ORIGINAL ARTICLE

Bacterial and fungal profile of corneal ulcers- a prospective study

Gulnaz Bashir, Azra Shah*, Manzoor A Thokar, Sabia Rashid**, Saman Shakeel

Abstract : *Corneal ulceration continues to be one of the most important causes of ocular morbidity and blindness worldwide. Between April 1999 and May 2001, 80 patients with corneal ulceration were examined to find the causative microorganisms, the sensitivity pattern of bacterial isolates to antibiotics, the predisposing factors for ulcerative keratitis and the comparison between culture and gram staining results. Corneal ulceration was seen more in males than females, predominantly in farmers (61.25%) and trauma was the commonest predisposing factor, the agents being mainly organic agricultural materials. Of the 80 corneal ulcers, 32(40%) yielded pure bacterial growth while fungal growth was seen in 10(12.5%). Streptococcus pneumoniae was the commonest bacterium while Aspergillus fumigatus and Fusarium species were the commonest fungi isolated. Most of the bacterial isolates were sensitive to chloramphenicol and tetracycline followed by the quinolones. The overall sensitivity and specificity of Gram staining as compared to culture was 57.14% and 94.7% respectively.*

KeyWords: corneal ulcers, bacteria, fungi, antibiotic sensitivity, gram staining

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Introduction

Corneal ulceration, a break in the epithelium with underlying stromal necrosis is a leading cause of ocular morbidity and blindness worldwide,[1] especially in India.[2]

Almost any organism can invade the corneal stroma, if the normal corneal defense mechanisms, i.e. lids, tear film and corneal epithelium are compromised. Most of the organisms cultured from corneal infections are of the same species as normally found on the lids and periocular skin, in the conjunctival sac or the adjacent nasal passage. Their incidence may vary geographically[1] and consequently the therapeutic strategies may be variable.[3] Clinical features alone are inadequate to confirm infection or suggest a particular cause; laboratory studies are required to identify the specific causative organisms.[4]

Considering the importance of corneal ulceration as a worldwide cause of visual loss and paucity of data regarding the aetiological factors in Kashmir valley, this study, the first of its kind was undertaken. The purpose of this study was to evaluate the spectrum of aerobic microorganisms-bacteria and fungi causing corneal ulcers; to study the sensitivity pattern of bacterial isolates to antibiotics; to characterize the predisposing factors for ulcerative keratitis and to compare culture and gram stain results.

Materials and Methods

The study was conducted in the department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar over a period of 2 years (April 1999-May 2001). It included 80 patients of ulcerative keratitis attending the Departments of Ophthalmology, SMHS and SKIMS hospitals, Srinagar. Non-infected and viral cases (e.g, geographic ulcers, neurotrophic keratitis and chemical burns) were excluded. Each patient underwent detailed ophthalmic evaluation including history taking (with special reference to occupation, history of trauma and medication to eye) and examination.

Cotton swabs moistened with brain heart infusion broth were used to culture material from the conjunctivae and lid margins of both eyes and plated directly onto blood agar. Next, cornea in the affected eye was anaesthetized using 2% lignocaine and corneal specimens were taken from leading edge as well as the base of an active ulcer with a sterile surgical blade no:15. Whenever possible multiple scrapings were taken. Each scraping was inoculated onto blood agar in a row of C- streaks and then the material was spread evenly on two glass slides for Gram and Giemsa staining. With a new surgical blade, the material was plated onto chocolate agar, MacConkey agar, Sabouraud's dextrose agar with chloramphenicol and lastly sample collected with a moistened swab placed

Departments of Microbiology, Pathology and Ophthalmology** SKIMS. Srinagar, Kashmir.*

Address for Correspondence:
Dr Gulnaz Bashir, House No:27, Post Office Lane, Hyderpora, Srinagar, Kashmir(J&K)190014, India.
E-mail:drgulnazbashir@hotmail.com.

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in brain heart infusion broth. All media were incubated aerobically according to standard protocols.[5] Aerobic bacterial cultures were incubated at 37°C and examined at 24 hours and daily thereafter. Media were held for at least 7 days before they were considered negative. Brain heart infusion broth showing no growth after three days was considered negative. The inoculated fungal medium-Sabouraud's dextrose agar with chloramphenicol was incubated at 25°C, examined daily and discarded at 3 weeks if no growth was seen. Anaerobic culture was not done. Corneal ulcer was considered infected only if it met one of the following criteria[6]:-

a) Growth of the same organism on more than one culture medium.

b) Semi confluent growth on two or more C-streaks on one solid medium.

c) Heavy growth within the liquid medium confirmed by a positive stained corneal smear. Growth off the streak was taken as contaminant.

Bacterial identification was done on the basis of staining characteristics, colony character on different media and various biochemical tests. Antimicrobial sensitivity of the isolates was done on Mueller Hinton agar using Kirby Bauer disc diffusion method. Results were interpreted based on NCCLS guidelines. Fungal identification was done on the basis of colony character and microscopic appearance in lactophenol cotton blue preparation.

Statistical methods used for analyzing the data were Chi-square test and odd ratio analysis.

Observations

80 patients studied included 57 (71.25%) males and 23(28.75%) females with age ranging from 6-75 years (Mean 45.95±17.82). [Fig.1]. Ulceration was more common in patients belonging to rural areas (92.50%) as compared to urban areas (7.50%) and left eye was involved in more cases (55%) as compared to right eye (45%). No patient had bilateral eye involvement.

Of the 80 patients, 49 (61.25%) were farmers, followed by elderly-aged more than 60 years with no particular occupation (12.5%), labourers (8.75%), shopkeepers (8.75%), students (6.25%), businessmen (1.25%) and tailors (1.25%).

36 (46.25%) of the 80 patients had one or more predisposing factor contributing to ulcerative keratitis. Trauma was the most common ocular predisposing factor seen in 30 patients. The agents were mainly organic agricultural materials like paddy and maize stalks, grass, wooden sticks and materials like sand.

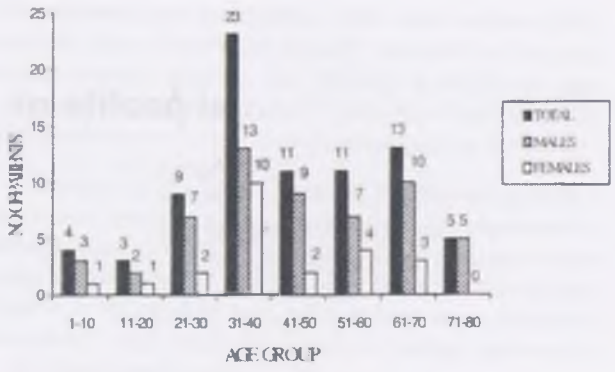

Fig. 1. Age and sex distribution of patients

Other predisposing factors were chronic dacryocystitis in 3(3.8%) patients, entropion, hypertension and steroid use in 1(1.35%) patient each. None of the patients wore contact lenses.

Many of the patients had sought medical and non-medical help before initial examination. 52(65%) patients had a history of topical antimicrobial instillation prior to presentation. These included antibiotics like gentamicin, tobramycin and ciprofloxacin; antifungals like natamycin;steroids and some herbal preparations. Of these 52 patients 24(46.15%) showed growth of microorganisms despite antimicrobial use. Of the 28 patients with no history of drug use, growth was seen in 18(62.28%).

42(52.5%) of the 80 corneal ulcers yielded a significant growth of microorganisms while 38(47.5%) showed no growth. 32(40%) exhibited pure bacterial growth while pure fungal growth was seen in 10(12.5%). None of the scrapings grew a mixture of organisms. Of the 32 bacterial isolates, 22 were gram positive cocci and 10 gram negative bacilli. Streptococus pneumoniae was the most commonly isolated bacterium in the series, accounting for 31.25% of all bacterial cultures. Other gram positive organisms isolated were *Staphylococcus epidermidis* (18.75%) followed by *Staphylococcus aureus* (9.37%) and *Micrococcus* (3.12%).

Pseudomonas aeruginosa was the most frequently isolated gram negative bacterium accounting for 12.5% of all bacterial cultures. This was followed by *Klebsiella aerogenes* (9.37%) and *Enterobacter aerogenes* (13.12%).

100% isolates of *Streptococcus pneumoniae* were sensitive to chloramphenicol, tetracycline and quinolones but resistant to aminoglycosides. Isolates of *Staphylococcus epidermidis* were most sensitive to chloramphenicol, tetracycline, quinolones and vancomycin but least sensitive to penicillin, ampicillin and cephalexin. All isolates of *Staphylococcus aureus* were sensitive to chloramphenicol, tetracycline, quinolones,

aminoglycosides and vancomycin. The single isolate of Micrococcus was sensitive to quinolones only.

Ceftazidime was found to be an excellent drug against Pseudomonas in vitro with 100% sensitivity while only 83.4% of the isolates were sensitive to chloramphenicol and carbenicillin. All Klebsiellae and the single isolate of Enterobacter aerogenes were only resistant to ampicillin.

All the gram positive cocci were sensitive to quinolones. 95% were sensitive to tetracycline and chloramphenicol whereas only 45% were sensitive to aminoglycosides. 90% of the gram negative bacilli isolated were sensitive to chloramphenicol and tetracycline while 80% were sensitive to aminoglycosides and quinolones.

70% patients with pneumococcal keratitis grew Strep. pneumoniae while 66.66% cases with pseudomonal keratitis grew Pseudomonas aeruginosa from the conjunctival sac and lid margins of the affected eye.

All the 10 fungi isolated were filamentous, none being yeast. They included 4(40%) isolates of Aspergillus fumigatus and Fusarium sp. each and 2(20%) of Curvularia sp. Definite history of trauma was obtained in only 20% of patients with fungal keratitis and no patient had received topical steroids. All the cases belonged to rural areas and 70% of them were farmers. Most of the patients were young males 26(32.5%) scrapings yielded positive results on Gram staining, out of which 24(92.3%) grew on culture. Of the 54(67.5%) scrapings with negative Gram staining results, only 18(33.3%) were culture positive. No gram negative bacilli were seen in Gram smears. Gram staining results were consistent with culture in 60(75%) cases,24 being smear and culture positive and 36 smear and culture negative. The likelihood of predicting growth in smear positive cases was 24 times more as compared to smear negative cases and the association ($p<0.05$) is highly significant. The overall sensitivity and specificity of Gram staining as compared to culture was 57.14% and 94.7% respectively. No difference was found between Gram and Giemsa staining in identifying fungi.

Discussion

Corneal ulceration was encountered in all age groups in our study with preponderance among physically active adults. The prevalence was higher in males than females. Similar observations were reported by Ormerod et al,[7] Asbell et al,[8] Polack et al,[9] Schonheyder et al[10] and Dart et al[11] in their studies.

In our study, ulcerative keratitis was common in farmers, which is explained by the fact that large number of our cases belonged to rural areas where farming is the predominant occupation. Men and women both work in farms hence are equally exposed to farming related corneal injuries. Our finding is similar to that reported by Upadhayay et al,[12] however, Carmichael et al[13] found ulcerative keratitis predominant in labourers.

Trauma as the most common predisposing factor for corneal ulceration in our study is also reported in other studies.[7,9,12,14,15] Corneal injury from rice and maize stalks represents the greatest risk factor in our study. Absent in this group was contact lens wear which is frequently implicated as a cause of corneal ulceration in the developed countries.[11,16]

Microorganisms were isolated from 52.5% of corneas that were cultured. This rate of recovery compares favourably with some studies[15,17,18,19,20] while high rate of recovery of organisms has been reported by others.[6,7,8,12,13,21,22] Streptococcus pneumoniae as the most frequently isolated bacterial pathogen in patients with corneal ulceration in our study has also been reported by Pahalkar et al[22] and Bharathi et al[1] in South India, Carmichael et al[13] in South Africa and Upadhayay et al[12] in Nepal. In the United States the incidence of Strep. pneumoniae as a corneal pathogen has fallen to less than 7% whereas Pseudomonas spp. has become the most frequently isolated pathogen in some series.[12] Pseudomonas has been reported as the predominant pathogen in all studies reporting substantial number of contact lens wearers.

45% of our bacterial isolates were sensitive to aminoglycosides while 96% and 91% of the isolates reported by Leisegang et al[6] and Asbell et al[8] respectively were sensitive to them. The reason may be probably a higher yield of Staphylococcus in these series as compared to ours in which Strep.pneumoniae is predominant which is usually resistant to aminoglycosides as compared to Staphylococci. In our study Pseudomonas aeruginosa was sensitive to usual antibiotics including carbenicillin and chloramphenicol which is different from the studies by Liesegang et al[6] and Brinser et al[23] where they have found Pseudomonas aeruginosa less sensitive to these drugs.

Fungal keratitis was observed in 12.5% of cases which is comparable with some studies[19] and higher than others.[12] All these patients had a significant risk of trauma. The commonest isolates were Aspergillus fumigatus and Fusarium species, followed by Curvularia sp. DunlopAA[20] has also found Aspergillus as the commonest fungal isolate followed by Fusarium and Curvularia but only in 13%, 7%, and 5% respectively. Aspergillus as the commonest fungus has been reported in some studies[12,14,19,25] while the finding of Fusarium as the commonest isolate is not different

from other studies.[6,9,15,24] *Curvularia sp.* formed 20% of the fungal isolates which is similar to that observed in other studies.[6,9,12,24]

Prior antimicrobial treatment is a significant factor for failure of isolation of organisms on culture,[20] however in most of the studies no such effect has been seen. It may be due to the use of inappropriate antimicrobials or poor patient compliance.[12,17] Similar results have been obtained in our study with culture positivity of 46.15% despite previous topical antimicrobial therapy. Streptococcus pneumoniae the commonest isolate in our series was resistant to aminoglycosides. The use of topical aminoglycosides by most of our patients prior to evaluation may have been responsible for high culture positivity of this organism. This emphasises the need for adequate broad spectrum antibiotics for all ulcers until an aetiological diagnosis is made.

Gram smear positivity of 32.5% in our study correlates well with the results of Carmichael et al.[13] (32%) while a higher slide positivity of 39.7%, 44% and 56.86% respectively is reported by Asbell et al.,[8] Schonheyder et al.[10] and Deshpande et al.[25] In our study Gram stain results were consistent with culture results in 75% of cases which is similar to findings of some authors[6,8,13] while others[20,26] have reported a lower percentage. Gram positive organisms were identified correctly on smears than gram negative organisms in our study, as has been reported by Liesegang et al.[6] and Dunlop et al.[20] We found no difference between Gram and Giemsa staining in identifying fungi. However Liesegang et al.[6] and Richard et al. [27] found Giemsa staining better than Gram's for fungal identification.

Summary and conclusion

Ulcerative keratitis was found to be more prevalent in rural areas with trauma being the most common ocular predisposing factor. Bacteria were more commonly isolated as compared to fungi. Pneumococcus was the commonest bacterial isolate while *Aspergillus fumigatus* and *Fusarium sp.* were the commonest fungal isolates. The overall sensitivity and specificity of Gram stain as compared to culture were 57.14% and 94.7% respectively. Chloramphenicol and tetracycline were found to be effective against most bacterial isolates in vitro followed by the quinolones.

Hence, we conclude that all the patients of keratitis should be subjected to microbiological evaluation and put on broad spectrum antimicrobials till culture results are available. Early recognition of the causative organisms and prompt use of specific antibiotics and antifungals will bring down the morbidity caused by this disease.

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