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Aspergillus flavus Keratitis: Experience of a Tertiary Eye Clinic in Turkey

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Abstract We investigated the clinical and mycological characteristics of four cases of mycotic keratitis caused by *Aspergillus flavus* that occurred from July 2014 to May 2015 at Çukurova University Hospital, Adana, Turkey. In a 10-month period, a total of 64 corneal smear/scrapings were examined from patients with suspected mycotic keratitis. Fungal cultures were positive in six of these patients, indicating a 9.4% incidence of mycotic keratitis in this region, including four cases of *A. flavus* and two cases of *Fusarium* spp. The predisposing factors, clinical presentation, and success of the therapeutic approaches were further evaluated. For all cases, topical voriconazole was the first choice of treatment. Surgical procedures were required to control infection in 3 of the 4 cases, including intrastromal voriconazole injection for two cases and keratoplasty for one case. Predisposing factors included trauma (two cases, 50%), contact lens use (one case, 25%), and previous ocular surgery (one case, 25%). The clinical presentations also differed, including a well-limited ulcer (one case), an ulcer with an irregular feathery margin (one case), and ulcers with satellite lesions (two cases). The mean duration between the time of presentation and definitive diagnosis by culture was 14 days (8–25 days). We observed that *A. flavus* keratitis can present with different underlying factors and clinical conditions. A combination of antifungal therapy and supportive surgical intervention may resolve infections caused by *A. flavus* in the cornea.

Keywords *Aspergillus flavus* · Mycotic keratitis · Voriconazole

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Introduction

Mycotic keratitis is a challenging condition in ophthalmological practice. Ocular trauma, particularly trauma caused by vegetative material, is reported as

the most common predisposing risk factor [1, 2]. Treatment options are limited, and many cases require surgery to maintain corneal integrity [3, 4]. Overall, the most common causes of filamentous mycotic keratitis worldwide are the *Aspergillus* and *Fusarium* genera [5, 6]. In the *Aspergillus* group, *Aspergillus fumigatus* followed by *Aspergillus flavus* (section *Flavi*) are the most commonly encountered opportunistic pathogens causing systemic infections and the leading causes of superficial infections in humans [7]. These fungi are predominantly associated with infections of the respiratory tract, sinuses, eye, and skin, and are prevalent in hot and arid climates, such as North Africa [8], the Middle East [9], and India [1]. For example, among 1737 culture-proven cases of mycotic keratitis in India, *Aspergillus* species were identified in 200 (11.5%) cases, and *A. flavus* (75%) was the predominant species [1].

The local epidemiology of mycotic keratitis, which may vary from country to country, as can the therapeutic modalities and the susceptibility patterns of causative agents (particularly the *Aspergillus* species) against a limited number of antifungal compounds. Understanding these differences can be important for ophthalmologists and may guide personalized therapy [1, 2]. In the current study, we present four cases with culture-proven *A. flavus* keratitis at Çukurova University, Balcalı Hospital, in Adana, Turkey. We discuss the predisposing factors, clinical manifestations, and epidemiological characteristics of the causative agents, and we describe the management strategies that have a high probability of successfully treating this disease.

Case 1

A 23-year-old woman presented with ocular pain, photophobia, and a mild disturbance of visual acuity in her left eye; these symptoms had begun 5 days previously. She had been wearing soft contact lenses for the past 5 years to correct myopia. During the last couple of months, she had not complied with the rules of contact lens use and had been sleeping in her contact lenses and using tap water to clean the lens case. A medical examination revealed that her visual acuity was 20/400 in the left eye. A slit lamp examination showed a corneal infiltration with irregular margins (Fig. 1a). A corneal scraping was performed to obtain samples for microbiological examination. A wide-spectrum antibiotic treatment [vancomycin (50 mg/mL) and amikacin (20 mg/mL) drops, hourly instillation] was initiated. In the subsequent days, the corneal infection progressed and visual acuity decreased to the hand motion level (Fig. 1b). Treatment was changed to an antifungal regimen (topical voriconazole, hourly, and systemic ketoconazole twice a day). The first scraping samples were negative for retrieval of any microorganism. Corneal collagen cross-linking with UV-A and riboflavin was performed at day 18 to control progressive corneal melting and deep invasion of the infection, and an intrastromal voriconazole injection was given at the same session. A second scraping was performed on day 20, and numerous fungal hyphae were found by cytological examination. Fungal culture for 4 days revealed *A. flavus*. Real-time PCR analysis was also positive for *Acanthamoeba* in the second sample analysis, indicating a mixed infection, so topical propamidine isethionate (Brolene® 0.1% eye drop, Sanofi-Aventis) and chlorhexidine (0.02%) were added to the treatment. The clinical findings regressed in the following days, and the patient recovered completely by the third month (Fig. 1c). The patient's visual acuity was 20/400 at the end of the 6-month follow-up.

Case 2

A 28-year-old man complained of redness, pain, and a white fleck in his left eye for the past 20 days. He was referred to our cornea clinic with a mild, but resistant, keratitis that had not resolved with several different topical antibiotics. His visual acuity was 20/200 in the affected eye. He was a worker at an iron-steel factory, and he described a minor ocular trauma involving splashed iron dust. A slit lamp examination showed a well-defined corneal ulcer with conjunctival inflammation (Fig. 2a). Mycotic keratitis was the main suspicion for this persistent corneal infection. *A. flavus* was identified after culture for 3 days. A topical voriconazole (10 mg/mL) drop was initiated hourly. His clinical response was rapid. His visual acuity improved to 20/50 at day 5 and 20/40 at day 7 (Fig. 2b). Repeated smear exams were negative. The clinical findings were stable at his 1-month follow-up examination. One year after presentation, the patient's

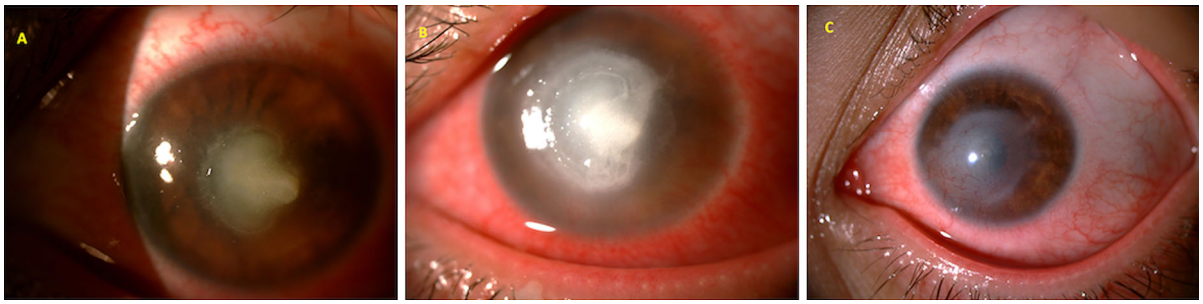

Fig. 1 A central corneal ulcer with feathery margins (**a**). At day 6, the lesion size and deep corneal vascularization have progressed (**b**). The corneal ulcer healing, with remaining scars and vascularization, at the end of the 6-month follow-up (**c**)

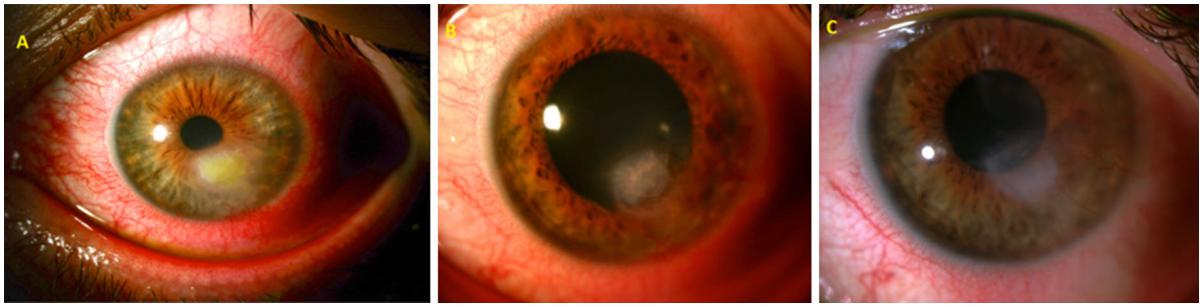

Fig. 2 A paracentral superficial ulcer (**a**). At day 7, corneal infiltration had regressed and a mild haze remained (**b**). The lesion was resolved completely, with residual scarring, at the end of 1 year (**c**)

visual acuity was 20/20, although a mild stromal haze remained (Fig. 2c).

Case 3

A 56-year-old woman was referred with a history of ocular trauma inflicted by an umbrella tip to the right eye 1 month previously. She had pain, redness, and low visual acuity, and her symptoms were unresponsive to topical antibiotic treatment. Upon examination, visual acuity was hand motion level in the right eye. A slit lamp examination revealed a corneal ulcer, satellite infiltrates, and hypopyon (Fig. 3a). Mycotic keratitis was the primary diagnosis, so a corneal scraping was examined for culture and cytological purposes. Empirical treatment included a topical voriconazole (10 mg/mL) drop hourly, with the addition of a vancomycin drop six times a day and an amikacin drop six times a day to the treatment regimen to cover possible bacterial contamination. *A. flavus* grew day 3 on culture. The patient's clinical signs progressed in the following days, despite the ongoing wide-spectrum topical antibiotic therapy (Fig. 3b). We then switched to an intrastromal voriconazole injection on day 12. None of these measures controlled the infection, so a penetrating keratoplasty was performed 24 days after the initial examination. Topical voriconazole (10 mg/mL) and cyclosporine A (drop 0.05%) were prescribed postoperatively. The corneal graft remained clear, and no infection recurred during the 6 months of follow-up. At the last visit, the patient's visual acuity was 20/200, and the patient had developed a cataract (Fig. 3c).

Case 4

A 50-year-old man had a history of lattice corneal dystrophy in both eyes. Penetrating keratoplasty had been performed on the left eye 2 years previously. He complained of pain, redness, and reduced visual acuity in his left eye over the last 2 days. Ophthalmologic examination revealed low visual acuity (20/400), as well as an abscess formation on the corneal graft (Fig. 4a). His clinical features were nonspecific at

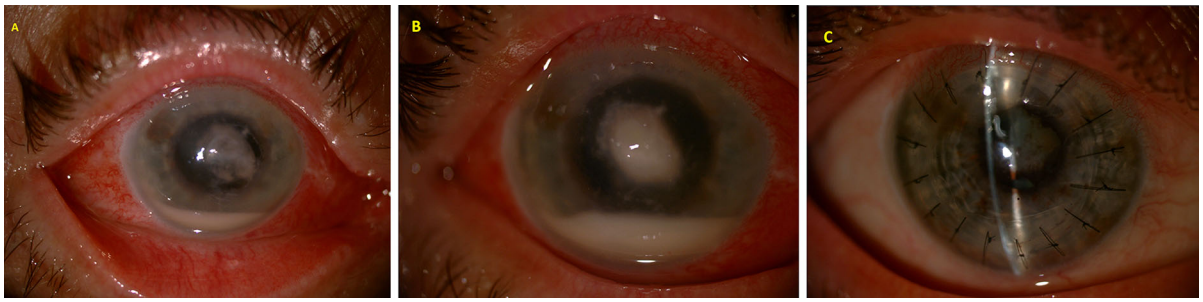

Fig. 3 A slit lamp photograph showing a central ulcer with an irregular edge, satellite infiltrations, and hypopyon (**a**). By day 5, a white abscess has formed, and the volume of hypopyon has increased (**b**). A clear corneal graft and complicated cataract were seen at the 6-month examination (**c**)

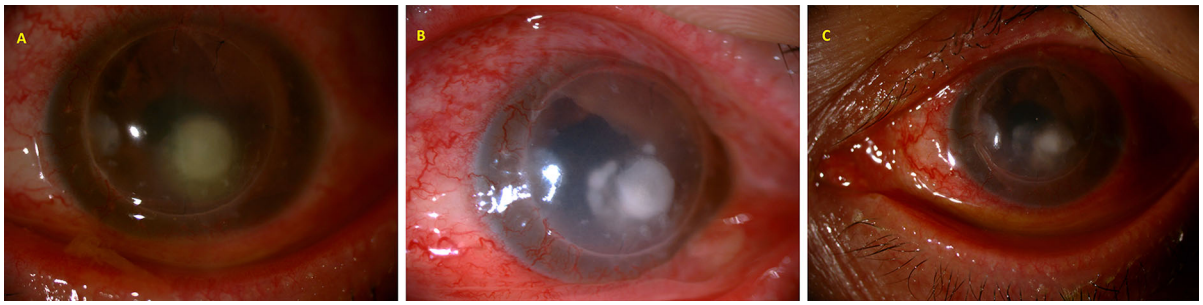

Fig. 4 At medical examination, *white* infiltration with an irregular margin on the corneal graft was observed (**a**). Multiple perilesional satellite infiltrations were detected 3 days after presentation (**b**). At day 10, clinical resolution was observed following intensive antifungal treatment (**c**)

presentation. Corneal smear samples were obtained for a microbiological examination. The initial suspicion was a mixed bacterial and fungal infection for this patient; therefore, a topical antibacterial treatment of vancomycin and amikacin was initiated to cover gram-positive and gram-negative bacteria. After a few days, the clinical picture had deteriorated rapidly. Visual acuity dropped to a hand motion level, corneal edema and infiltrate size increased, and hypopyon was visible. At this point, the clinical diagnosis was switched to mycotic keratitis because of the appearance of satellite lesions in the cornea (Fig. 4b). *A. flavus* was detected in cultures after 3 days of incubation. A topical voriconazole (10 mg/mL) drop was added to the treatment. Corneal collagen cross-linking, using ultraviolet light and riboflavin, was performed at day 6 to control melting and infection. A favorable clinical response was observed with this treatment, and the patient's visual acuity increased to 20/400 by day 10 (Fig. 4c). Repeated cytological examinations revealed no other pathogens. The antifungal treatment was reduced and stopped within 5 months. Visual acuity was 20/200, and the infection had subsided completely at the last visit.

Fungal Strains

All the corneal scraping and smear samples were inoculated onto Sabouraud glucose agar (SGA; Merck, Darmstadt, Germany), potato dextrose agar (PDA; Merck), blood agar (Biomark, Pune, India), brain–heart infusion agar (Merck), and Endo agar (HiMedia, Mumbai, India) in the form of a "C" streak. The SGA and PDA plates were incubated at 30 °C for 7 days, and the other plates were incubated at 37 °C for 3 days. Clinical samples were reported "positive" when the fungal growth was seen only the "C" streaks at least two media [2]. Table 1 lists all the fungal strains identified in this study, including their origin, identification number, and antifungal susceptibility data. All isolates were subcultured on SGA at 30 °C for 7 days. Morphological identifications were confirmed using sequence-based analysis

Table 1 MIC/MEC values for eight antifungal agents obtained by testing the susceptibility of four *Aspergillus flavus* keratitis isolates

Case number	MI no.	MIC/MEC (mg/L)							
		AMB	5-FC	FLC	ITC	VRC	POS	AFG	CAS
1	19,791	2	>64	>64	0.25	2	0.25	0.016	0.25
2	19,792	0.5	>64	>64	0.5	1	1	0.031	0.032
3	19,793	0.5	>64	>64	0.5	1	0.125	0.031	0.062
4	19,794	2	>64	>64	0.5	2	0.25	0.016	0.25

MIC minimum inhibitory concentration, *MEC* minimum effective concentration, *AMB* amphotericin B, *5-FC* 5-flucytosine, *FLC* fluconazole, *ITC* itraconazole, *VRC* voriconazole, *POS* posaconazole, *AFG* anidulafungin, *CAS* caspofungin, *MI* Macit Ilkit's working collection

of the rDNA internal transcribed spacer (ITS) and β-tubulin regions, as described previously [10].

Phylogeny, Identification, and Nomenclature of the Genus *Aspergillus*

The section *Flavi* represents a great diversity of species that cause infections in humans, such as *A. flavus*, *A. oryzae*, *A. tamarii*, *Petromyces alliaceus*, *A. nomius*, *A. quzitongi*, *A. beijingensis*, and *A. novoparasiticus* [11]. The β-tubulin gene, calmodulin, and, to a lesser degree, the ITS are considered the target genes for identifying *A. flavus* [12]. Molecular and phenotypic data indicate different genetic lineages within *A. flavus*. In addition, a higher molecular heterogeneity for *A. flavus* is reported, which further classifies this section into three main clades [13].

Antifungal Susceptibility Testing

In vitro antifungal susceptibility testing was performed against amphotericin B (AMB), flucytosine (5FC), itraconazole (ITC), voriconazole (VRC), posaconazole (POS), fluconazole (FLC), caspofungin (CAS), and anidulafungin (AFG) using the broth microdilution method of the European Committee on Antimicrobial Susceptibility [14]. The geometric mean MICs and MECs for the eight antifungals across all isolates, in increasing order, were as follows: AFG 0.02 mg/L, CAS 0.15 mg/L, POS 0.41 mg/L, ITC 0.44 mg/L, AMB 1.25 mg/L, VRC 1.50 mg/L, FLC >64 mg/L, and 5FC >64 mg/L.

Discussion

We described four cases of *A. flavus* keratitis observed in a 10-month period at a university hospital in the subtropical climate of Adana, Turkey. Our aim was to discuss the etiologic agents, epidemiological features, clinical characteristics, and antifungal susceptibility profiles of the corresponding fungi isolated from these patients. Although agricultural work has been considered the most commonly reported risk factor for *Aspergillus* keratitis [1], we identified several different risk factors in this case series, including trauma, wearing contact lenses, and previous corneal surgery.

Over the 10-month period, a total of 64 corneal smear/scrapings were examined from patients with suspected mycotic keratitis. Overall, a corneal ulcer was detected in 7 (10.9%) of the cases. The fungal culture was positive for six patients with corneal ulcer, indicating a 9.4% incidence of mycotic keratitis in this region, including four cases of *A. flavus* and two cases of *Fusarium* spp. Notably, several studies have reported a seasonal difference, with a high prevalence of mycotic keratitis occurring between June and September [1]. Similarly, 3 of the 4 cases in our study appeared in September.

A study by Manikandan et al. [1] indicated that most (88%) of the mycotic keratitis cases occurred in adults, ranging from 21 to 70 years of age, and co-existing disease was diagnosed in 16.5% of these patients. Consistent with that study, all the patients in our study were adults, ranging from 23 to 56 years of age, but our cases had no history of systemic comorbidities. In addition, ocular trauma with vegetative materials, including soil, leaves, or tree

branches, is the most reported source of mycotic keratitis, which is reported mainly in farmers and in agriculture-based tropical countries [1, 2].

In our study, the most difficult patient to treat (case 1) had a history of improper use of contact lenses, which had apparently led to serious complications, including mycotic keratitis [15]. In this patient, both *Aspergillus* and *Acanthamoeba* were detected coincidentally, both of which can cause sight-threatening complications. For this reason, the clinical course was more complicated in this case than in the others, and healing took 3 months.

Previously, Al-Wathiqi et al. [9] reported that the triazoles and echinocandins showed good activity against 92 clinical and 7 environmental *A. flavus* isolates. However, nearly 18% of the isolates showed a MIC >2 mg/L against AMB [9]. Consistent with those finding, Gonçalves et al. [11] tested 77 isolates belonging to *Aspergillus* section *Flavi* against 9 antifungals and reported that terbinafine and echinocandins demonstrated the lowest MICs and MECs, followed by posaconazole. Notably, AMB showed MICs ≥2 mg/L for 38 (49.4%) of the 77 isolates tested [11].

The treatment of mycotic keratitis is challenging because of the poor corneal penetration of medications and the limited efficacy of the available drugs. Voriconazole is a second-generation triazole that shows good corneal penetration [16]. Natamycin was not available, so we applied voriconazole therapy either as an empirical therapy or in culture-proven cases. Voriconazole is the drug of choice for treatment of *Aspergillus* spp. that show resistance to topical natamycin and amphotericin B [17]. Despite intensive antifungal treatment, perforation is not uncommon, so adjuvant treatments may be needed to prevent complications [18]. Intrastromal voriconazole injection can be used to achieve adequate intracorneal concentration of the drug [19, 20]. Amniotic membrane transplantation may also be warranted to control progressive melting [21].

Corneal collagen cross-linking is a novel therapeutic option for infectious keratitis. Cross-linking is effective as it has dual mechanisms: (1) it inactivates the pathogen by damaging nucleic acids and (2) it prevents enzymatic melting of the cornea [22, 23]. Several case reports confirm the beneficial effects of cross-linking as the primary treatment for small bacterial ulcers and as an adjuvant treatment for moderate to severe ulcers (of bacterial or fungal origin) [24, 25]. We performed cross-linking as an adjuvant treatment in cases 1 and 4. Symptomatic relief was clearly evident in the postoperative days, and the ulcers healed without complications. Based on these observations, cross-linking seems to be a safe and effective option for the management of mycotic keratitis.

In conclusion, we observed that *A. flavus* keratitis can present in response to several underlying factors and with different clinical conditions. In addition, our study suggests that mycotic keratitis caused by *A. flavus* may occur more often in a subtropical climate, such as in Adana, Turkey. A combination of antifungal therapy and supportive surgical intervention may successfully resolve the infection caused by *A. flavus* in the cornea.

Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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