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Case Report

Microbiology Section

A Rare Case Report of *Pythium insidiosum* keratitis in an Immunocompetent Patient

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ABSTRACT

Corneal ulcers are one of the major causes of monocular blindness after cataracts in developing nations and have a varied aetiology. One of the emerging causes of corneal ulcers closely resembling fungi in its morphology is the *Pythium insidiosum* which is known to infect a variety of animals, but in humans, only a few cases have been reported causing ocular, cutaneous/subcutaneous, vascular, and disseminated infections. This was a case of a 29-year-old male, non agriculturist by occupation who presented to the Ophthalmology Outpatient Department with a history of diminution of vision, redness and watering of the left eye in the last five days. On further investigations, he was diagnosed to have a corneal ulcer with *P. insidiosum* being the aetiological agent. The patient was managed surgically by penetrating keratoplasty and medically by combination therapy consisting of moxifloxacin eyedrops every 4th hour, voriconazole eye drops 2 hourly, natamycin eye drops 2nd hourly, oral fluconazole 150 mg thrice daily for one week.

Keywords: Corneal ulcer, Fungus-like organism, Keratitis, Parasitic oomycete

CASE REPORT

A 29-year-old male who was a non agriculturist by occupation presented to the Ophthalmology Outpatient Department with a history of diminution of vision, redness and watering of the left eye in the last five days. On ophthalmic examination, his visual acuity in the right eye was 6/18 and his left eye had the Perception of Light (PL)+Projection of Rays (PR) accurate. Examination of left eye anterior segment showed haziness of corneal stroma with central corneal ulcer measuring 6×7 mm. Corneal vascularisation was present from 4 O' clock to 7 O' clock position with 2 mm hypopyon.

There was no significant past or family history. The patient's total leucocyte count was 11,800 cells/mm^3, and his Erythrocyte Sedimentation Rate (ESR) was 34 mm/1st hr.

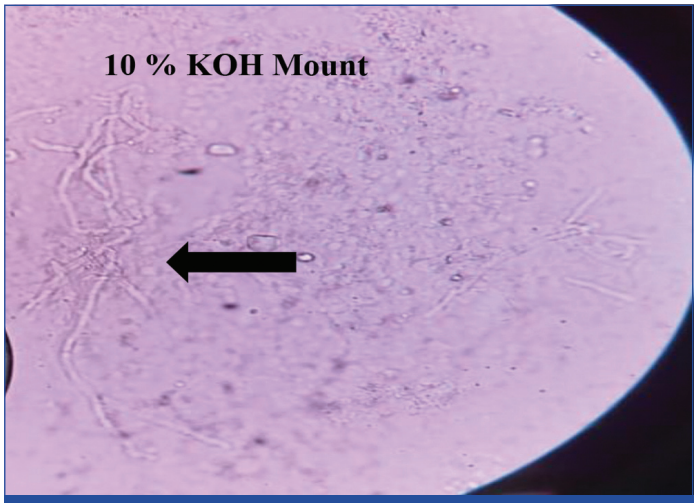
Corneal scrapings from the ulcer were sent to the microbiology lab for Gram staining, aerobic culture and antibiotic susceptibility testing, Potassium Hydroxide wet mount (KOH mount), and fungal culture, after which the patient was started empirically on moxifloxacin 0.5% eyedrops w/v every 4th hourly and voriconazole 1% w/v eyedrops every 2nd hourly. In direct microscopy with a 10% potassium hydroxide (KOH) mount, the sample showed hyaline, aseptate hyphae [Table/Fig-1]. Gram stain showed occasional White Blood Cells (WBC) with fungal hyphae [Table/Fig-2]. The corneal scrapings were inoculated on a blood agar plate and Sabouraud Dextrose Agar (SDA).

Blood agar plate showed two types of colonies:

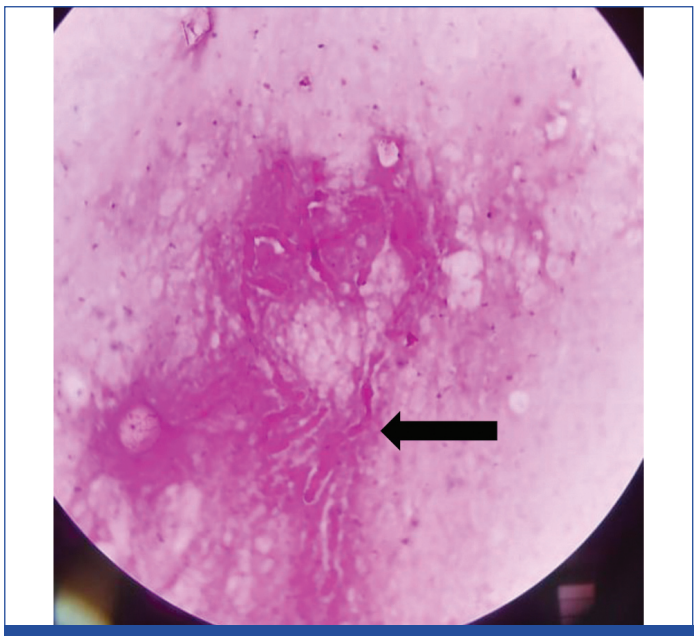
- Colony 1: A flat grey-white colony identified as Methicillin sensitive Coagulase-negative Staphylococcus (MSCoNS) sensitive to vancomycin, co-trimoxazole, erythromycin, clindamycin, linezolid, teicoplanin, cloxacillin, and doxycycline.
- Colony 2: Light grey feathery, partially submerged colonies that could not be scraped or picked.

On SDA it was flat, colourless to light brown leathery growth which was identified by slide culture as *P. insidiosum* with lactophenol cotton blue and later confirmed by the molecular methods (polymerase chain reaction) [Table/Fig-3a,b].

The patient underwent penetrating keratoplasty under local anaesthesia following which the topical medications were continued. The patient was administered a combination therapy consisting of moxifloxacin eyedrops every 4th hour, voriconazole eye drops 2 hourly, natamycin eye drops 2nd hourly, oral fluconazole 150 mg

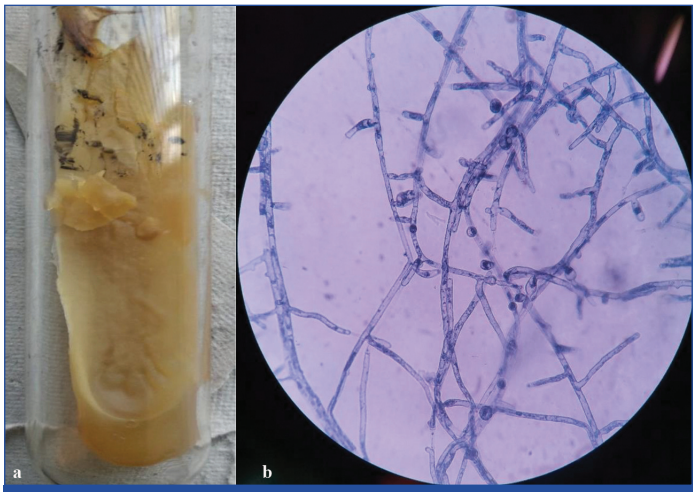


[Table/Fig-1]: Hyaline broad aseptate hyphae seen (black arrow); 10% KOH Mount (40X).



[Table/Fig-2]: Occasional WBC's with fungal elements (black arrow); Gram stain (100x).

thrice daily for 1 week. The patient was advised for a weekly follow-up but he was lost to follow-up.



[Table/Fig-3]: (a) On SDA flat, colourless to light brown leathery growth seen. (b) Slide culture illustrating the broad, branched, and sparsely septate hyphae of *Pythium insidiosum* (lactophenol cotton-blue, magnification 40X). (Images from left to right)

DISCUSSION

The aetiology of corneal ulcers differs in different geographical areas with bacterial aetiology most commonly reported from North America, Australia, Netherlands, and Singapore and that of fungal corneal ulcers (Aspergillus, Fusarium, *Scedosporium apiospermum*, phaeohyphomycetes, *Candida albicans*, and other Candida species) from India and Nepal, especially in the warmer, humid parts of the country. Fungal aetiologies account for only 5% to 10% of all corneal infections, most often precipitated by trauma to the cornea with subsequent exposure to plant or vegetable material [1,2].

Keratitis caused by a parasitic aquatic oomycete *Pythium* resembling fungal keratitis is also known as "parafungus" or "fungus-like organism", occuring in two forms which are an infective form of biflagellate zoospore in aquatic agricultural surroundings and a hyphal form in the ulcer, which belongs to the phylum Straminipila. However, the literature on incidence and prevalence of Pythium keratitis are very few due to the organism either being very rarely isolated or misdiagnosed as fungus and difficult to treat due to its poor response to conventional antifungal medication and surgical procedures such as penetrating keratoplasty. In 1884, the first case of systemic Pythiosis was reported by British veterinarians [3].

Its prevalence is high in tropical, subtropical, and temperate conditions due to its aquatic habitat and is more commonly seen in males, particularly field workers, and in this present case, the patient was not a field worker [4]. Thailand has recorded the most ocular pythiosis cases, however recently in 2015, India has been highlighted due to the nine reported cases [5].

However, in the recent past, we have noted an increase in the number of keratitis cases as observed by studies done by Sharma S et al., who reported a total of 11 cases, Hasika R et al., in South India who reported 71 patients with microbiologically proven *Pythium* keratitis in 22 months durations and Agrawal S et al., who reported 10 patients over 18 month's period [4,6,7]. A recent study done by Vishwakarma P et al., in Eastern India with a study period of 36 months had reported 18 patients (1.4%) to be culture positive for *Pythium* among 1251 patients who were diagnosed to have keratitis [8].

It has been reported that *Pythium* keratitis is caused by exposure to aquatic agricultural surroundings where it is in the infective form of the biflagellate zoospore [6]. However, non agricultural exposure like a contact lens or exposure to dust particles is also documented to be a risk factor for *Pythium* keratitis [9].

In the present study, the patient was a non agriculturist and similar case presentations of non agricultural workers were seen in other studies like Gurnani B et al., 16 (53.3%), Agarwal S et al., where 40 patients and Bagga B et al., reported 36% of cases had no history of injury [3,10,11].

The laboratory evaluation of this infection depends on examination of corneal scrapings in 10% KOH mount (appear as septate or aseptate hyphae with perpendicular or obtuse lateral branching). Apart from knowledge of the typical growth patterns on media like blood agar, chocolate agar and SDA prompt diagnosis by zoospore formation by leaf incarnation method can also be done [9]. Molecular methods of diagnosing include Polymerase Chain Reaction (PCR), based on the amplification of the Internal Transcribed Spacer (ITS) region, ribosomal Intergenic Spacer (IGS) region, and gene Cytochrome Oxidase (COX2) [12].

In the present study, a molecular method like PCR was used to confirm the diagnosis. A study done by Sharma S et al., identified thirteen *Pythium* keratitis cases using molecular methods [4]. Salipante SJ et al., had reported a case of subcutaneous *Pythium insidiosum* infection using molecular methods (PCR screening and Deoxyribonucleic acid sequencing) [13].

Earlier, 1st line of treatment was antifungals in the form of 5% natamycin suspension hourly, 1% voriconazole, or 1% itraconazole hourly were considered as *Pythium* was considered as fungal aetiology. But now, topical antibacterials, most commonly 0.2% linezolid and 1% azithromycin hourly along with early therapeutic keratoplasty with a good 1 mm margin clearance shows a good response [9]. In this case, the patient was administered a combination therapy consisting of moxifloxacin eye drops every 4 hours, voriconazole eye drops 2 hourly, natamycin eye drops 2 hourly, and oral fluconazole 150 mg thrice daily. However, the patient was lost to follow-up.

CONCLUSION(S)

The infection may not be as rare as previously thought as it is usually misdiagnosed as fungal keratitis or unrecognised due to a lack of knowledge of the organism. Knowledge and awareness are required about *P. insidiosum* as it could be confused as one of the zygomycetes due to its aseptate hyphal elements. A detailed clinical history regarding risk factors and exposure, along with a thorough slit lamp examination with fluorescein dye will also aid in the proper diagnosis of the organism.

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REFERENCES

[1] Suwal S, Bhandari D, Thapa P, Shrestha MK, Amatya J. Microbiological profile of corneal ulcer cases diagnosed in a tertiary care ophthalmological institute in Nepal. BMC Ophthalmol. 2016;16(1):01-06.
[2] Virgile R, Perry HD, Pardanani B, Szabo K, Rahn EK, Stone J, et al. Human infectious corneal ulcer caused by Pythium insidiosum. Cornea. 1993;12(1):81-83.
[3] Gurnani B, Christy J, Narayana S, Rajkumar P, Kaur K, Gubert J. Retrospective multifactorial analysis of Pythium keratitis and review of literature. Indian J Ophthalmol. 2021;69(5):1095.
[4] Sharma S, Balne PK, Motukupally SR, Das S, Garg P, Sahu SK, et al. Pythium insidiosum keratitis: Clinical profile and role of DNA sequencing and zoospore formation in diagnosis. Cornea. 2015;34(4):438-42. Doi: 10.1097/ICO.0000000000000349. PMID: 25738236.
[5] He H, Liu H, Chen X, Wu J, He M, Zhong X. Diagnosis and treatment of Pythium insidiosum corneal ulcer in a Chinese child: A case report and literature review. Am J Case Rep. 2016;17:982.
[6] Hasika R, Lalitha P, Radhakrishnan N, Rameshkumar G, Prajna NV, Srinivasan M. Pythium keratitis in South India: Incidence, clinical profile, management, and treatment recommendation. Indian J Ophthalmol. 2019;67(1):42. Doi:10.4103/ijo.IJO_445_18. PMID: 30574890. PMCID: PMC6324135.
[7] Agarwal S, Iyer G, Srinivasan B, Agarwal M, Kumar SP, Therese LK. Clinical profile of pythium keratitis: Perioperative measures to reduce risk of recurrence. Br J Ophthalmol. 2018;102(2):153-57.

[8] Vishwakarma P, Mohanty A, Kaur A, Das S, Priyadarshini SR, Mitra S, et al. Pythium keratitis: Clinical profile, laboratory diagnosis, treatment, and histopathology features post-treatment at a tertiary eye care center in Eastern India. Indian J Ophthalmol. 2021;69(6):1544.
[9] Gurnani B, Kaur K. Pythium Keratitis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK573072/. [Updated 2021 Dec 20].
[10] Agarwal S, Iyer G, Srinivasan B, Benurwar S, Agarwal M, Narayanan N, et al. Clinical profile, risk factors and outcome of medical, surgical and adjunct interventions in patients with Pythium insidiosum keratitis. Br J Ophthalmol. 2019;103(3):296-300.
[11] Bagga B, Sharma S, Guda SJ, Nagpal R, Joseph J, Manjulatha K, et al. Leap forward in the treatment of Pythium insidiosum keratitis. Br J Ophthalmol. 2018;102(12):1629-33.
[12] Kulandai LT, Lakshmipathy D, Sargunam J. Novel duplex polymerase chain reaction for the rapid detection of Pythium insidiosum directly from corneal specimens of patients with ocular pythiosis. Cornea. 2020;39(6):775-78.
[13] Salipante SJ, Hoogestraat DR, SenGupta DJ, Murphey D, Panayides K, Hamilton E, et al. Molecular diagnosis of subcutaneous Pythium insidiosum infection by use of PCR screening and DNA sequencing. J Clin Microbiol. 2012;50(4):1480-83.

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