included T11 (13.3%) and T3 (6.7%)[33]. Results revealed that in few cases, contact with contaminated soil/water was reported as one cause of the disease, but most of the patients had the history of contact lens wearing. Amoeba keratitis is reported in patients using of almost all types of CLs, and most have infection in one eye. Seventy-five percent of the patients used CLs regularly; thirteen percent used unlimited CLs; six percent used hard lens and four percent used rigid gas-permeable CLs[34]. A study carried out by Behboodi et al. in 2001 shows that among 53%

of hospitalized patients with keratitis in Tootoonkaran Hospital in Rasht city, Iran, 35 cases (66 %) were farmer. Among all patients, 55% had a history of eye trauma and more than 50% of them had a history of trauma caused by hit with rice stem. Incidence of keratitis cases in summer season was higher than other seasons which may be related to the rice farming. The researchers advised farmers to use glasses during the work in rice fields[35]. Rumelt *et al.* found the *Acanthamoeba* cysts in corneal trachea taken from a 27-year-old plumber which was caused by a sharp metallic piece[36] (Table 1).

3. Clinical signs of AK

There are two clinical forms of AK. In the first form, the amoeba is limited to the epithelium, and the patient in this form has a chance for fast cure. In the second form, the amoeba invades to the basic tissues and causes irritation and severe necrosis. Considering the ability of *Acanthamoeba* in adhesion and invasion to the healthy corneal epithelium which causes inflammation in the cornea[46] the diagnostic probability of AK should be considered in all CL wearers with corneal irritation[47].

Patients with amoeba keratitis experience severe pain which is more than imagination. Pain is one of the most important signs, and most patients complain of the pain. In some cases, reduction of this pain is impossible even with strong pain killers. The possible reason is infiltration of parasite and white blood cells around the corneal nerves which appears as radial keratoneurosis in clinical examination.

According to the severity of corneal involvement, the clinic signs can be different from complete vision to no light sense and blindness. Complete vision will achieve with corneal transplantation after successful treatment. Many studies put emphasis on early diagnosis of amoebic keratitis in the prevention of eye vision defects[48].

Although the sense of foreign body, photophobia, eye redness, excessive tearing, blurred vision, sensitivity to light and mucosal discharge are mentioned as unspecific signs of the disease, but photophobia is the most specific sign for amoeba keratitis which is not seen in other kinds of keratitises.

4. Diagnostic methods

4.1. Culture and staining

In the cases that AK is suspected, materials from debridement are cultured on non nutrient agar supplemented with Escherichia coli or other *Entrobacteriacea*, and incubated. Light microscope shows that trophozoites have contractile vacuoles which fade and appear rapidly^[49]. If there was no debridement material available for culture, corneal scrap, corneal smear, contact lenses in saline or contact lense solutions can be used for culture. For preparation of smear from CL solution, it should be filtrated and centrifuged at 250 rpm and the sediment would use for slide preparation. Then slides can be examined with phase microscope. As the mixed infection may occur, different cultures may be helpful for differentiation.

Prepared slides and debridement materials can be stained with Wright Gimsa, Weathly trichrome or Gomory methenamine silver. Trophozoites after Wright Gimsa staining appear as purple bodies with a big nucleus and elongated pseudopodia, and cysts appear with two walls containing internal three angular or multi angular layer. With Weathly trichrome staining, the cytoplasm of the trophozoites appears green with red karyosome and the wall of cysts green[50]. Another stain which can be used for staining of *Acanthamoeba* cyst and trophozoites is Calcaflur white. By this staining, the walls of cysts appeared with green fluorescence and the trophozoites with red one[51]. This stain attaches to the chitin and cellulose in the cell wall of *Acanthamoeba* cysts (but cannot attach to the trophozoites cell wall) and almost can reveal all cysts present in the sample under fluorescence microscope by an average diameter of 10-25 micrometer[52].

The sampling of the cornea must perform, when active lesions in the stroma appear. Non nutrient agar for culture of cornea trachea would be used. Other staining methods like hematoxylin and eosin and periodic acid Schiff, methenamine silver stain, Calcaflur white, indirect antibodies with fluorescence against *Acanthamoeba*, methylene blue, Kongo red and Lugol's iodine can be used for cornea trachea. Also electronic microscopy is another tool for detecting of *Acanthamoeba* in tissue samples. Direct examination of samples is very important and if other assays did not show the presence of amoeba, the only direct sample will be reliable[53].

Table 1. Isolated Acanthamoeba from some patients in Iran.

			Total number of infected				
Agent	Genotype	Age of patient(s) (years)	patients/all patients	Number of female	Number of male	Contact lens wearer	Ref.
A can tham oeba spp	ND					+	[37]
A can than oeba	T4, T9, T11	15-37	18/138	15	3	+	[38]
A can that moeba	T4	16-27	11/62	10	1	+/-	[39]
Acanthamoeba and Vahlkampfia	Т3	35	1/1	1	0	+	[40]
A can than oeba	T4, T3, T2, T11	15-54	13/52	7	6	+/-	[41]
A can that moeba	T4	18-30	5/89	15	3	+	[42]
A can that moeba	ND	ND	10	ND	ND	+/-	[43]
A can tham oeba spp	T4, T11	ND	15/50	ND	ND	+	[44]
A can tham oeba spp	ND	14-36	23/27	21	4	+	[45]

ND= Not defined.

4.2. Confocal microscopy

In case of sever *Acanthamoeba* infection with high density of amoeba, microscopy can detect the amoeba in clinical samples. However, microscopic examination of *Acanthamoeba* has limitation like poor sensitivity. Confocal microscop is a scanner by which cornea can be scanned layer by layer in real time. This is a non-interventional method and is useful for early detection of *Acanthamoeba* in cornea tissue[54].

Detection with confocal microscopy had detectable rate 10 fold higher than with amoeba culture[55]. Confocal microscopy is rapid, and is useful for follow up the patients who did not receive the treatment for AK. Among the patients without history of surgery, small holes in cornea stroma are seen by confocal microscopy. *Acanthamoeba* cysts appear as shiny and small round shapes with 10-20 micrometer in diameter[56].

4.3. New diagnostic methods

Although trophozoites and cysts of *Acanthamoeba* can be detected by several methods, assuring the amoeba type is difficult[57]. Confocal microscopy should not replace with clinical examination or corneal sampling/biopsy or culture methods[58]. Besides, the morphological characterization and the isoenzyme analysis can be used for amoeba characterization. Nowadays, restriction enzymes of DNA makes the detection easier[59](Table 2).

5. Treatment

5.1. Medical treatment

Treatment of AK is very difficult, and the available medicines are effective in trophozoite form[94-95]. Therefore, it is important to

Table 2. Acanthamoeba genotypes identified from some parts of Iran.

Source of infection	Province/City	Genotypes	Detection method R	Ref.
Dust and biofilm	Tehran/Tehran	T4	Morphological and molecular [1	16]
Clinical and environmental specimens	s Tehran/Tehran	T4, T5, T11	Morphological and molecular [3	32]
(water, soil and animal-origin samples)				
Recreational water sources	Gilan	T4	Morphological and molecular [6	60]
Water sources	Kish Iland	T3, T4, T5, T11	Morphological and molecular [6	61]
Drinking water sources	East Azerbaijan/Kaleybar, Khodaafarin, Jolfa	T3, mixed T3/T4, T4, T5, T13	Morphological and molecular [6	62]
Soil	East Azerbaijan	T3, T4, T5, T11	Morphological and molecular [6	63]
Water sources	West Azerbaijan/SiminehRud, ZarinehRud,	T4	Morphological and molecular [6	64]
	Boukan, Shahindezh, Miandoab, Mahabad			
Water of therapeutic hot springs	Ardebil/Ardebil	T3, T4	Morphological and molecular [6	65]
Water of therapeutic hot springs	Ardebil/Ardebil	T4	Morphological and molecular [6	
Surface water resources	Guilan, Mazandaran, Alborz, Tehran/Tehran,	T4,T5	1 0	67]
	Karaj			
Waste water samples	Tehran/Tehran	T4	Morphological and molecular [6	681
Water sources	Mazandaran	T4, T2	Morphological and molecular [6	
Rivers	Tehran/Tehran	T4, T15	Morphological and molecular [7	
Recreational water	Tehran/Tehran	T4, T5	Morphological and molecular [7	
Tap water	Fars/Shiraz	T4, T5, T15	Morphological and molecular [7	
Water, soil, air	Khuzestan/Ahvaz	T4, T2, T5	Morphological and molecular [7	
Environment	Different cities of Iran	T2, T4	Morphological and molecular [7	
Tap water sources	Hormozgan	Acanthamoeba spp	1 0	75]
Tap water and environment sources	Isfahan	Acanthamoeba spp		76]
Surface resting waters	East Azerbaijan/Tabriz	T5	Morphological and molecular [7	77]
Surface water	South Khorasan/Birjand	Acanthamoeba spp		78]
Surface water	Tehran	T4, T5	Morphological [7	79]
Drinking water (hospitals)	13 cities of Iran	Acanthamoeba spp	Morphological [8	80]
Water sources	Shiraz	Acanthamoeba spp	Morphological [8	81]
Surface water	Arak	Acanthamoeba spp	Morphological [8	82]
Water sources	North Khorasan/Bojnurd	T4	Morphological and molecular [8	83]
Hot springs	Mazandaran	Acanthamoeba spp	Morphological [8	84]
Tap water	Ahvaz	Acanthamoeba spp	Morphological [8	85]
Surface water	Shiraz	Acanthamoeba spp	Morphological and molecular [8	86]
Water of pools	Sistan	T3, T4, T5		87]
Rivers	Mazandaran/Tonekabon	Acanthamoeba spp	Molecular [8	88]
Soil	Tehran/Tehran	T4	Morphological and molecular [8	89]
Geothermal rivers	Ilam/Dehloran, Tar	T4, T2	Morphological and molecular [9	90]
Soil	Mazandaran/Sari	$A can that moeba \ { m spp}$	Morphological [9	91]
Water	Qazvin/Qazvin	T4, T2	Morphological and molecular [9	92]
Water, soil and animal origin samples	Tehran/Tehran	T4	Morphological and molecular [9	93]

evaluate the efficacy of medications on both cystic and trophozoite forms. Killing the cysts in the tissue is important to eradicate the amoeba. Studies with cDNA-RFLP and real time methods revealed that for cyst transformation to trophozoite, three genes named hsp70, actin and EF-1 \alpha are responsible, which can open a new aspect in the treatment of AK[96]. Common treatments are based on previous in vitro studies and experiments. Investigations showed that there are no reliable in vitro assays for evaluating the drug efficacy on amoeba cultures. The main reasons may be resulted from several factors including the difference in the culture phase for drug evaluating, Acanthamoeba culture preparation method, accumulation of amoeba in culture medium and exposure time with used compounds. These are the probable reasons which may lead to unfair results of in vitro assays. Besides, conclusion from clinical reports of AK is not reliable because there are differences in infection period and the treatment in patients, and will be more complicated with incorrect diagnosis. Another problem is the administration of corticosteroids before starting of medical treatment. Finally, the corneal grafting time, the treatment and the period of medication are different as reported in several studies. On the other hand, in some cases, the treatment started with five or even more medicines at the same time (local and systemic), thus the evaluation and comparison are very difficult. Common medicines included brolene 0.1% (propamidine) drops, propamidine isethionate (0.1%), dibromopropamidine, cationic ant pollutant (polyhexamethylen biguanide, clorohexydine), aminoglycosides (neomycine, paramomycine), imidazoles (myconazole, ketoconazole)[97].

In 2007, Vural *et al.* showed that the damage due to AK were significantly decreased in groups treated with propolis or clorohexidine[98]. Bong *et al.* reported the first case of successful treatment of *Acanthamoeba* keratitis with voriconazole who was resistant to clorohexidine[99]. Polat *et al.* reported the therapeutic effect of miltefosine on AK[100]. Siddiqui *et al.* demonstrated that using phenodinamic agents like Sn porphyrin and exposing with an adequate source of ultraviolet radiation is useful against corneal invasion of *Acanthamoeba*[101].

5.2. Non medicine treatments

In the early stages of AK with injuries restricted to the cornea epithelium, debridement of cornea is useful. Debridement can help the medicine penetrate to the cornea faster, and also can decrease the load of amoeba in cornea tissue. Debridement of corneal tissue is the simple way to remove necrotic tissue from the cornea. If the damaged tissue locates under the epithelium, the surgeon will remove the damaged tissue by sterile scalpel No15 under local anesthesia. Removed tissue and prepared slides will send to the pathology laboratory. This is not only a diagnostic method but also a treatment procedure in the early stage of infection as it can remove necrotic tissues. Depending on the patient's condition, the procedure can be repeated once in 1-2 d to two weeks once. This assay is very

effective in healing of damaged cornea and is suggested strongly in the early stage of *Acanthamoeba* infection.

It is important that the medication should continue a long time after infection for prevention of relapses considering resistance of cyst form to drug. Patients need 3-5 months of treatment[12].

5.3. Cryotherapy

In this case that the injured cornea is exposing to cold. Cryotherapy, could decrease the cell metabolism, inflammation, pain, spasm and vascular contractions. Also, this technique is used for damaging remained in tissue before cornea graft. This therapy can also prevent relapse. Anyway, as cryotherapy can cause severe damage in cornea tissue and is not effective enough, this assay is not routinely use for treatment of AK[102].

5.4. Layer keratectomy

In this procedure, the lesion and infected areas are picked up layer by layer. This technique is used for patients with ineffective medicinal treatment, and it can help to a faster healing. The aim of this method is to increase the efficacy of medicines and decrease the amoeba load in injured tissues[103].

5.5. Corneal graft

The proper time for corneal graft is controversial. Considering the presence of *Acanthamoeba* even in apparently healthy parts of the cornea, it is necessary to perform the corneal graft a long time after treatment and complete healing of the cornea. If the corneal graft performs before complete healing of *Acanthamoeba* infection, it will relapse again and will cause the graft rejection, and even worse the patient may lose his vision. Only in case of the presence of holes in cornea or the probability of its occurrence and after complete healing of cornea tissue, corneal graft can be performed. Demirci *et al.* reported the first successful corneal transplantation in patients with the treatment for AK[104].

There are some reports of relapsing after a corneal graft in inflammatory eyes, which were resulted from graft before eradication of infection, graft rejection, stromal injuries, leaking, opening of the wound, glaucoma and cataracts[105].

5.6. Conjunctival flap

The conjunctival flap could decrease the pain, and relieve other symptoms of AK. In the cases with corneal melting in AK, conjunctival flap can be the best solution to increase the survival of the integrity of the eye. In some cases, the amoeba remains under the conjunctival flap and causes cornea holing and reduce the depth to anterior chamber, but in cases with a long healing time, conjunctival flap after keratectomy will bring in a fast cure[106].

6. Discussion

Acanthamoeba has widely distributed in a variety of environments including ocean sediments, tap water, ponds, hydrotherapy pools, lakes and hot springs[107] (Table 1). Acanthamoeba keratitis has no endogenous origin[108]. The diagnosis of AK is very difficult and has a prolonged treatment period, which in untreated cases leads to blindness[109]. Acanthamoeba infection is equal in men and women[67,110]. Using of CLs is the main reason for catching AK[111] (Table 2). AK is mostly seen among young and healthy individuals, and most of them (80%) have a history of wearing CL, and it is seen in people who never use CLs as well[112]. CLs are able to cause small scratches on the cornea surface and if the lens or container is contaminated with amoeba, it can lead to penetration of Acanthamoeba to the injured part[113]. The reason that AK has frequently found in CL wearers is the abundance of amoeba in environment and the ability of amoeba in growth at different environments. Studies revealed that 70% of CL containers were contaminated with Acanthamoeba among individuals who did not have Acanthamoeba infection[114]. Since Acanthamoeba can survive in mineral waters, AK has seen among people who use non-sterile waters and salt pills for disinfecting or keeping their CLs in these solutions[41]. Also studies showed that Acanthamoeba can enter the eyes with contaminated water, dust and dirty fingers through small scratches and after cornea trauma and may lead to AK. Although the most cases of AK in the United States are seen among CL wearers, and in India the most cases of AK frequently are caused after eye traumas. In the Middle East the oldest cause of AK was trauma but nowadays the main cause is the using of CLs because of increasing demand for improving vision or cosmetic reasons. Amazingly, in some cases, AK is diagnosed in people who have no history of CL wearing or eye trauma. Thus using of CLs or eye trauma is not the only reason for AK[61]. Studies have shown that the most common genotype of Acanthamoeba in most of the countries in the Middle East is T4 genotype[114-115]. Also early diagnosis and using of antiparasite agents like propolis, ultraviolet radiation[69], and corneal graft in drug resistant patients[38] are effective tools to decrease and control of AK. For prevention of AK in CL wearers, the contamination of lens solutions should be paid attention to. Hands should be washed before wearing the lens, and the lenses must be removed before bathing/swimming, and sterile saline should not be used to contain lens. Despite of increasing knowledge in the diagnosis and treatment of AK, it still is hard to diminish the risk of this disease. Considering that there is no ultimate treatment (both medicine and surgery) for AK, it seems that prevention is more important than treatment. Early diagnosis and appropriate treatment of AK is very important in prognosis and controlling the infection specially for keeping vision health.

We summarized several aspects of *Acanthamoeba* keratitis with emphasize on Middle East and Iran. As this amoeba is widespread all around the world, the prevention of infection is the first priority of public health and needs more attention from health programmers and governments. Acquaint with epidemiological and clinical aspect

of the disease can help the young physicians to rapid diagnose and choose the proper treatment.

Conflict of interest statement

The authors report no conflict of interest.

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