Letter to the Editor

Study of microbial keratitis in central India

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Corneal ulceration is a leading cause of ocular morbidity and blindness worldwide, especially in developing countries such as India. Infection is the major cause of corneal ulceration [1]; therefore, prompt diagnosis and appropriate antimicrobial therapy is the hallmark of preventing corneal scarring and blindness. This study was undertaken in an attempt to identify the possible predisposing factors and etiological agents of corneal ulceration and to compare and correlate between direct microscopy and culture in the detection of etiological agents.

The age, sex, occupation and various predisposing factors leading to corneal ulceration were recorded for 74 patients. Clinical examination was performed with the aid of a slit lamp. To localize the ulcer properly, fluorescent stain was applied to the affected eye. Corneal scrapings, which were collected using a sterile Bard Parker blade (No. 15) from the base and advancing margins of the ulcers, were inoculated directly on sheep blood agar, chocolate agar, brain heart infusion broth, Sabouraud's dextrose agar, and 2% agar without any additive nutrients. Smears were prepared for Gram staining and wet mount for 10% potassium hydroxide (KOH) and calcofluor white (CFW) staining (Himedia Laboratories, Mumbai, India).

The results showed a male preponderance (67.5%) of cases and that the majority of patients in both sexes were in their sixties. Agricultural workers were mostly affected (41; 55.4%); followed by housewives (10; 13.5%), unemployed males (8; 10.8%), traders (7; 9.5%), children (2; 2.7%), and professionals (1; 1.4%). The most common predisposing factor was trauma (43; 58.1%), followed by local antibiotic use (7; 9.6%), ocular disease (4; 5.4%), systemic disease (2; 2.7%), ocular surgery (2; 2.7%), and topical steroid use (1; 1.3%). No specific history was observed in 15 (20.3%) cases. Correlation of type of trauma with microbial infection is shown in Table 1. Vegetative matter was found to be the predominant predisposing traumatic factor, and this was statistically significant ($p < 0.05$). Vegetative matter also caused more fungal (10; 33.3%) than bacterial (8; 26.7%) cases of keratitis, although this was not statistically significant ($p > 0.05$).

Infectious etiology was identified in 39 of 74 cases (52.7%). Bacterial infection (20; 27.0%, culture only) was more frequently detected than fungal infection (19; 25.7%, culture and microscopy); however, this was not statistically significant. Among the 20 diagnosed cases of bacterial keratitis, both culture and Gram staining were positive in 12 (60%) individuals, whereas the remaining 8 cases were only culture-positive. Among 19 fungal keratitis cases, sensitivity of microscopy was 100% (KOH and/or CFW) and that of culture 78.9% (15 cases). The 100% sensitivity of microscopy could be attributed to the CFW stain, as KOH alone detected only 11 cases (52.9%). The higher positivity of CFW in detecting the fungal elements over KOH was statistically significant ($p < 0.05$). Bacterial and fungal pathogens isolated are presented in Table 2. Rare fungi such as *Colletotrichum dematium*, *Acrophialophora fusispora* and *Phoma* were isolated.

Elderly people engaged in agricultural work were more affected because of exposure to trauma.

Table 1. Correlation of type of trauma and microbial infection

Type of trauma	Cases studied n (%)	Positive cases		
		Bacterial n (%)[1]	Fungal n (%)[1]	Total n (%)
Vegetative matter	30 (69.8)	8 (26.7)	10 (33.3)	18 (78.3)[2]
Soil / Dust / Stone	7 (16.3)	2 (28.6)	2 (28.6)	4 (17.4)[2]
Animal matter (cow tail)	2 (4.6)	0	1 (50.0)	1 (4.3)[2]
Metallic foreign body	2 (4.6)	0	0	0
Chemical dye	1 (2.3)	0	0	0
Cotton fiber	1 (2.3)	0	0	0
Total	**43**	**10 (23.2)**	**13 (30.2)**	**23 (53.5)[1]**

[1] Percentages in brackets are calculated from the respective value of the second column (number of cases studied)
[2] Percentages in brackets are calculated from column total 23

Deficiency and weakness of normal host defenses with advancing age presumably renders the corneal stroma more susceptible to infection and permits normally saprophytic organisms to become pathogenic invaders. Agricultural workers are at higher risk of trauma caused by organic matter such as rice paddy stalks, dust, grass, wood, and animal products; similar observations have been recorded in studies from southern India and Nepal [2-4]. Predisposing factors were reported in 59 (79.7%) cases, and of these, trauma was the major cause (58.1%). Trauma was also the major cause for positive cases (59.0%), which is similar to reports from other studies (39.2% to 65.4%) from India and other countries [3-5]. Injury caused by vegetative matter (18; 78.3%) was statistically significant the most frequent cause of keratitis, and resulted in more fungal than bacterial infections. Other studies have also reported the same correlation [5,6]. Local antibiotic use was the second most common factor (5; 12.3%) of the positive cases where mycotic keratitis was more common (3; 42.8%) than bacterial keratitis (2; 28.6%). Rural people of this region are generally illiterate and of low socioeconomic status. Furthermore, in addition to lacking access to improved medical facilities, they are often advised by non-accredited practitioners in the village to use local antibiotics for a long time. In trauma cases, this advice might have prepared a bed for the microbial growth.

Considering positivity, cases of bacterial keratitis were considered positive by culture only, whereas both microscopy and culture were used to identify positive cases of mycotic keratitis. In our study, 39 (52.7%) cases were positive for suppurative keratitis; this number correlates well with the results obtained by Shakarchi *et al*. (58.6%) [7]. The predominant organism identified in cases of bacterial keratitis was *Staphylococcus aureus* (9; 45.0%) followed by *Streptococcus pneumonae* (5; 25.0%). Many authors have reported *Staphylococcus aureus*/*Streptococcus pneumonae* as the predominant organisms [2,3,8,9] causing bacterial keratitis. A comparison of the recent reports with the earlier findings in Western countries shows a shift of the predominant bacterial pathogen causing keratitis from *Staphylococcus aureus* to *Pseudomonas* sp from 1992 onward. However, in our country and in those with similar climatic conditions, such as Bangladesh, Nepal, southern Australia and subtropical Africa, *Staphylococcus aureus* and *Streptococcus pneumonae* have been the predominant bacteria. Keratitis is likely the result of the entry of these bacteria into the devitalized corneal tissue via the different factors described earlier, either from the normal flora of the sac or from the neighboring areas such as the nose, throat and ear, due to poor hygiene. Poor hygiene and climatic conditions might be important risk factors responsible for higher incidence of *Staphylococcus aureus* and *Streptococcus pneumonae* keratitis in developing countries.

All 19 (25.7%) cases diagnosed as fungal keratitis were positive by microscopy, *i.e.*, KOH

Table 2. Bacterial and fungal pathogens isolated from culture positive cases

Organism	No. of cases	Percentage (%)
Staphylococcus aureus	9[1]	45
Streptococcus pneumonae	5	25
Coagulase negative *Staphylococci*	3	15
Pseudomonas aeruginosa	1	5
Acinetobacter baumanii	1	5
Mixed isolate (*Streptococcus species* and *Pseudomonas species*)	1	5
Aspergillus spp	4	26.7[2]
Aspergillus flavus	3	75.0
Aspergillus fumigatus	1	25.0
Fusarium spp	4	26.7[2]
Fusarium solani	3	75.0
other *Fusarium* spp	1	25.0
Phoma spp	2	13.3[3]
Colletotricum dematium	1	6.7[3]
Acrophialophora fusispora	1	6.7[2]
Cladosporium sp	1	6.7[4]
Alternaria sp	1	6.7[4]
Candida krusei	1	6.7[5]

[1]In one patient two strains of *S. aureus* were isolated.
[2]Hyaline filamentous fungi (60.0%)
[3]Coelomycetous fungi (20.0%)
[4]Dematiaceous fungi (13.3%)
[5]Yeast like (6.7%)

and/or CFW, but only 15 (78.9%) by culture. KOH alone would have missed eight cases. Therefore, the use of CFW with KOH appears to have significant advantage although Sharma *et al*. [10] reported CFW to be only marginally better than KOH. Among 15 culture positive cases, seven were positive by KOH only. This data suggests that cases examined by KOH alone should be also cultured, as otherwise 42.1% of these cases would have been missed. Hence CFW+KOH will give better microscopic diagnosis whereas culture and KOH is the alternative. Prevalence of fungal keratitis in our study was 25.7%, (19/74) whereas other studies have shown that the prevalence of fungal keratitis ranged from 7.3% to 44% [3,6,9,11,12]; the hot humid climate contributes to its higher prevalence in eastern, western, southern and central India as compared to northern India. Hyaline filamentous fungi (*Aspergillus*, *Fusarium*, *Acrophialophora fusispora*) were most frequently isolated (60.0%), followed by coelomycetous fungi (*Phoma* spp and *Choletotrihcum dematium*; 20.0%), dematiaceous fungi (*Cladosporium* sp, *Alternaria* sp; 13.34%), and yeast such as *Candida krusei* (6.7%). *Aspergillus* spp and *Fusarium* spp were the most commonly isolated fungal pathogens from cases of fungal keratitis in the tropics [9]. Studies from southern India [9] have reported *Fusarium* spp to be more common than *Aspergillus* spp, whereas investigations from northern India [12], Nepal [3] and coastal regions of India such as Goa [11] have reported *Aspergillus* spp as the predominant fungi. These discrepancies might be explained by the differences in the natural habitat of the fungi in the various environments. Moulds with enteroblastic conidia adhering in dry chains, for example *Aspergillus* spp and *Paecilomyces* spp, are more frequently isolated in the northern regions of the country where the environment is dry and dusty

than in the more humid southern territories. As observed by Khairalla [13], the high proportion of corneal infection caused by *Aspergillus* spp in drier climates may be due to the fact that *Aspergillus* spp can tolerate hot dry weather conditions . Central India has a mixed climate with both hot dry periods similar to those that occur in the northern part of the country and hot humid periods like the weather experienced in the south, which may explain the higher predominance of both *Aspergillus* spp and *Fusarium* spp in the central region.

To conclude, direct microscopy (KOH and CFW) should be used in laboratories for the early diagnosis and treatment of mycotic keratitis cases to prevent ocular morbidity and blindness.

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