CLINICO-MICROBIOLOGICAL PROFILE OF INFECTIVE KERATITIS IN A TERTIARY CARE HOSPITAL, EASTERN INDIA

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ABSTRACT

Background: Infective Keratitis (Microbial Keratitis) is infection of the cornea caused by a wide spectrum of microbial agents. Its main clinical presentation is Corneal ulcer that is defined as a loss of corneal epithelium. According to World health organization (WHO), corneal diseases are among the major causes of vision loss and blindness in the world today, second only to cataract in overall importance. **Aim:** To isolate and identify the pathogenic organism **Method:** The study was conducted in 45 patients diagnosed with corneal ulcer attending Ophthalmology OPD, MKCG Medical College and Hospital, Berhampur over a period of 2 months. The material was scraped from the leading edge and base of the ulcer and was inoculated onto Blood agar, Mac conkey agar and Sabouraud Dextrose agar for culture and onto 2 slides for Gram's stain and 10% KOH wet mount. The susceptibility testing was done by Kirby Bauer's disc diffusion method. Results: Out of total 45 patients,35were males. Majority of the patients belonged to age group of 50-60 years. Pain, Redness, Hypopyon was most commonly seen in Bacterial keratitis. In Fungal Keratitis, Redness (80%), Blurred vision (80%) was seen. Most common occupation was Farmers (66.6%). Trauma was the most common risk factor (23 isolates). Majority were bacterial isolates (29 isolates,64.4%) followed by fungal (5 isolates ,11.1%). Predominant isolate was *Staphylococcus aureus* (68.9%). All the gram- positive isolates showed 100% senitivity to Linezolid and Vancomycin. **Conclusion:** Knowledge of local prevalence of etiological agents of IK and their susceptibility patterns helps in guiding ophthalmologists to select appropriate antibiotic for empirical therapy

Keywords: Keratitis; Bacterial keratitis; Staphylococcus aureus; MRSA.

INTRODUCTION

Infective Keratitis (Microbial Keratitis) is infection of the cornea caused by a wide spectrum of microbial agents which is a potentially sight threatening condition [1,2]. According to World health organization (WHO), corneal diseases are among the major causes of vision loss and blindness in the world today, second only to cataract in overall importance [3].

WHO has perceived that corneal blindness due to microbial keratitis is emerging as principal reason for visual inability and that it is "Silent Epidemic" happening unnoticed around the world. Its main clinical presentation is corneal ulcer that is defined as a loss of corneal epithelium with infiltration and suppuration of underlying stroma accompanied with signs of inflammation with or without hypopyon. The etiological and epidemiological features of Infective keratitis depend on host factors, geographical location, and climate also tends to vary with time. Hence, epidemiological features, risk factors and etiological agents that occur in a specific region are important in rapid diagnosis of the disease and timely institution of specific therapy. In order to start specific therapy, it is necessary to do microbial investigations which includes microscopy and culture for identification.

DOI: 10.31878/ijcbr.2019.54.04

eISSN: 2395-0471
pISSN: 2521-0394

MATERIAL AND METHODOLOGY

Study design: Prospective study

Study place: The study was conducted in 45 patients diagnosed with corneal ulcer attending Ophthalmology OPD, MKCG Medical College and Hospital, Berhampur

Ethical approval: Consent was taken for collection of corneal scrapings and the study procedures were approved by Institutional ethics committee

Time frame: over a period of 2 months from January to March 2019

Inclusion criteria: All Suspected Bacterial and Fungal infected corneal ulcers

Exclusion criteria: Marginal keratitis, Interstitial keratitis, suspected viral ulcers.

Sample size: 45 samples

Methodology : History was taken like age, sex, occupation, history of trauma, surgery and antibiotic intake. Corneal scrapings were taken after complete ocular examination under slit lamp biomicroscope, with strict aseptic precautions using a sterile (Number-15) Bard-Parker blade. Before the collection, 4% paracaine

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was instilled without preservative. Consent was taken for collection of corneal scrapings

The material was scraped from the leading edge and base of the ulcer and was inoculated onto Blood agar, Mac Conkey agar and Sabouraud Dextrose agar for culture and onto two slides for Gram's stain and 10% KOH wet mount [4]. All the inoculated Bacteriological media were incubated at 37^0C.

Identification of the organisms was done by following standard protocols. The susceptibility testing was done by Kirby Bauer's [5] disc diffusion methods as per Clinical and Laboratory Standards Institute guidelines. Cefoxitin (30 microgram disk) was also given to study Methicillin Resistance Staphylococcus aureus by disk diffusion method according to CLSI guidelines [6].

Inoculated Sabouraud Dextrose Agar (SDA) was incubated at 27 ^{0} C and were examined daily until three weeks for growth. Fungi were identified by their colony characteristic on Sabouraud dextrose agar and morphological character on Lactophenol cotton blue mount.

RESULTS

Out of a total of 45 patients, 35(77.7%) were males, and 10(22.2%) were females. The most common occupation was Farmers (66.6%) followed by labourers (22.2%), Housewife (11.1).

Table 1:Age group of the patients (n=45)

Age group (years)	Number of patients (%)
10-20	2 (4.4)
21-30	3 (6.6)
31-40	4 (8.8)
41-50	6 (13.3)
51-60	15 (33.3)
61-70	7 (15.5)
71-80	8 (17.7)

Majority of the patients belonged to age group of 50-60 years (33.3%).

Pain (93.1%), Redness (89.6%), Hypopyon (72.4%), Lacrimation (86.2%) was most commonly seen in Bacterial keratitis. In Fungal keratitis, Redness (80%), Blurred/Diminished vision (80%), Pain (60%) was seen (Table 2).

Table 2: Clinical features

Clinical features	Bacterial keratitis (N=29)	Fungal keratitis (N=5)
Symptom		
Redness	26/29(89.6%)	4/5(80%)
Pain	27/29(93.1%)	3/5(60%)
Lacrimation	25/29(86.2%)	3/5(60%)
Photophobia	27/29(93.1%)	2/5(40%)
Blurred/Diminished vision	20/29(68.9%)	4/5(80%)
Signs		
Lid oedema	20/29(68.9%)	1/5(50%)
Hypopyon	21/29(72.4%)	2/5(40%)
Conjunctival congestion	16/24(66.6%)	3/5(60%)
Irregular feathery margins	12/24(50%)	4/5(80%)

Table 3: Risk factors (n=45)

Risk factor	No. of isolates
Trauma	23 (51.1)
H/O antibiotic intake	5 (11.1)
Prior surgery	6 (13.3)
H/O Diabetes mellitus	4 (8.8)
Contact lens wear	3 (6.6)
Pre-existing ocular disease	4 (8.8)

Trauma was the most common risk factor (23 isolates, 51.1%) followed by History of Prior surgery (13.3%).

Table 4: Types of isolate (n=45)

Organism isolated	Number of isolates (%)
Bacterial	29 (64.4)
Fungal	5 (11.1)
Sterile	11 (24.4)

Majority were Bacterial isolates (29 isolates,64.4%) followed by fungal (5 isolates ,11.1%).

Table 5: Bacterial isolates (n=29)

Bacteria isolated	Number (%)
Staphylococcus aureus	20 (68.9)
Enterococcus spp.	3 (10.3)
Pseudomonas aeruginosa	4 (13.7)
Acinetobacter baumanii	2 (6.8)

Predominant isolate was *Staphylococcus aureus* (68.9%) followed by *Pseudomonas aeruginosa*. (13.7%).

Table 6: Fungal isolates (n=5)

Fungus isolated	Number (%)
Fonsacea pedrosoi	1 (20)
Fusarium	2 (40)
Aspergillus fumigatus	1 (20)
Candida albicans	1(20)

Majority of the isolates were filamentous fungi (80%).

Table 7: Sensitivity Pattern of Gram- positive cocci

Bacterial isolate	Staphylococcus aureus	Enterococcus spp.
Linezolid	20/20 (100%)	3/3 (100%)
Vancomycin (30 µg)	20/20 (100%)	3/3 (100%)
Ciprofloxacin (5 µg)	11/20 (55%)	2/3 (66%)
Cefoxitin (30 µg)	10/20 (50%)	-
Gentamycin (10µg)	13/20 (65%)	1/3 (33%)
Moxifloxacin (5 µg)	14/20 (70%)	2/3 (66%)

All the gram- positive isolates showed 100%senitivity to Linezolid and Vancomycin. Out of 20 isolates of Staphylococcus aureus 14 isolates were sensitive to Moxifloxacin (70%) followed by Gentamycin (13 isolates,65%),Ciprofloxacin(11 isolates,55%) .

Table 8: Sensitivity pattern of Gram-negative bacilli

Bacterial isolate	Acinetobacter baumanii	Pseudomonas aeruginosa
Ciprofloxacin(5mcg)	1/2(50%)	1/4 (25%)
Gentamycin(10mcg)	2/2(100%)	2/4(50%)
Piperacillin tazobactam(100/10mcg)	-	3/4(75%)
Levofloxacin(5mcg)	2/2(100%)	1/4(25%)
Moxifloxacin(5mcg)	1/2 (50%)	3/4(75%)
Tobramycin(10mcg)	1/2 (50%)	2/4 (50%)

Pseudomonas aeruginosa was sensitive to Piperacillin tazobactam (3 isolates,75%), Moxifloxacin (3 isolates,75%).

DISCUSSION

At birth the eyes are sterile but soon become invaded by microorganisms. The interior structures remain sterile. Almost any bacterial species can infect the cornea if the integrity of the natural anatomic barriers or defence mechanisms is compromised [4]. Despite therapeutic advances in the treatment of IK, it continues to be a major cause of blindness, especially in developing nations.

Higher incidence was seen in age group 50-60 years (33.3%) in our study similar to Gopinathan et.al [8].

History of male preponderance (77%) has been reported by our study which is similar to study done by Bashir et.al. [9] (65%). However, Al Yousouf N et.al [10] and Kotigadde S et.al [11] reported higher incidence among women. The male predominance is due to their outdoor activities in the age group of 50-60 years thus predisposing to trauma and corneal infections.

Farmers and labourers are in constant contact with vegetative matter and thus prone to corneal ulcers. This may be the reason for highest incidence of corneal ulcers in farmers and labourers as compared to various other occupation similar to study done by Srinivasan et al[12].

Majority of the research work recorded trauma as a major risk factor in India. Trauma (51.1%) was the most common predisposing risk factor in our study similar to Srinivasan et.al [12]. However, Shaefer et.al [13] (Switzerland) and Green et.al [14] (Australia) have reported contact lens as the most common risk factor for development of keratitis. This difference can be explained as the study was carried out in a developed country, where more people use contact lenses and history of occupational trauma is uncommon due to increased awareness and occupational safety measures.

In the present study, the Patient presented with clinical features of Pain 93.1%, Redness 89.6%and Hypopyon 72.4% in Bacterial keratitis. In Fungal keratitis, Blurred/ Diminished vision 80%, Pain 60%and Redness 80% was seen which can be compared to the study done by Ibrahim et al [15] and Thomas et.al [16] (Red eye – Bacterial 89.22% fungal 87%, Pain – Bacterial 90.32 % Fungal 87.55 %, Photophobia-Bacterial 67.74% Fungal 86.67%, Poor vision –Bacterial 71.67% Fungal-93.49%, Hypopyon (Bacterial 36% Fungal 16%).

In our study we observed culture positivity in 34 (75%) of the 45 cases of clinically diagnosed infective keratitis and 11 cases were sterile which matches with reports of Srinivasan et.al [12]. The culture negativity may be due to that these patients may have received prior antibiotic treatment.

Majority of the cases had corneal infection by single agent the most common being bacterial in 29 cases (64.4%). However, Sirisha et al [17], concluded that fungal corneal ulcer (49%) is more common than bacterial corneal ulcer (21%).

Bacterial keratitis was predominantly caused by Gram positive bacteria. The incidence of gram-positive cocci (79%) coincides with the study done by Tewari et.al [18]. Predominance of *Staphylococcus aureus* was seen which is similar to a study done from Gangetic West Bengal [19]. However, *Streptococcus pneumoniae* was the predominant species in the study done by Bharathi et.al. [20].

P. aeruginosa is the predominant gram-negative bacteria that causes Corneal ulceration. In our study *P. aeruginosa* accounted for 13.7% of bacterial isolates which matches with the results of Kaliamurthy et al [21] (9.7%). but G. Singh et al [22], Asbell PA et.al. [23], Houang E et.al [24] isolated higher number of *Pseudomonas aeruginosa* isolates which may be due to climatic conditions difference.

Fungal growth was seen in 11% of total corneal ulcers. Filamentous fungi are the major fungal pathogens in corneal ulcer in our study. Yeast like fungi have low predominance in fungal corneal ulcers. Fusarium species was the predominant fungal isolate in our study similar to the study done by Sirisha et.al [17]. However, Laspina et al [25] found that *Acremonium* species was the most commonly identified fungi (40%) followed by *Fusarium* species (15%) which can be attributed to difference in geographic location and environmental factors between India and Paraguay.

Both gram-positive and gram-negative isolates showed varied susceptibilities to selected antibiotics. Antibiotic resistance among ocular pathogens is increasing in parallel with the increase seen among systemic pathogens and likewise may have serious consequences such as development of sight-threatening complications of keratitis, endophthalmitis, orbital cellulitis, or panophthalmitis [23]. Our antibiotic sensitivity results were quite comparable to studies done by Sharma et.al [26], where the most common effective drug was Ciprofloxacin (75%) followed by Gentamicin. In the present series, only 70% of organisms Staphylococcus aureus were susceptible to moxifloxacin, the 4th generation fluoroquinolone. Researchers have documented significantly increasing resistance rates to moxifloxacin among *Staphylococcus aureus*. Thirteen (56.5%) of staphylococcal isolates were MRSA. Majority of isolates of P. aeruginosa were sensitive to Moxifloxacin (75%) which is similar with the reports of Kaliamurthy J. et.al [27].

Finding of low resistance levels to these newer fluoroquinolones highlights the need to use them for first line monotherapy in BK. However, Moss et al [28] reported 100% sensitivity of moxifloxacin and Gatifloxacin against both gram-positive and gram-negative bacteria.

CONCLUSION

Understanding the geographical pattern of the pathogenic organisms responsible and the identification of risk factors, helps to create a broad strategy for the diagnosis and management of corneal ulcers and helps in guiding ophthalmologists to select appropriate antibiotic for empirical therapy. Confirmation by microbiological diagnosis is very essential in order to limit the ocular morbidity and prevent complications.

Conflict of interest : Nil

Source of funding : **Nil**

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