ORIGINAL ARTICLE


Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study

M Jayahar Bharathi, R Ramakrishnan, S Vasu, R Meenakshi, R Palaniappan
Department of Microbiology, Aravind Eye Care System, Tirunelveli, Tamil Nadu, India

Correspondence Address:
M Jayahar Bharathi
Department of Microbiology, Aravind Eye Care System, Tirunelveli, Tamil Nadu
India

Source of Support: None, Conflict of Interest: None

PMID: 14750619

Abstract

Purpose: To study the epidemiological characteristics and laboratory diagnosis of fungal keratitis seen at a tertiary eye care referral centre in South India.

Methods: A retrospective review of all culture-proven fungal keratitis seen over a 3-year period, September 1999 through August 2002.

Results: Fungal aetiology were confirmed in 1095 (34.4%) of 3183 corneal ulcers. The predominant fungal species isolated was Fusarium spp (471; 42.82%) followed by Aspergillus spp (286; 26%). Males (712; 65.08%) were more often affected (P< 0.0001). A large proportion of the patients (732; 66.85%) were in the younger age group (21 to 50 years). A majority (879; 80.27%) came from rural areas (P<0.0001), and most patients (709; 64.75%) were farmers (P<0.0001). Ocular trauma (1009; 92.15%) was a highly significant risk factor (P<0.0001) and vegetative injuries (671; 61.28%) were identified as a significant cause for fungal keratitis (P<0.0001). 172 (15.71%) patients had concurrent diabetes mellitus. The sensitivity of 10% potassium hydroxide (KOH) wet mount preparation was higher (99.23%) than Gram-stained smear (88.73%) (P<0.0001). Incidence of fungal keratitis was higher between June and September.

Conclusion: Agricultural activity and related ocular trauma were principal causes of mycotic keratitis. A potassium hydroxide (KOH) wet mount preparation is a simple, and sensitive, method for diagnosis.

Keywords: Fungal keratitis, microscopy, culture, risk factors, epidemiology

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes.\textsuperscript{1,2,3,4,5,6,7} Corneal infection of fungal aetiology is very common\textsuperscript{8,9,10,11} and represents 30\% to 40\% of all cases of culture-positive infectious keratitis in South India.\textsuperscript{12,13,14} Fungal keratitis is a major ophthalmic problem.\textsuperscript{15} Filamentous fungi are responsible for a larger proportion of these corneal infections in tropical climates than in temperate climates, particularly following trauma with vegetative matter. In tropical climatic conditions as in South Florida,\textsuperscript{8} Bangladesh,\textsuperscript{10,11} South India,\textsuperscript{12,14,16} and Nepal\textsuperscript{17} the incidence of fungal keratitis is reported to be from 17\% to 40\%. In temperate climates such as Britain\textsuperscript{18} and Northern United States,\textsuperscript{19} the proportion of fungi causing suppurative keratitis is very low. Similarly, at the high altitude of Johannesburg, South Africa, the incidence is not more than 2.3\%.\textsuperscript{20,21} At least 70 genera of fungi have been associated with fungal keratitis\textsuperscript{22} Of these, Fusarium species and Aspergillus species are responsible for 70\% of cases.\textsuperscript{23,24,25}

There are several studies of fungal corneal ulcer from South India.\textsuperscript{12,14,26,27,28} The aetiological and epidemiological pattern of corneal ulceration varies significantly with patient population, health of the cornea,\textsuperscript{29} geographic region, climate and also tends to vary over time. Hence, an understanding of the current status of the regional epidemiological features, risk factors, the presence of ocular and/or systemic co-morbidities, occupational status and knowledge of region-wise aetiological agents is important in the prevention and appropriate management of fungal keratitis.

The purpose of this study was to evaluate the specific pathogenic agents and study epidemiological characteristics of fungal keratitis presenting at a tertiary referral centre in the southern most part of India.

\section*{Materials and Methods}

\section*{Patients}

A retrospective analysis was performed of all patients with culture-proven fungal keratitis seen over a period of 3 years, September 1999 to August 2002. A total of 3183 consecutive patients with corneal ulceration seen at a tertiary eye care referral centre in south India were
The sensitivity of 10% KOH wet mount preparation was higher (99.23%) than Gram-stained smear (88.73%) in the detection of fungal filaments. A detailed ocular examination using standard techniques, an ophthalmologist took corneal scrapings under aseptic conditions from each ulcer using a sterile Bard-Parker blade (No 15).[12] The procedure was performed under magnification of slit lamp or operating microscope after instillation of 4% lignocaine (lidocaine) without preservative.[12] The scraping material obtained from the leading edge and the base of each ulcer was initially inoculated directly onto solid media such as sheep's blood agar, chocolate agar, or Sabouraud's dextrose agar in a row of C-shaped streaks. Deep inoculation in liquid media such as brain heart infusion broth without gentamicin sulphate and thioglycolate medium was also done.[12] The material obtained by scraping was spread onto labeled slides in a thin, even manner for 10% KOH wet mount, Gram's staining, and Giemsa staining.[12] In cases of suspected actinomycete keratitis, Kinyoun's method of acid-fast staining was performed.[12] When KOH smears were positive for amoebic cysts further corneal scrapings were performed and the materials were inoculated onto non-nutrient agar.[12] All patients received a slitlamp biomicroscopic examination by an ophthalmologist. The size of the epithelial defect after staining with 2% fluorescein was measured with the variable slit on the biomicroscope and recorded in millimeters.[12] In similar fashion the size and depth of the stromal infiltrate was recorded. A sketch of each ulcer was drawn on the form using standardised frontal and cross-sectional diagrams. The presence or absence of a hypopyon was recorded and the height measured in millimetres. Associated ocular conditions such as blepharitis, conjunctivitis, dacyrocystitis, spheroidal corneal degeneration, dry eyes, bullous keratopathy, pre-existing viral keratitis, lid abnormalities, Bell's palsy, lagophthalmos, trichiasis, suture infiltrates and adherent leucoma were noted.[12] The use of contact lenses and topical corticosteroids and other systemic combinations were also recorded.[12]

Clinical procedures

All patients received a slitlamp biomicroscopic examination by an ophthalmologist. The size of the epithelial defect after staining with 2% fluorescein was measured with the variable slit on the biomicroscope and recorded in millimeters. In similar fashion the size and depth of the stromal infiltrate was recorded. A sketch of each ulcer was drawn on the form using standardised frontal and cross-sectional diagrams. The presence or absence of a hypopyon was recorded and the height measured in millimetres. Associated ocular conditions such as blepharitis, conjunctivitis, dacyrocystitis, spheroidal corneal degeneration, dry eyes, bullous keratopathy, pre-existing viral keratitis, lid abnormalities, Bell's palsy, lagophthalmos, trichiasis, suture infiltrates and adherent leucoma were noted.[12] The use of contact lenses and topical corticosteroids and other systemic combinations were also recorded.[12]

Laboratory procedures

All inoculated media were incubated aerobically.[12] The inoculated Sabouraud's dextrose agar were incubated at 27°C, examined daily, and discarded at 3 weeks if no growth was seen. The inoculated blood agar, chocolate agar, thioglycolate broth, and brain heart infusion broth were incubated at 37°C, examined daily, and discarded at 7 days if no growth was seen. Broth tubes were held upright in racks. The inoculated non-nutrient agar plates were incubated at 37°C for 5 days, if there were no signs of growth. All laboratory methods followed standard protocols.[12] Microbial cultures were considered significant if growth of the same organism was demonstrated on more than one solid phase medium, and/or if there was confluent growth at the site of inoculation on one solid medium, and/or if growth of one medium with consistent with direct microscopy findings (that is, appropriate staining and morphology with Gram-stain) and/or if the same organism was grown from repeated scraping.[12] Meticulous care was taken in collection of material and its aseptic transfer to the appropriate culture media.[12] Fungus grown on the primary isolation medium was subcultured onto an SDA medium and incubated for a period of 15 days to facilitate sporulation.[13] Following adequate growth of the fungal isolate on SDA, identification was done based on its macroscopic and microscopic features.[13] Pearson's chi-square test was used for the statistical analysis wherever required.

Results

During the study period of three years, 3183 patients with the clinical diagnosis of corneal ulceration were evaluated at our institute. 1095 (34.4%) of 3183 patients grew fungus, 1043 (32.77%) had bacterial growth, 33 (1.04%) had sporulation.

Microbial cultures were considered significant if growth of the same organism was demonstrated on more than one solid phase medium, and/or if there was confluent growth at the site of inoculation on one solid medium, and/or if growth of one medium with consistent with direct microscopy findings (that is, appropriate staining and morphology with Gram-stain) and/or if the same organism was grown from repeated scraping. Following adequate growth of the fungal isolate on SDA, identification was done based on its macroscopic and microscopic features. Pearson's chi-square test was used for the statistical analysis wherever required.

During the study period of three years, 3183 patients with the clinical diagnosis of corneal ulceration were evaluated at our institute. 1095 (34.4%) of 3183 patients grew fungus, 1043 (32.77%) had bacterial growth, 33 (1.04%) had sporulation.

Microbial cultures were considered significant if growth of the same organism was demonstrated on more than one solid phase medium, and/or if there was confluent growth at the site of inoculation on one solid medium, and/or if growth of one medium with consistent with direct microscopy findings (that is, appropriate staining and morphology with Gram-stain) and/or if the same organism was grown from repeated scraping. Following adequate growth of the fungal isolate on SDA, identification was done based on its macroscopic and microscopic features. Pearson's chi-square test was used for the statistical analysis wherever required.

Discussion

The sensitivity of 10% KOH wet mount preparation was higher (99.23%) than Gram-stained smear (88.73%) in the detection of fungal filaments. The sensitivity of clinical diagnosis of fungal keratitis made by ophthalmologist was 94.1% and the correlation was highly significant (P<0.0001).

In our study cohort the fungal keratitis was seen more often between June and September (Table - 1) though this was not statistically significant.
Fungi are ubiquitous eukaryotic microorganisms. Fungi that infect the cornea are broadly classified as yeast or molds. Yeasts are unicellular fungi characterised by an oval or round structure, the blastoconidium. Molds are organisms with filamentous structure (hyphae) and a tangled mass of hyphae which constitutes the mycelium. Filamentous fungi may be classified as septate or non-septate. Fungi reproduce sexually by the formation of spores and asexually by forming conidia or sporangiospores. The disease-causing fungi in the cornea usually are in an asexual phase of their life cycle when they are cultured from infected cornea.[25],[33],[34]

The incidence of fungal keratitis in this study was 34.4%. Similar reported incidence in other regions of India are 7.3% in North India,[35] 32% in East India,[36] 38.9% in West India[37] and 32% - 39.8% in South India.[12],[13] This regional variation could be because fungal keratitis is expected to be more common in the tropical and subtropical regions than in the temperate regions. Fusarium spp was the predominant species in this study, similar to the reports from South Florida[8] and Ghana.[9] This is in contrast to most reports of Aspergillus spp from India,[22],[35],[38],[39] and Candida spp in other parts of the world.[40],[41],[42] The demalecates fungal cornea are frequently reported as causes of keratitis in many tropical and subtropical regions.[43]

The incidence of fungal keratitis was significantly higher in males, in individuals from rural area and following corneal injury. The calculated odds are 1.47 times in males (95% confidence interval(CI) 1.26-1.71), 2.2 times in individuals from rural areas (95% CI, 1.87-2.65); 1.4 times in farmers (95%CI; 1.19 -1.61); 7.7 times following injury (95% CI, 6.12 - 9.85); 23.6 times following injury with vegetative matter (95% CI, 19.07 - 29.3). Male predominance for corneal ulcers has been described in many studies.[8],[12],[13],[17],[22],[44] We observed that younger people, aged 21-50 years, are more often affected by fungal keratitis compared to those above 50 years, who are affected by bacterial corneal ulcers (unpublished data). Higher incidence of fungal keratitis has also been reported among farmers.[12],[13],[17],[22] We have also reported 70% incidence of Nocardia spp keratitis in farmers.[45]

Corneal trauma has always been identified as a cause of microbial keratitis,[12],[17],[46] and Schaefer et al have also identified co-existing ocular diseases as a major predisposing factor.[47] Predisposing ocular conditions and use of corticosteroids associated with development of fungal keratitis accounted for 86 (7.85%) patients. 172 (15.71%) patients had diabetes mellitus. Association of fungal keratitis with use of corticosteroids and diabetes mellitus has been reported earlier.[13],[22],[39],[44]

The clinical picture of fungal keratitis is polymorphic. It varies from individual to individual, and largely depends upon the type of fungus, severity of the invading pathogen, liberation of toxin, resistance of host tissue and age of the patient.[48],[49] The unique biomicroscopic appearance is a dry, raised, grayish white lesion and/ or as stromal infiltration with a feathery or hyphae border,[23],[44],[49] seen in 826 (75.43%) and 786 (71.78%) of our patients respectively. Satellite lesions seen in 110 (10%) patients also assisted in diagnosis.[23],[48],[49],[50] Other lesions that helped in diagnosis were heaped up or cheesy hypopyon in 609 (55.6%) patients, immune ring in 15 (1.37%) patients, corneal perforation in 15 (1.37%) and posterior corneal abscess in 11 (1%) patients. A dendritic pattern was observed in 46 (4.2%) patients in our study. It occurs in early stages of the fungal corneal infection. Sometimes these features may cause misdiagnosis and prompt a treatment with antiviral drugs or even corticosteroids.[23] The sensitivity and specificity of clinical diagnosis of fungal keratitis made by an ophthalmologist using slitlamp biomicroscope was 94.1% and 94.58% respectively. There was a high correlation between clinical and culture-based diagnosis of fungal keratitis and there was no significant difference between the two (P=1).

The observation on smears and cultures of all 3183 cases highlight the value of the traditional method of KOH wet mount preparation in the diagnosis of microbial keratitis. The sensitivity of KOH wet mount preparation (99.23%) was higher than that of Gram-stained smear (88.73%) in the detection of fungal filaments. In the Hyderabad study the sensitivity and specificity of KOH wet mount preparation in the detection of fungal filaments was 81.2% and 83.8%[51] respectively. The value of 10% KOH wet mount preparation in the diagnosis of fungal keratitis lies in its ability to clear the scraping of cellular debris, thereby rendering hyphal fragments more refraction on microscopic examination.[52] The staining quality of Gram-stain is often variable, hyphal elements frequently appear as linearly stained precipitates and it is usually not possible to determine whether they are coenocytic or septate. If the stained smear of scraping from an ulcer is thick, the hyphae will be interspersed through necrotic tissue and their identification may be difficult or impossible[52] KOH has been used as 10 to 20% suspension, alone,[51] with ink[53] or with lactophenol cotton blue[54] with variable sensitivity.[6],[51],[55] Additional benefit of KOH preparation is its ability for rapid detection and early diagnosis of Nocardia and Acanthamoeba.[56],[57]

The incidence of fungal keratitis was higher during paddy harvesting and also during the time of year when agriculture activity was greater. In our study it was more prevalent during June through September. The peak incidence correlates with windy and dry weather during the month of June through September. In coastal Karnataka, higher incidence is reported in October, June and January[38] and in Hyderabad, higher incidence of fungal keratitis is reported during the winter (October to January) and monsoon (June to September) seasons.[13] A hot, humid, windy climate and an agriculture-based occupation of a large population make fungal keratitis more frequent in tropical zones.[13]

In summary, this study presents the epidemiological and laboratory findings of the largest series of fungal keratitis among the population in our geographic region. Fungal keratitis continues to be a cause of concern to ophthalmologists. Predominance of agricultural activity is the principal causative factor. KOH wet mount preparation is as sensitive as other conventional methods in the diagnosis. Diagnosis can be delayed in the absence of proper mycological laboratory techniques.

References


---

**Figures**

[Figure - 1]

**Tables**

[Table - 1], [Table - 2], [Table - 3]

---

**This article has been cited by**

1. Clinical aspects and prognosis of mixed microbial (bacterial and fungal) keratitis
   Ahn, M., Yoon, K.-C., Ryu, S.-K., Cho, N.-C., You, I.-C.
   Cornea. 2011; 30(4): 409-413 [Pubmed]

2. Pathogenic spectrum of fungal keratitis and specific identification of Fusarium solani
   He, D., Hao, J., Zhang, B., Yang, Y., Song, W., Zheng, Y., Yokoyama, K., Wang, L.


4. Clinical signs in dematiaceous and hyaline fungal keratitis
   Oldenburg, C.E., Prajna, V.N., Prajna, L., Krishnan, T., Mascarenhas, J., Vaitilingam, C.M., Srinivasan, M., (...), Lietman, T.M.

5. Development of a novel ex vivo model of corneal fungal adherence
   Zhou, Q., Chen, H., Qu, M., Wang, G., Yang, L., Xie, L.

6. Ocular mycosis at a referral center in Saudi Arabia: A 20-year study
   Jastaneiah, S.S., Al-Rajhi, A.A., Abbott, D.

7. Comparative study on the incidence and outcomes of pigmented versus non pigmented keratomycosis
Sengupta, S., Rajan, S., Reddy, P.R., Thiruvenkadakrishnan, K., Ravindran, R.D., Lalitha, P., Vaitilingam, C.M.

8 Melanized fungi in human disease
Revankar, S.G., Sutton, D.A.
Clinical Microbiology Reviews. 2010; 23(4): 884-928

9 Keratomycosis in the area of Tunis: Epidemiological data, diagnostic and therapeutic modalities | [Les kératites fongiques dans la région de Tunis: Caractéristiques epidemiologiques, modalités diagnostiques et thérapeutiques]
Anane, S., Ayed, N.B., Malek, I., Chebbi, A., Lajri, S., Bougula, H., Kaouech, E., (...), Chaker, E.
Annales de Biologie Clinique. 2010; 58(4): 441-447

10 Current efforts and the potential of nanomedicine in treating fungal keratitis
Gratieri, T., Gelfuso, G.M., Lopez, R.F., Souto, E.B.

11 Comparative study of Gram stain, potassium hydroxide smear, culture and nested PCR in the diagnosis of fungal keratitis
Badiee, P., Nejabat, M., Alborzi, A., Keshavarz, F., Shakhba, E.
Ophthalmic Research. 2010; 44(4): 251-256

12 Comprehensive review of the effects of diabetes on ocular health
Skarbez, K., Priestley, Y., Hoepf, M., Koevary, S.B.

13 Mycotic keratitis in the elderly - a 32-year review | [Ceratite infecciosa em idosos - revisão de 32 anos]

14 Epidemiology and etiologic diagnosis of infectious keratitis in Uberlandia, Brazil
2010; 20(3): 498-503

15 Fungal keratitis: 84 cases report in Southern Pakistan
Narsani, A.K., Gul, S., Dabir, S.A., Jatoi, S.M., Khanzada, M.A., Kumar, M.

16 Voriconazole for the treatment of refractory Aspergillus fumigatus keratitis
Mehta, H., Mehta, H.B., Gang, P., Kodial, H.

17 Comparative bacteriology of acute and chronic dacryocystitis
Bharathi, M.J., Ramakrishnan, R., Maneksha, V., Shivakumar, C., Nithya, V., Mittal, S.
Eye. 2008; 22(7): 953-960

18 Use of Chlorazol Black E Mounts of Corneal Scrapes for Diagnosis of Filamentous Fungal Keratitis
Thomas, P.A., Kaliamurthy, J., Jesudasan, C.A.N., Geraldine, P.

19 Normalized diagnosis and treatment of infectious keratitis
Shi, W.-Y., Xie, L.-X.

20 Mycotic keratitis: An overview of diagnosis and therapy
Shukla, P.K., Kumar, M., Keshava, G.B.S.
Mycoses. 2008; 51(3): 183-199
26 Keratomycosis in and around Chandigarh: A five-year study from a north Indian tertiary care hospital
Chander, J., Singla, N., Agnihotri, N., Arya, S., Deep, A.
Indian Journal of Pathology and Microbiology. 2008; 51(2): 304-306

27 Clinical features and confocal microscopic imaging characteristics of Fusarium keratitis
Zhang, J., Wang, L., Sun, S., Zhang, Y.

28 Aqueous and vitreous concentrations following topical administration of 1% voriconazole in humans
Archives of Ophthalmology. 2008; 126(1): 18-22

29 Medical and surgical management of keratomycosis
Mann, S.S., Singh, J., Kaira, D., Panhar, J.K.S., Gupta, N., Kumar, P.
Medical Journal Armed Forces India. 2008; 64(1): 40-42

30 Saprophytic and pathogenic fungi of plants causing fungal keratitis in humans
Jeya, M., Udhaya, V., Vasudevan, R.
Asian Journal of Microbiology, Biotechnology and Environmental Sciences. 2007; 9(3): 507-510

31 Fungal keratitis and contact lenses: An old enemy unrecognized or a new nemesis on the block?
Eye and Contact Lens. 2007; 33: 415-417

32 Fungal keratitis in London: Microbiological and clinical evaluation
Galarreta, D.J., Tuft, S.J., Ramsay, A., Dart, J.K.G.
Cornea. 2007; 26(9): 1082-1086

33 The value of aetiology in the diagnosis of infectious keratitis
Shi, W.-Y., Liu, M.-N., Wang, T.

34 Letter to the editor
Jain, A.K., Bansal, R., Rajwanshi, A.

35 Epidemiology and treatment of fungal corneal ulcers
Ou, J.I., Acharya, N.R.
International Ophthalmology Clinics. 2007; 47(3): 7-16

36 Application of gene chip technique in identification of pathogens spaces in fungal keratitis
Zhang, Y., Wang, L., Li, Z., Sun, S.

37 Microbial keratitis in West Anatolia, Turkey: A retrospective review
Yilmaz, S., Ozturk, I., Masten, A.

38 Microbial keratitis in South India: Influence of risk factors, climate, and geographical variation
Bharathi, M.J., Ramakrishnan, R., Meenakshi, R., Padmawathy, S., Shivakumar, C., Srinivasan, M.
Ophthalmic Epidemiology. 2007; 14(2): 61-69

39 Retrospective study of suppurative keratitis in 1054 patients
Zhong, W.-X., Sun, S.-Y., Zhao, J., Shi, W.-Y., Xie, L.-X.

40 Dematiaceous fungi
Revankar, S.G.
Mycoses. 2007; 50(2): 91-101

41 In vivo confocal microscopy in fungal keratitis
Brasna, E., Bounier, T., Dupas, B., Degrange, S., Podliak, T., Bounier, T., Borderie, V., Baudouin, C.

42 Fungal keratitis in Melbourne
Bhartiya, P., Daniell, M., Constantinou, M., Islam, F.M.A., Taylor, H.R.
Clinical and Experimental Ophthalmology. 2007; 35(2): 124-130

43 Evaluation of impression smear in the diagnosis of fungal keratitis
Jain, A.K., Bansal, R., Felcida, V., Rajwanshi, A.

44 Harmful fungi in both agriculture and medicine
De Lucca, A.J.
Revista Iberoamericana de Micología. 2007; 24(1): 3-13
<table>
<thead>
<tr>
<th>ID</th>
<th>Reference</th>
</tr>
</thead>
</table>
To report the epidemiological features and laboratory results of 1,352 cases of fungal keratitis diagnosed at the L.V. Prasad Eye Institute (LVPEI) in south India. The medical and microbiology records of 1,352 culture proven cases (1,354 eyes) of fungal keratitis diagnosed at LVPEI were analyzed. There was a higher incidence of fungal keratitis during the monsoon and winter than summer. A fungal cause was established by smears of corneal scrapings in 1,277 (95.4%) eyes. The epidemiological characteristics and the causative agents of fungal keratitis at the National Ophthalmology hospital, the biggest eye care hospital in North Vietnam were described by using questionnaire and analyzing of ITS region sequences. In 2008, 687 fungal keratitis patients were diagnosed and 363 fungal strains were isolated from these patients. References.

This website uses cookies. By continuing to use this website you are giving consent to cookies being used. For information on cookies and how you can disable them visit our Privacy Policy.

AGREE & PROCEED