Review Article

Pythium insidiosum keratitis - A review

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Pythium insidiosum is an oomycete and is also called "parafungus" as it closely mimics fungal keratitis. The last decade saw an unprecedented surge in Pythium keratitis cases, especially from Asia and India, probably due to growing research on the microorganism and improved diagnostic and treatment modalities. The clinical features such as subepithelial infiltrate, cotton wool-like fluffy stromal infiltrate, satellite lesions, corneal perforation, endoexudates, and anterior chamber hypopyon closely resemble fungus. The classical clinical features of Pythium that distinguish it from other microorganisms are reticular dots, tentacular projections, peripheral furrowing, and early limbal spread, which require a high index of clinical suspicion. Pythium also exhibits morphological and microbiological resemblance to fungus on routine smearing, revealing perpendicular or obtuse septate or aseptate branching hyphae. Culture on blood agar or any other nutritional agar is the gold standard for diagnosis. It grows as cream-colored white colonies with zoospores formation, further confirmed using the leaf incarnation method. Due to limited laboratory diagnostic modalities and delayed growth on culture, there was a recent shift toward various molecular diagnostic modalities such as polymerase chain reaction, confocal microscopy, ELISA, and immunodiffusion. As corneal scraping (10% KOH, Gram) reveals fungal hyphae, antifungals are started before the culture results are available. Recent *in vitro* molecular studies have suggested antibacterials as the first-line drugs in the form of 0.2% linezolid and 1% azithromycin. Early therapeutic keratoplasty is warranted in nonresolving cases. This review aims to describe the epidemiology, clinical features, laboratory and molecular diagnosis, and treatment of *Pythium insidiosum* keratitis.

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Pythium insidiosum is an aquatic oomycete that is commonly found in tropical/subtropical regions.[1] They are classified under kingdom Straminipila, phylum Oomycota, class Oomycetes, and family Pythiaceae.[2] It closely resembles fungus due to its zygomycetous branching features but typically lacks ergosterol in its cell wall.[3] Additionally, unlike fungus, asexual reproduction occurs through sporangia containing biflagellate zoospores.[3] While it exists in two forms, such as mycelium and zoospore in freshwater, infection is acquired through motile zoospores pathogenic to humans, horses, and dogs.[1,4] Different forms of infections include cutaneous, vascular, systemic, gastrointestinal, and ocular infections.[1]

Ocular infections are gaining importance in recent days owing to their highly virulent nature, poor visual prognosis due to lack of standard treatment regimen, high recurrence rate, and associated grave ocular morbidity.[2] Most of the challenges in its management are attributed to the delayed diagnosis by routine microbiological methods and its closely mimicking nature to the fungus that complicates the crucial initial treatment regimen. Various reports on ocular pythiosis

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kick-started after its discovery in 2015 by a team in South India.[5] Even today, it is believed that many of the Pythium infections in India and elsewhere might go unrecognized due to a lack of awareness about identification techniques.[5] The classical clinical features of Pythium, such as reticular dot infiltrates, tentacular projections, and peripheral furrowing, are observed in a significantly less number of cases. Still, their presence can help in early clinical diagnosis.[6] The recommended medical management is antifungals,[7] antibacterials,[8] and steroids post keratoplasty.[2] Antifungals still find a place in the treatment of Pythium keratitis because the microorganism can only be confirmed by culture on nutritional agar and lack of rapid diagnostic modalities such as polymerase chain reaction (PCR) and confocal microscopy at all centers.[2] Bagga *et al*.[3] demonstrated that antibacterials have higher efficacy than antifungals, and linezolid and azithromycin can be considered first-line treatment. All the major studies have embarked on early therapeutic keratoplasty (TPK) in Pythium keratitis to salvage the globe and prevent irreversible vision-threatening sequelae.[9] Although there is adequate literature with evidence on clinical characteristics and diagnostic techniques, treatment dilemma still exists due to various reports on management strategies.[2,3,7,10,11] This review aims to summarize and present the updated literature on pathogenesis, clinical signs, various lab diagnostic techniques, critical appraisal of different medical/surgical management, and special mention on pediatric ocular pythiosis.

Literature search

A comprehensive systematic literature search was undertaken using PubMed, Google Scholar, ePub, and Cochrane Library database. The literature search was performed using keywords such as *Pythium insidiosum*, Pythium keratitis, *Pythium insidiosum* keratitis, Pythium AND (review) AND (treatment or update) AND (Zoospore). All relevant review articles, original articles, case series, and reports were reviewed. The search was not limited by the year of publication, and a manual literature search was also performed from an authentic database.

Epidemiology

Dutch investigators in 1901 were the first to describe Pythium infection in horses, and later finally in 1987, De Cock *et al*.[12] formally described *Pythium insidiosum* based on its sexual sporulation.[1] It is classified in the Phylum Straminipila, Class Oomycetes, Order Pythiales, and Family Pythiaceae.[13] In humans, it can manifest as vascular, ocular subcutaneous, or cutaneous and disseminated infection.[14] The first case of Pythium keratitis was reported in Thailand, where it is considered endemic because of the climatic conditions.[15] Though cases have been reported from Australia,[16] Israel,[17] the USA,[18] China,[19] and other parts of the world, most of the cases have been primarily reported from the tropical and the subtropical areas such as India and Thailand.[7,11,20] Clinical, microbiological, and histopathological resemblance to fungal keratitis makes it difficult to diagnose until there is a high grade of suspicion, probably contributing to the underreporting.[7,11,20] Based on the ITS region or cytochrome oxidase II gene, *Pythium insidiosum* is classified into three clades related to their geographic location. Clade I (ATH) contains isolates from America, Clade II (BTH) from Asia and Australia, and Clade III (CTH) is from Thailand and the USA.[21] History of exposure to contaminated water or vegetative matter has been identified as a significant risk factor; however, almost 50% of patients were noted to be housewives, software professionals, or people from urban locales.[2] Varied clinical features such as subepithelial or superficial stromal infiltrate radiating in a reticular pattern, full-thickness infiltrates, and peripheral guttering have been described. It clinically resembles fungal keratitis, making the diagnosis difficult.[2,3] In the most extensive series of 114 patients, the reported mean age was 41 ± 14.3 years, with 43% male; 40.4% were farmers, and 56.6% were homemakers/office goers. In 55.3% of patients, no predisposing factor was identified. A median logMAR visual acuity of 2.78 (IQR: 1.5–2.78) with a range of 0.10–3.00 was noted in 92.1% of patients with a median size of corneal infiltrate being 5.5 × 6 mm (range: 1–10 mm both ways) at presentation.[3] Despite multiple treatment options being recommended, a standardized management protocol is still lacking; early surgical intervention with or without surgical adjuncts is still preferred the most.[2,3,11,22] In a recent study evaluating the outcome of medical management of 69 eyes, 38 resolved, and a significant difference was found between duration from onset of symptoms to presentation, infiltrate size, deeper corneal involvement, and host not responding to medical management and requiring surgical intervention.[23] Other studies have reported a high recurrence rate of 51.8% and 54.2% following a therapeutic graft compared to only 7.1% in those undergoing a therapeutic graft with intraoperative adjunct therapy, namely cryotherapy to the host edge and/or topical application of absolute alcohol-soaked sponge.[7,24]

Pathogenesis of *Pythium Insidiosum* Keratitis

Since 1974, *Pythium insidiosum* has been a known pathogen in plants and animals.[25] Later, it was found to cause life-threatening infections in humans, called Pythiosis.[1] The Pythium species asexually produce motile, biflagellate, 9-10-µm diameter zoospores, which are responsible for the infections. The pathogenesis of *Pythium insidiosum* is relatively imprecise.[26] Although the clinical features of *Pythium insidiosum* keratitis are relatable to fungal infection, the pathogenesis differs. Fungus usually causes infection in immunocompromised hosts, unlike Pythium, which affects healthy and young individuals.[27] Thus, understanding pathogenesis leads to a better perspective on treatments options and prognosis.

Predisposing risk factors and etiology

There are several risk factors for acquiring *Pythium insidiosum* infection. Agricultural or water-associated leisure activities are considered the major predisposing factors for pythiosis as zoospores develop in swampy areas.[28] When they contact the zoospores in the water, persons with open wounds quickly get infected with *Pythium insidiosum*.[28] Authors have also noted hot pools (New Zealand study) and swimming pools (Northern Australian study)[16] to be the source of Pythium. As mentioned previously, *Pythium insidiosum* causes infection more often in immunocompetent individuals. Agarwal noted infection in healthy young and middle-aged individuals, mostly software professionals and housewives.[11] Tanhehco *et al*.[17] noted contact lens use and water exposure as a predisposing factor for Pythium infection in a 21-year-old Israeli man. In Thailand, Thanathanee *et al*.[20] found thalassemia patients susceptible

to the infection due to iron overload. Open wound, host body temperature, and low CO_2 content increase the susceptibility for *Pythium insidiosum* infection.[28] Hung *et al*.[29] reported *Pythium insidiosum* in immunocompromised individuals with Crohn's disease. In the majority of cases of Pythium keratitis, there is a history of preceding trauma. Very rarely, the patient is not aware of trauma.[2] Hasika *et al*.,[7] in their analysis, reported various risk factors such as dust fall, insect fall, vegetative matter injury, and exposure to dirty water. Gurnani *et al*.[2] reported bathing in pond water as another significant risk factor. Vishwakarma *et al*.[30] reported cement and agricultural injury as other contributory risk factors.

Pathogenesis

The pathology of fungal infections, upon contact, is mediated by the interaction of adhesins and the breached epithelial surfaces. They are recognized by the pattern recognition receptors expressed on the host epithelial surface and stimulate further tissue damage. In *Pythium insidiosum*, the infective propagules are the zoospores; when they contact the denuded epithelial surface in the wet environment, they secrete a sticky amorphous substance called glycoproteins mediates strong adhesion of zoospores to the epithelial surface. Stimulated by the host body temperature, zoospore develops a germ tube (hyphae) that extends into the infected tissue and directly invades the blood vessels, leading to easier penetration into body tissues. They are attracted by the low CO_2 levels in the skin and cornea, causing fulminant systemic pythiosis and rapid corneal stromal destruction, respectively.[1] The detailed mechanism of pathogenesis is depicted in Fig. 1.

Salient pathological features of *Pythium insidiosum* keratitis

Several authors described the virulence factors and the susceptibility of the hosts. Often, Pythium keratitis is misdiagnosed as fungal clinically and morphologically.[31] It is unresponsive to antifungals due to lack of ergosterol in the cell membrane.[7,31] Rapid progression of the disease despite the host immune response is not clearly explained in the literature. Mordoch and Parr *et al*.[32] tried to demystify the pathogenesis through the mechanism of the formation of zoospores. Within 1 h of induction, zoospores are formed and rapidly encyst producing germ tubes within 24 h, resulting in large quantities of mycelium, and thus responsible for the fulminant course of the disease. Krajaejun *et al*.[31] identified *Pythium insidiosum* genes involved in oxidative stress response, Cu-Zn superoxide dismutase, thioredoxin, and glutaredoxin. He also expressed sequence tags and identified 16 putative proteins having homology to virulence factors of fungus. Worasilchai N *et al*.[33] identified beta-D glucan and *Pythium insidiosum* IgG antibodies as potential markers in vascular pythiosis. Krajaejun *et al*.[31] also identified calmodulin and heat shock transcription factors through expressed sequence tags, responsible for pathogens' growth and thermal adaptation inside the host. Lelievre L *et al*.[34] described the susceptibility of thalassemia patients to *Pythium insidiosum* due to the expression of gene encoding ferrochelatase, an enzyme required for the final step of heme biosynthesis. The unbound iron is attracted to the microbial heme and stored as a source for further metabolic uses. Torto-Alalibo *et al*.[35] in 2005 described proteases as a potent virulent factor for causing keratitis in humans.

Clinical Features

Symptoms

The patients usually present with typical symptoms of corneal ulcer, such as pain, redness, watering, discharge, photophobia, and blurring of vision.[9,36]

Signs

Typical features

Pythium keratitis is an oomycete that causes vision-threatening infectious keratitis. It closely mimics fungal keratitis and requires a high index of clinical suspicion to distinguish it from fungal keratitis on slit-lamp examination. The typical clinical features that distinguish it from fungal keratitis are patchy reticular dot-like subepithelial and stromal infiltrate, multifocal infiltrates, cotton wool-like stromal infiltrate with hyphated edges, peripheral furrowing, early limbal spread, and peripheral corneal thinning with guttering and tentacular projections.[2] Fig. 2 depicts the hallmark features of Pythium keratitis.

Atypical features

The past decade has seen an upsurge in clinical cases of Pythium due to a high index of clinical suspicion, improved diagnostic modalities, and growing research considering treatment of this devastating entity.[2,3] The atypical features mimic other infectious keratitis and have been discussed under the following heads.

Clinical features resembling Fungal keratitis: Epithelial defect, creamy white stromal infiltrate, stromal edema, feathery margins, satellite lesions, stromal melt, endothelial plaque, ring infiltrate, hypopyon, corneal perforation, and rapid progression of ulcer despite medical treatment closely mimic fungal keratitis.[2,3,7,30]

Clinical features resembling Atypical Mycobacterium species: Epithelial defect, dry-looking greyish white stromal infiltrate, stromal edema, Descemet membrane folds, and crack windshield appearance.[37]

Clinical features resembling Acanthamoeba keratitis: Stromal infiltrate with radial keratoneuritis. The diagnosis can be easily missed in the case of mixed Acanthamoeba and Pythium cases and warrants a high index of suspicion, especially in contact lens users.[19,38]

Clinical features resembling Bacterial keratitis: Epithelial defect, thick creamy white stromal infiltrate, stromal edema, early stromal melt, endothelial exudates, hypopyon, and corneal perforation. On a cursory look, it can be misdiagnosed as bacterial keratitis. The history of contact lens use (Pseudomonas) also misleads the diagnosis.[17,39]

Special scenarios such as pediatric pythium keratitis

The clinical features of pediatric Pythium keratitis are no different than adults. The particular focus should be to eliminate the infective foci, cyanoacrylate glue with bandage contact lens (BCL)[6] to safeguard anatomical integrity in cases of stromal melt, salvage vision, and prevent amblyopia in pediatric children. A previous case report demonstrated radial keratoneuritis as a feature of pediatric Pythium. However, to date, only limited cases (4) of pediatric Pythium have been reported in the literature. Thus, it is difficult to say whether

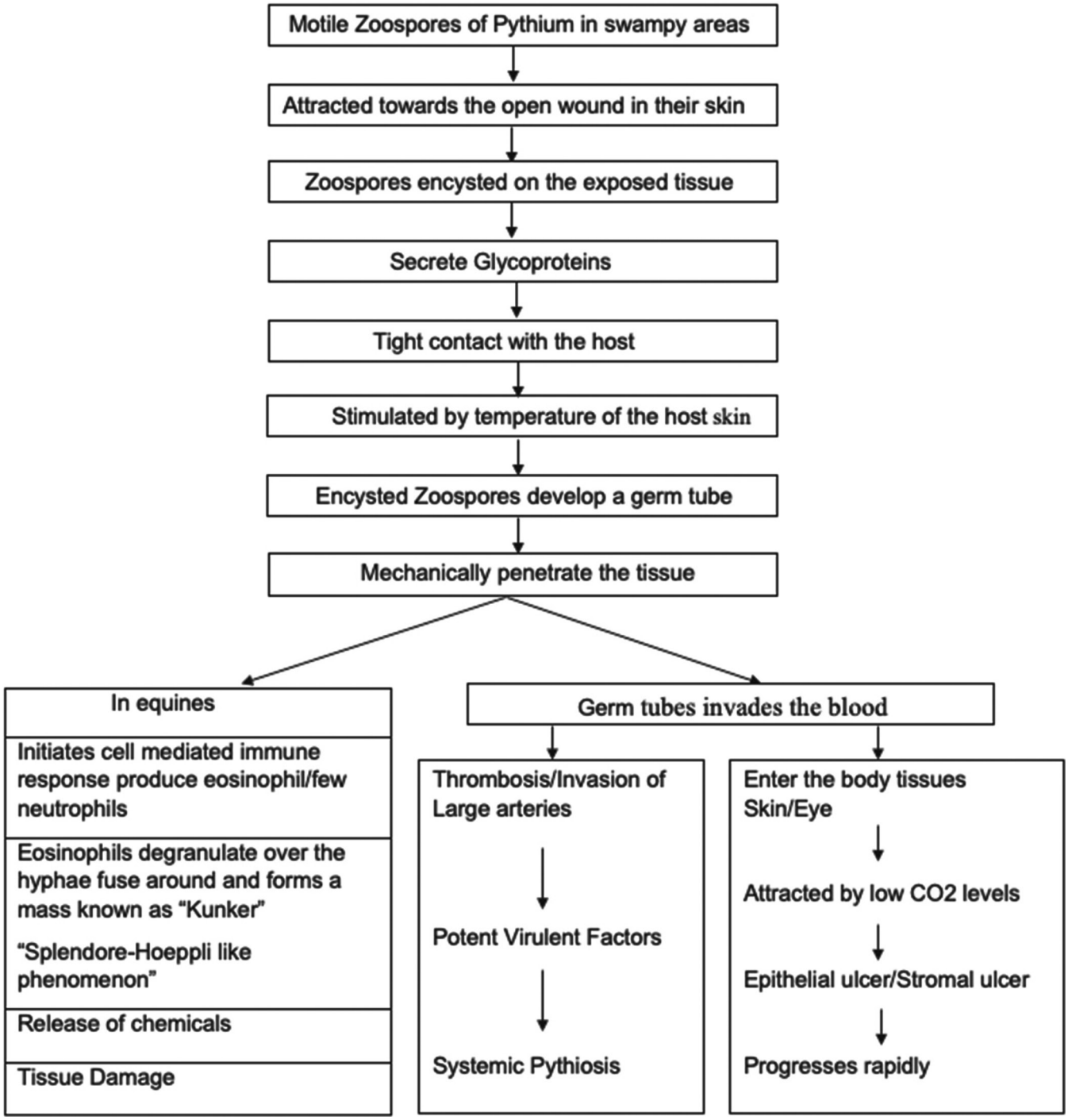

Figure 1: Image depicting the detailed mechanism of pathogenesis of *Pythium insidiosum* keratitis

it is a typical feature. Badenoch *et al*.[16] reported a case of a 3-year girl child who presented with central diffuse corneal infiltrate, stromal thinning, and keratic precipitates at the back of cornea along with hypopyon typically resembling fungal keratitis on presentation. He *et al*.[19] reported Pythium in a 7-year-old boy with a nasal peripheral corneal white stromal ulcer with surrounding diffuse infiltration along with multiple radial keratoneuritis at almost 360°. Two weeks later, the child presented with an endothelial plaque and corneal perforation. Chatterjee and Aggarwal.[40] reported a case of a 7-year-old boy with Pythium who presented with dense grayish-white stromal infiltrates with feathery margins, tentacular projection, and subepithelial pinhead-shaped infiltrates. In another recent report by Gurnani *et al*.[6] they reported a 9-year-old boy with dry-looking mid stromal corneal infiltrate with a feathery margin having stromal edema. After 5 days of treatment, the ulcer progressed and had features suggestive of Pythium, such as tentacular projections and peripheral furrowing. A detailed literature review of pediatric Pythium keratitis is listed in Table 1.

Systemic Pythiosis associations

1. Paroxysmal Nocturnal Hemoglobinuria (PNH)[41]
2. Thalassemia Hemoglobinopathy Syndrome[42]
3. Chronic Arterial Insufficiency[43]
4. Aplastic Anemia[44]
5. Cavernous sinus Thrombophlebitis[45]
6. Skin and subcutaneous tissue ulceration and granulomas[46]
7. Aneurysms[41]
8. Thrombosis[45]
9. Vasculitis[47]

Laboratory Diagnosis, Histopathology, and Microbiology of Pythium Keratitis

Laboratory diagnosis

Corneal scrapings are collected under topical anesthesia by using 0.5% proparacaine with the help of a Kimura spatula or a Bard–Parker blade. The specimen for diagnosis includes two scrapings for smear examination (one each for Grams stain and 10% potassium hydroxide wet mount) followed by a subsequent sequential scraping for culture on 5% sheep blood agar and potato dextrose agar (PDA). The blood agar plates are incubated in a 5% CO_2 incubator at 37°C, and PDA is incubated at 27°C and are observed for the macroscopic morphology. The growth of flat, feathery-edged, partially submerged, colorless, or light brown small hair-like projections of Pythium species growth is seen as "C"-shaped streaking areas under overnight incubation. A further incubated plate shows hyaline and submerged colony morphology. The direct smear morphology reveals the typical long, sparsely septate hyaline with numerous vesicles, and a ribbon-like folding pattern of fungal hyphae was observed.[7]

Histopathology

Hematoxylin and Eosin (H and E), Periodic Acid-Schiff (PAS) stain, and Gomori Methenamine Silver (GMS) stain are used to diagnose the fungal-like Pythium filaments from formalin-fixed, paraffin-embedded (FFPE) samples. The fungal hyphae morphology is usually seen as a short, longitudinal, rare septate hyphae and transverse hyphae with a diameter similar to that of the septate hyphae seen. H and E and PAS stains show necrotic eosinophilic granulomas around the hyphae. With H and E stain, the filaments of Pythium turn pale pink to ghost-like structures. Moreover, the Pythium diagnostic slides are stained with 0.5% and 1% PA for 0.5, 2, 3, and 5 min and Schiff's reagent is applied without modification for 10 min. The filaments turning pink are labeled as positive, and no staining is labeled as negative. With GMS, filaments of Pythium appear brown, and the stroma shows a greenish hue. These filaments exhibit varied morphology on GMS, such as septate or aseptate, narrow or broad, short or long, and may or may appear obtuse to perpendicular.[48]

The potassium iodide–sulfuric acid (IKI-H2SO4) stain is cost-effective, simple, sensitive, and specific for diagnosing the oomycete of Pythium. The Pythium hyphae are seen as blue/bluish-black and labeled as positive, and yellow/yellowish brown is considered negative staining.[48] Another recent novel diagnostic stain was trypan blue, which can be employed for early identification of septate and aseptate Pythium hyphae, especially in low-resource areas such as rural setups with a lack of advanced diagnostic modalities.[49]

Fig. 3 depicts the various stain and culture growth of *Pythium insidiosum* keratitis.

Molecular diagnostics tests

Polymerase chain reaction

Limitations of accurate and delayed diagnosis based on different staining techniques, growth in cultures, and zoospores have led to an increased interest in various molecular techniques. The molecular-based diagnosis, including nested polymerase chain reaction (PCR) and DNA sequencing, are widely employed for the species identification from formalin-fixed paraffin-embedded (FFPE) samples, clinical specimens, and cultures. PCR amplification targeting the internal transcribed

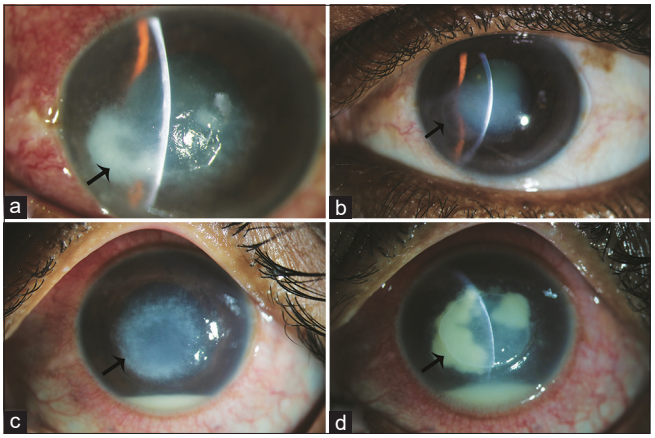

Figure 2: (a) Slit-lamp image depicting the case of confirmed Pythium keratitis having anterior to mid stromal infiltrate with tentacular extensions. (b) Slit-lamp image depicting the image of the same case after resolution on medical treatment. (c) Slit-lamp image depicting the case of confirmed Pythium keratitis having anterior to mid stromal infiltrate having tentacular extensions extending till posterior stroma. (d) Slit-lamp image depicting worsening of infection as observed by increased density of endo-exudates with cotton wool-like fluffy infiltrates

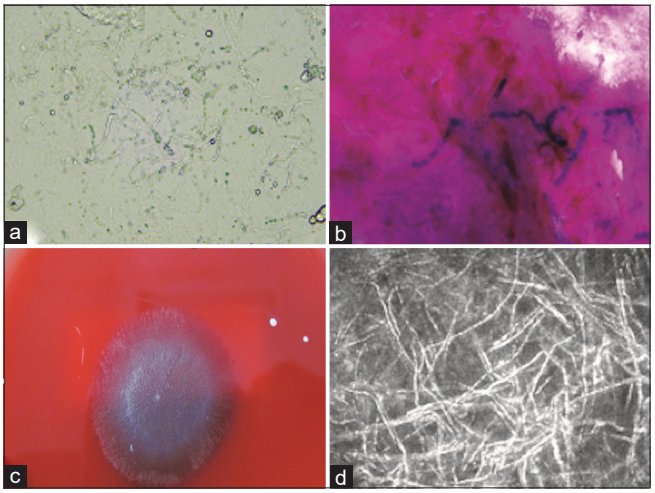

Figure 3: (a) Shows a 10% KOH wet mount demonstrating the presence of long, sparsely septate hyaline hyphae. (b) Shows the gram stain image depicting the thick cell wall, a few septate, and ribbon-like folding patterns of fungal hyphae. (c) Shows a 5-day old culture of P. insidiosum at 37°C grown on 5% sheep blood agar. (d) Shows a confocal microscopy image depicting thin, hyperreflective, occasionally branching structures with varying angles

Table 1: Review of literature of pediatric Pythium keratitis

Author	Age/ gender	Presentation	Clinical Features	Investigation	Treatment	Outcome
Badenoch *et al*.[16]	3 Y/F	9 days - history of use of public swimming pool, vegetative trauma in the swimming pool	Central corneal ulcer, hypopyon. Stromal thinning	Gram stain - polymorphonuclear cells, poorly staining hyphae. Chocolate agar- filamentous organism after 24 hours, on blood and non-nutrient agar plates. P. insidiosum was suspected	Voriconazole (1%) and Polyhexamethylene biguanide (0.02%) drops four times and oral voriconazole (100 mg) twice a day. TPK after 3 days was performed, and Fluorometholone (0.1%) drops postoperatively	Stable graft, no inflammation, and visual acuity of 20/80.
He *et al*.[19]	7 Y/M	Grittiness, photophobia for 5 days, twig injury	3×2 mm nasal peripheral corneal white stromal ulcer, diffuse infiltration along with multiple radial keratoneuritis at almost 360°	Acridine orange hydrochloride and lactophenol blue showed a thick cell wall, sparsely septate, with vesicles inside. Numerous refractile filaments on confocal microscopy. Culture reports negative, Later corneal button/hypopyon revealed white-yellowish clusters in the potato dextrose agar petri dish and culture tube and confirmed to be P. insidiosum	Antifungal therapy including topical Natamycin and Fluconazole eye drops every half hour, topical Fluconazole ointment every night, and oral Voriconazole 100 mg Bid. Later, TPK and antifungals (Natamycin, Fluconazole)	Cornea covered with conjunctiva with neovascularization, hand movement vision till 3 months of follow-up
Chatterjee *et al*.[40]	7 Y/M	10 days history of ulcer. No history of trauma	Central corneal ulcer. Significant corneal thinning after scrapping.	Microscopy of 10% KOH wet mount and Gram stain revealed aseptate fungal filaments. Rescrapping- broad aseptate hyaline filaments with ribbon-like folds and right-angled bends	Initially, Natamycin 5%, followed by the addition of Voriconazole 1%. Later treated with 1% azithromycin (hourly), 1% voriconazole (hourly), 1% atropine eye drops (TDS), oral azithromycin 250 mg OD for 3 days each week. Cyanoacrylate adhesive and a bandage contact lens to prevent corneal perforation	Dense vascularized stromal scar, hand movement close to his face vision. The child is awaiting penetrating keratoplasty
Gurnani *et al*.[6]	9 Y/M	Pain, redness, and decreased vision 5 days post stick injury	Dry-looking mid stromal infiltrates with feathery margins, suggestive of fungal keratitis. Later rapid progression of infiltrates and localized corneal melt	Corneal scraping and smear examination with 10% KOH and Gram staining revealed long slender hyaline hyphae with sparse septations. Later, blood culture confirmed Pythium growth.	Initially antifungals (5% Natamycin, 1% Itraconazole). Later after growth confirmation, hourly 0.2% Linezolid and 1% Azithromycin eye drops. cyanoacrylate glue, and bandage contact lens	Final BCVA recovered to 20/40

spacer region (ITS) in the ribosomal RNA regions of *Pythium insidiosum* has been used. The molecular phylogenetic relationship of *Pythium insidiosum* targets following genes have been explored, including ribosomal DNA (rDNA), exonuclease1 (exo1),[49,50] and cytochrome oxidase subunit II (cox II).[21,51-53] In several studies, rDNA (18S rRNA, internal transcribed spacer 1 (ITS1), 5.8S rRNA, ITS2, and 28S rRNA) are the main target region used for phylogenetic studies.[21,51,53-56] Over the last decade, ITS regions have been extensively used in phylogenetic studies. Recent studies found a better candidate gene like Cytochrome oxidase subunit II (cox II) for investigating the genetic relationship due to its higher interspecific and lower intraspecific divergences among the *Pythium insidiosum* strain.[21,57]

Kulandai *et al*.[58] standardized a novel duplex PCR targeting18S rRNA gene and ITS region of *Pythium insidiosum* to aid direct detection of the organism from the clinical specimens. This single-step qualitative duplex PCR is highly specific for Pythium with a 92.8% sensitivity. It is cost-effective and rapid compared to PCR-based DNA sequencing, which requires additional DNA sequences. Appavu *et al*.[59] used the cytochrome oxidase II gene and the ITS region to identify *Pythium insidiosum* from corneal specimens and correlated them with the clinical outcome. Most of their strains belonged to either clade BTH or CTH, with none being in ATH. However, no significant difference was reported between the clinical outcome and the genotype of *Pythium insidiosum*. The use of immunoperoxidase staining using *Pythium insidiosum* antibodies through various sources has been reported to have higher sensitivity and specificity as compared to the routine histopathological stains.[60] However, Kosrirukvongs *et al*.[61] compared nested

Table 2: Detailed literature review of various case reports of *Pythium insidiosum* keratitis

Author	Age, Gender, Visual acuity	Risk factor	Anterior segment findings	Investigations	Treatment	Outcome
Virgile *et al.*, Cornea, 1993[70]	31, Female, CF	Sickle cell trait	2/3 depth corneal infiltrate with 3 mm hypopyon	Gram stain-Gram positive diplococci Corneal biopsy-Staph epidermidis SDA- Pythium growth	Intravenous gentamicin 70 mg every 8 hourly, Tobramycin 15 mg/ml hourly, Cefazolin 1 gm every 8 h	Corneal perforation-Later TPK and anterior segment reconstruction
Murdoch and Parr, Aust NZJO, 1997[32]	28, Male, 6/36	Hot pool bath	6 mm stromal infiltrate, Hypopyon Later perforation	Corneal scraping-Fungal hyphae	Intensive antifungals; 5-fluorocytosine orally 150 mg/kg per day, ketoconazole orally 200 mg bd, natamycin drops 5% 1-hourly, and miconazole ointment 2% 4-hourly. Later, Prednisolone and finally TPK	Evisceration due to recurrence 2 days after 10 days
Lekhanont, Cornea, 2009[15]	22, Female	Contact lens	5.4×5.2 mm Central ulcer, underlying dense stromal infiltrates, subepithelial and superficial stromal opacity in a reticular pattern along with radial perineural-like infiltrates	Corneal scrapings and contact lens case cultured. Gram stain of the corneal scraping revealed many white blood cells with no organism. KOH wet mount negative preparation.	Vancomycin (50 mg/mL) and topical Ceftazidime (50 mg/mL) hourly around, TPK, 2 Regrafts,	Enucleation
Tanhehco *et al.*, Eye Contact Lens, 2011[17]	21, Male	Contact lens, Tap water	Corneal stromal infiltrate at the inferior limbus, and endothelial plaque, and a layered hypopyon	Cornea cultures- negative; Culture of the contact lens- Enterobacter In vivo, confocal microscopy showed cystic structures suggestive of Acanthamoeba Corneal button examination revealed branching hyphae that stained well with both Periodic acid-Schiff and Gomori methenamine-silver stains raising the suspicion for a Zygomycete	Topical antibiotics, antifungals and chlorhexidine, oral voriconazole, antiglaucoma drugs Later TPK	Repeat TPK later enucleation
Barequet *et al.*, Semin Ophthalmol, 2013[39]	24, Male	Contact lens, Swimming pool water	Severe corneal abscess	Initial cultures showed the presence of septate mold, unclearly identified PCR assay depicted homology to *Pythium insidiosum*.	Intensive fortified topical antibiotics and natamycin Topical and intravenous voriconazole	Good outcome, no recurrence after 5 years
Hung and Leddin, Clin Gastroenterol Hepatol, 2014[29]	51, Male,	Crohn's disease	Central corneal ulcer	Culture negative, Corneal biopsy negative, Pathological analysis of enucleated globe revealed Pythium	Adalimumab 80 mg weekly. Later TPK	Enucleation
Lelievre *et al.*, Am J Trop Med Hyg, 2015[34]	30, Female, 20/200	Contact lens	Central corneal infiltrate with subepithelial and superficial stromal	May Grünwald Giemsa staining and culture on chocolate PolyViteX agar, Schaedler broth with globula	Topical 1% voriconazole and 0.25% amphotericin B, antibiotic treatment (Bacitracin and	Failed graft, 20/2000

Contd...

Table 2: Contd...

Author	Age, Gender, Visual acuity	Risk factor	Anterior segment findings	Investigations	Treatment	Outcome
			infiltration, reticular pattern with feathery edges, satellite lesions, and Wessely ring in the right eye	r extract, and Sabouraud with antibiotics agar Confocal microscopy-fungal filaments Button culture positive for Pythium	Colimycin eye drops; one drop per hour). Oral voriconazole 200 mg two times per day at day 3, Topical and intravenous caspofungin Later TPK	
Ramappa *et al.*, Cornea 2017[37]	42, Female, 20/180	Trivial Injury	Central dense, dry-looking grayish-white mid stromal infiltrate, tentacular projections, peripheral pinhead size lesions	Scarping - broad, aseptate hyphae with ribbon-like folds suggestive of Pythium Confocal microscopy - fungal filaments	Topical 0.2% Linezolide, 1% Azithromycin, Atropine, and oral 500 mg 3 days Azithromycin	Corneal scarring after 3 weeks
Rathi *et al.*, Cornea, 2018[45]	70, Male	Tap water	Total corneal ulcer with thinning	Gram stain - gram-positive cocci KOH - fungal hyphae Confocal - Fungal hyphae Button culture on SDA - Hyphae MRI - Cavernous sinus thrombophlebitis PCR - *Pythium insidiosum*	Topical antibacterials + antifungals. Later PTK	Lid sparing exenteration
Neufeld *et al.*, CJO, 2018[71]	51, Male, 20/100 vision	Contact lens/ Crohn's disease	Epithelial defect, stromal infiltrate	Corneal biopsy - negative Confocal microscopy - negative GMS - septate hyphae PCR - *Pythium insidiosum*	Topical Propamidine, Amphotericin B, Chlorhexidine, Moxifloxacin, Ciprofloxacin, Natamycin, topical, and Voriconazole. Later TPK	Enucleation
Raghavan *et al.*, BMJ case reports, 2018[38]	21, Male, 6/36	Contact lens, dust fall	4*5 mm patchy mid stromal infiltrates	Gram stain - hyphae KOH, BA, SDA, NNA, BHI - negative Confocal - Acanthamoeba PCR - Pythium	Topical PHMB, Dexamethasone, Natamycin, Voriconazole, Moxifloxacin, Homide, Linezolid	Good outcome 6/18 vision
Bernheim *et al.*, Int J Inf D, 2019[72]	21, Male	Contact lens, swimming pool	Corneal abscess, peri-lesional infiltrates	PCR, Mass spectrometry - Pythium	Antibiotics, antifungals, collagen cross-linking	Good outcome
Maeno *et al.*, AJO case reports, 2019[27]	20, Male 20/28	-	Paracentral corneal hyphated ulcer	Smear - fungal filaments In vitro disc diffusion assay - showed more sensitivity to antibiotics	Intravenous Liposomal Amphotericin B 100 mg, Minocycline, Linezolid, Chloramphenicol. Later TPK	Good outcome, 20/25
Natarajan *et al.*, Asian Journal of Ophthalmology 2020[73]	2 Cases, 45 Male HM, 62 Male	- Thai fis	Case 1-8-mm corneal stromal infiltrate with reticular edges, reaching the temporal limbus	Case 1- Smear showed sparsely septate fungus-like filaments with ribbon-like folding. The culture showed growth of Pythium	Case 1-0.2% fortified Linezolid and 1% Azithromycin eye drops hourly. TPK, cryotherapy of the host margins, and absolute alcohol	Tarsorrhaphy, corneal scarring, and later PKP after 10 months

Contd...

Table 2: Contd...

Author	Age, Gender, Visual acuity	Risk factor	Anterior segment findings	Investigations	Treatment	Outcome
	CF	h	Case 2- Peripheral guttering ulcer 2-3 mm wide with concentric spread (5 to 11 o'clock hours). Central edge sloping, peripheral edges steep. Dense infiltrate at the base with 60-70% stromal thinning	insidiosum ,which was reconfirmed with polymerase chain reaction (PCR) test Case 2-Corneal scraping showed sparsely septate filaments suspicious of Pythium Confocal microscopy- multiple, linear, hyper-reflective, lattice-like structures in the area of infiltrate	application of the edges Case 2-0.2% linezolid and 1% azithromycin eye drops hourly and oral Azithromycin 500 mg once a day Cyanoacrylate glue with a bandage contact lens over thinned out area Later TPK with absolute alcohol	Corneal scarring
Kate *et al.*, Ocul Immunol Inflamm, 2021[74]	54, Male PL	-	Peripheral Ulcerative Keratitis like picture with corneal thinning and perforation	Gram stain-revealed gram-positive cocci Culture negative Excised corneal button revealed-*Pythium insidiosum* (confirmed by DNA sequencing and zoospore formation) histopathology showed filaments suggestive of Pythium species	Initial treatment-Topical vancomycin 5% and ciprofloxacin 0.3% Later corticosteroids were added, which worsened the picture Therapeutic keratoplasty for corneal perforation Later anti-pythium drugs	

PCR with culture and immunostaining and found PCR to be the most sensitive.

Confocal microscopy

The use of in vivo confocal microscopy has also been described to diagnose *Pythium insidiosum*; however, the features are not characteristic or specific and cannot be used to distinguish from fungi. They appear as beaded, thin hyperreflective branching structures with a mean angle of 78.6° and varying from 90 to 400 μm in length. However, confocal microscopy could have a role in detecting an early recurrence.[62] [Fig. 3]

Serological tests

The serological diagnosis rests on the detection of antibodies in the serum. The various serological tests implicated from *Pythium insidiosum* detection are western blot, enzyme-linked immunodiffusion assay (ELISA), immunodiffusion, and hemagglutination. The sensitivity and specificity of ELISA for immunodiffusion are 100% and 61%, respectively.[63-66]

Treatment

Medical management

Antifungals

Earlier antifungals such as 5% natamycin, 1% voriconazole, and 1% itraconazole were considered first-line drugs as Pythium was wrongly grouped as a fungal species.[67] The majority of the previous case reports and studies show limited success with antifungals. Hasika *et al*.[7] and Bagga *et al*.[3] in their respective studies also proved the limited role of existing antifungals in Pythium keratitis management. Before culture results, Pythium hyphae completely mimic fungal hyphae and it becomes challenging to decide on the drug of choice based on the clinical picture alone. Thus, antifungals can be started before culture results, and once the culture results are available can be switched to targeted treatment.[2] In clinically suspicious cases with reticular dot infiltrate, tentacular projections, and peripheral furrowing, before the culture results, the clinician can start the patient on a combination of antifungal and antibacterial (0.2% linezolid and 1% azithromycin) treatment.[2]

Antibacterials

Based on *in vitro* susceptibility[3] and *in vivo* studies on rabbit model[8,68] of Pythium keratitis, the patient with a microbiologically confirmed diagnosis can be started on the recommended regimen of a combination of topical linezolid 0.2% (prepared from IV preparation) one hourly and azithromycin eye ointment 1% twice a day, along with oral azithromycin 500 mg once a day (for 2 weeks). These cases should be followed up every three days initially and then once a week to assess the response. Clinically, resolution [Fig. 1] of keratitis is manifested by the[23] decrease in the number and density of tentacular extensions with a reduction in the cellularity of surrounding stroma. On follow-up, a formation of peripheral guttering surrounding the infiltrate is usually observed along with an increase in deep vascularization. In cases with significant corneal thinning, cyanoacrylate glue application should be planned to avoid corneal perforation. Medical treatment would result in the resolution of infection in nearly 50%–60% of cases with a median duration of 3 months.[23] The patients should be closely monitored for the development of a limbal and deep stromal extension of infection during regular follow-ups.

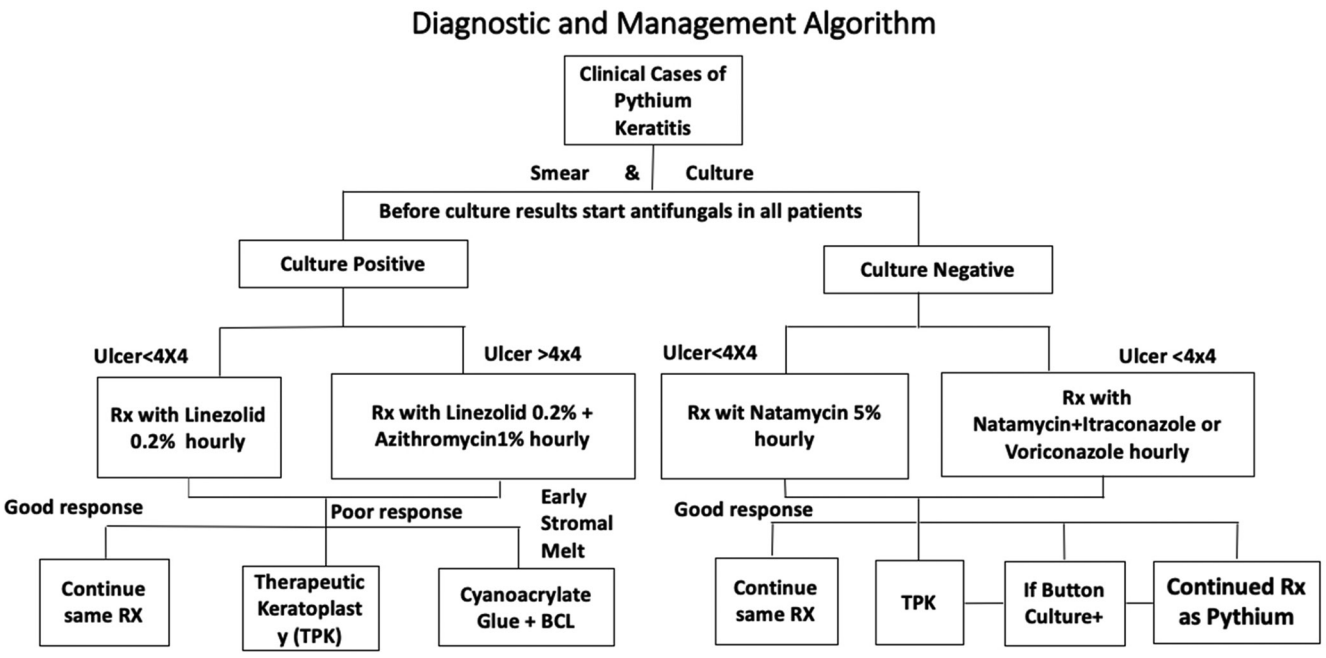

Figure 4: Depicts a proposed diagnostic algorithm for diagnosis and management of Pythium keratitis

Surgical treatment

Therapeutic keratoplasty

Due to the rapid proliferating capacity of the microorganism, high virulence rate, limited response to existing drugs, and high recurrence rate, medical treatment of Pythium keratitis may not be sufficient to control or eradicate the infection.[9] In such cases, therapeutic keratoplasty (TPK)[7,11] should be planned along with the initiation of medical treatment. The patients with early stromal melt, descemetocele, corneal perforation, nonresolving endoexudates, and scleral involvement should undergo early TPK within 10–14 days. While planning therapeutic keratoplasty [Fig. 2], it is essential to take at least a 1.5-mm larger size of trephination than the size of the corneal infiltrate. There are high risks of recurrences of infection after keratoplasty, as reported by Agarwal *et al*.[11,24] Hasika *et al*.,[7] in their analysis of 71 cases, showed that TPK is the mainstay of treatment for Pythium keratitis.

Optical penetrating keratoplasty (OPK)

Post TPK, when the patient is infection-free and there are no signs of recurrence for 6–8 months under steroid cover, the patient can be taken for optical penetrating keratoplasty as a visual rehabilitation procedure.[2,9]

Enucleation and evisceration

High virulence, the rapid proliferation of microorganisms, and the tendency for early limbal spread have tested clinicians' patience in difficult clinical scenarios. Evisceration has been reported as a treatment modality in endophthalmitis secondary to Pythium keratitis. The patient developing panophthalmitis should undergo enucleation.[30,69]

Adjunctive treatment modalities

Cryotherapy with ethanol

To avoid recurrence of infection, adjunctive measures such as cryotherapy on the host bed with ethanol have been tried with moderate success. The mechanism by which cryotherapy is helpful by dehydration and ischemic infarction of cells and accumulation of toxic metabolites within cell walls, which destroys cells. Ethanol promotes apoptosis, increases cell lysis, and alleviates the proliferation of cells, thus resulting in reduced cell life. Agarwal *et al*.,[24] in their study, used cryotherapy with ethanol in six of their subjects having anterior chamber angle and scleral involvement. Only one subject had a recurrence, thus labeling it as an effective treatment modality.

Cyanoacrylate glue with bandage contact lens (BCL)

Cyanoacrylate glue is reported to have antibacterial properties. It can have a synergistic effect in combination with antibacterials such as linezolid and azithromycin. Gurnani *et al*.[6] reported successful management of a 9-year-old child with early stromal melt with cyanoacrylate glue, BCL, and antibacterials. Thus, in patients with rapid stromal melt, pediatric patients with early corneal thinning, descemetocele, and corneal perforation glue with BCL are essential adjunctive treatment modalities for anatomical and functional rescue.[6,30] Fig. 4 depicts the diagnostic and management algorithm for *Pythium insidiosum* keratitis based on a detailed literature review. We propose that clinicians can employ this protocol to enhance treatment outcomes while dealing with this devastating entity.

Postoperative management, rehabilitation, and counseling

After TPK, there should be a continuation of 0.2% linezolid and 1% azithromycin 6–8 times per day for a minimum of 1 month, presuming the eye to be free of infective foci. The initial hourly application should be avoided for the duration as it may result in drug toxicity. The antibacterials should be tapered based on clinical response and assisted with adjuvant drugs such as 1% homatropine to reduce ciliary spasm and 0.5% timolol to prevent secondary glaucoma. Different centers follow different protocols for initiating postoperative steroids.

Table 3: Depicts detailed literature review of significant studies on Pythium keratitis

Study	Study period	Number of eyes	Healed with Medical treatment	Required TPK	Repeat TPK	Globe salvage	Evisceration/ Phthisis bulbi
Kunavisarut *et al.*, J Med Assoc Thai, 2003[75]	1988-1998	8	0	100%	12.5%	12.5%	87.5%
Thanathanee *et al.*, Cornea 2013[20]	May-July 2009	5	0%	100%	20%	80%	20%
Sharma *et al.*, Cornea 2015[5]	Phase 1-2010-2012 Phase 2-2014	13	0%	100%	7.6%	84.6%	15.3%
Agarwal *et al.* BJO 2017[11]	2014-2016	10	0%	100%	80%	0%	20%
Agarwal *et al.* BJO 2018[24]	Jan 2014-July 2017	46	2.1%	91.30%	23.91%	84.78%	15.21%
Bagga *et al.* BJO 2018[3]	Jan 2014-Dec 2016	114	11.4%	85%	-	96.4%	1.75%
Hasika *et al.*, IJO 2019[7]	Jan 2016- Nov 2017	71	4.2%	67.60%	54.2%	43.7%	28.1%
Permpalung *et al.*, Med Mycol, 2019[76]	Jan 2010 - Jul 2016	30	-	76.6%	-	53.34%	46.66%
Bagga *et al.* Ophthalmology Jan 2021[23]	Jan 2017-Oct 2018	69	55.10%	44.90%	24.63%	-	-
Gurnani *et al.*, IJO 2021[2]	Oct 2017-Mar 2020	30	20%	63.30%	20%	90%	10%
Vishwakarma et, IJO 2021[30]	Jan 2016-Dec 2018	18	0%	83.30%	22.2%	72.2%	27.7%
Puangsricharern *et al.*, CO, 2021[69]	2006-2019	26	7.6%	80.7%	30.7%	42.3%	57.7%
Nonpassopon *et al.*, Cornea 2021[77]	2009-2019	6	0%	100%	-	83.33%	16.67%
Sane *et al.*, Cornea 2021[78]	Oct 2016-Dec 2019	21	82.35%	19.04%	-	85.71%	4.76%
Zhang *et al.*, CJO, 2021[79]	June 2017-June 2019	6	0%	100%	33%	66%	33%

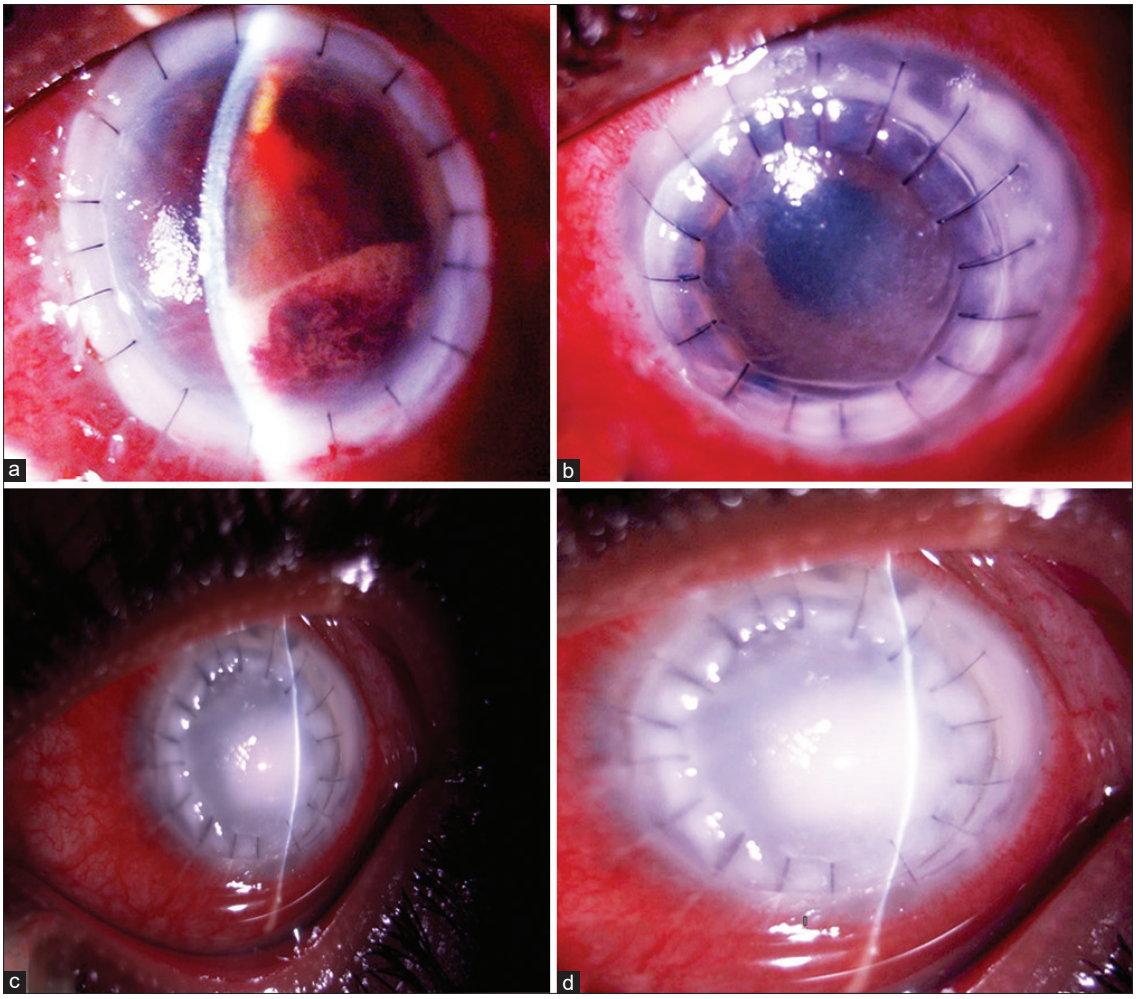

Figure 5: (a) Slit-lamp image depicting graft reinfection with anterior chamber exudates. (b) Slit-lamp image depicting graft reinfection with host rim margin infiltrate. (c) Slit-lamp image depicting graft reinfection with full-thickness infiltrate of host and the donor cornea. (d) Slit-lamp image depicting graft reinfection with full-thickness infiltrate of host and the donor cornea

If the patient is culture-positive and postoperative button culture is also positive for Pythium, it is suggested to wait for at least 3 weeks before initiating steroids, and if the patient is culture-positive and postoperatively button culture is negative, it is recommended to start steroids at least after 2 weeks of TPK. Topical 0.1% dexamethasone and 1% prednisolone can be used in tapering doses for graft survival. The regimen can be two hourly for the first 2–3 days, six times for 15 days, followed by 4/3/2/1 times three months each. Postoperatively, there are high chances of cataract and glaucoma and need to be managed effectively. Graft survival after TPK, performed in cases with worsening keratitis, had inferior outcomes and needs regrafting in terms of endothelial keratoplasty or penetrating keratoplasty for visual rehabilitation. The patient should be counseled regarding the prognosis of the disease and the need for regrafting and follow-up in the future.[2,9]

Differential Diagnosis

Clinically, microbiologically, and histopathologically, it resembles fungal keratitis, and the suspicion of P. insidiosum should be high for a refractory case of fungal keratitis. Pythium has been reported in patients following contact lens wear and needs to be differentiated from acanthamoeba keratitis.[15] In the early stage of the disease, the subepithelial reticular infiltrates can be confused with prominent corneal nerves, especially if there is a history of contact lens use and exposure to contaminated water. In addition, it is often similarly associated with severe pain.[11] The presence of cellulose in the cell wall similar to Acanthamoeba encouraged assessment and revealed *in vitro* sensitivity of Pythium to polyhexamethylene biguanide (0.01% and 0.02%).[11] Ramappa *et al*.,[37] in their case report, showed that early clinical features of Pythium such as dry stromal infiltrate and satellite lesions can also mimic atypical mycobacterial keratitis. Few of the previous reports have managed Pythium keratitis as bacterial keratitis owing to its presence in the form of cotton wool-like fluffy stromal infiltrate.[2] In late-stage with stromal melt, Lt also mimics the clinical picture of necrotizing viral keratitis.[9]

Complications

Though rare, infectious keratitis caused by Pythium leads to significant globe-threatening complications and visual morbidity.[7,15,20] The need for evisceration was reported as high as 57.5% by Puangsricharern *et al*.[69] Delayed presentation, an advanced disease with increased density of Pythium hyphae at presentation, or posterior stromal involvement were noted to be risk factors for globe removal. However, their initial management following diagnosis of Pythium keratitis included the use of topical and systemic antifungal agents; therapeutic penetrating keratoplasty was considered and performed in advanced keratitis not responding to medical management or in eyes with limbal involvement. Poor response to medical treatment, progressive corneal melt, perforation, and infiltrates extending up to the limbus have been significantly more in patients with infiltrates more than 4–6 mm in size.[2,23] A recurrence of infection requiring regraft has been reported to range from 20% to 71%. However, surgical adjuncts such as cryotherapy and topical absolute ethanol intraoperatively have helped reduce this incidence to 7.1%.[2,3,7] Early diagnosis, use of topical antibiotics, and early surgical intervention with or without surgical adjuncts have also helped improve overall outcome, with less than 10%–12% eyes requiring evisceration, in recent reports.[7,62,69] The other complications reported include cataract, secondary glaucoma, choroidal detachment, endophthalmitis, scleritis, and phthisis.[7,11,22,23] Extending beyond ocular morbidity, ocular pythiosis leading to fatal cavernous sinus thrombophlebitis has been reported by Rathi *et al*.,[45] thus necessitating timely diagnosis and early intervention. Fig. 5 depicts the various complications encountered while dealing with Pythium keratitis. Tables 2 and 3 illustrate a comprehensive review of all the major case reports and studies on *Pythium insidiosum* keratitis, respectively.

Prognosis

The major literature available on Pythium keratitis has reported poor prognosis in the majority of the cases resulting in regraft, graft infection, enucleation, and Phthisis bulbi. The prognosis depends on infiltrate size, depth, extent, delay in presentation to the clinician, misuse of steroids, lack of diagnostic modalities, and delay in initiating targeted antibacterials. Superficial corneal infiltrates not involving the visual axis, if managed aggressively and promptly, usually have a good anatomical and functional outcome. Full-thickness infiltrates involving visual axis, early limbal spread, peripheral furrowing presence of endoexudates, and hypopyon requires early TPK, and the prognosis is usually guarded in these cases. Patient developing complications such as panophthalmitis, endophthalmitis, retinal detachment, choroidal detachment, and recurrent graft infection has an extremely guarded prognosis. Early TPK with good margin clearance can have good anatomical and rarely good functional outcomes. A timely TPK can manifest as early corneal opacification, which can be later managed with optical penetrating keratoplasty for visual restoration.[2,3,6,7,9,11,23,30]

Future Directions

Detailed literature review lacks multicentric and randomized controlled trial on Pythium keratitis. Thus, in the future, the focus should be entailed toward trials to reach conclusive evidence regarding diagnosis and treatment. Recently, newer laboratory stains and diagnostic modalities have been fined for Pythium, which have helped in the early detection of the microorganism. Diagnosis and treatment is still an area of active research, and more energy should be focused on innovations and diagnostic research on Pythium keratitis.

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