

# BRIEF REPORTS

## Polymerase Chain Reaction Diagnosis in Fungal Keratitis Caused by *Alternaria alternata*

Consuelo Ferrer, PhD,  
Gonzalo Muñoz, MD, PhD,  
Jorge L. Alió, MD, PhD, José L. Abad, and  
Francisca Colom, MD, PhD

**PURPOSE:** To contribute toward assessing the effectiveness of polymerase chain reaction as a rapid method in diagnosis of torpid keratitis caused by opportunistic fungi.

**METHODS:** Interventional case report. A 50-year-old man with a corneal abscess in the right eye treated for a period of 6 months with different combinations of broad-spectrum antibiotics and steroids was referred to our center. Corneal scraping was taken for microbiological study, including classic cultures and polymerase chain reaction.

**Amplified DNA was sequenced to identify the pathogen. RESULTS:** Polymerase chain reaction amplification was negative for *Acanthamoeba* species and positive for fungi. The sequence analysis showed *Alternaria alternata* as the causal agent in 24 hours. Cultures confirmed the identification in 10 days.

**CONCLUSION:** Polymerase chain reaction amplification with subsequent DNA-typing was revealed to be a useful method for detection of ocular pathogens such as *A. alternata* involved in cases of torpid keratitis, even in the presence of broad-spectrum antimicrobial therapy. (Am J Ophthalmol 2002;133:398–399. © 2002 by Elsevier Science Inc. All rights reserved.)

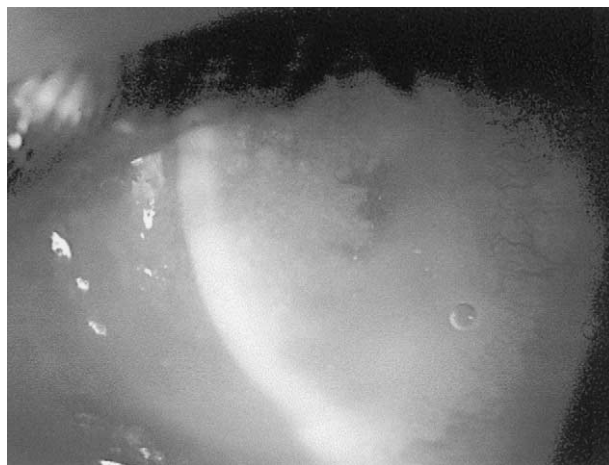
**T**HE MICROBIOLOGICAL DIAGNOSIS OF FUNGAL KERATITIS is usually challenging. Gram and Giemsa stains of corneal scrapings have low sensitivities of about 50% to 80%,<sup>1,2</sup> and fungal cultures from corneal samples often take more than 3 days to become positive. The necessity of

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From the Dpto. Biología Molecular, Instituto Oftalmológico de Alicante, Alicante, Spain (C.F.), the Instituto Oftalmológico de Alicante, Alicante, Spain (G.M., J.L.A., J.L.A.), and the Div. Microbiología, Universidad Miguel Hernández, Alicante, Spain (F.C.).

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Inquiries to Consuelo Ferrer, PhD, Dpto. Biología Molecular, Instituto Oftalmológico de Alicante, Avenida de Denia 111, 03015 Alicante, Spain. fax: 34 965 160468; e-mail: cferrer@oftalio.com

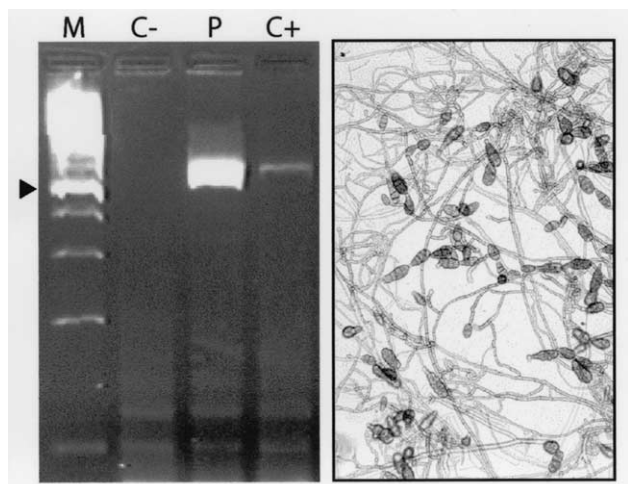


**FIGURE 1.** Right eye shows complete opacification of the cornea with deep stromal infiltrates and hypopyon.

a rapid diagnostic method has prompted the development of a culture-independent test such as the polymerase chain reaction.<sup>3,4</sup> This work reports the efficiency of polymerase chain reaction diagnosis in a case of *Alternaria alternata* torpid keratitis.

A 50-year-old agricultural worker complained of pain and decreased visual acuity to light perception in his right eye. Examination showed hyperemia and an opacified cornea with ulceration and deep stromal infiltrates (Figure 1). Hypopyon and posterior synechiae were present, but an ultrasound study showed no involvement of the posterior segment. The patient had a history of corneal chemical trauma and herpetic keratitis 2 years previously. Complaints had started 6 months before our initial examination, and the patient had been treated with systemic and topical steroids and broad-spectrum antibiotics, including systemic and topical fluconazole, topical polymyxin B, and fortified tobramycin and cefazolin, with no improvement of the condition.

Corneal samples were inoculated into several media, including thioglycolate, blood, chocolate, CLED, MacConkey, and Roiron and Sabouraud's dextrose. Gram and Giemsa stains were also done. Two polymerase chain reactions were performed: one to amplify a fragment of the *Acanthamoeba* species 18S rDNA<sup>5</sup> and the other to amplify the ITS/5.8S region of fungal DNA.<sup>4</sup> Amplified DNA from polymerase chain reaction was sequenced and analyzed by



**FIGURE 2.** Diagnosis by polymerase chain reaction and culture. (Left) Gel shows the polymerase chain reaction result with fungal primers. M: ladder marker GeneRuler 100bp DNA Ladder Plus (500 bp; black triangle); C-: control negative (ddH<sub>2</sub>O); P: corneal scraping of the patient; C+: control positive (*Candida albicans* DNA). (Right) Light microscopy of *Alternaria alternata*. Typical dematiaceous conidia (×400).

using the BLAST alignment program of GenBank database (National Institutes of Health, Bethesda, Maryland).

*Acanthamoeba* species DNA amplification by polymerase chain reaction was negative. The ITS/5.8S rDNA fragment amplification with specific fungal primers was positive, which showed a band about 570 bp. The result was obtained 8 hours after the sample was taken (Figure 2, left). Fungal identification was carried out by ITS/5.8S region sequencing 24 hours after the sample was obtained. A DNA database comparison of the sequence obtained demonstrated that it was identical to the *A. alternata* ITS/5.8S rDNA region.

Gram stain did not show cells suggestive of fungal conidies. After 6 days of incubation, filamentous fungal growth was detected in Roiron broth. Microscopic observation and subculture on Sabouraud dextrose agar allowed the definitive identification of *A. alternata* in 10 days (Figure 2, right).

Soon after polymerase chain reaction identification of *A. alternata* as causative agent, a new antifungal treatment was started, including systemic and topical fluconazole and topical amphotericin B. Unfortunately, it was too late to save the cornea and the patient was scheduled for penetrating keratoplasty.

The role of timely and aggressive medical intervention is of utmost importance to preserve the vision in the case of fungal keratitis. The polymerase chain reaction-based test is a rapid method to detect and identify fungal pathogens in ocular samples even in the presence of broad-spectrum antimicrobial therapy. Compared with standard laboratory techniques, it offers a significant reduction in the time

required to establish the diagnosis. This case points to the need for a rapid presumed diagnosis to initiate antifungal therapy as soon as possible.

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## Corneal Edema With Dislocated Anterior Polar Cataract

Urmi N. Shah, MBBS, MS,  
Prashant Garg, MBBS, MS, and  
Geeta K. Vemuganti, MD

**PURPOSE:** To report an unusual manifestation of anterior polar cataract.

**METHODS:** Observational case report. A 35-year-old woman presented with localized inferior corneal edema associated with a white nodular lesion on the iris. The anterior lens capsule was deficient centrally with scrolled edges and an underlying subcapsular opacity. The nodule was removed surgically and subjected for histopathologic examination.

**RESULTS:** The histopathologic examination of the white nodular lesion showed parallel collagen fibers that were positive for collagen on the Masson trichrome stain. Correlating with clinical features, these findings were suggestive of dislocated anterior polar cataract.

**CONCLUSION:** Anterior pyramidal cataract may dislocate spontaneously and present as an anterior chamber foreign body and may even lead to corneal endothelial cell loss. (*Am J Ophthalmol* 2002;133:399–401. © 2002 by Elsevier Science Inc. All rights reserved.)

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From the Cornea Service (U.N.S., P.G.), and the Ophthalmic Pathology Center (G.K.V.), L.V. Prasad Eye Institute, Hyderabad, India.

Supported by Hyderabad Eye Research Foundation, Hyderabad, India. Inquiries to Prashant Garg, MBBS, MS, L.V. Prasad Eye Institute, L.V. Prasad Marg, Banjara Hills, Hyderabad 500 034, India; fax: +91 40 3548271; e-mail: prashant@lvpeye.stph.net