Review Article

Pheohyphomycosis of the eye: A microbiological review

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ABSTRACT

Ophthalmic infections of fungal origin, namely mycotic keratitis, mycoticendophthalmitis, etc., are commonly encountered in the tropical and sub-tropical countries including India. Mycotic keratitis, in particular, presents an important ophthalmic problem causing visual disability due to its protracted course and unfavorable responses. Determination of various host and environmental factors that put an individual at risk for development of fungal infections in the eye may reduce the time to their diagnosis. Even though *Aspergillus* and *Fusarium* are the most common species causing keratitis, pigmented dematiaceous fungi remain an important cause of mycotic keratitis. Majority of fungal keratitis responds to medical therapy if diagnosed on time and treatment started immediately. Failure of medical therapy may be due to infection of the cornea by certain pathogens or late presentation, which requires surgical treatment in conjunction with anti-fungal therapy post-operative. We, therefore, searched for literatures using the keywords "pigmented fungi, pheohyphomycosis, keratitis, ophthalmology" through GoogleScholar and reviewed the microbiology of pheohyphomycosis of the eye and its treatment in the light of improved culture and diagnostic methods.

Key words: Keratitis, mycology, ophthalmology, pheohyphomycosis, pigmented fungi

INTRODUCTION

Ophthalmic infections of fungal origin are commonly encountered in the tropical and sub-tropical countries including India. Mycotic keratitis presents an important ophthalmic problem causing visual disability due to its protracted course and unfavorable responses. The incidence of fungal keratitis has been reported to range between 25.6-36.7% in various parts of India.[1] In addition to this, endophthalmitis of fungal origin has been encountered and results of studies in India as well as abroad indicate the incidence to vary between 3-8%.[2] Even though *Aspergillus* and *Fusarium* are the most common species causing keratitis, pigmented dematiaceous fungi remain an important cause of mycotic keratitis.[1] These fungi are, in fact, responsible for a wide variety of clinical syndromes, from local infections due to trauma, to disseminated infection in immuno-compromised patients. They are unique owing to the presence of melanin in their cell walls, which imparts the characteristic dark colour to their spores and hyphae.[3] In this paper, literatures on current knowledge of the incidence, risk factors, management, and outcomes of ophthalmic infections due to pigmented fungi in comparison with non-pigmented fungi are retrieved through Google Scholar and are reviewed.

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RISK FACTORS

Fungal keratitis, including both pigmented and non-pigmented fungi, is encountered significantly more in males ($P < 0.0001$) than females.[4-6] A large group of patients are young adults. In a study conducted by Chowdhary *et al.*,[4] 36% of patients who were diagnosed with mycotic keratosis were in the age-group of 31-40 years. Other studies also showed that more than 60% of the patients were between

the ages 2150 years.[5,6] Patients from rural areas are more commonly affected ($P < 0.0001$) and a majority of them are farmers.[6] Bharathi *et al.*,[6] identified corneal trauma as the predominant predisposing factor and the correlation between trauma and fungal keratitis was highly significant ($P < 0.0001$). A retrospective analysis of 557 cases of mycotic keratitis due to dematiaceous fungi also showed corneal trauma to be a predisposing factor in 47.70% of the patients.[7] Other predisposing risk factors seen areuse of contact lens, presence of co-existing ocular diseases and application of topical corticosteroids.[4,6] In a study conducted by Gopinathan *et al.*,[5] from a referral eye care center at South India, a higher incidence of fungal keratitis was observed during the monsoon and winter than summer. On the other hand, Wilhelmus studied the seasonality of 52 episodes of dematiaceous fungal keratitis occurring along the Gulf of Mexico and observed a relative increase in the diagnosis of dematiaceous fungal keratitis during late summer and persisted throughout autumn.[8]

INCIDENCE AND AGENTS OF PHEOHYPHOMYCOSIS OF THE EYE

The primary factor unifying the dematiaceous fungi is the dark pigmentation of their hyphae. At least 20 species of fungi belonging to 11 different genera have been implicated as causes of keratitis. Dematiaceous fungi have been reported to be the third most frequent cause of mycotic keratitis (behind *Aspergillus*and *Fusarium*) and may also cause infections of the orbit or intra-ocular infections. These fungi exhibit a brown-to-olive-to-black color in the cell walls of their vegetative cells, conidia, or both and colonies thus appear olive-to-black.[9]

Pheohyphomycotic keratitis

Dematiaceous fungi have been reported to be the third most frequent cause of mycotic keratitis and its incidence ranges approximately from 03% to 30%.[4-7,10-15] *Curvularia*, one of the genera of septatedematiaceous fungi, is reported to be the most common pigmented fungus implemented as a causative agent of dematiaceous keratomycosis.[4,10,16] The most common species of *Curvularia* isolated from mycotic keratosis is *C lunata*.[16,17] *C brachyspora*, *C senegalensis*, *C pallescens*, *C geniculata*, and *C prasadii* have also been implemented in the causation of keratitis.[9,16,18,19] However, a retrospective study conducted by Anita Panda *et al.*,[12] observed the isolation rate of *Alternaria spp.* (10.20%) to be more than that of *Curvularia spp.* (07.4%). Like other septatedematiaceous fungi, *Alternaria* is usually non-pathogenic and causes an opportunistic infection. In an unusual case, it causes infection of the cornea with perforation. An isolation of *Alternaria alternata* from a diabetic patient who was diagnosed with keratitis[20], a patient wearing rigid gas-permeable contact lens[21] and a corneal transplant[22] has been reported. Ferrer *et al.*,[23] recovered *Alternaria infectoria* from a patient who developed keratitis and endophthalmitis after eye-perforating trauma from a lemon tree branch. Anandi *et al.*,[24] reported a repeated isolation of *Bipolarishawaiiensis* from a corneal ulcer which followed an injury. A case of mycotic keratitis caused by *Bipolarisspicifera* has also been reported.[25] *Ulocladiumatrum* keratitis, without any predisposing event, has been described in a 43-year-old man from Australia.[26]

Lari *et al.*,[27] described a case of corneal chromoblastomycosis with *Cladophialophoracarrionii* in a 69-year-old woman who presented with right eye pain and decreased visual acuity, 4 weeks after clear corneal cataract surgery. *Cladorrhinum spp.* has also been isolated from a case of keratomycosis in a 12-year-old boy which followed the introduction of a small piece of straw into the cornea.[28] *Fonsecaeapedrosoi*, the most common causative agent of cutaneous chromoblastomycosis, has been described to cause corneal chromoblastomycosis by several authors.[29,30] *Exophialajeanselmei* has been recovered from a Saudi man who presented with a fungal corneal abscess.[31] Meanwhile, Patel *et al.*,[32] also reported a corneal infection that was found to be caused by another species of *Exophiala*, *E dermatitidis*in, a 52-year-old man who had laser *in situ* keratomileusis. *Exserohilumrostratum*, another known pathogen of cutaneous and subcutaneous pheohyphomycosis, has also been implicated to cause corneal pheohyphomycosis spontaneously[33] as well as following organic trauma.[34,35] Qiu and Yao reported a case of mycotic keratitis concurrently infected by *Exserohilummcginnisii* and*Candida parapsilosis* in a Chinese female patient.[36] *Exserohilumlongirostratum* has also been described by Bouchen *et al.*,[37] to cause mycokeratosis. Rishi and Font[38] described a case of keratitis caused by *Phoma spp.* in a 72-year-old man, who developed a non-healing corneal ulcer with brownish pigmentation. *Phialophoraverrucosa* has also been shown in conjunction with *Candida tropicalis*, and *Propionibacterium acnes* to penetrate intact lens capsule from infected iris.[39]

A series of corneal ulcers (25 cases) with growth of *Aureobasidiumpullulans* on two or more culture media or growth in one medium with consistent direct microscopy findings has also been reported from a tertiary eye care center of Eastern Nepal.[40] Freda *et al.*[41], from Brazil, reported a case of corneal wound infection after phacoemulsification by a pigmented fungus, *Phialemoniumcurvatum*. Meanwhile, Hong *et al.*,[42] from Korea, encountered a case of keratitis caused by *Phialemoniumobovatum* after penetrating injury to the cornea. *Scedosporiumapiospermum* has also been described as an agent of corneal pheohyphomycosis by several authors.[43-47]

Saha *et al.*,[48] isolated *Lasiodiplodiatheobromae* from corneal scraping of a 32-year-old woman with a history of vegetative trauma. The isolate was identified from culture using D1/D2 region of Large Sub Unit (LSU): 28S ribosomal deoxyribonucleic acid (rDNA)-based molecular technique. Tananuvat *et al.*[49] also identified various

pigmented fungi from cases of mycotic keratitis by DNA sequencing of amplified polymerase chain reaction (PCR) products, which included *Hyphodontia sp.*, *Botryosphaeria sp.*, *Cladosporiumcolocasiae*, *Cladosporiumoxysporum*, and *Curvulariaaffinis*.

Pheohyphomycotic endophthalmitis

The most common pathogenic agents of mycotic endophthalmitis may be *Candida*, *Fusarium*, and *Aspergillus fumigatus* but several dematiaceous fungi have been reported to be responsible for the same. Pathengay *et al.*[50] reported two cases of endophthalmitis due to *Curvularia spp.* in two Hispanic men who underwent repair of corneal laceration. Two cases of late endophthalmitis caused by *Exophialajeanselmei* after cataract surgery have been encountered.[51] An intra-ocular infection due to *Exophialadermatitidis* has also been encountered in a patient who underwent corneal transplantion for congenital hereditary endothelial dystrophy.[52] Rao *et al.*[53] reported an endogenous endophthalmitis due to *Alternaria spp.* in an immunocompetent host while Newell *et al.*[54] described chronic postoperative endophthalmitis caused by *Bipolarisaustraliensis*. A series of cases (14 out of 17 patients) with *Bipolarishawaiiensis* endophthalmitis occurred after intra-vitreal injection of triamcinolone derived from a single lot which, subsequently, was found to be contaminated with the same pathogen.[55] Cases of endophthalmitis induced by *Phialophora verrucosa*[56] and *Phialophora richardsiae*[57] have been reported. Zarkovic *et al.*[58] described a case of traumatic endophthalmitis due to *Scedosporiumapiospermum* while LaRocco and Barron[59] reported an endogenous *Scedosporiumapiospermum* endophthalmitis. A case of bilateral endogenous fungal endophthalmitis has also been reported to result from disseminated *Scedosporiumprolificans* in a woman who underwent lung transplantation. The patient was on chronic immunosuppressive therapy and cultures of both bronchial brushings and vitreous tap revealed *S. prolificans*.[60] Acase of *Phaeoacremoniumparasiticum* endophthalmitis has also been documented in a patient who had a penetrating injury 5 years prior to the presentation.[61]

Pheohyphomycotic sub-retinal abscess

Matthews *et al.*[62] reported a unique case of pheohyphomycosis sub-retinal abscess in a patient with arthropathy and lung pathology. Culture of vitreous sample showed a non-diagnostic non-infectious chronic vitritis whereas direct biopsy of the white sub-retinal mass in the peripheral nasal area revealed an abscess containing unidentified pigmented fungi.

Pheohyphomycotic dacryocystitis

Sodhi and Kaur[63] encountered two cases of dacryocystitis caused by the genus *Curvularia*in immunocompetent patients. The infection was localized to the lacrimal sac only and presented in the form of acute to chronic dacryocystitis.

Pheohyphomycotic conjunctivitis/scleritis

Moss *et al.*[64] reported a dematiaceous fungal infection of the conjunctiva in an immunocompetent man who presented with an enlarging pigmented mass of the conjunctiva. The lesion mimicked a conjunctival melanoma and on performing wide excision, it revealed a pigmented fungus. Reddy *et al.*[65] also reported the same from two patients who presented with a pigmented conjunctival mass, one resembling necrotizing scleritis with uveal prolapse and the other resembling a pigmented ocular surface tumor. Surgical excision of each lesion histopathologically displayed pigmented dematiaceous fungi. Li *et al.*,[66] encountered a case of sub-conjunctival mycetoma which yielded *Exophialadermatitidis* in a healthy middle-aged woman with recalcitrant ocular inflammation and black depositsin her tears, which is to be differentiated from a similar clinical entity termed *melanodacryorrhea* (black tears) caused by extra-ocular extension of uvealmelanoma.

Secondary optic atrophy due to pheohyphomycosis elsewhere

Smith *et al.*,[67] reported a case of right optic nerve atrophy secondary to neural compression by a *Curvularialunata* mucocoele in the pituitary fossa. The patient presented with poor vision of his right eye and transphenoidal resection of the mucocoele was performed which revealed *Curvularialunata*.

CLINICAL PRESENTATION

The sensitivity of clinical diagnosis of fungal keratitis on comparison with microbiological diagnosis may reach 95%.[6]

A study conducted by Bharathi *et al.*,[6] showed that majority of the patients with diagnosis of mycoticcorneal ulceration, including both pigmented and non-pigmented fungal keratitis, presented with typical clinical features. Dry, thick, and raised corneal surface (75.43%), stromal infiltrates with feathery margins (71.78%), typical satellite lesions (10.05%), dentritic pattern (4.2%), and white immune ring in the mid-periphery of cornea (1.37%) were observed. Hypopyon was present in 55.62% patients, deep stromal infiltration in 37.53% patients, corneal perforation in 1.37% patients and corneal abscess in 1% patients. Eyes with non-pigmented keratitis are associated with significantly larger ulcers and poorer vision at presentation compared to those with keratomycosis due to pigmented fungi ($P = 0.01$).[68]

Garg *et al.*,[7] on the other hand, studied 88 cases of fungal keratitis caused by dematiaceous group of fungialone and established that only 24 eyes (27.27%) presented with the characteristic macroscopic pigmentation. This finding is almost double of that observed by Sengupta *et al.*[68] (14.50%), who also reported that 53 eyes (61.3%) had the classical clinical picture of yellow-white, dry raised infiltrate with feathery hyphate edges at initial examination. Treatment of

mycotic keratitis with corticosteroids and antibiotics may lead to the development of corneal ulcer after 1 week of treatment and when left untreated for up to 3 months, it may progress to phthisis bulbi.[25,40] The healing response to anti-fungal treatment in terms of visual acuity of dematiaceous fungal keratitis is significantly good when compared with *Fusarium* and *Aspergillus* mycokeratosis.[1] However, Sengupta *et al*.,[68] showed similar response to medical therapy and similar visual outcome of pigmented fungal keratitis when compared with non-pigmented keratitis.

In Vivo Confocal Microscopy (IVCM) and Anterior segment Optical Coherence Tomography (AS OCT) examination can demonstrate various signs of fungal keratitis like the presence of small, round, hyper-reflective cells surrounded by hypo-reflective irregular areas and highly reflective dendritic shaped cells at the level of the epithelium, etc. Martone *et al*.[69] demonstrated the association of the isolation of *Alternariaalternata* with these clinical signs and observed a significant reduction of the inflammatory cells and a hyper-reflective scar-like tissue after a month of anti-fungal treatment. They also documented the healing process and concluded that IVCM and AS OCT could, therefore, be useful for the early diagnosis and monitoring of treatment of fungal keratitis.

MICROBIOLOGICAL ANALYSIS

Criteria for diagnosis of keratitis include growth on multiple culture media or a positive microscopy with growth in single culture medium, whereas those of orbital and intra-ocular infections include growth in culture with positive microscopy.[9]

Specimen collection

Corneal scrapings are taken from the corneal ulcers by ophthalmologists under aseptic conditions using a heat-sterilized platinum spatula. The procedure may be performed under magnification of slit-lamp or operating microscope after instillation of 4% lignocaine (lidocaine) without preservative. Very little material is usually obtained because of the risks of corneal thinning or perforation. Physicians should be instructed to first inoculate the specimen directly onto a non-inhibitory medium, such as Sabouraud's dextrose agar (SDA), and then to place some material on a sterile glass slide (in the center) for KOH mount or staining. The scraping should be placed in two or three places on the plate, using an X- or C-shaped motion. The inoculated plate should be kept at room temperature and transported immediately to the laboratory.[70]

Vitreous or vitreous humor is the clear, gelatinous material that fills the space between the lens of the eye and the retina. When taken by physicians, vitreous is often diluted by irrigation fluid due to which it should be concentrated by centrifugation and the sediment should be used to inoculate media and to make smears. Specimens should be placed onto SDA, inhibitory mould agar, and/or brain heart infusion (BHI) agar with 10% sheep blood and inoculated at 30°C. Media containing cycloheximide should be avoided.[70]

Cultural and non-cultural techniques

Pheohyphomycotic keratitis

Of the 1,095 culture-positive mycotic keratitis cases studied by Bharathi *et al*.,[6] 10% KOH wet mount preparation had a sensitivity of 99.23% in the detection of fungal filaments (including both pigmented and non-pigmented fungi), whereas that of Gram-stained smear was 88.73%. On considering pigmented fungi alone Garg *et al*.,[7] reported that septate branching fungal filaments were identified under light microscopy in 88.63% of the 88 cases studied.

Of the 12 dematiaceous fungal keratitis studied by Gajjar *et al*.,[1] 11 (91.6%) samples showed growth on SDA within 24 hours. Colony morphology of all the dematiaceous fungi isolated showed the presence of a peculiar color. Pink or light brown colony in the case of *Curvularia spp*., yellow for *Papulaspora spp*., and dark brown for *Exserohilum spp*. were observed. A sample of *Lasiodiplodiatheobromae* obtained from the scraping material was grown for 48 hours and a gray fluffy growth with abundant aerial mycelia was visible. They also observed that SDA did not support sporulation of *Curvularia spp*. and *Lasiodiplodia spp*. in all the samples. *Curvularialunata* and *Exserohilumrostratum* were identified using their growth characteristics and typical spores, and this was confirmed using the Internal Transcribed Spacer (ITS) sequence. ITS sequences are the most widely sequenced DNA region in fungi and by using this, all the other dematiaceous isolates were identified as *Lasiodiplodiatheobromae*, *Cladorrhinumbulbilosum*, and *Cladosporiumcladosporioides*. The ITS sequence misidentified only one isolate as *Chaetomium spp*., which was later identified as *Papulaspora spp*. on the basis of its typical microscopic features.

Of the ten PCR confirmed dematiaceous fungi described by Tananuvat *et al*.,[49] five isolates were obtained by conventional fungal culture. The number of true positives and false negatives were, thus, 5 each on comparison of results of culture and PCR in the diagnosis of dematiaceousmycotic keratitis. Ferrer *et al*.,[23] also reported the application of PCR and molecular methods to provide a rapid diagnosis, which resulted in the administration of specific and effective therapy.

Pheohyphomycotic endophthalmitis

A vitreous sample obtained by pars planavitrectomy (PPV) is superior to that by vitreous tap. Small *et al*.,[55] detected 7% (1/14) fungus by cytologic or culture examination from initial vitreous tap, whereas vitreous samples obtained by PPV, by comparison, resulted in fungus-positive cytologic results in 43% (6/14) of eyes and positive culture results in 36% (5/14) of eyes.

ANTI-FUNGAL TREATMENT

Mycotic keratitis is managed by medical or surgicalmeans. Medical therapy consists of non-specific measures and the use of specific anti-fungal agents. Cycloplegics are used to relieve the iridocyclitis (anterior uveitis) that usually accompanies mycotic keratitis and broad-spectrum anti-bacterials may be needed to combat secondary bacterial infection.[71] In a study conducted by Tananuvat *et al*.,[49] four out of the ten dematiaceous fungal keratitis responded to medical treatment alone. Untimely diagnosis of fungal keratitis often leads to failure of medical therapy that requires surgical management like penetrating keratoplasty or enucleation.[72,73]

There are currently no Clinical and Laboratory Standards Institute (CLSI) interpretative criteria or guidelines for mould susceptibility by microbroth dilution.[74] The minimum inhibitory concentration (MIC) results are, therefore, reported as μg/mL without any interpretations in most studies. Success of therapy is determined by symptomatic improvement of the patient's ocular status and/or negative results on repeated culture. In a comparative study performed by Sengupta *et al*.,[68] on the incidence and outcomes of pigmented versus non-pigmented keratomycosis, both groups responded favorably to medical therapy (78.1% vs. 69.1%) with scar formation and showed a significant improvement in mean visual acuity compared with that at presentation. This study also showed that location of the ulcer was the only factor that had significant predictive value for visual outcome.

Minimum inhibitory concentration (MIC)

Konidaris *et al*.,[22] performed an anti-fungal susceptibility testing for a strain of *Alternariaalternata* isolated from a case of infected corneal transplant. The test was done by E-test strips (bioM´erieux S.A., Marcy-l'Etoile, France) on Roswell Park Memorial Institute (RPMI)-1640 3-(N-morpholino) propanesulfonic acid (MOPS) agar plates, which revealed an MIC of 0.047 μg/mL for amphotericin B, 16 μg/mL for flucytosine, 0.023 μg/mL for itraconazole, 0.125 μg/mL for voriconazole, 0.064 μg/mL for posaconazole, and 0.125 μg/mL for caspofungin. Saracli *et al*.,[43] described the *in vitro* susceptibility testing they performed from an isolate of *Scedosporiumapiospermum*, which showed an MIC value of 16 μg/mL against amphotericin B,0.125 μg/mL against itraconazole, and 4 μg/mL against fluconazole.

The anti-fungal susceptibility testing performed by Gajjar *et al*.,[1] for dematiaceous fungi causing keratitis provided MIC values against various drugs: *Cladorrhinumbulbilossum*, *Lasiodiplodiatheobromae*, and *Papulaspora spp*. showed MIC values of 0.064 μg/mL, 0.064 μg/mL, 0.32 μg/mL, 0.016 μg/mL against natamycin, amphotericin B, fluconazole and itraconazole, respectively. Two strains of *Exserohilumrostratum* also showed geometric mean of MIC against natamycin (2 μg/mL), amphotericin B (1 μg/mL), fluconazole (32 μg/mL), and itraconazole (0.25 μg/mL). Four strains of *Curvularialunata* showed geometric mean of MIC value against natamycin (12.5 μg/mL), amphotericin B (16 μg/mL), fluconazole (128 μg/mL), and itraconazole (128 μg/mL).

A *Curvularialunata* strain isolated from a case of fungal endophthalmitis showed an MIC value against fluconazole (4 μg/mL), voriconazole (0.12 μg/mL), ketoconazole (0.12 μg/mL), amphotericin B (0.12 μg/mL), itraconazole (0.03 μg/mL), and 5-flurouracil (>128 μg/mL).[74]

Routes of drug administration

Anti-fungal drugs administration may be through topical,[21,44,45,47,64] intra-stromal[72,75], or intra-cameral[48] routes for the management of keratitis. Systemic routes, oral or intravenous, may also be used in addition to local therapy.[23,35,36,76,77] Endophthalmitis may be managed by intra-vitreal administration of drugs.[58,61]

Clinical outcomes of anti-fungal drug therapy

The clinical outcomes of various drugs used for the treatment of pheohyphomycosis of the eye are described below:

Fluconazole

Twenty-two out of the 25 cases of *Aureobasidiumpullulans* keratitis responded well to treatment with topical and intravenous fluconazole.[40] The therapeutic response of four patients who had proven infection of the ocular structures with *Curvularia spp*. treated with fluconazole orally and/or topically was good.[78] However, a case of *Lasiodiplodiatheobromae* keratitis demonstrated resistance to fluconazole.[48]

Itraconazole

A patient with a recurring corneal infection caused by *Fonsecaeapedrosoi*, who was treated with a therapeutic penetrating keratoplasty in combination with systemic itraconazole for 5 months showed no recurrence thereafter.[29] A patient with mycotic keratitis due to *Scedosporiumapiospermum* was cured clinically after itraconazole treatment and surgical intervention.[43] However, a case of *Lasiodiplodiatheobromae* keratitis demonstrated resistance to itraconazole.[48]

Voriconazole

Treatment of an *Alternaria* keratitis with oral and topical voriconazole was followed by a steady resolution within 10 days of therapy.[79] Therapeutic response to topical voriconazole of *Scedosporiumapiospermum* keratitis was good.[44,45,47] A case of traumatic endophthalmitis due to *Scedosporiumapiospermum* was successfully treated with a combination of intra-vitreal and systemic voriconazole.[58]

Miconazole

Cladorrhinumsppisolated from a case of keratomycosis in 1979 was successfully treated with miconazole.[28]

Amphotericin B and Other Polyenes

Topical administration of amphotericin B is effective against keratitis caused by *Alternaria*, *Fonsecaeapedrosoi*, and *Exophiala dermatitidis*.[20,30,32] However, Ozbek *et al*.,[79] reported recalcitrance of *Alternaria* keratitis to treatment with topical amphotericin B. There have also been reports on therapeutic failure of amphotericin B treatment of keratitis caused by *Phialemoniumcurvutum* and *Scedosporium apiospermum*.[41,43] Natamycin when administered topically healed a dematiaceous fungal conjunctivitis that mimicked a melanoma, whereas corneal ulcers of 25 *Aureobasidiumpullulans* keratitis cases showed negligible improvement to topical natamycin.[40,64] *Bipolarishawaiiensis* keratitis has been shown to respond successfully to treatment with nystatin ointment.[24]

Caspofungin

Neoh *et al*.,[72] reported the use of intra-stromal caspofungin to treat *Alternaria* keratitis in a case in which clinical resolution was not fully achieved despite the use of topical caspofungin in addition to extensive use of topical, intra-stromal, and oral voriconazole. Relapse of the corneal pathology was not seen 15 months follow-up.

Combined drug therapy

There are several reports on the successful outcome of treatment of dematiaceous fungal keratitis with a combination of Azole and Polyene group of drugs. *Alternariaspp* keratitis has been successfully treated with a topical amphotericin B in addition to orally administered ketoconazole[80]; systemic amphotericin B and topical fluconazole[23,77]; intra-stromal voriconazole with concurrent topical caspofungin.[75] Symptomatic improvement of *Alternaria* keratitis has also been seen with a topical therapy of natamycin and amphotericin B.[21] A combination of topical natamycin and oral itraconazole has been reported to cure keratitis caused by *Bipolaris spicifera*[25] and *Exserohilum rostratum*[35] and that caused by *Exserohilummcginnisii* has been treated with a topical amphotericin B and oral itraconazole with therapeutic success.[36] Intra-cameral amphotericin B in conjunction with topical voriconazole cured *Lasiodiplodoatheobromae* keratitis that was refractory to treatment with topical voriconazole alone.[48] *Pheoacremoniumparasiticum* endophthalmitis has a good therapeutic outcome on treatment with an intra-vitreal amphotericin B and oral voriconazole.[61]

CONCLUSION

Incidence of pheohyphomycosis of the eye, especially keratitis, due to pigmented fungi may be increasing as compared to previous data. *Curvularia* is the commonest pigmented fungi isolated from mycotic keratitis. Cases of endophthalmitis, dacryocystitis, and scleritis due to pigmented fungi have been encountered. Factors increasing the risk of dematiaceous fungal infection of the eye are similar to those of non-pigmented fungal infections.

In routine mycology laboratory, conventional diagnostic procedures like KOH mount, gram-stained smear, and culture are still essential tools to identify causative organisms even though it may be less sensitive than the PCR. Moreover, culture allows for testing of anti-fungal susceptibility and will allow growth of fungal organisms not detectable with the primers used in the PCR assay. However, PCR may be considered as an alternative, or adjunctive in cases that the causative pathogen cannot be identified by a routine work-up.[49] In addition to PCR, DNA typing is also a useful tool in the diagnosis of fungal infections. The most widely sequenced DNA region in fungi is the ITS region, and the International Sub-Commission of fungal bar coding has proposed the ITS region as the prime fungal bar code for species identification.[81]

Pigmented fungal keratitis has similar response to medical therapy and similar visual outcome compared to non-pigmented keratitis. Central ulcers which resulted from both pigmented and non-pigmented fungal keratitis have a poor visual outcome.[68] Voriconazole is effective against most reported cases of keratitis.[79] Combined anti-fungal drug therapy may be adopted when mono-therapies fail to eradicate the infection. A combination of Azole and Polyene group of drugs may be an ideal treatment protocol especially for non-healing fungal keratitis. *In vitro* anti-fungal susceptibility profile provides valuable information in selecting a proper anti-fungal agent for treatment. However, these results should be interpreted with caution because *in vitro* studies are only limited approximations of the *in vivo* situations. Various host factors and routes of drug administration may affect the outcome of therapy. Intra-vitreal administration of anti-fungal drugs must be an ideal approach to treating fungal endophthalmitis. Mycotic keratitis, however, may be managed through topical, intra-stromal, or intra-cameral administration of drugs in addition to systemic use. Studies of correlation of *in vitro* data with clinical outcome are needed for a moredefinitive evaluation of the predictive value of MICs for filamentous fungi. Mycotic infection of the eye usually responds to medical therapy when diagnosed and treated on time. Cases with late diagnosis often lead to unresponsive medical management.

Majority of the studies on the epidemiology and management of pheohyphomycotic infection of the eye are based on retrospective analysis of data[5,7,8,12,55] and limited size of study populations.[1,49,55] We, therefore, recommend a prospective, randomized, comparative long-term study of various anti-fungal agents to assess their efficacy and the

risks and benefits associated with each anti-fungal in addition to formulating a treatment protocol for non-healing fungal keratitis.

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