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Abstract

Corneal diseases are one of the main causes of vision loss and blindness. Ocular morbidity in turn is mainly caused by Fungal Keratitis, throughout the world and especially in Asia. Isolation of the etiological agent and accurate diagnosis are important for the successful treatment of this disease. In extreme cases of infection the fungi penetrate the Descemet's membrane ultimately leading to tissue necrosis. Under such conditions, corneal transplantation is the most effective cure. The corneas that are excised are stored in Mc Carey Kaufmann (MK) media. The objective of this study was to analyze the epidemiological and microbiological features, pre disposing factors associated with Fungal Keratitis. During the study period of June 8 to July 31 2009, 100 clinically suspected cases of fungal keratitis were chosen. Corneal scraping samples were collected for analysis and in cases where the fungi had penetrated deeper into the tissue corneal buttons or the abscessed iris samples were collected. Analysis of the results indicated that males were more susceptible to the disease over females, the occupational status being the major determinant. Manual labor (30%), followed by agriculture (21%) were the two highest affected groups. The duration of the symptoms as well as a detailed history of the patients was documented. Under the microbiological findings, both filamentous fungi and yeasts were recovered from the cultures, though filamentous fungi predominated (98.55%). Beyond understanding the epidemiological aspects of fungal keratitis, further study should aim at assessing the efficacy of the anti fungal activity of the drugs used in the treatment of the disease amongst varying populations. This can be done to analyze the most effective drug in order to avoid a penetrating keratoplasty procedure.

INTRODUCTION

According to World Health Organization, corneal diseases are a significant cause of vision loss and blindness. Fungal keratitis refers to the inflammation of the eye's cornea through infection by a fungal organism. The cornea forms the anterior portion of the eye which covers the pupil. In humans the cornea has a diameter of about 11.5 mm and a thickness of 0.5–0.6 mm in the center and 0.6–0.8 mm at the periphery. The layers of the cornea include Epithelium, Bowman's layer, Corneal Stroma, Descemet's membrane and the Endothelium. Fungal Keratitis is the leading cause of ocular morbidity throughout the world and is a major blinding eye disease in Asia; Whitcher et al. (2001). Owing to varying environmental conditions, the occupational status and risk factors such as use of antibiotics and steroids among the infected patients, it is evident that the incidence of fungal keratitis broadly ranges from 6% and 56%; Ginrich (1962), Srinivasan et al. (1997) and Thomas (1994). One report from South India found that 44% of all central corneal ulcers are caused by fungi; Tanure et al. (2000). Lack of awareness about the disease, misdiagnosis, and inappropriate treatment are some of the factors contributing to this scenario.

The precipitating event for fungal keratitis includes a range of factors such as the clinical history, presence of systemic and ocular illness, occupation status, nature and agent of trauma. Factors such as age, gender, duration of symptoms, therapy received prior to presentation should be taken into account when a smear is obtained, to analyze the fungus. Across the world, the single most commonly reported fungus isolated from mycotic keratitis is Aspergillus species; Foster (1992). In India a greater number and diversity of dematiaceous moulds were cultured; Curvularia species were most frequently isolated; Leck (2002).

Fungi are saprophytic and occasionally a part of the normal external ocular flora. They gain access in to the corneal stroma through a defect in epithelial barrier which may be due to external trauma, a compromised ocular surface or previous surgery. Once in the stroma, they multiply and cause tissue necrosis through an intact Descemet's membrane. It is believed that once the organisms gain access into the anterior chamber or to the iris and lens, eradication of the organism becomes extremely difficult. Degradation of laminin in the basement membrane of the cornea has been shown to be caused by the conidia of Aspergillus fumigatus; Sharma (2001).

In our paper we aim to identify different fungal pathogens causing fungal keratitis, determine the incidence, epidemiological features, risk factors, laboratory results of 100 clinically suspected cases of fungal keratitis.

Materials and Methods

Epidemiological features of fungal keratitis:

2.1.1 **Patients**

100 clinically suspected fungal keratitis cases seen at the Cornea out-patient department at L. V Prasad Eye Institute in Hyderabad, South India between June 8 to July 31, 2009 were chosen for this study with informed consent. The parameters evaluated included age, gender, occupational status, risk factors, duration of symptoms and spectrum of fungal species. Documentation of a detailed history of the patient including demographic features, duration of symptoms, predisposing factors, therapy received prior to presentation and associated ocular and systemic diseases was performed.

2.1.2 **Clinical procedures**

A common protocol allowing the diagnosis of bacterial, fungal, parasitic keratitis was used in all cases. The standard protocol is addressed in the following section. Under topical anesthesia (4% lignocaine hydrochloride) and slit-lamp magnification, corneal scraping were obtained by qualified cornea specialists from the base and edges of the ulcer using a sterile surgical blade No. 15 on a Bard Parker handle.

2.1.3 **Laboratory procedures**

The samples collected were inoculated directly onto sheep blood agar, chocolate agar, nonnutrient agar, Sabouraud dextrose agar, potato dextrose agar, thioglycollate broth and brain heart infusion broth. The scrapings were inoculated in a row of C-shaped streaks on all solid media. Sabouraud and potato dextrose agar plates were incubated at 25^{o}C to enhance the growth of fungi, and remaining plates were incubated at 37^{o}C. Blood agar plates were incubated under both aerobic and anaerobic conditions, chocolate agar was incubated with 5% carbon dioxide, thioglycollate broth and brain heart infusion broth were incubated aerobically, and nonnutrient agar was incubated aerobically with an added live *Escherichia coli* suspension. All media were incubated for 7 days, except for Sabouraud dextrose agar and potato dextrose agar, which were incubated for 2 weeks.

As part of standard protocol for the microscopic evaluation of corneal scraping, Grams and Giemsa staining were performed and observed under bright field microscope, while for KOH/CFW staining, one drop of 10% KOH and one drop of 0. 1% Calcoflour white- sigma, USA (CFW) with 0. 1% Evans blue solution was added onto the corneal scrape, cover slip was placed on it and observed under a fluorescent microscope.

All media were examined daily for growth of organisms (bacteria, fungi and Acanthamoeba) for a period of 7-14 days. In clinically suspected cases of fungal keratitis, the Sabouraud dextrose agar and the potato dextrose agar media were incubated for further 7 days. The culture was considered significant when there was growth of the same organism in two or more media, confluent growth of an organism at the site of inoculation in one solid medium and growth of an organism in one medium with consistent direct microscopic finding or growth of the same organism on repeated scrapings. The rationale was to exclude impact created by other organisms and hence identify the correct organism. The presence of the organism can be confirmed only if there is growth in both media. Similarly, there should be growth only at the site of inoculation; growth elsewhere, indicates contamination. Both direct microscopy and the culture should correlate with each other so that the organism can be identified with certainty.

2.1.4 **Fungal Identification:**

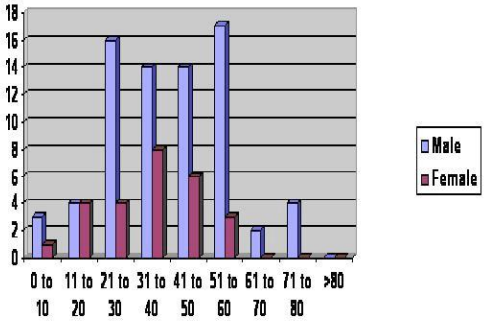
All media are observed for the growth of fungi and the colony morphology recorded. Fungal growth on media other than SDA or PDA was sub cultured on PDA for the formation of typical fungal growth and sporulation. Filamentous fungal species are identified based on rate of growth, colony morphology (color, texture, consistency) and pattern of sporulation. The type of spores is studied by examining a lacto phenol cotton blue preparation of the colony. Prolonged incubation (up to one month) may sometimes be required to obtain appropriate spores for identification. Slide culture may also be required to study the pattern of sporulation.

3.

RESULTS:

3.1 Epidemiological characteristics of fungal keratitis

3.1.1 **Figure 1: Age/Sex Distribution of the patients -** The graph below shows the age and sex distribution of 100 clinically suspected patients of Fungal Keratitis.



3.1.2 **Table 1**: Duration of Symptoms in patients at presentation to LVPEI: The table below shows the symptoms found in 100 clinically suspected patients of Fungal Keratitis.

Duration (day's)	Number of People	Percentage
0-5	14	14
6-10	19	19
11-30	43	43
>30	10	10
unknown	14	14

3.1.3 **Table 2: Occupational Status of patients:**

Occupation	Number	Percentage
Agriculture	21	21
Manual Labor	30	30
Household	11	11
Student	9	9
Desk Job/Tradesman	17	17
Unemployed/Unknown	12	12

3.1.4 **Pre-Disposing Factors:**

3.1.4.1 Table 3: Traumatic agents in 50/100 patients

Agents	Number	Percentage
Sand/ dust/ cement	10	20
Wood/ Stick	9	18
Leaf/ Thorn	4	8
Limestone	1	2
Vegetative matter	3	6
Salt	1	2
Finger Nail	2	4
Miscellaneous/ Unknown	20	40

3.1.4.2 Table 4: Others, Risk factors in 50/100 patients

Risk Factors	Number	Percentage
OCULAR		
Corneal Scrapping/ Pterygium	3	6
Prior Ulcer	10	20
Antibiotic Use	8	16
Contact Lens	1	1
Non resolving Keratitis	4	8
Glaucoma	2	4
SYSTEMIC FACTORS		
Herpes	2	2
Diabetics	4	4

3.1.5 A. **Epidemiological Characteristics:**

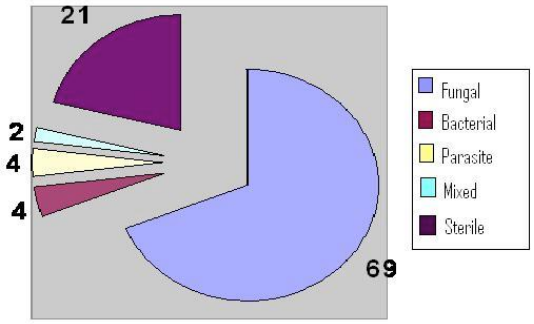
During the study period, 100 patients were evaluated. Among them, there were 74 males and 26 females, the male to female ratio being 2.8:1. Males were affected more than females, the highest affected age group in males being 51 to 60 years (17%), closely followed by the 21 to 30 years age group (Figure 2). People beyond 60 years of age and below 10 years of age seem less likely to be affected. The occupational status of 88 people was known and the two major contributing activities was found to be manual labor (30%), followed by agriculture (21%) (Refer Table 2). Students contributed to 9% of the total occurrence of fungal keratitis. Table 1 gives information on the duration of symptoms in patients. While around 14 patients (14%) presented within 0 to 5 days following onset of symptoms and 19 patients (19%) within 6 to 10 days, the maximum number presented themselves

(43%) after 11 to 30 days of the onset of the symptoms. A detailed history of 80 patients was obtained and among them traumatic agents contributed to 50% of the total. The predisposing factors are mainly classified into two tables (Tables 3 and 4), the first dealing with the agents causing trauma and the latter dealing mainly with the ocular and systemic risk factors. Under traumatic agents, injury due to sand/dust/cement (20%) was found to be the highest risk factor. Injury caused by wooden stick (18%) was the next major contributing factor. Unknown agents (foreign bodies) were responsible for 40% of the occurrence. Under ocular risk factors, prior or pre-existing ulcers (20%) were found to play a major role in triggering off fungal keratitis. Prolonged use of antibiotics and corticosteroids (16%) was another factor found to be responsible. The systemic factors, which include Herpes labielis lesions and Diabetes mellitus, had equal percentage of occurrences (4%).

3.1.6 B. **Microbiological Findings:**

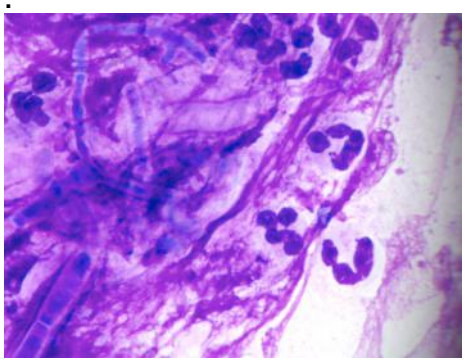
Among these 100 patients, Corneal Scrapings were collected from 70 of them. From the remaining 30 patients, other samples such as corneal biopsies/AC exudates/Foreign bodies/Abscised Iris and Vitreous Biopsies were retrieved (Refer Table 7 and 8). From these samples, pure fungus was found to grow in (69%) of them, pure bacterial growth was present in 4 (4 %) of them. Acanthamoeba grew in 4 (4 %) and 21 cases (21 %) were found to be sterile. Mixed growth was observed in 2 (2 %) of the cases (Refer figure 3). Samples where collected from patients and were subjected to staining by three methods namely KOH/CFW, Gram's and Giemsa. KOH smear was obtained in 95 cases, Gram's Stain in 97 cases and Giemsa Stain in 94 cases. Scanty material was the primary reason due to which all the three smears could not be obtained from all patients. Both filamentous fungi and yeasts were recovered from the cultures, though filamentous fungi where more predominant (98.55%) than yeast (1.45%) (Refer Table 6). Among the filamentous fungi, 80.88% isolates were found to belong to the hyaline group and 19.12% of the isolates were found to belong to dematiaceous. The spectrum of hyaline fungi was dominated by Aspergillus spp. (58. 18%), in which A.flavus (65. 625%) was the highest, followed by Acremonium (20%). In the dematiaceous group, the percentage of unidentified dematiaceous species (69%) was the highest to be isolated.

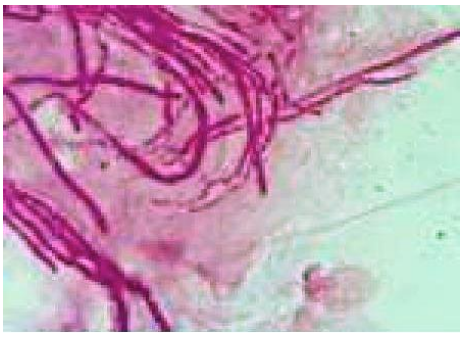
3.1.7 Figure 2-1, 2-2: **The spectrum of division of organisms among the 100 clinically suspected cases.**

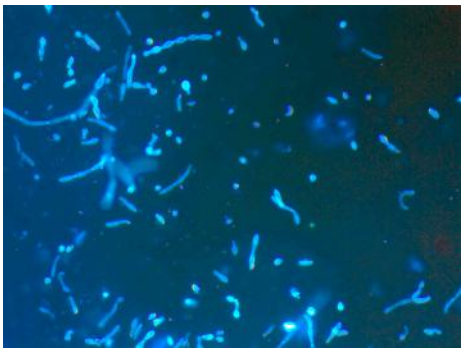


Fungal Isolates	Number	Percentage (%)
Filamentous Fungi	68	98.55
Hyaline Fungi	55	80.88
Aspergillus spp.	32	58.18
A.flavus	21	65.625
A.fumigatus	5	15.625
A.niger	1	3.125
A.terreus	5	15.625
Fusarium spp.	6	10.90
Penicillium spp.	1	1.81
Acremonium spp.	11	20
Unidentified hyaline	5	9.09
Dematiaceous Fungi	13	19.12
Curvularia spp	3	23.08
Stemphylium spp	1	7.69
Unidentified dematiaceous	9	69.23
Yeasts (Candida albicans)	1	1.45

3.1.8 Figure 2-3**: Direct microscopy images of fungi – KOH+CFW, Gram's, Giemsa stain .**







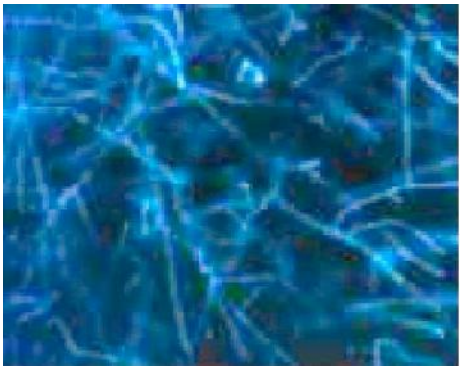
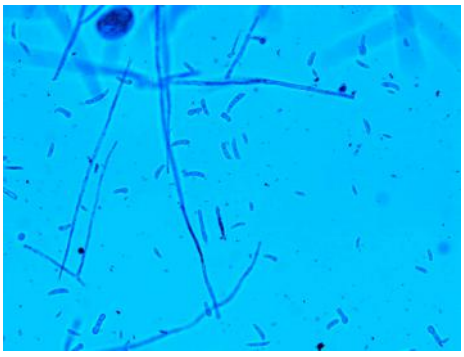
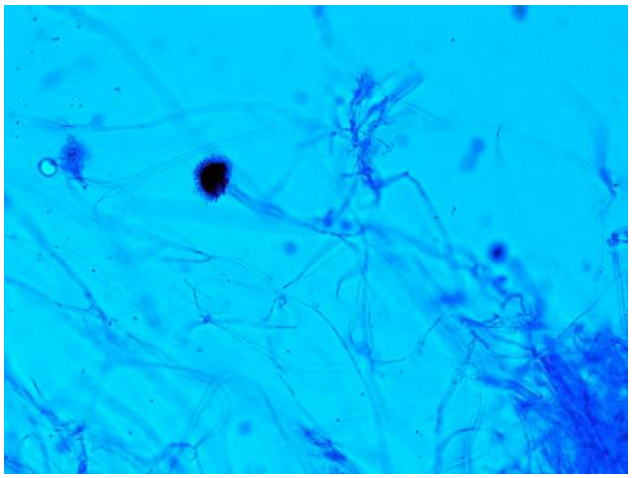
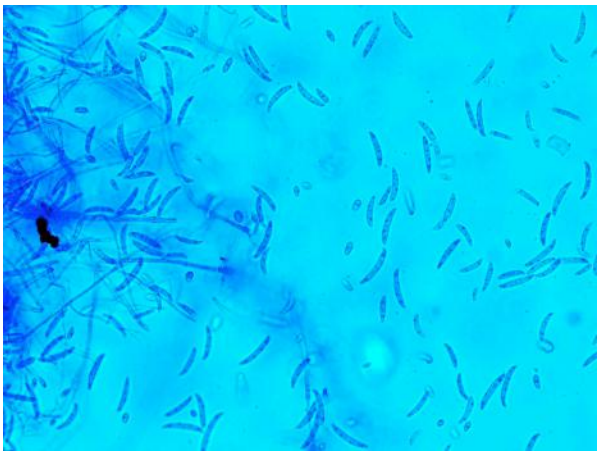


Figure 2-4: **Lacto phenol cotton blue slides used for identification of fungi under microscope – Acremonuim spp., Aspergillus spp., Fusarium spp.**







4.

DIDISCUSSIONS:

Fungus is known to cause disease in man since 1839, when Lagenback (1839) first described the fungus causing thrush but ocular infection due to fungus was first described by Leber in 1879, who demonstrated Aspergillus of cornea causing hypopyon. Fungi are ubiquitous eukaryotic microorganisms. Reported incidence of fungal keratitis is 7.3% in North India (Chander et al. (1994)), 32% in East India (Dutta et al. (1981)), 38.9% in West India (Varenkar et al. (1998)), and 32%-39.8% in South India (Srinivasan et al. (1997)). Fungal keratitis is reported more frequently from regions with a warm, humid climate of tropical and subtropical regions than temperate regions. Fusarium species and Aspergillus species are most commonly reported.

Fungal Keratitis continues to be a major cause of visual loss in developing countries. An understanding of the regional epidemiological features, risk factors and etiological agents is

important in the prevention and appropriate management of this disease entity.

In our study, the incidence of fungal keratitis is 69% which is higher than those reported in literature (6% to 56% ; %; Ginrich (1962), Srinivasan et al. (1997) and Thomas (1994).). This variation could be due to the inclusion of only highly suspected cases of mycotic keratitis and the study period (June to July). It has been reported that in Hyderabad the incidence of fungal keratitis is higher in winter (October-January) and monsoon (June-September), which accounts for our results. The predominance of fungal infections is found more among males with the age group ranging from 21 to 60 years. These results could be due to the greater involvement of men in outdoor activities and agriculture based occupation.

Trauma was identified as the major risk factor contributing to 50% of the total cases, which is similar to other reported cases; Whitcher et al. (2001), Sharma (1993) and Gopinathan et al. (2002). Of the non-traumatic risk factors, antibiotic use and pre-existing corneal ulceration has found to have a major impact on fungal keratitis.

It is of interest to note that most of the patients presented themselves only after 11 to 30 days after the onset of symptoms. The clinical features of fungal keratitis are pleomorphic. The early stages of fungal ulcers appear like a dendritic ulcer of herpes simplex virus origin. These features sometimes cause misdiagnosis and prompt treatment with anti viral drugs or corticosteroids; Agarwal et al. (1994). Thus patients who presented themselves are those cases in which empirical treatments have failed at primary and secondary centers and have been referred here, being a tertiary center for further management.
While coming to the microbiological findings, all the three smears, namely KOH+CFW, Gram's and Giemsa have shown to be effective in the detection of fungal elements, all having a sensitivity of above 90%, which is in correlation with other findings; Leck et al. (2002).

Among the 69% of pure fungal isolates, 98.55% were filamentous fungi and 1.45% was identified to be yeast. The higher incidence of filamentous fungi can be attributed to the
enormous amount of spores in tropical and sub-tropical regions rather than temperate climates; Prasad et al. (1982).

In our study, Aspergillus species dominated the spectrum of hyaline fungi (58.18 %), which is in accordance to other studies in India; Bharathi et al. (2003). Among the dematiaceous fungi, the unidentified dematiaceous group (69 %) was predominant. The species were unable to be identified due to lack of sporulation. Similar incidents of unidentified fungal species have been quoted in literature; Srinivasan et al. (1997) and Liesgang et al. (1980).

Corneal ulcers pose a challenging problem to ophthalmologists, as their etiology is of unknown origin and their course unpredictable, prognosis uncertain, a specific therapy still being a confused issue, as effective specific antifungal broad spectrum antibiotics are still not available. Infact the current treatment available is unsatisfactory, the available antifungal agents being fungistatic, requiring prolonged course of therapy; Srinivasan (2004). Fungi considered to be ocular pathogens are rarely encountered among the systemic mycoses, hence therapeutic principles valid for systemic fungal infections may not apply to the cornea; O'Day (1987). Thus further study aimed at determining fungal sensitivity of antifungal drugs should be carried out to determine the most effective drug in order to avoid a penetrating keratoplasty procedure.

REFERENCES:

Whitcher, J.P. et al (2001) Corneal blindness: a global perspective. Bull World Health Origin 79, 214-221.

Ginrich, W.D. (1962) Keratomycosis. JAMA 179, 602-608.

Srinivasan, M. et al. (1997) Epidemiology and aetiological diagnosis of Corneal Ulceration in Madurai, South India. British Journal of Ophthalmology 81, 965-971.

Thomas, P.A. (1994). Mycotic keratitis. An underestimated mycosis. Journal of

Medical and Veterinary Mycology; 32: 235-56.

Sharma, S. et al. (1993). The current status of Fusarium species in mycotic keratitis in South India. Indian Journal of Medical Microbiology 11, 140-147.

Tanure, M.A.G. et al. (2000). Spectrum of fungal keratitis at Wills Eye Hospital, Philadelphia, Pennsylvania. Cornea 19(3), 307-312.

Foster, C.S. (1992). Fungal Keratitis. Infectious Disease Clinics of North America 6, 851-857.

Leck, A.K. et al. (2002). Aetiology of suppurative corneal ulcer in Ghana and South India, and epidemiology of fungal keratitis. British Journal of Ophthalmology 86. 1211-1215.

Lagenback, C.J.M. (1839). Comptzy Weimer 12, 144 (Quoted from Modern Ophthalmology Vol. 2, By Arnold Sorsby 19; 3.

Lebez, T.H. (1879). Graefe's Archive for Clinical and Experimental Ophthalmology 25. 285-301.

Chander, J. et al. (1994). Prevalence of fungal corneal ulcer in Northern India. Infection 22. 207-209.

Dutta, L.C. et al. (1981). Study of fungus keratitis. Indian Journal of Ophthalmology 29. 407-409.

Varenkar, M.P. et al. (1998). Study of mycotic keratitis in Goa. Indian Journal of Medical Microbiology 16. 58-60.

Sharma, S. (2001). Keratitis. Bioscience Reports 21. 419-444.

Gopinathan, U. et al. (2002). The Epidemiological Features and Laboratory Results of Fungal Keratitis: A 10 year Review at a Referral Eye care center in South India. Cornea 21(6). 555-559.

Bharathi, M.J. et al. (2003). Epidemiological characteristics and laboratory diagnosis of fungal keratitis: a three year study. Indian Journal of Ophthalmology 51. 315-321.

Liesgang, T.J. et al. (1980). Spectrum of microbial keratitis in South Florida. American Journal of Opthalmology 90. 38-47.

Prasad, S. et al. (1982). Mycotic infections of the cornea. Indian Journal of Ophthalmology 30. 81-85.

Agarwal, V. et al. (1994). Current perspectives in infectious keratitis. Indian Journal of Ophthalmology 42. 171-191.

Srinivasan, M. (2004). Fungal Keratitis. Current opinions in Ophthalmology 15. 321-327.

O'Day, D.M. (1987). Selection of appropriate antifungal therapy. Cornea 6. 238-245.