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Fungal Keratitis: Recent Advances in Diagnosis and Treatment

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Abstract

Fungal keratitis or fungal corneal ulcer is potentially blinding infection of cornea, is considered one of the major cause of ocular morbidity, particularly in developing countries. It is a common cause of infectious keratitis, especially in tropical and subtropical countries. Fungal keratitis is notoriously challenging to diagnosis and difficult to treat. Delay in diagnosis may result in irreversible sequelae of corneal fungal infections, which can be preventable. Fungal keratitis often have worse treatment outcomes than bacterial keratitis, Delayed diagnosis and scarcity of effective antifungal agents are the major factors for poor outcome. In the recent years considerable advancement in the diagnosis and treatment has been occurred. In this chapter, we will discuss the recent advances in diagnosis and management of fungal keratitis with a brief discussion on pathogenesis and future therapeutic models.

Keywords: infectious keratitis, fungal keratitis, confocal microscopy, polymerase chain reaction, metagenomics deep sequencing, voriconazole, posaconazole

1. Introduction

Infectious keratitis is an inflammation of the cornea caused by microorganism. It is most commonly associated with bacterial, fungal or viral microorganisms that invade into the corneal stroma, resulting in inflammation and destruction of these structures; ultimately leading to visual impairment and blindness. Fungal keratitis (FK) or keratomycosis is one of the most challenging to diagnose and difficult to treat. The prevalence of fungal keratitis is variable depending upon the geographic location. It is more common in tropical and subtropical areas and relatively rare in temperate countries. It is reported about 1–60% of all cases of microbial keratitis in various studies [1–3]. A recent review including 37 countries reported highest proportion in Vietnam (59.58%) followed by Paraguay (58%) [2–4]. The fungi that commonly cause infection of the cornea include *Fusarium*, *Aspergillus*, *Curvularia*, *Bipolaris*, and *Candida* [1, 2, 5].

Most of the currently available antifungal medications have limitations, such as poor bioavailability and limited ocular penetration, especially in cases with deep keratitis [6–8]. This results in slow resolution of fungal infections. In addition, fungi can penetrate deeper into corneal stroma and Descemet membrane, therefore more difficult to eradicate. Surgical excision of the infected cornea is required to control the infection in nonresponsive cases [9–12]. In recent years, considerable research is being continue in the field of management of fungal keratitis and several

newer antifungal agents and drug delivery techniques are being evolved to overcome these limitations and improve outcome. In this chapter, we discuss the recent advances in diagnosis and treatment of fungal keratitis with a brief discussion on pathogenesis and future considerations.

2. New aspects of pathogenesis

Pathogenesis of FK has not been fully elucidated. Recent studies and advances have contributed in better understanding of the complicated process and host immune response.

2.1 Risk factors

The common risk factors for fungal keratitis are trauma with vegetative matter or objects contaminated with soil, contact lenses, ocular surface disease, lacrimal duct occlusion, fungal skin infections, long-term use of antibiotics or steroids locally or systemically [2, 13–17]. Other relatively rare risk factors include history of eye surgery, herpes simplex virus keratitis, eyelid abnormalities, etc. [18, 19].

Still in developing countries, the most common risk factor for fungal keratitis is ocular trauma but in developed countries, contact lens emerged as more common risk factor. This change has been occurred due to industrialization of farming and increase use of contact lens in developed world. In a large case series of 695 cases with fungal keratitis reported from 10 tertiary eye care centres across the United States over a 7-year period, 283 (40.71%) cases involved the use of contact lens [1]. Similarly Keay et al. in a multicentre case series of 733 cases from 11 tertiary care centres across the United States reported that 37% cases were associated with refractive contact lens wear, 25% were associated with ocular trauma, and 29% were associated with ocular surface disease [20].

In a study, the storage of the anti-microbial agent alexidine in its plastic containers at higher than room temperatures was found as the reason for decreased effectiveness [21]. This temperature difference in the plastic containers led to decreased concentration of the agent in solution (2.8 times less) and a corresponding higher concentration in the walls of its plastic containers (3.1 times higher) [22]. The lens type and its soaking time significantly influences the fungicidal activity of cleaning and storage solutions and poor compliance significantly increase the risk of contamination [23, 24].

2.2 Causative fungi

A review article found about 144 species of fungi from 92 genera as causative agents in keratitis, showing largest diversity; whereas 77 species from 42 genera of bacteria, 12 species from 4 genera of protozoa and only 4 types of viruses were implicated in infectious keratitis. However, in the majority of cases of FK the causative organism belong to a few genera: *Fusarium*, *Aspergillus*, and *Candida* [25]. Other fungi implicated in mycotic keratitis are *Curvularia*, *Alternaria*, and *Penicillium* [2, 13, 14]. The rarely reported fungal pathogens include *Lasiodiplodia theobromae*, *Cylindrocarpus* species, *Metarhizium anisopliae*, *Paecilomyces* species, and *Pythium insidiosum* [15, 26–29].

2.3 Host immune response

Fungal infections initiates with adhesion of fungal cells with epithelial surfaces. Fungi produce various surface proteins to contribute to the adhesion to the corneal

epithelium, which has potential fungal binding sites such as laminins, fibronectins, and collagens [30, 31]. Alterations of the corneal surface due to trauma or other predisposing condition result in easy invasion of organisms deeper into underlying layers, which leads to an innate and adaptive immune-mediated inflammation, resulting in subsequent tissue necrosis of the surrounding area, consequently leads to further tissue damage, scarring, and opacification of the cornea.

2.3.1 Cytokines and innate immunity

The contact between fungi and host, result in expression of pattern recognition receptors (PRRs) on host epithelial and immune cells, which recognize the fungi. PPRs are Toll-like receptors (TLRs, including TLR2 and TLR4), C-type Lectin receptors (CLRs, including Dectin-1, Dectin-2 and Mincle). Dectin-1 recognizes β -glucan in fungal cell wall, while Dectin-2 and Mincle recognize mannan of cell wall [32]. *Candida albicans* and *Aspergillus fumigatus* encounter during fungal keratitis have been reported to be sensed by TLR [33–35]. Activated TLRs in corneal epithelium induce production of CXC chemokines and recruit neutrophils (are more than 90% of the infiltrating cells). Neutrophils are predominant source of mature interleukin-1 β (IL-1 β) and acidic mammalian chitinase (AMCase) in corneas, which can inhibit the hyphal growth [36, 37]. The increased expression and the activation of PPRs in response to *A. fumigatus* with resultant increased secretion of inflammatory cytokines (IL-1 β , IL-6, IL-8, IL-17 and IL-23) in human corneal epithelial cells and neutrophils is reported [38]. Increased production of reactive oxygen species (ROS) in response to increased levels of IL-1 β , TLR4, Dectin-1 and LOX-1, facilitates the fungal killing [39, 40].

Leal et al. found that neutrophils produced NADPH oxidase to control the growth of fungi. The antifungal activity of neutrophils depended on CD18, and inhibiting thioredoxin, an antioxidant increased the sensitivity of fungal hyphae to neutrophil-mediated killing in vitro [41]. The expression of PPRs, promote the production of pro-inflammatory cytokines, as well as the recruitment of neutrophils that can also cause serious inflammatory damages to cornea leading to opacification [32, 40, 42]. In fungal keratitis, the levels of pro-inflammatory IL-1 β , IL-6, IL-8, IL-17, IL-23 and IFN- γ in aqueous humor were significantly higher in comparison to the non-keratitis control group [38]. A study among a Han Chinese population of patients with FK compared controls found a particular TLR4 allele that was associated with an increased risk of developing FK [43].

Fungi can produce enzymes that degrade physical barriers and facilitate tissue invasion. The mycotoxins produced from *Fusarium* species can inhibit immunity, break down tissues, and promote the fungal survival in host. Corneal epithelial cells can be destroyed by some cytosolic proteins and peptide toxins produced by fungi [44]. The protease and phospholipase activities detected in *A. flavus* and *F. solani* isolated from human eyes and their role in causation of ulceration in fungal keratitis, are reported in several studies [45, 46].

2.3.2 Autophagy

Autophagy is a lysosome-mediated degradation process, which regulates intracellular homeostasis of eukaryotes by mediating the degradation of proteins and organelles [47]. It can be activated in response to starvation, stress, hypoxia, tumor, and infection [48]. Autophagy is proved to be involved in immune responses, a previous study found that autophagy maintains the cellular and immune homeostasis during the *Candida albicans* infection [49]. Autophagy can regulate IL-1 β release in human primary macrophage to resist the fungal infection [50].

A study by Li C et al. reported that the progression of FK caused by *A. fumigatus* result in increased expression of autophagy and the severity of *A. fumigatus* keratitis, aggravated with inhibition of autophagy. The induction of autophagy reduced the severity of keratitis via regulating the recruitment of PMNs, balancing the pro-inflammatory and anti-inflammatory cytokines release, and possibly affecting the differentiation of neutrophils. Autophagy may become a novel target for the treatment of FK in future. Further studies may add our understanding regarding the protective role of autophagy in FK [51].

2.4 Fungal biofilm

Biofilm formation is one of the primary mechanisms through which fungi evade the immune response and establish infection. Clinical isolates of *Fusarium*, *Candida* and *Aspergillus* have been shown to form biofilms. A study reported that *F. solani* formed a biofilm in vitro by 24 h while other species (*Cladosporium sphaerospermum* and *Acremonium implicatum*) formed at 48 h. A time-dependent decrease in efficacy for all six antifungal agents (amphotericin B, voriconazole, itraconazole, fluconazole, terbinafine, and natamycin) is reported with increase in minimum inhibitory concentration (MIC) of all six antifungal agents tested with the development of biofilm [52]. This suggests that an ability to disrupt the biofilm may prove useful in increasing antifungal efficacy.

3. Recent advances in diagnosis

3.1 Clinical diagnosis

Fungal keratitis can be diagnosed based on characteristic clinical features. Patients with keratitis usually present with sudden onset of pain, photophobia, watering and discharge and reduced vision. In fungal keratitis, symptoms are much milder than the signs [53].

A fungal keratitis classically presents as a dry, raised lesion with crenate or feathery borders, presence of satellite lesions and a hypopyon. An immune ring of Wessely may be visible due to deposition of immune complexes and inflammatory cells around the ulcer (**Figures 1–5**). However, a study reported that Clinicians could correctly distinguished the microbial kingdom for 54 (73%) of 74 culture-positive infections, including 41 (79%) of 52 bacterial keratitis, 5 (38%) of 13 fungal

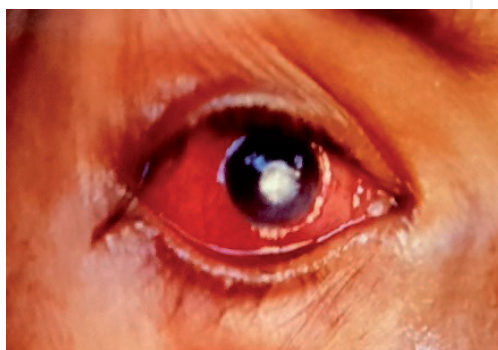


Figure 1.

Plaque-like ulcer with slightly defined margins, marked conjunctival injection and chemosis; fungal isolate-*candida albicans*.



Figure 2.
A dry looking lesion with greyish white raised exudate appearing as plaque with hypopyon in a 56-year-old male with fungal keratitis from Aspergillus.

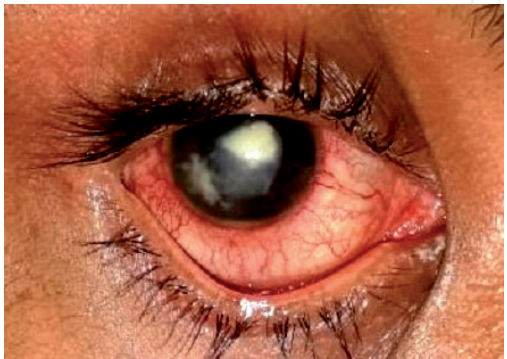


Figure 3.
A greyish white infiltrate with feathery borders and a satellite lesion in a case fungal keratitis caused by Fusarium.



Figure 4.
Severe fungal keratitis with feathery edges in case Fusarium Keratitis.



Figure 5.
Corneal thinning and necrosis in severe fungal keratitis caused by Fusarium in a 48-year-old male with history of topical steroid instillation.

keratitis, and 8 (89%) of 9 amoebic keratitis correctly [54]. In a photographic survey, clinician were able to distinguish between bacterial and fungal aetiologies 66% of the time. In 39 cases of fungal keratitis, the clinicians predicted genus in 27% of cases and species in 7.9% of cases [55].

3.1.1 Confocal microscopy

In vivo confocal microscopy (IVCM) of the cornea has been emerged as clinically useful non-invasive technique for early diagnosis of FK. It produces images from the cornea with a resolution of one micrometer (μm), which is enough for imaging of microorganisms larger than one μm , such as *Acanthamoeba* cysts and fungal hyphae [56]. This provides rapid and reliable diagnosis however, a clinical consensus in the interpretation of IVCM images is still lacking.

IVCM can directly visualize filamentous fungi within the whole cornea of patients. Confocal microscopy in vivo uses serial images to create optical sections through the full-thickness of the living cornea. It allows rapid identification of fungi and can be used to differentiate between fungal species.

Brasnu et al. diagnosed all the cases of suspected fungal keratitis (five out of five) caused by different fungal species using IVCM with sensitivity equal to the direct microscopy and culture [56]. They analyzed IVCM images of keratitis obtained using the Heidelberg Retina Tomograph (HRT) II confocal microscope (Heidelberg Engineering, Heidelberg, Germany) in five patients (four patients with *Fusarium solani* and one patient with *Candida albicans* infection), and three donor corneas with *Aspergillus fumigatus*, *F. solani*, and *C. albicans* infection. *F. solani* hyphae seen as high contrast lines 3–5 microns (μm) in diameter, 200–300 μm in length, with a branching angle of 90° in IVCM images from patients as well as from the infected donor cornea. *A. fumigatus* hyphae seen as numerous high-contrast lines 200–300 μm in length and 3–5 μm in width, with the branching angle 45° in the infected donor cornea. *C. albicans*-infected patient's cornea revealed numerous high-contrast elongated particles measuring 10–40 μm in length and 5–10 μm in width. *C. albicans*-contaminated donor cornea revealed numerous characteristic high-contrast elongated particles measuring 10–40 μm in length and 5–10 in μm in width, consistent with *Candida* pseudofilaments [56].

The hyper-reflective elements seen on IVCM must be differentiated from the basal corneal epithelial nerves, which have a more regular branching pattern. Stromal nerves, on the other hand, are much larger in diameter (25–50 μm). There are now several studies reported the use of IVCM in diagnosis and monitoring of treatment of fungal keratitis with reported sensitivity of 80–94% [57–60].

IVCM is a noninvasive in vivo technique useful for early identification of fungal elements, monitoring and guidance of treatment, and determination of the depth of infection. The limitation of IVCM are that technique is extremely user-dependent, need a skilled operator and experienced viewer. The dense corneal infiltrates or scarring could preclude proper tissue penetration and visualization.

3.2 Laboratory diagnosis

Conventional methods for the diagnosis of fungal keratitis include staining of tissue scrapings with Gram-stain, 10% potassium hydroxide (KOH) wet mount, lactophenol cotton blue, Giemsa, or calcofluor white. Reported sensitivity of Gram staining is in the range of 36–50% [61]. KOH is a rapid and an inexpensive and one of the most commonly performed procedures for detection of fungi with a sensitivity of 61–94% and specificity of 91–97% for detecting fungus (**Figure 6**).

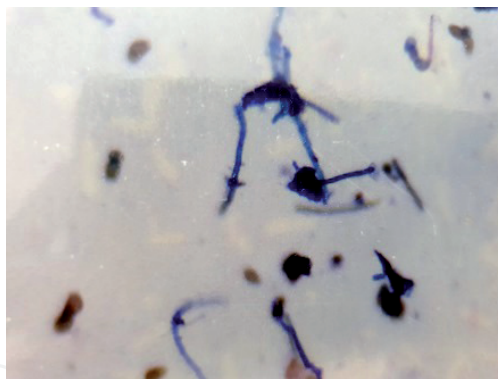


Figure 6.
Fungal hyphae in KOH wet mount counterstained with methylene blue.

Lactophenol cotton blue mounts had reported sensitivity of 85% and specificity of 90–91% [62]. Sabouraud dextrose agar medium is considered as a culture medium of choice for isolating fungi however it cause delay in diagnosis. Initial growth occurs within 72 hours in 83% of cultures and within 1 week in 97% of cultures [63]. Sometimes it may be necessary to wait for two weeks to confirm no growth in culture. Over the last decade, a number of newer methods have been devised for detection of fungi.

3.2.1 Polymerase chain reaction

Polymerase chain reaction (PCR) involves repeated cycles of denaturation, amplification, and replication, in which segments of deoxyribonucleic acid (DNA) are continuously multiplied. Specific DNA primers are employed to indicate the presence of the microorganism in question [64]. PCR has emerged as a sensitive and specific test for the diagnosis of fungal keratitis. Several techniques of PCR have been evolved and currently used for identification of fungi.

Traditional PCR by using single pair of primer to amplify the target genomic sequence is simple and efficient technique, but generation of nonspecific products can affect the results. In Nested PCR, two pairs of primers are used; one set of primer is an amplified sequence, and the other is complementary to the sequence amplified by the first one. It is more specific than traditional PCR; amplifies only the specific sequences looked for; but identify a set of fungal pathogens, not a single specific species.

In multiplex PCR Multiple primer, pairs are used. Advantage is Rapid amplification of multiple sequences, conserves template DNA, and minimizes expense; recognizes many pathogens at once. In real time PCR, one set of primers is used; amplified sequence is linked with a fluorescent probe, which emits light when bound to the amplified product. It is more specific, sensitive, and reproducible but not ideal for multiplexing [65–67].

PCR reported higher sensitivity in comparison to culture and stains for both bacteria and fungi [68, 69]. Zhao et al. reported significantly higher positive detection rate of PCR for fungal keratitis (84.5%) as compare to the positivity rate for culture (35.3%) and stain (64.7%) [69]. A higher sensitivity of PCR for infectious keratitis compared to culture (98% versus 47%), but a slightly lower specificity (83% versus 100%) is reported in this study [69].

The PCR is rapid test, it takes 4–8 h, and only a small clinical sample is needed for diagnosis [7]. The limitation of PCR is that it is expensive, not readily available and specificity is lower than culture. Extraction of artifacts and amplification of

non-pathogenic DNA can lead to over diagnosis [66]. However, it can be used to detect fungal DNA in corneal scrape material, to start antifungal therapy at an early stage of the keratitis.

3.2.2 Metagenomic deep sequencing

Metagenomic deep sequencing (MDS) is a new technique for the diagnosis of FK; with next generation sequencing rapid and accurate diagnosis is possible. Next generation sequencing is high-throughput sequencing methods where billions of nucleic acid fragments can be sequenced simultaneously and independently. MDS is an unbiased approach that interrogates all genomes in a clinical sample and identify any organism whereas PCR is a targeted test the clinician must know the suspected causative organism.

It has been shown to enhance detection of common and unusual pathogens from the intraocular fluid of patients with infectious uveitis and other systemic infections [70–72]. A study by Seitzman et al. in a case series of nine patients of infectious keratitis diagnosed by conventional methods reported that MDS detected all the microorganisms identified by culture or PCR. MDS was able to identify parasitic, fungal, bacterial, and viral infections as a single assay. The pathogenic organisms ranged in size from smaller genomes (*herpes simplex virus-1* and *adenovirus*) to larger genomes (*Acanthamoeba* and *Aspergillus*). In one case, the MDS identified the organism not supposed to be a cause of infectious keratitis. The case was culture positive for *Purpureocillium lilacinum* was identified as the second most abundant organism and, the most abundant organism in the sample was *Auricoccus indicus*, which is not known to cause ocular infections and not even listed in the University of California San Francisco's mass spectrometry's database for identifiable organisms [73].

4. Recent advances in medical treatment

Polyenes (Amphotericin B and Natamycin) and azoles (fluconazole, itraconazole, ketoconazole, miconazole, voriconazole, and posaconazole) constitute two major classes of antifungal drugs used to treat ocular fungal infections including fungal keratitis. In Comparison to antibacterial agents, antifungals have a lower efficacy due to their mechanism of action (usually fungistatic, with dose dependent fungicidal action), lower tissue penetration, and the indolent nature of the fungal infection [74]. Still for the management of fungal keratitis, the traditional anti-fungal drugs like natamycin and fluconazole in topical and oral form are used most commonly. In recent years, other new drugs and drug delivery system to increase bioavailability of drugs have been evaluated. Anti-fungal agents are summarized in **Table 1**.

4.1 Natamycin

Natamycin is first antifungal agent approved for FK by Food and Drug Administration in the 1960s. After that, many antifungal agents have been evaluated, no single agent has emerged as the best and most cost effective agent [7]. Cochrane systematic review in 2008 and 2012, found no evidence that any single drug, or combination of drugs, is more effective in the management of fungal keratitis. The trials included in this review were of variable quality and were generally underpowered [75, 76].

Agent	Route of administration/ Dose	Indication	Limitations
Polyenes			
Amphotericin B	Topical 1.5-5 mg/ml IS 5-10 µg IC 5-10 µg/0.1 ml	First line therapy for Candida species. Good to moderate activity against filamentous fungi. Deep keratitis with partial response to topical therapy	Not commercially available. Side effects: cataract, transient iritis and corneal oedema
Natamycin	Topical 5% (50 mg/ml) suspension	First choice for Fusarium, Good activity against Aspergillus, less effective against Candida species	Low corneal penetration
Azoles			
Imidazoles			
Econazole	Topical 2%	Effective against Fusarium, Aspergillus, and Candida species	Not commercially available for ophthalmic use
Miconazole	Topical 10 mg/ml SC 1.2 to 10 mg	Effective against candida Adjuvant with topical therapy in patients with low compliance	Less effective than polyenes
Ketoconazole	Topical 1-2% Oral 200-400 mg/day	Broad spectrum As an adjuvant in deep keratitis	Less effective systemic toxicity (gastric intolerance, hepatotoxicity)
Triazoles			
Fluconazole	Topical 0.2 % SC 2 mg/1 ml Oral 100-400 mg/day oral	Effective against yeast, less effective against filamentous fungi Good intraocular penetration used as adjuvant with topical agents	Filamentous fungi exhibit resistance liver enzyme monitoring
Itraconazole	Topical 1% Oral 200-400 mg/day	Effective against Aspergillus, Candida, less effective against Fusarium As adjuvant with topical therapy in deep keratitis/ intraocular involvement by yeasts	Less effective than natamycin Lower bioavailability, and penetration into ocular tissues than other azoles
Voriconazole	Topical 1-2% IS 50 µg/0,1 m IC 50µg/0,1 m Oral 200 mg **BID	Broad spectrum, FK resistant to polyenes/ first-line triazoles. Deep keratitis and Intraocular involvement	Less effective than natamycin Side effects- blurred vision change in colour perception; liver enzyme monitoring during oral use
Posaconazole	Topical 100 mg/ml; 40 mg/ml Oral 200 mg **QID/ 400 mg BID	Broad spectrum, FK resistant to polyenes/first-line triazoles.	Limited information

Agent	Route of administration/ Dose	Indication	Limitations
Pyrimidines			
Flucytosine	Topical 10 mg/ml	Synergistic effect with topical Amphotericin B in FK due to yeasts	Narrow spectrum/ low penetration into ocular tissues
Echinocandins			
Capsosungin	Topical 0.5%	Yeasts resistant to polyenes and first-line triazoles	Limited information
Micafungin	Topical 0.1%	Yeasts resistant to polyenes and first-line triazoles	Limited information
Allyamine			
Terbinafine	Topical 0.5 % Oral 250 mg/day	Active against Aspergillus, Fusarium, Scedosporium and Candida.	Limited information
*IC: Intracameral, IS: Intrastromal, SC: Subconjunctival. **BID: Twice a day, QID: for times a day.			

Table 1.
Summary of antifungal agents used in Fungal Keratitis

Natamycin is a polyene antifungal drug, it binds preferentially to ergosterol on the fungal plasma membrane and causes localized membrane disruptions by altering membrane permeability. Natamycin is currently considered the most effective medication against Fusarium and Aspergillus [7]. Cochrane systematic review in 2015 found that there is evidence that natamycin is more effective than voriconazole in the treatment of fungal ulcers. However, the trials included in this review were of variable quality and were generally underpowered. Future research should evaluate treatment effects according to fungus species [77].

Several studies reported that fungal keratitis due to fusarium responded better to Natamycin as compare to itraconazole and voriconazole [77]. NTM is the treatment of choice for filamentous keratitis, especially that due to Fusarium species. However its poor penetration into corneal stroma, limits its use in deep stromal keratitis. In deep keratitis or with involvement of intraocular structures, natamycin should be associated with other antifungal agents using a different route of administration.

4.2 Amphotericin B

Amphotericin B is the first broad-spectrum antifungal agent, produced by the actinomycetes, *Streptomyces nodosus*. It acts by binding to ergosterol and by promoting oxidative action on cells, thus altering their metabolic functions. This binding also results in formation of pores or channels in the fungal cell membrane and increasing cell permeability. Its binding to cholesterol in human cells is responsible for its side effects. It is effective against *Aspergillus* and *Candida* species but less effective against *Fusarium* species [74]. It is administered as a topical solution in concentration of 1.5 to 5 mg/ml.

Amphotericin B has poor ocular penetration after intravenous administration and is toxic to human cells at a higher dose. Due to systemic (nephrotoxicity) and ocular toxicity (punctate epithelial erosions and greenish discoloration of the cornea), amphotericin B is not currently a first line agent in treating fungal keratitis.

In a study, Morand K, et al. compared the commercial 0.15% Amphotericin B with a liposomal formulation and found that the liposomal form was more stable and less toxic. The liposomal formulation also increased the potential amount of loaded drug by 3-fold compared with the conventional form [78].

4.3 Fluconazole

Fluconazole is a synthetic bistriazole available in oral, topical, and IV preparations. It has good intraocular penetration with low side effect. It shown to have excellent absorption from the gastrointestinal tract. Its plasma concentrations with oral use reach almost the same levels as with intravenous administration. Intraocular Penetration is effective, with aqueous concentrations similar to those in the plasma [74, 79]. Topical 0.2% fluconazole is effective against *Candida* keratitis with deep lesions. Oral fluconazole in a dose of 200 to 400 mg per day effective as an adjuvant with other topical antifungal agents.

4.4 Voriconazole

Voriconazole, a newer-generation triazole, with excellent ocular penetration and broad spectrum. Most of fungal isolates commonly implicated in keratitis were found to be susceptible to voriconazole.

Voriconazole has been reported to be effective in the treatment of fungal keratitis caused by different species and in cases not responding to other antifungal like natamycin and amphotericin [79–85]. Voriconazole has good intraocular penetration following oral administration. Advantage of oral administration is that it may provide steadier drug levels at the site of infection. Theil et al. compared aqueous samples after topical and oral voriconazole found that topical administration of voriconazole resulted in highly variable aqueous concentrations with troughs well below the minimum inhibitory concentration at which 90% of fungal isolates are inhibited (MIC₉₀). Whereas, oral voriconazole provided relatively constant therapeutic concentration [84]. Many case reports reported successful treatment with topical voriconazole in conjunction oral or intravenous voriconazole [85, 86].

4.5 Posaconazole

Posaconazole is a new triazole, a synthetic structural analogue of itraconazole. It is available as an oral suspension (40 mg/ml), administered at a dose of 200 mg four times daily or 400 mg twice daily. Now also available as delayed release tablets (100 mg) and injection (18mg/ml). In vitro and in vivo studies showed that posaconazole has a broad spectrum against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., and *Fusarium* spp. etc. and effective against most agents resistant to itraconazole and fluconazole [87–91].

Evidence on its use in ocular infections is still limited, but initial results are encouraging. Sponsel et al. also describe a case of keratitis by *Fusarium solani* resistant to Amphotericin B and natamycin but successfully treated with topical drop (100 mg/ml prepared from an oral solution) associated with oral posaconazole 200 mg 4 times daily [89]. However, comparative controlled studies with first-line antifungal agents are still lacking. Altun et al. reported successful treatment with posaconazole in two cases with recalcitrant fungal keratitis that were resistant to conventional antifungal drugs (systemic and topical fluconazole or voriconazole and amphotericin B and topical natamycin were all ineffective) [90]. Posaconazole use resulted in rapid resolution of infection in these cases without significant toxicity. Posaconazole can be useful in cases of fungal keratitis that are resistant to standard antifungal therapy. However,

the use of topical posaconazole as monotherapy needs to be evaluated as well as the optimum effective concentration has to be standardized. Two different concentration of the topical preparation is used in the above studies.

4.6 Echinocandins

Echinocandins (caspofungin and micafungin) are semisynthetic lipopeptides act by inhibiting the synthesis of glucan in the fungal cell wall causing osmotic imbalance and cell lysis. Matsumoto et al. have reported successful use of topical 0.2% micafungin in cases of refractory fungal keratitis [92]. In another study by Matsumoto et al. using topical micafungin 1 mg/ml reported found an efficacy comparable or superior to fluconazole in the treatment of keratitis by *Candida albicans* and *Candida parapsilosis* [93]. Topical caspofungin has been used in the cases of fungal keratitis refractory to voriconazole [94]. There are limited data on the use of echinocandins to treat fungal keratitis in humans.

5. Recent advances in surgical treatment

Surgical intervention may be an option for patients with refractory FK not responding to medical treatment and severe fungal infections. Penetrating keratoplasty is considered the most common surgical intervention for serious fungal keratitis and cases with perforation or impending perforation. Recent advances have added more options such as targeted drug delivery at the site of infection in the form of intrastromal injections, collagen cross-linking and rose Bengal aided photodynamic therapy.

5.1 Intrastromal voriconazole

The efficacy of topical, as well as systemic, voriconazole is well established. Intrastromal voriconazole has been found as an effective approach for targeted drug delivery in the management of deep FK not responding to standard topical therapy [95–97]. Targeted drug delivery overcomes the issue of poor bioavailability of drugs in cases of deep fungal keratitis. It provides a depot of drug, close to the infected area. However, risk of introducing a new infection, inadvertent anterior chamber entry while performing the procedure in a hazy cornea are associated.

5.2 Intracameral amphotericin B

Intracameral Amphotericin B is another approach for targeted drug delivery, indicated when medical treatment with topical and systemic antifungal has failed, especially in cases with deep mycosis, endothelial plaque and presence of hypopyon with inflammation of the anterior chamber. The concentration injected, ranges between 5 and 10 µg/0.1 ml [98, 99].

5.3 Penetrating Keratoplasty

Penetrating Keratoplasty (PK) is indicated for treatment of refractory or severe fungal keratitis, corneal thinning and perforation in FK [100]. A retrospective study including 52 eyes which underwent PK for corneal perforations secondary to FK, reported improved visual acuity in 46 eyes (88.5%) and clear grafts in 44 eyes (84.6%) at final follow-up [101]. The common complications of PK are graft rejection, recurrence of infection, and secondary glaucoma. Following PK, oral and

topical antifungal medications are usually continued for 2 weeks and if pathology reports presence of fungus on the margin of the cornea sample, treatment continues for 6–8 weeks.

Cyclosporine has been recommended after PK in cases of fungal keratitis as it has been suggested to have dual antifungal and anti-immune properties [102]. However; evidences at present are limited, further studies are required to evaluate the risk and benefit of cyclosporine patients undergoing corneal transplant for fungal keratitis.

5.4 Amniotic membrane transplantation

Amniotic membrane transplantation (AMT) has emerged as an option to delay or prevent PK secondary to fungal keratitis. Amniotic membranes have been used to facilitate ocular surface reconstructions in other ocular surface conditions. AMT support re-epithelialization of tissue, and the active components present in the membrane like nerve growth factors are thought to reduce pain [103]. In a study, 23 culture-proven, acute fungal keratitis patients with non-healing corneal ulcers, or impending corneal perforation underwent AMT to prevent PK or to promote re-epithelialization. Following AMT, 25% of patients with persistent positive culture for fungus required PK. The final visual outcome was BCVA > 20/400. It improved in 17, did not changed in four and worsened in two patients [104].

In an inflamed eye, there is increased risk of infection to be introduced into the anterior chamber or vitreous after PK and the use of corticosteroids, to prevent corneal graft rejection, may increase the risk of recurrence of fungal infection. Delay in PK can avoid these complications.

5.5 Lamellar Keratoplasty

Lamellar keratoplasty (LK) is emerged as an alternate surgical procedure for fungal keratitis in which only diseased layers of the corneal surface are excised and replaced by donor cornea. In a study from China, reported the leading indication for LK in 2008 was infectious keratitis, and fungal keratitis constituted 67% of the infectious keratitis cases [105]. In another study, 55 antifungal refractory patients underwent LK with intensive topical and oral antifungal medication. In 93% of the patients, the fungal infection was eradicated. The remaining four patients were treated by a secondary PK. Visual acuity ranged from 20/20 to 20/63 with a few complications after 6–18 months follow-up [106].

5.6 Corneal collagen cross-linking (riboflavin with ultraviolet-A irradiation)

Corneal collagen cross-linking (CXL) has been found successful in halting the progression of keratoconus by using riboflavin and UV-A light. In recent years, role of CXL in infectious keratitis is investigated in several studies with conflicting results on the efficacy of CXL in infectious keratitis [107–113]. Specifically the term photoactivated chromophore cross-linking (PACK-CXL) is used for CXL to treat infectious keratitis [108].

CXL may act in cases of fungal keratitis by a direct antifungal effect and by halting the ongoing melting, thus helping to avoid emergency keratoplasty [109–111]. Said et al. found that although PACK-CXL did not shorten the time to corneal healing, it prevented corneal melting [107]. PACK-CXL is found to be useful in fungal keratitis [108–110]. Abbouda et al. reported halting of corneal melting with PACK-CXL in one case while the other developed perforation [112]. The safety of CXL is of concern because the ultraviolet (UV) -A could damage intraocular structures. Spoerl

et al. analyzed the expected damage compared with acceptable damage thresholds. During standard CXL of a cornea with a 400- μm thickness, the irradiances of the UV light reaching the iris, lens, and retina are less than the damage thresholds, and only the microbes, the corneal endothelium, and the keratocytes are at risk [113]. Minor complications after CXL, like transient limbitis and a transient increase in the size of the hypopyon in the first 24 h after CXL reported to be regress subsequently [107].

5.7 Rose Bengal photodynamic therapy

Photodynamic therapy (PDT) has been used in treatment of choroidal neovascularization in age-related macular degeneration, corneal neovascularization and in infectious keratitis due to *Acanthamoeba* [114]. PDT involves the activation of photosensitizers using light of varying wavelengths. Rose Bengal photodynamic therapy (RB-PDT) involved a photochemical process using Rose Bengal, excited with green light (wavelength: 500–550 nm) to generate reactive oxygen species (ROS), which, react with various intracellular components to cause cell death. In an in vitro study, Arboleda et al. have demonstrated RB PDT to be successful in fungal keratitis [115].

In a pilot clinical study by Naranjo et al., RB-PDAT was performed in 18 patients with progressive infectious keratitis unresponsive to standard medical therapy. RB-PDAT was considered successful in 13 individuals, defined as control of infection without the need for a therapeutic PK [116]. Amescua G et al. in an vitro and in a case study evaluated the efficacy of rose bengal photodynamic antimicrobial therapy (PDAT). They found that Riboflavin CXL demonstrated no inhibition of fungal isolate growth, whereas rose bengal PDAT inhibited fungal isolate growth within the irradiation zone. In addition, a case with resistant fusarium keratitis was treated successfully [117].

6. Future perspective

6.1 New targets in immunology

In a study, the role of vitamin D receptor (VDR) in innate immunity being discovered, may be a new target of treatment that can be explored for FK [118]. Liposomes-encapsulated mannan extracts from *C. albicans* stimulate the production of antibodies protective against candidiasis in mice [119]. Probiotics, such as *L. rhamnosus*, *L. acidophilus*, *L. pyogenes*, *L. casei* GG and Bifidobacterium, reported to be protective from candidiasis by eliciting protective immune and non-immune responses in mice [120]. These experimental studies may further facilitate researches to develop fungal keratitis vaccine and use of probiotics in ocular surface for diseases prevention.

6.2 Ocular novel drug delivery system

Recently, many efforts have been made to improve topical ocular drug delivery by designing various novel drug delivery systems (NDDS), including liposomes, nanoparticles, nanoemulsions, nanosuspensions, micelles, nanofibers, etc.

Several in vitro and in vivo experimental studies have reported encouraging results with NDDS. In a study, the liposomal formulation of the antifungal drug voriconazole found to exhibit a sustained drug release profile, and an 8-fold increase in the amount of drug retained in the cornea after 1 hour of exposure compared with the conventional suspension formulation [121]. The nanoparticle formulation of amphotericin B showed a sustained and controlled drug release for

up to 11 hours, while the conventional drug formulation (0.15%) released the entire drug in only 4 hours. Nanoparticle formulation has also shown better pharmacokinetic properties, including 1.5-fold increase in half-life compared to the conventional solution formulation [122]. The microemulsion formulations of fluconazole showed a controlled release profile, releasing 50–80% of the drug in 12 hours, compared to the conventional drug solution, which released almost the entire drug in the first 6 hours [123]. In future, these newer formulations can be very useful in management of fungal keratitis.

6.3 Antimicrobial peptides

Antimicrobial peptides (AMPs) have significant potential for use as antimicrobial agents for ocular or other infections [124]. AMPs, also known as host defense peptides, are naturally produced, small, cationic, amphiphilic peptides ranging in length from 12 to 50 amino acids. They are present on the surfaces of the eyes and in tears. More than 500 AMPs have been reported, including large molecules (RNases and S100A proteins); small peptides α and β defensins in human cationic antibacterial protein (CAP) 18, and α 37 amino acids; proteins like lysozyme and peptidoglycan recognition protein with significant bactericidal activity. The cations carried by AMPs can bind to the anion surface of the bacterial plasma membrane, causing the perforation of cell membrane and subsequently microbial death. AMPs also prevent microbial adhesion to and access into host cells and cause digestion of fungal cell wall by lysozyme [124, 125].

In vitro studies have shown AMPs Pc-C and Pc-E reduced binding of *Aspergillus fumigatus* to cells; CAP37 inhibits candida infection by fungicidal activity [124, 125]. Wu et al. evaluated in vivo application of synthetic β -sheet forming peptide (IKIK) 2-NH₂ and (IRIK) 2-NH₂ for treatment of FK in comparison with amphotericin B [126]. It was found that topical solutions of the designed peptides were safe, and as effective as the clinically-used Amphotercin B. Many other AMPs such as Clavanin A, Chitinase 3-like 1, and CXCL 10 and S100 proteins may have role in prevention of infection.

7. Conclusion

Early diagnosis and treatment of fungal keratitis remains a challenge. A better understanding of pathogenesis can broaden the approach to management. Recent advances in techniques such as in vivo confocal microscopy and the evolution of PCR and MDS can be useful in rapid and accurate diagnosis. Newer antifungal agents and newer methods of targeted drug delivery system can be helpful in treating refractory cases and improving outcome. New evolving technique like PACK-CXL and RB-PDT can be useful as adjuvant therapy.

New researches are continue to investigate the new aspects of pathogenesis, to device the novel drug delivery system to overcome the poor ocular penetration of antifungal drugs and enhance their efficacy and evaluate newer antifungal drugs. In recent years focus on modifying the immune response to the infection, thereby reducing the corneal melting and scarring which lead to poor vision, may have the greatest potential to improve visual outcomes.

Conflict of interest

The authors declare no conflict of interest.

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