The Epidemiological Features and Laboratory Results of Fungal Keratitis

A 10-Year Review at a Referral Eye Care Center in South India

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Purpose. 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. Methods. The medical and microbiology records of 1,352 cases of fungal keratitis diagnosed at the LVPEI between January 1991 to December 2000 was retrospectively reviewed for demographic features, risk factors, seasonal variation, and laboratory findings. Results. Males (962) were affected significantly more (p < 0.0001) than females (390). Of 1,352 patients, 853 (64.4%) were in the younger age group (16–49 years). Ocular trauma predisposed to infection in 736 (54.4%) of 1,354 eyes. 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 potassium hydroxide preparation (KOH), Calcofluor white (CFW), Gram-, and Giemsa-stained smears revealed fungus in 1,219 (91.0%), 1,224 (91.4%), 1,181 (88.2%), and 1,139 (85.1%) eyes, respectively. Fusarium (506, 37.2%) and Aspergillus species (417, 30.7%) predominated in the hyaline fungal spectrum (1,133) and Curvularia species (39, 2.8%) were the highest among the dematiaceous isolates (218). Conclusions. To the best of our knowledge, this review presents the epidemiological features and laboratory results of the largest series of fungal keratitis ever reported in the literature. Keratomyocysis is predominant in young adults with trauma as the major predisposing factor. With fungal keratitis being a major ophthalmologic problem in the tropical regions of the world, data available on the epidemiological features of a large series would greatly help medical practitioners at primary and secondary health care centers in the management of the disease. A simple KOH preparation of corneal scraping alone is highly beneficial in confirming the diagnosis.

Key Words: Fungal keratitis—Epidemiology—Etiology.

Fungal keratitis continues to be a cause of concern to ophthalmologists. Lack of awareness about the disease, misdiagnosis, and inappropriate treatment are some of the factors contributing to this scenario. Thus, apart from identifying typical clinical features, an understanding of the risk factors and the assistance of simple laboratory investigations would largely aid in the diagnosis of this disease entity. Although specific clinical features have been described for bacterial, fungal, and parasitic corneal infections, prolonged use of wrongly administered antimicrobial agents and/or corticosteroids and patient or parasite related factors could result in a considerable overlap of the identifying features. The incidence of fungal keratitis, risk factors, and the type of agent causing it may vary among different geographic regions, as previously reported. Hence, documentation of patient’s clinical history with regard to the nature of the agent causing the trauma, the presence of ocular and/or systemic illness, use of antimicrobials or steroids, and occupational status is essential to the understanding and management of the disease. Since laboratory procedures have become easily available at the reference centers, microbiologic investigation of corneal scrapings avoids delay in instituting appropriate medical therapy in these cases.

Individual case reports and small series have presented data on the fungal spectrum and treatment outcome. However, there seems to be inadequate information about the epidemiological and etiological features of a large series of fungal keratitis from representative geographic regions. It has therefore become increasingly important to gather sufficient data that would project the gravity of the problem of fungal keratitis of a particular geographic area in terms of the epidemiology and etiology, the documentation of which would serve as a useful guide for practicing ophthalmologists.

This review summarizes the epidemiological features and laboratory findings of 1,352 culture-proven cases of fungal keratitis (1,354 eyes) diagnosed at the L.V. Prasad Eye Institute (LVPEI), Hyderabad, India, over a 10-year period (January 1991 to December 2000) and is the largest series ever reported in the literature.

MATERIALS AND METHODS

The computerized corneal ulcer database was used to analyze the medical and microbiology data of 1,352 patients (1,354 eyes).
with culture-proven fungal keratitis diagnosed at the LVPEI between January 1991 and December 2000. The parameters evaluated included the age, gender, predisposing factors, duration of symptoms at presentation, treatment received prior to presentation at this center, seasonal variation, and laboratory results.

All patients with infectious keratitis presenting to the LVPEI underwent a detailed investigation that includes (1) the documentation of a detailed history of the patient (demographic features, duration of symptoms, predisposing factors, therapy received prior to presentation, and associated ocular and systemic diseases), (2) ocular examination using a slit-lamp biomicroscope, and (3) microbiologic workup comprising direct smear examination and culture of corneal scrapings taken under local anesthesia from the bed and advancing edges of the ulcer.

The corneal scrapings were stained by the Gram and Giemsa methods and also viewed as wet mount preparations using 10% potassium hydroxide preparation (KOH) and calcofluor white (CFW) stains. The material was also inoculated onto a variety of solid and liquid media that support the growth of bacteria, fungi, and Acanthamoeba.8,9 All media were incubated for a period of 14 days at appropriate atmospheric conditions and examined daily for the growth of organisms.

A definitive diagnosis of fungal keratitis was made if

1. Corneal scrapings revealed fungal elements in smears,
2. Fungus grew in more than one medium in the absence of fungus in smears,
3. Fungus grew on a single medium in the presence of fungus in smears,
4. Confluent growth of fungus appeared at the inoculated site on a single solid medium.

A fungus grown on the primary isolation medium was subcultured onto a potato dextrose agar (PDA) medium and incubated for a period of 10 days to facilitate sporulation. Following adequate growth of the fungal isolate on PDA, the identification was carried out based on its macroscopic and microscopic features.

Medical treatment with antifungal agents was initiated if corneal scrapings revealed the presence of fungus by direct microscopy. However, in the absence of visualization of fungus in smears, treatment was initiated if fungal growth appeared on culture and in accordance with the criteria listed above. Pearson’s χ² test was used to carry out the statistical analysis wherever required.

RESULTS

Epidemiological Features

Among the 3,399 patients with culture-proven infectious keratitis, 1,352 (39.8%) patients were diagnosed as having fungal keratitis. Among them, one patient had two episodes of infection (with different fungi) and another patient had a bilateral infection (with the same fungus), accounting for 1,354 infected eyes. Among the 1,352 patients who presented to our institute, 950 (70.3%) were males and 390 (28.8%) were females; the male-to-female ratio was 2.5:1. Males were significantly more affected than females (p < 0.0001). Of 1,324 patients, 50 (3.8%) were younger than 16 years of age, 853 (64.4%) were 6 to 49 years of age, and 421 (31.8%) were older than 49 years of age, the majority of patients thus belonging to the younger age group. The average age at presentation was 40.4 ± 15.3 years. The occupational status of 1,328 patients was available as shown in Table 1. Of these, 642 (48.3%) patients were engaged in outdoor activities and 389 (29.3%) were indoors.

A history of recent injury to the affected eye was obtained in 736 (54.4%) of 1,354 eyes. The object causing the trauma was not reported in 276 (37.5%) of 736 trauma cases. Injury with plant or animal material led to fungal infection in 188 (13.9%) and 28 (2.1%) eyes, respectively. A history of dust falling into the eye was identified in 155 (11.4%) eyes. Other objects responsible for injury are listed in Table 2. Among patients for whom both occupational status and history of trauma were documented, those working outdoors (268, 51.3%) were more likely (p < 0.0001) to have incurred trauma (107, 36.8%) than those indoors.

Ocular and systemic factors likely to have compromised the cornea were recorded in 158 (11.7%) and 109 (8.0%) eyes, respectively. Prior ocular surgery was documented in 87 (6.4%) of 1,354 eyes. Among systemic factors recognized, diabetes mellitus was reported in 87 (6.4%) eyes. Other ocular and systemic factors analyzed are given in Table 3. Table 4 depicts the duration of symptoms experienced by patients (documented in 1,327 patients) prior to their presentation to LVPEI. While 891 (65.8%) patients reported within 1 to 2 weeks of the onset of symptoms, the remaining (436, 32.2%) presented between 2 weeks to more than 3 months following the onset of symptoms. Preceding their initial visit to our institute (referred from primary and secondary health care centers), 950 (70.3%) patients had received antibacterial, antifungal, antiviral agents, and/or steroids topically, either alone (592) or in combination (358), as shown in Table 5.

Figure 1 illustrates the seasonal variation in the occurrence of fungal keratitis analyzed over a period of 10 years. Our data revealed that in India, fungal keratitis was reported more often during the winter (October to January) and monsoon (June to September) seasons than in summer (February to May). However, the difference in the occurrence of fungal keratitis between these seasons was not found to be statistically significant.

Microbiological Findings

In 1,354 eyes with fungal keratitis, fungi alone were the etiological agents in 1,158 (85.5%) cases, while bacteria and parasites coexisted with fungi in 194 (14.3%) and two (0.1%) eyes, respectively. Corneal scrapings collected from 1,339 of 1,354 (98.9%) eyes were also subjected to staining by all four methods (KOH, CFW, Gram, and Giemsa) and inoculated for culture. Due to inadequacy of the material, corneal scrapings of 15 eyes were processed only for culture and not for smears.

Fungal elements were visualized by direct microscopy of corneal scrapings in 1,277 (95.4%) of 1,339 eyes by one or more of the staining methods: the KOH preparation, CFW preparation, Gram-stained smears, and Giemsa-stained smears revealing fungus in 1,219 (91.0%), 1,224 (91.4%), 1,181 (88.2%), and 1,139 (85.1%) of the cases, respectively. However, fungal elements were
not detectable in corneal scrapings of 62 of 1,339 (4.6%) eyes in which a definitive diagnosis of fungal keratitis was made based on the growth of fungus on culture media alone.

In the present series, among the 1,360 fungal isolates recovered from 1,354 eyes, the filamentous fungi were the etiological agents in 1,351 (99.3%) and yeasts in nine (0.7%) eyes (Table 6). Two fungal species each were isolated from nine eyes, accounting for 1,360 isolates. Among the filamentous fungal isolates, 1,133 (83.8%) belonged to the hyaline (nonpigmented) group and 218 (16.1%) to the dematiaceous (pigmented) group of fungi, and the remaining were unidentified. The hyaline fungal spectrum was predominated by Fusarium species (506, 37.2%), followed by Aspergillus species (417, 30.7%). Among the dematiaceous fungal isolates, Curvularia species were the most common (39, 2.8%).

**DISCUSSION**

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. The present report has therefore attempted to elucidate these features with particular reference to patients that presented over a 10-year period to the Cornea Services of the L.V. Prasad Eye Institute, a tertiary eye care center in south India.

At this center, the incidence of fungal keratitis between January 1991 and December 2000 was observed to be 39.8%. From reports available in the literature from different parts of the world, it is evident that the incidence of fungal keratitis ranges from 6% and 56%.1,5,10 Mycotic keratitis is expected to be more common in...
the tropical and subtropical locations than in the temperate regions. A hot, humid climate and an agriculture-based occupation of a large population make fungal keratitis more frequent in tropical countries.

The predominance of fungal corneal infections in the younger age group (16–49 years) and among males could be attributed to their greater involvement in outdoor activities, thus more prone to corneal injury with external agents. A few investigators have made similar observations.\textsuperscript{11–14}

Trauma has been identified as the major risk factor in 54.4% of the infected eyes. A similar source (51.5%) was reported for adult keratomycosis earlier.\textsuperscript{14} Several reports on fungal keratitis describe an antecedent injury of the cornea caused by leaves, paddy grain, cow tail, tree branch, and metal pieces.\textsuperscript{5,16–17} Ocular and systemic illnesses have been reported to be one of the major predisposing factors for fungal keratitis in children, unlike adults,\textsuperscript{18} leading to a compromised state and a greater predisposition to fungal keratitis as described earlier.\textsuperscript{19} In the current series, previous ocular surgery and diabetes mellitus were each associated with keratomycosis in only 6.4% of the eyes.

While 891 eyes were examined at the LVPEI between 1 and 2 weeks of the onset of symptoms, a delay of 3 weeks to more than 3 months was recorded in 436 eyes. Our experience indicates that patients who report late for medical advice frequently present with large ulcers that are often refractory to antifungal therapy and therefore more likely to undergo corneal transplantation. At this institute, a retrospective review of fungal keratitis (88 cases) caused by dematiaeuse fungi\textsuperscript{20} indicated that patients who presented late to clinics without receiving appropriate treatment until then responded poorly to antifungal agents administered at a later stage. Larger ulcer size and involvement of deeper tissue layers are factors limiting successful management of fungal keratitis despite appropriate treatment.

Of 950 patients on prior therapy, 291 (30.6%) were on a combination of antifungals, antibiotics, antivirals, or steroids. This indicates the tendency of general practitioners and ophthalmologists at the primary and secondary eye care centers to prescribe a cocktail of drugs and to refer patients to tertiary eye care centers only when empirical treatment failed. Only 6.4% (61) patients were treated elsewhere with antifungal agents alone. Many of them had received insufficient dosage, demonstrating the tendency of medical practitioners to treat the condition inadequately. Long-term use of antibiotics and corticosteroids is believed to compromise the cornea and worsen the disease condition in fungal keratitis.\textsuperscript{7,21}

Therefore, the value of microbiologic investigation in establishing a definitive cause of fungal keratitis cannot be underestimated. General physicians and individual practicing ophthalmologists also need to be aware of the availability of referral eye care centers where diagnostic facilities are provided.

The utility of laboratory investigations in making a definitive diagnosis of infectious keratitis has been addressed previously.\textsuperscript{22} Fungal keratitis may mimic other infectious keratitis (bacterial or parasitic) if appropriate therapy was delayed or inappropriate therapy was administered. In our series, direct microscopic examination of smears of corneal scrapings alone gave a clue to the diagnosis of fungal keratitis in all except 4.6% of the eyes in which a definitive diagnosis of fungal keratitis was possible only based on cultures (fungi were not detected in smears).

A laboratory investigation carried out at this center\textsuperscript{9} that compared different staining techniques demonstrated that KOH preparation compared well with the other techniques in the diagnosis of fungal keratitis. The same study recommended that all smear-positive cases be treated with antifungal agents despite negative culture results. In the current review, among all staining techniques used, a simple KOH preparation was comparable with the CFW stain. Considering the cost effectiveness, easy availability of reagents, ease of preparation of the reagent, and sensitivity of the method, the KOH preparation continues to be an ideal technique for revealing fungal elements in smears of corneal scrapings.

Yeasts appeared to play a minor role in the cause of mycotic keratitis because these organisms were responsible for keratitis in only nine of 1,354 eyes. While filamentous fungi are more frequently encountered than yeasts in the tropics, the latter has been isolated with greater frequency in the west where traumatic keratomycosis is uncommon.\textsuperscript{7,16} Our series and reports from south India,\textsuperscript{3,12} Florida,\textsuperscript{7,12} and Ghana\textsuperscript{23} have identified species of \textit{Fusarium} and \textit{Aspergillus} as common corneal pathogens. \textit{Curvularia} species were the foremost among the pigmented spectrum, which corroborates earlier reports.\textsuperscript{7,24} Of the 1,360 fungal isolates recovered, 316 (23.2%) were not identifiable owing to lack of sporulation. Similar incidences of unidentified fungal species have been reported.\textsuperscript{5,7}

Fungal keratitis is more frequently reported in India in winter with a humid climate favoring fungal growth.\textsuperscript{14,19,25} The data from our center also point toward a relatively higher occurrence of fungal keratitis during the winter (October to January) and monsoon (June to September) seasons compared with summer (February to May). In India, the period between October and January is cool and dry and represents the harvest months. The increased incidence of fungal keratitis over a major part of the year in India may be attributable to the enormous amount of fungal spores prevalent in the environment.\textsuperscript{26} Nigeria experiences a climate similar to that of India and has reported a greater number of cases of fungal keratitis during winter and the hot, humid season.\textsuperscript{27}

In this report, we have attempted to highlight the fact that a high index of suspicion is the key to early diagnosis of fungal keratitis and that a simple investigative technique contributes significantly to the detection of a fungal etiology in corneal scrapings. An understanding of the risk factors, identification of the typical presenting features, and a KOH wet mount preparation can confirm the diagnosis of fungal keratitis. This report on the epidemiological and etiological data of a large series of patients with fungal keratitis provides significant insights into the understanding of this potentially devastating corneal disease.

REFERENCES