Clinical Characteristics and Outcomes of Rare Fungal Keratitis Caused by

Verticillium dahliae

Qing Huang, MD.1 Wenlu Zhang, BD.2 Yu Sun, MD.2 Xiaofeng Li, BD.2 Xiaoyu

Zhang, MD.2 Xiuhai Lu, MD.2 Xiaolin Qi, MD, PhD.2

1 Shandong First Medical University & Shandong Academy of Medical Sciences

2 Eye Hospital of Shandong First Medical University, State Key Laboratory

Cultivation Base, Shandong Provincial Key Laboratory of Ophthalmology, Shandong

Eye Institute, Shandong First Medical University & Shandong Academy of Medical

Sciences, Jinan, China

Corresponding author: Xiuhai Lu, Xiaolin Qi

Address: Eye Hospital of Shandong First Medical University, 372 Jingsi Road, Jinan

250021, China

Email: xiuhaulu@163.com, qinglianqxl@163.com

Telephone: 086-0531-81276007

Fax: 86-531-8127-6090
Abstract

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

**Methods** Clinical data of 7 patients diagnosed as fungal keratitis cause by V. 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 worker, 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 hyphaes 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 V. 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.

**Key Words:** Fungal keratitis, Verticillium dahliae, In vitro antifungal susceptibility test, DNA sequence
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Data availability All data in this study were available.

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

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

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 [1-3]. 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 V. 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 V. 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 Shandong Eye Hospital and adhered to the tenets of the Declaration of Helsinki. Clinical data of 7 patients diagnosed as fungal keratitis cause by V. dahliae from December 2018 to December 2020 in our hospital were retrospectively analyzed. The diagnostic criteria included [4-5]: (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. Amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin, micafungin and 5-fluorocytosine (provided by Shandong Boke Biological Co., Ltd.) were tested for in vitro antifungal susceptibility, and the minimum inhibitory concentration (MIC) values were reported [6].

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.

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 chlamydosores 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 internal transcribed spacer (ITS) region was sequenced and DNA sequences were determined at Kingdom clinical trial center (Guangzhou, China). 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). 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 ug/ml, respectively.

**Treatment and Outcomes**

All seven patients were hospitalized and received 0.2 mg/ml intravenous drip once a day, and topically polyene (5% natamycin eye drops or 0.25% amphotericin B eye drops) and imidazole (0.5% fluconazole eye drops or 10mg/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.

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 (Fig.1).

Discussion
Fungal keratitis is the leading cause of infectious corneal disease worldwide, especially in some developing countries with warm and wet climates [7-9]. 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 [10-11]. In the recent years, the incidence of uncommon fungal keratitis caused by rare species with diverse morphology has greatly increased [12-19]. The current study described the first confirmed series of fungal keratitis cause by V. dahliae with detailed descriptions of clinical
characteristics and treatment outcomes.

V. dahliae, known as soil-borne pathogens that causes vascular wilt diseases in a wide range of plant hosts [1-3]. Therefore, agricultural environments and trauma caused by plants are the most common factors associated with keratitis caused by V. dahliae. The onset of keratitis caused by V. 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 3-6mm. 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 V. dahliae were moderately reflective and thin, their diameter was mainly 2-3μm, and their thickness was uniform; the angles between the hyphae were small, mostly acute; and chlamydomspore-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 V. 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 V. 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 V. 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 V. 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.

References

1. Dung JKS. Verticillium Wilt of Mint in the United States of America. Plants (Basel). 2020 Nov 18;9(11):1602.

2. Shaban M, Miao Y, Ullah A, Khan AQ, Menghwar H, Khan AH, Ahmed MM, Tabassum MA, Zhu L. Physiological and molecular mechanism of defense in cotton against Verticillium dahliae. Plant Physiol Biochem. 2018 Apr;125:193-204.
3. Carroll CL, Carter CA, Goodhue RE, Lawell CCL, Subbarao KV. A Review of Control Options and Externalities for Verticillium Wilts. Phytopathology. 2018 Feb;108(2):160-171.

4. Shi W, Wang T, Xie L, Li S, Gao H, Liu J, Li H. Risk Factors, Clinical Features, and Outcomes of Recurrent Fungal Keratitis after Corneal Transplantation. Ophthalmology. 2010, 117(5):890-6.

5. Xie L, Zhai H, Zhao J, Sun S, Shi W, Dong X. Antifungal susceptibility for common pathogens of fungal keratitis in Shandong Province, China. Am J Ophthalmol. 2008 Aug;146(2):260-265.

6. Lu X, Wang X, Zhang L, Li X, Qi X. Rare Fungal Keratitis Caused by Coprinellus Radians. Mycopathologia. 2020 Apr;185(2):389-394.

7. Mahmoudi S, Masoomi A, Ahmadikia K, Tabatabaei SA, Soleimani M, Rezaie S, Ghahvechian H, Banafsheafshan A. Fungal keratitis: An overview of clinical and laboratory aspects. Mycoses. 2018 Dec;61(12):916-930.

8. Watson SL, Cabrera-Aguas M, Keay L, Khoo P, McCall D, Lahra MM. The clinical and microbiological features and outcomes of fungal keratitis over 9 years in Sydney, Australia. Mycoses. 2020 Jan;63(1):43-51.

9. Hsu HY, Ernst B, Schmidt EJ, Parihar R, Horwood C, Edelstein SL. Laboratory Results, Epidemiologic Features, and Outcome Analyses of Microbial Keratitis: A 15-Year Review From St. Louis. Am J Ophthalmol. 2019 Feb;198:54-62.

10. Wei ZY, Liang QF. [Progress of clinical diagnosis and treatment in fungal keratitis]. Zhonghua Yan Ke Za Zhi. 2020 Aug 11;56(8):631-636.
11. Xie L, Zhong W, Shi W, Sun S. Spectrum of fungal keratitis in north China. Ophthalmology. 2006 Nov;113(11):1943-8.

12. Permpalung N, Worasilchai N, Chindamporn A. Human Pythiosis: Emergence of Fungal-Like Organism. Mycopathologia. 2020 Oct;185(5):801-812.

13. Ghosh A, Kaur H, Gupta A, Singh S, Rudramurthy SM, Gupta S, Chakrabarti A. Emerging Dematiaceous and Hyaline Fungi Causing Keratitis in a Tertiary Care Centre From North India. Cornea. 2020 Jul;39(7):868-876.

14. Buchta V, Nekolová J, Jirásková N, Bolehovská R, Wipler J, Hubka V. Fungal Keratitis Caused by Colletotrichum dematium: Case Study and Review. Mycopathologia. 2019 Jun;184(3):441-453.

15. Wang L, Yu H, Jiang L, Wu J, Yi M. Fungal keratitis caused by a rare pathogen, Colletotrichum gloeosporioides, in an east coast city of China. J Mycol Med. 2020 Apr;30(1):100922.

16. Shin JY, Kim HM, Hong JW. Keratitis caused by Verticillium species. Cornea. 2002 Mar;21(2):240-2.

17. Sahay P, Goel S, Nagpal R, Maharana PK, Sinha R, Agarwal T, Sharma N, Titiyal JS. Infectious Keratitis Caused by Rare and Emerging Micro-Organisms. Curr Eye Res. 2020 Jul;45(7):761-773.

18. Showail MJ, Kus JV, Tsui GK, Chew HF. Fungal keratitis caused by Metarhizium anisopliae complex. Med Mycol Case Rep. 2017 Jun 23;17:28-30.

19. Tabatabaei SA, Tabatabaei M, Soleimani M, Tafti ZF. Fungal keratitis caused by rare organisms. J Curr Ophthalmol. 2017 Aug 26;30(1):91-96.
2019. Patel DV, Zhang J, McGhee CN. In vivo confocal microscopy of the inflamed anterior segment: A review of clinical and research applications. Clin Exp Ophthalmol. 2019 Apr;47(3):334-345.

21. Chidambaram JD, Prajna NV, Larke N, Macleod D, Srikanthi P, Lanjewar S, Shah M, Lalitha P, Elakkiya S, Burton MJ. In vivo confocal microscopy appearance of Fusarium and Aspergillus species in fungal keratitis. Br J Ophthalmol. 2017 Aug;101(8):1119-1123.

22. Wang JY, Wang DQ, Qi XL, Cheng J, Xie LX. Modified ulcer debridement in the treatment of the superficial fungal infection of the cornea. Int J Ophthalmol. 2018 Feb 18;11(2):223-229.
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.

Fig. 2 A 10% potassium hydroxide smear (magnification of × 400), and B Calcofluor White Staining (magnification of ×400) showed a large number of complete hyphae, which were thin and regular in width.

Fig. 3 A and B confocal microscopy showed a large number of hyphae were densely distributed. The hyphae were moderately reflective, upright, and slender. Their diameters were mainly 2-3 μm, there were many branches, and the angles between the hyphae were small, mostly acute.

Fig. 4 A Colony morphology of SDA medium cultured at 28°C for 8 days showed that the colonies grew slowly and were white, powdery, or fluffy, and B the reverse was light orange. C SDA medium was cultured at 28°C for 8 days and stained with lactophenol cotton blue staining (a magnification of × 400).