

# *Acanthamoeba keratitis* – Importance of the Early Diagnosis

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## ABSTRACT

*This is a case report of Acanthamoeba as a causative agent of keratitis at the Department of Ophthalmology, University Hospital Rijeka. Delay in treatment led to an advanced stage of the disease with multiple complications. Initially, presented symptoms were similar to those of herpetic keratitis. In the mean time progression of the disease led to a cloudy cornea with a stromal ring infiltrate, poor vision, elevated intraocular pressure, a mature cataract and finally corneal melt. Corneal scrapings were obtained from a 28 years old female patient, wearing soft contact lenses and with poor lens hygiene. Acanthamoeba cysts were identified by the cytological examination. Culture of the corneal scraping had confirmed Acanthamoeba as the etiological agent. Making the diagnosis of Acanthamoeba is difficult. We presented this case of delayed diagnosis and unfavorable outcome as a reminder that clinical suspicion remains the most important diagnostic tool. Contact lens wears with a new diagnosis of herpes simplex keratitis are in high risk group, especially those with significant pain or poor response to conventional therapy. We also wanted to point out the possibility of an early, prompt and inexpensive diagnosis with the cytological examination.*

**Key words:** *Acanthamoeba keratitis; contact lenses; diagnosis; cytological techniques*

## Introduction

*Acanthamoeba spp.* is ubiquitous, free-living ameba that is found commonly in the environment, including water (e.g. tap, bottled water, swimming pools, hot tubs, lakes, rivers, thermal and brackish waters or in the sea), soil, sewage systems, cooling towers, heating/ventilation/air conditioning systems<sup>1</sup>. *Acanthamoeba spp.* has two stages in the life cycle: the motile trophozoite (8–40 µm) and the dormant cyst (8–29 µm)<sup>2</sup>. In an unfavorable surrounding the trophozoite is turning into the cyst which can evade extreme environmental conditions such as: hyperosmolarity, glucose starvation, desiccation, extreme temperatures (from –20 to + 42°C) and extreme pH<sup>3</sup>.

*Acanthamoeba spp.* can cause granulomatous meningoencephalitis in immunocompromised patients, but usually infects the eye causing *Acanthamoeba keratitis* (AK), which is a progressive and potentially blinding corneal infection<sup>3</sup>. Although AK is a rare infection of the cornea,

its frequency is increasing, since its first description in mid- 1970s<sup>4,5</sup>. The first reported cases were associated with some degree of ocular trauma; but with increasing use of contact lenses, especially soft contact lenses, a dramatic increase in cases of AK was observed<sup>6</sup>. The expected incidence of AK is one per 30 000 hydrogel contact lens wearers per year (or 0.33 per 10 000)<sup>7</sup>.

Effective treatment began in the mid- 1980s through the use of propamidine isothionate 0.1% (Brolene)<sup>8</sup>, because treating complications of AK are mainly unsuccessful<sup>9,10</sup>. Significant progress in medical treatment of AK was not made until early 1990s with the introduction of cationic antiseptic agents: chlorhexidine (0.02%) and polyhexamethylene biguanide (PHMB, 0.02%)<sup>11,12</sup>.

The clinical picture of AK is remarkable for its variability. Clinical characteristics of AK, especially the irreg-

ular epithelial lesions and the stromal infiltrative keratitis may resemble herpes simplex keratitis. Many patients are initially diagnosed and treated for this infection<sup>13,14</sup>. Until recently, the correct diagnosis was made only after detailed histological examination of corneal tissue removed at the time of transplantation. Nowadays the diagnosis can be quickly confirmed by scraping the cornea, staining of the obtained material and examining it with a standard light microscope<sup>15,16</sup>. To our knowledge this is the first published case of AK diagnosed cytologically in Croatia. Therefore we wanted to point out the possibility of an early diagnosis with the cytological examination and to emphasize its importance. There is a clear association between delay in the diagnosis and the outcome of AK<sup>17,18</sup>. Unfortunately, in our case the infection was recognized in the advanced stage of the disease.

### Case Report

A 28-year-old female, high myopic patient was wearing soft contact lenses for 15 years. She was cleaning her lenses with tap water and keeping them in saline solution during the night. She was using two weeks lenses, but kept them wearing for a month.

Initially, the symptoms in her left eye were similar to those of herpes simplex keratitis and she was treated, for two months, with antiviral and antibacterial agents that led only to temporary improvement of her condition. At the time of hospitalization, cornea was markedly blurred with the stromal ring infiltrate and visual acuity of hand motion on the left eye (Figure 1). She suffered severe pain spreading to the forehead with photophobia and excessive lacrimation.

Corneal scrapings were obtained with a sharp, metal instrument. Samples were taken for cytological staining with a routine May-Grunwald-Giemsa (MGG) stain and

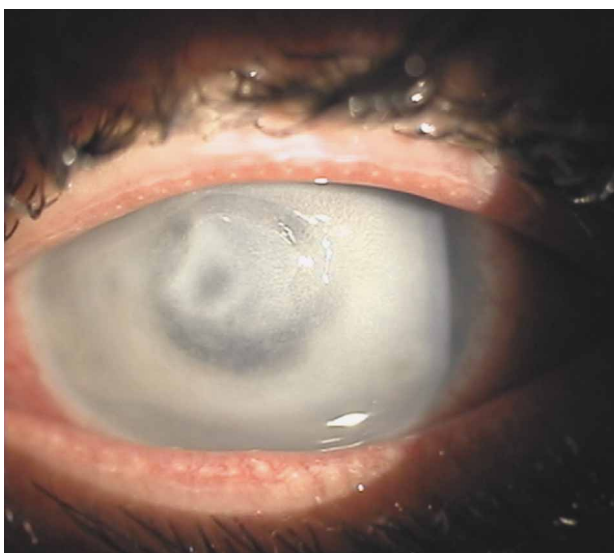


Fig. 1. Slit-lamp photo of the left eye. Blurred cornea with the stromal ring infiltrate.

for culture on non-nutrient agar overlaid with *Escherichia coli*. The material for cytological examination was placed on slides, set aside to dry and sent to the cytological laboratory. Additional material for culture was put into a saline solution and sent immediately to the laboratory where plates were examined microscopically for the presence of amoeba daily.

To our knowledge this is the first case of AK diagnosed cytologically in Croatia. *Acanthamoeba* cysts (Figure 2) and a trophozoite (Figure 3) were identified from the cytological corneal scraping, stained with a routine MGG stain and examined with a standard light microscope at 1000x magnification. After 7 days, the culture confirmed *Acanthamoeba* as the etiological agent.

The patient was initially treated with topical chlorhexidine (0.02%) intensively every two hours in combination with tobramycin (Tobrex) for a week, followed by a

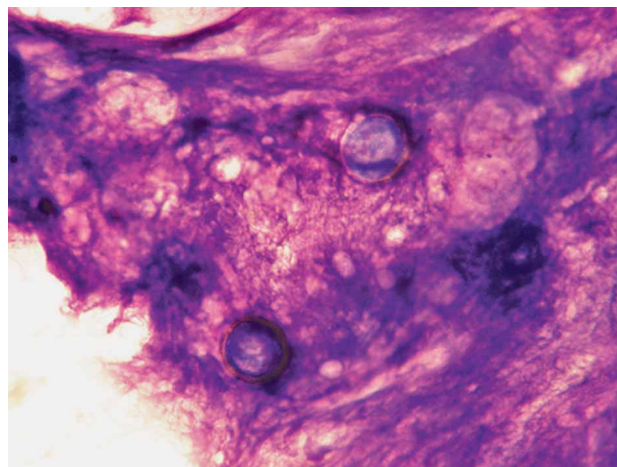


Fig. 2. *Acanthamoeba* cysts identified from the cytological corneal scraping, taken from the left eye (May-Grunwald-Giemsa stain,  $\times 1000$ ).

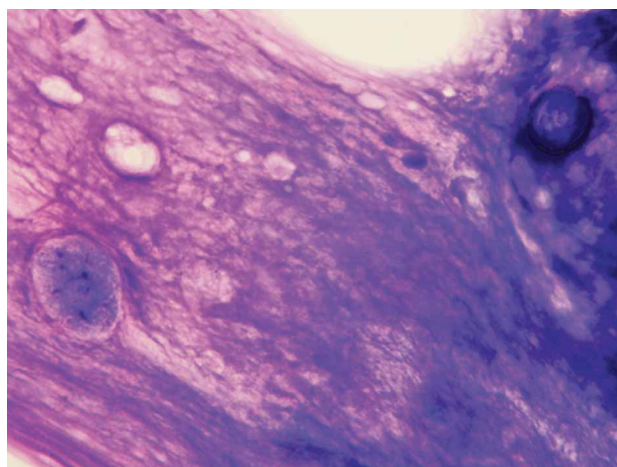


Fig. 3. The same cytological specimen with a trophozoite on the left side of the slide and a cyst on the right (May-Grunwald-Giemsa stain,  $\times 1000$ ).

gradual reduction of applied medications. After her dismissal, propamidine isotreonat (Brolene) was added. Two months after that, a secondary glaucoma developed, so timolol (Timalen) was introduced to the previous therapy. In the mean time, patient developed a mature cataract.

Besides from previously mentioned secondary glaucoma and a cataract, the corneal ulcer progressed to the full thickness corneal melt. This serious complication developed during the process of preparing for corneal transplantation.

Eight months after the diagnosis, the patient had sclerokeratoplasty with cataract surgery and intraocular lens (IOL) implantation. Despite the recommended therapy with topical steroids, an inflammatory process was still present. Six months after the surgery, visual acuity of hand motion was due to the optic nerve atrophy which developed as a complication of secondary glaucoma.

## Discussion and Conclusion

The clinical picture of AK is remarkable for its variability. Pain disproportional to the clinical findings is an indicative symptom. Most commonly the initial appearance is a pseudo-dendritic epithelial lesion that is often mistaken for herpes simplex keratitis<sup>13,14</sup>. A pattern of radial perineural infiltrates is virtually pathognomonic<sup>19</sup>. The ring-shaped stromal infiltrate is characteristic for the advanced stage of the infection<sup>20</sup>. As the disease progresses, there may be progressive loss of corneal stroma with possible perforation<sup>21</sup>.

Impairment of the corneal epithelium is the most important step in the development of the infection<sup>22</sup>. Prevaling risk factor for development of AK is contact lens wearing. Poor hygiene constitutes significant additional risk<sup>23</sup>.

Diagnosing AK is difficult. The first and the most important step is to suspect it, especially in contact lens wears with the corneal involvement resembling to herpes simplex keratitis, presenting with the pain disproportional to the clinical findings<sup>1,23</sup>. Once the diagnosis is suspected, confirmation is also challenging.

*Acanthamoeba* is cultured on non-nutrient agar overlaid with *Escherichia coli*. Not all microbiological labora-

tories are equipped to provide this culture. At the time when we needed this diagnostic test, the Institute of Public Health in Rijeka was not able to conduct it. The material had to be sent to the Croatian National Institute of Public Health in Zagreb.

Corneal scarapings stained with different stains and examined cytologically with bright-field microscopy may also reveal cysts and trophozoites but very few cytological laboratories are willing to accept the material. We sent our slides to the cytological laboratory of General Hospital in Pula. All these obstacles in the attempt of achieving the diagnosis are making this process difficult for a routine diagnosis.

Recently, confocal microscopy is being used for diagnosis of *Acanthamoeba* in vivo<sup>24,25,26</sup>. The major advantage of this method is that it can be performed even when there is a low index of suspicion. This method is helpful in monitoring the patient's response to treatment. However, the expense of equipment limits its usage. Amplification of *Acanthamoeba* ribosomal DNA with polymerase chain reaction (PCR) is another new method for detection of the organism<sup>27</sup>. PCR is very sensitive diagnostic method but it needs special equipments and technical expertise.

Beside all the above and due to a relatively low incidence, AK is rarely considered in the earliest stage of the disease. It is well appreciated that early diagnosis and treatment of AK is critical for a good visual outcome. With the introduction of cationic antiseptic agents (PHMB and chlorhexidine), the possibility of successful therapy has been achieved<sup>28</sup> and the importance of the early diagnosis as well as an interdisciplinary approach were pointed out<sup>17,18,29</sup>. We presented this case of delayed diagnosis and unfavorable outcome as a reminder that clinical suspicion remains the most important diagnostic tool. Many technical obstacles are present in achieving the diagnosis particularly in contact lens wears with a new diagnosis of herpes simplex keratitis. Patients with significant pain or poor response to conventional therapy are in high risk group. We want to point out the possibility of an early, prompt and inexpensive diagnosis with the cytological examination.

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## AKANTATAMEBNI KERATITIS – VAŽNOST RANE DIJAGNOSTIKE

### SAŽETAK

Ovo je prikaz slučaja akantamebnog keratitisa (AK), dijagnosticiranog na Očnoj klinici, KBC Rijeka, u uznapredovanoj fazi bolesti sa mnogobrojnim komplikacijama. U početku, simptomi su sličili herpetičnom keratitisu, no u međuvremenu su doveli do prstenastog zamućenja rožnice, izrazitog pada vidne oštrine, povišenog intraokularnog tlaka, mrežnice, a na kraju do otapanja rožnice. Učinjen je bris rožnice 28-godišnjoj pacijentici koja je koristila mekane kontaktne leće. Kontaktne leće prala je u tekućoj vodi, a preko noći ih je držala u fiziološkoj otopini. *Acanthamoeba* ciste utvrđene su u citološkom preparatu brisa rožnice. Kultura brisa rožnice potvrdila je *Acanthamoebu* kao etiološkog uzročnika keratitisa. Dijagnoza AK je teška. Prikazali smo ovaj slučaj AK u uznapredovanoj fazi bolesti, sa nepovoljnim rezultatom, kao podsjetnik na to da klinička sumnja ostaje najvažnije dijagnostičko oruđe. Najrizičnija skupina pacijenata su nosioci mekih kontaktnih sa novo otkrivenim herpetičnim keratitismom, naročito oni sa izrazitom boli i oni koji ne reagiraju na konvencionalnu terapiju. Htjeli smo ukazati na važnost citološkog pregleda kao rane, brze i jeftine dijagnoze.