Epidemiological features and laboratory results of bacterial and fungal keratitis: a five-year study at a rural tertiary-care hospital in western Maharashtra, India

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INTRODUCTION This study was conducted to determine the epidemiological pattern and risk factors associated with corneal ulcers in rural areas of western Maharashtra, India, and to identify the bacterial and fungal agents responsible for causing keratitis.

METHODS A total of 852 patients with corneal ulceration were included in the study. Sociodemographic data and information pertaining to risk factors were collected. Corneal scrapings obtained from these patients were processed for bacterial and fungal agents using standard techniques.

RESULTS Out of the 852 patients studied, 537 (63.02%) were culture positive. A majority of the culture-positive patients were farmers (52.32%), and ocular trauma was the most common predisposing factor (60.15%). Among these patients, fungal isolates (57.91%) were more frequent than bacterial isolates (42.08%). The most common fungal isolate was *Fusarium* spp. (35.04%) followed by *Aspergillus* spp. (18.00%). *Streptococcus pneumoniae* was the predominant bacterial isolates (32.74%) followed by *Staphylococcus* spp. (17.25%).

CONCLUSION Corneal trauma from plant parts or organic matter was found to be the most common risk factor associated with corneal ulceration in the rural areas of western Maharashtra. Epidemiology and aetiology of microbial keratitis vary from region to region, and therefore, careful history taking and proper identification of aetiological agents are necessary for the institution of appropriate therapy.

Keywords: aetiology, corneal scraping, corneal ulceration, microbial keratitis, ocular trauma
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INTRODUCTION

Globally, it is estimated that ocular trauma and corneal ulceration result in 1.5–2 million cases of corneal blindness annually.[1] 90% of such patients hail from developing countries, where the lesion has now been recognised as a silent epidemic.[2] A national survey by the Government of India (1991–2001) estimated that corneal lesions were responsible for 9% of blindness in the country.[3]

Almost any microorganism can invade the corneal stroma if the normal corneal defence mechanisms are compromised.[4] A wide spectrum of microbial organisms can produce corneal infections and, consequently, the therapeutic strategies adopted for its treatment may be varied.[5] As there is no definite pathognomonic clinical feature, it is difficult to ascertain 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 to ensure specific therapy for keratitis.[6]

Considering the importance of corneal ulceration as a major cause of visual loss, many studies have reported the prevalence of microbial pathogens and identified the risk factors predisposing to corneal infections.[7-9] However, no such study is available from the rural parts of India. This study was conducted to determine the risk factors and other epidemiological characteristics for patients presenting with corneal ulceration at a rural tertiary care hospital in western Maharashtra, India and to identify the specific bacterial and fungal pathogens causing keratitis in this group.

METHODS

A total of 852 patients presenting to the Department of Microbiology at Rural Medical College, Pravara Institute of Medical Sciences, Loni, Maharashtra, India, with corneal ulceration were studied over a period of five years between December 2004 and December 2009. Ulceration was defined as a loss of the corneal epithelium, with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.

After detailed ocular examination, corneal scrapings were obtained from the patients by an Ophthalmologist under aseptic conditions from the margins of the ulcer using a sterile Baird-Parker blade (no. 15) (Niraj Industries, Faridabad, India). The scrapings were used for the preparation of 10% potassium hydroxide mounts and Gram staining (Fig. 1). For bacterial cultures, the material obtained from scrapings was directly inoculated on blood agar and chocolate agar. Sabouraud dextrose agar (SDA) with chloramphenicol was used for fungal

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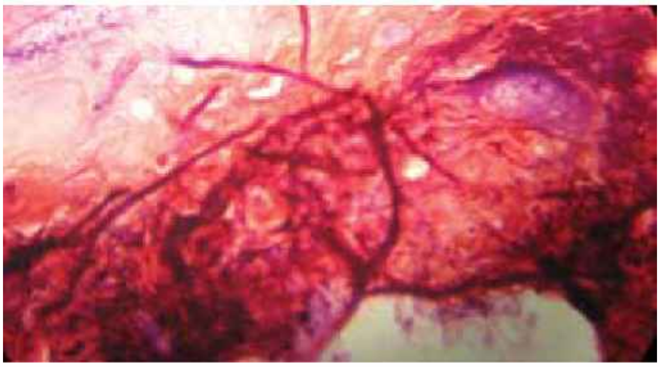

Fig. 1 Photomicrograph of corneal scraping shows Gram-positive fungal hyphae (Gram stain, × 1,000).

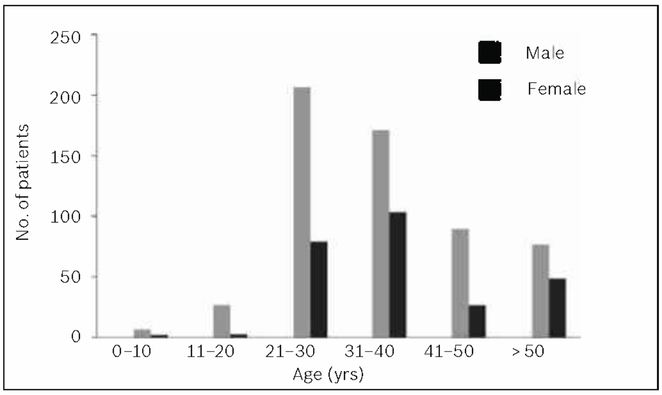

Fig. 2 Graph shows age and gender distribution of patients with corneal ulcer (n = 852).

cultures. A C-shaped streak was made on culture plates to ensure that growth was from the specimen and not laboratory contaminants. Blood agar and chocolate agar were incubated at 37°C aerobically, while for fungal cultures, two sets of SDA were inoculated and kept at 25°C and 37°C, respectively. Blood agar and chocolate agar plates were examined daily and discarded after seven days if no growth was seen. SDA plates were examined daily for three weeks. Any growth observed was identified using standard bacteriological and mycological techniques.[10] Microbial cultures were considered significant if any/all of the following criteria were satisfied: (1) growth of the same organism was demonstrated on more than one solid medium; (2) confluent growth was seen at the site of inoculation on one solid medium; (3) growth of one medium was consistent with direct microscopy findings (appropriate staining and morphology with Gram stain); (4) the same organism was grown from repeated scraping material.[11]

Data related to sociodemographic features, duration of symptoms, predisposing factors, history of corneal trauma, traumatic agents and associated ocular conditions were collected from the medical records of patients with culture-proven bacterial and fungal keratitis.

RESULTS

A total of 852 patients with a clinical diagnosis of corneal ulcer were enrolled in the study, among whom 582 (68.31%) were male. The majority of male patients presenting with corneal ulcers were in the age group 21–30 years, while most females were aged 31–40 years (Fig. 2). Microbial growth was seen in 537 (63.02%) patients, of which 311 (57.91%) isolates were of fungal origin and 226 (42.08%) were bacterial isolates (Table I). Culture-positive patients were further assessed based on occupation and predisposing risk factors (Table I). Maximum incidence of corneal ulceration was seen in farmers (n = 281, 52.32%) followed by labourers (n = 123, 22.90%) (Table I).

502 (93.48%) patients had a definite history of trauma, foreign body, cataract surgery or diabetes mellitus. Only 35 (6.52%) patients who were positive for both bacterial and fungal cultures did not have any positive history predisposing to fungal infection (Table I). The fungal and bacterial isolates obtained on culture are presented in Tables II and III, respectively. *Fusarium* spp. (Fig. 3) was most commonly isolated (35.04%), followed by *Aspergillus* spp. (18.00%) among the fungal isolates (Table II). Out of 311 positive fungal cultures, 283 were pure fungal isolates, nine were mixed with other fungi and 19 were mixed with bacteria. Among the bacterial isolates, Gram-positive cocci were the predominant isolates, with *Streptococcus pneumoniae* being most common (32.74%). *Pseudomonas* spp. was the predominant bacterial isolate among Gram-negative bacilli (Table III). Out of 226 bacterial cultures, 168 were pure isolates, 39 were mixed with other bacteria and 19 were mixed with fungi.

Table I. Findings on patients with positive cultures.

Finding	No. (%)
Patients with corneal ulceration (n = 852)	
Positive cultures	537 (63.02)
Fungal isolates	311 (57.91)
Bacterial isolates	226 (42.08)
Occupation of culture-positive patients	
Farmer	281 (52.32)
Labourer	123 (22.90)
Tradesmen/professional	51 (9.49)
Domestic worker	34 (6.33)
Unemployed/unknown	29 (5.40)
Student/children	19 (3.53)
Predisposing factors in culture-positive patients	
Trauma	323 (60.15)
Foreign body	84 (15.64)
Cataract surgery	51 (9.49)
Diabetes mellitus	44 (8.19)
Unknown	35 (6.52)

Fig. 3 Photomicrograph shows septate hyphae and bean-shaped macroconidia of *Fusarium* spp. (Lactophenol cotton blue stain, × 400).

Table II. Fungal isolates identified from patients with corneal ulcers.

Isolate	Pure isolates	Mixed with other fungi	Mixed with bacteria	Total No. (%)
***Fusarium* spp.**	101	-	8	109 (35.04)
***Aspergillus (A.)* spp.**	52	2	2	56 (18.00)
A. niger	22	-	1	23 (7.39)
A. flavus	16	1	-	17 (5.46)
A. fumigatus	14	1	1	16 (5.14)
***Candida (C.)* spp.**	32	-	5	37 (11.89)
C. albicans	16	-	3	19 (6.10)
C. tropicalis	9	-	2	11 (3.53)
C. parapsilosis	5	-	-	5 (1.60)
Others	2	-	-	2 (0.64)
***Penicillium* spp.**	26	-	-	26 (8.36)
***Alternaria* spp.**	21	-	-	21 (6.75)
Mucor	14	-	-	14 (4.50)
Rhizopus	12	-	-	12 (3.85)
***Curvularia* spp.**	6	-	-	6 (1.92)
***Bipolaris* spp.**	4	-	-	4 (1.29)
Unidentified	15	7	4	26 (8.36)
Total	283	9	19	311

Table III. Bacterial isolates identified from patients with corneal ulcers.

Isolate	Pure isolates	Mixed with other fungi	Mixed with bacteria	Total No. (%)
Gram-positive cocci	118	21	4	143 (63.27)
Streptococcus pneumoniae	68	5	1	74 (32.74)
Staphylococcus (S.) spp.	28	9	2	39 (17.25)
S. epidermidis	17	7	-	24 (10.61)
S. aureus	11	2	2	15 (6.63)
Streptococcus spp.	22	7	1	30 (13.27)
α-haemolytic streptococci	17	3	1	21 (9.29)
β-haemolytic streptococci	-	2	-	2 (0.88)
γ-haemolytic streptococci	5	2	-	7 (3.09)
Gram-negative cocci				
Moraxella spp.	7	1	1	9 (3.98)
Gram-negative bacilli	43	17	14	74 (32.74)
Pseudomonas spp.	16	-	2	18 (7.96)
Klebsiella spp.	8	5	5	18 (7.96)
Proteus spp.	6	-	-	6 (2.65)
Enterobacter spp.	4	7	4	15 (6.63)
Alkaligenes spp.	4	5	2	11 (4.86)
Acinetobacter spp.	3	1	1	5 (2.21)
Citrobacter spp.	2	1	-	3 (1.32)
Total	168	39	19	226

DISCUSSION

A variety of factors determine clinical outcome in microbial keratitis, and epidemiological patterns may vary from country to country and between different geographical regions within a country. Corneal trauma and ulceration is the second leading cause of unilateral blindness. In contrast to visual loss from cataract, which affects mainly the older age groups, corneal ulceration is seen in all ages. In this study, corneal ulceration was more common in men (68.31%), although earlier studies by Basak et al,[9] Srinivasan et al[12] and Kumari et al[13] have reported a comparable gender distribution and Al-Yousuf[7] has reported a higher incidence in women. The higher incidence rates seen in men in this study may be due to the higher risk of their exposure to injury during fieldwork compared to women. Ulceration was more frequent among both men and women during the middle decades of life (Fig. 2), perhaps due to these age groups being maximally active outdoors.

Out of the 852 patients with corneal ulcer, the cultures of 537 (63.02%) patients had positive microbial growth and 315 (36.97%) cultures were negative, with no definite laboratory diagnosis. A possible reason for these negative cultures may be that patients were already on topical medication when they arrived at the hospital. Various other studies have also reported high negative culture rates.[14,15] Fungal isolates (57.91%) were more common in this study than bacterial isolates (42.08%), which was similar to the findings of Basak et al.[9] Contrary to this, Upadhaya et al[8] have reported more bacterial isolates from corneal ulcers.

An analysis of the patient group based on occupation revealed the patterns of employment in the region, with a majority of patients working in the agricultural sector, mainly in sugarcane fields. Corneal injury from sugarcane leaf was a major risk factor in this patient group. In developing countries, ocular injury from plant parts such as paddy stalk and jute plants is a major predisposing factor, unlike in developed countries where causes such as the use of contact lens are reported to be common.[7,9] This is explained by the fact that most fungi isolated from the cultures of patients with corneal ulcer in this study are also commonly present as atmospheric saprophytes and can initiate an ulcer even with a small abrasion.

Fusarium spp., followed by *Aspergillus* spp., was the most commonly isolated fungal pathogen in this study, similar to the findings of Srinivasan et al,[12] although other studies have reported *Aspergillus* spp. as the most common fungal pathogen isolated from patients with corneal ulcers.[9,13,16] This may be due to differences in the climate and natural environment of the areas studied. In this study, *S. pneumoniae* was the most common bacterial species isolated, followed by *Staphylococcus* spp.; this is similar to some reports,[3,5,7] but is at variance with the findings of Deshpande and Koppikar,[17] who reported *Pseudomonas aeruginosa* as the most common bacterial isolate in their study.

Corneal ulceration, a major cause of blindness in developing countries, can be caused by bacteria, fungi, parasites and viruses, and for this reason, microbial keratitis of one form can be confused with another. The epidemiology and aetiology of microbial keratitis may also vary from region to region. The lesion is an ocular emergency due to the rapid progression of corneal infection, with a threat of visual loss and potential corneal perforation. Careful history taking and proper identification of the causative organism are therefore necessary for the timely institution of appropriate therapy for patients presenting with corneal ulcers. It is of the essence that suspected microbial keratitis be scraped for smear and culturing before antibiotic treatment is initiated for such patients.

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