

FUNGUS AS AN ETIOLOGY IN KERATITIS- OUR EXPERIENCE IN SRMC

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ABSTRACT

PURPOSE: To evaluate the role of fungus in the etiology in patients with corneal ulcers attending Ophthalmology OPD in SRMC, a tertiary care centre in Chennai.

METHODS: All patients with keratitis who presented from July 2006 to 31st May 2008 were evaluated. They were examined by slit-lamp biomicroscopy and corneal scrapings which were taken for cultures and smears by using standard protocols.

RESULTS: During this period, 300 patients with corneal ulcerations were registered and 45(15%) diagnosed as microbial keratitis were evaluated. Out of 45 corneal ulcers cultured, 10(22%) were found to be bacteria, 20(44%) were found to be fungi, 3(6.7%) were found to be mixed with bacteria and fungi, and the remaining 12(26.7%)

were found to be culture negative. Both gram positive cocci and gram positive bacilli were seen, with one case of gram negative bacilli (*Pseudomonas* spp). The predominant fungal pathogens isolated were *Aspergillus fumigatus* 8 (40%) followed by *Fusarium solani* 7 (35%). cases of *Curvalaria* spp(2), and one each of *Hormonema dermatioidis*, *Alternaria alternata* and *Scytallidium infestans*.

CONCLUSION: Fungal infections occurred with an increased frequency when compared to the bacterial infections, the predominant fungal pathogens being *Aspergillus fumigatus* followed by *Fusarium solani*. The findings of our study show that there is a region wise variation in the predominance of corneal pathogens. This has an important public health implication for the initiation of therapy.

Key words : Fungal, keratitis, microbial

INTRODUCTION :

Microbial keratitis is the most common serious ocular infection and may be caused by a variety of bacteria, fungi, viruses or parasites.

Corneal infection is a leading cause of ocular morbidity and blindness worldwide. Corneal ulceration is a major cause of monocular blindness in developing countries (1,2). A recent report on the causes of blindness world wide consistently lists corneal scarring second only to cataract as the major etiology of blindness and visual disability in many of the developing nations like Asia, Africa and the Middle East (3).

Almost any microorganism can invade the corneal stroma, if the normal corneal defense mechanisms, i.e. lids, tear film and corneal epithelium are compromised (4). A wide spectrum of microbial organisms can produce corneal infections and consequently the therapeutic strategies may be variable (5). One of the key elements in this effort is a proper understanding of the microbiological and clinical characteristics of this disease entity which will enable the ophthalmologist to initiate appropriate antimicrobial therapy (5).

Considering the importance of corneal ulceration as a world wide cause of visual loss, there are surprisingly few

studies evaluating the aetiological factors predisposing a population to corneal infection (6,7,8). The majority of bacteria cultured from infections of the cornea are of the same species that normally are present in the conjunctival sac, on the lids or periocular skin, and in the adjacent nasal passages. Their incidence may vary geographically (9,10,11). The purpose of this study was to evaluate the current microbial pathogens of all infectious corneal ulcers seen at a tertiary referral centre in south India, during a period of 23 months and compare these profiles with other series.

MATERIALS AND METHODS:

Clinical specimen and method of collection:

Corneal scrapings taken from patients suffering from keratitis (fig. 1) were subjected to microbial culture when at least one of the following was present:

- Size: infiltrate with >2mm epithelial defect
- Location: infiltrate > 2 mm from the limbus
- Depth: infiltrate > 20% of the corneal thickness
- Associated findings: anterior chamber reaction > grade 2
- Organic trauma
- Atypical ulcerations: younger individuals and children
- Failure to regress with in 24 hrs

After a detailed ocular examination corneal scrapings were collected under aseptic conditions from each ulcer by an ophthalmologist after instillation of 4% paracaine eye drop without preservative, using a sterile Bard Parker blade (No 15). The procedure was performed under magnification of slit-lamp or operating microscope. The scraping material obtained from leading edge and base of each ulcer was initially directly inoculated onto the surface of solid media such as sheep's blood agar, chocolate agar and Sabouraud's

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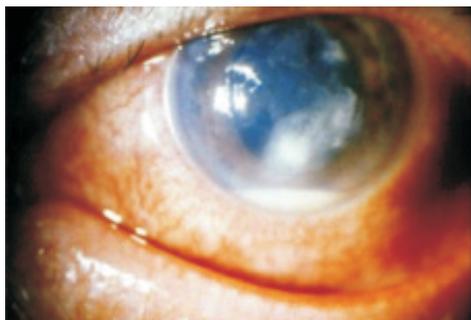


Fig. 1 : Showing the ulcer on the cornea



Fig. 2 : KOH mount showing narrow septate hyphae of the corneal scraping. (magnification 20 X).

dextrose agar in a row of C- shaped streaks (6,12). The material obtained by the next scraping was spread onto labeled slides in a thin, even manner for 10 % potassium hydroxide (KOH) wet mount (Fig 2) and Gram staining. Meticulous care was taken in the collection of material and transferring it to the appropriate culture media aseptically.

LABORATORY PROCEDURES :

All inoculated media were incubated aerobically. The inoculated media - blood agar, chocolate agar were incubated at 37°C and were evaluated at 24 hours and at 48 hours and later discarded if there was no growth. The inoculated fungal media-Sabouraud’s dextrose agar was incubated at 25°C, examined daily, and discarded at 3 weeks if no growth was seen. Microbial cultures were considered positive only if at least one of the following criteria were met (5).



Fig. 3 : showing the C shape inoculation and the growth along the inoculum.

- a. The growth of the same organism was demonstrated on two or more solid media on the C-streak; or there was semiconfluent growth at the site of inoculation on one solid medium, (Fig 3)
- b. The same organism was grown from repeated scraping,
- c. It was consistent with clinical signs,
- d. Smear results were consistent with cultures.

Cultures for *Staphylococcus epidermidis* and *Corynebacterium spp.* were considered positive only if there was moderate growth on at least two solid media. The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory criteria (12).

The etiological agent was identified using KOH mount and gram’s stain and preliminary report was given to the clinician based on which, the treatment was started with the appropriate antifungal agent. The diagnosis was then confirmed with culture on appropriate culture media.

FUNGAL CULTURE IDENTIFICATION :

Macroscopic appearance

The major macroscopic features remarkable in species identification are the growth rate, color of the colony, and thermo tolerance.

Microscopic appearance

The basic microscopic morphology is different for different species. Microscopic structures are unique to certain species and constitute the key features for species identification together with the surface color of the colony.

Results

From July 2006 to 31st May 2008, 300 cornea cases were registered out of which 45 cases were diagnosed as microbial keratitis and evaluated.

Out of 45 corneal ulcers cultured, 10(22%) were found to be bacteria, 20(44%) were found to be fungi, 3(6.7%) were found to be mixed with bacteria and fungi, the remaining 12(26.7%) showed no growth (Fig 4).

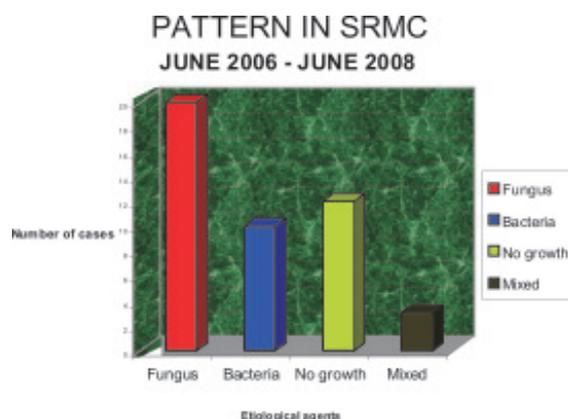


Fig. 4 : Showing the pattern of organisms isolated

Both Gram positive cocci and Gram positive bacilli were seen, with one case of Gram negative bacilli (*Pseudomonas* spp.).

In fungal corneal ulcers most common organism noted was *Aspergillus fumigatus* (8) followed by *Fusarium solani* (7). 2 cases were identified as *Curvalaria* spp, and 1 each of *Homonema dermatioidis*, *Alternaria alternata* and *Scytallidium infestans*.

DISCUSSION :

Fungi gain access into the corneal stroma through a defect in the epithelium, then multiply and cause tissue necrosis and an inflammatory reaction. The epithelial defect usually results from trauma (eg, contact lens wear, foreign material, prior corneal surgery). The organisms can penetrate an intact descemet membrane and gain access into the anterior chamber or the posterior segment. Mycotoxins and proteolytic enzymes augment the tissue damage.(5)

Corneal trauma is the most frequent and major risk factor for fungal keratitis. In fact, the physician should have a high level of suspicion in a patient with a history of corneal trauma, particularly with plant or soil matter.

Fungal keratitis is a major blinding eye disease in Asia. One report from South India found that 44% of all central corneal ulcers are caused by fungi (13). This high prevalence of fungal pathogens in South India is significantly greater than that found in similar studies in Nepal(17%), Bangladesh (36%), Ghana (37.6%), and south Florida (35%) (14,15,16,17,18).

In China, the incidence of fungal keratitis has increased during the past decade (19).

In temperate climates, such as Britain and the northern United States, the incidence of fungal keratitis remains very low (18,20,21).

There is a geographical variation in the incidence of fungal keratitis. In our institution the incidence of fungal keratitis was found to be 44%. This is similar to the studies from other parts of South India(22, 23) whereas reported incidence in states of northern India are less (7.3%-32%) (24,25).

A study from Goa which is again in the south western part of India had reported a prevalence of 38.9% (26). This regional variation could be because the climate in the southern part of India is hot and humid for most part of the year.

Aspergillus spp was the predominant etiological agent causing keratitis in our study, similar to the reports from other parts of India (27,28), Other rare isolates reported were *Curvalaria* spp, *Homonema dermatioidis*, *Alternaria alternata* and *Scytallidium infestans*. The dematiaceous fungi are reported as causes of keratitis in many tropical and subtropical regions(29).

Of the 45 patients who had microbial keratitis, only 28 had good compliance and were reviewed regularly. Conservative management was successful for 25 patients who got a visual acuity in the range of > 6/60 to 6/9 after best correction. Surgical management for 3 patients who underwent keratoplasty, after the refraction got a visual acuity of CF 3mtr to 6/36.

CONCLUSION:

Globally, the incidence of keratomycoses is rising. Fungal infections is occurring with an increased frequency when compared to the bacterial infections, the predominant fungal pathogens being *Aspergillus fumigatus* and *Fusarium solani* respectively. The findings of our study show that there is a region wise variation in the prevalence of the corneal pathogens.

Ongoing research towards rapid diagnosis and specific drug therapy could minimize the morbidity caused by this preventable disease. Various antifungal drugs including itraconazole, fluconazole and voriconazole, have been applied recently for the treatment of keratomycosis.

Our study emphasizes the fact that early diagnosis and intervention can significantly decrease permanent corneal scarring and vision loss in patients with infective keratitis.

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