

Epidemiological and Microbiological Profile of Infectious Corneal Ulcers in Tertiary Care Centre, Kumaon Region, Uttarakhand

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Abstract- Purpose: To determine the epidemiological pattern, clinical presentation and risk factors involved in infectious corneal ulceration in Tertiary Care Centre, Kumaon region, Uttarakhand and to identify the specific microbial agents responsible for corneal infections.

Methods: All patients with suspected microbial keratitis presenting to the OPD, Department of Ophthalmology, GMC, Haldwani from November 2012-August 2014 were evaluated. Sociodemographic data and information pertaining to the risk factors, clinical presentation were recorded. After diagnosing infective corneal ulcer clinically, corneal scraping and cultures were performed.

Results: Over the study period, 76 patients with suppurative keratitis were evaluated. Ocular trauma was the most common predisposing factor in 55 (72.4%) patients ($p = 0.001$), followed by co-existing ocular disorder in 7 (9.2%) patients. Cultures were positive in 52 (68.4 %) patients. Among these culture positive vases, 31 (40.7%) patients had pure fungal infection, 19 (25%) had pure bacterial infections and 2 (2.6%) had mixed fungal and bacterial infections. The most common fungal pathogen was *Aspergillus* spp representing 18 (54.5%) of all positive fungal cultures, followed by *Fusarium* spp in 11 (33.3%) instances. The most common bacterial isolate was *Staphylococcus aureus*, representing 9 (42.8%) of all the bacterial culture followed by *Coagulase negative Staphylococci(CONS)* 6 (28.6%). *Nocardia* spp was detected in 1(1.3%) patient.

Conclusion : Infective Keratitis in Kumaon region, Uttarakhand most often occurs after a superficial trauma with vegetative or organic materials. Fungal ulcers were more common than bacterial ulcers. *Aspergillus* spp and *Staphylococcus aureus* were the most common fungus and bacteria respectively. These findings have important public health implications for the treatment and prevention of suppurative corneal ulceration in this region of India.

Index Terms- Suppurative Keratitis, Microbial keratitis, infectious keratitis, corneal ulceration

cause of monocular blindness after unoperated cataract in some developing countries in the tropics.³⁻⁶ 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.^{7,8} The reported incidence of corneal ulceration in India is 1130 per million population.⁹

The prevalence of blindness directly resulting from complications of suppurative keratitis is estimated to be 5%; the associated ocular morbidity is the result of several factors and patients management is directly affected by the lack of diagnostic facilities and initiation of appropriate antimicrobial therapy. Specific treatment requires quick and accurate identification of the causative microorganisms.¹⁰ Corneal blindness is responsible for 1.5 to 2 million new cases of monocular blindness every year in which ocular trauma and corneal ulceration are significant factors.⁶

The epidemiological pattern and causative agents for suppurative corneal ulcer varies significantly from country to country, and even from region to region within the same country. It is important to determine the "regional" aetiology within a given region for comprehensive strategy for the diagnosis and treatment of corneal ulcer.¹⁰ Infectious corneal ulcer is associated with some predisposing factors. Ocular trauma is a far more common predisposing of infectious Keratitis in developing countries, whereas pre-existing ocular disease and contact lens are common risk factors in developed countries.¹

In a vast agricultural country like India, particularly where primary health care and referral systems are weak, minor eye injuries sustained in agricultural farms often lead to infectious corneal ulceration and loss of vision.¹¹

The purpose of this study was to evaluate all suppurative keratitis seen at Tertiary Care Center, Kumaon region, Uttarakhand over a period of 2 years from November 2012 to August 2014. We also attempted to search for the antecedent risk factors predisposing to the development of suppurative keratitis and to identify the specific microorganisms responsible for infections. This will help in management of such cases in this region.

I. INTRODUCTION

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.¹ Bacteria and fungi is the main cause of unilateral corneal scar.² *Suppurative Keratitis* is the second most common

II. MATERIALS AND METHODS

Patients

All patients with infectious corneal ulcers presenting to the OPD, Department of Ophthalmology, GMC, Haldwani, Uttarakhand from November 2012 to August 2014 were included

in the study. Patients were seen consecutively after the initial clinical diagnosis of corneal ulceration was made. 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. Typical viral ulcers and non-healing ulcers were excluded as were Mooren's ulcers, marginal ulcers, interstitial keratitis, sterile neurotrophic ulcers, and any ulcers associated with autoimmune conditions. A standardised form was filled out on each patient documenting socio-demographic information as well as clinical information including duration of symptoms, previous treatment, predisposing ocular conditions, and associated risk factors.

Clinical procedures

The visual acuity was measured in a standard manner. All patients were examined under slit lamp biomicroscope by ophthalmologist. The size of the ulcer was measured after staining with wet sterile fluorescein paper strip using the variable slit on the slit lamp and recorded in millimetres, also pictures of which recorded with the help of mobile slit lamp adaptor. In a similar way, size of the stromal infiltrate and depth of the lesion was recorded. Ulcer margin, floor, thinning, satellite lesions, pigmentation on the ulcer surface, any impacted lesions, pigmentation on the ulcer surface, any impacted foreign body were noted. The presence of hypopyon was recorded and its height was measured in millimetres. Any associated ocular condition like trichiasis/entropion, Blepharitis, Bell's palsy, lagophthalmos, chronic dacryocystitis, dry eyes, corneal anaesthesia, bullous keratopathy, spheroidal degeneration of the cornea, any surgery on the cornea, use of contact lens, or ocular leprosy was also noted. The use of topical medications including topical corticosteroids were also noted.^{9,12}

Corneal scraping was performed under strict aseptic conditions by an ophthalmologist using a sterile Bard-Parker blade (No15).^{12,13} The procedure was performed under magnification of a slit lamp or binocular surgical loupe following instillation of preservative free 2% lignocaine hydrochloride. Material obtained from scraping of the leading edge and base of each ulcer was inoculated directly in the media and smeared onto two slides, one stained with Gram stain and the other with 10% Potassium hydroxide (KOH) for direct microscopic evaluation.¹⁰

Laboratory procedures

All bacterial cultures were incubated aerobically at 37 °C. Cultures on blood agar and chocolate agar were evaluated at 24 hours and at 48 hours and then discarded if there was no growth.

Fungal cultures inoculated onto SDA were incubated at 27 °C, examined daily, and discarded at 2 weeks if no growth was present. Cultures on non nutrient agar overlaid with *E. coli* were examined daily for the presence of *Acanthamoeba* spp and likewise discarded at 1 week if there were no signs of growth.

Microbial cultures were considered positive only if growth of the same organism was demonstrated on two or more solid media; or there was semiconfluent growth at the site of inoculation on one solid medium associated with the identification of the organism of appropriate morphology and staining characteristics on Gram or Giemsa stained corneal smears.¹⁰ The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory criteria. Fungi were identified by their colony characteristics on SDA and by the morphological appearance of the spores in lactophenol cotton blue stain. If by microscopy in KOH mount preparation, hyphae were observed in corneal smear, but failed to grow in culture, the causative organism was reported as fungal. All laboratory methods followed standard protocols which have been discussed in detail in a previous report.^{4,9,10,12}

III. RESULTS

Epidemiological Characteristics

76 patients with the clinical diagnosis of corneal ulcer with or without hypopyon were enrolled for this study. 44 (57.9%) patients were males and 32(42.1%) were females. In both groups, keratitis occurred most frequently (60:78.9%) in the age group 21-65 years. Majority of the patients (59:77.6%) were from rural areas. The majority (53:69.7%) were labourers/farmers or homemakers, usually working in paddy fields in this region. A majority of the patients (33:43.4%) were seen between 2-3 weeks of their illness at our hospital and 17(22.4%) patients reported after 4 weeks. There was a significant increase in patients during the month of November and December (20:26.3%) and July and August (17:22.4%). (Table 1) Previous treatment was already taken by 67 (88.1%) patients before their first visit to us.

Of the patients who were on some form of topical medication (n=67), 53(69.7%) were taking antibiotic drops (ciprofloxacin, tobramycin, ofloxacin, chloramphenicol, gentamycin), 10(13.2%) patients were taking antifungal eye drops (natamycin or itraconazole), 3(3.9%) were on topical corticosteroids and 2(2.6%) were on some local drugs.

Table 1. Demographic characteristics of suppurative corneal ulcers seen at a tertiary care centre in Kumaon region, Uttarakhand		
Demographics	Particulars	n (%)
Sex	Male	44(57.9)
	Female	32(42.1)
Age in Years	<20	9(11.8)
	21-40	23(30.3)
	41-65	37(53.9)
	>65	7(9.2)
Residence	Rural	59(77.6)
	Urban	17(22.4)
Occupation	Labourers/farmers	27(35.5)

	Homemakers	26(34.2)
	Business/professionals	9(11.8)
	Students/children	12(15.8)
	Others	2(2.6)
First presentation	Within 1 st weeks	26(34.2)
	2 nd – 3 rd week	33(43.4)
	≥ 4 week	17(22.4)
Seasonal Variation	Jan-Feb	7(9.2)
	Mar-Apr	11(14.5)
	May-June	13(17.1)
	July-Aug	17(22.4)
	Sep-Oct	8(10.5)
	Nov-Dec	20(26.3)

Predisposing factors

A history of recent corneal injury was obtained in 55(72.4%) patients (p = 0.001) 18(23.7%) patients had corneal injury with vegetative trauma, 11(14.5%) patients had animal injury. Other significant agents were sand/stone, wooden material, flying insect, dirty wire...etc. Ocular problems predisposing to corneal ulcer were present in 7(9.2%) patients. Among them were chronic dacryocystitis, entropion, and trichiasis. 1(1.3%) patient was diabetic patient and 1(1.3%) was using steroids inadvertently. There was no specific history in 12(15.8%) patients. (Table 2) The risks for suppurative keratitis associated with these predisposing conditions were presumptive.⁹

Table 2. Predisposing factors of suppurative corneal ulcers seen at a tertiary care centre in Kumaon region, Uttarakhand

Predisposing factors	n (%)
Trauma	55(72.4)
Vegetative trauma	18(23.7)
Animal matter	11(14.5)
Sand/stone	7(9.2)
Wooden material	7(9.2)
Miscellaneous	12(15.8)
Co-existing ocular disorder	7(9.2)
Co-existing systemic disease	1(1.3)
Inadvertent use of steroids	1(1.3)
No specific history	12(15.8)

Microbial Diagnosis

Cultures were positive in 52(68.4%) corneal ulcers. 31(40.7%) patients had pure fungal growth, 19(25%) had pure bacterial growth, 2(2.6%) cases had mixed bacterial and fungal growth and 1(1.3%) was positive for *Nocardia* spp. The remaining 24(31.5%) were culture negative. (Table 3) *Staphylococcus aureus* was the most commonly isolated bacterial organism (9:42.8%) of all positive bacterial cultures. The other isolated gram-positive organisms were *Coagulase negative Staphylococci*(CONS) (6:28.6%) and *Streptococci pneumoniae* (2:9.5%). *Pseudomonas* spp, the most frequently occurring gram-negative organism was isolated from 1(1.3%) culture. The other gram negative organisms were *Acinobacter* spp, *Citrobacter* spp and *Nocardia* spp.(Table 4) Out of the fungal isolates (33:43.4%), 33(57.6%) grew *Aspergillus* spp, 11(33.3%) grew *Fusarium* spp and 2(6.1%) grew *Penicillium* spp. Yeast form, *Candida* spp was positive in 1(1.3%) case. (Table 5)

Table 3. Growth pattern of micro-organisms responsible for corneal ulcers in Kumaon region, Uttarakhand

Type of micro-organism cases	Percentage	No. of
Definite bacterial growth	19	25
Definite fungal	31	40.7
Mixed microbial growth	2	2.6
Patients with positive cultures	52	68.4
Patients with negative cultures	24	31.5
Total No. of suppurative corneal ulcers	76	100

Table 4. Identification of the bacterial isolates from corneal ulcers in Kumaon region, Uttarakhand

Bacteria with fungus	Total(%)	Pure Isolates	Mixed
Gram Positive Organisms			
<i>Staphylococcus aureus</i>	8	1	9(42.8)
<i>Streptococcus pneumoniae</i>	2	0	2(9.5)
<i>Streptococcus pneumoniae</i>	6	0	6(28.6)
<i>CONS</i>	16	1	17(80.9)
Subtotal (%)			
Gram negative organisms			
<i>Pseudomonas</i> spp	1	0	1(4.8)
<i>Pseudomonas</i> spp	1	0	1(4.8)
<i>Acinobacter</i> spp	0	1	1(4.8)
<i>Citrobacter</i> spp	1	0	1(4.8)
<i>Nocardia</i> spp	3	1	4(19.04)
Subtotal (%)			
Total No. of bacterial corneal ulcers	19	2	21(100)

Table 5. Identification of the fungal isolates from corneal ulcers in Kumaon Region

Fungi with bacteria	Total(%)	Pure isolates	Mixed
<i>Aspergillus</i> spp	18	1	19(57.6)
<i>Aspergillus</i>	5	1	6(18.2)
<i>fumigatus</i>	6	0	6(18.2)

<i>Aspergillus flavus</i>	3	0	3(9.1)
<i>Aspergillus niger</i>	4	0	4(12.1)
<i>Aspergillus</i> spp	11	0	11(33.3)
<i>Fusarium</i> spp	0	1	1(3.03)
<i>Candida</i> spp	2	0	2(6.1)
<i>Penicillium</i> spp.			
Total No. of fungal corneal ulcers	31	2	33(100)

IV. DISCUSSION

In this study, the commonest age group affected was between 41-65years (37:53.9%), followed by 21-40 years (23:30.3%), <20 years(9:11.8%) and >65 years(7:9.2%) This has a considerable socioeconomic impact because this age group are bread earners of the family. Similar age group was also most commonly affected in south India and western Orissa.^{9,14} While in eastern study and Bangladesh study, the commonest age group was 21-40 years.^{1,15}

The majority of the corneal ulcer patients (53:69.7%) were agricultural workers/daily wage earners (27:35.5%) or homemakers/housewives (26:34.2%), involved in agricultural activities. Housewives/homemakers (26:34.2%) were more affected in our study because in this region, they were also involved more in agricultural activities and cutting grasses from the forest for animals..etc. But in other studies labourers/farmers contributed ranging from 54-70% and housewives/homemakers 11-13%.^{9,15,16} Undoubtedly ocular injury (55:72.4%) was the most common predisposing factor, followed by co-existing ocular disorder (7:9.2%).

Unlike south India (60%), 26(34.2%) patients in this study presented during the first week of their illness.^{9,12} Before their first presentation at our hospital, 67(88.1%) patients had consulted health care provider of some kind or local dai or quack. Similar to south india and eastern india, most eye medications are sold over the counter without a prescription till date. A significant increase in the number of cases of suppurative keratitis was observed during harvesting seasons of November-December (20:24.3%) and July-Aug (17:22.4%). Others have noted an increase incidence of fungal keratitis during the dry, windy, harvesting seasons compared with the wet, humid months of the year,10,17-19 and few others have reported an increase during the hot and humid months.²⁰⁻²²

In this study 52(68.4%) of 76 corneal scrapings were culture positive. This is similar to reports in Ghana,¹⁰ south india,^{9,12} and eastern india,¹⁵ 57.3%, 68.4%, 70.6% and 67.7% respectively. Among these, 31(40.7%) cases were positive for fungus, 2(2.6%) patients with mixed infection. So a total of 33(43.4%) were treated as fungal infection. This is similar to south india reports by Leck et al,¹⁰(44.1%), Bharathi et al,¹² but lower than Srinivasan et al study⁹(51.9%) and Basak SK et al study¹⁵(59.3%). It is also lower than the study in Assam, eastern india where the incidence of fungal keratitis was 32%. Mixed infections both by bacteria and fungi was similar to Bharathi et al (2.4%) report from south india,¹² but less as compared to reports by Srinivasan et al(5.1%),⁹ Leck et al (5.5%),¹⁰ and Basak SK et al(9.5%).¹⁵

21(27.6%) were bacteria positive, 25% had pure bacterial infection and 2.6% had a mixed infection. This reduction in bacterial corneal ulcers in general at the referral centres might be attributed to more successful treatment of bacterial corneal ulcers in the periphery since the introduction of topical fluoroquinolones in the late 1990s.²² Of the 21 bacterial isolates, 42.8% was *Staphylococcus aureus*, which was similar to studies in West Bengal.^{15,23} The most common infecting bacteria was *Streptococcus pneumoniae* in Nepal and south India,^{4,8,9} and *Pseudomonas* spp in Ghana and Bangladesh.^{10,17}

The most commonly isolated fungal pathogens in the current series were *Aspergillus* spp. Of 33 fungal isolates, 57.5% were *Aspergillus* spp, 33.3% were *Fusarium* spp and 6.1% were *Penicillium* spp. In our study, *Candida* spp (1:3.03%) was less compared to other studies.^{4,12} *Aspergillus* species were predominant in Mumbai, parts of south India, north india, Nepal and Bangladesh.^{4,12,17,24-27} Other studies in south india reported *Fusarium* spp to be more common than *Aspergillus* spp.^{10,13,28} *Fusarium* spp have also been found to be the principal fungal pathogen in Florida, Paraguay, Nigeria, Tanzania, Hong Kong and Singapore.^{4,29-36} These phenomenon may be explained by differences in climate and the natural environment.

In summary, suppurative keratitis continues to be a cause for concern among the ophthalmologist in Kumaon region, Uttarakhand and it is evident that the predominance of agricultural activity in this hilly Kumaon region is the principal causative factor. This “regional” information is important with regard to empirical management, as many eye clinics in the locality do not have microbiology facilities. It will also help us to formulate guidelines for prevention of suppurative keratitis in the population at risk.

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