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BACTERIAL AND FUNGAL ISOLATES IN CORNEAL ULCERS CASES AT TERTIARY CARE CENTRE

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ABSTRACT

Background: Corneal blindness is the fourth most common cause of blindness in the world (5.1%) and the significant causes are ulceration of cornea and trauma of eyes. The importance of microbiological evaluation of an etiological component and antibiotic sensitivity is very important. **Aim:** This study was conducted to evaluate sociodemographic risk variables, assess the diagnostic value of Gram staining, the responsible microorganisms, and examine the antibiotic sensitivity profile of bacterial isolates in this tertiary care centre. **Materials and Methods:** This study was conducted in a tertiary care hospital and was observational, descriptive, and cross-sectional in nature. Before collecting data, Institutional Ethics Committee (IEC) approval was obtained. 100 patients with corneal ulcers having an infectious aetiology were included in the study. **Results:** Male to female ratio was 1.2:1. The highest incidence of corneal ulcers was observed in industrial labours (32%), followed by housewives (24%). Staphylococcus epidermidis (30%), Staphylococcus aureus (20%) followed by Pseudomonas aeruginosa (11%) were the major organisms isolated in corneal ulcers. Sensitivity, Specificity, PPV and NPV of Gram staining with culture as a gold standard in present were 66.32%, 97.11%, 97.89% and 64.75%, respectively. Bacterial isolates were sensitive to Sparfloxacin (88%) followed by Ciprofloxacin (72%) and Netilmycin (64%). Bacterial isolates were resistant to Penicillin (83%) followed by Carbenicillin (70%) and Piperacillin (69%). **Conclusion:** Gram stain findings, culture data, and in-vitro tests of antibiotic sensitivity from microbiological work up can all be very helpful in determining the best course of treatment for bacterial keratitis.

Keywords: Colour blindness, keratitis, Trauma.

INTRODUCTION:

After cataract, glaucoma, and age-related macular degeneration(AMD), corneal blindness is the fourth most common cause of blindness in the world (5.1%). The significant causes of

corneal blindness are ocular trauma and ulcerations of cornea.[1] It is important to treat microbial keratitis medically and should be considered an emergency. A wide range of microbiological pathogens are to blame for the corneal infection.[2]

The World Health Organization (WHO) claims that microbial keratitis, which causes corneal blindness, is a "silent pandemic" that is occurring unreported all across the world.[1,3] The loss of corneal epithelium accompanied by clinical signs of inflammation, either with or without hypopyon, is known as corneal ulceration. Due to the frequently quick progression of this to the potential of blindness, it is an ocular emergency. Depending on the type of injury, the environment, and other sociodemographic factors of the person, several types of causal organisms are present. Antibiotics must be used appropriately and promptly to stop the progression of the disease and avoid consequences[5].

The identification of the microbial agent responsible for the disease is accomplished using microscopy (gram staining) and laboratory investigations, which involve the culture of corneal scrapings. Even though culture is the highest standard, it can take up to 14 days to complete.[12]

At light of the aforementioned facts, this study was designed to evaluate the sociodemographic risk variables, assess the diagnostic value of Gram staining, the responsible microorganisms, and examine the antibiotic sensitivity profile of bacterial isolates in this tertiary care centre.

MATERIALS AND METHODS

This study was conducted in a tertiary care hospital and was observational, descriptive, and cross-sectional in nature. Before collecting data, Institutional Ethics Committee (IEC) approval was obtained. 100 patients with corneal ulcers having an infectious aetiology were included in the study. 25 of these patients were serious cases and needed to be admitted. Patients were excluded if they declined to take part, had Mooren's ulcer, viral ulcers that were not secondary infected, or if they were newborns. Based on the treatments received, before admission to the hospital, the patients were divided into two groups, definitive and probable. The patients who could produce the antibiotics or knew the name of antibiotic were included in the definitive group, whereas those who had had therapy but were unable to specify the antibiotic they had received or had taken steroids, or had received no treatment were included in the probable group. Before collecting any data, written informed consent was obtained. Standard operating procedures were developed, along with follow up until the very end. Each patient's evaluation was done, according to protocol, which includes a thorough history, particularly in light of risk factors and previous antibiotic use treatment, a comprehensive

clinical evaluation, and ophthalmic standard evaluation and laboratory practises as per protocol. Under slit lamp examination, the ophthalmologist took sterile surgical blade No. 15 or a small sterile needle scrapings from the ulcer's edges and centre. The scraping was done several times. The material is extracted from the base of the ulcer as well as from the leading edge of an active ulcer. The conjunctival specimens were collected using swabs moistened in Brain Heart Infusion broth. The lower cul-de-sac is completely wiped. Before being inoculated in Chocolate agar (CA) and Sabourauds Dextrose agar with Chloramphenicol (SDA), the sample was first placed in Brain Heart infusion Broth (BHI) and Blood agar . From corneal scrapings and conjunctival swabs, lactophenol blue wet mounts, smears for gramme stain, giemsa stain, kinon C cold carbafuchsin stain for Nocardia, and Ziehl-Neelsen (20%) for mycobacteria were made. Gram staining was used for identification of bacteria, and lactophenol blue wet mounts and Giemsa staining were used for identification of fungi. BHI (Brain Heart infusion broth), BA (Blood agar), CA (Chocolate agar), SDA (Sabouraud Dextrose agar) was used for the inoculation of corneal scrapings and conjunctival swabs. After 24 hrs, the sample was reported as having no bacterial growth, if no growth was observed in blood agar, chocolate agar and brain heart infusion broth. Colony morphology was noted if growth was present and to study the morphology of the organism, a gram stained smear was prepared from the colony. Standard Kirby Bauer disk diffusion method on Mueller - Hinton agar was used for screening of cultures. Gram positive Staphylococcus aureus, Gram negative Escherichia coli and Pseudomonas aeruginosa standard strains were used as controls for antibiotic susceptibility testing. As per NCCLS standards 1997[4], the sensitivity results were evaluated as resistant, intermediate and sensitive. Data was recorded in Microsoft excel and analysed with SPSS. v.10. Frequency and proportion were the descriptive statistics used. To summarise the results, tables and figures were used. To evaluate the diagnostic test, sensitivity and specificity were used.

RESULTS:

Table 1: Age and gender distribution of patients.

Age group (Years)	Number of males (%)	Number of females (%)	Total (%)
0 to 11	1 (1%)	1 (1%)	2 (2%)
12 to 21	8 (8%)	9 (9%)	17 (17%)
22 to 31	8 (8%)	6 (6%)	14 (14%)
32 to 41	10 (10%)	8 (8%)	18 (18%)

42 to 51	9 (9%)	7 (7%)	16 (16%)
52 to 61	10 (10%)	8 (8%)	18 (18%)
>61	9 (9%)	6 (6%)	15 (15%)
Total	55 (55%)	45 (45%)	100 (100%)

Table 1 shows that males were 55% and females were 45% out of 100 cases of corneal ulcers. Male to female ratio was 1.2:1. Predominant age was between 21 to 51 years. There was 1 case each in both males and females below the age of 11 years. In males, there were more proportion of corneal ulcers in each age group when compared to females.

Table 2: Occupation profile distribution of patients.

Occupation	Number	Percentage (%)
Farming	8	8
Labour	32	32
Housewife	24	24
Student	21	21
Senior Citizen	9	9
Others	6	6

Table 2 shows that the highest incidence of corneal ulcers was observed in industrial labours (32%), followed by housewives (24%), students (21%), senior citizens (9%) and farmers (8%).

Table 3: Injury profile distribution of patients

Type of injury (n=80)		Number(Percentage)
Foreign body	Total	40(40%)
	Vegetative	10(10%)
	Others	30(30%)
Trauma	Total	40(40%)
	Surgical	5(5%)
	Others	35(35%)

Table 3 shows that out of 80 cases, 40 cases had injury due to foreign body and trauma each. Vegetative foreign body injury was observed in 10% cases. Surgical trauma was observed in 5% of cases. History of eye injury within 3 months was observed in 86 cases.

Table 4: Corneal isolates distribution.

Bacterial Isolates	Number	Percentage (%)
Staphylococcus epidermidis	22	30

Staphylococcus aureus	15	20
Streptococcus pyogenes	5	7
Streptococcus pneumoniae	4	5
Streptococcus viridians	4	5
Pseudomonas aeruginosa	8	11
Klebsiella pneumonia	4	5
Escherichia Coli	4	5
Fungal Isolates		
Fusarium	2	3
Candida species	2	3
Acremonium	2	3
Paecilomyces	2	3
Total growth positive	64	64
Mixed growth	10	10
No growth	36	36

Table 4 shows that the major organisms isolated in corneal ulcers were Staphylococcus epidermidis (30%), Staphylococcus aureus (20%) followed by Pseudomonas aeruginosa (11%). Streptococcus pyogenes (7%), Streptococcus pneumonia (5%), Streptococcus viridians (5%), Klebsiella pneumonia (5%) and Escherichia Coli (5%). Out of total isolates from 100 cases, 64% isolates showed growth and 36% showed no growth and 10% showed mixed growth.

Table 5: Evaluation of microscopy of gram stained corneal smears compared with culture results.

Smear and culture results	Number	Percentage (%)
Only smear +ve culture –ve	2	2
Smear –ve culture +ve	18	18
Smear +ve culture +ve	32	32
Total smear +ve	35	35
Total culture +ve	48	48
Smear –ve culture –ve	30	30

Table 5 shows that Out of total 100 cases, 48% showed growth on culture while 35% cases were smear positive. Both were positive in 32% cases and both were negative in 30% cases. Only in two cases (2%), smear was positive but culture was negative. In 18% cases, culture

were positive but smear results were negative.

Sensitivity, Specificity, PPV and NPV of Gram staining with culture as a gold standard in present were 66.32%, 97.11%, 97.89% and 64.75%, respectively. Out of 100 cases,19% conjunctival swab showed growth and 81% corneal swab showed growth. Out of 19 conjunctival swabs, 5 cases were S.aureus, 14 cases were S.epidermidis. Out of 5 cases of S.aureus, 4 cases were isolated in corneal scrapings. Out of 14 cases of S.epidermidis, 10 were isolated in corneal scrapings. Overall sensitivity ranged from 17% to 92% and resistance ranged from 12% to 85%. Bacterial isolates were sensitive to Sparfloxacin (88%) followed by Ciprofloxacin (72%) and Netilmycin (64%). Bacterial isolates were resistant to Penicillin (83%) followed by Carbenicillin (70%) and Piperacillin (69%). The most often isolated organism was Staphylococcus epidermis, which is extremely sensitive to Ciprofloxacin, Netilmycin, and Sparfloxacin. Staphylococcus aureus was extremely sensitive to Netilmycin, Sparfloxacin and Piperacillin. Streptococcus species was extremely sensitive to Erythromycin, Ciprofloxacin, Sparfloxacin and Cephalothin. Pseudomonas aeruginosa was sensitive to Ciprofloxacin, Tobramycin and Kanamycin while E. coli and K. pneumoniae sensitive to Netilmycin, Kanamycin and Sparfloxacin.

DISCUSSION

In Sharma et al.[6] study, it was reported that incidence of infectious keratitis was observed in 61.58% males and 38. 42% females with Male: female ratio of 1.6:1 with most common age group of 41-50 years (28.46%). In Joshi et al.[7] study, it was reported that 66.9% males and 33.08% female cases with most common age group between 30-60 years. Similar results were observed in Seal et al[8] and Bashir et al[9] studies. Male to female ratio was 1.8:1 with mean age of 35 years was observed in Sunitha R Bhandari et al[10] study. In the present study, male to female ratio was observed to be 1.2:1 with mean age of 36 years. In outdoor activities, males were more exposed to the risk factor like trauma, so, males reported higher incidence. In present study, the highest incidence of corneal ulcers was observed in industrial labours (32%), followed by housewives (24%), students (21%), senior citizens (9%) and farmers (8%). Similar results were observed in Sunitha R Bhandari et al[10] study which was highest incidence was found in industrial labourers (38.5%) followed by housewives (23.25%). In Ibanga et al.[11] study, it was reported that higher incidence in labours (29%) and students (19%) while in Venkatesh et al.[12] study, it was reported that higher incidence in farm labourers (89.02%). In Bashir et al.[9] study, it was also reported that highest incidence was in farmers. The present study results was slightly different from other study results as study was

conducted in urban area, so, most cases were of industrial labours. In present study, out of 80 cases, 40 cases had injury due to foreign body and trauma each. Vegetative foreign body injury was observed in 10% cases. Surgical trauma was observed in 5% of cases. Study conducted by Seal et al[8]. it was reported that 44% cases were due to ocular trauma while in Jampla et al[13]. study, it was reported that 14% cases were with trauma and 3% cases with post-operative trauma. In Sunitha et al study[10], trauma was cause for 45% cases and surgical trauma was cause for 4% cases. These findings were concurrent with the findings of Jampla et al.[13] In Sharma et al[6]. study, it was reported that the results were concurrent with present study as vegetative cause of trauma reported in 33% cases and 45% has a history of trauma. In present study, the major organisms isolated in corneal ulcers were Staphylococcus epidermidis (30%), Staphylococcus aureus (20%) followed by Pseudomonas aeruginosa (11%). Streptococcus pyogenes (7%), Streptococcus pneumonia (5%), Streptococcus viridians (5%), Klebsiella pneumonia (5%) and Escherichia Coli (5%). In Sunitha et al study[10], Staphylococcus epidermidis (34%), Staphylococcus aureus (22%) followed by Pseudomonas aeruginosa (14%) were commonest organisms isolated from growth. Joshi et al[7]. reported A. fumigatus (53%), staphylococcus (15%) and Streptococcus (13%) as commonest organisms. In Sedhu et al[14]. study, it was also reported that Aspergillus and S. Pneumoniae were the most common isolates. In study conducted by Venkatlakshmi et al[15]., it was reported that findings were concurrent with present study as they reported, S. Epidermidis (47%) and S. Aureus (21%) as the most common isolates. Jampla et al[13]. reported P. Aeruginosa (24%) and S. Aureus (14%) as the most common bacterial isolates and Candida as a fungal isolates (44%). In Sunitha et al[10] study, bacterial isolates were found in 46 cases and fungal isolates were in 4 cases. In Jampla et al.[13] study, it was reported that fungal isolates were in 12% cases. In study conducted by Seal et al[8]., it was reported that10% mixed growth, 48% bacterial growth and 28% fungal growth. In Ranjini et al.[16] study, it was reported that Fusarium and Aspergillus as the most common fungal isolates and S. aureus and P. Aeruginosa as the most common bacterial isolates. In present study, sensitivity, specificity, PPV and NPV of Gram staining with culture as a gold standard were 66.32%, 97.11%, 97.89% and 64.75%, respectively. Similar results were observed in Sunitha et al[10] study, sensitivity, Specificity, PPV and NPV of Gram staining with culture as a gold standard were 64.44%, 96.43%, 96.67% and 62.79%. In contrast to the present study, in Jampla et al.[13] study, it was reported that Gram stain's sensitivity of 16.67% (95% CI: 4.84% to 37.40 %), specificity of 83.33% (95% CI: 36.10 % to 97.24 %), PPV (80%) and NPV (20%) in the case of bacterial infection. Out of 100 cases,19% conjunctival swab showed growth and 81%

corneal swab showed growth. Out of 19 conjunctival swabs, 5 cases were S.aureus, 14 cases were S.epidermidis. Out of 5 cases of S.aureus, 4 cases were isolated in corneal scrapings. In Sunitha et al[10] study, out of 14 cases of S.epidermidis, 10 were isolated in corneal scrapings were reported in present study. 4 (5.41%) cases were S. aureus, 9 (12.3%) cases were S. epidermidis, out of 13 conjunctival swabs. Out of these 9 isolates of S. epidermidis from conjunctival swabs, the same organism was isolated from corneal scrapping on culture in 6 cases (8.21%) and similarly, out of 4 isolates of S Aureus from conjunctival swabs the same organisms was obtained from corneal scrapping in 3 cases (4.10%). In present study, Bacterial isolates were sensitive to Sparfloxacin (88%) followed by Ciprofloxacin (72%) and Netilmycin (64%). Bacterial isolates were resistant to Penicillin (83%) followed by Carbenicillin (70%) and Piperacillin (69%). The most often isolated organism was Staphylococcus epidermis, which is extremely sensitive to Ciprofloxacin, Netilmycin, and Sparfloxacin. Staphylococcus aureus was extremely sensitive to Netilmycin, Sparfloxacin and Piperacillin. Streptococcus species was extremely sensitive to Erythromycin, Ciprofloxacin, Sparfloxacin and Cephalothin. Pseudomonas aeruginosa was sensitive to Ciprofloxacin, Tobramycin and Kanamycin while E. coli and K. pneumoniae sensitive to Netilmycin, Kanamycin and Sparfloxacin. Similar results were observed in Sunitha et al[10] study. All Gram +ve bacteria were sensitive to moxifloxacin. It was reported as 89.33% sensitivity was seen in S. epidermidis isolates to Ciprofloxacin, Chloramphenicol, and Gatifloxacin. S. aureus strains were sensitive to Chloramphenicol (75%) and moxifloxacin (100%). Among the Gram -ve isolates, Pseudomonas aeruginosa exhibited good sensitivity to Ofloxacin (100%) and Chloramphenicol (100%) in Venkatlakshmi et al study.[15] Gram-negatives were sensitive to colistin and grampositives to vancomycin and aminoglycosides. Gram-ve isolates were susceptible in highest percentage to moxifloxacin amikacin and meropenem (84.26% each) followed by gatifloxacin. Among gram-positive isolates, moxifloxacin showed sensitivity of 91.66% and gatifloxacin showed sensitivity of 94.43%. Moxifloxacin showed highest sensitivity against P.aeruginosa. All yeast isolates were sensitive to tested antifungal drugs. Overall, highest sensitivity showed by amikacin (92.06%) followed by gatifloxacin and gentamicin were reported in Seal et al[8] which was similar to the results of Ranjini et al.[16] study.

Conclusion:

Gram stain findings, culture data, and in-vitro tests of antibiotic sensitivity from microbiological work up can all be very helpful in determining the best course of treatment for bacterial keratitis.

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