Clinical and microbiological spectrum of corneal ulcer in eastern region of Uttar Pradesh

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Abstract
Aim: To find clinical microbiological spectrum and predisposing factors of corneal ulcer in eastern Uttar Pradesh.
Materials and Methods: This was a hospital based prospective study conducted in Regional Institute of Ophthalmology, IMS, BHU, Varanasi, over a period of three years from May 2015 to April 2018.Suspected microbial keratitis were stained with fluorescent paper strip and examined under cobalt blue light. Scraping of the edge and bases were directly inoculated on Sabouraud's Dextrose Agar and Blood Agar. Plates were examined daily in 1st week then every 3rd day for next 3 weeks.
Results: Maximum cases were seen in the age group of 25-50 years (63.79%), with male preponderance (60.30%). Trauma to the affected eye was major predisposing factor for microbial keratitis (74.10%). The major etiological agent in our study was fungal corneal ulcers followed by bacterial ulcer. *Aspergillus spp*. (53.84%) was most common filamentous fungi followed by *Fusarium spp*. (23.07%). Among bacteria most common species was *Staphylococcus aureus* 57.89% followed by *Streptococcus pneumoniae and Pseudomonas aeruginosa* present in 21.05% and 15.78% respectively.
Conclusions: The major aetiological agent in our study was fungal corneal ulcers followed by bacteria ulcer. Filamentous fungi were most common cause of fungal corneal ulcer with Hyaline fungi (80.76%) and Phaeoid fungi (11.53%).

Keywords: Aspergillus, Bacterial Growth, Corneal ulcer, Keratitis, Keratomycosis.

Introduction
Microbial keratitis is a leading cause of ocular morbidity and blindness worldwide including India.[1-3] Corneal ulcer is a potentially sight threatening ocular condition and the leading cause of monocular blindness. It can be caused by various pathogens i.e., bacteria, fungi, virus and parasites. Bacteria and fungi is the main cause of unilateral corneal scar.[4] Suppurative Keratitis is the second most common cause of monocular blindness after unoperated cataract in some developing countries in the tropics. The incidence of microbial keratitis varies from 11 per 100,000 persons/year in the United States to 799 per 100,000 persons/year in Nepal. The reported incidence of corneal ulceration in India is 1130 per million population.[5-9]

The Etiological and Epidemiological features of infective keratitis [IK] depend on host factors, geographical location and the climate. Several risk factors like age, sex, immune status and socio-economic background determine the pathogenesis of IK.[10]

Materials and Methods
This prospective observational study was conducted in department of Ophthalmology. Sir Sundar Lal Hospital, I.M.S BHU Varanasi India. A total of 58 patients with infective keratitis were enrolled during the study period from April 2017 to August 2018.

With complain of blurring of vision, watering, photophobia, redness which is presumed to be microbial keratitis were included in the study either associated with ocular injury, foreign body, contact lens users or installation of corticosteroid drugs.

Following were exclusion criteria:
1. Viral ulcers
2. Non healing ulcer
3. Mooren ulcer
4. marginal keratitis
5. Descemetocele
6. Interstitial keratitis
7. Neurotrophic keratitis
8. Any ulcer associated with autoimmune disease.
9. Ulcer with impending or actual perforation.

Suspected microbial keratitis were stained with fluorescent paper strip and examined under cobalt blue light. Ulcer gives green fluorescent. 2% paracaine was instilled to desensitize the affected eye. Corneal scraping was done with a sterile Bard-Parker blade (15 No.) by using slit lamp microscope. Material obtained from scraping of the edge and bases were directly inoculated on Sabouraud's Dextrose Agar and Blood Agar. Sample were also taken on labelled slide for 10% KOH wet mount and Gram's stains. Corneal scraps were directly inoculated in culture media in "C" shaped manner.

Blood agar was incubated at 37°C and SDA were incubated at 25°C in BOD incubator. Petri dishes with solid media were kept in such way that lid of the Petri dish was below and solid media above, to prevent the condensation of moisture onto the media. Most of the fungi grew within 1st week of incubation. Culture was declared negative if there is no growth after 28 days of continuous incubation.

Only growth on the inoculated "C" region on solid media was considered significant. Plates were examined daily in 1st week then every 3rd day for next 3 weeks. Plated

were observed in both obverse and reverse view. Subculture was done on SDA from primary growth.

Results

Out of these 58, male and female were 35(60.3%) and 23(39.7%) respectively with M:F ratio 1.52:1. This study showed slightly more preponderance for males. 63.79% of the affected persons belong to young age group (25-50 years) while only 10.34% cases have age group <25 years and rest 25.86% belong to age >50 years. The mean age of the study population was 49.46 years.

Trauma to the affected eye was major predisposing factor for microbial keratitis. As in our study, 74.10% of the person has a history of ocular trauma. The most common traumatizing agent was vegetative matter in 17 cases (41.46%) followed by mud, dust and soil in 13 cases (31.70%), animal matter in 4 cases(6.89%) followed by metallic foreign body in 3 cases (7.31%), wood pieces in 2 cases (4.87%) miscellaneous 2(4.87%). (Table 1)

Table 1: Distribution according to traumatic agents

Traumatic Agent	No. of Cases	Percentage
Vegetative matter	17	41.46
Mud, dust and soil	13	31.70
Animal matter	4	9.75
Metallic foreign body	3	7.31
Wood pieces	2	4.87
Miscellaneous	2	4.87

The occupations of the patients reflected a cross section of the work force in Varanasi and the surrounding agricultural area. The majority were farmers or hired agricultural workers or labourer, usually working in wheat, rice, sugar cane, pulses etc fields. In our study 29 (50%) cases where farmer, 11(19%) where labourer, 8(13.8%) cases were domestic or household worker, 7(12.06%) of them were Tradesman or professionals, only 2(3.44%) of the were students and 1(1.72%) miscellaneous.

Corneal ulcer is most common in Rural area 70.7% cases as compared to Urban area i.e 29.3% this is because of locality of Varanasi, were most of the population belong to Rural area. According to Socioeconomic Condition most of the patients belong to the Upper Lower class (55.2%), then Lower class i.e (32.8%), followed by Lower Middle (8.6%) and Upper Middle (3.4%).

A total of 19 patients (32.75%) were positive for bacteria. Pure bacterial growth is positive in 10 patients (17.24%) and mixed microbial growth is present in 9 patients (15.51%). Out of 19 isolates 16(27.58%) were Gram positive and 3(5.17%) were Gram negative. *Staphylococcus aureus* was the most common isolated bacterial organism representing 11(57.89%) of all positive bacterial case. In our study *Streptococcus pneumoniae* being the second most common isolated bacteria representing 4(21.05%) of all positive cases. Other Gram positive bacteria which were positive in our cases of corneal ulcer's are *Corynebacterium* 1(5.26%) and *Bacillus* 1(5.26%). The most frequently occurring Gram negative organisms is *Pseudomonas aeruginosa* 3(15.78%) of all positive cases. [Table 2 & Fig. 1].

Table 2: Distribution according to bacterial growth

Blood agar culture	Frequency	Percent
Staphylococcus. aureus	10	17.2
Streptococcus .pneumoniae	4	6.9
Pseudomonas	3	5.2
Bacillus	1	1.7
Corynebacterium.Diphtheroid	1	1.7
negative	39	67.2
Total	58	100.0

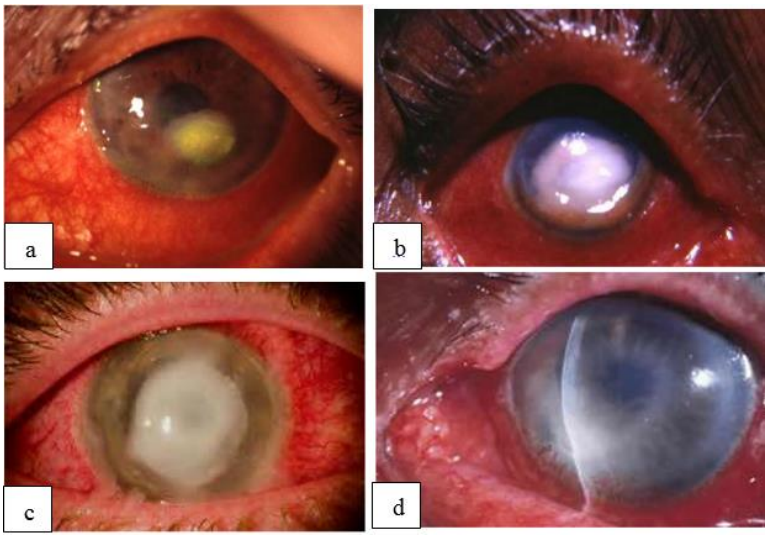

Fig. 1: Clinical photograph showing bacterial keratitis caused by (a): *Staphylococcus aureus;* (b): *Streptococcus pneumoniae;* (c): *Pseudomonas areoginosa;* (d): *Corynebacterium diphtheroid*

Out of 58 patients 26(44.8%) cases were positive for fungal culture, out of which *Aspergillus flavus* is positive in 7(12.1%) cases while *Aspergillus fumigatous* in 4(6.9%) cases, *Aspergillus niger* in 3(5.2%)cases, *fusarium spp*. in 6(10.3%) cases, *Curvularia* in 1(1.7%) cases, *Phaeoid* in 2(3.4%) cases, *Candida* in 2(3.4%)cases, *Penicillum* in 1(1.7%) cases and rest 32(55.2%) were culture negative. [Table 3 & Fig. 2].

Table 3: Distribution according to fungal growth on SDA culture

SDA Culture	Frequency	Percent
A. Fumigatus	4	6.9
A. flavus	7	12.1
A. niger	3	5.2
Fusarium spp.	6	10.3
Phaeoid	3	5.2
Candida	2	3.4
Penicillum	1	1.7
Negative	32	55.2
Total	58	100.0

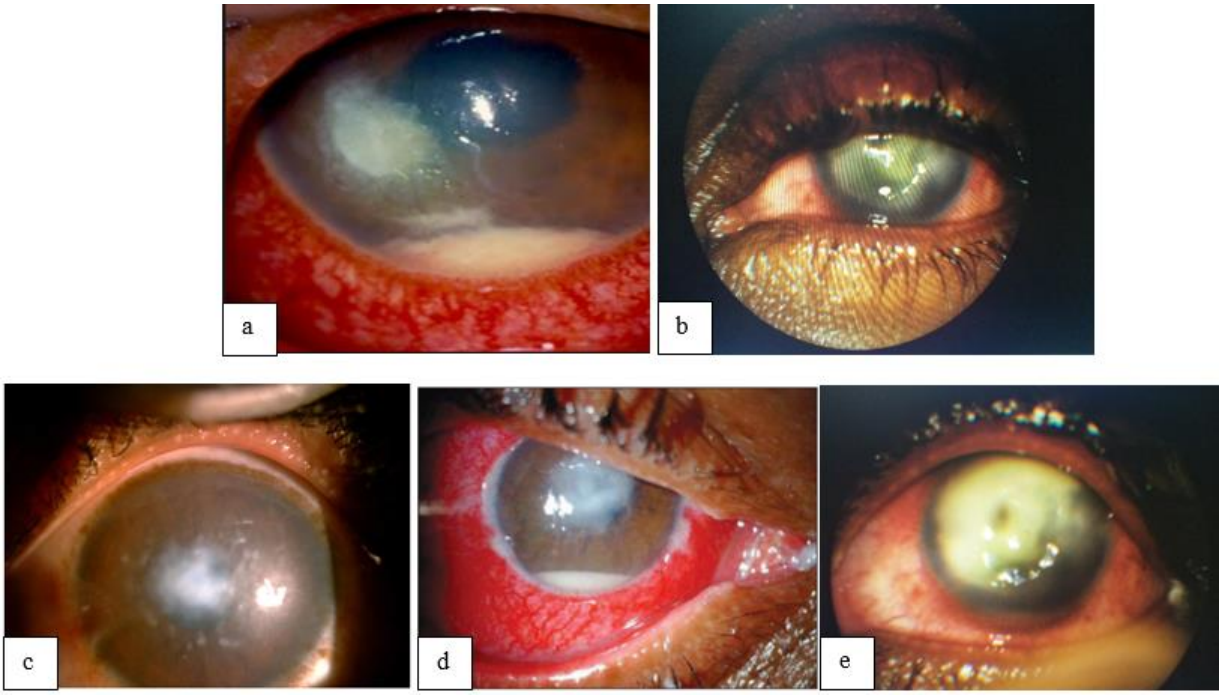

Fig. 2: Clinical photograph showing Fungal keratitis caused by (a): *Aspergillus flavus;* (b): *Aspergillus niger;* (c): *Aspergillus fumigatus;* (d): *Fusarium spp;* (e): *Phaeoid*

Discussion

Infective keratitis (microbial keratitis) is infection of the cornea caused by a wide spectrum of microbial agents and should be considered a medical emergency.[11] A rapid aetiological diagnosis helps in initiating an aggressive specific treatment which could prevent untoward sequels.[12]

With the overall decline in causes of blindness like trachoma, onchocerciasis and leprosy, the World Health Organization (WHO) has perceived that corneal blindness due to microbial keratitis is emerging as a principal reason for visual inability[13] and that it is a "silent epidemic" happening unnoticed around the world.[14]

In this study males clearly had higher incidence of corneal ulcers (60.3%) than females. This is in conformity with several studies conducted elsewhere like the madurai study by Srinivasan et al,[3] where in the ratio of male to female was 1.6:1. This could be explained by the fact that they are more involved in outdoor occupation and hence exposed to unfavourable circumstances.

74.10% of all patients with a history of trauma implicated vegetative matter as the most common traumatic object. This was followed by mud, dust soil, animal matter, metallic foreign body, wood pieces. Any programme of prevention obviously must address this high rate of corneal injury associated with corneal ulceration.

The majority of ulcer patients were agricultural workers, home makers, or labourers (82.8%), an occupation profile similar to Nepal (72%) but in marked contrast with

Ghana where only 16.1% of the patients were involved in agricultural activity. Most of the Eastern Zone in Indian, patients were engaged in heavy labour, either in a Farming, in construction, or in physical transport of heavy materials. So most of them belongs to Rural area (70.7%) and of Upper Lower class (55.2%).

Use of topical antibiotic and steroid instillation, ocular disorder and systemic illness are the predisposing factors, of them most common is topical antibiotic and steroid instillation which promotes fungal proliferation and invasion. Excess and misuse use of both systemic and topical antibiotics, inappropriate dosing and prolonged duration of therapy continue to fuel an increase in antibiotic resistant ocular pathogens. As in our study, 34.5% and 53.4% corneal ulcer patients had already taken corticosteroid and antibiotics respectively.

The lower rate of isolation was attributed to the more widespread availability of topical medications and their use in the majority of patients before evaluation. In our study about 31(53.44%) cases have already used antibiotics drops before evaluation. These may leads to decrease spectrum of bacterial in causing corneal ulcer.

Most common isolated fungi was hyaline (n=15) including *Aspergillus spp*. 13(50%) and *Fusarium spp*. 6(23.07%) followed by phaeoid fungi. *Aspergillus spp*. was most preponderance in fungal corneal ulcer as shown by other studies in Indian subcontinent. In phaeoid fungi, *Curvularia spp*. was the most common fungi in fungal corneal ulcer.

Conclusion

The major aetiological agent in our study was fungal corneal ulcers followed by bacteria ulcer. Filamentous fungi were most common cause of fungal corneal ulcer.

Pure bacterial growth is positive in 17.24% and mixed microbial growth is present in15.51%. Vegetative material was most common traumatic agent. Farmer's and labourer's are more prone to corneal ulcer.

Conflict of Interest: None.

Ethical Clearance: Ethical Clearance obtained from IMS, BHU

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How to cite this article: Shahi R, Sarin D, Maurya OPS, Tilak R, Prajapati M, Clinical and microbiological spectrum of corneal ulcer in eastern region of Uttar Pradesh. *Indian J Clin Exp Ophthalmol* 2019;5(2):182-5.