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Original Research Article

Ocular Pathogen Causing Bacterial Keratitis in a Tertiary Care Rural Hospital

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ABSTRACT

Introduction: Corneal blindness is a major public health problem in India. Morbidity can be significantly reduced when the treatment modality is guided by the knowledge of the causative organism & its antimicrobial susceptibility.

Aims & Objectives:

1. To isolate the bacteria causing keratitis & study the antibiotic sensitivity pattern of isolates.
2. To study predisposing factors causing keratitis.

Materials & Methods: A prospective study was employed from December 2010 to December 2012 from which a total of 97 patients with keratitis were included in the study. Corneal scrapings collected were transported and microbiologically processed using standard operating procedure.

Result: Out of 97 cases, keratitis showed the highest 30 (31%) cases were in the age group of 41-50 years with male preponderance. Out of 44 positive cultures 21 (21.64%) were bacteria, 21(21.64%) were fungi & 2(2.06%) were mixed. The most important predisposing factor in the present study was trauma in 86 cases (88.65%). Among the isolates 6 (26.08%) were Staphylococcus aureus, 3 (13.04%) were CONS, 6 (26.08%) were Pseudomonas, 4 (17.39%) were Klebsiella, 2 (8.69%) were each of Proteus mirabilis & Proteus vulgaris. Gram positive cocci were sensitive to Erythromycin, Gentamicin with resistance to Ciprofloxacin & Cefoxitin. Gram negative isolates were sensitive to Ciprofloxacin, Gentamicin & resistant to Amoxclave.

Conclusion: Routine microbiological examination of corneal ulcer is necessary to analyze & compare the changing trends in the microbial etiology & their susceptibility pattern to formulate a proper & appropriate antibiotic response against corneal ulcer.

Key words: keratitis, CONS, antibiotics.

INTRODUCTION

Corneal blindness is a major public health problem in India and infections constitute the most predominant cause. A recent report on the causes of blindness worldwide constituently lists corneal scarring second only to cataract as major a etiology of blindness and visual disability in many of the developing nations in Asia. [(1,2)]

Microbial keratitis is defined as a loss of corneal epithelium, with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon. [(3)] Keratitis is a potentially vision threatening condition. [(4)] It may be caused by bacteria, fungi, viruses or parasites. [(5)]

The microbial causes of keratitis vary considerably between continents and countries. [(6)] The hallmark of treating keratitis is the prompt institution of appropriate antimicrobial therapy to minimize corneal scarring and visual loss. [(7)] Morbidity can be significantly reduced

when the treatment modality is guided by the knowledge of causative organism and its antimicrobial susceptibility test. [8]

MATERIALS & METHODS

The present study was carried out in the Department of Microbiology of tertiary care rural hospital. A total of 97 clinically suspected patients of keratitis attending Ophthalmology OPD of our hospital from December 2010 to December 2012 were studied. Detailed clinical history of patients such as, age, sex, occupation, duration of symptoms, if any was recorded. In the affected eye, the integrity of the corneal epithelium was checked by using 2% sodium fluroscein solution or 1% sodium fluroscein strips. It is kept in lower fornix for 2 seconds and then removed. The patient is asked to blink, the stain spreads and then the area is visualized using slit lamp by the ophthalmologist and ulcer details were noted.

Corneal scrapings taken by sterile Bard-Parker surgical blade no.15 from the margins as well as base of the anaesthetized cornea & smear prepared for Gram staining & 10 % KOH preparation. For aerobic cultivation, all solid agar (Blood agar for both aerobic & anaerobic, Mac Conkey Agar, Chocolate Agar) were inoculated on the surface without cutting the agar & C-shaped inoculations were made at sites (multiple). [9] Thioglycollate broth used for transport of anaerobic specimens. Any turbidity appeared in brain heart infusion were identified by Gram Stain & subsequently sub-cultured into Mac Conkey & blood agar. For anaerobic cultivation, the Anaerobic jar containing the blood agar plates were incubated at 37ºC & examined after 48-72 hrs. Culture plates showing no growth were further incubated anaerobically for 5 days & examined on alternative days before discarding. [10] Characterization & identification of both aerobic & anaerobic organisms were done by studying the colony morphology, gram staining, motility, & biochemical reactions. All the isolated bacteria were tested against different antimicrobial agents by standard disc diffusion method (Kirby Bauer disc diffusion method) in accordance with CLSI guideline. For anaerobic bacteria, blood agar plates were incubated an aerobically at 37ºC for 48 hours. [11]

RESULTS

The specimens from all 97 cases were subjected to bacteriological, mycological and parasitological investigations and following observations were made. Maximum incidence was seen in males in age group of 31-40 years (22.68%) and 41-50 years(21.64%) followed by 61-70 years(11.34%). Minimum incidence was recorded for males and females of 21-30 years (2.06%), below 20 years, above 70 years (3.09%) and 51-60 years (1.03%).

Table 1: Showing age and sex wise distribution of cases of keratitis

Age (yrs.)	Male	Female	Total
Below 20	5	0	5(5.15%)
21-30	4	2	6(6.18%)
31-40	22	5	27(27.83%)
41-50	21	9	30(31%)
51-60	6	1	7(7.22%)
61-70	11	9	20(20.6%)
above 70	4	3	7(7.22%)
Total	**70**	**27**	**97**

The most important predisposing factor in the present study leading to keratitis was trauma (88.65%) followed by topical antibiotic use (69.07%). There was no h/o contact lens use by the patient in this study period.

Table 2: Showing distribution of predisposing factor of keratitis

Predisposing factor	Number of cases (n=97)	
	Total number of cases studied (%)	Number of positive cases (%)
History of corneal trauma	86(88.65)	44 (45.36%)
Topical antibiotic	67(69.07)	28 (28.86%)
Surgery (cataract)	1 (1.03%)	0
Use of contact lens	0	0
Use of herbal medicine	11 (11.34%)	10 (10.30%)
Other local / systemic conditions	5 (5.15%)	4 (4.12%)
No significant history	4 (4.12%)	3 (3.09%)

Table 3, shows that among the various etiological isolates, fungi were the most prevalent (32%), followed by bacteria (23.8%). No parasitic isolates were found during the study period.

Table 3: Etiological agents in cases of keratitis

Isolated organisms	No. of cases	Percentage
Bacterial isolates	23	23.8
Fungal isolate	31	32
Parasite isolate	0	0
Mixed (Bacterial+fungal)	2	2.06
Total	**52**	**53.60**

In our study, *Staphylococcus aureus* 6 cases (26.08%) and *Pseudomonas aeruginosa* 6 cases (26.08%) were the predominant bacterial isolates, followed by *Klebsiella* spp 4 cases (17.39%), *Coagulase negative staphylococcus* 3 cases (13.04%), 2 cases (8.69%) of each *Proteus mirabilis* and *Proteus vulgaris*.

Table 4: Bacterial pathogen isolated from cases of keratitis

Bacterial isolates	No. of cases	%
Total Gram positive isolates	9	39.13%
Staphylococcus aureus	6	26.08%
Coagulase negative staphylococcus spp.	3	13.04%
Total Gram negative isolates	14	60.86%
Pseudomonas aeruginosa	6	26.08%
Klebsiella spp.	4	17.39%
Proteus mirabilis	2	8.69%
Proteus vulgaris	2	8.69%
Total	**23**	**100%**

In the table 6, 23 out of 31 clinically diagnosed bacterial keratitis yielded growth in culture, thus showing the statistically significant correlation between the clinically diagnosed and culture positive bacterial keratitis. ($\chi^2 = 52.18$, $P < 0.0000001$)

Table 6: Correlation of clinically diagnosis and culture in bacterial keratitis

Clinical diagnosis	Number	Growth in culture for bacteria	
		Present	Absent
Bacterial keratitis	31	23	08
Fungal keratitis	66	02	64
Total	**97**	**25**	**72**

In the table 7, *Staphylococcus aureus* was the most common gram positive isolate which was found sensitive to Erythromycin, Gentamicin, Co-trimoxazole. Most of the Gram positive isolates were resistance to Penicillin, Cefoxitin and Ciprofloxacin.

Table 7: Showing antibiotic sensitivity pattern of Gram positive organisms

Isolates Antibiotics	Staphylococcus aureus (S/R)	Coagulase negative staphylococcus (S/R).
Penicillin	2/4	1/2
Erythromycin	5/1	2/1
Cefoxitin	2/4	1/2
Gentamicin	4/2	2/1
Ciprofloxacin	2/4	1/2
Co-trimoxazole	4/2	2/1
Cefuroxime	3/3	1/2

In the table 8, it was found that most gram negative isolates were sensitive to Amikacin, Imipenem, Gentamicin, Piperacillin, Piperacillin/Tazobactam, Ciprofloxacin, and Cefepime, whereas most were resistant to Ampicillin, Ampicillin/ Clavulanic acid.

Table No 8: Showing antibiotic sensitivity pattern of gram negative organisms

Isolates/ Antibiotics	Pseudomonas aeruginosa (n=6) (S/R).	Klebsiella aerogenes (n=4) (S/R).	Proteus species (n=4) (S/R).	Total (S/R).(n=14)
Ampicillin	-	1/3	1/3	2/6
Amikacin	6/0	-	-	6/0
Gentamicin	5/1	2/2	2/2	9/5
Piperacillin	5/1	-	-	5/1
Piperacillin+ Tazobactam	5/1	-	-	5/1
Imipenem	6/0	-	-	6/0
Cefepime	4/2	2/2	2/2	8/6
Cefuroxime	-	1/3	2/2	3/5
Ceftriaxone	-	1/3	2/2	3/5
Ampicillin+ Clavulanic acid	-	1/3	1/3	2/6
Ciprofloxacin	5/1	3/1	3/1	11/3

Result in our study shows that, 19.09% cases of bacterial keratitis and 37.93% cases of fungal keratitis, 50% of mixed infection deteriorated or had some complication.

DISCUSSION

Keratitis is emerging as a major cause of preventable blindness throughout the world. The incidence of keratitis varies from country to country and region to region. This variation can be due to seasonal

and climatic differences and also due to occupation involved in different parts of the world. With this knowledge, the present study was undertaken to know the clinical pattern of keratitis and its correlation with microbiological investigation along with various predisposing factors.

Highest incidence of keratitis was observed in the age group of 41-50 years (31%) and 31-40 years (27.8%) in the present study. Among these, maximum incidence of bacterial keratitis was observed in age group of 41-50 years. The incidence was more in males (72.16%) than in females (27.8%) and male to female ratio was 2.59:1. Corneal infection among males could be attributed to their greater involvement in outdoor activities, thus being prone to corneal injury with external agents. Younger age groups in the both the sexes are more physically active and are at risk for corneal injury. [7,5] These findings were similar to studies done by Tewari A, et al from Ahmedabad in 2012[12] showed that maximum patients were from age group 21-40 (52.6%) years followed by patients in the age group 41-60 (39.3%) years. Males were mostly affected (68%) than females (32%). Male to female ratio was 2.12:1 which coincides with the findings of present study. Kumar A, et al. from Gujarat in 2011 [13] showed that the frequency of keratitis was greater in male (61%) than in female (39%) and is significantly higher among those aged < 50 years (73.62%). This age preponderance was similar to studies of Bharati et al [14] Srinivasan et al, [4] Basak S, et al [15] and Gopinathan et al, [16] where the middle decades of life were affected the most.

In this study, a history of injury to the cornea (45.36%) was identified as the principal predisposing factor for the development of corneal ulcer, representing 18 cases of bacterial (78.26%) and 28 cases of fungal (90.32%) keratitis respectively. This is similar to the findings of studies done by Gopinathan U, et al. (54.4%), [16] S. Ahmed, et al (59.18%) [17] Higher findings were showed by study done by Tewari A, et al. Ahmedabad (90%), [12] BasakS et. al from West Bengal (82.9%), [15] Bharathi MJ, et al from South India (70.88%). [14] In this study, most of the patients were farmers (58.76%), followed by labourers (14.43%), carpenters and students (5.15%), housewives and unemployed (9.27%). This occupational profile is similar to the study done by Bharathi MJ, et al (59.03%), [18] Basak S, et al (57.7%), [15] Shrinivasan M, et. al. (56.4%), [2] and Narsani A, et. al. (55.15%). [19] In this study, corneal injury with vegetative matter predisposing to corneal infection was found to be higher (74.19%) than other agents. Out of 71 cases of h/o trauma with the vegetative matter 28 (39.43%) developed fungal keratitis whereas 12 (16.90%) developed bacterial keratitis.

In 23.8% cases, bacteria were identified from culture. Patient in whom antibiotic therapy was initiated before clinical examination, the diagnosis was difficult to be obtained and 53 cases did not show any culture isolates (including viral keratitis cases). Gram nagative bacteria were predominant (60.86%) in 14 cases, mainly *Pseudomonas* species (26.08%) were isolated followed by *Klebsiella* species, *Proteus* spp. Gram positive bacteria accounted for 9 cases (39.13%), mainly *Staphylococcus* species were isolated. Mixed infection was noted in 2 cases. Filamentous fungi were more common in which *Aspergillus* species were predominant (74.19%), followed by *Fusarium* spp. (9.67%). In this study, 53.6% cases were microscopy and culture positive, and 46.39% cases had no definite laboratory diagnosis. Possible reasons for this could be some patients were already on topical medication when they arrived, defective scraping technique by ophthalmologist on call, and/ or problems in microbiology transport and handling.

In our study, 6 cases each of *Staphylococcus aureus* (26.08%) and *Pseudomonas aeruginosa* (26.08%) were the predominant bacterial isolates, followed by 4 cases of *Klebsiella* spp (17.39%), 3

cases of Coagulase negative staphylococcus (13.04%) and 2 cases (8.69%) of each *Proteus mirabilis* and *Proteus vulgaris*. These results were consistent with the work done by Basak S, et.al. [(15)] Tewari A, et al., [(12)] Narsani AK, et al, [(19)] Sothi S, et al, [(20)] who also found *Staphylococcus aureus* and *Pseudomonas* spp. as the most common bacterial isolates.

A clinical diagnosis of bacterial keratitis was put on the basis of presence of keratitis, conjuctival hyperaemia, congestion, mucopurulent exudates and presence of hypopyon. 23 out of 31 clinically diagnosed bacterial keratitis yielded growth in culture, thus showing the statistically significant correlation between the clinically diagnosed and culture positive bacterial keratitis. (χ^2 = 52.18, P< 0.0000001)

Staphylococcus aureus, the most prevalent bacterial isolate was 83.33% sensitive to Erythromycin, 66.7% sensitive to Gentamicin and Cotrimoxazole, 50% to Cefuroxime and 33.34% sensitive to Penicillin, Cefoxitin, Ciprofloxacin, whereas the Coagulase negative staphylococcus spp. were 66.67% sensitive to Erythromycin, Gentamicin, and Cotrimoxazole, 33.34% sensitive to Ciprofloxacin, Penicillin, Cefoxitin, Cefuroxime. Recently, Afshari, et al reported an increase in the resistance of gram positive keratitis isolates to ciprofloxacin from 12% to 22% over 10 months period in consecutive years. Biradar S, et al from Karnataka [(21)] showed that 49% of *Staphylococcus aureus* were resistance to two or more antibiotics. 14% of *Staphylococcus aureus* were resistant to Methicillin. All *Pseudomonas* spp. (100%) were sensitive to Amikacin and Imipenem, 83.33% to Gentamicin, Piperacillin, Piperacillin/Tazobactam and Ciprofloxacin, 66.7% to Cefepime. *Klebsiella* spp. were 75% sensitive to Ciprofloxacin, 50% to Gentamicin, Cefepime and 25% to Ampicillin, Cefuroxime, Ceftriaxone, Ampicillin/Clavulanic acid. *Proteus* spp. were sensitive 75% to Ciprofloxacin, 50% to Gentamicin, Cefepime, Cefuroxime, Ceftriaxone and 25% to Ampicillin and Ampicillin/Clavulanic acid. Biradar S, et al from Karnataka [(21)] showed that, most of the bacterial isolates were sensitive to Amikacin, Gentamicin and Ofloxacin. Barathi M J, et al from South India [(18)] showed that, the gram negative isolates were susceptible in highest percentage to Amikacin (93.51%), followed by Gatifloxacin (92.66%), Ofloxacin(88.72%), Ciprofloxacin (86.64%). Out of 97 cases of keratitis 45 (46.39%) cases did not turn up for follow up. Out of remaining 52 patients, who were followed for a week or more, 29 cases (29.8%) had good visual outcome with visual acuity better than the level at admission.

Among the sterile culture in 15.55%, final visual outcome was poor or deteriorated. 42.85% of bacterial keratitis and 34.48% of fungal keratitis improved with prompt treatment with antibiotics, antifungal therapy respectively. 19.09% of bacterial, 37.93% of fungal and 50% of mixed keratits deteriorated in spite of treatment.

Statistical analysis revealed that very poor visual outcome was significantly correlated with the history of pre-existing ocular/ systemic conditions. In the present study, complications of microbial keratitis were noted in 23 cases. Amongst them, 18 cases showed corneal scarring, 4 cases showed corneal thinning with descematocele, 1 case showed endophthalmitis and 1 case showed perforation. Evisceration was done in 2 cases. 18 cases were referred for keratoplasty to the higher centre. Study done by Schaefer F, et al [(22)] showed that, among the patients treated with standard therapy 4% had poor clinical outcome. Amongst those patients, 2 developed corneal perforation and 1 developed corneal abscess. Out of 13% of patients, who did not benefit from the standard treatment, 13% had poor clinical outcome, out of which one had thinning of cornea and other, developed irregular astigmatism. Complications were

mostly associated with initial treatment with corticosteroid self medication, poor patient compliance and patients with ocular pathology and systemic diseases like leprosy, diabetes mellitus, postoperative keratitis, bell's palsy with exposure keratitis.

However, in our study, 29.8% cases were treated with appropriate treatment.

CONCLUSION

Microbial keratitis is rare in the absence of predisposing factors and it is frequently associated with h/o trauma. The epidemiology and aetiology of microbial keratitis is specific to the region. Identifying the at risk population, screening for predisposing factor and educating the people at risk about protective measures such as wearing of goggles during harvest season and importance of early consultation to a trained ophthalmologist and treating the co-existing ocular diseases may reduce the occurrence of microbial keratitis. This regional information of aetiological agent is very important as this help us to have a high degree of clinical suspicion in starting the appropriate initial treatment before getting the microbiological confirmation. Routine microbiological examination of corneal ulcer is necessary to analyze & compare the changing trends in the microbial etiology & their susceptibility pattern to formulate a proper & appropriate antibiotic response against corneal ulcer.

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