

Comparative Study of Bacterial Profile of Patients with Bacterial Corneal Ulcer with Bacterial Profile of Conjunctiva of Controls - A Prospective Study

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ABSTRACT

Introduction: The normal ocular flora has an essential role in preventing as well as causing ocular infection. In addition to the other ocular parts, conjunctiva harbour natural flora which might play an important role in the development of corneal ulcer with the infliction of trauma to the cornea which might be in the form of vegetative material, use of contact lens, use of topical steroid etc. The present study was carried out to find the relation between conjunctival bacterial flora in the development of corneal ulcer.

Material and method: A total of 100 cases of bacterial corneal ulcer were studied at the Regional Institute of Ophthalmology, GMCH from June 2012 to May 2016. 64% of cases were males, 36% females. The most common age group affected was the 3-5th decade. Most of the cases were from rural areas (67%) with agriculture being the most common occupation (58%). Ocular trauma (69%) was the most common predisposing factor. Culture was positive in 69.4% of cases.

Result: The most common organism causing bacterial corneal ulcer was found to be *Stap. Epidermidis* (38.3%). *Pseudomonas auregonisa* (13.6%) was the most common gram negative organism. There were 100 controls with 23 controls below the age of 17 years.

Conclusion: The most common organism encountered in the controls among both the paediatric and adult group was *Stap. Epidermidis*.

Keywords: Bacterial Profile, Bacterial Corneal Ulcer, Conjunctiva of Controls

INTRODUCTION

Bacterial corneal ulcer is a medical emergency and a potentially sight threatening condition which can result in loss of vision without prompt and adequate treatment due to corneal scarring and or endophthalmitis.¹ According to the WHO, corneal disease is the major cause of blindness globally after cataract and glaucoma.²

Bacterial keratitis is defined as a suppurative corneal infiltrate and overlying epithelial defect associated with presence of bacteria on corneal scraping and/or that was cured with antibiotic therapy.³

The severity of the corneal infection depends on the pathogenicity of the infecting bacteria and underlying condition of the cornea.³

Bacterial corneal ulcer is rare in the absence of predisposing factors.⁴ The major risk factors include ocular trauma, ocular surface diseases and the use of contact lens. While ocular trauma is the main predisposing factor in the developing

nations, its the dramatic increase in the use of contact lenses that is the major predisposing factor for bacterial keratitis in the developed world.⁵

The spectrum of corneal pathogens show wide geographic variation, while viral infections are the leading cause of corneal ulcer in the developed world, bacteria, fungi and acanthamoeba are important aetiological agents in the developing world.⁶

The presence of micro-organisms in the normal human conjunctiva was established in the 19th century.⁷ The conjunctival sac is sterile at birth but acquires a microbial flora soon after birth and some of the commensals become resident flora in the conjunctiva and have a potential to turn into pathogens⁸ and cause bacterial keratitis, when the normal corneal defence mechanism of lids, tear film and corneal epithelium are compromised.

The present study was carried out to analyze the bacteriological profile of corneal ulcer cases and compare them with that of normal conjunctiva to see if any similarities exist as the commonsals in the conjunctiva are potential pathogens in the compromised cornea. The knowledge of the bacterial profile of the conjunctiva can help in initiating prompt treatment.

MATERIAL AND METHODS

The present study was a prospective case control study from June'12 to May'14 carried out in the laboratory of RIO Guwahati, which is in the Northeastern region of India. It is a tertiary eye care hospital under the Govt. of Assam and mainly caters to the people of Assam especially the economically weaker section of the society.

A detailed history and clinical examination using slit lamp biomicroscopy and assessment of the visual acuity was carried out in all the cases presenting in the outpatient dept. of RIO with corneal ulcer. All procedures performed in studies

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How to cite this article: Reepa Borah Choudhury, Dipak Kumar Das, Barnali Das, Jyoti Bhuyan. Comparative study of bacterial profile of patients with bacterial corneal ulcer with bacterial profile of conjunctiva of controls - a prospective study. International Journal of Contemporary Medical Research 2017;4(10):2127-2130.

involving human participants were in accordance with the ethical standards of Guwahati Medical College and Hospital institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standard

Exclusion criteria:- Patients suspected of having corneal or fungal or acanthamoeba infection by direct visualization under microscopy and /or culture were excluded.

A total no. of 100 cases with suspected bacterial corneal ulcer were selected and their consent taken. In these patients corneal scrapping was taken under topical anaesthesia, using a sterile no. 15 Brad Parker Blade or a kimura spatula. Scrapping was taken from the edges and base of ulcer after debridement of necrotic debris. The material obtained was examined microscopically using Grams stain and 10% KOH stain for rapid detection under microscope.

Scrapped material was also inoculated in Blood agar with 10% CO₂ and Chocolate agar with 5% CO₂ and Sabouraud agar for culture. The plates were incubated and examined for a maximum of 2 weeks and 3 weeks for bacterial and fungal cultures respectively. A single colony of a virulent organism or at least three colonies of an organism that usually is not considered to be highly pathogenic on the ocular surface were considered to be positive cultures (T Bourcier). The organisms cultured were then identified by conventional methods and all fungal positive cases were excluded.

In the control groups a total of 100 people were included. All these were clinically normal individuals with no evidence of infection or inflammation of the eyes and included mostly medical students, school students, paramedical staff, doctors. Swab was taken from the inferior tarsal and fornical conjunctiva and Gram stain and KOH mount and culture was done to study the flora of normal conjunctiva.

STATISTICAL ANALYSIS

Descriptive statistics like mean and percentages were used for the analysis. Mean and percentages were used for the interpretation of data.

RESULTS

Of the 100 cases, 64 were males and 36 were females, the age ranged from 4yrs to 80 yrs with maximum no. of cases in the 3-5th decade(table-1). Corneal ulcer was more commonly observed in rural population (67%). Agriculture was the most common occupation among the cases (58%) and ocular trauma was the most commonly observed predisposing factor (69%). The months of Nov.-April was the most common period in which the patients presented. Gram staining was able to detect bacteria in 48% of cases. Culture positivity was noted in (69.4%). 83.3% of infections involved gram positive bacteria and 16.3% gram negative bacteria. 3% of cases were polymicrobial in nature. The most commonly observed organism was Staphylococcus epidermidis (38.3%) (table-2a and b). Gram positive bacteria was noted in 100% of the corneal trauma cases, whereas Gram -ve bacteria (94%) was mostly associated with contact lenses. The visual acuity of the cases at presentation ranged from 6/6 to no

light perception. The antibiotic used was Moxifloxacin and fortified Moxifloxacin. 92% of cases had a good response.

Among the control group there were 23 children below 17yrs and 77 adults, with ranging from 18-80 yrs. With 10 females and 13 males and 37 females and 40 males below 17 yrs and above 18 yrs respectively. Of the 200 eye examined, 46 were of children and 154 of adults. The number of eyes that were culture positive was 32 in children and 106 in adults(table-3). The most commonly observed organism in both the paediatric (53.1%) and adult group (50.9%) was Stap. Epidermidis (table-4).

DISCUSSION

As corneal ulcer is a sight threatening condition, the need for prompt diagnosis and efficient management is of utmost importance. Whereas gram stain provides a clue to the type

Age distribution (yrs)	Male	Female	Total
0-10	02	00	02
11-20	03	01	04
21-30	09	06	15
31-40	20	14	34
41-50	13	07	20
51-60	10	04	14
61-70	05	04	09
71-80	02	00	02

Table-1: Table showing the age distribution between male and female cases of bacterial keratitis

Gram +ve bacteria	Pure isolates	Mixed with other bacteria	Total no. of bacteria isolates
Stap. epidermidis	25	03	28
Stap. pneumoniae	09	-	09
Stap. Aureus	06	01	07
αHaemolytic Streptococci	02	-	02

Table-2(a): Species of bacteria isolated from corneal ulcer cases

Gram -ve bacilli	Pure isolates	Mixed with other bacilli	Total no. of bacilli
Cornybacterium *	07	-	07
Propionibacterium acnes	06	-	06
Bacillus	01	-	01
Pseudomonus species	08	02	10
Haemophylus influenzae	02	-	02
Serratia	01	-	01

Table-2(b): Species of bacilli isolated from corneal ulcer cases

	Children below 17 yrs	Adults
No. of patients	23	77
No. of eyes examined	46	154
No. of sterile eyes	14	48
No. of eyes with positive culture	32	106
No. of anaerobic culture	03	14

Table-3: Results of culture among controls

	Children	Adults
Streptococcus epidermidis	17(53.1%)	54(50.9%)
Diphtheroids	11(34.3%)	22(20.7%)
Corynebacterium	01(3.1%)	13(12.2%)
species Streptococcus species	05(15.6%)	02(1.8%)
Staphylococcus aureus	1(3.1%)	5(4.7%)
Haemophilus influenzae	-	3(2.8%)
Propionibacterium	4(12.5%)	20(18.8%)

Table-4: Species of bacteria isolated from conjunctiva of controls

of bacteria and enables initiating empirical treatment but culture remains the gold standard for diagnosis of the type and strain of bacteria.

Majority of patients with corneal ulcer were male(64%) similar to other Indian authors^{3,9,10,12} with 3-5th decade being the most commonly affected age group. Similar to Basale et al⁹ Ravinder et al¹⁰ in contrast to MJ Bharathi et al¹¹ in South India who found people above 50 yrs to be more commonly affected. The patients were mainly from rural areas (67%) and fell in the lower socio economic group (61%), where agriculture was the most common occupation (58%). Ocular trauma was found to be the most common predisposing factor. These findings are consistent with observations made by other Indian^{9,10,12} as well as data reported from Middle Eastern Countries.^{13,14,15} Whereas contact lens use is the major predisposing factor in the Western world.^{3,16,17,18}, the use of contact lens was seen only in 12% of our cases whereas Jayram et al (1%)¹² from South India and Basak et al (0.3%)⁹ from West Bengal found the association with contact lens to be non-significant.

The months of November to April was the most common time of the year where most of the patients presented. All these findings suggest that in this part of India, the population most susceptible to corneal ulcer are the males in the lower socioeconomic group where agriculture being the main occupation and as such these group of people are prone to injury by both animate and inanimate matter in the harvesting season leading to corneal injury and subsequent ulceration.

In our study all the cases presented with unilateral corneal ulcer. In 48% of cases Gram staining was able to detect bacteria and culture was positive in (69.6%) of cases similar to T. Bouecier et al (68,21) 83.3% of cases 68.2% were gram positive and 16.3% were gram negative and 4% of the cases were polymicrobial. The most common organism observed was Staphylococcus epidermidis 38.3% similar to other Indian authors.^{6,19,20} However MJ Bharti et al¹¹ and Srinivasan et al²¹ in South India found Staphylococcus Pneumoniae to be the most common organism, whereas Basak et al⁹ in West Bengal found Staphylococcus to be the most common organism.

Staphylococcus epidermidis was also observed to be the most common bacteria cultured from corneal ulcer cases in the West.^{3,16}

The most common Gram negative organism observed in our study was Pseudomonas auregonisa (13.6%) similar to other

Indian^{6,9} and western authors.^{3,16}

All the cases of ocular trauma was associated with Gram positive organism similar to other studies³ and 94% of patients using contact lens was associated with Gram negative organism predominantly Pseudomonas auregonisa similar to other studies.^{3,8,23}

The conjunctiva is normally sterile at birth but becomes gradually colonised soon after.²⁴

Several studies have found Staphylococcus epidermidis to be the most common bacteria in both children and adults as quoted by Thomas R. Singer et al²⁵ however there are exceptions. Locather Khdrzo D et al²⁶ found Staphylococcus aureus to be the most common organism.

Saudakoff P S et al²⁷ found diphtheroids to be the most common organism observed. Some studies noted diphtheroids to be the most common in adults but T.S. Singer et al²⁵ found no difference in the incidence of diphtheroids on adults and children, however they noted Streptococci to be higher in children. In our study we found that Staphylococcus epidermidis followed by diphtheroids to be most common in both children and adults and Streptococcus to be more common in children than adults as noted by T.S. Singer et al.²⁵

Propionibacteria was noted to be the most commonly observed anaerobic bacteria similar to findings of Brooke et al.²⁸ However the percentage of Propionibacteria was slightly higher in adults than children.

CONCLUSION

In conclusion Staphylococcus epidermidis being the most common bacteria cultured from both the infected cornea and healthy conjunctiva proves that a similarity exist between the bacteria most responsible for infections keratitis is usually a commensal normally found in the conjunctiva which in times of corneal stress can cause infection and lead to a sight threatening condition. Thereby the knowledge of the common bacteria residing in the conjunctiva can help in the prompt management of infectious keratitis and halt the progression of disease.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 01-10-2017; **Accepted:** 31-10-2017; **Published:** 10-11-2017