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Research Paper

Study of Fungal Infections in Clinically Suspected Cases of Keratitis

Medical Science

KEYWORDS : Fusarium sp., Aspergillus sp., Fungal keratitis.

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ABSTRACT

Objective: This study was conducted to identify the aetiological fungal agents and to determine the epidemiological characteristics of fungal keratitis.

Methods: 300 patients with suspected fungal keratitis were studied from July 2011-March 2012.The corneal specimens were collected and processed.The patients with 10% KOH mount and culture positive fungal keratitis were analyzed.

Results: In 54 samples, fungus were isolated. Out of that, 53(98.14%) were filamentous fungi while 1(1.85 %) was yeast. The hyaline fungi like Fusarium species 28(51.85%),Aspergillus flavus 7(12.96%), Aspergillus fumigates 1(1.85%), Acremonium sp.1(1.85%) and other less common were Paecilomycetes sp., Rhizopus sp., Absidia sp. The dematiaceous fungi like Curvularia sp. 3(5.55%), Bipolaris sp. 2(3.70%) and other less common were Fonsecaea pedrosoi,Aureobasidium pullulans.

Conclusion: Our study shows that the Fusarium sp. were the most common followed by Aspergillus sp. Laboratory diagnosis is essential for treatment to avoid complication.

Introduction:

Ophthalmic mycoses are being increasingly recognized as an important cause of ocular morbidity as well as blindness and keratomycosis is the most frequent presentation. [1] The incidence of mycotic keratitis is more than 50% of all the culture-proven cases of keratitis. [1] Corneal trauma has been listed as the most common risk factor for mycotic keratitis in most of the studies. Other predisposing factors could be prolonged use of topical corticosteroids or antimicrobial agents, systemic diseases such as diabetes mellitus, pre-existing ocular diseases and use of contact lenses. [1] The etiological and epidemiological pattern of corneal ulceration varies significantly with patient, health of cornea, geographical region and also tends to vary over a period of time. [2]

Early diagnosis of fungal keratitis and its treatment is important in preventing complications. We conducted this study to identify the aetiological fungal agent, laboratory findings and treatment outcomes.

Material and methods:

A total of 300 patients with suspected fungal corneal ulcers were investigated for fungal etiology in the Department of Microbiology, Smt N.H.L.M.M.C.,Ahmedabad. The Patients with evidence of keratitis due to bacteria, virus and other aetiology were excluded from this study.

In all these patients, the corneal scrapings were processed. Direct microscopic examination was performed with 10% KOH mount and Gram's stain for fungus. The samples were inoculated directly on duplicate set of Sabouraud's dextrose agar (SDA) and were examined daily for 4 weeks. These media were checked for fungal growth in the form of yeast or mycelia, daily during the first week and twice a week for the subsequent 3 weeks. Any growth obtained was further identified by standard laboratory techniques. [3] However, the media were labeled as sterile if no growth was observed even at the end of 4 weeks and were discarded. The cultures were considered positive if at least one of the following criteria was fulfilled: [4]

1. The growth of the same organism was demonstrated on one or more solid media and/or if there was confluent growth at the site of inoculation on at least one solid medium.
2. The growth on one medium was consistent with direct microscopic findings.
3. The same organism was grown from repeated corneal scrapings.

Results:

Out of the total 300 patients, 67(22.33%) specimens were direct KOH positive and fungus were isolated from 54 samples.13 samples were direct KOH positive but fungus could not be isolated. Both hyaline and dematiaceous fungi were isolated. Among total fungal isolates 45(83.33%) were hyaline fungi, 8(14.81%) were dematiaceous fungi and 1(1.85%) was yeast-like.

Out of 300 patients, 62(20.66%) were in the age group 41-50 years followed by 55(18.33%) in age-group 51-60 years. There were 200(66.66%) males in the study. Most common predisposing factor observed in the study for keratitis was trauma which was seen in 24.33% (73/300) of patients.

Among the reasons for corneal ulcers, trauma due to unknown foreign body 70.37% (38 patients) followed by wooden objects 22.22%(12 patients) was the leading cause followed by tree branch injury in 12.96% (07 patients) and soil particle 9.25% (05 patients).

Table 1:Distribution of fungal isolates.

Fungal isolates	No. (%)
Hyaline filamentous fungi	
Fusarium sp.	28(51.85)
Aspergillus flavus	07(12.96)
Aspergillus fumigates	01(1.85)
Acremonium sp.	01(1.85)
Paecilomycetes sp.	01(1.85)
Scopularopsis sp.	01(1.85)
Rhizomucour sp.	02(3.70)
Rhizopus spp.	03(5.55)
Absidia sp.	01(1.85)
Dematiaceous fungi	
Curvularia sp.	03(5.55)
Bipolaris sp.	02(3.70)
Fonsecaea pedrosoi	02(3.70)
Aureobasidium pullulans	01(1.85)
Yeast like	
Candida sp.	01(1.85)

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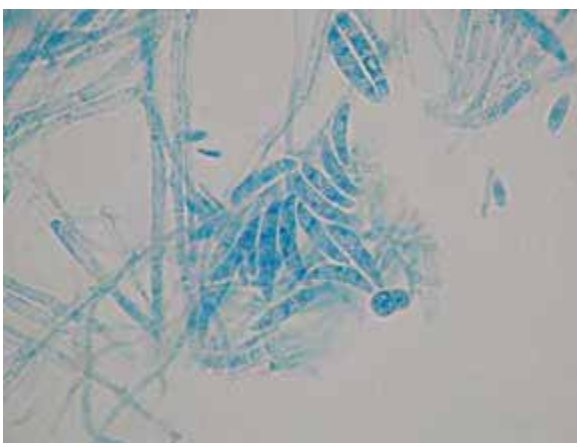
Macroscopic and Microscopic Morphology of Fungal Growth:

Fusarium species :

Macroscopic morphology : After 5-6 days,colonies are white to cream,woolly tocottony. A variety of colours may be produced,from gray on white surface to yellow on brown, buff pink on violet.

Microscopic morphology: The thin, septate hyaline hyphae are seen. Conidiophores are absent.

Macroconidia typically fusiform which form sporodochia, mostly 3 septate. Microconidia abundant, produced on elongated conidiophores. Chlamydospores frequent, smooth, terminal or intercalary.



LCB mount
Figure .1 Fusarium species

Aspergillus flavus:

Macroscopic morphology : After 3-6 days, the colony is mat like, flat with yellow to yellow-green surface pigment. The reverse is colourless to light yellow.

Microscopic morphology: The hyaline,septate hyphae with thick walled,hyaline conidoaphores.

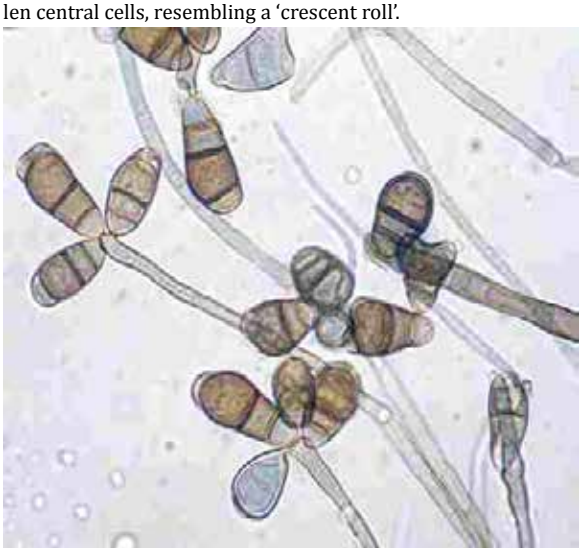
The vesicles are large and globose with biseriate phialides. Foot cells are present.

LCB mount
Figure. 2 *Aspergillus flavus*

Curvularia species:

Macroscopic morphology : After 5-6 days, colonies are velvety with dark brown to black pigment. The reverse of the colony is dark brown to black.

Microscopic morphology : Thin walled,septate, dematiaceous hyphae with darkly pigmented conidiophore which is geniculated and multicellular poroconidia with transverse septa,produced in whorls. Large dark conidia with darker swollen central cells, resembling a 'crescent roll'.



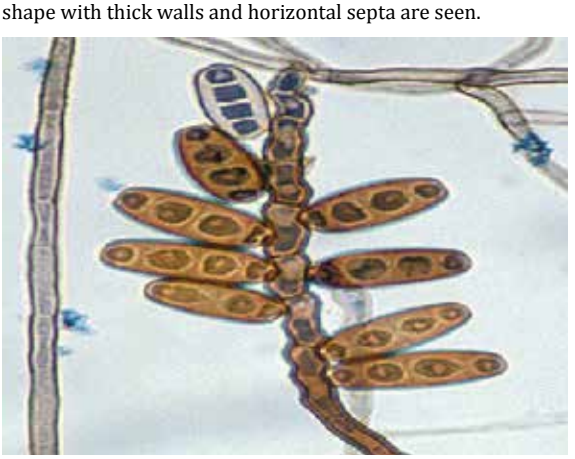
LCB mount
Figure. 3 Curvularia species

Bipolaris species:

Macroscopic morphology : After 4-5 days, colonies are dark coloured and cottony with a gray to brownish black surface and a black reverse.

Microscopic morphology : The dark brown , septate hyphae with darkly pigmented ,septate geniculate conidiophores resembling a 'bent knee' where poroconidia are produced.

Sympodially produced multicellular 3-5 cells conidia, pea pods shape with thick walls and horizontal septa are seen.



LCB mount
Figure.4 Bipolaris species

Discussion:

Though *Fusarium spp.* is a saprophyte but its traumatic implantation may results in keratomycosis. If untreated, it can result in blindness.The most frequently implicated fungi in mycotic keratitis appear to vary depending on the geographical location. In the present study, *Fusarium* species have been reported as the most frequent cause. Although in several parts of the world *Aspergillus fumigatus* is the commonest infective agent, *Fusarium* species and *candida albicans* have also been reported as the predominant agents. [5-8] Dematiaceous fungi have been reported as the third most common cause of keratitis in a number of other studies. [5],[9]

In the present study, 13 samples remained sterile on culture despite positive direct KOH microscopic findings. These were considered as positive because the direct microscopic findings corroborated with the clinical findings of the patients.

In the present study, hyaline fungi like *Fusarium species*(51.85%) have been reported as the most frequent cause followed by *Aspergillus flavus* 7(12.96%)followed by *Aspergillus fumigates*

1(1.85%), *Acremonium spp.*1(1.85%), *Paecilomycetes spp.*1(1.85*%), Rhizopus spp.*3(5.55%), *Rhizomucour* spp.2(3.70%) and *Absidia spp*.1(1.85%)and dematiaceous fungi like *Curvularia spp*.3(5.55%) is more common followed by *Bipolaris spp.* 2(3.70%) and *Fonsecaea pedrosoi* 2(3.70%) and *Aureobasidium pullulans* 1(1.85%) were isolated.

Conclusion:

Mycotic keratitis continues to be an important cause of ocular morbidity, mostly in the persons inhabiting rural areas, involved in outdoor, agricultural activity.

Therefore, we conclude that early institution of antifungal therapy following meticulous examination of corneal scrapings by direct microscopy may limit ocular morbidity and disastrous sequelae among these patients. So early laboratory diagnosis is essential to avoid complication.

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