ORIGINAL ARTICLE

Microbial profile of suppurative keratitis a prospective study

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ABSTRACT

This is a prospective study on the microbial profile of suppurative keratitis conducted at Regional Institute of Ophthalmology, Trivandrum for a period of 2 years from March 2006 to February 2008. The objective was to identify the specific causative organisms from patients with suppurative keratitis in a tertiary referral eye care center in South Kerala. All consecutive cases of clinically diagnosed corneal ulcers were evaluated. Corneal scrapings were performed and processed for direct microscopy and culture on appropriate media. A total of 1221 cases of corneal ulcers were evaluated. Causative organism was isolated in 343 cases (28.09%). Among them, 240 cases (69.9%) were fungi and 97 cases (28.27%) were bacteria. *Acanthamoeba* was isolated in six cases (1.74%). Among fungi *Fusarium* species and *Aspergillus* species were predominant (31.66%) followed by *Penicillium* species (24.58%). Among bacteria, Gram-positive cocci (26.80%) were predominant followed by *Pseudomonas* (24.74%).

Key words: *Acanthamoeba*, *Aspergillus*, *Fusarium*, keratitis

AIM OF STUDY

Corneal infection is a leading cause of ocular morbidity and monocular blindness worldwide, more so in developing countries like ours. A wide spectrum of microbial organisms can produce infective keratitis.[1,2] Proper understanding of the pathogen helps to give appropriate antimicrobial therapy early, thus reducing ocular morbidity.

The purpose of this study was to identify the microbial profile of suppurative keratitis.

MATERIALS AND METHODS

This study was conducted in a teaching hospital in South India over a period of 3 years. This study was also approved by Institutional Ethics Committee. All consecutive cases of clinically diagnosed suppurative keratitis who attended Regional Institute of Ophthalmology, Trivandrum were included in this study. Suppurative keratitis was defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated signs of inflammation with or without hypopyon. All culture positive cases where a pure isolate was obtained, was analyzed. All cases with polymicrobial isolates were excluded from the analysis. Suspected viral keratitis, interstitial keratitis, and autoimmune keratitis were also excluded. After a detailed ocular examination and slit lamp biomicroscopy, corneal scrapings were collected from each ulcer after instillation of local anesthetic drops by using no: 15 Bard parker blade under aseptic conditions. The procedure was performed under a slit lamp or operating microscope. The scraping material obtained from the leading edge and base of ulcer, was inoculated onto the surface of solid media such as blood agar, chocolate agar, MacConkey agar and Sabourauds dextrose agar (SDA). The material obtained from next scraping was then spread onto two glass slides in a thin even manner for 10% KOH mount and Gram-staining. Whenever *Acanthamoeba* cysts were seen on wet film, scrapings were inoculated into nonnutrient agar. Modified acid-fast bacilli staining was performed in suspected actinomycetes infections. If microscopy showed plenty of pus cells and no organism grown in routine culture for next 48 h, then glucose broth sub culture was done to wane off the effect of antibiotics.

Laboratory procedures

All laboratory methods followed standard accepted protocol. Inoculated media for bacterial isolation were incubated aerobically at 37°C and were evaluated at 24 h, 48 h and later discarded after 72 h if there was no growth.

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Sabourauds media were incubated at 27°C and examined daily and discarded if no growth was seen after 21 days. Inoculated nonnutrient agar plates were incubated at 37°C after overlaying with *Escherichia coli* broth culture. These were examined daily for the presence of *Acanthamoeba* species and discarded after 7 days if there are no signs of growth.

Microbial culture positivity on solid media was correlated with the direct microscopy result. Laboratory findings were always correlated with clinical findings.

Specific identification of bacterial pathogen was based on microscopic morphology, staining characteristics and biochemical properties using standard laboratory criteria. Fungi were identified by their colony characteristics on SDA and by their morphological appearances in wet mount with lacto phenol cotton blue stain and slide culture method.[3]

RESULTS

From March 2006 to February 2008, there were 1221 cases of clinically diagnosed corneal ulcers. Of 1221 cases evaluated, isolation of microorganism was done in 343 cases (28.09%).[4,5]

Among culture positive cases fungi were predominant, in 240 cases (69.9%). Ninety-seven cases were found to be bacteria (28.27%). *Acanthamoeba* were isolated in 6 cases (1.74%) [Table 1].

Of the 240 fungi isolated, *Fusarium* species and *Aspergillus* species were equal in numbers 76 each (31.66%). This was followed by *Penicillium* species, 59 cases (24.58%). In decreasing order of frequency following fungi were also isolated-*Aureobasidium pullulans* (5), *Dreschlera* species (4), *Sporothrix schenckeii* (3), *Curvularia* spp. (3), *Nigrospora* species (2), *Acremonium* spp. (2), *Epicoccum* spp. (1) and *Candida* and other yeasts (9)[6] [Table 2].

Among 97 bacteria isolated, Gram-positive organisms were predominant (52.5%) followed by Gram-negative (47.42%). Most commonly isolated organism was *Streptococcus pneumoniae* 26 cases (26.8%). This was followed by *Pseudomonas aeruginosa* in 24 cases (24.74%), *Staphylococcus aureus* in 13 cases (13.4%), *E. coli* in 12 cases (12.37%), *Klebsiella* in 10 (10.31%) cases (8.24%), and coagulase negative staphylococci in 8 cases (8.24%). *Moraxella* and *Acinetobacter* spp. were infrequently found (two each) [Table 3].

In this study, 343 of 1221 corneal scrapings (28.09%) were culture positive for a single isolate. Poly microbial isolates were not included in this analysis (16.07%).

Table 1: Comparison of various isolates

Isolates	Number (%)
Fungi	240 (69.9)
Bacteria	97 (28.27)
Parasites (*Acanthamoeba*)	6 (1.74)
Total	343

Table 2: Comparison of various fungal isolates

Isolates	Number (%)
Fusarium spp.	76 (31.66)
Aspergillus spp.	76 (31.66)
Penicillium spp.	59 (24.58)
Aureobasidium pullulans	5 (2.08)
Dreschlera spp.	4 (1.66)
Sporothrix schenckeii	3 (1.25)
Curvularia spp.	3 (1.25)
Nigrospora spp.	2 (0.83)
Acremonium spp.	2 (0.83)
Epicoccum spp.	1 (0.41)
Candida and other yeasts	9 (3.75)
Total	240

Table 3: Comparison of various bacterial isolates

Isolates	Number (%)
Streptococcus pneumonia	26 (26.8)
Pseudomaonas aeruginosa	24 (24.74)
Staphylococcus aureus	13 (13.4)
Escherichia coli	12 (12.37)
Klebsiella spp.	10 (10.31)
Coagulase negative staphylococci	8 (8.24)
Moraxella spp.	2 (1.03)
Acinetobacter spp.	2 (1.03)
Total	97

Among the 1221cases studied 903 (73.95%) had sought previous medical help. They were on treatment with various topical medications from local ophthalmologists, general physicians or from pharmacists. Most of these cases were culture negative even though organisms were seen on microscopy.

DISCUSSION

Microbial keratitis is a sight threatening condition with ocular morbidity that requires prompt and appropriate management. To minimize complications and permanent sequelae, timely antimicrobial treatment must be initiated on the basis of "clinical and microbiological evaluation."

Microbial culture and direct microscopic detection always supplement the clinical diagnosis and provide

supportive evidence for planning appropriate therapy. Suppurative keratitis is frequently caused by bacteria and fungi. The causative agents for suppurative corneal ulcer vary significantly from country to country and from region to region within the same country. Knowledge of the local etiological agent within a given region is of great value for the diagnosis and treatment of corneal ulcer.[7,8]

The reduction in corneal ulcers of bacterial etiology in general at a referral center can be attributed to more successful treatment of bacterial ulcers in the peripheral centers with topical antibiotics.

Acanthamoeba was the only parasite isolated in all reported series. Frequency of *Acanthamoeba* in our series was six cases (1.74%). Interestingly, none of these patients had a history of contact lens use.

CONCLUSIONS

- Fungal keratitis is much more frequent in our region compared with other parts of the country.
- *Fusarium* and *Aspergillus* are the common fungi responsible for corneal ulcer in this region followed by *Penicillium* species.
- <30% of corneal ulcers are bacterial in origin and pneumococcus is the commonest causative agent.
- *P. aeruginosa* is the most common Gram-negative organism causing corneal ulcers.
- *Acanthamoeba* is the only parasite isolated and *Acanthamoeba* keratitis is relatively more common in this region.

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