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Research Article

STUDY OF PREDISPOSING FACTORS AND AETIOLOGIC DIAGNOSIS OF INFECTIOUS KERATITIS

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Abstract- Introduction: Microbial keratitis is a potentially vision-threatening ocular infection that may be caused by bacteria, fungi, viruses or parasites. The etiological and epidemiological features of keratitis depend on host factors, geographical location and the climate. Several risk factors like age, sex, immune status and socio-economic background determine its pathogenesis. Therefore, knowledge of above features plus local organisms and resistance patterns help in rapid identification and appropriate selection of antimicrobial therapy. Aims and objective: To analyse the aetiology and predisposing factors associated with microbial keratitis. Material and methods: Corneal scraping from 100 suspected cases of infectious keratitis were included in the study. They were processed by standard microbiological procedures for isolation and speciation of different microbial agents. Result: Of the 100 patients investigated bacterial growth was found in 26 (26%) patients while fungal growth in 39 (39%) patients. Filamentous fungi isolated predominantly (95%), of which *Aspergillus* spp (25.6%) was commonest followed by *Fusarium* spp (17.9%), *Penicillium* spp (10.2%) *etc. C. albicans* were found in 2 (5%) patients. Of the positive bacterial culture *Staphylococcus aureus* (26.9%) were predominant followed by CONS and *Pseudomonas aeruginosa* (15.3%), *E.coli* (11.5%) etc. Trauma was commonest predisposing factor. Conclusion: An understanding of the epidemiological features, risk factors, and etiological agents for microbial keratitis in a specific region is important in rapid recognition, timely institution of therapy, optimal management, and prevention of this disease.

Keywords- *Keratitis, Microbial, Mycotic, Bacterial, Trauma*

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Introduction

Microbial keratitis is a serious condition that could result in corneal scaring, corneal perforation, and blindness. It can be caused by different microbial agents like bacteria, fungi, viruses and parasites. Although any organism can invade the corneal stroma if the corneal protective mechanisms such as blinking, tear dynamics and epithelial integrity are compromised; microbial cause of suppurative corneal ulcer differ considerably from region to region [1]. Shifting trends in the microbiological profile of keratitis have been reported in studies in some parts of the world [2, 3]. In developing countries like India, keratomycosis is considered to be one of the leading cause for ocular morbidity. Opportunistic fungal pathogens, more importantly *Aspergillus* and *Fusarium* are commonly reported from such cases. Corneal trauma with plant or animal material widespread use of broad-spectrum antibiotics and steroids, the frequent and prolonged use of contact lenses and post-operative infections are common predisposing factors responsible for increasing keratomycosis [4]. The appropriate empiric therapy is often based on the local microbial distribution pattern along with the patients demographic and risk factors profile. Therefore, local epidemiological studies are necessary when planning a corneal ulcer management strategy. Further, it is equally important to perform meticulous laboratory investigations including microscopy and culture of corneal scraping for identification of various microbial agents. Hence, the present study was designed to determine both bacterial and fungal aetiology of infectious keratitis along with their predisposing factors from our tertiary care hospital.

Aims and objective

To determine bacterial and mycotic etiology of infectious keratitis
To isolate & identify bacterial and fungal agents from corneal scraping of keratitis patients in a tertiary care Hospital
To identify the risk factors predisposing to corneal infections.

Material & methods

The present study was conducted in the Department of Microbiology at a Tertiary care Hospital and Research Centre. Corneal scrapings were obtained from suspected cases of infectious keratitis attending ophthalmology OPD and/ or admitted in ophthalmology ward over a period of two years 2013-2014. A standardised proforma was collected for each patient documenting sociodemographic information and clinical findings. Associated local factors and predisposing systemic conditions were checked. Any kind of pre-existing ocular problem, previous treatment history, use of topical steroids and contact lenses also documented. After detailed ocular examination by an ophthalmologist, corneal scraping was collected from each patient, after installation of proparacaine eye drops, using no.15 Bard Parker blade under aseptic conditions. Samples were sent to microbiology laboratory for isolation & identification of causative agent.

Laboratory procedure

Samples were inoculated on various bacterial & fungal culture media and smeared onto two slides one for Gram stain & other for 10% KOH wet preparation.

Bacterial culture

Material obtained from scraping was inoculated directly on the culture media like blood agar, chocolate agar, McConkey agar, in a row of C shaped streak. Liquid media such as thioglycolate & brain-heart infusion broth also inoculated. All the inoculated media were incubated at 37°C for 24-72 hrs. Microbial culture was considered positive when the growth of the same organism was demonstrated on two or more solid media on C -streak, confluent growth at the site of inoculation, consistent with clinical sign, consistent smear observation or same organism isolated on repeated scrapping.

The specific identification of bacterial colonies was performed on the basis of Gram staining by microscopy & biochemical properties using standard laboratory criteria.

Fungal culture

Material obtained from scraping was directly inoculated on Sabouraud dextrose agar (SDA) without/ and with antibiotics *i.e.*, Chloramphenicol & Gentamicin and incubated at 25°C & 37°C separately over a period of four weeks. Fungus identification was done based on the growth rate, colony morphology, reverse & obverse surface colour of SDA. Species identification was done by Lactophenol cotton blue (LPCB) mount of culture positive fungi. Yeast identification was done by standard conventional methods like Germ tube test, chlamydospore production and by using chrome agar.

Results

A total 100 patients of keratitis were studied. Of these 66% were male and 34% were female [Fig-1]. Maximum numbers of patients were from the age group 21-40 years (48%) followed by patients in age group 41-60 years (25%) [Table-1].

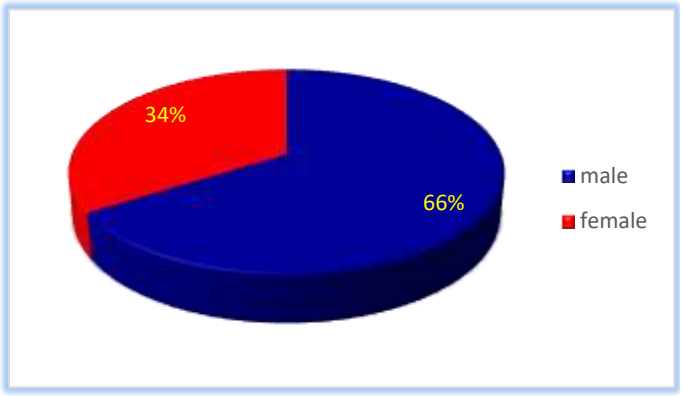

Fig-1 Gender wise distribution of keratitis patients

Table-1 *Age wise distribution of patients with keratitis*

Age in years	Number of patients (%)
< 20	15 (15%)
21- 40	48 (48%)
41-60	25 (25%)
>60	12 (12%)

Table-2 *Micro-organisms isolated from keratitis patients*

Type of Microorganism	Number (%)
Bacterial isolates	26 (26%)
Fungal isolates	39 (39%)
No organism isolated	35 (35%)
Total	100

Following the culture of corneal scraping from above mentioned 100 keratitis patients, micro-organisms were isolated from 65 patients. Of these bacterial growths was found in 26 (26%) patients while 39 (39%) patients showed fungal growth [Table-2].

Fungal growth was observed in a total 39 samples. Among this filamentous fungi (95%) were isolated predominantly while two were yeast like *C.albicans* (5%). Filamentous fungi isolated mainly comprise of hyaline fungi (74.3%) while dematiaceous fungi were (20.5%). *Aspergillus* spp (10) were found to be commonest, of which *Aspergillus fumigatus* (4) and *Aspergillus niger* (3) were predominant. Other fungal growth isolated included *Fusarium oxysporum* (7), *Penicillium* spp (*P.marneffei* 2/3), *Zygomycetes etc.* Dematiaceous fungi isolated were *Bipolaris* spp (1), *Fonsecaea pedrosoi* (1), *Cladosporium* spp (2), *Acremonium* spp (2) *Colletotrichum* spp (1) and *Pseudallescheria boydii* (1)etc. Dimorphic fungi like *Sporothrix schenckii* (2) also isolated. Dematiaceous fungi *Bipolaris* spp and *Colletotrichum* spp were confirmed as species *Bipolaris Spicifera* and *Colletotrichum gloesporioides* by Postgraduate Institute of Medical Education and Research, Chandigarh.

Table-3 *Fungal pathogen isolated from keratitis cases*

Type of fungus	Name of the fungal isolate	Number (%)
A. Filamentous fungi		
Hyaline	*Aspergillus spp*	10 (25.6%)
	Aspergillus fumigatus	4 (10.2%)
	Aspergillus niger	3 (7.6%)
	Aspergillus flavus	2 (5.1%)
	Aspergillus terreus	1 (2.5%)
	Aspergillus glaucus	1 (2.5%)
	Fusarium oxysporum	7 (17.9%)
	Penicillium spp	4 (10.2%)
	Aureobasidium pullulans	3 (7.6%)
	Sporotrix schenckii	2 (5.1%)
	Zygomycetes	1 (2.5%)
	Paecilomyces	1 (2.5%)
	Total	29 (74.3%)
Dematiaceous fungi	*Bipolaris spp*	1 (2.5%)
	Fonsecaea pedrosoi	1 (2.5%)
	Cladosporium spp	2 (5.1%)
	Colletotrichum spp	1 (5.1%)
	Pseudallescheria boydii	1 (5.1%)
	Acremonium spp	2 (5.1%)
	Total	8 (20.5%)
B. Yeast	*Candida albicans*	2 (5.1%)
Total		39

Table-4 *Bacterial pathogens isolated from keratitis cases.*

Name of the bacterial isolate	Number (%)
Gram positive cocci (13)	
Staphylococcus aureus	7 (26.9%)
Coagulase negative Staphylococci (CONS)	4 (15.3%)
Streptococcus pneumoniae	2 (7.6%)
Gram negative bacilli (10)	
Pseudomonas aeruginosa.	4 (15.3%)
Escherischia coli	3 (11.5)
Klebsiella spp.	2 (7.6%)
Aeromonas hydrophilia	1 (3.8%)
Gram positive bacilli (3)	
Corynebacterium spp	1 (3.8%)
Bacillus cereus	1 (3.8%)
Nocardia	1 (3.8%)
Total	26

Out of total number of positive bacterial cultures, 13 were Gram positive cocci (GPC), 10 were Gram negative bacilli (GNB) and 3 were Gram positive bacilli (GPB). *Staphylococcus aureus* (7) were isolated more commonly and 80% of which found sensitive to the methicillin. Among the Gram negative bacilli *Pseudomonas* spp.(4) were isolated predominantly, followed by *E.coli* (3), *Klebsiella* spp (2) and *Aeromonas hydrophilia* (1). Gram positive bacilli like *Bacillus cereus*, *Corynebacterium* spp and *Nocardia* were isolated. A summary of the bacterial pathogens isolated during the study is shown in [Table-4].

Table-5 *Predisposing factors*

Predisposing factors	Number (%)
Trauma	58 (58%)
Vegetative trauma	26
Stone particle, dust, foreign body	21
Animal tail	8
Nail	3
Insect entry into the eye	11 (11%)
Corneal abscess	9 (9%)
Chronic dacrocystitis	5 (5%)
Post-cataract surgery	2 (2%)
Ectropion as a sequel e of Leprosy	1 (1%)
Neuroparalytic case	1 (1%)
Application of mud & Ghee into the eye	1 (1%)
No apparent factor/other	12 (12%)

The most common predisposing factor observed in the present study was trauma which was seen in (58/100) of the keratitis patients. However, vegetative trauma was the leading cause (26). Other traumatic factors involved, stone, foreign body injury (21), animal tail injury (8), nail injury (3) etc. corneal ulcer due to insect entry into eye were reported in 11 patients, 9 patients were having corneal abscess while 5 patients gave history of chronic dacrocystitis. Two patients followed the post cataract surgery. One patient of leprosy developed the corneal infection due to ectropion.

Discussion

This study was designed to identify specific pathogenic agents and to determine demographic characteristics and predisposing factors of corneal ulcer patients attending our tertiary care hospital. In the present study, corneal ulceration was seen in all age groups with preponderance (48%) among physically active adults in age group of 21-40yrs [Table-1]; higher in males (66%) than in females (34%) [Fig-1]. Geethakumari, *et al*., Sedhu, *et al*., Bashir, *et al.,* study [5-7] also found male preponderance while Yousuf, *et al.,* [8] have found females more compared to males as cases of keratitis. These findings are better explained as males are engaged more in outdoor activities. Prevalence of microbial keratitis differs from 22-71% depending upon different geographical area and different seasons can also affect its prevalence. In this study micro-organisms were isolated in 65 (65%) of the 100 cases of suspected infectious keratitis, among these 26 (26%) were bacterial and 39 (39%) were fungal origin. Similarly, Geetakumari, *et al* (Bacterial 27.4%, fungal 69.78%), Basak, *et al* (bacterial 24.8%, fungal 67.7%), Shrinivasan, *et al* (bacterial 32.3%, fungal 57%) [5,9,10] found more fungal as compared to bacterial isolates. Shanthi, *et al* and Renato, *et al* [11,12] found higher bacterial isolates 77.7% & 47.1% respectively. The reduction in bacterial keratitis may be attributed to more effective and successful treatment of bacterial ulcers with new generation topical antibiotics. The reported incidence of mycotic keratitis is 17-36% worldwide, whereas it is about 44-47% in India [4]. *Aspergillus* spp found to be the most predominant fungal pathogen in the temperate region of Northen India, Nepal and Bangladesh [13]. In the present study also *Aspergillus* spp (25.6%) were the predominant fungal pathogen isolated followed by *Fusarium* spp (17.9%), *Penicillium* spp (10.2%). This is comparable to many other studies [4,13,14]. However, *Fusarium* was predominant fungal pathogen in many other regions of Florida, Nigeria, Tanzania, Singapore, South India and Ghana [1, 15, 16]. Similarly, Geetakumari, *et al*, Palanisamy, *et al* also reported higher rate of isolation of *Fusarium* [5,17]. *Aspergillus fumigates* was isolated more frequently as compared to other species. Bharthi, *et al*, Saha, *et al* and Assudani, *et al* reported higher prevalence of *A. flavus* [18, 19,20]. *Candida* spp are uncommon cause of mycotic keratitis in almost all studies including the present study and preferably in patients with systemic disorders or pre-existing ocular abnormalities [21], though Saha, *et al* have recorded a prevalence of 19% [22]. *Dematiceous fungi* (20.5%) isolated were, *Cladosporium* spp (2), *Acremonium* spp (2), *Bipolaris spicifera* (1) *Colletotrichum gloesporioides* (1), *Fonscecea pedrosii* (1) and *Pseudalscheria boydii* (1). Dimorphic fungi like *Sporothrix schenchii* were isolated from 2 cases.

The microbiological profile of microbial keratitis varies across the countries. According to studies in Hong Kong, Taipei, Bangkok *Pseudomonas aeruginosa* was the most prevalent bacterial pathogen accounting for 29-42% of keratitis cases. This was different from studies conducted in France, Switzerland & Turkey where the prevalence of *Staphylococcus* was as high as 52 to 60% [23]. In our study *Staphylococcus aureus* (26.9%) was the predominant bacterial species, followed by *Pseudomonas aeruginosa* and CONS (4 each 15.3%), *E.coli* (3), *Klebsiella* spp and *Streptococcus pneumoniae* (2 each). This coincides well with study of Gangetic West Bengal [9]. However, in other studies from Shanghai and South India, Kashmir; *Streptococcus pneumoniae* was the predominant bacterial pathogen [7,13,17,24,25,]. Gram positive bacilli differ in various studies which have demonstrated 4-12.5% incidence [9, 17, 25]. In the present study Bacillus cereus, *Corynebacterium* spp and *Nocardia* were isolated. Trauma was the predominant predisposing factor (58%) in most of the infectious keratitis cases. Of this injury due to vegetable matter was leading cause. This matches with the study in Paraguay, Gangetic West Bengal, South India, Madurai and Ghana [23]. However, Tewari *et al* reported higher number of keratitis cases due to trauma with wooden objects [26]. In our study, trauma with the vegetative matter while working in the field leads to mycotic keratitis due to *P. marnefeii, Fusarium oxysporum* and *Cladosporium* spp. *Sporothrix schenkii* was isolated in patient of sugarcane injury. One patient of buffalo tail injury developed *A. fumigates* infection. All these cases support that injury due to vegetative or animal matter predisposes mycotic keratitis.

Homemade remedies to treat the corneal ulcer were found to be dangerous in many patients. In present study one patient who applied Ghee and mud into the eye developed staphylococcal infection. Corneal ulcer due to insect entry into eye were reported in 11 patients [*Cellatotrichum* spp was isolated in a patient with a corneal ulcer due to insect entering into the eye], Schaefer, *et al* have identified co-existing ocular diseases as a major predisposing factor [27]. In this study, 9 patients were having corneal abscess while 5 patients gave history of chronic dacrocystitis. Two patients followed the post cataract surgery. One patient of leprosy developed the corneal infection due to ectropion.

Conclusion

A rising trend in the incidence of fungal keratitis has been observed in present study. This regional information is of great value in the clinical diagnosis and better patient management. Microbiological culture and direct microscopic examination for detection of the causative organism always supplement the clinical diagnosis and provide supportive evidence for planning the appropriate therapy.

Application of research: Molecular techniques can be useful in prevention of misidentification of rare isolates. Optimizing the prevention strategies and management of microbial keratitis by use of microbiological diagnosis needs to be emphasized

Research Category: Corneal infection

Abbreviations:

LPCB: Lactophenol cotton blue, CONS: Coagulase negative Staphylococci

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