REVIEW ARTICLE

Keratomycosis:Clinical diagnosis, Medical and Surgical Treatment

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Keratomycosis is a major cause of visual disability in developing countries(1). It is common in our country because of the tropical climate and a large agrarian population at risk. Since the first report of keratomycosis, by Professor Theodor Leber, fungi have been increasingly implicated in the cause of corneal ulcer (2,3), there has been a dramatic rise in frequency of these infections in the last two decades possibly because of the indiscriminate use of antibiotics and corticosteroids in ophthalmology practice (3, 4). An increased clinical awareness has also partially contributed to its frequent reporting (4).

In northern India, fungal keratitis has a prevalence of 8.4% (5) while it has been reported as high as 46.3% from southern India (6). Keratomycosis can be caused by as many as 60 species of fungi. The predominant etiological agents vary in different geographical areas. However, Aspergillus spp. is the commonest isolate in India (5,6).

Risk factors for keratomycosis

Fungi are opportunistic agents of infection and become pathogenic under conditions of impaired immunodefense. Fungal infection in the absence of precipitating event is unusual

Trauma is the most common precipitating factor in most of the cases(7). The nature of injury often is vegetative in origin, which may consist of trauma with plant twigs, rice-husk, cotton plant etc. Trauma leads to destruction of the epithelium and Bowman's membrane, impairing barrier to infection. The underlying stroma becomes, excessively hydrated and possibly altered in such a way to constitute a more favorable site for fungus to grow. Keratomycosis caused by filamentous fungi is an occupational hazard of farmers and agricultural workers(8). The seasonal variation noted in most series most likely represent occupational injuries associated with harvesting. Alternately, mycotic infection especially Candidal spp.may develop in pre existing lesions like herpetic scars, neurotrophic keratitis which alters local ocular immuno defense (9).

The time interval from trauma to clinical features can be 24-48 hours but often is as long as 10 to 21 days, depending on the organism, the size of inoculum and host resistance (10).

The **topical corticosteroids therapy** has been associated with increased incidence and worsening of fungal keratitis (11-13). The increased incidence is probably due to altered local immune response and increased rates of conjunctival colonization by fungi. Additionally, it indirectly promotes fungal replication and corneal invasion by interfering with host's inflammatory response. The systemic use of corticosteroids may predispose to fungal keratitis by causing immunosuppression (12).

Candida spp. commonly colonize conjunctive and eye lid margins of normal individuals(14). However, it may produce keratitis in patients with impaired immune response. The local ocular resistance may be lowered by atopic disease, eye-lid malposition, dry-eye conditions, neurotrophic or herpetic keratitis predisposing to fungal keratitis(15).

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Pathogenesis

The pathogenic mechanisms of fungi include direct physical damage caused by invasion and growth of fungal elements, damage from infiltrating leucocytes and damage produced by fungal toxins and enzymes. Clinical manifestations may occur as early as 24 to 46 hrs or may be delayed for 10 to 20 days, potentially allowing extensive fungal replication before detection by the host (16). The invasion of mycelia usually occurs parallel to collagen lamellae but may be perpendicular with more virulent organisms (17). This leads to disruption of normal collagen fiber arrangement. The surface mannoproteins of hyphae or pseudohyphae also inhibit the attachment of neutrophils, escaping phagocytosis (18). The mycotoxins released by fungi have antibacterial, antiviral, antitumour and antiphagocytic effects that suppress the immune function. Some fungi like Candida also produce a variety of proteases e.g aspartyl acid proteases , neutral and carboxyl proteases that contribute to invasiveness of the organism (19).

Infiltration of host leucocytes forms important component of the disease process. Ring abscesses composed of PMN leucocytes; plasma cells and rarely eosinophil around fungal hyphae are characteristic (20). Leucocytes are also present in areas of corneal damage without hyphae.Fungal hyphae are large enough to preclude ingestion by neutrophils; however attempts at phagocytosis result in extracellular release of lysosomal enzymes and oxygen metabolites which digest the stromal collagen (21). Fusarium spp. induces little cellular infiltration and can grow extensively, spreading deeper across the descemet's membrane into the anterior chamber.

Clinical Features

Kaufman and Wood (2) described the salient clinical features of filamentous fungal keratitis in 1965. Though there are no pathognomonic clinical features, some lesions can be suggestive of the diagnosis.

Filamentous keratomycosis usually presents as a slowly progressive corneal ulcer usually involving the central cornea. The early biomicrcoscopic features consist of fine or coarse granular infiltrates within the epithelium and anterior stroma, with minimal cellular reaction. The epithelial surface has a dry, rough texture and dirty gray-white colour. The epithelium may be elevated and intact or occasionally it may be ulcerated. The lack of marked stromal inflammation permits direct visualization of pigment and delicate feathery branching hyphae with surrounding stromal infiltrate. Mild inflammation may contribute to irregular edge of feathery infiltrates by spreading of leucocytes along the interlamellar spaces.

There may be multifocal suppurative microabscesses or satellite lesions. The extension of hyphate margins beyond the ulcer edge present a distinctive feature and a useful diagnostic criterion in distinguishing fungal from bacterial ulcers.

Typical corneal infection with fungus produces a violent ocular reaction. Even when the ulcer appears to be superficial, there are usually folds in Descemet's membrane and there is appreciable ciliary flush and flare in anterior chamber. Hypopyon is invariably present in fungal keratitis and usually result from sterile reaction to fungus and its toxins. However, fungi may invade the anterior chamber through intact Descemet's membrane and result in a fixed hypopyon.

Satellite lesions are discrete stromal infiltrates that surround the ulcer and are separated by clear cornea. Many Descemet's folds may occur associated with deep stromal inflammation. An endothelial plaque, composed of fibrin and leucocytes, usually is located under the stromal lesion and may be present in the absence of hypopyon.

Microabscesses, satellite lesion and ring infiltrates are non-specific and represent an immune response. Early cases of fungal keratitis may be mistaken for persistent corneal erosions, herpes simplex keratitis or if eccentric staphylococcal marginal ulcers.

In advanced cases, the entire cornea becomes homogenously yellow-white, and can resemble any microbial keratitis. Stromal ulceration and necrosis may

lead to perforation and endophthalmitis. This is especially a threat with Fusarium solani keratitis in association with inappropriate use of topical corticosteroids.

In contrast to filamentous keratitis, yeast keratitis often causes a small oval ulceration with an expanding, discrete, sharply demarcated, dense, yellow-white stromal suppuration lacking delicate features of filamentous organisms. These more commonly resemble a gram-positive bacterial keratitis, such as staph. aureus or S. pneumoniae. Severe and chronic yeast keratitis may develop a wet, necrotic stromal inflammation with features indistingushable from other microbial keratitis.

Laboratory Diagnosis of Keratomycosis

Most cases of mycotic keratitis are amenable to treatment if effective topical antifungal therapy is started early. The major hurdle to this lies in definitive laboratory diagnosis in clinically suspected case of oculomycosis. False negative diagnostic results may result in delay in institution of anti fungal therapy or use of corticosteroids with disastrous results.

Different modalities of diagnosis are discussed as under: -

1. Collection of sample
2. Direct microscopic examination
3. Culture
4. Recent advances-polymerase chain reaction, confocal microscopy, Immunoflourescence staining etc.

1. Collection of sample

A failure to collect adequate sample material from the relevant site is mainly responsible for the false negative reports in a clinically suspected fungal corneal ulcer. The material for culture and microscopic examination must be obtained from the lesion itself. Unlike bacterial keratitis in which actively propagating organisms are located in the leading edge of the ulcer, the mycelial elements in fungal lesions are best obtained by scrapping the central deeper area. This may be accomplished by using a scalpel blade, platinum spatula or a needle. The debris and necrotic area should be thoroughly removed and discarded. The material obtained is used for culture and preparing wet-mount or stained smears. Recent studies have shown improved means of recovering isolates in culture with direct rubbing of the lesion with either a calcium alginate swab moistened with trypticase soy broth, or a rayon swab moistened with thioglycollate broth (22). Multiple samples especially from the base and leading edge of the infiltration should preferably be taken using a slit lamp or an operating microscope. In case of deep keratitis, a keratectomy biopsy or loosening deeper corneal material with a disposable needle is necessary.

2. Direct Microscopic Examination

It is the most valuable and rapid diagnostic tool for detecting the fungal elements in corneal scrapings. However, this method requires considerable expertise in interpretation besides being tedious and time-consuming.

Various techniques used are

- Wet-mount- KOH smear 10% -20% with or without Indian pink or lactophenol cotton blue (LPCB).
- Smear examination using different stains.The stains used are:
  - a. Gram's stain
  - b. Giemsa stain
  - c. Gomori's methenamine silver stain
  - d. PAS (periodic acid shiff staining)
  - e. Calcoflur white [CFW]
  - f. Flourescein conjugated lectins
  - g. Acridine orange
- Histopathological examination of keratectomy biopsy.

KOH Wet mount

KOH is one of the oldest methods of demonstrating fungi in corneal scrapings. It has been traditionally used by the dermatologists to detect fungal elements in wet skin prepration. Potassium hydroxide partially digests the proteineous components such as host cells and stromal collagen, leaving polysaccharide containing fungal cell walls intact.Classically scrapings of the lesion are mixed with several drops of 10% aqueous solution and examined under microscope. The initial examination is done under low magnification and then under higher

magnification. If the smear examination is to be undertaken later , it is best to surround the preparation with a small well of lubricating petroleum jelly and top this with cover slip to prevent evaporation and crystallization of KOH. When the tissue specimen appear too thick for microscopy, the coverslip is gently pressed to spread the tissue and examination delayed for some time to allow lysis of debris and host cells by the alkali.

Glycerol 10% or thiomersol 0.1% can be added to preserve the sample for longer periods. Mycelia are seen as refractive filaments on microscopic examinations. The use of counter stain such as ethylene blue Indian pink or CFW facilitates recognition of hyphal structures with better appreciation of morphology of smaller fungal filaments.

The sensitivity of this method is highly variable, ranging between 33-94%. Liesegang and coworkers found sensitivity of 33% in culture proven cases (23).

In a recent study done by Sharma *et. al.* KOH smear (without CFW staining) examination was compared with Gram's stain, lactophenol blue under bright field and flourescence microscopy. An overall sensitivity of 81.2% was detected when compared with culture positivity for KOH smear and 93.7% for CFW. Specificity of both methods was identical- 83.8% (24).

It is less expensive, easy to prepare and less time consuming. Moreover it is also useful in diagnosis of Nocardia and Acanthamoeba. However, it is not reliable in early fungal ulcers, cannot be stained or preserved for longer duration and has high rate false positive artifacts.

a) Gram's staining

Gram 's stain is the most common initial stain used for early identification of fungi. It only stains the fungal protoplasm; the cell walls are not stained. Most fungi appear gram positive on staining. Yeasts typically stain dark blue .The fungal elements are identified by high dry magnification ;oil immersion is not necessary. The sensitivity of the staining technique is varies from 55-75% (10,25).

This method however has many shortcomings. The staining quality of the smear is often variable and mycelial elements frequently appear as stain precipitates. In addition, proteinaceous debris and thick smears may reduce the contrast between the fungal elements and the background; thus, interfering with fungal identification. Moreover, it is difficult to identify fungus in necrotic smears and artifacts are very common.

b) Giemsa Stain

It is classically used to evaluate cytology of conjunctival scrapings. It stains hyphae purplish blue. It has sensitivity and disadvantages comparable to Gram's stain.

c) Gomori-Methenamine Silver Stain

It is the most selective method for identifying fungal elements in tissue. The technique depends on the reduction of silver by oxidized carbohydrate components of cell wall to stain the fungus black. A light green counter stain produces a pale, transparent background against which hyphae may be more easily recognised. Corneal scrapings previously stained with other stains can also be reexamined using this technique. It is more sensitive and reliable than KOH, Gram's and Giemsa stains. The negative test is more reliable. The artifacts are uncommon (26).

d) Periodic acid Shiff staining (PAS)

PAS stain is frequently used in histopathological diagnosis of fungus. It causes hydrolysis and oxidation of cell wall polysaccharides staining hyphae bright red. Hyphal elements can be readily recognized even in the thick clumps of epithelial cells. It may also be used on a KOH wet-mount read as negative for hyphae, offering maximum utilization of limited material available for immediate analysis.

e) Acridine orange

It has been reported as a duct and simple fluorescent stain for fungi in histologic sections of suspected mycotic lesion. It can also be used as a rapid stain for smear and scrapping as well as with KOH in wet mount preparation with fluorescent microscope; a brilliant yellow-orange hue is seen against a dark background. It avoids misinterpretation of artifacts, such as vegetable fibers,

often a problem with KOH wet mount. However, certain fungi like Nocardia are known not to accept the stain at all (27).

f) Gridley's stain

It is a modification of PAS technique and enhances visualization of fungal protoplasm. The fungi may range in colour from purplish pink to dark blue against a yellow background.

g) Calcofluor white

It is a fluorochrome with affinity for chitin and cellulose. It is a useful adjunct in detection and confirmation of fungi.

3. Culture techniques:

The basic technique of laboratory isolation of fungus is inoculation of clinical material into neo-peptone dextrose agar medium known as Saboraud's Agar.

Characteristics of good media

- It should be fresh
- It should not contain inhibitory compounds, such as cycloheximide as these may prevent the recovery of organisms known to be pathogenic in human cornea that are saprophytes or non-pathogens in other sites.

Collection of inoculum

- The sample is collected under topical anesthesia.
- The epithelium over the lesion, if intake, should be removed.
- The surface of the lesion is cleared off the debris and dead cells.
- Base and the margins of the ulcer are scrapped vigorously with 24 G needle, BP knife or Kimura spatula.
- The inoculum can be directly plated on the culture media by making a row of "C" marks, reversing the edge of spatula with each "C" so that all material on the spatula is transferred to plate.
- The spatula is flamed and procedure separated until several such rows of "C" have been made on each agar plate.
- For inoculation into liquid media, the spatula is briefly immersed directly into the culture fluid or wiped onto a cotton swab that is then inoculated into the medium.

Sabouraud's Agar

It is the most popular medium for fungal isolation, which inhibits growth of saprophytic fungi. Agar contains 50µg/per ml of gentamicin to inhibit bacterial contamination. The plates are maintained at room temperature (25°C).

Emmond's Modification of Sabouraud's Agar

It has similar composition as Sabouraud's Agar but has a pH of 7.0 and no cyloheximide. It is preferable for ocular specimen as is supports the growth of even saprophytic fungi, which become opportunist pathogens in certain conditions.

Liquid brain heart infusion medium

It is used as an adjunct to solid fungal media

a) However, preparation and storage of this medium is inconvenient and the identification of fungal contaminants is not possible. The isolates must be transferred to solid media for sporulation and identification. Use of Sabouraud's agar (Emmond's modification) maintained at room temperature is probably the most sensitive method for isolating fungi (8, 10,23).

Blood agar plate kept at room temperature is slightly less sensitive (23). The brain heart infusion also provides excellent recovery of fungi (28). However, the recovery rate declines when same media are incubated at 37°C.

Positive cultures in Sabouraud's agar (Emmond's modification) are seen in 90% of cases. Microscopic evidence may appear with in 24 hrs. Initial macroscopic growth occurs within 72 hrs. in 83% and within 1 week in 97% of cultures. However, It is not unusual for oculomycoses to take 5 to 7 days to grow. All fungal cultures should be kept for at least 1 week before being declared negative. Once fungal growth has appeared on primary isolation medium, colonies should be subcultured promptly to fresh medium for isolation and identification in pure.

3.Histopathological Techniques

Histopathological evaluation for fungus is done of specimens obtained by superficial keratectomy or corneal buttons removed during therapeutic PK. It can reveal presence of fungal elements in 75% of patients (29). The histopathological findings include loss of corneal epithelium, Bowman's layer, and variable amounts of corneal stroma or deep stromal abscess. The

inflammatory cell infiltrate is typically granulomatous, although non-granulomatous and purulent inflammatory reactions may also occur. Hyphal elements are seen parallel to collagen lamellae; a perpendicular orientation however indicates an increased virulence (17). The hypopyon encountered is sterile as Descemet's' membrane acts as a relative barrier to invasion of anterior chamber. Few fungi such as Fusarium can however pass through an intact Descemet's membrane (30,31).

4.Recent advances in diagnostic techniques

Polymerase Chain Reaction

Polymerase chain reaction is known as the most sensitive and specific test to detect a specific DNA sequence. It is being developed as a rapid test for early diagnosis and therapy of different life threatening systemic mycosis both in immuno-competent as well as in immunocompromised host. It was used for diagnosis of Fusarium keratitis in an animal model. PCR was performed with primer directed against a portion of Fusarium cutinase gene, and the presence or absence of this amplified target sequence detected by agarose gel. It was shown to have a sensitivity of 89% compared to 21% for culture (32). However, further studies are needed to refine the technique, improve its sensitivity and specificity.

Confocal microscopy

It is a new non -invasive method of imaging the human cornea in-vivo. It can be used as a safe diagnostic tool in determing the presence of fungal hyphae in vivo in early cases of suspected keratomycosis (33). However, cost of the instrument limits its widespread use.

Principles of management

The treatment of fungal corneal ulcers has been a challenge to the ophthalmologist because of paucity of antifungal drugs, poor corneal penetration and toxicity of the available drugs. In addition, an in vitro susceptibility testing as guide to therapy is lacking (34). At present, only broad generalizations about susceptibility to antifungal agents are probably applicable. Recently, Etest has been described as a promising test for determining fungal susceptibility. It uses both dilution and diffusion methods to quantify antifungal susceptibility in terms of discrete minimum inhibitory concentration (MIC) and has a potential to be an alternative to the conventional methods (35).

The available antifungal drugs reach fungi static but rarely fungicidal, levels in tissue. The objective of antifungal therapy is to inhibit fungal growth over a long period so that body's defense mechanisms can manage the fungus. If these defense mechanisms are crippled by immunosuppression antifungal therapy may have to be maintained for many months while other microbial infections are watched for and tested.

In contrast to the rapid response seen in bacterial corneal ulcer, fungal keratitis respond slowly requiring treatment for extended period of time. This prolonged treatment and drug toxicity precludes their use in suspected cases of fungal keratitis without laboratory confirmation. Only in vision threatening cases, when clinical findings are strongly suggestive, the use of these agents without culture is warranted.

Treatment may be started if cytology report is supporting clinical features. Usually, topical 5% natamycin is advocated as drug of first choice for superficial keratomycosis. If a smear unequivocally reveals septate hyphal fragments, suggesting filamentous fungi, there is general agreement that 5% natamycin is drug of choice (36,37). It is administered hourly during daytime and 2 hourly at night for several days. The dosage can be gradually reduced. The topical treatment is continued for at least 6 weeks (36). If Natamycin is unavailable, 0.15% amphotericin -B can be used. When yeast or pseudohyphae are present in smear, treatment with topical 0.15% Ampotericin-B is indicated (4,38). Amphotericin B is less efficacious against filamentous fungi and is best served for those situations in which natamycin is unavailable. For ocassional strain of Candide albicans, resistant to amphotericin-B, topical natamycin, fluconazole or miconazole appears useful alternatives. Subconjunctival antifungals are poorly tolerated and are generally avoided.

Systemic antifungals are usually indicated in deep keratitis, scleritis and endophthalmitis. Ketaconazole is

the most frequently used oral antifungal. However, fluconazole has a better penetration into the cornea after systemic administration and may be associated with less side-effects (39). Topical steroids should be avoided in the initial management of fungal keratitis. They should not be considered until after at least 2weeks of antifungal treatment with clear evidence of control of the infection (40). A careful follow- up is thereafter required to document improvement.

Recently, intracameral amphotericin B (7.5-10 microgram in 0.1 ml.) has been successfully tried on three patients of culture proven Aspergillus flavus keratitis with good clinical response (41). No corneal or lenticular toxicity was reported in this series.

Adjunctive therapy

Keratomycosis is commonly complicated by associated elevated intraocular pressure secondary to uveitis. Topical cycloplegia with homatropine or cyclopentolate 1% makes pupil mobile and prevent development of posterior central synechiae, and reduce the possibility of pupillary block glaucome. A topical β-blocker or a carbonic anhydrase inhibitor best manages the elevated intraocular pressure.

Surgical Management

Surgery has an important role in the management of keratomycosis. In fact, ocular mycosis is primarily a surgical disease in most parts of the world because of the delay in initiating a medical treatment or non-availability of antifungal drugs (40). About one third of fungal infections result in either medical treatment failures or corneal perforations requiring surgery. The various surgical interventions undertaken in the management are:

- Corneal scrapping usually done as an initial diagnostic procedure not only provides material for smear and culture, but also promotes drug absorbtion by removing epithelial barrier (3).
- Debridement-It is the simplest and important surgical intervention. This helps in removal of necrotic material, debulking of organisms and promoting drug penetration. It should be repeated every 24 to 48 hours (3).
- Conjunctival flaps are used in cases unresponsive to medical management (42).
- Therapeutic penetrating keratoplasty [PK] is indicated in early treatment of deep keratomycosis unresponsive to medical treatment associated with impending corneal perforation (8,43).The ideal timing of graft is 5 ds. after intensive medical course. PK eliminates residual infection and restores the architectural integrity of the globe. The trephination should include a clear corneal zone of 1 to 1.5mm to reduce the risk of recurrence (44). Cryo of sclera and subconjunctival miconazole (10-20 mg/ml) should be given if scleritis is present. The corneal button should be cultured for residual fungi. The aqueous fluid should also be cultured. Post-operatively, topical antifungals should be continued for 6 to 8 weeks. Topical steroids should be avoided in early post-operative period.

Excimer laser lamellar keratectomy

It has been tried in ablation of experimental septate Fusarium keratitis. This was found to be useful in sterilizing early and localized infection. However, advanced infection with deep stromal involvement and suppuration could not be eradicated (45). It is therefore recommended only for superficial and well-circumscribed lesions not responsive to medical management.

Conclusion

Keratomycosis is one of the most difficult forms of microbial keratitis for the ophthalmologists to diagnose and treat. The diagnostic work up is tedious and the topical antifungals are not as effective as antibiotics in bacterial keratitis. The treatment is prolonged and is often complicated by secondary glaucoma and medical failures. However, a high index of clinical suspicion and a judicious management approach will go a long way in reducing visual disability.

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