Clinical Characteristics and Outcomes of Rare Fungal Keratitis Caused by *Verticillium dahliae*

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Received: 18 February 2021 / Accepted: 28 October 2021 / Published online: 17 November 2021
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Abstract

*Purpose* To observe clinical characteristics and treatment outcomes of fungal keratitis caused by *Verticillium dahliae*.

*Methods* Clinical data of 7 patients diagnosed as fungal keratitis caused by *Verticillium dahliae* were retrospectively analyzed. The clinical manifestations, mycology, in vitro antifungal susceptibility, treatment regimens, and prognoses of the patients were evaluated.

*Results* All 7 patients were farm workers, of which 5 cases were caused by plant trauma. The corneal ulcer had a round shape and a relatively limited range with the diameters mainly in the range of 2–7 mm. The stromal infiltration was mild, and had no pseudopodia, mossiness, or endothelial plaques. Intact hyphae were detected in corneal scrapings and confocal microscopy, isolates were identified by morphology and by sequencing the internal transcribed spacer region of ribosomal DNA. In vitro antifungal susceptibility testing showed that the most sensitive antifungal drug was Amphotericin B. In the 6 patients with an ulcer less than 2/3 of the corneal thickness, the ulcer healed after 18 days of antifungal treatment only in one eye. The other five patients underwent corneal ulcer debridement or conjunctival flap covering surgery. The remaining one patient with ulcer depth more than 2/3 of the corneal thickness underwent lamellar keratoplasty.

*Conclusion* Fungal keratitis caused by *Verticillium dahliae* has typical signs of a mild inflammatory response, and is not sensitive to antifungal drugs. It is recommended that patients undergo corneal ulcer debridement as soon as possible to promote rapid healing of the ulcers.

Keywords Fungal keratitis · *Verticillium dahliae* · In vitro antifungal susceptibility test · DNA sequence

Introduction

More than 105 species that belong to 50 genera of fungi pathogens have been reported to cause human fungal keratitis. The most common pathogens include...
hyphomycetes (Aspergillus app., Fusarium app., and Scedosporium app.) and yeasts (Candida albicans and C. parapsilosis) [1]. But some rare pathogens causing human fungal keratitis are emerging in recent years, including Pythium spp., Acremonium spp., Cladosporium spp., Curvularia spp., Bipolaris spp., Microsporidia spp., Pseudallescheria spp., Colletotrichum spp., and others [2]. Verticillium dahliae, a fungus in the phylum Ascomycota and the genus Verticillium, is a soil-borne pathogen that infects plant roots by forming hyphopodia; it causes severe verticillium wilt diseases, which lead to enormous financial losses in the production of cotton and other field crops [3–5]. Our clinical work finds that trauma caused by plants, such as scratches from cotton branches, may lead to fungal keratitis due to the infection of human corneal tissues with Verticillium dahliae; such ulcers have unique biological characteristics, such as a limited ulcer area and mild stromal infiltration, but with slow healing and easy prolongation. A retrospective study was conducted to evaluate the clinical characteristics and treatment outcomes of fungal keratitis cause by Verticillium dahliae, hoping to provide valuable information regarding early diagnosis and timely antifungal treatments in these circumstances.

Materials and Methods

Patients

This study was approved by the Institutional Review Board of Eye Hospital of Shandong First Medical University and adhered to the tenets of the Declaration of Helsinki. Clinical data of 7 patients diagnosed as fungal keratitis cause by Verticillium dahliae from December 2018 to December 2020 in our hospital were retrospectively analyzed. The diagnostic criteria included [6, 7]: (1) corneal scraping examination revealed fungal presence in smears; (2) in vivo confocal microscopy (HRT3; Heidelberg Engineering, Dossenheim, Germany) revealed reflective hyphae structures; (3) fungal pathogen culture showed that the colonies grew slowly and were white, powdery or fluffy; (4) the fungi were identified as Verticillium dahliae by DNA sequences.

The medical history included symptoms associated with the patient’s complaint, the cause of the disease (e.g. foreign body scratching or plant trauma), the onset time, medication, and changes in disease condition. Best corrected visual acuity (BCVA), intraocular pressure (IOP), clinical characteristics were recorded. In vivo confocal microscopy was used to observe hyphal morphology. RTVue optical coherence tomography (OCT; Optovue, Fremont, California, USA) was used to clarify the depth of corneal ulcers.

The internal transcribed spacer (ITS) region was sequenced and DNA sequences were determined at Kingdom clinical trial center (Guangzhou, China). The Primer sequence are ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The accession No. are MN518385.1 and HQ839784.1. The resulting sequences were deposited in the GenBank database. Species identification was performed by searching databases using the BLAST sequence analysis tool (https://www.ncbi.nlm.nih.gov/BLAST/).

In vitro antifungal susceptibility testing was performed by Sensititre Yeast One™ (AccuMed International, Chicago, IL, USA). Colorimetric microdilution method was used, the quality control (QC) isolates Candida krusei (ATCC 6258) and Candida parapsilosis (ATCC 22,019) were included as positive growth controls, and MIC ranges were within established limits (Table 1). Amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin, micafungin and 5-fluorocytosine (provided by Shandong Boke Biological Co., Ltd.) were tested for vitro antifungal susceptibility, and the minimum inhibitory concentration (MIC) values were reported [8].

Results

Demographics and Clinical Characteristics

The patients were 3 males and 4 females, aged 47–74 years (mean, 57 ± 9.0 years). All 7 patients were farm worker. Five cases were caused by plant trauma, of which 3 cases scratched by cotton branches and 2 cases by corn leaves, and the other two cases were without inducement. None of the 7 cases had a history of topical steroid use.

Foreign body sensation, red eye, photophobia, tearing, and decreased visual acuity in the affected
eye were noted by all 7 patients. The onset time, and duration of hospitalization ranged from 7 to 20 days (mean, 13.8 ± 2.2 days), and 14 to 30 days (mean, 17.4 ± 1.8 days), respectively.

The corneal ulcer were all located in the center of the cornea and had a round shape and a relatively limited range. The boundary between the ulcers and the surrounding normal cornea was relatively clear. There were no typical manifestations, such as pseudopodia, mossiness, satellite ulcers, or endothelial plaques, and only one patient (with a ulcer approximately 7 mm in diameter) was complicated with a 3-mm hypopyon (Fig. 1). The diameter of the ulcers was mainly in the range of 2–7 mm, with diameters less than 3 mm in 2 cases, between 3 and 6 mm in 4 cases, and more than 6 mm in 1 case. RTVue OCT showed that the ulcer was less than 1/3 of the corneal thickness in 5 patients, approximately half of the corneal thickness in 1 patient, and approximately 2/3 of the corneal thickness in 1 patient (Table 2).

Intact separate and branched hyphae were detected in the 7 specimens (with 10% potassium hydroxide smears or calcofluor white staining). The hyphae were thin, with relevantly uniform diameters. No chlamydospores were detected (Fig. 2).

Scanning of the ulcer area of the 7 patients using confocal microscopy showed the distribution of hyphae-like structures. The hyphae were moderately reflective, upright, and slender, and some had bamboo-like changes. Their diameters were mainly 2–3 μm, there were many branches, and the alignment was disordered. The angles between the hyphae were small, mostly acute. The field of vision with densely distributed hyphae was clean, and some spore-like structures were visible (Fig. 3).

### Mycology

Seven strains of filamentous fungi were isolated from the 7 specimens submitted for examination, with a positive culture rate of 100%. The colonies grew slowly and were white, powdery, or fluffy, and the back was colorless or light orange. Microscopically, the conidiophores were slender and solitary or had multilayered branches with spiral growth (verticillated) and sharp ends. The angles between the conidiophores and the hyphae were mostly acute, and most of the phialides had no basal septum. The conidia were single-celled and transparent, with smooth walls and an elliptical to oval shape, and grew singly or in clusters at the end of the phialide (cephalophores) (Fig. 4).

The minimal inhibitory concentrations (MICs) for Amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin, micafungin and 5-fluorocytosine were 2 ~ > 8, > 256, > 16, 4 ~ > 8, > 8, 0.25 ~ > 8, 0.06 ~ > 8, 0.015 ~ > 8, > 64 μg/ml, respectively.

### Treatment and Outcomes

All seven patients were hospitalized and received 0.2 mg/ml fluconazole sodium chloride injection intravenous drip once a day, and topically polyene (5% natamycin eye drops or 0.25% amphotericin B

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### Table 2

| Antifungal drugs | Candida krusei ATCC 6258 | Candida parapsilosis ATCC 22,019 |
|------------------|--------------------------|-------------------------------|
| Amphotericin B   | 0.5–2                    | 0.25–2                        |
| Anidulafungin    | 0.03–0.12                | –                             |
| Caspofungin      | 0.12–1                   | 0.25–2                        |
| Fluconazole      | 8–64                     | 16–128                        |
| 5-fluorocytosine | 4–16                     | 8–32                          |
| Itraconazole     | 0.12–1                   | 0.25–1                        |
| Micafungin       | 0.06–0.25                | 0.12–0.5                      |
| Posaconazole     | 0.06–0.5                 | 0.12–1                        |
| Voriconazole     | 0.06–0.5                 | 0.12–1                        |

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eye drops) and imidazole (0.5% fluconazole eye drops or 10 mg/ml voriconazole eye drops).

Surgical treatment was used when drug therapy was shown to be ineffective after approximately one week. Ulcer debridement was performed when the ulcer depth was ≤ 1/3 corneal thickness. Once the depth reached 1/2 corneal thickness, conjunctival flap could be combined. Lamellar keratoplasty (LKP) was chosen when the ulcer depth was more than 2/3 the corneal stroma but not reach the corneal endothelium (Table 2).

The ulcer in one patient healed after 18 days of antifungal treatment, and the best corrected visual acuity improved by 4 lines compared to the pretreatment assessment. Four patients underwent corneal ulcer debridement combined with intrastromal injection of 10 mg/ml voriconazole, followed by antifungal treatment, and the ulcer healed after an average time of 7.5 days. One patient underwent conjunctival flap covering surgery followed by antifungal treatment, and the ulcer healed after 8 days. The corneal ulcer depth of 1 patient was more than 2/3 of the corneal thickness, and the condition did not improve after 1 week of medical treatment. The patient underwent lamellar keratoplasty, with no infection detected at the postoperative follow-up visits (Table 2, Fig. 1).

Fig. 1 A Slit-lamp examination of the left eye showing a 1.5 × 1.5 mm² gray-white corneal ulcer with the depth ≤ 1/3 corneal thickness, B the ulcer healed after antifungal drug therapy. C Slit-lamp examination of the left eye showing a 3 × 3 mm² gray-white corneal ulcer with the depth ≤ 1/3 corneal thickness, D the ulcer healed after surgical debridement. E Slit-lamp examination of the left eye showing a 6 × 7 mm² gray-white corneal ulcer with the depth up to 2/3 corneal thickness, F lamellar keratoplasty was performed after ineffective antifungal drug therapy.
Table 2 Details of 7 patients diagnosed as fungal keratitis caused by *Verticillium dahlia*

| Patient No | Gender (male / female) | Age (years) | Diameter of Corneal Ulcer (mm) | Depth of Corneal Ulcer | Systemic Antifungal Drugs and Duration | Topical Antifungal Drugs and Duration | Topical Antibacterial Drugs and Duration | Surgical Treatments | Outcomes |
|------------|------------------------|-------------|--------------------------------|-----------------------|----------------------------------------|----------------------------------------|------------------------------------------|---------------------|----------|
| 1          | Female                 | 54          | 3                               | < 1/3                 | Fluconazole Sodium Chloride Injection 7 days | Fluconazole eye drops 7 days | Gatifloxacin eye drops 18 days | _                    | Healed after 18 days |
| 2          | Female                 | 51          | 5                               | 1/2                   | Fluconazole Sodium Chloride Injection 7 days | Voriconazole eye drops 7 days | Gatifloxacin eye drops 15 days | Conjunctival Flap Covering | Healed after 8 days |
| 3          | Male                   | 55          | 3                               | < 1/3                 | Fluconazole Sodium Chloride Injection 7 days | Voriconazole eye drops 7 days | Gatifloxacin eye drops 14 days | Amphotericin B eye drops 28 days | Healed after 7 days |
| 4          | Female                 | 62          | 4                               | < 1/3                 | Fluconazole Sodium Chloride Injection 7 days | Voriconazole eye drops 7 days | Gatifloxacin eye drops 15 days | Amphotericin B eye drops 28 days | Healed after 8 days |
| 5          | Male                   | 58          | 7                               | > 2/3                 | Fluconazole Sodium Chloride Injection 7 days | Voriconazole eye drops 7 days | Gatifloxacin eye drops 14 days | Amphotericin B eye drops 30 days | DALK Healed after 7 days |
| 6          | Male                   | 74          | 4                               | < 1/3                 | Fluconazole Sodium Chloride Injection 7 days | Fluconazole eye drops 7 days | Gatifloxacin eye drops 14 days | Amphotericin B eye drops 28 days | Healed after 7 days |
| 7          | Female                 | 47          | 5                               | < 1/3                 | Fluconazole Sodium Chloride Injection 7 days | Voriconazole eye drops 7 days | Gatifloxacin eye drops 15 days | Natamycin eye drops 28 days | Healed after 8 days |

CT = Corneal Thickness; DALK = Deep Anterior Lamellar Keratoplasty
Discussion

Fungal keratitis is the leading cause of infectious corneal disease worldwide, especially in some developing countries with warm and wet climates [8–11]. More than 100 species have been reported as pathogens of fungal keratitis, *Fusarium* species are the most commonly isolated pathogens, followed by *Alternaria* and *Aspergillus* species, in Shandong Province, China [12, 13]. In the recent years, the incidence of uncommon fungal keratitis caused by rare species with diverse morphology has greatly increased [1, 2, 14–19]. The current study described the first confirmed series of fungal keratitis cause by *Verticillium dahliae* with detailed descriptions of clinical characteristics and treatment outcomes.

*Verticillium dahliae*, known as soil-borne pathogens that causes vascular wilt diseases in a wide range of plant hosts [3–5]. Therefore, agricultural environments and trauma caused by plants are the most common factors associated with keratitis caused by *Verticillium dahliae*. The onset of keratitis caused by *Verticillium dahliae* is slow, ranging from 7 to 20 days (mean: 13.8 ± 2.2 days). The most pronounced eye signs were that a moderate inflammatory response at the ulcer and the absence of typical manifestations, such as pseudopodia, mossiness or immune ring. The ulcer diameters were small, and 4 patients in this study
had ulcers with diameters in the range of 3-6 mm. The range of the ulcers was relatively limited, and the boundary with the surrounding normal cornea was relatively clear. The above characteristics were consistent with the slow growth of colonies and the limited colony expansion found in fungal culture. Additionally, confocal microscopy examinations showed that the hyphae of *Verticillium dahliae* were moderately reflective and thin, their diameter was mainly 2–3 μm, and their thickness was uniform; the angles between the hyphae were small, mostly acute; and chlamydospore-like structures were rarely detected, which can be used for preliminary differentiation from *Fusarium*, *Aspergillus*, etc. [20, 21].

In vitro antifungal susceptibility testing showed that Amphotericin B was the optimal option for treating keratitis caused by *Verticillium dahliae* (MIC 2- > 8). Unfortunately, Amphotericin B eye drops are not commercially available in China at present, which greatly limits the clinical application of Amphotericin B. On the other hand, keratitis caused by *Verticillium dahliae* is less sensitive to commonly used antifungal drugs, such as fluconazole (MIC > 256) and voriconazole (MIC 4- > 8). Therefore, in this study, one patient who received drug treatment had a prolonged condition for 18 days before the ulcer healed completely. Conversely, in the 5 patients who underwent corneal ulcer debridement and conjunctival flap covering surgery, the ulcers healed rapidly, at 7–8 days after surgery. We believe the reason for the different healing durations is that the corneal ulcers caused by *Verticillium dahliae* infection were small, and the depth of the infiltration was shallow (the depth of the corneal ulcer in 6 patients was less than 1/3 of the corneal thickness), which is a perfect indication for corneal ulcer resection. Corneal ulcer debridement removes the superficial fungal infection of the cornea, which shortens the ulcer healing time and reduces the disease course [22].

In summary, keratitis caused by *Verticillium dahliae* has typical signs of a mild inflammatory response, small ulcers, a limited ulcer range, and shallow infiltration and is not sensitive to antifungal drugs. Therefore, the disease is prone to prolongation and heals slowly. It is recommended that patients undergo corneal ulcer debridement as soon as possible to promote rapid healing of the ulcers.

**Acknowledgements** We sincerely thank our colleagues at Eye Hospital of Shandong First Medical University for their valuable discussions and help.

**Funding** This study was supported by National Natural Science Foundation of China (81870639 and 81900907), Taishan Scholar Program (201812150), Science &Technology Development Program of Shandong Province (2016WS0511).

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest and no ethical issue.

**Data availability** All data in this study were available.

**Ethical approval** This study was approved by the Institutional Review Board of Eye Hospital of Shandong First Medical University, Jinan, China.
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