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Rapid Diagnosis of *Acanthamoeba* Keratitis by Iodine Staining of Corneal Scrapings

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ABSTRACT

Background: Early and accurate diagnosis of *Acanthamoeba keratitis* is crucial for appropriate clinical management, preventing permanent corneal damage, scarring, or even perforation. We aimed to introduce a rapid diagnostic method for *A. keratitis*.
Methods: Corneal scrapings from 21 patients with culture- and PCR-confirmed *A. keratitis* were evaluated using Giemsa and iodine staining. All cases were referred to Farabi Eye Hospital, a tertiary care center in Tehran, Iran, for specialized evaluation and management.
Results: *Acanthamoeba* was detected in 42.33% of samples stained with Giemsa and 100% of samples stained with iodine.
Conclusion: Iodine staining is a simple, cost-effective, and rapid method for diagnosing *A. keratitis*.

Keywords: *Acanthamoeba keratitis*, iodine staining, diagnosis

Introduction

Acanthamoeba is a free-living protozoa that has been isolated from soil, air, fresh water, and sea water environments (1). Human infection with *Acanthamoeba* is rare and opportunistic and has been reported to involve the skin and central nervous system in immunosuppressed patients and patients with acquired immune deficiency syndrome and occasionally the cornea in relatively healthy patients by causing *A. keratitis* (2).

A. keratitis is a chronic infection of cornea that is as a serious health problem for more than 3 decades and it is unknown for many physicians, pathologists, and laboratorists. It is largely restricted to contact lens wearers who have experienced corneal trauma, implicating contact lens wear (3, 4). The disease is characterized by severe pain because of radial neuritis and inflammation with redness and photophobia, but this condition is also

noted for its wide variety of clinical presentations (5-7).

Accurate and early diagnosis of *A. keratitis* is essential for proper clinical management and prevention of irreversible corneal damage, including scarring or even perforation. Delayed diagnosis and treatment can have catastrophic consequences, potentially leading to permanent vision loss. Although clinical observation remains the primary diagnostic approach, *A. keratitis* is frequently misdiagnosed - most commonly as herpetic keratitis - in over 50% of cases due to overlapping clinical manifestations (5, 8, 9). This diagnostic error often results in significant delays in initiating appropriate therapy.

The diagnosis of *Acanthamoeba* in corneal infections presents considerable challenges. In tissue sections, the amoebae closely resemble keratocytes, neutrophils, and monocytes, frequently leading to incorrect diagnostic interpretations (10). This morphological similarity makes definitive diagnosis both difficult and time-consuming.

Early detection is particularly crucial for epithelial-stage *A. keratitis*, as these infections typically respond to treatment within 4-8 weeks. In contrast, deep stromal infections often require prolonged medical therapy lasting six months or more and may eventually necessitate surgical intervention. Thus, the depth of corneal involvement serves as a critical determinant of both treatment duration and clinical outcome.

Current diagnostic protocols in ophthalmology centers primarily rely on microscopic visualization of amoebae in stained corneal smears (using Giemsa, periodic acid-Schiff (PAS), calcofluor white, or acridine orange stains), culture from corneal scrapings, or in refractory cases, lamellar corneal biopsy. However, these methods have significant limitations, as *Acanthamoeba* organisms often demonstrate strong morphological similarities to macrophages and other mononuclear cells in these staining preparations.

Non-nutrient agar seeded with Escherichia coli or *Enterobacter aerogenes* remains the preferred medium for culturing *Acanthamoeba*. Clinical specimens for culture typically include corneal scrapings, corneal epithelial biopsies, contact lenses, and contact lens cases. However, even in cases of advanced *A. keratitis*, culture yields positive results in fewer than 60% of cases (11), highlighting the clear need for complementary diagnostic methods.

While newer diagnostic approaches based on molecular techniques like PCR enable rapid detection, they remain limited by their dependence on specialized expertise, specific laboratory requirements, and high costs. With the rising incidence of *A. keratitis*, there is an urgent need to develop simpler, faster, and more accessible diagnostic methods.

In this study, we evaluated the diagnostic utility of iodine and Giemsa staining for detecting *Acanthamoeba* in corneal scrapings from cases where infection had been previously confirmed by both culture and PCR.

Methods

Patients

Seventy patients presenting with corneal ulcers and suspected *A. keratitis* were enrolled. All cases were referred to Farabi Eye Hospital, a tertiary care center in Tehran, Iran, for specialized evaluation and management.

Techniques

Following corneal scraping collection, samples were divided for parallel processing. Each sample was simultaneously inoculated onto non-nutrient agar plates overlaid with *Escherichia coli* for culture and submitted for PCR analysis. All samples that tested positive by either culture or PCR subsequently underwent staining evaluation using both Lugol's iodine and

Giemsa stains through two distinct methods.

For the direct smear technique, corneal scrapings were smeared onto clean glass slides and allowed to air dry. The slides were then fixed with absolute methanol before staining with either Lugol's iodine or Giemsa solution for exactly three minutes. The prepared slides were carefully examined under light microscopy at 400× magnification to identify characteristic *Acanthamoeba* cysts.

The concentration technique involved a different approach. Corneal scrapings were suspended in 1 mL of methanol and centrifuged at 1,500 RPM for five minutes. After centrifugation, the resulting pellet was resuspended in one drop of either Lugol's iodine or Giemsa solution. This concentrated suspension was then transferred to a clean slide as a wet mount preparation and similarly examined under 400× magnification for cyst identification.

This dual-method approach allowed for comprehensive evaluation of staining effectiveness while controlling for potential variations in sample preparation. Both techniques were performed on all positive samples to ensure methodological consistency and to compare the relative sensitivity of each approach. The standardized protocols minimized technical variability and allowed for direct comparison between the two staining methods.

Results

Among the 21 culture- and PCR-confirmed *Acanthamoeba*-positive samples, we observed significant differences in detection rates between staining methods. Giemsa staining successfully identified the parasite in only 9 samples (42.33%), while Lugol's iodine staining demonstrated 100% detection efficacy (Figure 1).

Our comparative analysis revealed substantially improved diagnostic accuracy when using the centrifugation method versus direct air-dried smears. The concentration technique through methanol fixation and centrifugation yielded more reliable and consistent results compared to simple air-drying preparation. This enhanced performance was particularly evident in samples with lower parasite loads, where the concentration method improved visualization of characteristic cyst morphology.

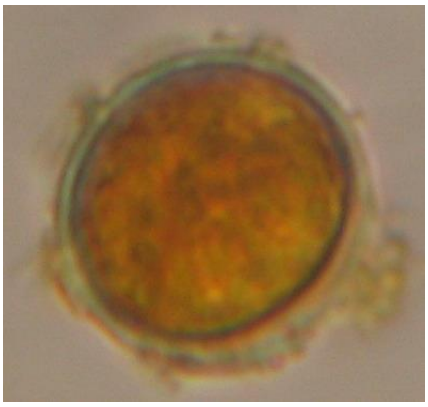

Figure 1: Photograph showing *Acanthamoeba* cyst with Iodine staining; cyst wall is clear, cytoplasm yellow, nucleus is obvious, and chromatin is red color

Discussion

There are different staining to confirm *Acanthamoeba* infection such as Gram, Giemsa, periodic acid-Schiff (PAS), calcofluor white, acridine orange, methylen blue, Gomori methenamine silver, Hematoxiline eosine stains. However, each of these staining have some weakness in detecting *Acanthamoeba*. One of the most important aspect of them is actual similarity of amebae to macrophage or other mononuclear cells. So, in many laboratory examination, it can be misdiagnosed with mononuclear cells or degenerated epithelial cells, especially in staining with Gram and Giemsa. Phase contrast microscope although is very helpful tool, but is not available in many of ophthalmologic centers. On the other hand, it is not possible to culture *Acanthamoeba* in all laboratories. In addition, in contrast to confirmation of

Acanthamoeba infection by PCR, it does not grow in culture media (12, 13).

In the present study, Iodine staining was applied for detecting *Acanthamoeba* in corneal scrapings. This method is not only inexpensive and easy but also it can be performed in all laboratories. With Iodine staining, amebae cyst stains well; cyst wall would be clear and obvious, cytoplasm yellow, nucleus is obvious, and chromatin is red color (Figure 1). Other background cells such as epithelial cells would be brown or black. The culture of the parasites tacks nearly 2 weeks. Therefore, culturing is time wasting and may cause delay in diagnosis and treatment.

One of the most important aspects of this staining that, no morphological changes in cyst or trophozoite shape of the parasite would be expected, so this technique is appropriate for morphological studies. In addition, results of this method are fully well-matched with PCR. Compatibility of Iodine staining with PCR is promising in that it can be used in all laboratories easily, rapidly and with low expense.

Conflicts of interest

There is no conflicts of interest.

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