

A Case of *Alternaria alternata* Keratitis Isolated from Corneal Tissue

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= Abstract =

A 71-year-old man presented with pain in the left eye that revealed a 3×3 mm deep corneal stromal infiltrate, with a 2×2 mm epithelial defect. The patient started topical moxifloxacin, voriconazole 2%, and natamycin for 2 weeks. However, the treatment was not effective and the corneal infiltration worsened. Subsequently, the patient underwent therapeutic penetrating keratoplasty. Thick brown/gray mold colonies on Potato Corn Meal Tween 80 agar was isolated from excised corneal tissue and on slide culture many septated, and club-shaped ascospores were revealed. Histological findings also showed numerous hyphae scattered in corneal tissue. *A. alternata* colonies were confirmed by 18S rRNA sequencing. Intracameral voriconazole was injected every other day for 2 weeks to eliminate remaining fungi on the deep corneal stroma. The remaining corneal infiltration was improved one month after the injection. During 5 months postoperative follow up, the infection did not recur. In conclusion, deep corneal infection of *A. alternata* was effectively treated with intracameral voriconazole injection.

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Key Words: *Alternaria alternata*, Cornea culture, Intracameral voriconazole injection

INTRODUCTION

Fungal keratitis is a potentially sight-threatening corneal infection due to the difficulties associated with its diagnosis and treatment¹. Delayed diagnosis is common because the process is slow and

initial symptoms are not severe. Gram and Giemsa staining of corneal scrapings is commonly used for initial and rapid identification of fungi. However, these methods have low sensitivity; recent studies have reported that positive identification of fungi occurs in approximately 27~33% of cases². Fungal keratitis also poses a therapeutic challenge. There

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are limited commercially available antifungal agents; furthermore, they do not work as effectively as antibiotics for bacterial infections because of poor corneal penetration. The largest series of fungal keratitis has been reported from India, and the most common fungal isolates were *Fusarium* and *Aspergillus* species (36.6% and 30.4%, respectively), followed by dematiaceous fungi (15.7%)³.

Alternaria species belong to a group of dematiaceous fungi and are ubiquitous in the environment as saprophytes of humans. *Alternaria alternata* is a common isolate that readily causes opportunistic infections in immunocompromised patients who have received long-term treatment with immunosuppressants such as steroid and tacrolimus. *Alternaria* species have been reported to cause cutaneous and subcutaneous infections, onychomycosis, sinusitis, visceral infections, and osteomyelitis⁴. However, corneal infection caused by *Alternaria* is less common and cases reporting infection in corneal tissue are rare. This present report describes a case of keratitis caused by *A. alternata* isolated from surgically excised corneal tissue.

CASE REPORT

A 71-year-old man presented with ocular pain and decreased visual acuity in the left eye. The patient had received pterygium surgery in the left eye 10 years previously. At presentation, best corrected visual acuity (BCVA) was 20/25 in the right eye and 20/70 in the left eye, and the intraocular pressure was 10 mmHg in both eyes. Slit-lamp biomicroscopy of the left eye revealed a nasal, juxtalimbal, 3×3 mm deep stromal infiltrate with a 2×2 mm epithelial defect. The feature of corneal infiltrate was irregular feathery margin and a dry rough texture. The sclera adjacent to the corneal lesion was white, avascular, and thin. Dark blue

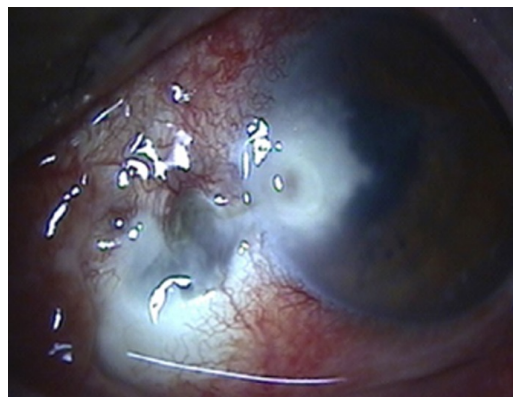


Fig. 1. Slit-lamp biomicroscopy image showing a nasal, juxtalimbal, 3×3 mm deep corneal stromal infiltrate, with a 2×2 mm epithelial defect. The sclera adjacent to the corneal lesion was white, avascular, and thin. Dark blue uveal tissue showed through the thin sclera. The conjunctiva was moderately injected.

uveal tissue showed through the thin sclera and the conjunctiva was markedly injected (Fig. 1). There was a severe anterior chamber reaction and the patient had a history of long-term topical corticosteroid use to treat chronic ocular surface inflammation of bare sclera originated from previous pterygium excision. Gram and Giemsa stainings of bacterial and fungal cultures from corneal scrapings were performed but did not reveal anything suggestive of fungal infection.

Clinical findings of insidious onset, irregular feathery margin and a dry rough texture, suggested fungal infection. The patient received initial empirical treatment for bacterial and fungal coverage; the treatment comprised hourly doses of topical moxifloxacin, voriconazole 2%, and natamycin and topical corticosteroid was discontinued. The cultures were obtained, no fungi could be identified after seven days. Within the first two weeks of intensive topical treatment, clinical signs and symptoms did not improve and the corneal infiltration progressed



Fig. 2. Thick black, brown or gray mold colonies on Potato Corn Meal Tween 80 agar.

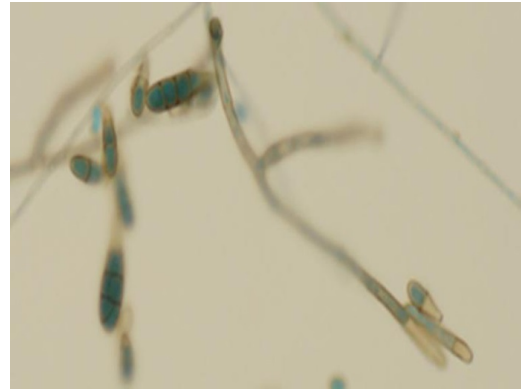


Fig. 3. Slide culture showing numerous yellow-brown and septated club-shaped ascospores (400 \times).

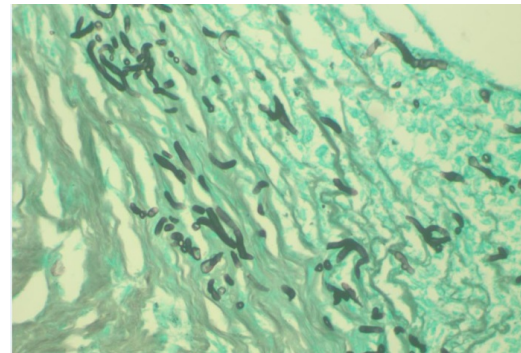


Fig. 4. Gomori methenamine silver staining showing numerous hyphae scattered in the epithelial and stromal layers of corneal tissue (400 \times).

into deep stroma. Subsequently, the patient underwent therapeutic penetrating keratoplasty (PKP). The infected cornea was removed and submitted to the clinical microbiology and surgical pathology laboratory for bacterial and fungal identification. Corneal grafts were sutured in place with 10~0 nylon. While the bacterial culture showed negative, four days after PKP was performed, fungal culture on Potato Corn Meal Tween 80 agar showed brown /gray mold colonies and a slide culture revealed many yellow-brown, septated, club-shaped ascospores, consistent with the findings for *A. alternata* (Figs. 2 & 3). H&E and Gomori methenamine silver stains also showed numerous hyphae scattered in the epithelial and stromal layers of corneal tissue (Fig. 4). The colonies of *A. alternata* in the

culture were confirmed by 18S rRNA sequencing as following. DNA was extracted with Genomic cell/tissue spin mini kit (Genotech, Daejeon, Korea). The 18S rRNA gene was amplified with the primer pair of TIS1 (TCCGTAGGTGAACCTGCGG). TIS4 (TCCTCCGCTTATTGATATGC) PCR was performed under the following conditions: initial denaturation at 95 $^{\circ}$ C for 5 min, followed by 30 cycles of denaturation (95 $^{\circ}$ C for 45 sec) and annealing (55 $^{\circ}$ C for 45 sec), extension (72 $^{\circ}$ C for 45

Alternaria alternata strain ATCC 46561 JQ070079.1

Score 968 bits(524) Identities 524/524(100%) gap 0/524(0%)

Query 1 CGGGTATCCCTACCTGATCCGAGGTCAAAGTTGaaaaaaGGCTTAATGGATGCTAGAC 60
|||||
Sbjct 525 CGGGTATCCCTACCTGATCCGAGGTCAAAGTTGAAAAAAGGCTTAATGGATGCTAGAC 466

Query 61 CTTTGCTGATAGAGAGTGC GACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAAT 120
|||||
Sbjct 465 CTTTGCTGATAGAGAGTGC GACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAAT 406

Query 121 TACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGCCAACACCAAGCAAAGCTT 180
|||||
Sbjct 405 TACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGCCAACACCAAGCAAAGCTT 346

Query 181 GAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGC 240
|||||
Sbjct 345 GAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGC 286

Query 241 GTTCAAAGATTGCATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTGCTG 300
|||||
Sbjct 285 GTTCAAAGATTGCATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTGCTG 226

Query 301 CGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTG 360
|||||
Sbjct 225 CGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTG 166

Query 361 TTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTGCCTAGTGGTGGGCGAACCCA 420
|||||
Sbjct 165 TTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTGCCTAGTGGTGGGCGAACCCA 106

Query 421 CCAAGGAAACAAGAAGTACGAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCC 480
|||||
Sbjct 105 CCAAGGAAACAAGAAGTACGAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCC 46

Query 481 GAGAGGTTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCG 524
|||||
Sbjct 45 GAGAGGTTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCG 2

Fig. 5. The DNA sequences of PCR product of 18 S rRNA.

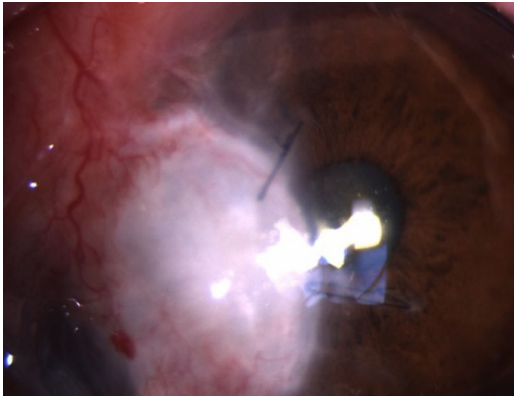


Fig. 6. Slit-lamp biomicroscopy showing a clear cornea 5 months after penetrating keratoplasty.

sec), and a final extension step at 72°C for 10 min. The PCR products were purified, and sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA). All loci were sequenced in both the forward and reverse directions with the same primers as those used for the PCR. Sequence similarity searches were performed using the BLAST tool at the NCBI database (<http://www.ncbi.nlm.nih.gov/blast>) (Fig. 5).

Although the transplanted corneal graft was well attached with clearance, deep corneal infiltration remained on the host's posterior corneal stroma. Intracameral voriconazole (100 µg/0.1 cc) and subconjunctival voriconazole (300 µg/0.3 cc) were injected under aseptic conditions. Intracameral and subconjunctival voriconazole were injected every other day for 2 weeks. One month after the injection of antifungal agents was administered, the periphery of the corneal graft showed decreased infiltration. All topical antifungal agents were tapered and discontinued 2 months after surgery. The cornea remained clear five months postoperatively; there was no recurrence of fungal infection (Fig. 6) and BCVA in the left eye was 20/100.

DISCUSSION

Fungal keratitis is a sight-threatening disease because, in contrast to bacteria, fungi can penetrate Descemet's membrane and reach the anterior chamber. Once fungi reach the anterior chamber, elimination of fungi becomes difficult because topical antifungal agents have poor corneal penetration abilities. Furthermore, there are very few antifungal agents available commercially. Consequently, the treatment of fungal keratitis is difficult and the prognosis is poor.

Alternaria species are a type of Dematiaceous fungus that produces melanin-like pigments. They are ubiquitous in the environment and frequently isolated from soil, plants, food, and air⁵. Opportunistic infections by these fungi can occur in immunosuppressed patients, particularly in bone marrow transplanted patients^{6,7}. *Alternaria* infection accounts for 3.3~8.7% of fungal keratitis cases⁸⁻¹²; it is less commonly associated with corneal infections. Hsiao et al. reviewed 25 previous case reports using the Medline database¹³ and summarized the risk factors of *Alternaria* keratitis. *Alternaria* species infection was found to be associated with corneal trauma in 9 of 25 (36%) cases, previous corneal disease or surgery in 10 (40%) cases, and contact lens usage in 4 (16%) cases. Topical corticosteroids had been used in 14 of 25 (56%) of these cases, before diagnosis of fungal keratitis. Similarly, the patient in the present case had received long-term treatment with topical corticosteroids. The use of topical corticosteroid may therefore potentially result in the development and worsening of fungal keratitis. Mohd-Tahir et al. performed a retrospective review of all medical and microbiology records for all cases treated with fungal keratitis from January 2007 until December 2011¹⁴. They

also reported the use of topical steroids was the second most common predisposing risk factor.

Fungal keratitis caused by *A. alternata* is a difficult form of microbial keratitis to diagnose and treat successfully. In these cases, early diagnosis and prompt treatment is necessary to achieve a successful outcome. Although Gram and Giemsa stainings can rapidly identify the fungi, these techniques have low sensitivities. Fungal cultures have higher sensitivities, but result in a longer time to diagnosis (>3 days). Alternative diagnostic methods with both high sensitivity and rapid diagnostic abilities are therefore required. In the present case report, both fungal culture and 18S rRNA sequencing from excised corneal tissue revealed *A. alternata* infection. Diagnosis of *A. alternata* infection is faster using polymerase chain reaction (PCR) compared to fungal culture. In this case, PCR using corneal scrapings taken from the first visit was not performed and the diagnosis was therefore delayed. The PCR method has the potential to be a useful diagnostic tool because it enables early diagnosis and thereby facilitates prompt treatment.

In this case, the definitive diagnosis from corneal tissue was essential to choose the appropriate drug and its delivery method. Topical natamycin has been used for *Alternaria* keratitis, but it has limited effect because of its poor corneal penetration ability through intact epithelium. Ozbek et al. reported a case of *Alternaria* keratitis that was cured using systemic and topical voriconazole after treatment with topical natamycin and amphotericin B had failed⁵. However, in our presented case, deep seated infection could not be treated with topical antifungal eyedrops. With the definitive diagnosis, we decided using intracameral voriconazole treatment. This present report also indicated that topical and intracameral use of voriconazole may be an effective

treatment option for *Alternaria* keratitis resistant to natamycin and amphotericin B. Voriconazole has variable penetration abilities. The corneal epithelium serves as a barrier to the antifungal agent; therefore, in cases where the epithelium is intact, it is not possible to administer the agent to the desired level. The effectiveness of intracameral voriconazole injection for *Alternaria* keratitis has never been reported. In the present case, intracameral voriconazole injection with PKP resulted in the complete elimination of the fungi.

To our knowledge, this is the first case to report a successful outcome using intracameral voriconazole injection for *Alternaria* keratitis. In conclusion, deep corneal infection of *A. alternata* was confirmed by laboratorial diagnosis and effectively treated with intracameral voriconazole injection.

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