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Original Research Paper

Microbiology

MICROBIAL PROFILE OF ULCERATIVE KERATITIS AT TERTIARY LEVEL HOSPITAL

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ABSTRACT **Background:** Keratitis is the term applied for inflammations of the cornea. Ulcerative keratitis is a common potentially sight threatening ocular infection that may be caused by bacteria, fungi, viruses or parasites. Corneal ulcer is an ocular emergency that requires prompt management to ensure the best visual outcome for the patient. Otherwise, it can result in scarring and blindness. **Aim and objectives:** To study Microbial causes and to identify risk factors, and corelation of clinical features of ulcerative keratitis with etiological agents **Materials and methods:** Total 325 corneal ulcer cases were studied. Corneal scrapings were collected and subjected for examination by Gram's stain, 10% KOH, bacterial and fungal culture. Antibiotic susceptibility of the bacterial pathogens was performed by Kirby Bauer disc diffusion method as per CLSI guidelines with various groups of antibiotics. MIC for vancomycin were determined for study isolates by Epsilometer test. **Results:** Totally 325 infectious corneal ulcers were studied in detail. Aetiological agents were isolated in 115 (35.38%) cases. Majority of the isolates were fungal agents (44.18%), belonging to the Aspergillus (21.05%), Fusarium (14.47%). Bacterial corneal ulcer was less common (25.49%). The predominant bacterial pathogen isolated was Staphylococcus epidermidis, followed by Pseudomonas aeruginosa. no Acanthamoeba were identified. The sensitivity of potassium hydroxide staining was almost 100% in culture-proven fungal cases. Trauma is the leading cause for the corneal ulcers. Majority of the bacterial isolates were susceptible to amikacin. **Conclusion:** Ulcerative keratitis being a sight threatening disorder, early suspicion, rational use of laboratory diagnostic procedures, identification of the causative organisms and timely institution of appropriate antimicrobial therapy based on the prevailing sensitivity pattern of the isolates could save the eye from this preventable cause of blindness.

KEYWORDS : Ulcerative keratitis, MIC, Corneal scrapings

INTRODUCTION:

Ulcerative Keratitis is defined as patients having corneal ulcer with loss of the corneal epithelium, underlying stromal infiltration and suppression associated with signs of inflammation with or without hypopyon.[1]

Corneal ulcer is an ocular emergency that can result in scarring and blindness and requires prompt management to ensure the best visual outcome for the patient.

Ulcerative keratitis is a common, potentially sight threatening ocular infection that may be caused by bacteria, fungi or viruses. Predisposing factors such as trauma, contact lens wear, dry eye, glaucoma, epithelial defect, systemic disease and immunosuppressant may alter the defence mechanisms of the eye and permit bacteria to invade cornea. The clinical picture of keratitis caused by various microorganisms is indistinguishable therefore laboratory diagnosis plays a significant role in the treatment and outcome of Ulcerative Keratitis. [1] [2] [3]

The most common bacterial agent is *S. aureus*. Filamentous moulds especially Fusarium spp. and *Aspergillus spp.*, and yeast, most commonly *Candida spp*. produce a lesion that may resemble bacterial infection, delaying diagnosis and appropriate treatment. Herpes simplex virus can cause an ulcerative lesion called dendritic keratitis because of the branching pattern of the lesions. Reactivated varicella zoster virus can produce a similar keratitis, if it affects the ocular branch of the trigeminal nerve. In people who wear contact lenses, *Acanthamoeba spp*. (a free-living amoeba) can produce ulcerative lesions that are frequently very painful.[4]
Laboratory diagnosis includes microscopy and culture of corneal scrapings for identification of the microbial agent to start the specific antimicrobial therapy. Given the rapid progression and virulent nature of many infectious agents, any corneal inflammation should be considered a threat to vision, requiring prompt treatment and the empirical therapy is based on the prevailing antibiotic susceptibility profiles, which can be subsequently modified based on the culture and sensitivity results. Antimicrobial susceptibility tests are mandatory to monitor the efficiency of available antimicrobial agents and the emergence of drug resistance among bacterial and fungal isolates.[5]

In view of these observations, the present study was undertaken to identify microbial causes of infective Keratitis. This will help to start the specific therapeutic measures for better outcome.

AIM AND OBJECTIVES:

To study microbial causes and to identify risk factors, and corelation of clinical features of ulcerative keratitis with etiological agents of ulcerative keratitis

MATERIALS AND METHODS:

This is a prospective descriptive study of 325 corneal scraping specimens received during a study period of 18 months in the Department of Microbiology of a tertiary care centre, Mumbai from patients presenting with corneal ulcers at the Outpatient of the Department of Ophthalmology irrespective of age and gender.

Inclusion Criteria: -

All age group patients visiting outpatient department of ophthalmology with ulcerative keratitis.

Exclusion criteria:-

Not willing for consent Peripheral ulcerative keratitis Systemic autoimmune diseases Severe systemic debilitating illness

Methodology:

After taking informed consent, a proforma was filled out for each patient documenting age, sex, occupation, residence, predisposing factors and treatment history. Corneal scraping from both the leading edge and the base of each ulcer was collected under aseptic conditions by an ophthalmologist under the magnification of a slit-lamp beam after instillation of 0.5% proparacaine, using a no.15 Bard Parker blade.

Scrapings were smeared onto a glass slide and stained with Gram stain. 10% KOH mount was performed and examined for fungal elements and Acanthamoeba cysts. Scrapings were inoculated directly onto blood agar, chocolate agar and MacConkey agar and 2 sets of Sabouraud dextrose agar with chloramphenicol in row of C-shaped streaks. All bacterial cultures were incubated at 37°C, with the chocolate agar in a CO2 jar and examined at 24 hrs, 48 hrs and 72 hrs. Fungal cultures were incubated at room temperature and 37°C and incubated up to 6 weeks. Isolates were identified using standard protocol

Microbial cultures were considered positive only if growth of the same organism was on >2 media. Bacterial isolates were identified by standard protocol and were tested for antibiotic susceptibility using Kirby-Bauer disc diffusion method on Mueller Hinton agar and were interpreted in accordance with the CLSI guidelines [6][7]. MIC for vancomycin was determined by Epsilometer test as per CLSI guidelines.

Following adequate growth of the fungal isolate on SDA, identification was done based on its macroscopic and microscopic features. (? LPCB)

OBSERVATIONS AND RESULTS:

A total of 325 patients with the clinical diagnosis of ulcerative keratitis were enrolled for this study. Epidemiological characteristics of the population are recorded. A maximum of patients was from the age group 20–40 years (74.2%) followed by patients in the age-group 41–60 years (22%). Male predominance is noticed. Male female ratio was 2.2:1. The occupation profile of the study group mainly consisted of Farmers (50.3%), followed by laborers (16.41%), carpenter (13.6%), housewives (4%) and others (10.6%).

Predisposing factors were present in 105 cases; trauma (77.14%), local corticosteroids (5.71%), chronic dacryocystitis (4.29%), and blepharitis, endophthalmitis, surgery of same eye and diabetes mellitus in (2.86%), entropion (1.43%) others (8.57%).

Total 325 out of which 44.18% were fungal keratitis and 25.49% were bacterial keratitis and (30.33%) samples didn't give any growth. There was no cases of Viral keratitis and Amoebic keratitis.

Table 1: Bacterial isolates

PATHOGENS	FREQUENCY (%)
GRAM POSITIVE	
Staphylococcus epidermidis	33.33%
Staphylococcus aureus	20.51%
Enterococcus	7.69%
Micrococcus	5.1%
GRAM NEGATIVE	
Pseudomonas aeruginosa	12.82%
E. coli	7.69%
Non fermenter gram negative bacilli	7.69%
Enterobacter	2.56%
Klebsiella pneumoniae	2.56%
Citrobacter koseri	2.56%

Table 2: Fungal isolates

FUNGAL ISOLATES	FREQUENCY (%)
Aspergillus	21.05%
Fusarium species	14.47%
Filamentous fungi	5.26%
S. apiospermum	2.63%
C. albicans	1.31%
Bipolaris species	1.31%
S. rostrata	1.31%

In bacterial keratitis, 76.6% of people treated with conservative treatment whereas 23.07% treated with surgical methods. In fungal keratitis, 85.5% of people treated with conservative treatment whereas 14.47% treated with surgical methods.

DISCUSSION:

As there is no definite pathognomonic clinical feature, it is difficult to establish the aetiology of corneal ulcer merely on the basis of clinical features. Hence microbiological evaluation is a must in order to attain a definitive diagnosis and ensure specific therapy

In the present study incidence of corneal ulcer was seen most commonly in age group 21-40 years (72.5%) followed by 41-60years (22%) which is comparable with studies conducted by Amrutha KB et al,[8] Waghmare AS et al,[9] Mehta S et al,[10] Gotekar R. B et al,[11] whereas the study by Vasudha CL et al [12] showed 41-50 years as most common age group (38.8%), the study by Gotekar R.B et al [11] showed 56-70 years as the most affected age group (35%).

Agricultural workers (50.3%) were most affected in the present study due to exposure to the vegetative injuries because of their nature of work. This was in line with studies by Mehta et al[10], Vasudha CL[12], Jayashree MP et al[13]. This study shows that there is significant association between occupation and bacterial keratitis.

Predisposing factors such as corneal trauma in our study was seen in 77.14%. This was comparable with studies by Waghmare AS et al.,[9] Sharmila Raut et al.,[81] Metha S et al.,[10] Suwal S, 17 Gotekar R. B et al.,[11] Jayashree MP et al [13]

In our study, *Staphylococcus epidermidis* (33.33%) was the most common gram-positive bacteria followed by *Staphylococcus aureus* (20.51%), *Enterococcus* (7.69%) and *Micrococcus* (5.1%) species.

Among gram negative bacteria, *Pseudomonas aeruginosa* (12.82%) was the most common followed by E. coli (7.69%), *Non fermenter gram negative bacilli* (7.69%), *Enterobacter* (2.56%), *Klebsiella pneumoniae* (2.56%) and *Citrobacter koseri* (2.56%). Many studies have reported the common bacterial causes of microbial keratitis as *Pseudomonas aeruginosa, S. aureus, S. epidermidis*. Gopinathan et al have reported S. epidermidis as the predominant bacterial pathogen.[14]

Amikacin was found to be the most effective antibiotic covering 100% of isolates in the present study. This observation was similar to the studies by Chien Fan-Fong et al (2007) [15]and Chalita.M.R. R, et al, (2004) [16] where Amikacin was found to be effective against 93% - 95% of isolates. In the study of Savitri Sharma, et al, 1999, 71.7% isolates were sensitive to Chloramphenicol, 67.7% to Norfloxacin, 69 % to Ciprofloxacin and 70.6% to Gentamicin. Most common fungal isolate was *Aspergillus species* followed by *Fusarium species*. Similar findings were seen in studies

conducted by Vasudha CL et [12], Waghmare AS et al [9], while the study by Sharmila Raut et al showed *Aspergillus* as most common fungal isolate (37.4%)

100% responses were obtained from both antibiotic and surgical treatment with well-equipped medical practices.

CONCLUSION:

Corneal ulceration was predominant in adult males of rural background, with vegetative matter induced ocular trauma being the major predisposing factor. Fungal corneal ulcers were more common than bacterial corneal ulcers. A simple KOH preparation was highly beneficial as a rapid screening test for diagnosis. The vital role of microbiological evaluation in the management of infectious corneal ulcer is clearly evident, since the clinical features alone are not adequate to confirm infection. It is important to create awareness among people especially from rural background with regard to trauma as a major predisposing factor for corneal ulcers. Precise identification of the causative organisms and timely institution of appropriate antimicrobial therapy based on the prevailing sensitivity pattern of the fungal and bacterial isolates could prevent blindness. Such institutional studies will help in the formulation of antibiotic policy for a particular geographical area.

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