Case Report

Aspergillus Flavus Keratitis after Deep Anterior Lamellar Keratoplasty

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Purpose: To report the clinical, microbiologic, confocal scan and histopathologic features of Aspergillus flavus keratitis which developed immediately after deep anterior lamellar keratoplasty (DALK).

Case Report: A 28-year-old woman underwent DALK using the big-bubble technique for keratoconus. The operation was uneventful, yielding a bare Descemet's membrane (DM) followed by transplantation of a corneal graft devoid of DM and endothelium. Four days after keratoplasty, mild infiltrates were noticed in the inferonasal margin of the graft, which rapidly progressed to involve the adjacent recipient cornea. Confocal scan findings suggested filamentous fungal keratitis, leading to initiation of topical and systemic antifungal medications followed by immediate replacement of the graft. Histopathologic examination disclosed keratitis caused by a filamentous fungus, which was determined by microbiologic cultures to be Aspergillus flavus. Early diagnosis and appropriate management resulted in complete recovery from this potentially devastating infection.

Conclusion: Aspergillus Flavus can cause graft ulcers immediately after DALK. Confocal scan proved to be a valuable tool for early diagnosis and prompt intervention to control this otherwise devastating infection.

Keywords: Aspergillus Flavus; Fungal Keratitis; Deep Anterior Lamellar Keratoplasty; Anwar's Big-Bubble Technique

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INTRODUCTION

Fungal keratitis after corneal transplantation may result from contaminated donor material or intraoperative infection with fungal elements.[1] Infection may also develop postoperatively due to certain predisposing factors namely epithelial defects, suture-related problems, application of topical corticosteroids and use of broad-spectrum antibiotics that may alter normal ocular flora allowing fungal species to grow.[2]

Most cases of fungal keratitis after both lamellar and penetrating keratoplasty are caused by Candida species.[3,4] Other causative agents are Cladosporium, Cryptococcus, and Aspergillus species.[5-7] Although Aspergillus keratitis has been reported after refractive surgery,[8,9] to the best of our knowledge, there is no report on graft ulceration caused by Aspergillus flavus following deep anterior lamellar keratoplasty (DALK). Herein, we present a keratoconic eye

that underwent graft replacement immediately after successful Anwar's big-bubble DALK due to graft infection with Aspergillus flavus. Rapid diagnosis using confocal scan and appropriate intervention together with the nonpenetrating nature of the corneal transplantation contributed to a favorable outcome despite the devastating nature of the infection.

CASE REPORT

A 28-year-old immunocompetent woman with advanced keratoconus underwent DALK in her left eye using the big-bubble technique. The surgical technique has previously been described in detail.[10] Briefly, the periorbital skin was scrubbed with 10 % povidone iodine solution and the 5% solution was applied into cul-de-sacs for 1 minute before starting the operation. The recipient trephine size was 8.0 mm. The operation was uneventful, a bare Descemet's membrane (DM) was successfully achieved, and a 0.25-mm larger donor cornea devoid of endothelium and DM was fixed to the recipient bed using 16 separate 10-0 nylon sutures. The donor cornea, rated only for lamellar keratoplasty, was preserved at 4˚C Optisol-GS (Bausch & Lomb, Irvine, CA, USA) containing gentamicin and streptomycin for 3 days. No cultures of the donor corneoscleral rim were taken following use of the donor tissue. At the conclusion of the operation, betamethasone 4 mg and cefazolin 50 mg were injected subconjunctivally.

On postoperative day 1, near-total epithelial defect and mild graft edema (due to low graft quality) were noted; the recipient DM was completely attached to the overlying stroma. Topical betamethasone 0.1%, chloramphenicol 0.5%, and hypertonic sodium chloride 5% eye drops every 6 hours together with preservative-free artificial tears every 3 hours were started. The postoperative course was initially uneventful with gradual resolution of the epithelial defect and stromal edema together with an increase in visual acuity until postoperative day 4 when subtle infiltrates were noticed at the inferonasal margin of the graft, an area which was last to be re-epithelialized. The infiltration involved the anterior and middle stroma with an extension of about 90 degrees.

Within just four hours, the condition deteriorated rapidly to involve the adjacent recipient cornea (Fig. 1A). Confocal scan, which was performed immediately, revealed several high contrast hypha-like structures measuring 3.6 µm in width and branched at 45˚ angles within the anterior and middle stroma (Fig. 2). With a diagnosis of filamentous fungal keratitis, topical betamethasone was discontinued; natamycin 5% eye drops every 30 minutes and oral fluconazole 100 mg twice a day were initiated.

Due to the progressive nature of the infection we decided to proceed with graft exchange.

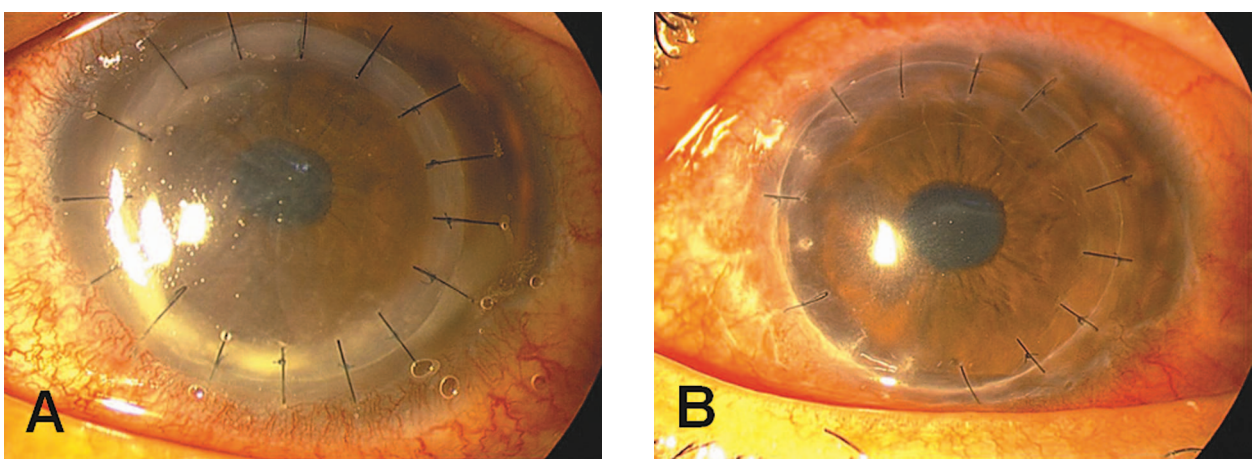

Figure 1. Infiltrations are visible in the inferonasal margin of the graft involving the adjacent recipient cornea (A). Two months after graft replacement, the second graft and recipient bed are clear (B). Note that some sutures were prematurely removed due to rapid healing response at the involved area.

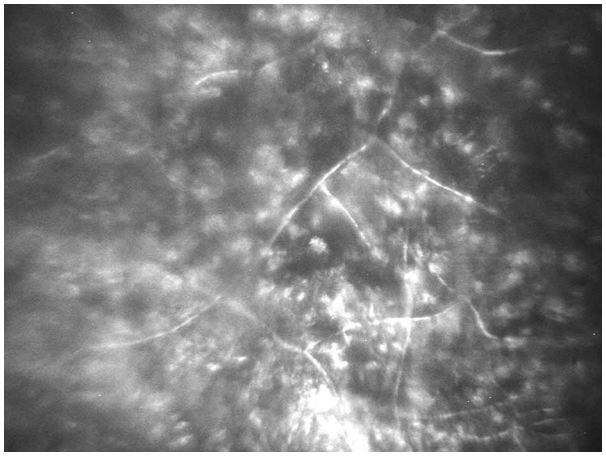

Figure 2. Confocal scan image; note the presence of several branched and high contrast fungal hyphae amongst inflammatory cells in the corneal stroma (magnification ×500).

The infected graft was simply removed after cutting the sutures and replaced on the same day with another 0.5 mm larger full thickness donor cornea. During regrafting, the recipient DM appeared clear and intact. The involved recipient rim was removed, followed by irrigation with balanced salt solution and 5% povidone iodine solution. At the conclusion of surgery, subconjunctival amphotericin B 1500 micrograms in 0.5 cc was injected in the inferonasal quadrant. The removed donor lenticule was bisected; half was embedded in Blood agar for microbiologic culture and the other half was fixed in 10% formalin and sent for histopathology.

Postoperatively, topical and systemic antifungal agents were continued, while topical betamethasone 0.1% was withheld for two weeks and then resumed twice a day. Antifungal agents were gradually tapered off over the next 6 weeks as the epithelial defect and stromal edema gradually reduced and active infiltration was no longer observed. At final follow-up, 2 months after graft replacement, best-corrected visual acuity had increased to 20/60 and the second graft was clear with no evidence of recurrence (Fig. 1B). Based on postoperative eye bank reports, the recipient of the fellow cornea from the same donor, maintained a clear graft with no infectious complications.

Histopathology and microbiology

After fixation of the corneal specimen in formalin, the tissue was processed and thin sections were prepared and subsequently stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Gomori's methenamine silver (Grocott). The stained sections were examined using a light microscope (Olympus BX41, Japan) which revealed an epithelium-free cornea with intact Bowman's layer and stromal infiltration of large numbers of neutrophils. Fragments of PAS-reactive and Grocott-positive septate fungal elements were noted, appearing especially close to one margin of the corneal specimen (Figures 3A and 3B).

Microbiological cultures grew velvety, yellow to brown colonies after 3 days. Scotch tape preparation of the colonies demonstrated heavy walled, uncoloured, coarsely roughened conidiophores, less than 1 mm in length. The size of the conidia themselves varied from 3.5 to 5.0 mm (Fig. 3C). Based on gross colony

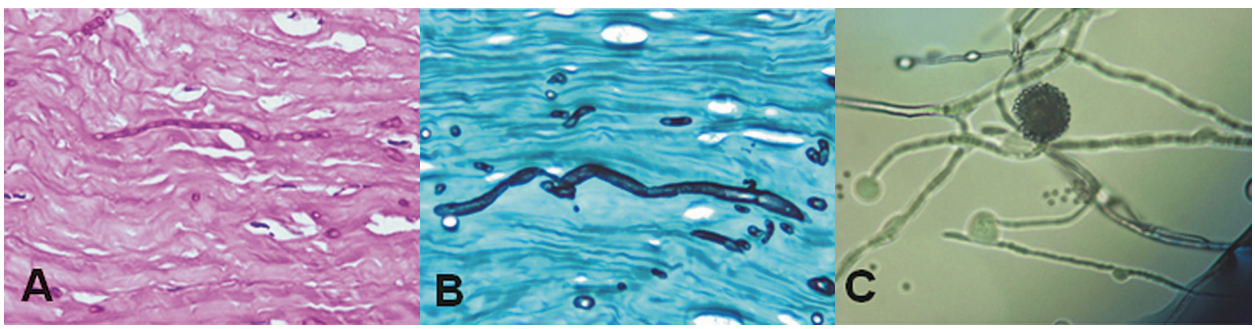

Figure 3. PAS-reactive (A) and Grocott-positive (B) septate fungal elements within the stroma of the removed graft (magnification ×1000). Scotch tape preparation of the fungal colonies (C) shows spherical vesicles and spherical chains of conidia (magnification ×400).

morphology, color and microscopic features, the isolate was identified as Aspergillus flavus.[11]

DISCUSSION

Fungal graft ulcers, following both lamellar and penetrating keratoplasty, are most commonly caused by Candida species[3,4] but also on rare occasions, by other fungi including Cladosporium, Cryptococcus and Aspergillus species.[5-7] Candida keratitis which usually develops 1 to 2 weeks following DALK, is believed to be transmitted through a contaminated donor.[3,4] This is in contrast to the condition in our patient in whom Aspergillus flavus keratitis developed immediately after corneal transplantation.

Transmission of the infection from the donor seems unlikely. Although cultures had not been taken from the storage medium or the corneoscleral rim, the recipient of the donated fellow cornea from the same donor maintained a clear graft. However, this transmission route is a possibility that should not be disregarded. Contamination could have occurred intraoperatively or immediately after surgery. The latter possibility is supported by the site of the ulcer, which was the last area to be re-epithelialized.

The short interval from surgery to infection and rapid progression can be attributed to the high virulence of Aspergillus flavus, which is second only to Aspergillus fumigatus as the cause of invasive human aspergillosis including chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis.[11] Aspergillus flavus is an important cause of keratitis, accounting for 80% of cases of Aspergillus species related keratitis.[12-14] Reported predisposing factors for Aspergillus flavus keratitis are trauma, generally with plant material, and corneal refractive as well as cataract surgery.[14-18]

In fungal keratitis, early diagnosis and timely intervention play an important role in achieving good results and preventing complications such as scleral and/or anterior chamber involvement. Confocal scan is a valuable, non-invasive technique for early diagnosis, yielding images very similar to fungal structures in the initial phase of the disease.[19] High-contrast lines measuring 200-300 µm in length and 3-5 µm in width, with 45˚ branching have previously been reported as confocal scan features of Aspergillus keratitis.[20] We observed similar findings on confocal scan imaging which helped us diagnose the causative agent and provide timely intervention by administration of frequent topical natamycin eye drops and systemic fluconazole, along with replacement of the infected graft.

Aspergillus flavus is most sensitive to natamycin, followed by amphotericin B and terbinafine, however sensitivity to ketoconazole is rather low.[12] Poor corneal penetration and ocular irritation prevent administration of topical amphotericin B in large doses for a long duration.[12] Furthermore, resistance to both amphotericin B and itraconazole have emerged.[11]

Immediate graft exchange was considered in this case because of rapid progression of the ulcer. Although we did not encounter any toxicity after irrigation with 10% povidone iodine solution, use of amphotericin B 50 µg/ml may be safer when working close to the corneal endothelium. Intraoperatively, it was noted that the DM had remained intact while the adjacent recipient cornea was involved. One advantage of DALK is the prevention of post-keratoplasty endophthalmitis by leaving an intact DM which prevents penetration of microorganisms into the anterior chamber from an infected donor. DALK is essentially an extraocular procedure as compared to penetrating keratoplasty,[21] therefore the risk of introducing infectious organisms from contaminated donor tissue into the anterior chamber and secondary endophthalmitis is low. This advantage has previously been observed in cases of post-DALK interface keratitis caused by Candida species.[3,4]

In summary, this case report introduces Aspergillus flavus as a cause of graft ulcer developing immediately after DALK. The favorable outcome achieved, in spite of the devastating nature of the infection, is attributed to rapid diagnosis using confocal scan and timely medical and surgical intervention. Additionally, the nonpenetrating nature of lamellar corneal

transplantation probably played an important role in minimizing intraocular penetration of the microorganism.

Conflicts of Interest

None.

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