A study of Mycotic keratitis in a tertiary care hospital in North-East Karnataka

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Abstract
Introduction: Fungal keratitis is a major blinding eye disease in Asia, has been known since 1879, when Leber reported first case. The acuteness of problem in Northeast Karnataka area is due to hot and dry environment with agriculture as major occupation.

Objectives: To study the prevalence of fungi causing keratitis and to identify the causative agents.

Material & Methods: A total of 130 non-repetitive corneal scrapings collected during a period of 1 year were included in the study. All the corneal scrapings samples were subjected to Gram’s staining to see for the presence of bacteria, yeasts and other fungi, examined in 10% KOH mount. The scrapings were also inoculated on SDA slants with antibiotics and with or without cycloheximide and incubated at room temperature & at 37° C, respectively. Slants were observed for fungal growth daily for upto 3 weeks. The identification of the fungal growth was done on the basis of cultural characteristics and microscopic morphology on LPCB staining.

Results: Out of 130 samples, 50 samples were found positive for fungal culture. Among 50, 46 were positive for fungi in KOH mount microscopy and 4 were negative. Of the 50 fungal cultures, 26 were Aspergillus spp, 10-Fusarium, 8-Penicillium & 6-Candida. Among the samples positive for fungal growth, 74% were from patients of 21-40 yrs of age group. Males are affected more common than females. The major predisposing factors here are trauma (76%), cataract surgery (8%), diabetes mellitus (6%) and use of contact lens (4%).

Conclusion: Mycotic keratitis should be suspected in patients with a corneal lesion and must be ruled out before commencing steroids or antibiotics in order to save the sight. The findings of the study suggests that 21-40 yrs age group are more prone for mycotic keratitis and it is more commonly seen in males in this region. History of trauma is the major predisposing factor observed. Fungal Culture is more sensitive than KOH mount method for diagnosis. In present study Aspergillus spp is the predominant organisms found to infect injured cornea while Candida is the least.

Keywords: Mycotic, Keratitis, corneal scrapings, KOH mount.

Introduction
Fungal keratitis is a major blinding eye disease in Asia; the disease has been known since 1879, when Leber reported first case of fungal keratitis.¹

According to WHO, corneal diseases are a major cause of vision loss and blindness secondary only to cataract in overall importance.²

One study from South India reported that 44% of all central corneal ulcers are caused by fungi.³

More than 70 species of fungi are reported as pathogenic to human cornea.

Filamentous fungi are associated with infections following trauma with vegetable-contaminated matter, whereas yeasts infections typically occurs in eyes with preexisting ocular surface diseases.

The most frequently isolated pathogen varies with the geographical area studied.⁴⁻⁵

Across the world single most commonly reported fungus isolated from mycotic keratitis is Aspergillus spp.⁶

The symptoms of fungal keratitis may not present as acutely as with other forms of microbial keratitis but the signs of fungal keratitis are commonly seen as in other forms of microbial keratitis and include-suppuration, conjunctival irritation, epithelial defect, stromal infiltration and anterior chamber hypopyon.

The disease should be suspected in bacterial corneal ulcers, indolent and refractory to antibiotic therapy and with history of ocular trauma with vegetable contaminated matter.

The other important predisposing factors are foreign bodies, cataract surgeries, diabetes mellitus, prolonged use of steroids and use of contact lenses.

The acuteness of problem in India is due to dry & hot environment with agricultural economy, poverty, illiteracy & ignorance.⁶⁻⁷⁻⁸

As keratomycosis is more likely to produce perforation in comparison to bacterial keratitis it should be recognised by clinical features & some laboratory examinations.⁹⁻¹⁰

The aim of the study is to determine the prevalence & causative factors of mycotic keratitis.

Material & Methods
The study was conducted at Basaveshwar Teaching & General hospital attached to MR Medical College, Kalaburagi, for duration of 1 year (Jan 2014- Jan 2015).

Total of 130 non-repetitive corneal samples received at the laboratory were accessed for the fungal etiology.

The samples were subjected to KOH mount examination and Gram’s staining within an hour of collection for presumptive diagnosis as per the standard protocols.¹¹⁻¹³

Samples were also inoculated on 4 SDA slants with antibiotics and with or without cycloheximide in
duplicate and incubated at room temperature (25°C-30°C) and 37°C respectively.

The slants were observed daily for a period of up to 3 weeks for fungal growth.

Identification was done by cultural characteristics of colonies and microscopical morphology on LPCB staining. In mixed growth immediate subcultures and isolation was done at an early stage.

_Candida spp._ if isolated were identified in addition by standard tests like germ tube test and sugar fermentation tests.

**Results**

Out of 130 samples examined, 50 samples were found positive for fungal growth.

There was no case of polymycotic infection.

Among 50, 46 (92%) were KOH positive for fungal elements and 4 were negative.

Of 50 samples 74% patients were in the age group of 21-40 yrs and males were more commonly affected than females in ratio 3.5:1 (Table-1).

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>17</td>
<td>4</td>
<td>21</td>
<td>42</td>
</tr>
<tr>
<td>31-40</td>
<td>14</td>
<td>2</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>41-50</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>51-60</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>&gt;60</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>39 (78%)</td>
<td>11 (22%)</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

The most common fungal isolates causing mycotic keratitis belong to _Aspergillus_ 26 (52%) followed by _Fusarium_ 10 (20%), _Penicillium_ 8 (16%) and _Candida spp_ 6 (12%) (Table 2).

**Table 2: Showing number of fungal isolates**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number(50)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td><em>Candida</em></td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

The most common predisposing factor among the patient under the present study was found to be external trauma and was present in 38 (76%) of cases. The other predisposing factors were cataract surgery 4 (8%), Diabetes mellitus 3 (6%), use of contact lens 2 (4%) and others 3 (6%) included chronic use of antibiotics, steroids and immunocompromised state each one.

**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.

In various published reports, mycotic keratitis has been found to account for 6-56% of all cases of ulcerative keratitis.\(^{[12]}\)

A hot, dry climate and agriculture based occupation of a large population make fungal keratitis more frequent in tropical countries.

The predominance of fungal corneal infections in the youngest age group (21-40 yrs) and among males could be attributed to their greater involvement in outdoor activities and active farming practices, thus more prone for corneal injury with external agents.

A study done by Rosa et al has made similar observations.\(^{[13]}\)

Trauma was the major predisposing factor in 76% cases, which is not very different from that reported for fungal keratitis in general.\(^{[13]}\)

Several reports on fungal keratitis describe an antecedent injury of the cornea caused by leaves, paddy grains, cow tail, tree branch and metal pieces.\(^{[14]}\)

Long term use of antibiotics and corticosteroids is believed to compromise the cornea and worsen the disease condition in fungal keratitis.\(^{[15]}\)

Therefore the value of microbiologic investigations in establishing a definitive cause of fungal keratitis cannot be underestimated.

In our study out of 50 samples, 46 samples showed KOH positive & only 4 were KOH negative.

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.

The value of 10% KOH wet mount preparation in the diagnosis of fungal keratitis lies in its ability to clear the scrapings of cellular debris, there by rendering hyphal fragments more refractile on microscopic examination.

A study from south India reported equivalent sensitivities when 10% KOH smear (90%) compared with calcofluor white (91%) stain.\(^{[16]}\)

As all the fungi isolated from corneal ulcers are prevalent as saprophytes in the atmosphere even a small abrasion can initiate the ulcer.

In our study, incidence of _Aspergillus spp_ was greatest i.e. 52% which corroborates with the findings of various workers\(^{[17,18]}\) and _Candida spp_ is least frequent causative agent.

A study from Gopinath et al. (2002) from India have reported _Candida_ as a rare fungal corneal pathogen.\(^{[19]}\)

Fungal keratitis from yeast infections commonly occurs in host corneas with compromised immune response.
defense. This may result from the use of topical steroids, antibiotics, antivirals, epithelial defect, contact lens wear, previous corneal surgery or atopic diseases.\(^{(20)}\)

In our study *Aspergillus spp* is common isolate. Our results and reports from South India, Florida and Ghana\(^{(21)}\) have identified various species of *Fusarium* and *Aspergillus* as common corneal pathogens. Many other several studies have got same results as ours.\(^{(22,23)}\)

**Conclusion**

Mycotic keratitis should be suspected in patients with a corneal lesion and should be ruled out before commencing steroids or antibiotics in order to save the sight.

KOH mount microscopy method is more reliable in early detection and fungal culture is most sensitive method for diagnosis.

The most common age group prone for mycotic keratitis in the region under study is between 21-40 yrs and commonly seen in males.

Trauma is the most important predisposing factor. *Aspergillus spp* is the predominant organism followed by *Fusarium, Penicillium* and *Candida*.

**References**