

Brief Report

Amoebic keratitis in Iran (1997-2007)

**M Rezaian*¹, *Sh Farnia*¹, *M Niyati*¹, *F Rahimi*²

¹*Dept. of Medical Parasitology and Mycology, School of Public Health, Medical Sciences/University of Tehran, Iran*

²*Farabi Hospital, Medical Sciences/University of Tehran, Iran*

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Abstract

Background: Amoebic keratitis introduced as a painful corneal infection which sometimes lead to poor vision and blindness. The main goal of this study was to report amoebic keratitis during ten years from 1997-2007 in patients who was suspected to have amoebic keratitis and referred to Parasitology laboratory, School of Public Health, Tehran University of Medical Sciences, Iran. Other aim was to assess the major risk factor for developing this sight-threatening disease. Comparison of lens culture and corneal scrapes culture also was performed.

Methods: During 1997-2007, 142 patients referred to Dept. of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran. Details of each patient such as age, sex, history of contact lens wear, type of contact lens, clinical symptoms were recorded in questioners. Keratitis was diagnosed on the basis of culture of lenses and/or corneal scrapes on non-nutrient agar overlaid with *Escherichia coli* and direct microscopy of lenses and/or corneal scrapes.

Results: Among 142 patients, 49 (34.5%) had amoebic keratitis. 73.46% of these patients were from Tehran but there were a few cases from other cities. The commonest age was between 15-25 yr (75.5%) and more female (37:12) were identified then male. It is worth to mention that 44 patients (89.79%) were contact lens wearers who among them 41 patients (93.18%) wore soft contact lens and only three patients suffer from amoebic keratitis because of wearing hard contact lens. Other finding of this study demonstrated that the most common sign of the patients was severe pain combined with photophobia.

Conclusion: This study indicates that *Acanthamoeba* keratitis continue to rise in Iran. This is due to increase frequency of lens wearers as well as consideration of ophthalmologist to *Acanthamoeba* as an agent of keratitis and improvement of laboratory methods. Another finding of this research was the confirmation of soft contact lens as a major risk factor. It is recommended to educate contact lens wearers for regular disinfection. Besides, culture of corneal scrapes was negative in most of cases, so lens culture were performed which had a much better result.

Keyword: *Acanthamoeba*, *Keratitis*, *Iran*

Introduction

A *canthamoeba* is one of the most prevalent amphizoic protozoa which has a wide distribution in nature such as water, air, soil, eye wash solution, tap water, swimming pools and so on (1, 2). So we can isolate *Acanthamoeba* from almost anywhere.

Several species of *Acanthamoeba* can cause serious human infections such as Granuloma-

tose Amoebic Encephalitis (GAE) which is rare but fatal disease, especially in immunocompromised patients (2). There are no reports of GAE cases in Iran up till now.

Another disease due to *Acanthamoeba* in human is amoebic keratitis. *Acanthamoeba* keratitis is a painful, devastating corneal infection which frequently progresses slowly and if not diagnosed and treated early, loss of vision, corneal perforation and in some cases blindness

ensues (2, 3). Keratitis due to *Acanthamoeba* was first described by Dr. Jones in the early 1970's (4, 5). Review of literature shows that in the early to mid 1980's there was a remarkable growth in the number of this sight-threatening disease (6). This was because of increased number of soft contact lens wearers. Eventually, the correlation of amoebic keratitis and wearing contact lenses was confirmed (6-8). It should be emphasized that 85% of amoebic keratitis develop in contact lens wearers (9). Indeed, contaminated contact lens is one of the most predisposing factors for developing amoebic keratitis. Nowadays, contact lens combined with corneal minor trauma remains the two major risk factors for developing disease (8, 10). The cases of amoebic keratitis reported by Khan in 2003 were more than 1350 cases worldwide (11). It is worth mentioning that in 2007 the cases of keratitis related to genus *Acanthamoeba* are estimated to be more than 3000 cases (2). These reports show that the frequency of amoebic keratitis continues to rise (6, 11) and because of difficulties for early and fast diagnosis and the lack of effective drugs, it is important to instruct contact lens users proper lens handling and regular disinfection by multi-purpose systems such as hydrogen peroxide(3). Decades ago in Iran, there were a few amoebic keratitis cases reported by ophthalmologists on the basis of clinical manifestations. However, it is worth mentioning that a wide range of organisms can lead to similar presentations, so diagnosis on the basis of clinical features can be confusing to the physicians.

Due to increasing number of contact lens wearers combined with high clinical suspicions in patients with no response to antimicrobial drugs, laboratory methods such as direct microscopic smears and culture of corneal scrapes or lenses were set up at the Dept. of Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran. It should be mentioned that although molecular methods support the diagnosis of amoebic keratitis, but the gold standard is culturing the organism (1).

Although, during 1997-2007 an increasing number of positive amoebic keratitis incidences were observed in patients referred to the Parasitology laboratory, but it is estimated that the cases of this progressing keratitis is more than the reported number. It is important to alert ophthalmologists to consider amoebic keratitis in contact lens wearing patients who experience severe pain (6).

Investigation of Maghsood *et al.* showed that there are three species responsible for amoebic keratitis in Iran (12). These species are as follows: *A. castellanii*, *A. griffini*, *A. palestinensis*. *A. castellanii* was the most frequent isolated species among patients.

The aim of this study was to report amoebic keratitis in patients with diagnosed keratitis during 1997-2007 and assess the major risk factors for developing the disease in addition to compare the culture of lens and corneal scrapings for detection of *Acanthamoeba* organism.

Materials and Methods

Patients

This research was a cross-sectional, descriptive study. The patients were the individuals with clinically diagnosed keratitis referred to the Dept. of Parasitology, School of Public Health, Tehran University of Medical Sciences, during 1997-2007. The samples were corneal scrapes taken by ophthalmologist, lenses, lens cases and lens solutions. For each patient we filled out a questioner. The data of questioner included details of patient's age, sex, history of contact lens wear, contact lens type, history of eye surgery or trauma, clinical picture and diagnosis methods.

Examination of Samples

All patients' samples such as corneal scrapings, corneal swabs, contact lenses or even lens solution were examined by both direct microscopic smears and by plate culture methods (1). Direct microscopy was performed by preparation of smears from contact lenses, corneal scrapings or lens solution onto clean glass slides and

searched by magnification 40 for detection of cysts or trophozoites of amoeba.

In the next step, we used non-nutrient agar coated with gram negative bacteria such as old culture of *Escherichia coli* as a suitable bacterial food source. The bacteria can be used on the agar surface. It is better to spread the bacteria on the agar surface as a lawn.

Non-nutrient agar contained agar in Page's Amoeba Saline (PAS). The component of page saline is NaCl, KH₂ PO₄, Na₂ HPO₄, CaCl₂. 6H₂O and MgSO₄. 7H₂O. The pH of this medium should be adjusted to 6.9 by KOH. Then, inoculation of corneal scrapings or contact lenses was performed in separate plates. Plate cultures were sealed with parafilm for prevention of environmental contamination. Plates were incubated in room temperature and observed daily at 40x magnification for detecting *Acanthamoeba* trophozoites or cysts. The plates were discarded at 4 weeks if no signs of amoeba growth were seen. Positive culture was defined as the presence of Amoeba usually after 4-7 d.

Results

During a ten year study, 142 patients were examined to investigate *Acanthamoeba* as a casual agent for keratitis. Diagnosis was made on the basis of culture and direct microscopy. These methods indicate that in 49 patients (34.5%), *Acanthamoeba* was the cause of this disease. Most of the amoebic keratitis were from

Tehran (73.46%) and the commonest age was between 15-25 yr (75.5%) (Fig.1). Besides, More females (37:12) than males were identified. Other interesting finding was that 44 patients (89.79%) were contact lens wearers who among them 41 patients (93.18%) wore soft contact lens and only three patients suffer from amoebic keratitis because of wearing hard contact lens.

Other finding demonstrated that the most common sign of the patients was severe pain combined with watering, and photophobia (Table 1). In addition, five patients had other risk factors such as eye surgery and history of trauma. Eighty percent of these patients were in the age range of >35 yr. It should be mentioned that in two patients the ocular infection were too intensive, so that we could detect the amoeba by direct microscopy of lens. It was interesting that in one of them the culture of corneal scrapes was negative even after 15 d. In addition, results indicate that lens culture have more sensitivity through corneal scrapes culture, since lens culture became positive after 4-5 d, in contrast to corneal scraping culture which were negative even after 20 d. Results show that among patients whom we culture both lens and corneal scrapes, lens culture became positive in 100% of cases. However, the increased frequency of amoebic keratitis was seen since 1997 in Iran. From this year to 2002, totally 20 cases of amoebic keratitis were recorded while from 2003 to 2007 this number changed to 29 cases.

Table 1: Frequency of signs & symptoms in 49 amoebic keratitis patients referred to the Dept. Medical Parasitology, School Of Public Health, Tehran University of Medical Sciences from 1997 to 2007

Signs/symptoms	No.	%
Corneal severe pain	42	85.7
Corneal watering	40	81.6
Photophobia	31	63.2
Corneal redness	15	30.6
Corneal burning	13	26.5
Corneal opacity	13	26.5
Corneal irritation	6.0	12.2
Corneal ulcer	3.0	6.1
Poor vision	1.0	2.0

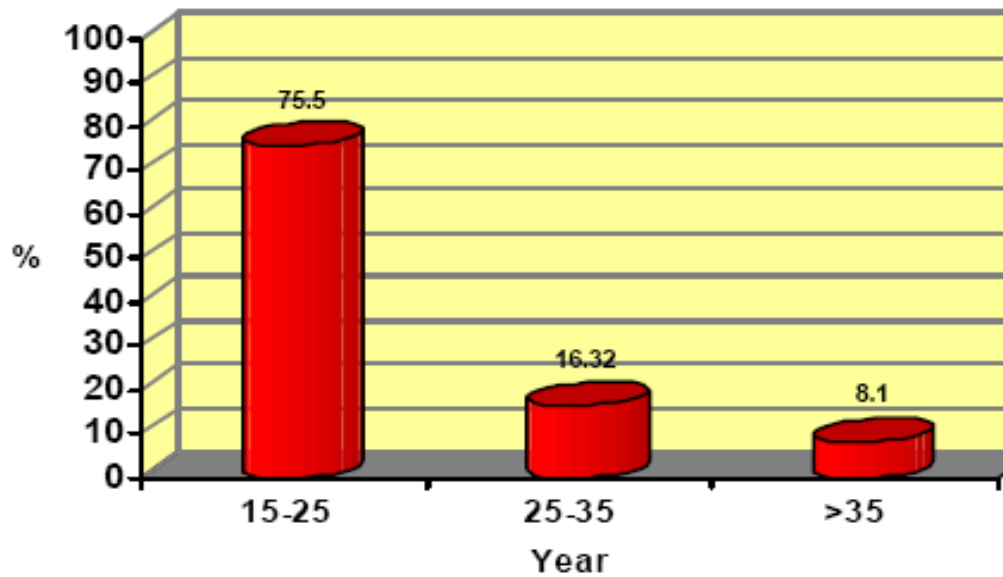


Fig. 1: The age range (yr) and percentage of amoebic keratitis patients referred to the Dept. of Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran from 1997 to 2007

Discussion

Keratitis due to *Acanthamoeba* is a rather rare but sight-threatening corneal infection. Our patients came from different parts of Iran but most were from Farabi Hospital in Tehran. Amoebic keratitis researches attract many researchers, since it is an ocular infection which is rising and can lead to blindness. Only early detection and accurate treatment of this infection will lead to good outcome (1-3).

However, the increased frequency of amoebic keratitis which we inform since 1997 in Iran is probably due to consideration of ophthalmologist to amoebic keratitis as an agent of keratitis and improvement of laboratory methods for detection of amoeba in addition to increase rate of contact lens wearers. Previously, ophthalmologist take only corneal scrapes as a patients sample for laboratory investigation but since culture of corneal scrapes were negative in most of cases, lens culture were performed which had a much better result. We can estimate that the frequency of keratitis patients were much more then the reported number. This is due to detection of amoeba only by culturing of cor-

neal scrapes in some patients. This study suggested that the sensitivity of lens culture is much more then corneal scrapings culture. This is probably due to sampling of ophthalmologist or progression of amoeba into the deeper layers of cornea (3). The commonest age range was from 15 to 25 (yr) and nearly all of them were contact lens wearers. Indeed, we can conclude that since younger patients especially women wore contact lenses for more beauty, they have a higher risk for amoebic keratitis. In contrast, most amoebic keratitis in older individuals is as a result of trauma or eye surgery.

Another main finding of this study was the confirmation of soft contact lens as a major risk factor. This is in agreement with Walochnik study as well as Khan and Panjwani works (11, 13, 14). The findings of the above researches shows that soft contact lens in addition to minor corneal trauma are the major risk factors for initiating the disease. In fact, poor contact lens hygiene, home-made solution, inadequate disinfection or swimming with lenses is amongst the risk factors for amoebic keratitis (6). The results indicate that soft contact lens wearers show a higher risk then hard contact lens wear-

ers for developing amoebic keratitis. Actually, *Acanthamoeba* can attach to soft contact lenses readily and these lenses act as a carrier for transferring the amoeba to the eye. It should be mentioned that in India as opposed to other countries most of amoebic keratitis patients do not have a history of wearing contact lens. In contrast, the majority of patients had corneal trauma as a major risk factor (6). However, several reports indicate that contact lens wear was the cause of 80%-86% keratitis, especially in developing countries (6, 15).

Briefly, contact lenses especially the soft ones are the commonest risk factor along with poor hygiene. It is recommended to educate contact lens wearers for regular disinfection.

Methods for rapid detection are extremely important such as direct microscopic smears which provide immediate information about the agent. In two patients the infection was too intensive which we detect a large amount of cysts on the surface of lens. Although some studies reported that culturing of *Acanthamoeba* from eye is difficult, but other researches emphasized that culture is still a gold standard for detection of *Acanthamoeba* (1). Difficulties due to culturing are due to the distribution of amoeba to the deeper layers.

It should be emphasized that although molecular method such as PCR- techniques provide rapid and accurate identification but they are inapplicable partly due to expense especially in developing countries (2).

In conclusion, this study shows that amoebic keratitis continue to rise in Iran and ophthalmologist should consider *Acanthamoeba* keratitis as an agent of keratitis specially in patient with history of contact lens wear. Laboratory methods such as culture of lens together with severe pain and photophobia are very useful for confirmation of clinical diagnosis.

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