RESEARCH ARTICLE

CORNEAL ULCER: A PROSPECTIVE CLINICAL AND MICROBIOLOGICAL STUDY

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ABSTRACT
Background: It is important to study the epidemiologic features and predisposing factors of corneal ulcer and subsequently to find out its causative agents and their antimicrobial susceptibility patterns in a given community, climate and culture.
Aims & Objectives: This prospective study of 100 cases of corneal ulcer was undertaken to bring out the bacterial and fungal prevalence among different age groups.
Materials and Methods: Corneal scrapings were collected from all patients. One corneal swab and three corneal scrapings were collected. Direct examination of samples was done by potassium hydroxide wet mount and gram stained smear and then inoculated onto blood agar, MacConkey's agar and Saboraud's dextrose agar media. Identification of fungal growth finally was done based on its macroscopic and microscopic features. Bacterial colonies were identified by Gram staining and standard biochemical tests and antimicrobial susceptibility testing was carried out for each bacterial isolate.
Results: Out of total 100 specimens of corneal ulcer, only 55% cases were found to be culture positive in which bacteria were more frequently isolated than fungi. *Staphylococcus aureus and Aspergillus* spp were the most frequent bacterium and fungus. The incidence was higher in males and in age group of >40-60 years. While *S. aureus* was found to be most sensitive to vancomycin, *Staphylococcus epidermis* was most sensitive to cefazoline.
Conclusion: *S. aureus* and *Aspergillus* spp were the most common isolate to be associated with corneal ulcer, and the incidence was higher in rural population, especially farmers, who were constantly exposed to vegetative matter.
Key Words: Corneal Ulcer; Prevalence; Bacteria; Fungi; Predisposing Factors

Introduction

Corneal ulcer is a potentially sight threatening ocular condition and a leading cause of monocular blindness in developing countries.[1,2] Corneal ulcers have attained an important place in causing blindness (9%), particularly in equatorial and tropical countries like India.[3,4] It can be caused by exogenous infections i.e. by viruses, bacteria, fungi or parasites. Sometimes it is allergic in nature or it can be due to endogenous infections.[5] The frequency of fungal keratitis has increased over the past 20 to 30 years[5,6], especially with the advent of corticosteroid therapy. The steroids allow the fungi to prosper and gain a more substantial foothold in the cornea.[6,7] Secondary fungal keratitis occurs in immunocompromised persons. It has been realized that a significant percentage of suppurative keratitis is caused by fungi.[3] Etiologic and epidemiologic pattern of corneal ulceration varies with the patient population, geographic location and climate, and it tends to vary somewhat over time.[8] Infectious corneal ulcer is associated with some predisposing factors, such as poor socio-economic status of people at large, illiteracy, social taboos, ignorance and malnutrition which makes the problem much more serious. Ocular trauma is a far more common predisposing factor of infectious keratitis in developing countries; whereas pre-existing ocular disease and contact lens usage are common risk factors in developed countries.[9,10] The cornea is continually subjected to challenge by a variety of influences present in the environment, including micro-organisms. During evolution, it has developed several means of preventing infection and colonization from these airborne organisms, but any breach in the security leads to colonization by these omnipresent invaders. The bacterial, viral and other inflammations are quite satisfactorily being controlled by modern therapeutic medications. The present study is undertaken to analyze the epidemiologic features, and predisposing factors of corneal ulcer and subsequently to assess the etiological factors, causative agents and their antimicrobial susceptibility patterns.

Materials and Methods

This prospective study was carried out in departments of Microbiology, G. R. Medical College, Gwalior. All the specimens received from out patients of the Department of Ophthalmology, J.A. Group of Hospitals of G. R. Medical College, Gwalior from April 2007 to March 2009 were processed for isolation and identification of all pathogens, according to the standard microbiological techniques.[14] This research work was executed after approval from the ethical committee of GRMC, Gwalior.

The demographic data and medical history were taken from each patient; including age, gender, occupation, history of diabetes mellitus, history of trauma or foreign body entering eye, use of contact lens and long term use of steroids. Corneal scrapings were collected from all patients using sterile Kimura spatula or hypodermic needle after using preservative free topical Anesthetic. One corneal swab and three corneal scrapings were done gently from the margin as well as from the base of the ulcer with all aseptic precautions. Corneal swab was taken by rubbing the ulcerated area of the cornea with sterile cotton swab soaked in sterile normal saline before instillation of local anaesthetic.[11] The material scraped was initially spread onto a labelled slide to prepare a 10% potassium hydroxide wet mount. The second scraped material was directly inoculated onto sabouraud's dextrose agar media and the last scraping was used to prepare a smear for gram staining.[12,13] The swab was inoculated onto blood agar (BA), Mac Conkey's agar (MA) and Saboraud's dextrose agar (SDA) media. BA and MA media were incubated at 37˚C for 24 hours and the plates were observed the following day, but extended to 48 hours if there was no bacterial growth within 24 hours. SDA medium was incubated at 25˚C, and observed daily for the first 7 days, and on alternate days for the next 21 days, for observing slow growing fungi. Identification of fungal growth finally was done based on its macroscopic and microscopic features. Isolated colonies were subjected to Gram staining and biochemical tests for identification. Identification was carried out according to the standard biochemical tests.[14] Antimicrobial susceptibility tests were carried out on isolated and identified colonies of bacteria using commercially prepared antibiotic disk (HiMedia) on Mueller Hinton agar plates by the disk diffusion method, as per Central Laboratory Standards Institute (CLSI) guidelines.[15]

Results

A total of 100 specimens of corneal ulcer were included in the study. Only 55% cases were found to be culture positive and 45% cases were found to be culture negative. Out of the total culture positive cases, bacteria were more frequently isolated than fungi. *Staphylococcus aureus* (19%) was most frequent bacterium whereas *Aspergillus* spp (25%) were most frequent fungii. *Staphylococcus aureus* was maximally involved in infective corneal ulcer. In traumatic corneal ulcer, *Aspergillus* spp was found to be most common causative micro-organism. Peripheral ulcerative keratitis (PUK) was sterile in nature (Table 1).

Table-1: Type of micro-organisms in relation to corneal ulcer

Micro-organisms		Type of corneal ulcer			
		Infective	Traumatic	PUK	Total
Bacterial	*S. aureus*	14	5	0	19 (19%)
	S. epidermis	2	0	0	2 (2%)
	S. pneumoniae	2	0	0	2 (2%)
Fungal	*Aspergillus* spp.	4	21	0	25 (25%)
	Fusarium spp.	0	6	0	6 (6%)
	Candida spp.	1	0	0	1 (1%)
Sterile		19	17	9	45 (45%)

Table-2: Frequency of corneal ulcer in relations to age and sex

Age group (years)	Male		Female		Total	
	N	%	N	%	N	%
0-20	5	9.6	3	6.25	8	8
>20-40	14	26.9	2	25	26	26
>40-60	19	36.5	17	35.4	36	36
>60-80	8	15.3	9	18.7	17	17
>80-100	6	11.5	7	14.5	13	13
Total	52	52	48	48	100	100

Table-3: Antibiotic susceptibility patterns of bacterial isolates

Microorganisms	Antibiotics													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
S. aureus (19)	12	9	5	12	19	9	0	7	8	0	0	3	16	6
S. epidermis (2)	1	0	0	0	0	0	0	0	0	1	1	0	2	0
S. pneumoniae (2)	0	0	2	0	0	0	2	0	0	2	0	0	2	0

1: Amikacin; 2: Gentamicin; 3: Ciprofloxacin; 4: Oxacillin; 5: Vancomycin; 6: Gatifloxacin; 7: Clindamicin; 8: Imipenem; 9: Chloramphenicol; 10: Ceftrioxone; 11: Cefaperzone + Sulbactam; 12: Pipercillin; 13: Cefazoline; 14: Moxifloxacin

The frequency of incidence of corneal ulcer was observed in relation to age as shown in table 2. We found the relationship of corneal ulcer between sex, rural-urban population, middle-higher income group and month wise distribution. The prevalence rate was higher in male (52%) patients compared to female (48%). 78% and 22% cases belonged to rural and urban population respectively, in which 36% cases were of farmers, 23% cases of laborer, 18% cases were of unemployed and 23% cases of others. As per socio-economic status distribution, 76% cases belonged to lower income group and 19% and 5% cases belonged to middle and higher income group respectively. As per month wise distribution, maximum cases were found in June (16%) followed closely by July (14%). On the basis of clinical description, infective type of corneal ulcer was found in 42% cases, followed by traumatic (49%) and peripheral ulcerative keratitis (9%) respectively.

Staphylococcus aureus was found to be most sensitive to vancomycin, cefazoline, ciprofloxacin, amikacin and resistant to moxifloxacin. *Staphylococcus epidermis* was most sensitive to cefazoline and resistant to gatifloxacin, *Streptococcus pneumoniae* was sensitive to ciprofloxacin, ceftriaxone and moxifloxacin and resistant to amikacin. (Table 3)

Discussion

The successful management of bacterial corneal ulcers is based on prompt identification of the causative organism and effective treatment with an appropriate antibiotic which remains as a challenge to ophthalmologists. Our study shows that 55% samples were positive for growth of either bacteria or fungus. Our results were consistent with similar studies carried out by Pichare *et al* and Ly CN *et al* which showed positive culture in 39% and 42% of their patients, respectively.[16,17] The lower positive culture results in their cases might be attributed to previous antimicrobial therapy. Microbiological study revealed that *Staphylococcus aureus* was found in maximum number of cases (19/23) in infective corneal ulcer and *Aspergillus* spp growth in (25/32) in traumatic corneal ulcer. This finding is in accordance with those of Dunlop and co-worker.[18] When factors such as age and sex of the patient were considered, we found the occurrence of corneal ulcer to be higher in males and in the patients in the age group >40-60 years. These findings correlate with that of Reddy *et al* and Bharthi *et al* .[19,20] The greater frequency in person of 40-60 years of age group may be due to fact that these persons are more involved in outdoor working, and majority of them belong to poor social status where hygienic conditions were not maintained properly. In our study, 78% cases were found to be in rural population, whereas 22% cases were found to be in urban population. Bharthi and colleagues, in their study, noted cases of corneal ulcer to be 54.07% rural and 45.95% urban.[20] The greater prevalence of all the types of corneal ulcer in rural population may be due to the fact that in this group of population, persons have more ignorance towards health and have more poverty as compared to urban class society. Also, rural population is much more exposed to cultivation and forestry as compared to urban population. The maximum incidence of corneal ulcer was found to be more common in farmers (36%) than the laborers (23%) in our study. Srinivasan M *et al,* in their study, summarized that the occupational incidence of corneal ulcer in general, and not group wise as in present study.[21] Bharthi *et al* found that among the microbial keratitis patients, agriculture worker were 42.38% and laborers were 23.58%.[20] This can be due to the constant exposure of farmers to various types of vegetative injuries. They are mainly involved in agricultural activities and are away from the health care facilities whereas laborers were involved due to poor hygienic conditions and poor nutrition are more prone to corneal ulcer. In our study, maximum no of corneal ulcer cases were found to be in lower income group (76%). The corneal ulcer is a disease of poverty, as all the types of corneal ulcer were seen more in poor class. Prashant Bhushan *et al*[22] also reported the general incidence of corneal ulcer to be higher in low socioeconomic class. Our study shows seasonal variation in presentation of cases. Incidence was maximum in months of June and July. This can be explained by the fact that these months are the harvesting time in most places. This necessitates more number of persons working for a longer time in fields contaminated with fungal spores.[23] This predisposes to an increase in the incidence of keratomycosis. Our findings are in accordance with those of Shukla *et al* and Sharma et al.[24,25] The maximal susceptibility of *Staphylococous aureus* was against vancomycin, cefazoline, ciprofloxacin, and amikacin respectively, whereas *Staphylococcus edidermis* was found to be most susceptible to Cefazoline and *Streptococcus pneumoniae* was found to be most susceptible to ciprofloxacin, ceftriaxone and moxifloxacin respectively. These findings are similar to study done by P. Manikandam et al.[26]

Conclusion

S. aureus and *Aspergillus* spp. were the most common isolate to be associated with corneal ulcer, and the incidence was higher in rural population, especially farmers, who were constantly exposed to vegetative matter.

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