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Research Article

Fungal Keratitis - A Brief Overview

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Abstract

Fungal keratitis is a fungal infection of the cornea. It primarily affects the corneal epithelium and stroma, although the endothelium and anterior chamber of the eye may get involved in more severe disease. Fungal keratitis remains a diagnostic and therapeutic problem for ophthalmic clinicians because these fungal infections of the cornea frequently result in corneal melting, visual impairment and devastating ocular damage if they are not diagnosed early and treated promptly and effectively. Hence there is a need for more sensitive and specific diagnostic methods for early detection of fungal keratitis. Also effective antifungal agents with good corneal tissue penetration are desired for improved clinical outcomes.

Keywords: Diagnostic methods; Fungal keratitis; Treatment; Visual impairment

Introduction

Keratitis is an inflammation of the cornea and is often caused by bacteria, fungi, viruses and parasites [1]. In tropical countries, including India, keratitis is the most frequently encountered fungal infection, although the orbit, lids, lacrimal apparatus, sclera, conjunctiva and intra-ocular structures may also be involved [2]. Fungal keratitis (also termed as keratomycosis, Mycotic keratitis) is a fungal infection of the cornea. It primarily affects the corneal epithelium and stroma, although the endothelium and anterior chamber of the eye may get involved in more severe disease. It was first described by Leber in Germany in1879 [3]. Since then it has been recognized as a major public health problem causing visual loss and blindness, especially in developing countries, and presently it represent 40-50% of culture proven infectious keratitis [4,5]. Fungal keratitis remains a diagnostic and therapeutic problem for ophthalmic clinicians because these fungal infections of the cornea frequently result in corneal melting, visual impairment and devastating ocular damage if they are not diagnosed early and treated promptly and effectively [6]. Hence there is a need for more sensitive and specific diagnostic methods for early detection of fungal keratitis. Also effective antifungal agents with good corneal tissue penetration are desired for improved clinical outcomes [7]. This review was aimed to discuss briefly about etiological agents, pathophysiology, diagnosis and management of fungal keratitis.

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Epidemiology of Fungal Keratitis

The epidemiological variation and wide geographical distributions are related to the economic and climate factors [8]. Its incidence ranges from 6% to 20% of all microbial keratitis, and is seen in about 50% in culture-proven cases [9,10]. Traditionally, it is considered a disease of rural areas and is frequently caused by trauma with vegetative material. However its incidence is increasing in urban areas due to widespread use of contact lenses and topical steroid usage [11].

It is seen that there is preponderance of filamentous fungi in tropical areas while temperate climates show higher percentages of yeast infections [12,13].

Predisposing factors

Various known risk factors for fungal keratitis are enumerated below:

- Trauma to the eye with vegetative matter is the most common predisposing factor. Trauma could predispose to ulcerative keratitis in 23-55% of cases as per the incidence predicted in various studies [13]. A typical history of young adult engaged in agricultural or outdoor work gets trauma with vegetative matter and develops an ulcerative lesion in 10-15 days is seen in cases of fungal keratitis. The peak months of disease correspond to harvesting season, when chances of injury with infected vegetative matter are very high.
- Environmental factors like humidity, rainfall and wind greatly influence the occurrence of filamentous fungal keratitis [14].
- Contact lens overuse especially with hydrogel contact lenses [15].
- Corneal surgery such as penetrating keratoplasty, clear cornea (sutureless) cataract surgery, photorefractive keratectomy, or Laser *In Situ* Keratomileusis (LASIK) [16].
- Chronic Keratitis due to herpes simplex, herpes zoster, or vernal keratoconjunctivitis [16].
- Long term topical medications like corticosteroids and antibiotics [13,16].
- Allergic conjunctivitis due to airborne or bacterial toxins in tears or chemical agents causing injury to eye [14].
- Long term illnesses like diabetes, atopic diseases, cancer, long term systemic steroids or cytotoxic drugs [16].
- Pre-existing ocular conditions namely insufficient tear secretion, defective eyelid closure etc., [16,17].

However, for keratitis caused by yeasts (*candida*), three important risk factors are more commonly associated such as chronic ocular surface disease, contact lens wear and use of topical steroids [18].

Etiological agents

More than 105 species of fungi have been implicated as the etiological agents of fungal keratitis [19]. These may be hyaline or pheoid hyphomycetes and yeast like fungi. However, majority of cases are caused by *Aspergillus*, *Fusarium*, *Curvularia* and *Candida spp.* In tropical areas, *Aspergillus* and *Fusarium spp.* are common agent while in temperate regions *Candida albicans* is the usual isolate in fungal keratititis [20]. The commonly encountered fungal agents are enumerated in table 1 [19-21].

Hyaline hyphomycetes	*Aspergillus*	*A. fumigatus, A. flavus*
	Fusarium	*F. solani, F. oxysporum*
	Acremonium	*A. potronii, A. kiliense*
	Penicillium	*P. spinulosum, P. citrinum*
	Scedosporium	*S. apiospermum*
	Paecilomyces	*P. lilacinus, P. variotii*
	Zygomycetes (rarely)	*Absidia, Rhizopus*
Phaeoid hyphomycetes	*Curvularia*	*C. lunata, C. geniculata, C. senegalensis*
	Bipolaris	*B. spicifera, B. hawaiiensis*
	Exserohilum	*E. rostratum, E. longirostrata*
	Fonsecaea	*F. pedrosoi*
	Colletotrichum	*C. dematium*
	Aureobasidium	*A. pullulans*
Yeast like fungi	*Candida*	*C. albicans, C. tropicalis, C. krusei*
	Trichosporon, Rhodotorula, Saccharomyces	Rarely seen
Dimorphic fungi	*Histoplasma, Blastomyces, Cryptococcus*	Very rare

Table 1: Fungal agents responsible for fungal keratitis.

Pathogenesis of fungal keratitis [8-10]

Fungal keratitis occurs due to interaction between different factors like invasiveness and toxicity of fungal agent, host factors, hypersensitivity reaction and underlying defect of neutrophils. Fungi are not able to penetrate the intact corneal epithelium, unless the eye is severely immunocompromised. Hence any trauma particularly by organic matter facilitates the penetration of the fungal inoculum into corneal stroma [22]. Spores germinate to produce hyphae and hyphal forms traverse through the stroma and reach the Descemet's membrane. Satellite lesions are formed around the main site of involvement. Organism can perforate the entire cornea and reach the anterior chamber, giving rise to hypopyon. Leukocytic infiltrate with feathery borders in corneal stroma are characteristic of fungal keratitis. Once, invasion occurs, the intrinsic virulence of fungi, helps them to proliferate within the corneal tissue, resisting the host defense and producing tissue damage. Toxins and enzymes such as hemolysins, exotoxins and proteases, liberated by the fungi contribute to the tissue damage accentuated further by the host inflammatory response. Organisms that infect preexisting epithelial defects belong to the normal microflora of the conjunctiva and adnexa as seen in *candida* infection [23,24]. Also, *Candida albicans* produces phospholipase A and lysophospholipase on the surface of blastospores, facilitating the entrance to the tissue [24].

Clinical features

Patient often presents with symptoms like foreign body sensation, increasing eye pain or discomfort, blurring of vision, redness in the eye and photophobia [23]. Slit lamp examination reveals breach in corneal epithelium. As the disease progresses, central shaggy-edged ulcer with satellite lesions, hypopyon, and endothelial plaque is seen [25]. Stromal keratitis caused by *candida spp*. may be more localized and have collar- button configuration with small ulceration and expanding infiltrate. In fungal keratitis caused by filamentous fungi, firm elevated slough, 'hyphae margins extending beyond the ulcer edge into the normal cornea, multifocal granular (or feathery) grey-white 'satellite' stromal infiltrates, immune ring (Wessley's ring), Descemet's folds, endothelial plaques along with corneal abscess seen [26,27]. In cases caused by phaeoid fungi, pigmented infiltrate may be an important diagnostic clue. These are the basic features seen in fungal keratitis, however clinical manifestations vary according to the etiological agent. Fusarium infection is severe and can cause perforation, deep extension and malignant glaucoma and can destroy the eye completely within few weeks while *Aspergillus* keratitis is less severe and more amenable to therapy than *Fusarium* keratitis [27,28]. There may be mixed infection particularly due to *acanthamoeba spp.* (in contact lens wearer) or bacterial agents.

Diagnosis of Fungal Keratitis

Early diagnosis and prompt treatment of fungal keratitis is necessary to prevent vision loss and other complications.

Sample collection

Clinical samples collected for laboratory diagnosis include corneal scraping, corneal biopsy and anterior chamber aspirate. Corneal scrapings are collected with sterile Kimura spatula or no. 15 Bard-Parker surgical blade aseptically using local anaesthetic (4% xylocaine) drops. Material is collected both from the base as well as from the edge of the ulcer, after retracting the lids properly and after cleaning any discharge or debris from the vicinity of the ulcer [29]. Corneal biopsy from lesion with 4 mm or larger trephine can be taken, but it is an invasive procedure requiring minor operation theatre. Biopsy is indicated in cases with strong clinical suspicion of fungal keratitis, at least twice negative smear and culture report and no clinical improvement on empiric antibiotic therapy. Biopsied material is sent for culture and smear examination, and also for histopathological examination [29]. Anterior chamber aspirate is required in cases with strong clinical suspicion of intra ocular infection or in cases with progressive corneal damage and persistent hypopyon. The aspirate is collected via limbus using 22 gauge needle in sterile tuberculin syringe [29].

Processing of samples

Corneal material is used for 10% KOH wet mount, gram staining and culture. Sensitivity of KOH wet mount ranges from 33 to 92% while of gram stain ranges from 60-75% in detecting the causative organism [30]. Other staining techniques like hematoxylene and eosin staining, Periodic Acid Schiff (PAS) staining, Gomori's methenamine silver staining has also been recommended. KOH preparation and staining are used to obtain rapid information about the pathogen [31]. However, if fluorescein microscopy is available, acridine orange and calcofluor white are the stains of choice [31]. Corneal material is inoculated on two sets of Sabouraud dextrose agar containing antibiotics but without cycloheximide (incubated at both 25°C and 37°C), one set of blood agar and one set of Brain-Heart Infusion (BHI)

agar in 'C' or 'S' shaped streak and incubated at 37°C for four weeks [19]. Growth occurring on streaked area is considered significant and processed further. Mycelial isolates are identified by their colony morphology, LCB (Lactophenol Cotton Blue) stain and by slide culture. Yeasts can be speciated by tests like chlamydospore formation on cornmeal agar, germ tube production, sugar fermentation and assimilation tests, urease test, reduction of tetrazolium medium and other biochemical tests [32]. Growth of fungi in culture is considered significant if it correlates with the clinical presentation, if growth of the same fungus is demonstrated on two or more culture media, growth seen at the site of inoculation and is consistent with microscopy. Although culture is considered as the gold standard for FK diagnosis, the major limitations are the length of time consumed for confirming the culture growth because the fungi often have a slow growth, and a high chance of culture media contamination.

Other laboratory tests

Diagnosis of fungal keratitis by conventional culture technique may be problematic because of the very small sample obtained by scraping the corneal ulcer. Therefore, recent methods for the identification of fungi have been under study and include immunofluorescence staining, electron microscopy, and confocal microscopy. Confocal microscopy may help in correctly diagnosing early stages of fungal keratitis and in monitoring disease progress at the edges and depth. It may also help guide timely decision for keratoplasty and may be helpful in determining when to stop medication [33]. Serological method for detection of antigen or antibody in serum is not useful because of the localized infection, and can be used only in immunocompromised patients when infection becomes systemic [32].

Molecular methods like Polymerase Chain Reaction (PCR) assay, RT-PCR (Real Time PCR), PCRSSCP (Single Stranded Conformational Polymorphism) and PCR-RFLP (Restriction Fragment Length Polymorphism) techniques have also been standardized for fungal identification [34]. Of these, PCR is most commonly used for diagnosing fungal keratitis because it offers increased sensitivity and significant reduction in the time required to establish a diagnosis. Its sensitivity ranges between 89 to 94%, whereas, specificity ranged between 50% to 88% [35,36]. It can detect fungal DNA for the majority of fungal corneal ulcers with negative culture, and by using the DNA sequencing or specific primers, novel organisms can be detected in culture-negative cases [36]. Real time PCR can also quantify the load of the organism and can be used in the treatment follow up. However, there are certain limitations with molecular methods such as differentiating between active and latent infections, viable and nonviable organisms especially after treatment, cost and false positive results may also occur during the DNA sequencing due to contamination [37].

Histologic findings

Histopathologic examination of corneal buttons can reveal the presence of fungal elements in fungal keratitis patients. Vertical oriented fungal elements in regard to stromal lamellae and penetration of the Descemet membrane by fungal elements depicts an aggressive organism and a higher risk for contamination of the globe [32].

Imaging studies

Ophthalmic B-scan ultrasound may be necessary if there is suspicion of posterior segment involvement to rule out concurrent fungal endophthalmitis.

Treatment of Fungal Keratitis

Delay in appropriate treatment may lead to consequences like blindness and corneal perforation for which surgical procedure is required. Treatment on the basis of correct identification of fungi and its antifungal susceptibility has better outcomes than empirical treatment with broad-spectrum antibiotics. Therapy depends on the etiological agent as well as depth of lesion. For superficial keratitis, topical agents like natamycin (5%) or amphotericin B 0.15% is usually selected as first-line therapy, however deep lesion involving corneal stroma necessitates addition of systemic therapy, such as oral ketoconazole/itraconazole, or fluconazole [28,38]. Topical voriconazole is increasingly favored among ophthalmologists because of its wide spectrum of coverage against yeasts and filamentous agents of fungal keratitis [39]. Topical and oral posaconazole have also been used with success in therapy of *Fusarium* keratitis, resistant to voriconazole [40,41]. We should see for clinical signs of improvement like diminution of pain, disappearance of satellite lesions, rounding out of the feathery margins of the ulcer and hyperplastic masses or fibrous sheets in the region of healing fungal lesions [42]. Negative cultures during treatment do not indicate eradication of infection and there may be active proliferation of the fungi deep in the stroma; hence, long term therapy is recommended. Topical therapy is applied hourly for minimum 6 weeks in superficial keratitis, and therapy is usually extended for 12 weeks in deep keratitis or in keratitis caused by filamentous fungi [43]. Topical corticosteroid therapy is contraindicated for any of the eye ailment in fungal keratitis because even low dose steroids may lead to bad prognosis [43].

If corneal infection progresses inspite of vigorous antifungal therapy or in patients with impending perforation with presence of a descemetocele, surgical debridement like penetrating keratoplasty (full thickness corneal grafting) is done [44]. In this procedure, at least 0.5 mm of clear corneal tissue is excised all around the infected area in order to decrease the chances of recurrence. In some cases where infection does not extend through the entire thickness of the cornea, lamellar keratoplasty can be considered [45]. Hypopyon, corneal perforation, corneal infection extending to limbus, or lens infection are major risk factors for recurrence of infection [44]. Table 2, is highlighting the management protocol of fungal keratitis.

Filamentous fungi		
Superficial keratitis	1st choice	Natamycin 5% ointment
	2nd choice	Amphotericin B 0.15% drops
Deep keratitis		Oral itraconazole or fluconazole along with topical therapy
Yeast like fungi		
Superficial keratitis	1st choice	Amphotericin B 0.15% drops
	2nd choice	fluconazole 2%/itraconazole 1%/ voriconazole 1% drops
Deep keratitis		Oral itraconazole or fluconazole along with topical therapy
Non-responders or presence of descemetocele		Penetrating keratoplasty along with topical and systemic antifungals

Table 2: Treatment commonly used in fungal keratitis [42,45].

Newer modality of treatment

Corneal collagen cross linking is an exciting new technique using the photosensitizer riboflavin (vitamin B2) and Ultraviolet A (UVA) irradiation at 370 nm to induce increasing corneal tissue strength and rigidity [46]. UV irradiation onto riboflavin saturated cornea generates oxygen and superoxide anion radicals, which induce intra and/or interhelical collagen cross-links and also inactivate microbial pathogens [47]. Several studies demonstrated *in vitro* antimicrobial activity of corneal collagen cross linking against various pathogens [48-50]. Riboflavin/UVA adjuvant to antifungal therapy seems to be a promising technique for management of fungal keratitis. Further randomized clinical trials are required to establish the risks and benefits of this technique.

Conclusion

Fungal keratitis is a leading cause of visual loss and its remains a diagnostic and therapeutic challenge for ophthalmologists. Early diagnosis and prompt treatment of this condition is critical for saving visual loss. As there is often delay or misdiagnosis of fungal keratitis, aggressive diagnostic efforts and maximal therapeutic strategies should be exercised in cases having high suspicion or in failure of keratitis to respond to antibacterial therapy. New areas of research and development into both diagnostic and therapeutic methods may lead to prompt initiation of specific treatment and improved prognosis with better management of the fungal infection.

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