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Nigrospora oryzae causing human corneal keratitis: A case report

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[ABSTRACT]

Purpose: We report a rare case of microbial keratitis caused by *Nigrospora oryzae*.
Observations: A 72-year-old Japanese woman was injured by plant debris and developed oval corneal ulcers and hypopyon in the anterior chamber. After 5 days, she complained of pain, redness, and vision loss in her left eye and was treated with antibacterial eye drops and an ointment (1.5 % levofloxacin hydrate, cefmenoxime hydrochloride, and sterilization and disinfection eye drops; SAN-IODE and ofloxacin ophthalmic ointment). Examination revealed a worsening oval corneal ulcer with Descemet's folds and a faint hypopyon. Considering the infection from soil or plants and the poor response to intensive antibacterial eye drops, topical antifungal eye drops, i.e., 1 % voriconazole eye drops, and 1 % natamycin ointment were applied. Direct microscopy of the corneal scraping with Gram staining was performed and the result was negative. Cultures from corneal scrapings showed the growth of dark colonies after several days. The colony was identified as *Nigrospora oryzae* by sequencing of the fungal internal transcribed spacer region. Pain and vision loss improved with improvement in corneal ulcers. The antifungal treatment was administered for 37 days. Discontinuation of the eye drops after 1 month did not result in keratitis recurrence. At the final follow-up at 70 days, the best-corrected visual acuity was 20/25, with persistent small corneal opacity.
Conclusions and importance: Here, we report a case of fungal keratitis caused by *Nigrospora oryzae*. Microbiological identification of the causes of rare infections is difficult in clinical laboratories, necessitating the use of advanced molecular techniques based on amplification and sequencing of appropriate phylogenetic markers. *Nigrospora oryzae* responds to topical voriconazole and natamycin.

1. [Introduction]

Although human infections with *Nigrospora* species, particularly *Nigrospora sphererica*, have been reported, corneal ulcers, respiratory allergies, onychomycosis, deep fungal infections, and skin infections have been observed in both immunocompetent and immunocompromised patients.[1–5] *N. oryzae* is a plant pathogen[6,7] that has caused opportunistic pulmonary infections in humans for the first time.[8] Here, we report a rare case of keratitis caused by *N. oryzae* in a non-immunocompromised host.

2. [Case report]

A 72-year-old Japanese woman was injured by plant debris in her right eye. Three days after the injury, she visited another clinic complaining of foreign body sensation and pain. A corneal ulcer was identified, and she was prescribed antibacterial eye drops (1.5 % levofloxacin hydrate, cefmenoxime hydrochloride, SAN-IODE; Santen Pharma) administered every hour along with ofloxacin ophthalmic ointment. After two days of antibacterial treatment, as the corneal ulcer worsened and a hypopyon was detected in the anterior chamber, the patient was referred to our hospital for further evaluation and treatment. The patient had no relevant medical history.

Slit-lamp examination revealed mild conjunctival hyperemia, an oval corneal ulcer 2 mm in diameter, surrounding anterior stromal haze with Descemet's folds, and a faint hypopyon (day 6; Fig. 1). She could not open her eyes sufficiently because of severe pain; thus, pain and visual acuity could not be measured. Cell cultures were obtained from the corneal scrapings. Based on her medical history, slit-lamp examination, corneal ulcer triggered by plant trauma, and poor response to antibiotic therapy, topical fungal therapy with 1 % voriconazole (Vfend; Pfizer Inc., New York, New York, USA) eye drops was administered every hour, along with 1 % natamycin ophthalmic ointment (Senju Pharmaceutical Co., Ltd.) once before sleep. Antibacterial eye drops

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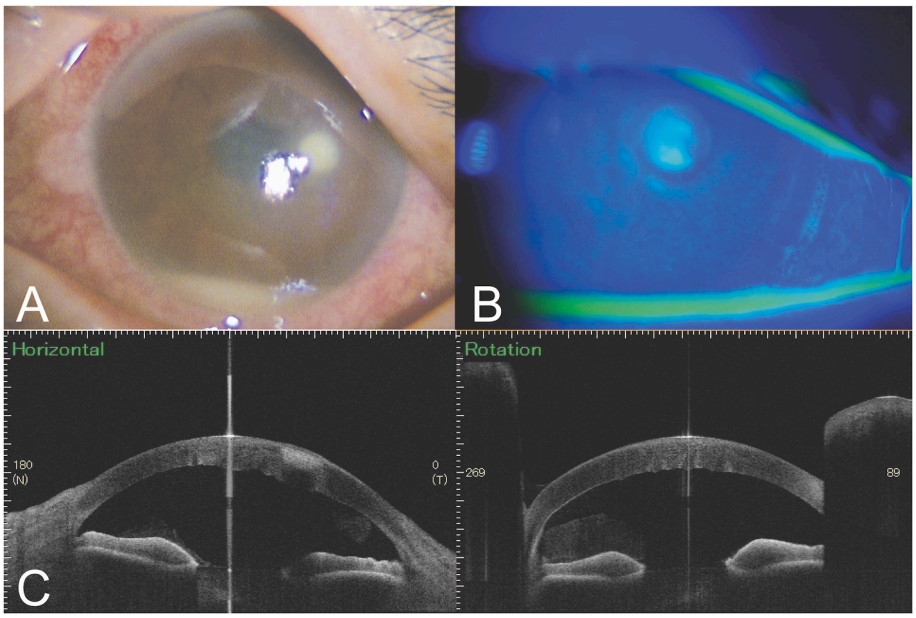

Fig. 1. Clinical observations on day 1. A: The slit-lamp image shows an oval corneal ulcer surrounded by anterior stromal haze with Descemet's folds. B: Slit-lamp image after fluorescein staining, showing ulcers and diffuse superficial punctate keratitis. C: Anterior-segment optical coherence tomography showing an ulcer confined to the corneal stroma.

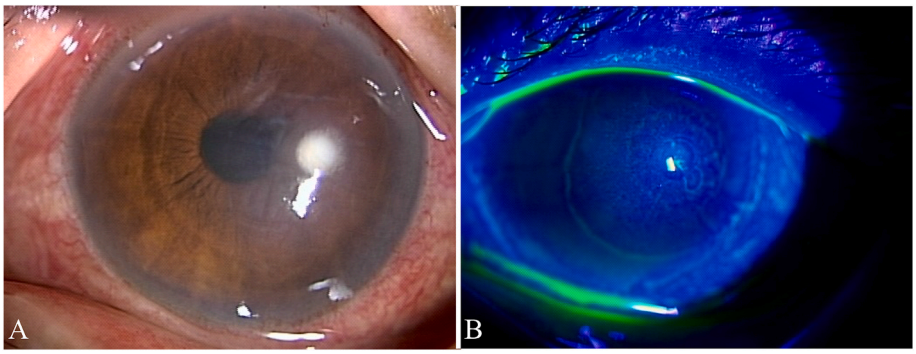

Fig. 2. Clinical observations on day 5. A: Close-up slit-lamp image showing a marginally smaller corneal ulcer and no hypopyon. B: Slit-lamp image after fluorescein staining showing less-defined ulcer margins and improvement in diffuse superficial punctate keratitis.

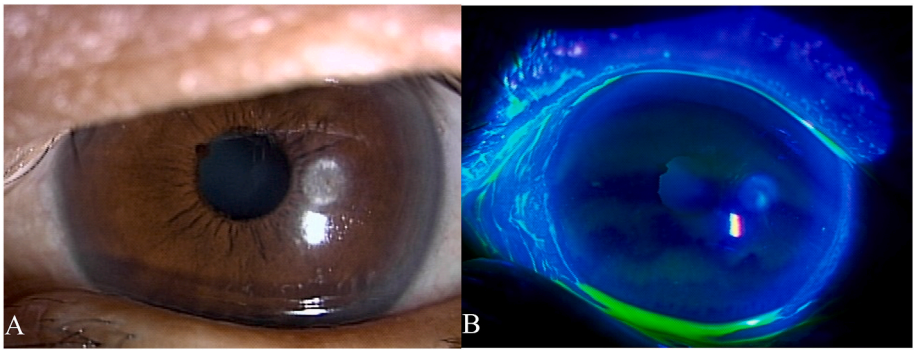

Fig. 3. Clinical observations on day 65. A: Slit-lamp image showing persistent corneal opacity with resolved conjunctival hyperemia, faint hypopyon, and no Descemet's folds. B: Slit-lamp image after fluorescein staining showing resolved ulcers and superficial punctate keratitis.

were administered four times daily to prevent superinfection.

After five days, her visual acuity and intraocular pressure were 20/250 and 23 mmHg, respectively. Despite no apparent improvement in the subjective symptoms, the corneal ulcer size was slightly smaller, and no hypopyon was detected. Therefore, the dose of voriconazole eye drops was decreased to every 2 h, and SAN-IODE was discontinued (day 11; Fig. 2). At 21 days post-injury (day 21), a slit-lamp examination revealed corneal opacity without an epi defect. Visual acuity and intraocular pressure improved to 20/40 and 17 mmHg, respectively. Although the corneal opacity persisted, the pain and subjective vision

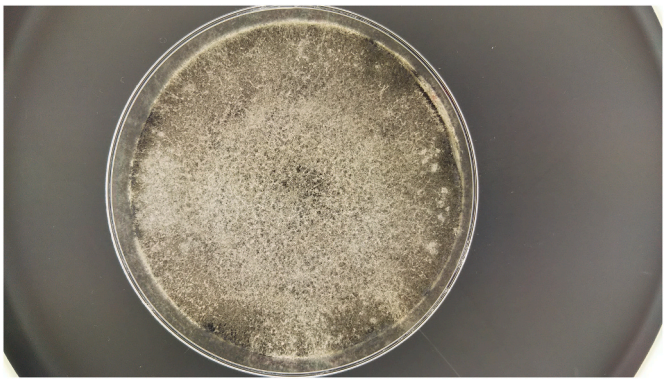

Fig. 4. Formation of a black colony. The fungal culture isolated from the corneal scrapings formed a black colony as shown.

improved. She was prescribed with 0.1 % fluorometholone eye drops thrice daily and maintenance doses of voriconazole eye drops and 1.5 % levofloxacin hydrate eye drops four and three times daily, respectively. On day 42, visual acuity improved to 20/20, and opacity decreased considerably. Therefore, voriconazole eye drops were discontinued, and fluorometholone and 1.5 % levofloxacin hydrate eye drops were maintained thrice per day until day 49. No recurrence of keratitis was observed on day 70 (Fig. 3).

Direct microscopy of corneal scraping with Gram staining yielded a negative result. After several days, the initial culture was positive for mold with black colonies (Fig. 4). Morphological examination showed that the conidiophores are colorless to light brown, curved and branching, with conidia being black and ranging from spherical to broadly ellipsoidal in shape (Fig. 5). We did not retest the initial sample or repeat corneal scraping to negate contamination or identify bacteria. Identification using MALDI-TOF MS was impossible because of the lack of equipment at our hospital. The fungal samples were sent to the Medical Mycology Research Center, Chiba University, and *N. oryzae* was identified by sequencing the internal transcribed spacer (ITS) gene of the strain (Fig. 6). Susceptibility testing was not performed due to poor sporulation.

The clinical presentations, related treatments, and outcomes are presented as a timeline (Fig. 7).

3. [Discussion]

Herein, we report a case of fungal keratitis in which *N. oryzae* was isolated from a corneal culture. The genus *Nigrospora* belongs to the family Apiosporaceae of the phylum Ascomycota and is predominantly a plant-pathogenic saprobic fungus with few reports of human infections. To the best of our knowledge, only two reports of *N. oryzae* infection in humans have been published to date. One report discussed pulmonary infection in patients with bronchogenic cancer[8] while another described isolation from human epidermal scrapings.[9] After conducting a literature review on January 12, 2024, using PubMed and Google Scholar with the keywords (keratitis, *Nigrospora oryzae*), we did not find any prior reports of *N. oryzae* causing corneal keratitis.

Although in a previous study *N. oryzae* showed decreased susceptibility to many antifungal agents (MIC >1 μg/mL),[9] other reports have demonstrated the inhibition of *N. oryzae* by voriconazole and anidulafungin, which are potent *in vitro* antifungal agents.[8] Three cases of keratitis caused by *Nigrospora* spp. have been reported,[1,4,5] with treatment details provided in only one instance.[5] Improvement was not achieved with oral ketoconazole and natamycin eye drops, but was achieved with oral administration of voriconazole and eye drops. As no antifungal susceptibility test was performed and susceptibility testing

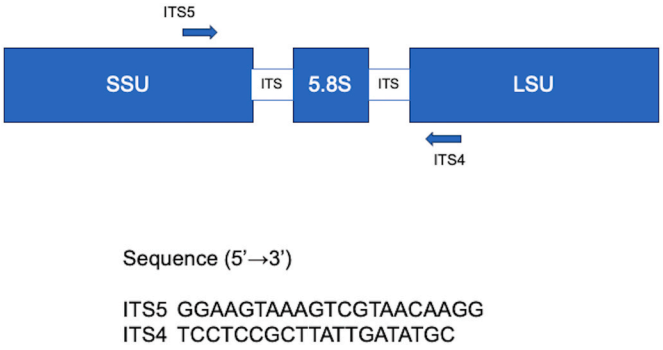

Fig. 6. Primers for amplification of the ITS region. The positions of the forward (right-pointing arrow) and reverse (left-pointing arrow) primers are shown on a map of the ITS regions. SSU, small subunit rDNA; LSU, large subunit rDNA; ITS, internal transcribed spacer.

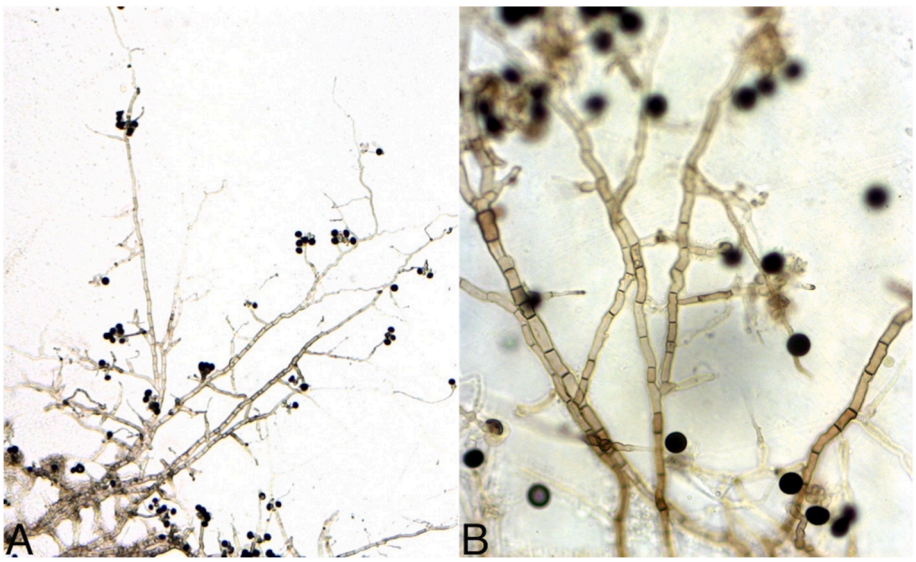

Fig. 5. Colony of *N.Oryzae*. A: Conidiophores and conidia were observed at 40 × magnification. B: The conidiophores appeared colorless to light brown and exhibit curved branching, while the conidia were black and range from spherical to broadly ellipsoidal in shape at 200 × magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

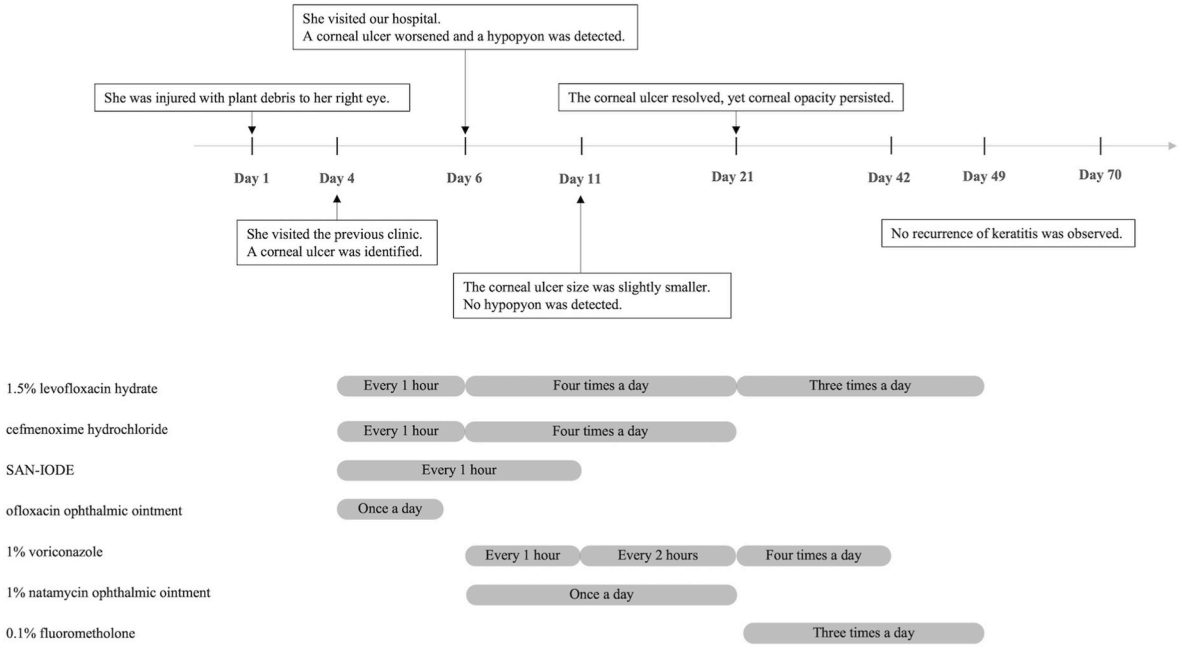

Fig. 7. Treatment timeline showing clinical presentations, related treatments, and outcomes.

for filamentous fungi is not covered by insurance in Japan, data on the susceptibility of the current fungal strains were unavailable. *N. oryzae* has been hypothesized to respond to topical voriconazole and natamycin.

N. oryzae causes opportunistic infection.[6] However, our patient had no history of immunosuppression. In the present case, the patient did not respond to intensive antibiotic treatment, and improvement was observed only after intense antifungal treatment. Furthermore, a black colony formed in the corneal scraping culture, and no other bacterial cultures were isolated. Although these facts suggest that *N. oryzae* can cause keratitis even in the absence of immunosuppression, we did not retest the initial sample or repeat the corneal scraping to negate contamination or identify the bacteria. Thus, the possibility of contamination of the *N. oryzae* culture cannot be excluded.

Although corticosteroids are considered harmful for the treatment of fungal keratitis,[10] they may reduce inflammation and subsequent scarring. Previous randomized trials have shown that corticosteroids are safe and effective for herpetic keratitis when used with appropriate antiviral therapy, and are safe for bacterial keratitis when used with broad-spectrum topical antibiotics.[11] In general, the efficacy of steroids against fungal keratitis has not been demonstrated, although observational human studies have found that steroids are harmful to fungal keratitis prior to diagnosis.[12] In the present case, antifungal eye drops were administered to treat fungal keratitis prior to corticosteroid use. Corticosteroids were initiated after the epithelial defect healed. We believe that corticosteroid eye drops helped reduce corneal scarring. However, further studies are required to evaluate the efficacy and safety of steroid eye drops in the treatment of fungal keratitis.

MALDI-TOF MS is used for the rapid, accurate, and cost-effective identification of cultured bacteria and fungi in clinical microbiology.[13,14] It is a novel method for identifying target strains by searching and collating with a known standard strain library,[15] and 93.2 % of fungi have been identified to the species level using MALDI-TOF MS.[16] This technique is particularly useful for identifying rare organisms, especially those whose morphological features are not well known or defined.[17] PCR gene sequencing has been used to identify pathogens associated with various corneal infections.[18] The overall sensitivity and specificity of PCR for the diagnosis of fungal keratitis range from 57 to 91 % and 79 %, respectively.[17] This technique is superior to conventional culture tests in terms of testing time and detection rate.[19,20] PCR gene sequencing may be useful for identifying fungi that cannot be identified using culture or MALDI-TOF MS, as in this case.

4. [Conclusions]

Herein, we report a case of corneal infection caused by *N. oryzae* in an immunocompetent patient. *N. oryzae* could not be identified using MALDI-TOF MS. However, the keratitis was successfully treated using voriconazole eye drops and natamycin. Plant-induced corneal injury can result in fungal keratitis, which is occasionally caused by rare fungi. Culturing fungi is often time consuming; therefore, a culture must be obtained from the outset.

5. [Patient consent]

Written informed consent was obtained from the patient for the publication of this case report and the accompanying images. A copy of the written consent form is available for review by the editor-in-chief of the journal.

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Conflicts of interest

T.T. has no financial disclosures.

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Authorship

All authors attest that they meet the current ICMJE criteria for authorship.

CRediT authorship contribution statement

Takuya Takayama: Writing – original draft. **Satoru Inoda:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Akira Watanabe:** Visualization, Resources. **Hidetoshi Kawashima:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The following authors have no financial disclosures: T.T.,

S.I.: Lecturer's fees from Kowa, Novartis, and DeepEyeVison. Inc, and grants from Novartis, outside this work.

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