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RESEARCH ARTICLE

PREDISPOSING FACTOR AND ETIOLOGICAL DIAGNOSIS OF CORNEAL ULCER

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ABSTRACT

Aims and Objectives: To identify the specific pathogenic organisms risk factors responsible for infection in corneal ulcer patient.
Materials and Method: The present study was undertaken on 100 patients of corneal ulcer attending the outpatient department of Navodaya medical college hospital and research centre, Raichur, with special reference to the etiology and predisposing factors, examination in detail for morphological features, microbiological work up, management and follow up.
Results: Out of 100 patients in 100 eyes, M: F ratio was 1.7:1. Most common age group affected was between 31 – 50 years for all types of infectious keratitis 37.5%, 44.1%, 37.5% and 49.99% for bacterial, mycotic, mixed and sterile keratitis. Socio-economically poor classes had 87% of keratitis. Keratitis occurred more frequently in the residents of rural areas 79%. Trauma was found to be the most common predisposing factor 60% followed by chronic dacryocystitis 6%,chronic steroid use 6%, lid disorders 6%, dry eye 5%, corneal degenerations / dystrophy 4%, contact lens use 1%, diabetes 1%, none 11%. Inferonasal cornea was involved with highest frequency in bacterial and central cornea in fungal keratitis. Staphylococcus epidermidis 37.04% was the most common bacterial isolate followed by staphylococcus aureus 24.07%. Fusarium sp. 33.3% and aspergillus sp. 33.3% were most common of fungal isolates.
Conclusion: Central corneal ulceration is a common problem in surroundings of Raichur and most often occurs after a superficial corneal injury with organic material. Bacterial keratitis is maginally higher than fungal keratitis. Staphylococcus epidermidis is most common bacterial and Fusarium spp. And aspergillus spp are the most common fungal isolate. Staining can be the efficient tool for starting the specific medical therapy in the management of corneal ulcer in the places where culture facilities are not available.

INTRODUCTION

Corneal ulcer is a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon (Srinivasan *et al.*, 1997). Diseases affecting the cornea are a major cause of blindness worldwide, second only to cataract in overall importance. The epidemiology of corneal blindness is complicated and encompasses a wide variety of infectious and inflammatory eye diseases that cause corneal scarring, which ultimately leads to functional blindness. In addition, the prevalence of corneal disease varies from country to country and even from one population to another. While cataract is responsible for nearly 20 million of the 45 million blind people in the world, the next major cause is trachoma which blinds 4.9 million individuals, mainly as a result of corneal scarring and vascularisation. Ocular trauma and corneal ulceration are significant causes of corneal blindness that are often underreported but may be responsible for 1.5–2.0 million new cases of monocular blindness every year (Whitcher John *et al.*, 2001; Madhukar K. Reddy 1994; Chirambo *et al.*, 1986; Chirambo 1976; Rapoza *et al.*, 1991; Brilliant *et al.*, 1985; Khan *et al.*, 1985; Gilbert *et al.*, 1995). The epidemiological pattern and causative agents for 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 etiology within a given region for comprehensive strategy for the diagnosis and treatment of corneal ulcer. The associated ocular morbidity is the result of several factors and patient's management is directly affected by lack of diagnostic facilities and initiation of appropriate antimicrobial therapy. Specific treatment requires quick and accurate identification of the causative micro organisms. These are crucial if a

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programme of prevention is to be considered and if appropriate therapeutic measures are to be instituted (Basak *et al.*, 2005; George Alexandrakis and Eduardo C. Alfonso 2000; Rossolini and Mantengoli 2005; Norina *et al.*, 2008).

MATERIALS AND METHODS

Patients

This prospective study was undertaken on 100 patients of corneal ulcer attending the outpatient department of Navodaya medical college and research centre, Raichur, with special reference to the etiology and predisposing factors, examination in detail for morphological features, microbiological work up, management and follow up. Inclusion criteria was all patients diagnosed with infective corneal ulcer. Exclusion criteria were : Typical viral ulcers, healing ulcers, mooren's ulcers, marginal ulcers, interstitial keratitis, sterile neurotrophic ulcers.any ulcer associated with autoimmune conditionsand if patient was on treatment. After selection of patients, standardized proforma was filled for each patient documenting, Age, Sex, Domicile, Education, Economic status, Occupation and Predisposing factors including history of Trauma, Diabetes mellitus and Operation (if any).Detailed clinical history was taken and any previous usage of medications were recorded.

Clinical procedure

After having obtained a written informed consent the sampling procedure was undertaken. Detailed systemic clinical examination of patient was done. Visual acuity of every patient was noted. Detailed clinical examinations including slit lamp biomicroscope with special reference to fluoresce in staining was done. Material was collected from conjunctiva, sac area, corneal ulcer scrapings. Corneal scrapings was performed under aseptic conditions on each ulcer taken after putting topical anaesthesia (4% lignocaine) using a flame sterilized kimura spatula or 15 No. blade. Material from corneal scraping was smeared on to separate glass slides: one for gram stain other for KOH mount. Material was sent for culture. Material obtained from scraping will be inoculated directly onto sheep's blood agar, chocolate agar, Mc Conkey's agar for bacterial culture and sensitivity. Also for fungal culture materials was inoculated on to Sabouraud's Dextrose agar. By convention to indicate the site of inoculums on a solid medium, harvested material is inoculated in the form of a 'C' streak on each medium from separate scrapings.

Laboratory procedure

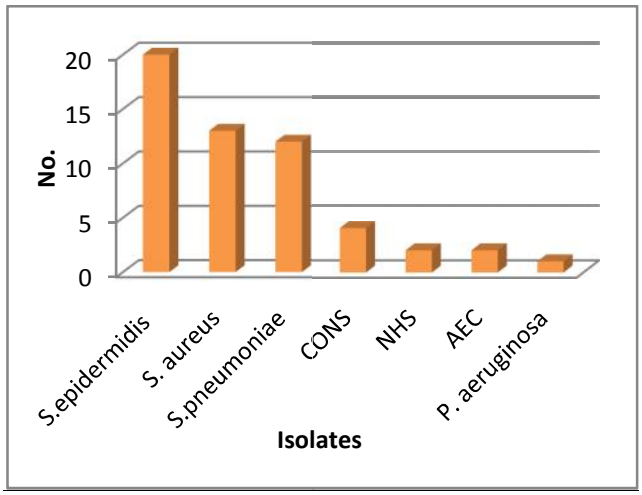
For bacterial culture, the specimens were cultured on dried plates of MacConkey's agar at 37^{o}C for 18-24 hours and on 5% Sheep Blood agar and Chocolate agar with 5-10% CO_2 atmosphere(candle jar) at 37^{o}C for 24 - 48 hours and the same swab was placed in BHI broth and incubated at 37^{o}C for 18-24 hours. The plates were examined for growth, If there was no growth, the plates were further incubated for up to 7 days to look for any slow growing or fastidious organism and reported as no growth if no growth even after 7 days of incubation. presence of growth only on the "C" streak was considered significant then the colony morphology was studied on the MA, BA and CA plates and processed further. BHI broth was examined for any turbidity and sub cultured on MA at 37^{o}C for 18-24 hours and BA with 5-10% CO_2 atmosphere (candle jar) at 37^{o}C for 24 - 48 hours were examined for growth, if there was no growth it is reported as no growth. For further identification isolated colony was inoculated appropriate media for biochemical tests. For fungal culture, the specimens (one more separate swab) was used to inoculate on Sabouraud Dextrose Agar tube and incubated at 25^{o}C, it was examined daily for any growth for the first week and twice a week for a period of four weeks. Rate of growth, Morphology of colony, Texture, Surface pigmentation, Microscopic examination like LPCB mount and slide culture were done to identify the fungi.

Systemic investigations

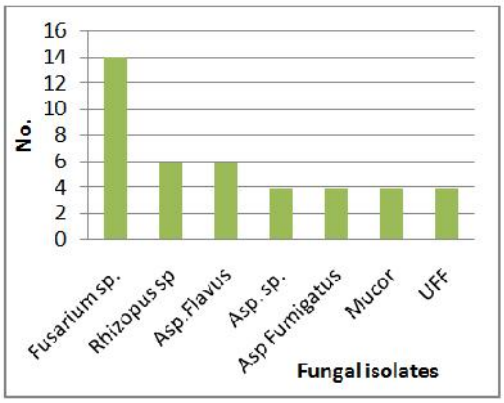
Haemoglobin,Total and differential leucocyte count, Erythrocyte sedimentation rate, Random blood sugar ,HIV testing ,Hbs Ag testing ,MRI head and brain (optional)

RESULTS

During 18 consecutive months (from November 2010 to may 2012), 100 patients were seen with a corneal infiltrate that was compatible with a diagnosis of infective keratitis. Male: Female ratio for infectious keratitis was 1.7:1. There was no significant difference in the eye involved. Involvement of right eye was slightly more (52%) compared to left eye (48%). In total 40 cases were diagnosed as bacterial keratitis, 34 cases as fungal keratitis and 8 cases as mixed (both bacterial and fungal), no growth was seen in 18 cases termed as sterile. The commonest age group was found to be between 31 – 50 years for all types of infectious keratitis 37.5%, 44.1%, 37.5% and49.99% for bacterial, mycotic, mixed and sterile keratitis respectively. Staphylococcus epidermidis was the predominant bacterial isolates in 20 cases (37.04%), followed by Staphylococcus aureus 13 cases (24.07%), Streptococcus pneumoniae 12 cases (22.22%), Coagulase negative staphylococci (CONS) in 4 cases(7.41%), Non Hemolytic streptococci (NHS), atypical E Coli in 2 cases (3.70%) each. Pseudomonas aeruginosa was isolated in contact lens user in 1 case (1.85%).



Graph 1. Bacterial isolates



Graph 2. Fungal isolates

Trauma was the most common (60%) predisposing factor followed by chronic dacryocystitis 6%, chronic steroid use 6%, lid disorders 6%, dry eye 5%, corneal degenerations/dystrophy 4%, contact lens use 1%, diabetes 1%, none 11%.The most common offending agent causing trauma leading to ulceration was vegetative matter. Out of 60 cases of trauma 29 (48.33%) cases had injury due to vegetative materials. The predominant fungal isolates was Fusarium sp. 14 cases (33.3%) followed by Aspergillus Flavus and Rhizopus sp. 6 cases (14.29%) each. Aspergillus sp., Aspergillus Fumigatus, Mucor isolated in 4 cases (9.52%) each. 4 cases (9.52%) of the isolates remained unidentified all which were filamentous fungi. Socio-economically poor classes (upper lower and low class) showed the highest percentage of keratitis 87%. Keratomycosis and bacterial keratitis occurred more frequently in the residents of rural areas 88.24% and 70% respectively. People associated with farm work were most commonly predisposed to the infectious keratitis 56 cases (56%). Central cornea was involved most commonly in fungal keratitis 29.41% and inferonasal 22.25% in bacterial keratitis. 65% > 5 mm^2 surface area of cornea involved. 63% visual acuity in the range of PL PR – 6/60 at the time of presentation signifies late presentation. A majority of cases showed involvement 1/3rd – 2/3rd of corneal thickness i.e., 60 cases (60%). Rolled out/irregular feathery margins were noted in 70.59% cases of keratomycosis. Over hanging margin was more common in bacterial keratitis 77.5% Hypopyon was present in 73.53% cases of keratomycosis which was higher than hypopyon positive cases of bacterial keratitis 67.5%. 64% cases of fungal keratitis showed thick and fibrinous hypopyon whereas 59.26% cases of bacterial keratitis showed liquid hypopyon.

Table 1. Occupation wise distribution

Sl. No.	Occupation	Bacterial No	Fungal No	Mixed No	Sterile No	Total No
1.	House wife	7	7	1	2	17
2.	Agriculture	12	15	3	5	35
3.	Coolie	8	8	2	3	21
4.	Clerical	3	1	0	3	7
5.	Students	5	1	1	1	8
6.	Business	4	1	0	2	7
7.	Others	1	1	1	2	5
Total		40	34	8	18	100

Table 2. Causes for corneal ulcer

Factors associated	Bacterial No.	Fungal No.	Mixed No.	Sterile No.	Total No.
Trauma	22	29	3	6	60
Chronic dacrocystitis	4	0	0	2	6
Dry eye	3	1	0	1	5
Steroid usage	1	2	2	1	6
Lid disorder	2	1	1	2	6
Contact lens	1	0	0	0	1
Corneal disorder	1	0	1	2	4
Diabetes	1	0	0	0	1
None	5	1	1	4	11
Total	40	34	8	18	100

Table 3. Modes of trauma

Agents	Bacterial No.	Fungal No.	mixed No.	Sterile No.	Total No.
Vegetative matter	8	18	1	2	29
Soil/sand/stone	8	2	0	1	11
Animal tail	1	8	1	2	12
Finger nail	2	0	1	0	3
Unknown	3	1	0	1	5
Total	22	29	3	6	60

Out of 36 KOH positives, 34 correlated with culture results, out of 64 KOH negatives 8 was positive for culture results .Out of 42 Gram stain positive result, 34 correlated with culture results and out of 58 Gram stain negative results, 14 positive with culture results. 41 out of 53 clinical diagnosed microbial corneal ulcers yielded growth in culture, 7 out of 47 diagnosed as fungal ulcer gave bacterial culture positive. Out of 47 cases of clinically diagnosed fungal corneal ulcer 35 showed fungal growth on Sabouraud's agar where as 7 out of clinically bacterial appearing ulcers were positive for fungal growth.

DISCUSSION

Microbial keratitis is a common, potentially sight threatening ocular infection that may be caused by bacteria, fungi, viruses or parasites. Bacterial keratitis rarely occurs in normal eye because of the human cornea's natural resistance to infection. However, predisposing factors such as corneal injury, contact lens wear, ocular adnexal dysfunction (including tear film deficiencies), corneal abnormalities and other exogenous factors, systemic disease and immunosuppression may alter the defense mechanisms of the outer eye and permit bacteria to invade the cornea. (Sharma *et al.*, 2003) Corneal ulcer showed a higher prevalence i.e., 72 cases (72%) in the economically active age group (21-60 years). Least incidence of corneal ulcer was noted in the extremes of age 3 (3%) cases in 1-10 years, 9 cases (9%) in 11-20 years, 5 cases above 71 years. Basak *et al.* (2005) showed 49.3% of keratitis cases were 21-40 years age group. Sharma *et al.* (2003) showed 83% of cases were 21-60 years age group with maximum incidence was

noted in the age group of 41-60years. Male to female ratio of 1.7: 1. M. Srinivasan *et al.* (1997) which showed overall ratio of male to female patients of 1.6 to 1. It can be correlated with active outdoor activities. Maximum incidence 87 cases (87%) in the socio-economically disadvantageous group. Out of 100 patients 79 (79%) were from rural areas and 21 (21%) were from urban residents.Farmers and agricultural laborers constituted 56% of cases this was followed by housewife /not working category who constituted 17 cases (17%) of corneal ulcers, followed by students 8 cases (8%), followed by clerical work and business who constituted 7% each. Similar reports were observed in studies by M Srinivasan *et al.* (1997), Bharathi *et al.* (2003), Upadhyay *et al.* (1991) Lack of awareness about eye protective measures, nature of the works which involves handling sharp or projectile objects in rural and agriculture area has predisposed particular population to corneal injury. History of corneal trauma predisposing to corneal ulceration was the most frequent predisposing factor noted, representing 22 cases of bacterial (55%) and 29 cases of fungal (85.29%) corneal ulcers respectively. Chronic dacryocystitis was noted in 6 cases (6%).Lid disorder was noted 6 cases (6%). 2 patients had facial palsy with lagophthalmos, 2 had entropion, 1 had trichiasis, 1 had distichiasis. Chronic topical steroid use was noted in 6 cases (6%). Dry eye conditions representing 5 cases (5%) were noted. Corneal dystrophy/degeneration was noted in 4 cases (4%). Contact lens use was seen in 1 case (1%), as very few percentage of contact lens user are found in this area.1 patient had uncontrollable diabetes with h/o recurrent bacterial conjunctivitis. The present results are similar to the work done by Srinivasan *et al.* (1997) showed trauma was most common (69.4%) followed by chronic dacryocystitis (4.60%) followed by lid disorder in (3%). Other causes noted were leprosy, diabetes, dry eye, corneal anaesthesia following herpes simplex or herpes zoster infections, corneal degeneration/dystrophy. Dr Suresh Prasad *et al.* (1982) in their study noted 92% of their cases gave history of corneal trauma Basak *et al.* (2005) and Norina *et al.* (2008) showed 54.61% and 50% of cases of trauma were due to vegetative materials respectively. Encouraging the people to use protective measures like protective glasses, caution about penetrating objects, immediate referral of patients in any ophthalmic disorder can reduce the burden of corneal ulcer drastically.

Central locations were the most frequently observed in fungal ulcers i.e., 10 cases (29.41%). Most common sites of the cornea involved is infero-nasal in bacterial keratitis 9 cases (22.50%. The next most commonly involved site was central in bacterial i.e., 8 cases (20%) and inferotemporal in 9 cases (26.47%) of fungal corneal ulcers. The incidence of anterior chamber involvement with hypopyon was slightly more in cases of fungal keratitis 25 cases (75.53%) compared to 27 cases (67.5%) of bacterial keratitis. In 40 cases (40%) bacteria were identified from the cultures. In 34 cases (34%) fungus was isolated. 8 cases (8%) showed mixed growth of both bacteria and fungus. 18 (18%) cases showed no growth. In our study staphylococcus epidermidis was the predominant bacterial isolate (37.04%), followed by staphylococcus aureus (24.07%), Streptococcus pneumoniae (22.22%), Coagulase negative staphylococcus (CONS) (7.41%), Non hemolytic streptococci (3.70%), Atypical E coli. (3.70%) Pseudomonas aeruginosa (1.85%). The results were consistent with the work done by Gopinathan *et al.* (2009) and Schaefer *et al.* (2001) who found the Staphylococcus epidermidis to be the most common bacterial isolate followed by staphylococcus aureus. But it differs with work done by Bharathi *et al.* (2003) and M. Srinivasan *et al.* (1997) who found the Streptococcus pneumoniae to be the most common bacterial isolate followed by staphylococcus species. This indicates regional variation in micrbiological profile of corneal ulcer and importance of microbiological culture from infective tissues. In our study the predominant fungal isolates was Fusarium sp. (33.3%) and Aspergillus spp. (33.3%) followed by Rhizopus spp (14.29%), Mucor (9.52)% unidentified filamentous fungi (9.52%). Filamentous fungi accounted for 76.18% of total 34 cases of fungal keratitis. Results in our study are similar to other studies carried out in tropical countries, where filamentous fungi specially the Fusarium sp. is the predominant fungal isolate from the corneal scraping and culture of fungal keratitis cases. Laila *et al.* (2010) showed in their study aspergillus was the most common 45.4% followed by fusarium species24.24%. Gopinathan *et al.* (2009) in their study showed fusarium spp 36.6% followed by aspergillus spp 25% of fungal isolates.

Direct smear examination was done by gram's staining and for fungi by 10% KOH mount and gram's stain. Gram stain had sensitivity of 70.8%, specificity of 84.6%, Positive Predictive Value 80.95 %, Negative Predictive Value 75.86 %. P value <0.001(X^2 test) .KOH had sensitivity of 80.95%, specificity of 96.5%, Positive Predictive Value 94.4 %, Negative Predictive Value 87.5 % . P value<0.001(X^2 test). Possible reason for low sensitivity may be due to inadequate specimens, insufficient microbiological investigation. However it can be seen that most cases will be guided reliably by their Gram stain results if culture facilities are not available. A clinical diagnosis of bacterial corneal ulcer was put on the basis of presence of corneal infiltrates, epithelial defects, conjunctival hyperaemia, cilliary congestion, mucopurulant exudates and presence of hypopyon. A provisional diagnosis of bacterial corneal ulcer was given to 53 cases (excluding viral or fungal appearing cases) on the basis of round corenal ulcer with over hanging margins conjuctival hyperemia, circum ciliary congestion and type of hypopyon. Out of 53 such cases only bacterial isolates were obtained from 41 cases with sensitivity of 85.42% and specificity of 76.92% Positive predictive value of 77.35%, Negative predictive value of 85.11%. P value <0.001. (X^2 test) The clinical diagnosis of fungal corneal ulcer was put on the basis of a dry looking ulcer with rolled out margins and feathery finger like extensions into the surrounding stroma, presence of large hypopyon and presence of satellite lesions and immune ring. 35 out of 47 clinically suspected fungal ulcers yielded growth in culture, giving a sensitivity of 83.33%% and specificity of 79.31%, Positive predictive value of 74.46%,Negative predictive value of 86.8%. P value <0.001. (X^2 test). Study done by Bharathi *et al.* (2003) the sensitivity of clinical diagnosis of bacterial keratitis made by ophthalmologist was 83% and of fungal keratitis was 94.1%.

Conclusion

Central corneal ulceration is a common problem in surroundings of Raichur and most often occurs after a

superficial corneal injury with organic material. Bacterial keratitis is maginally higher than fungal keratitis. Staphylococcus epidermidis is most common bacterial and Fusarium spp. And aspergillus spp are the most common fungal isolate. Staining efficiently establishes the diagnosis therefore can be used in the management of corneal ulcer to start the prompt treatment as corneal ulcer is a medical emergency. The microbiological profile helps the ophthalmologists to start the specific treatment directed against the causative organisms.

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