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Microbial Profile of Corneal Ulcers in a Tertiary Care Hospital in Eastern India

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Abstract
Background: *A corneal ulcer or keratitis is an open sore on the cornea. The cornea covers the iris and the round pupil much like a watch crystal covers the face of a watch. A corneal ulcer usually results from an eye infection, but severe dry eye or other eye disorders can cause it. Corneal ulcer is a major cause of ocular blindness and visual disability in different developing countries in Middle East, Africa and Asia, ranking only second to cataract. This is an endeavour to study about the microbiological figure and prevalence of infectious keratitis in a tertiary eye care hospital of Eastern India, and to test for the in vitro antimicrobial resistance of the bacterial isolates.*
Methods: *A total of 348 patients who visited a tertiary eye care hospital in Eastern India, with infected corneal ulcer were enlisted in this study. Their risk factors and socio-demographic data were documented. Corneal scrapings were assembled from the margin of the ulcer and sent for routine microbiological examination which included Gram stain and KOH mount. Culture was obtained from MacConkey agar, blood agar, chocolate agar and Sabouraud's dextrose (SDA) agar in multiple C shaped streaks. After overnight incubation, bacterial culture was proceeded by standard biochemical tests and antimicrobial sensitivity as stated in the clinical and laboratory standards institute (CLSI) guidelines. Inoculated SDA was observed everyday for up to 10 days and the growth was identified by its colony morphology, pigment production and lacto-phenol cotton blue mount examination.*
Results: *Of 348 patients, a microbial etiology was entrenched in 132 cases (37.5%). Of these, 84 (63.63%) were male. The 41-60 years age range was the most afflicted group. Of 132 positive cases, 58 (43.93%) were bacterial, 67 (50.75%) were fungal and 7 (5.30%) patients showed mixed variety. The most common isolated fungus was Fusarium which was detected in 44 (33.33%) cases, succeeded by Aspergillus spp in 13 (9.84%) patients. Staphylococcus aureus was the most prevailing isolated bacteria. All Gram positive cocci were susceptible to Vancomycin followed by Gatifloxacin, while all Gram negative bacilli were susceptible to Gatifloxacin.*
Conclusion: *These findings demonstrated the current trend in the microbiological etiology of patients with corneal ulcer in Eastern India, which has a significant public health involvement for the treatment in conjunction with prevention of corneal ulceration in developing world.*
Keywords: *Corneal Ulcer; Microbiological figure; Antibiotic Susceptibility.*

Introduction

Infectious keratitis is a leading cause of corneal blindness in developing countries.[1] Corneal ulceration results in 1.5–2 million new cases of corneal blindness annually, posing a major public health problem according to the World Health Organization (WHO) reports.[2] Fungi are the most common etiological agents which account for 30–40% whereas bacteria account for 13–48% of all cases of suppurative keratitis; this varies by geographical area.[3] These pathogens lead to corneal damage directly or by release of toxins and enzymes or by activating the host immune system.[4] An intact corneal epithelium acts as a barrier for the majority of microorganisms.

Microorganisms can penetrate through a breach in the epithelium either due to penetrating or perforating ocular trauma or due to surgery. Various risk factors have been implicated for increased incidence of fungal keratitis including widespread use of antibiotics and steroids, use of contact lenses, and postoperative infections.[5] Ocular morbidity such as corneal scarring and subsequent visual loss can be significantly reduced by prompt institution of appropriate therapy guided by the knowledge of the causative agents.

Aims & Objectives

The present study is an attempt to identify the prevalence of microbial keratitis in this area and to test for the *in vitro* antimicrobial resistance.

Materials & Methods

This study was conducted at a tertiary eye care hospital in Kishanganj, India for two years from July 2014 to June 2016. Patients with suspected microbial corneal ulcers were included and their socio-demographic data and risk factors were recorded. A corneal ulcer was defined as a corneal infiltrate associated with an overlying epithelial defect. Corneal scrapings were routinely collected from patients with corneal ulceration by an ophthalmologist viewing through a slit lamp. Scrapings were taken from the edge of the ulcer before administration of any antimicrobials using a sterile 15 number blade after instillation of topical 4% xylocaine.

Direct microscopy was performed by taking the scrapings on two glass slides, one for Grams staining and the other for KOH mount. To prepare the culture, corneal scrapings or buttons were inoculated bed side on blood agar, chocolate agar, MacConkey agar and Sabouraud's dextrose agar (SDA) in multiple C shaped streaks. After overnight incubation, bacterial culture was confirmed by growth on blood agar, chocolate agar and MacConkey agar followed by standard biochemical tests according to the clinical and laboratory standards institute (CLSI) guidelines.

Inoculated SDA was inspected daily for up to ten days and declared as fungal culture negative thereafter. Fungal growth on observation was grossly identified by its colony morphology, pigment production on reverse, and microscopically by lacto-phenol cotton blue stain. Diagnosis of fungal keratitis was done when any of the following criteria were met:

- Correlation between direct KOH examination and growth on SDA
- Growth on more than one C streak lines
- Similar growth on more than one media.

Results

Three hundred fourty-eight eyes of 348 patients were included in this study. Of these, 132 (37.93%) were positive for smear and culture. Of 132 patients, 84 (63.63%) were male. The age range of 41–60 years was the most affected group. Microbial etiology was bacterial in 58 (43.93%) and fungal in 67 (50.75%) cases. Seven subjects showed mixed growth – both bacterial and fungal (5.30%). Risk factors were trauma in 64 (48.48%) followed by diabetes mellitus in 42 (31.81%), corticosteroid therapy in 13 (9.48%) eyes and contact lens usage in 5 (3.78 %)

The most common fungus isolated was Fusarium in 44 (33.33%) eyes followed by *Aspergillus* spp found in 13 (9.84%) cases. Common bacterial isolates were *Staphylococcus aureus* in 26 (19.69%) subjects followed by *Streptococcus pneumoniae*

seen in 9 eyes (6.8%) from the Gram positive bacteria, and *Pseudomonas aeruginosa* in 11 (8.33%) cases followed by *Klebsiella pneumoniae* seen in 5 eyes (3.78%) from the Gram negative bacteria.

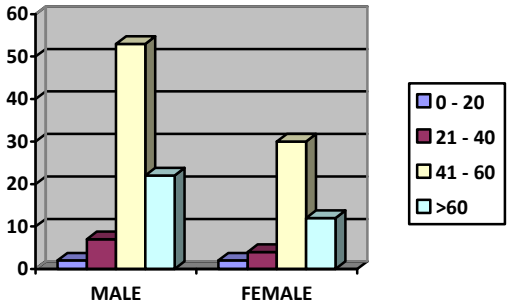
All Gram positive cocci were susceptible to vancomycin followed by gatifloxacin, chloramphenicol and moxifloxacin whereas all Gram negative bacilli were susceptible to gatifloxacin followed by moxifloxacin and ofloxacin. The highest resistance was seen to ciprofloxacin and gentamicin.

Socioeconomic factors and clinicial presentation of corneal ulcers showed that almost 58.33% of the culture positive cases were farmers and 72.72% of them were illiterate. Patient diagnosed via culture positivity for microbial etiology as the corneal ulcer cases presented with different clinical symptoms including ocular pain, redness of the eyes, decreased vision, white lesion and others (discharge, watering and foreign body sensation). Growth positivity for microbial etiology was statistically significant ($p < 0.05$) with trauma (28.9%) as an important clinical presentation among the positive cases.

Discussion

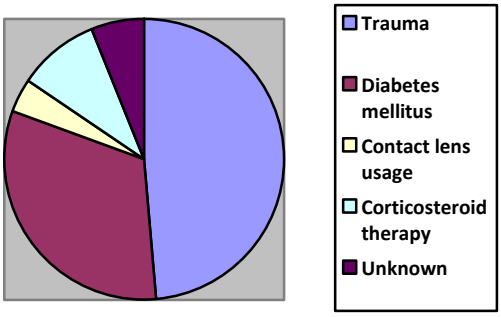
Suppurative keratitis and its complications constitute important causes of ocular morbidity often leading to blindness if early management is not instituted. A proper clinical history coupled with detailed clinical examination would be beneficial to identify the predisposing factors for corneal perforation in microbial keratitis.

In the present study, male subjects were more affected than female patients which is in agreement with the study done by Tityal et al.[6] The age range of 41–60 years was more affected consistent with the results of Cameron et al[7] in Sydney and Das et al[4] in Kolkata [Graph 1]. This could be attributed to the increased outdoor activity of men especially in the working age group. In contrast, in the study done in China, women were more affected and most of them were over the age of 60.[8] This could be due to higher employability of women particularly in the agricultural sector in China.



Graph 1. Age and gender wise distribution of corneal ulcer.

The most common associated risk factors in our study were trauma followed by diabetes mellitus, corticosteroid therapy and contact lens usage which is comparable with other studies.[1,9] While Yousuf et al[10] reported the use of contact lens as the major cause, Krishna et al[11] demonstrated injury to eye as the predominant risk factor followed by the foreign body induced microbial keratitis. The role of diabetes was negligible in the latter study (2%) [Graph 2].



Graph 2. Risk factors of corneal ulceration

In our study, out of 348 cases, 132 (37.93%) were culture positive, and fungi were recovered slightly more frequently than bacteria (67 vs 58 eyes, respectively) [Tables 1 and 2]. Srinivasan et al., isolated equal numbers of bacterial (47.1%) and fungal (46.8%) agents causing infectious keratitis with 5.1% cases having mixed infections [12].Katara et al.,[13] also reported a culture positivity of 40%, of which 26% were fungal isolates and the remaining 14% of samples had bacterial etiology. A comparison of the prevalence rates of microbial keratitis due to bacterial and fungal agents is shown in Table 3.

Among the fungal isolates, filamentous type was

more common compared to yeast and *Fusarium* spp was the most isolated species followed by *Aspergillus* spp. [Table 4]. Comparable results were obtained in studies done by Alkatanet al.,[14] and Idiculla et al.,[15] In contrast, Lack et al., observed a higher incidence of *Aspergillus* spp in their series [3]. In the current study, most of the fungal isolates (80%) were obtained during the months of July to September. In contrast, Krishna et al.,[11] reported maximum incidence of fungal keratitis in Bellary during the harvest months of January, February and June.

The difference in the isolation rates of these fungal pathogens can be explained by the differences in the climate and the natural environment of individual regions. Studies in the Eastern Indian region have shown a higher incidence of *Fusarium* as compared to studies in the northern or western India. *Fusarium* keratitis has a more aggressive course and is less responsive to treatment than *Aspergillus*. [16,17] Katara et al., in Gujarat showed *Aspergillus* as the dominant isolate.[13] The higher incidence of mycotic keratitis due to *Aspergillus* spp in their study may be due to the high tolerance of their spores to hot and dry weather conditions.[16] Furthermore, *Aspergillus* spp are more ubiquitous and can almost be found everywhere on every conceivable type of substrate including soil and decaying organic debris while *Fusarium* species are common plant pathogens and are mostly found in soil.[3]

Bacteria account for 65–90% of corneal infections with *Staphylococcus aureus, S. pneumonia and Pseudomonas aeruginosa* accounting for more than 80% of bacterial keratitis.[18] In our study, bacterial keratitis was predominated by Gram positive bacteria similar to other Indian studies by Gopinathan et al[19] and Das et al.[4] The most common bacterial isolate was *S.aureus* followed by *Pseudomonas*. A study conducted in Pakistan by Narsani et al., also showed higher isolation of Gram positive organisms with S. aureus being the most common (60%)[20]. This has been attributed to the climatic zone variation as Gram positive bacterial species are more frequently recovered in temperate zones and Gram negative species in tropical climates. While some studies have reported Pseudomonas as more common bacteria than *S. aureus*. [1,10] other studies showed coagulase-negative *Staphylococci* as the most common isolate [Table 4].[9,19,21]

The standard protocol for treatment of bacterial corneal ulcer in our patients was topical instillation of antibiotics.

As there are no standard CLSI guidelines yet for topical ocular antibiotics, proper interpretation of the drug sensitivity testing is not possible; antibiotic sensitivity pattern coupled with clinical improvement is needed to assess the efficacy of a particular antibiotic.

Regarding the antimicrobial susceptibility pattern, all Gram positive bacteria were sensitive to vancomycin. More than 70 percent sensitivity was seen in *S.aureus* isolates to gatifloxacin, gentamicin and chloramphenicol. All nine *S.pneumoniae* strains were sensitive to all antibiotics except gentamicin.

Among the Gram negative isolates, *Pseudomonas aeruginosa* exhibited good sensitivity to gatifloxacin (100%) followed by ciprofloxacin (90%) and moxifloxacin (90%) and was the least sensitive to chloramphenicol (30%). Enterobacter spp and Proteus spp were sensitive to all antimicrobials used [Table 5].

As we restricted our study to aerobic bacterial and fungal agents and did not include anaerobic bacterial, amoebic and viral agents causing keratitis, complete analysis regarding the microbial profile was not possible. Anaerobic organisms usually cause keratitis as mixed infection with aerobic organisms. Not many studies have been conducted in this regard due to the cost and no feasibility of maintaining anaerobic culture methods. Perry et al[22] reported a prevalence of 16.66% for anaerobic corneal infections. Further studies inclusive of all pathogens would give a comprehensive picture of infectious keratitis in our region.

Table 1 Number and percentage of type of isolates

Type	Number
Only bacteria	58(43.43%)
Only fungi	67(50.75%)
Mixed Both bacteria Bacteria and fungi	 2(1.51%) 5(3.78%)
Total number of isolates (bacteria, fungal and mixed)	132

Table 2. Pathogens isolated from patients with mixed infections

Fungi	Bacteria
Fusarium	*Strep. pneumoniae*
Curvularia	*S. aureus*
Fusarium	*S. aureus*
Aspergillus niger	*S. aureus*
Hyalohypomycetes	*S. aureus*

S, Staphylococcus; Strep, Streptococcus

Table 3. Prevalence rate of microbial keratitis in various studies

Study	Microbial keratitis	Bacterial keratitis	Fungal keratitis	Others
Ashok Kumar Narsari et al, 2009, HYD	71%	48.5%	26.36%	4.25%
Sadia Sethi et al, 2010, Peshawar	22%	13%	9%	0%
Renato MP et al, 2010, Sao Paulo, Brazil	53.5%	47%	6%	0.4%
Hitesh J Assugani et al, 2013, Gujarat	27%	13%	14%	0%
Present study	37.93%	43.93%	50.75%	5.30%

Table 4. Etiological distribution of microbial keratitis

Isolate	Number
Fungal	
Fusarium	44
Aspergillus	13
Hyalohyphomycetes	3
Curvularia	1
Unidentified dematacious fungi	2
Cladosporium	1
Aureobasidium	1
Pseudoallescheria boydii	1
Candida albicans	1
Bacterial	
Gram positive	
S. aureus	26
S. pueumoniae	9
CONS	4
Gram negative	
Pseudomonas aeruginosa	11
Klebsiella pneumoniae	5
Escherichia coli	1
Enterobacter spp	1
Proteus spp	1
Others	7
Nocardia spp	
Total number of isolates (bacterial, fungal and mixed)	132

CONS, Coagulase-negative staphylococci

Table 5. Antibiotic susceptibility pattern (%)

Isolates	G	Tob	Gat	Mo	Of	Cip	C	Va	A
S. aureus (26)	76	62	71	67	38	38	86	100	80
S. pneumoniae (9)	33	100	100	100	100	100	100	100	30
CONS (4)	80	75	100	100	100	60	85	100	34
Ps. Aeruginosa(11)	60	70	100	90	80	90	30	-	90
K. pneumonia (5)	80	86	100	100	100	67	100	-	70
E. coli (1)	100	100	100	100	0	0	100	-	80
Enterobacter (1)	100	100	100	100	100	100	100	-	86
Proteus (1)	100	100	100	100	100	100	100	-	100

S, Staphylococcus; CONS, Coagulase-negative staphylococci; K, Klebsiella; Ps, Pseudomonas; E, Escherichia; G, Gentamicin 10 mcg; Tob, Tobramycin 10 mcg; Gat, Gatifloxacin 5 mcg; Mo, Moxifloxacin 5 mcg; Of, Ofloxacin 5 mcg; Cip, Ciprofloxacin 5 mcg; C, Chloramphenicol 30 mcg; Va, Vancomycin 30 mcg: A, Amikacin

Conclusion

In conclusion, routine microbiological examination of patients with corneal ulcer is necessary in order to analyze and compare the changing trends of the etiology and their susceptibility patterns which would be beneficial in applying an appropriate antimicrobial treatment.

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Conflicts of Interest

There are no conflicts of interest.

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