Case Report

Clinical and mycological characteristics of keratitis caused by Colletotrichum gloeosporioides: A case report and review of literature

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Abstract

Introduction: Colletotrichum species are well-known plant pathogens, which have been increasingly reported as the cause of keratitis or subcutaneous lesions in humans. In this study, we reported a rare case of fungal keratitis from Iran and reviewed the literature.

Case Presentation: A 69-year-old man whose right eye was injured by herbal material was examined by slit-lamp biomicroscopy and mycology investigation of corneal scrapings was done. The grown filamentous fungal was identified as Colletotrichum gloeosporioides based on morphological characteristics and DNA sequence of the internal transcribed spacer region. The isolated strain was sensitive to amphotericin B, caspofungin, anidolafungin, micafungin, voriconazole, and relatively resistant to fluconazole, and itraconazole. Patient was successfully treated with voriconazole.

Conclusions: This report highlights that the early and accurate identification and therapy can be helpful to management keratitis caused by C. gloeosporioides.

Key words: Colletotrichum gloeosporioides; Keratitis; Molecular identification.


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Introduction

Fungal keratitis (FK) is one of the main causes of ocular morbidity [1]. Delay or inappropriate treatment can be potentially leading cause of blindness [2]. In recent years, the genus Colletotrichum has been recognized as an infrequent cause of FK [2-4]. Colletotrichum species are common plant pathogens in tropical and subtropical regions worldwide [3-5]. Trauma with plant materials is one of the main predisposing factors in keratitis caused by these fungi [6]. Among Colletotrichum genus, only a few species are known to be pathogenic for humans. The Colletotrichum spp. have been associated with human ophthalmic infections including C. dematium, C. coccodes, C. gloeosporioides, C. graminicola, C. crassipes, C. capsici [7]. Based on the literature, C. gloeosporioides considered as the second most common cause of FK after Colletotrichum dematium [8,9]. Though Colletotrichum infection in humans is rare, to avoid ophthalmic complications an early identification of pathogenic fungus and their antibiotic susceptibility can be helpful for management of the infection. Molecular techniques may help in accurate diagnosis of the agent [7]. Close resemblance of the curved conidia of Fusarium and Colletotrichum spp. may mislead inexperienced laboratory personnel [3]. Here, we review the literature and report a case of FK. To our knowledge, this is the first case report documenting the clinical and mycological characteristics of FK caused by C. gloeosporioides from Iran.

Case presentation

A 69-year-old male farmer presented to Farabi eye hospital in Tehran with a sudden loss of visual acuity. He mentioned a history of ocular trauma in right eye by...
herbal material 10 days earlier. On initial examination of the patient, visual acuity (VA) was counting fingers in the right eye and 20/25 in the left eye. Slit lamp examination of the affected eye revealed superficial corneal infiltration with a size of 4×3 mm and epithelial defect without hypopyon (Figure 1). Corneal scrapings are obtained using sterile surgical blades for mycological examination. One sample was used to perform potassium hydroxide (KOH) mount and routine Gram’s staining, while the second was inoculated into Sabouraud dextrose agar (SDA- Merck, Germany) supplemented with 0.5% chloramphenicol and sheep blood agar plates, which incubated at 28°C and 37°C, respectively. Microscopic examinations by KOH mount and gram’s staining of sample showed septate filamentous fungal fragments and did not show any bacterial cells. Based on clinical findings and the result of microscopic examination of corneal scraping, treatment was initiated with topical 0.1% voriconazole, vancomycin and ceftazidime hourly. Culture of corneal scrapings revealed brownish-black filamentous fungal colonies, consisting of numerous sclerotia on SDA (Figure 2). The microscopic examination of fungal colonies showed abundant cylindrical non-septate conidia with blunt ends and darkly pigmented with white aerial hyphae (Figure 3), which are features characteristic of the genus *Colletotrichum*. DNA was extracted using a DNA isolation kit (Gene All DNA extraction kit; Gene All, Germany) according to the manufacturer’s instructions and stored at -20°C prior to use. To confirm the *Colletotrichum* species identification, the Internal Transcribed Spacer (ITS) region was sequenced, as previously described [10]. Yieldsed sequence was subjected to Basic Local Alignment Search Tool (BLAST) program (http://www.blast.ncbi.nlm.nih.gov/Blast). The DNA sequence of the ITS gene matched that of *Colletotrichum gloeosporioides* species (GenBank MT130718) by showing 99% similarity with the ex-type strain of the species (GenBank MT065696). Additionally, *in vitro* antifungal testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 for filamentous fungi [11]. The minimal inhibitory concentrations (MICs) of amphotericin B, fluconazole, voriconazole, itraconazole, caspofungin, anidulafungin and micafungin were 0.5, >64, 0.5, 8, 0.5, 0.5 and 0.25 respectively. The patient was treated with voriconazole every hour, levofloxacin 4 hours and passing 42 days, the lesion turned into corneal scar and there were no signs of a recurrence after 2 months of treatment with voriconazole.

**Discussion**

*Colletotrichum* species are coelomycetous soil fungi that are an uncommon cause of keratitis worldwide [5, 6]. Traumatic insemination is important for the initiation of *Colletotrichum* infection [2]. Thorough search of literature using “Keratitis” and “*Colletotrichum*” showed *Colletotrichum* spp. keratitis is increasingly reported (Table 1). Fernandez et al. reported, FK due to *Colletotrichum* was in 10 of 360

**Figure 1.** Slit lamp photographs of right eye.

(A) Corneal ulcer with infiltration and the centrifugal, linear, circular spread of fungal infection through the corneal channels, (B) Corneal scarring after treatment with voriconazole.

**Figure 2.** Fungal colonies growth on Sabouraud dextrose agar.

(A) The growing fungus produced brown-gray concentrically ringed colonies. *Colletotrichum gloeosporioides* produced orange mucus beads centrically after 10 days at 30°C. (B) Reverse side showing brownish-black pigmentation.

**Figure 3.** (A) Lactophenol cotton blue tease mount from the colony. Hyphae with abundant cylindrical conidia with blunt ends (magnification X400); (B) brownish appressoria, which are characteristic features of *Colletotrichum gloeosporioides* (magnification X400).
cases (2.8%) [12]. In other series by Kalamurthy et al. [13] and Hung et al. [3], Colletotrichum was the cause of FK in 7 of 378 cases (1.9%) and in 7 of 65 cases (10.8%), respectively. The ocular trauma and contamination by organic materials were principal risk factors for Colletotrichum keratitis [12, 13]. Our case 22 had a history of ocular injury by herbal material during farm work, similar to other cases reported [8, 9, 14]. C. gloeosporioides spp. can be identified by nonseptate conidia, presence of appressoria, and (in the later stage) acervuli with setae but the morphological identification is time-consuming and technically challenging [7]. Due to the difficulty of the morphological identification of Colletotrichum spp., molecular approaches based on the nucleic acid sequence of ITS region are the more applicable and accurate method for isolates identification to the species level in the diagnostic laboratory [5, 15]. Most cases of FK caused by Colletotrichum species, particularly C. dematium and C. gloeosporioides respond well to natamycin, possibly

### Table 1. Summary of previously reported cases reports of *Colletotrichum gloeosporioides* keratitis.

<table>
<thead>
<tr>
<th>Authors (Reference)</th>
<th>Country, Year</th>
<th>No. of case</th>
<th>Age/ Sex</th>
<th>Predisposing factor and risk factor</th>
<th>Eye direction</th>
<th>Initial visual activity</th>
<th>Hypopyon</th>
<th>Medical therapy</th>
<th>Fungal identification method</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maria Borrás-Mánez et al. (5)</td>
<td>Spain, 2015</td>
<td>1</td>
<td>75 / M</td>
<td>trauma with plant material (orange tree branch)</td>
<td>NM</td>
<td>NM</td>
<td>Yes</td>
<td>Voriconazole, Keratoplasty</td>
<td>Microscopic, culture and molecular (sequencing)</td>
<td>Evisceration</td>
</tr>
<tr>
<td>S.T. Pote et al. (19)</td>
<td>India, 2017</td>
<td>1</td>
<td>45 / M</td>
<td>trauma with with stone chip</td>
<td>Right</td>
<td>6/60</td>
<td>Yes</td>
<td>Amphotericin-B, Voriconazole, Natamycin</td>
<td>Microscopic, culture and molecular (DNA sequencing of ITS region)</td>
<td>CI</td>
</tr>
<tr>
<td>Lipeng Wang et al. (15)</td>
<td>China, 2019</td>
<td>1</td>
<td>52 / M</td>
<td>trauma with plant material (apple tree branch)</td>
<td>Right</td>
<td>NM</td>
<td>NM</td>
<td>Natamycin, Levofloxacin, Voriconazole</td>
<td>Microscopic, culture, MALDI-TOF, DNA sequencing of ITS,</td>
<td>CI</td>
</tr>
<tr>
<td>Atsushi Shiraishi et al. (4)</td>
<td>Japan, 2011</td>
<td>3</td>
<td>71 / F</td>
<td>Trauma with plant material</td>
<td>Left</td>
<td>20/80</td>
<td>NM</td>
<td>Voriconazole, Pimaricin</td>
<td>Microscope, culture, DNA sequencing of ITS</td>
<td>CI</td>
</tr>
<tr>
<td>Ning Hung et al. (3)</td>
<td>Taiwan, 2020</td>
<td>5</td>
<td>79 / F</td>
<td>Trauma with plant material</td>
<td>Right</td>
<td>20/300</td>
<td>NM</td>
<td>Voriconazole, Natamycin, Miconazole, Levofloxacin</td>
<td>Microscope, culture, DNA sequencing of ITS</td>
<td>CI</td>
</tr>
<tr>
<td>J. Lamarca et al. (20)</td>
<td>Spain, 2015</td>
<td>1</td>
<td>56 / F</td>
<td>Trauma with plant material (orange tree branch)</td>
<td>Left</td>
<td>NM</td>
<td>Yes</td>
<td>Natamycin, Keratoplasty</td>
<td>Microscope, culture, PCR</td>
<td>CI</td>
</tr>
<tr>
<td>Ismail Zakariya-Yousef Brevel et al.(21)</td>
<td>Spain, 2019</td>
<td>1</td>
<td>45/ M</td>
<td>Trauma with plant material (orange tree branch)</td>
<td>Right</td>
<td>NM</td>
<td>Yes</td>
<td>Voriconazole</td>
<td>Microscope, culture</td>
<td>CI</td>
</tr>
<tr>
<td>Our case</td>
<td>Iran, 2020</td>
<td>1</td>
<td>69 / M</td>
<td>Trauma with plant material</td>
<td>Right</td>
<td>counting fingers</td>
<td>No</td>
<td>Voriconazole</td>
<td>Microscope, culture, DNA sequencing of ITS</td>
<td>CI</td>
</tr>
</tbody>
</table>

Abbreviations: M: male; F: female; NM: not mentioned; CI: clinical improvement.
due to its easy availability [12, 13]. However, Shiraishi et al. and Fernandez et al. reported that isolates of C. gloeosporioides in their study showed intermediate resistance to natamycin [4, 12]. In our reported case, simultaneous voriconazole regimen leads to complete regression of eye lesion. The excellent results have been reported following voriconazole treatment in cases of FK and endophthalmitis caused by a number of species of fungi [16, 17]. Shiraishi et al. [4] treated 3 cases of C. gloeosporioides keratitis with voriconazole, as well as Mitani et al. reported [2]. However, Márquez et al. [5] reported oral, topical, intravitreal and intrastromal voriconazole showing no improvement of eye lesion. In some case reported combination therapy (natamycin plus amphotericin-B/voriconazole) may be more effective than monotherapy [4]. There is a lack of consensus regarding the optimal therapy against Colletotrichum spp. ophthalmic infection in the literature [15]. Few reports mentioning the drug sensitivity test against C. gloeosporioides [4, 15]. Shiraishi et al. indicated that all three C. gloeosporioides, isolated cases of FK, were sensitive to amphotericin B, itraconazole, miconazole, micafungin, and voriconazole, and relatively resistant to fluconazole, fluconazole, and natamycin [4]. Also, Mitani et al. reported similar results [2]. The MICs of different antifungal drugs on the C. gloeosporioides isolated from case reports are shown in Table 2. Reported studies have indicated that the majority of Colletotrichum isolates have a different degree of resistance to antifungal drugs and therefore combination therapy may be more effective than monotherapy for complete cure of patients having Colletotrichum infections [18, 19].

In conclusion, this is a rare FK case caused by C. gloeosporioides in Iran. The results suggest that molecular investigations and antifungal susceptibility testing will be required to further understand the epidemiology and optimal management of FK caused by rare fungal pathogens in humans.

**Table 2. The MICs of antifungal drugs on the C. gloeosporioides isolated from reported case.**

<table>
<thead>
<tr>
<th>Authors (Reference)</th>
<th>Amphotericin B</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
<th>Itraconazole</th>
<th>Posaconazole</th>
<th>Miconazole</th>
<th>Caspofungin</th>
<th>Anidulafungin</th>
<th>Micafungin</th>
<th>5-Flucytosine</th>
<th>Natamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipeng Wang et al. (15)</td>
<td>0.25</td>
<td>64</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>-</td>
<td>0.25</td>
<td>0.12</td>
<td>0.06</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Atsushi Shiraishi et al. (4)</td>
<td>0.125</td>
<td>&gt;64</td>
<td>0.5</td>
<td>0.25</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>&lt;0.03</td>
<td>&gt;64</td>
<td>4</td>
</tr>
<tr>
<td>Our case</td>
<td>&lt;0.03</td>
<td>64</td>
<td>0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>-</td>
<td>0.25</td>
<td>-</td>
<td>&lt;0.03</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>16</td>
<td>0.125</td>
<td>0.03</td>
<td>-</td>
<td>0.25</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>&gt;64</td>
<td>2</td>
</tr>
</tbody>
</table>

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**Authors’ contributions**

AI, RDG, SJH and SK drafted the manuscript. AI, MS, MG, KA, MA, MG, ZA and SK conducted the clinical examinations and Laboratory tests. AI, MS, RDG, SJH, AB, and SK reviewed the manuscript and participated in the layout and design of the report. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The patient provided a signed written informed consent to undergo the treatment. This report was approved by ethics committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1398.256).

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