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Original Article

Bacterial and Fungal Profile of Infectious Keratitis: A Prospective Study

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Abstract

Background: Keratitis is the term applied for inflammations of the cornea. Microbial keratitis is a common, potentially vision-threatening ocular infection that may be caused by bacteria, fungi, viruses, or parasites. The purpose of the present study is to determine microbial etiology of suppurative keratitis and to identify the risk factors predisposing to corneal infections.

Materials and Methods: Fifty patients attending ophthalmology outpatient department and diagnosed with corneal ulcer needing microbiological investigations were included and subjected to microbiological intervention. All patients were subjected to slit-lamp biomicroscopic examination. After detailed ocular examination, corneal scrapings were collected under aseptic conditions. All laboratory methods followed standard protocols and microbial cultures were considered positive only if they fulfil the criteria.

Results: Corneal stains were found to be positive in 38 (76%) patients. Corneal cultures were found to be positive in 36 (72%) patients. 18 (36%) patients had bacterial growth, 18 (36%) had fungal growth, and the remaining 14 (28%) were found to be culture negative. That is, the bacterial and fungal infections occurred almost with equal frequency. The predominant bacterial pathogen isolated was *Streptococcus Pneumoniae* representing 16% followed by *Pseudomonas* 8%. The predominant fungal pathogens isolated were *Aspergillus* species (24%) followed by *Candida Albicans* (8%). The sensitivity of potassium hydroxide staining was almost 100% in culture-proven fungal cases. Trauma is the leading cause for the corneal ulcers, and most of the fungal ulcers are because of trauma due to vegetative matter.

Conclusion: Staining efficiently establishes the diagnosis, and therefore, can be used in the management of corneal ulcer to start the prompt treatment as corneal ulcer is a medical emergency. The microbiological profile helps the ophthalmologists to start the specific treatment directed against the causative organisms.

Key words: Bacteria, Fungus, Infectious keratitis

INTRODUCTION

Keratitis is the term applied for inflammations of the cornea. Corneal infections are known to be the second most significant cause of monocular blindness rated after unoperated cataract in some developing nations in particular and in the tropics in general. Microbial keratitis is a common, potentially vision-threatening ocular infection that may be caused by bacteria, fungi, viruses, or parasites.

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Emphasizing the importance of corneal ulceration as an important cause of visual loss, many studies have reported the prevalence of microbial pathogens and identified the risk factors predisposing a population to corneal infection in India and abroad.[1]

The etiological and epidemiological patterns of corneal ulceration have been found to vary with the patient population, health of the cornea, geographic location, and climate and also tend to vary over time. Hence, an understanding of the epidemiological features, risk factors, and etiological agents that occur in a specific region is important in rapid recognition, timely institution of therapy, optimal management, and prevention of this disease. To start specific therapy, it is necessary to do meticulous laboratory investigations, and this includes microscopy and culture of corneal scrapings for identification of the microbial agent.[2]

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More than one-third of eye injuries among children and adolescents result from their natural curiosity, immature motor skills, and tendency to imitate adult behavior without assessing the risks relevant to their actions. Although infectious keratitis is not common in this age group, it is potentially devastating as approximately considering that 30% of young victims of serious eye injuries end up with visual acuity lower than 20/200. Infectious keratitis is more prevalent in tropical developing countries with poor health-care systems than in developed countries.[3]

The diagnosis of keratitis is usually clinical, and the treatment is empirical, with the application of broad-spectrum topical antimicrobial agents until identification of the etiologic agent is completed. However, few studies have investigated the causative agents and risk factors of infectious keratitis in children and adolescents.[4,5]

There are several preceding studies establishing the microbial etiology of suppurative keratitis from various parts of the world.[6-9] However, little data are found in the technical literature from India in particular. Since microbial etiology varies geographically, the present study acquires significance on the backdrop of similar articles for other regions of India. The purpose of the present study is to determine microbial etiology of suppurative keratitis and to identify the risk factors predisposing to corneal infections.

MATERIALS AND METHODS

Fifty patients attending the ophthalmology outpatient department and diagnosed with corneal ulcer needing microbiological investigations with their consents were included and subjected to microbiological intervention.

Inclusion Criteria

All patients with presumed infection corneal ulcer attending ophthalmology department for 1 year were included in the study.

Exclusion Criteria

The following criteria were excluded from the study:

1. The patients who are not willing to give consent for investigations.
2. The patients with corneal ulcer involving peripheral cornea.

Method of Collection of Data

After a detailed history and thorough clinical examination, the patient was subjected to a protocol of investigations. Routine investigations such as hemogram and renal blood sugar were done. Briefly, all patients were subjected to slit-lamp biomicroscopic examination. After detailed ocular examination, corneal scrapings were collected under aseptic conditions from each ulcer after instillation of 4% lignocaine (lidocaine) without preservative using a sterile blade (no 15) or hypodermic needle. The procedure was performed under magnification of slit-lamp or operating microscope. The scraped tissue obtained from leading edge and base of each ulcer is directly surfaced on solid media such as Sheep blood agar, chocolate agar, and Sabouraud dextrose agar in a row of C-shaped streaks. The material obtained by the next scraping was spread onto labeled slides in a thin, even manner for 10 % potassium hydroxide (KOH) wet mount, gram staining, Giemsa staining. When KOH smears were positive for *Acanthamoeba* cysts, corneal scrapings were taken and the material was inoculated on non-nutrient agar. Meticulous care was taken in the collection of material and transferring it to the appropriate culture media aseptically.

Procedure

All inoculated media was incubated aerobically. The inoculated media - blood agar, chocolate agar, tryptone glucose beef extract agar, brain heart infusion agar, was incubated at 37°C and was evaluated at 24 h and 48 h and later discarded if there was no growth. The inoculated fungal media Sabouraud's dextrose agar was incubated at 27°C, examined daily, and discarded at 3 weeks if no growth was seen. The inoculated non-nutrient agar (*Escherichia coli* enriched) was examined for the presence of *Acanthamoeba* and discarded at 24 h if there was no growth. All laboratory methods followed standard protocols, and microbial cultures were considered positive only if one of the following criterions was fulfilled.

1. The growth of the same organism demonstrated on two or more solid media on the C- streak; or semi confluent growth at the site of inoculation on one solid medium.
2. Is consistent with clinical signs.
3. Smear results consistent with cultures.

RESULTS

A total of 50 patients with the clinical diagnosis of infectious keratitis were enrolled for this study. Epidemiological characteristics of the population are recorded. A maximum of patients were from the age group 21–40 years followed by patients in the age-group 41–60 years. Male predominance is noticed. About 70% patients hailed from urban areas. The occupation profile of the study group mainly consisted of housewives (21%), followed by farmers (16.9%), laborers (13.6%), and carpenters (12.3%).

All inoculated media was incubated aerobically. Different media showed different percent of growth [Tables 1-6]. Corneal stains were found to be positive in 38 (76%) patients.

Table 1: KOH Preparation

Impression	Frequency (%)
F-E	19 (38.0)
Nil	31 (62.0)
Total	50 (100.0)

KOH: Potassium hydroxide

Table 2: Sheep blood agar

Growth	Frequency (%)
Positive	16 (32.0)
Nil	34 (68.0)
Total	50 (100.0)

Table 3: Chocolate agar

Growth	Frequency (%)
Positive	4 (8.0)
Nil	46 (92.0)
Total	50 (100.0)

Table 4: Tryptone glucose beef extract agar

Growth	Frequency (%)
Positive	18 (36.0)
Nil	32 (64.0)
Total	50 (100.0)

Table 5: Brain heart infusion agar

Growth	Frequency (%)
Positive	2 (4.0)
Nil	48 (96.0)
Total	50 (100.0)

Table 6: Sabouraud dextrose agar

Growth	Frequency (%)
Positive	19 (38.0)
Nil	31 (62.0)
Total	50 (100.0)

Table 7: Microorganism isolated

Microorganism isolated	Frequency (%)
Nil	14 (28.0)
Aspergillus	12 (24.0)
Pseudomonas	4 (8.0)
C. Albicans	4 (8.0)
S. Pneumoniae	8 (16.0)
Pseudomonas+S. Aureus	3 (6.0)
Penicillium	2 (4.0)
S. Aureus	2 (4.0)
S. Epidermidis	1 (2.0)
Total	50 (100.0)

C. Albicans: Candida Albicans, S. Pneumonia: Streptococcus Pneumonia, S. Aureus: Staphylococcus Aureus, S. Epidermidis: Staphylococcus Epidermidis

Table 8: Culture proven

Microorganism	Culture proven
Fungal keratitis	18
Bacterial keratitis	18
Culture showed no growth	14

Table 9: Culture-proven fungal cases

Culture proven fungal cases	*n* (%)
Aspergillus	12 (66.6)
C. albicans	4 (22.2)
Penicillium	2 (12.2)

C. Albicans: Candida Albicans

Table 10: Culture-proven bacterial cases

Culture proven bacterial cases	*n* (%)
Pseudomonas	4 (22.2)
S. pneumonia	8 (44.4)
Other	6 (34.4)

S. Pneumonia: Streptococcus Pneumonia

Table 11: Cause of culture-proven fungal keratitis

Cause	Culture-proven fungal keratitis
Trauma due to vegetative matter	13
Others	05

The predominant bacterial pathogen isolated was *Streptococcus Pneumoniae* representing 16% followed by *Pseudomonas* 8%. The predominant fungal pathogens isolated were *Aspergillus* species (24%) followed by *Candida Albicans* (8%). Of this, 18 (36%) patients had bacterial growth, 18 (36%) had fungal growth, and the remaining 14 (28%) were found to be culture negative. That is, the bacterial and fungal infections occurred almost with equal frequency [Tables 7-10].

Trauma is the leading cause for the corneal ulcers, and most of the fungal ulcers are because of trauma due to vegetative matter [Table 11]. The incidence of the bacterial and fungal keratitis is almost the same in this area. The higher incidence of fungal ulcers may be related to the agricultural activities and the environmental conditions.

DISCUSSION

Considering the predominant predisposing factor of trauma in all types of microbial keratitis (bacterial - 46.6%, fungal - 81.9%, and *Acanthamoeba* - 95.5%), the probable reason for male preponderance is obvious. Ocular trauma was significantly more associated with outdoor occupation in this series.

It is interesting to note that a majority of our patients presented within 1 week of onset of symptoms. This indicates the easy availability of transport to patients and is in contrast to the situation in other developing countries such as Nepal where 19.3% of the patients took longer than 1 month to reach the hospital for treatment.[10]

Direct microscopic examination of corneal scrapings provides rapid diagnosis and forms the basis for instituting initial antimicrobial therapy which may be modified later according to culture reports.[11] The detection of fungi was much higher in the smears than it was for bacteria in our study.

In the study conducted by different researchers[12-15] regarding the utility of Gram-stain in the diagnosis of early and advanced bacterial keratitis,the sensitivity was found to be 36.0% and 40.0% respectively. The low sensitivity was attributed to the use of antibiotics prior to presentation by nearly 50% of the patients. The sensitivity of Gram's stain in the diagnosis of bacterial keratitis, as reported by other authors, is close to the overall sensitivity noted in their study (56.6%) which dropped on the correlation of the presence of similar bacteria in smears and cultures (45.7%).

Microorganisms were isolated in 72% of the cases. This figure is close to many other reports but is almost near to the reports from Nepal (80%) and from Bangladesh (81.7%).[16,17] The protocol of culture techniques followed in this study and the procedure of sample inoculation directly in the clinic leave virtually no scope for the role of laboratory-related reasons for low yield in culture.

A majority of our patients had a corneal infection by a single agent, the most common being bacterial of which *S. Pneumoniae* was found to be most predominant organism followed by *Pseudomonas*, which is very much similar to other studies from Asia and Africa.[18,19]

A review of literature of most of the studies from developed countries such as the USA (except southern USA) and Australia showed *Staphylococcus Epidermidis* or coagulase-negative staphylococci as the leading cause of bacterial keratitis.[20] It is not clear whether the tendency to consider *S. Epidermidis* or coagulase-negative staphylococci as a normal commensal of the conjunctiva may have led to underreporting in some of the studies.

A high prevalence of fungal keratitis caused by filamentous fungi in warmer climates has been widely reported.[20,21] The most common fungi isolated were *Aspergillus* followed by candida. Similar reported incidence in other regions of India is 7.3% in North India, 32% in East India, and 38.9% in West India. This regional variation could be because fungal keratitis is expected to be more common in the tropical and subtropical regions than in the temperate regions. This is in contrast to most reports of *Aspergillus* from India and Candida in other parts of the world.

The diatomaceous fungi are frequently reported as causes of keratitis in many tropical and subtropical regions.[5] In similar studies in South India, the incidence of fungal keratitis was nearly 38%.[9]

This study was developed primarily to determine the specific pathogens responsible for corneal ulceration, and an attempt was made to identify the epidemiological characteristics of the population at risk for corneal ulceration as well as those factors which predispose to the development of a corneal ulcer.

Comprehensive surveys such as this are necessary to assess the specific epidemiological characteristics of corneal ulceration which are unique for each region and population, to design an efficient public health program for the rapid referral, diagnosis, treatment, and ultimately the prevention of corneal ulceration in the population at risk.[11]

The present study was limited by the small sample size and a relatively short period of study. Further, detailed analysis is needed over a longer duration of time making use of modern investigative modalities such as immunochemistry, fluorescent microscopy, enzyme immunoassays, radioimmunoassays, and molecular biological techniques which have led to the modification of the conventional techniques, for rapid identification of the various etiological agents of ocular infections within 1–6 h. This will help us in further consolidating the findings of our study and help us in identifying the pathogen and institute prompt treatment.

CONCLUSION

1. Corneal stains were found to be positive in 38 (76%) patients.
2. Corneal cultures were found to be positive in 36 (72%) patients. Of this, 18 (36%) patients had bacterial growth, 18 (36%) had fungal growth, and the remaining 14 (28%) were found to be culture negative. That is, the bacterial and fungal infections occurred almost with equal frequency.
3. The predominant bacterial pathogen isolated was *S. Pneumonia* representing 16% followed by *Pseudomonas* 8%.
4. The predominant fungal pathogens isolated were *Aspergillus* species (24%) followed by *C. Albicans* (8%).
5. The sensitivity of KOH staining was almost 100% in culture-proven fungal cases.

6. Trauma is the leading cause for the corneal ulcers, and most of the fungal ulcers are because of trauma due to vegetative matter.
7. The incidence of the bacterial and fungal keratitis is almost the same in this area. The higher incidence of fungal ulcers may be related to the agricultural activities and the environmental conditions.
8. Staining efficiently establishes the diagnosis therefore it can be used in the management of corneal ulcer to start the prompt treatment as corneal ulcer is a medical emergency.
9. The microbiological profile helps the ophthalmologists to start the specific treatment directed against the causative organisms.

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