Microbiological study in cases of corneal ulcer and clinical outcome

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Abstract
Introduction: Cornea is anterior transparent layer of eye and important for image formation. Keratitis (corneal inflammation) and resulting corneal ulcer is important clinical challenge in ophthalmology. Prompt treatment requires identification of organism involved. Insight regarding types of organisms involved, patient demography, antibiotic sensitivity greatly helps in better patient management.

Materials and Methods: Patients with clinical diagnosis of ‘Corneal ulcer’, attending Department of Ophthalmology GMERS Medical College, Gandhinagar were selected for this observational study. Total 79 patients were studied. Detailed history and ocular examination were undertaken for each patient. Corneal scrapings from corneal ulcer were taken for pathological and laboratory study to identify type of organism and its antibiotic sensitivity. Data was arranged in tables for analysis. Diverse types of organisms and their frequency of occurrence was calculated. Patients were selected as per predefined criteria. Study recruitment was done after informed consent. Institutional ethics committee clearance was obtained.

Discussion: Corneal ulcer is important and serious ophthalmic problem contributing to significant portion of total blindness burden in our country. Understanding types and varieties of organisms gives insight regarding local community conditions. Antibiotic sensitivity study gives accurate guideline regarding precise use of antibiotic thereby shortening treatment period and minimizing antibiotic resistance. Comparison about various similar studies were carried out to elucidate findings.

Results: Patient demographics tables and graphs were prepared and analysis presented.

Keywords: Antibiotic, Community demography, Corneal ulcer, Organisms, Sensitivity.

Introduction
Cornea being the most anterior part of the eyeball, is exposed to atmosphere and hence prone to get infected easily. Most of the bacteria cannot easily penetrate the intact corneal epithelium. Also, other protective mechanisms, like eye lid movement and lysozymes in tear film, give additional protection. The disturbance in this system usually results in infective keratitis or commonly called ‘Corneal Ulcer’. Knowing the organism pattern greatly helps in management of this important clinical condition.

Materials and Methods
Study site and study population: Patients attending Department of Ophthalmology at GMERS Medical College and Hospital Gandhinagar.
Study design - A prospective Observationnel study.
Duration of Study: 2015 TO 2016
Sample Size: 79 cases with corneal ulcer
Inclusion criteria: Only the cases showing clinical signs of Corneal ulcer and reporting first time after their present episode were recruited for study.
Exclusion criteria: Patients who will be not willing to give consent for Participation and patients who had received topical ocular therapy elsewhere.

Methods of collection of data:
A total of 79 patients presenting with a corneal Infiltrate Compatible with a diagnosis of microbial keratitis selected from the OPD of Department of Ophthalmology of GMERS Medical College and Hospital Gandhinagar. A written informed consent was taken prior to investigating procedures. For the evaluation of the pre-disposing factor of corneal ulcer, the detailed history for demographic factors like age, sex, occupation and social status were recorded as per the predetermined proforma.

Corneal scraping was performed under aseptic conditions by an ophthalmologist using a sterile surgical blade No. 15. Scraping was performed at a slit-lamp biomicroscope after instilling topical 4% lignocaine (lidocaine) into the eye to ensure that adequate material was obtained and care taken to avoid perforation of the eye. Occasionally ulcers were very soft, sticky and had a mucoid consistency in which case they were too slippery and tenacious to be removed with a metal scraper. A disposable sterile cotton swabs was used (Jacob et al 1995). The scraping material obtained from leading edge and base of each ulcer was initially directly inoculated onto the surface of solid media such as blood agar, chocolate agar and Sabouraud dextrose agar in a row of C- shaped streaks taking care not to penetrate agar surface (Benson & Lanier 1992). The material obtained by the next scraping was spread onto labelled slides in a thin, even manner for 10 % potassium hydroxide (KOH) wet mount, Gram staining, Giemsa staining. After inoculation, the bacterial plates were incubated at 37°C for 24-48 hrs. then left for a week to detect slow growing bacteria, while the plates of Sabouraud dextrose agar were incubated at 28°C for a week to isolate fungi. Growth on the C-streaks is significant, while growth outside the C-streaks is likely to because of contamination. Gram staining is done to identify whether the organism grown is gram positive or gram negative. The organisms are further identified to genus and species.
level depending on motility and biochemical reaction. Antibiotic susceptibility testing was done. Identification of the micro-organisms was done using various biochemical reaction as well as routine tests. Biochemical tests were included to identify Gram positive (catalase, coagulase, etc.) and gram-negative bacteria (catalase, oxidase, indole, MR-VP, urease, citrate, TSI, O/F etc.). Data was arranged in tabulated form and percentages were calculated. Average and standard deviation were calculated for age.

Institute ethics committee clearance:
Institute Ethics Committee Clearance was obtained before the start of the study.

Table 1: Age wise distribution of cases in study group

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>9</td>
<td>11.4</td>
</tr>
<tr>
<td>20-40</td>
<td>25</td>
<td>31.6</td>
</tr>
<tr>
<td>40-60</td>
<td>35</td>
<td>44.3</td>
</tr>
<tr>
<td>&gt;60</td>
<td>10</td>
<td>12.7</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1: Bar diagram of occupation wise distribution of cases in Study group

Table 2: Predisposing factors wise distribution of cases in study group

<table>
<thead>
<tr>
<th>Predisposing Factor</th>
<th>Particular</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular Trauma</td>
<td>Vegitative Injury</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Injury by wooden object</td>
<td>8</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>Injury by unknown foreign body</td>
<td>5</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Stone Injury</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Thorn Injury</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Injury by animal tail</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Chemical Injury</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Total Ocular Trauma</td>
<td>35</td>
<td>44.3</td>
</tr>
<tr>
<td></td>
<td>Conjunctivitis</td>
<td>13</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Blepharitis</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>Dacryocystitis</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>19</td>
<td>24.1</td>
</tr>
<tr>
<td>Pre existing Trauma diseases</td>
<td>--</td>
<td>5</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Contact lens wearing diseases</td>
<td>--</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>Corticosteroid therapy</td>
<td>--</td>
<td>5</td>
<td>6.3</td>
</tr>
<tr>
<td>Post-operative</td>
<td>--</td>
<td>7</td>
<td>8.9</td>
</tr>
<tr>
<td>Viral Infection</td>
<td>--</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td>Not defined</td>
<td>--</td>
<td>79</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Microorganism isolated wise distribution of cases in study group

<table>
<thead>
<tr>
<th>Microorganism isolated</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>14</td>
<td>17.7</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>16</td>
<td>20.3</td>
</tr>
<tr>
<td>Fusarium</td>
<td>5</td>
<td>6.3</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>8</td>
<td>10.1</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>5</td>
<td>6.3</td>
</tr>
<tr>
<td>Corynaebacterium</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Fonsecaea Pedrosi</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>No growth</td>
<td>24</td>
<td>30.4</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>100</td>
</tr>
</tbody>
</table>
Discussion

In our study among the 79 corneal ulcer patients studied the commonest age group affected was between 40-60 yrs., followed by 20-40, >60 and <20 (Table 1). In a study conducted by Nath et al in 2011 the commonest age group was between 41-50 years which agreed with the present study. 69 In accordance with our results, Srinivasan et al. (1997) found that the most susceptible persons to keratitis were adults of 31-60 years (59% of 434 cases studied). Garg et al. (2000) found that the average age in keratitis was 46.5 years.

Our study was predominant by male patients (62%) as compared to female patients (38%) (Table 2). Similar male preponderance was found in other studies. Because of their work profile male are more exposed to outdoor activities which increases their vulnerability to the disease. Srinivasan et al. in 1997 studied 434 patients where males were affected more than females in the ratio of 2:1. Garg et al. in 2000 found male to female ratio of 4:1. A study conducted in 2005 by Butler et al. examined 190 patients of keratitis, out of which 54% were males and 46% were females. Males were more commonly affected than females in a study conducted by Bandyopadhyay et al.

Most patients with keratitis (out of 79 cases studied) were farmers (38%) followed by followed by Driver (19%), Housewife (17.7), service holder (12.7%), student (10.1%) and Unemployed (2.5) (Table 3). It is obvious that in the cases of males, occupational risk plays a key role in contracting the disease. The same result was seen in a study in India where the majority of 434 keratitis cases were farmers (56.4%) followed by housewives (12% of total cases) (Srinivasan et al. 1997). Similar findings were also reported in Egypt (Al ghali bi 2000) and China (Xie et al. 2001).

Trauma to the eye was the most significant risk factor of keratitis where 44.3% of the 79 cases were due to trauma followed by due to predisposing factor - 24.1 and due to others 24.1 (Table 4). Srinivasan et al. (1997) found also that the most predisposing factor for keratitis was corneal trauma in 284 (65.4%) out of 434 cases. Keay et al. (2006) also reported trauma as the most common risk factor in 106 (36.4%) out of 291 keratitis cases studied. Trauma was the commonest risk factor in a study conducted by Bandyopadhyay et al. in west Bengal. Risk factors other than trauma showed lower percentages (Table 4). Similar findings were reported by Panda et al. 1997, Wong et al. 1997, Yee et al. 1997, Al ghali bi 2000, Xie et al. 2001, and Keay et al. 2006.

Of the 79 cases of keratitis studied, 39.2% were due to bacterial infection, 30.4% were due to fungal infection while other 30.4% were negative for bacteria and fungi. This finding correlates with a study conducted by Basak et al. which showed 62.7% patients had fungal infection, 22.7% had bacterial infection while 14.1% had mixed infection. Srinivasan et al. in 1997 found 47.1% bacterial infection, 46.8% fungal infection and in 5.1% mixed infection. A study conducted by Leck et al. found that fungal isolates were 44% in southern India and 37.6% in Ghana as compared to 29.3% and 13.8% bacterial isolates from south India and Ghana respectively. Gopinathan et al. 2002 and Bharathi et al. 2003 found comparable results.

In our study among most common isolate was Staphylococcus aureus 16(20.3%) followed by Aspergillus 14(17.7%), Fusarium 5(6.3%), Streptococcus pneumoniae 8(10.1%), Candida albicans 4(5.1%), Pseudomonas 5(6.3%), Corynebacterium 2(2.5%) and no growth were 24(30.4%). Staphylococcus aureus was most common which is like the earlier study by Basak et al. However, The most common infecting bacteria was Streptococcus pneumoniae in Nepal and south India, and Pseudomonas spp in Ghana and Bangladesh. In our study, Staphylococcus aureus was 100% sensitive to ciprofloxacin and cefuroxime. Streptococcus pneumoniae was 50% sensitive to ciprofloxacin. Pseudomonas and Corynebacterium were 100% sensitive to erythromycin and cefuroxacin (Table 4). According to this study both second and fourth generation fluoroquinolones are equally effective against commonly found organisms in corneal ulcer. Ganapadhyay et al found no significant treatment difference between fluoroquinolone and fortified therapy in terms of final visual outcome, but concluded that fluoroquinolones have the advantage of decreased toxicity and duration of treatment.

In our study among fungal isolates, Aspergillus was the predominant isolate in West Bengal in a study conducted by Basak et al in 2005, 39.8 %. A study conducted by Despande & Koppikar, 1999 in Mumbai and parts of South India by Venugopal et al. 1989 showed comparable results. Comparable results were

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Staphylococcus aureus (n=16)</th>
<th>Streptococcus pneumoniae (n=8)</th>
<th>Pseudomonas (n=5)</th>
<th>Corynebacter (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>12</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>16</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Cotrimazole</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>16</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4: Sensitivity pattern of bacteriological isolates

reported in Nepal and Bangladesh by Upadhyay et al.,
1991 and Williams et al., 1987 respectively. But,
Fusarium spp. was found to be more common in other
studies in South India (Bharathi et al., 2003 (42.82%);
Leck et al., 2002 (39.9%); Srinivasan et al.,
1997(47.1%)). The Fusarium species preponderance
was also found in Paraguay, Florida, Hong Kong
and Singapore (Mino de Kasper et al, 1991; Liesegang &
These differences in predominance of fungal isolates
could be attributed to different climatic conditions.

Results
During study period, a total of 79 patients were
diagnosed with corneal ulcer and following parameters
were studied such as age, sex, occupation of patients
and predisposing factors for
corneal ulcer, identifying the causative bacteria and
studying the antibiotic sensitivity of these bacteria to
commonly used antibiotics.
1. In our study among the 79 corneal ulcer patients
studied the commonest age group affected was
between 40-60 yrs., followed by 20-40, >60 and <20
(Table 1).
2. Our study was predominant by male patients (62%)
as compared to female patients (38%) (Table 1).
3. Most patients with keratitis (out of 79 cases studied)
were farmers (38%) followed by followed by Driver
(19%), Housewife (17.7), service holder (12.7%),
student (10.1%) and Unemployed (2.5%) (Fig. 1).
4. Trauma to the eye was the most significant risk
factor of keratitis where 44.3% of the 79 cases were
due to trauma followed by due to predisposing
factor - 24.1 and due to others 24.1 (Table 2).
5. Of the 79 cases of keratitis studied, 39.2% were due
to bacterial infection, 30.4% were due to fungal
infection while other 30.4% were negative for
bacteria and fungi (Table 3).
6. In our study among most common isolate was
Staphylococcus aureus 16(20.3%) followed by
Aspergillus 14 (17.7%), Fusarium 5 (6.3%),
Streptococcus pneumoniae 8 (10.1%), Candida
albicans 4 (5.1%), Pseudomonas 5 (6.3%),
Corynebacterium 2 (2.5%) and no growth were
24(30.4%) (Table 3).
7. In our study, Staphylococcus aureus was 100%
sensitive to ciprofloxacin and cefuroxime.
Streptococcus pneumoniae was 50% sensitive to
ciprofloxacin. Pseudomonas and Corynebacterium
were 100% sensitive to erythromycin and
ciprofloxacin (Table 4).

Conclusion
The epidemiological patterns vary from one country
to the other and in different geographical areas in
the same country. Males are more prone to corneal ulcers
than females as they are more involved in outdoor
activities. Trauma is the leading cause for the corneal
ulcers and most of the fungal ulcers are because of
trauma due to vegetative matter. The incidence of the
bacterial & fungal keratitis is almost the same in this set
up. The higher incidence of fungal ulcers may be related
to the agricultural activities and the environmental
conditions. Microscopic evaluation of corneal smear and
culture remains the gold standard for the identification
of pathogens causing microbial keratitis. [6,7] Culturing,
however, allows sensitivity testing so that treatment
modifications can be made in an informed manner if the
clinical response to the initial treatment is inadequate.
Currently most commonly isolated organisms are
sensitive to second generation and fourth generation
fluoroquinolones currently being used. It is therefore still
justifiable that these can be used for treatment of
suspected bacterial keratitis as first line empirical
therapy. Persistent efforts should be put for continuous
surveillance and epidemiological characterization which
are imperative to treat and prevent morbidity and
blindness of population at risk in India.

Conflict of interest: None

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