Suppurative corneal ulceration in Bangladesh
A study of 142 cases examining the microbiological diagnosis, clinical and epidemiological features of bacterial and fungal keratitis

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Abstract
Suppurative keratitis is an important preventable cause of blindness, particularly in the developing world. This study analyses 142 cases of suppurative keratitis referred to Chittagong Eye Infirmary, Bangladesh. Some 53.5% of cases were bacterial and 35.9% were fungal. The five most common pathogens were: Pseudomonas sp. 24%, Strep-tococcus pneumoniae 17%, Aspergillus sp. 13%, Fusarium sp. 7% and Curvularia sp. 6%. Gram stain and culture results were consistent in 62.6% of cases. Previous antibiotic treatment was a significant factor for failure of culture isolation and less so for Gram stain failure. On Gram stain, 55.9% of pseudomonal cases were missed, but only 2% of fungal cases were missed. Over all, Gram stain had a sensitivity of 62% and positive predictive value of 84% for bacterial cases, and 98% and 94% for fungal cases, respectively. Fungal ulcers were typically filamentous, but an antecedent history of trauma was not common. The most frequent injury was due to rice grains, but the inoculum appeared to be introduced during eye washing with contaminated water. Pseudomonal ulcers occurred most frequently in the monsoon season, and Fusarium cases were seen only in the hot, dry season.

Key words: Bacteria, corneal ulcers, culture, epidemiology, fungus, Gram stain, keratitis.

Corneal opacification has become the second most common cause of visual disability in the world. Suppurative keratitis is an important preventable cause of blindness in the developing world. As championed by Jones, the attending doctor ideally needs the assistance of a trained microbiological service which can identify the corneal pathogens and report antimicrobial sensitivities. In reality, much of the developing world is denied this service.

The range of pathogens known to cause corneal ulcers is very broad and shows geographic variation. The tropics are noted for the increased prevalence of Gram-negative isolates. Filamentous fungi may account for a third of all cases of suppurative keratitis in the tropics.

Williams et al. established a low-cost microbiology laboratory in Chittagong Eye Infirmary and Training
### Table 1. Microbiological results

(a) Corneal smear microscopy and culture both positive

<table>
<thead>
<tr>
<th>Bacterial pathogens</th>
<th>Fungal pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Aspergillus fumigatus</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Aspergillus flavus</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>Fusarium solani</td>
</tr>
<tr>
<td>Streptococcus pyogenes GpA</td>
<td>Fusarium dimerum</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Curvularia fallax</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>Curvularia sp.</td>
</tr>
<tr>
<td>Moraxella sp.</td>
<td>Lasiodiplodia theobromae</td>
</tr>
<tr>
<td>E. coli</td>
<td>Scedosporium sp.</td>
</tr>
<tr>
<td>Total</td>
<td>Epicoccum sp.</td>
</tr>
</tbody>
</table>

(b) Corneal smear microscopy negative but culture positive

<table>
<thead>
<tr>
<th>Bacterial pathogens</th>
<th>Fungal pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas sp.</td>
<td>Fusarium solani</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td></td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td></td>
</tr>
<tr>
<td>‘Coliform’</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
</tr>
</tbody>
</table>

(c) Corneal smear microscopy positive but culture negative

<table>
<thead>
<tr>
<th>Bacterial organisms seen</th>
<th>Fungal hyphae seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive diplococci</td>
<td>2</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td>1</td>
</tr>
<tr>
<td>Gram-positive rods</td>
<td>1</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>2</td>
</tr>
<tr>
<td>Gram-negative coco bacilli</td>
<td>1</td>
</tr>
<tr>
<td>Gram-negative diplobacilli</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
</tr>
</tbody>
</table>

(d) Corneal smear microscopy and culture both negative — Total 15

Complex in 1983. A pilot study at this time and a later study of 127 cases has identified the prevalence of corneal pathogens in the region and demonstrated the value of Gram stain to allow rational selection of antimicrobial agents.

This study from the same base hospital aims to investigate further the value of the Gram stain as a first line investigation, particularly for demonstrating fungal elements. It also aims to give epidemiological information (prevalence, associated history, seasonality and clinical features) that may aid the clinician successfully to treat suppurative keratitis when Gram stain or culture results are not available.

**Patients and methods**

Inpatients and outpatients at the Chittagong Eye Infirmary with the diagnosis of suppurative keratitis were studied. Exclusion criteria were: herpetic ulcers, neuroparalytic keratitis, interstitial keratitis, ulcers associated with autoimmune conditions (e.g., Mooren's ulcer) and of course patient refusal.

Patient information, clinical histories and clinical features of infection were recorded at presentation and updated during treatment.

**Microbiological methods**

Representative areas of suppuration in the cornea were sampled using a sterile ‘Kimura’ platinum spatula or a scalpel blade. A local anaesthetic without preservative was used. Smears were fixed with 95% methanol before Gram staining by standard methods. The culture, identification and antibiotic sensitivity methods used were those described previously. Facilities for anaerobic, mycobacterial and acanthamoeba culture were not available. Some bacteria and all fungi were sent for identification and antifungal sensitivity testing was performed by Dr Y Clayton at St John’s Institute for Dermatology, St Thomas’ Hospital.

**Significance testing**

Means were compared using Students’ t test and proportions were compared using the $\chi^2$ test.
Results

One hundred and fifty-one cases were referred over an 11-month period. Nine were excluded under the aforementioned criteria. Eighty-nine cases (63%) showed consistent microscopy and culture results, while 27 cases (19%) showed growth on culture but no organisms were seen on Gram stain. In 11 cases (8%), organisms were seen on Gram stain, but not isolated on culture. In 15 cases (11%), no organisms were seen on Gram stain and culture was negative.

Table 1 outlines the corneal scrape results as defined by the mode of isolation.

Seventy-six patients (54%) had bacterial keratitis and 51 patients (36%) had fungal keratitis. In six patients both bacterial and fungal organisms were isolated. The bacteria isolated in these cases were Staphylococcus aureus (three cases) and Streptococcus pneumoniae (three cases). These bacteria may be components of the conjunctival flora. Their role in ulcer pathogenesis is unclear, but the fungal organisms were considered to be the major pathogens. These cases were therefore included with the other fungal cases for further analysis.

The five most commonly encountered pathogens were: Pseudomonas sp. 34 cases (24%), Streptococcus pneumoniae 24 cases (17%), Aspergillus sp. 19 cases (13%), Fusarium sp. 10 cases (7%) and Curvularia nine cases (6%). Note that of the 27 cases which showed growth on culture but no organisms on Gram stain, only one case was fungal, and 19 cases were pseudomonal (eight of these were P. aeruginosa). Table 2 evaluates microscopy by Gram stain as a diagnostic technique against the culture results as a standard. For fungal ulcers the sensitivity, specificity and predictive value of Gram stain microscopy is much higher than that for bacterial ulcers.

Comparison of the clinical features of bacterial and fungal corneal ulcers

The presenting features of bacterial and fungal ulcers were compared to identify characteristics which aid clinical differentiation. Table 3 shows eight features examined and the results obtained for each aetiological group. The main findings are:

1. Fungal ulcers had a significantly longer history compared with bacterial cases (mean durations 16.6 and 10.8 days respectively, \( P < 0.01 \)).

2. Pseudomonal ulcers had significantly larger mean epithelial defect and corneal infiltrate diameters (both \( P < 0.01 \)) compared with those caused by S. pneumoniae although there was no significant difference between mean duration of symptoms of the two groups. Pseudomonal ulcers also had a significantly larger mean defect diameter compared with that of the fungal group in total (\( P < 0.05 \)).

3. Pneumococcal ulcers had a significantly larger mean hypopyon height compared with the pseudomonal group: 1.9 mm and 1.3 mm respectively (\( P < 0.01 \)).

4. Forty-six per cent of pneumococcal ulcers showed corneal thinning of greater than half its thickness compared with 26% in the pseudomonal group.

5. Ulcers with a dry, raised, necrotic or fluffy surface were seen significantly more frequently in the fungal group compared with the bacterial group (23% v. 2.9%; \( P < 0.01 \)).

6. Endothelial rings were also seen more frequently in the fungal group (13% v. 7.4%).

7. Dacryocystitis in the affected eye was diagnosed in 33% of the patients with pneumococcal ulcers. This relationship is highly significant; the condition was seen in only four other cases in the series.

Other features examined were endothelial plaques, corneal vascularisation, intraocular pressure and corneal satellite lesions. Endothelial plaques were seen in only eight cases with no obvious difference between the groups. Satellite lesions were seen in only two cases, one bacterial and one fungal. Increased vascularity was observed in severe infections and when the ulcer was more peripheral. Raised intraocular pressure was found in only seven cases, two bacterial and five fungal.

Epidemiology

Table 4 shows the mean age of patients in each group, sex ratios, occupations and history of ante-
Table 3. Clinical features

<table>
<thead>
<tr>
<th>Feature</th>
<th>Pseudomonas n=34</th>
<th>Pneumococcus n=24</th>
<th>Other bacterial n=10</th>
<th>Aspergillus n=19</th>
<th>Fusarium n=10</th>
<th>Curvularia n=6*</th>
<th>Other fungal n=13</th>
<th>All bacterial n=68</th>
<th>All fungal n=48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean duration of symptoms in days</td>
<td>10.1</td>
<td>10.5</td>
<td>13.8</td>
<td>13.5</td>
<td>12.4</td>
<td>24.7</td>
<td>21.0</td>
<td>10.8</td>
<td>16.6</td>
</tr>
<tr>
<td>Mean diameter of epithelial defect (mm)</td>
<td>4.9</td>
<td>3.5</td>
<td>4.1</td>
<td>4.3</td>
<td>4.6</td>
<td>3.7</td>
<td>4.1</td>
<td>4.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Mean diameter of corneal infiltrate (mm)</td>
<td>6.6</td>
<td>4.4</td>
<td>7.5</td>
<td>5.5</td>
<td>5.9</td>
<td>4.9</td>
<td>5.6</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Mean height of hypopyon (mm)</td>
<td>1.3</td>
<td>1.9</td>
<td>2.0</td>
<td>1.6</td>
<td>1.8</td>
<td>0.9</td>
<td>1.2</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Percentage of eyes with corneal thinning &gt;1/2 thickness</td>
<td>26%</td>
<td>46%</td>
<td>50%</td>
<td>42%</td>
<td>30%</td>
<td>50%</td>
<td>38.5%</td>
<td>37%</td>
<td>40%</td>
</tr>
<tr>
<td>Percentage of eyes with dry, raised or fluffy ulcer surface</td>
<td>6%</td>
<td>0</td>
<td>0</td>
<td>26%</td>
<td>20%</td>
<td>33%</td>
<td>15%</td>
<td>29%</td>
<td>23%</td>
</tr>
<tr>
<td>Percentage of eyes with dacryocystitis</td>
<td>0</td>
<td>33%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16.5%</td>
<td>7.7%</td>
<td>11.8%</td>
<td>4.2%</td>
</tr>
<tr>
<td>Percentage of eyes with endothelial ring</td>
<td>6%</td>
<td>13%</td>
<td>0</td>
<td>26%</td>
<td>0</td>
<td>0</td>
<td>7.7%</td>
<td>7.4%</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

*No information for three cases hence, n=6.

cedant eye injury. Few children were seen; 23 patients were under 18 years and only two were under 10 years. The main epidemiological findings are:

1. Patients with pneumococcal ulcers tended to be older than those with pseudomonal infections (mean ages 52.5 and 29.8 years respectively; P<0.01).
2. The sex ratio for the series of 142 cases was 2.2 males to one female. The M:F ratio was higher in the pneumococcal group, 7M:1F.
3. No clear association between aetiology and agricultural occupation was found, but those with fungal ulcers were more likely to perform domestic work, e.g., housewives and servants (46% compared with 26% for bacterial cases).
4. A high percentage of patients gave no history of eye injury. Sixteen of those with pseudomonal ulcers (47%) had been injured with rice grains or leaves; 14 involved only grains. Three patients with Curvularia sp. infections reported injuries with vegetation, but more cases are needed to assess the significance of this finding.
5. The relationship between aetiology and date of onset was explored: 65% of the pseudomonal cases occurred during the monsoon period, July to September; and all of the Fusarium cases occurred in the first six months of the year, the hot-dry season. The other groups were evenly distributed throughout the 11 month study period.

Discussion

In this series, the Gram stain recovery rate of 70.4% (100 of 142) of cases is consistent with previous studies, range 57% to 70%, as is the culture recovery rate of 81.7% (116 of 142), range 60% to 84%. In 11 cases (7.7%), organisms were seen on Gram stain, but not isolated on subsequent culturing. Possible reasons for this include previous antibiotic treatment, inadequate specimens or insufficient microbiological investigations. Five of the eight bacterial cases in this category (Table 1c) had received antibiotics, a high percentage (63%) compared with prior antibiotic usage in the whole bacterial group (18%). In two of the three fungal cases (Table 1c) insufficient material was obtained for culture. A higher prior antibiotic usage (73%) was also reported in the 15 cases in which no pathogen was found on Gram stain or culture.

Of the 27 cases which showed growth on culture but no organisms on the original Gram stain, 19 cases (70.4%) were pseudomonal. In fact, more Pseudomonas sp. were seen in this mode (Table 1b) than when consistent with microscopy and culture (Table 1a; 15 cases). In this series, Gram stain is therefore a poor predictor of pseudomonal infection and we would advise appropriate antibiotic cover if the clinical history is suggestive and/or culture results are pending.

With reference to Table 2, however, it can be seen that most cases will be guided reliably by their Gram stain results if culture facilities are not
suppurative corneal ulceration in Bangladesh

available. This is not to say that culture investigations are not warranted. Williams et al. highlighted the usefulness of culture for the monitoring of the prevalence and antibiotic sensitivities of corneal pathogens.4

More significant is the fact that only one known case of fungal keratitis (2%) was missed on Gram stain, later to be identified on culture. A recovery rate of 98% is much more than predicted by Jones (78%),2 or Upadhyay et al. (55.4%).10 The value of the Gram stain, in our hands, to identify fungi cannot be overstressed, particularly as antifungals are expensive and in short supply and therefore cannot be used in a broad-spectrum treatment protocol, despite the high incidence of fungal keratitis.

Over 20 years ago, Jones observed that each geographic region has a differing prevalence of corneal pathogens.11 In accord, Asbell and Stenson recorded the changing prevalence of pathogens across America and also noted the change of prevalence within a region, with a change of socioeconomic status of the referral population.5 The observed trend is an increased incidence of Gram-negative and fungal isolates in the southern parts on America.12

This series shows that fungal keratitis accounts for 35.9% of presentations of suppurative keratitis against 53.5% of bacterial cases. The most common isolate was the Gram-negative organism Pseudomonas sp. (24%). Eighteen of the 34 Pseudomonal isolates were identified as P. aeruginosa. Other studies in the tropics – Bangladesh,4,13 India,14 and South Africa7,8 have shown S. pneumoniae and S. aureus to predominate. The association of pseudomonal infection with rice grain injuries in the wet, rice-growing season may account for this difference. There is also a high concentration of Gram-negative rods isolated from pond and canal water in this region. This water may be used to wash foreign bodies from eyes, or be used as initial local treatment ("holy water").1

Keratomycosis in tropical and subtropical regions is predominantly caused by filamentous fungi rather than yeast species.15 The three major fungal pathogens in this study were filamentous species; Aspergillus sp. 37% (19 of 51), Fusarium sp. 20% (10 of 51), and Curvularia sp. 18% (nine of 51). There was only one case of Candida keratitis. Lasiodiplodia and Scedosporium have been reported to cause keratomycosis. Dichotomothrophoropsis nymphaearum has been reported as a corneal pathogen only in this region, and only in one case.16

Clinical features

The microbial cause of corneal ulceration cannot generally be diagnosed clinically. Conclusions from this study must be tempered by the small number of cases observed for each pathogen, but the following are features we believe are worthy of mention. The fungal cases showed a longer history before presentation. The high prevalence of filamentous fungi meant that the ulcers took on the clinical appearance typical of the genera; a dry, raised,
necrotic or fluffy surface, with an endothelial ring surrounding the ulcer, as opposed to the ‘collar-button’ configuration typical of a yeast keratitis.15

Of the two most prevalent bacterial pathogens, the pneumococcal ulcers had smaller epithelial defect and infiltrate diameters, larger hypopyons and more severe corneal thinning than pseudomonal ulcers. Both had equal duration of symptoms before presentation. The association of pneumococcal ulcers with dacryocystitis is well recognised17 and re-emphasised in this series.

This study confirms that in the tropics, corneal ulcers tend to occur in adult males working outdoors. Interestingly, fungal ulcers were seen frequently in domestic workers and Curvularia showed a female predominance. Of patients with fungal ulcers, 65% reported no history of trauma. This is contrary to what was predicted, as the fungal inoculum has been thought to be due to a injury with vegetable matter.15,18

The most frequent injury in this series was due to rice grains or leaves. These have been shown to carry Bacillus sp. and fungi of the Mucorales family.3 The rice grain injuries caused more bacterial ulcers (31%) than fungal (13%) and the most frequent pathogen was Pseudomonas sp., indicating that the inoculum more likely occurred during the washing of the injured eye with contaminated water carrying the Gram-negative rod. The implications for population education are obvious.

This study also indicates that the absence of trauma (e.g., with vegetation) does not exclude a fungal pathogen. Practitioners should also be aware of the increased incidence of pseudomonal cases during the monsoon period and the increased incidence of Fusarium during the dry-hot beginning of the year.

Suppurative keratitis is relatively uncommon in the United Kingdom; 67 (only two fungal) over 20 months were reported in Moorfields by Coster in 1981.6 In the same year, Rahman saw 508 cases (22.6% fungal) at the Islamia Eye Hospital, Bangladesh.19 In many areas of the developing world trachoma, onchoceriasis, leprosy and other infectious causes of ocular disease are endemic.10 It is well recognised that normal commensals can become pathogens in the compromised cornea. This may explain the increased incidence of suppurative keratitis, but other significant factors include the use of contaminated local remedies after minor trauma, uncontrolled medicines including steroids, poor availability of medical care and late referral to specialist care.

In the early 1980s, 200 to 300 cases of corneal ulcers were seen annually at the Chittagong Eye Infirmary. Intractable infection was responsible for about 60% of all eyes enucleated. Williams et al. have shown that the establishment of a low-cost microbiology laboratory to identify corneal pathogens and their antibiotic sensitivity has reduced this enucleation rate significantly.4 It is hoped that, using information from this laboratory to define the prevalence of pathogens and the pattern of disease in the region, we may be able to help medics in the region, without access to these facilities, to initiate early and effective treatment for suppurative keratitis.

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References