International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor (2018): 7.426

Clinical Analytical Study of Microbial Keratitis in a Tertiary Care Institution

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Abstract: ***Aim: To study the epidemiologic characteristics, risk factors, etiology, relevance of gram stain and culture, response to treatment and outcome of microbial keratitis in the general adult population. Material and Methods: Study Design: A prospective, nonrandomized, analytical clinical Study of 100 cases presenting with suspected microbial keratitis presenting to the Cornea clinic, Department of Ophthalmology, Tirunelveli Medical College Hospital were examined according to a dedicated corneal ulcer protocol. Study period: August 1, 2011 and July 31, 2016. Inclusion criteria: >16 years of age, corneal infiltration, microbiological tests of scrapings Exclusion criteria: viral keratitis, keratitis of noninfective etiology and children below 16 years of age. Corneal ulcer scrapings were subjected to gram stain and culture and sensitivity. The clinical outcomes were tabulated. Results and Discussion: The 40-50 years group was the most affected. There was no sex preponderance. Staphylococcus spp were the most common organism isolated. Pseudomonas was the most common gram negative bacterium isolated. Gatifloxacin (0.3%) was the most effective antibiotic according to sensitivity reports. Fortified cefazidime (50 mg/ml) and amikacin (20mg/ml) were effective on gram negative bacteria when added to topical gatifloxacin. Poor outcomes were significantly associated with older age (p=0.021708), large ulcers(p=0.000032), central ulcers(p=0.00001) and presence of hypopyon (p=0.000012). Conclusion: Topical gatifloxacin (0.3%) is the antibiotic of choice for initial mono therapy of bacterial keratitis. Fortified amikacin (20mg/ml) or fortified ceftazidime (50mg/ml) must be added to gatifloxacin 0.3% if gram negative bacilli are identified. Modification of initial antibiotic based on antimicrobial sensitivity is needed only when worsening of the ulcer occurs clinically.***

Keywords: Corneal Ulcer, Hypopyon, Fortified Antibiotics, Microbial Keratitis

1. Introduction

The World Health Organization has recognized corneal blindness resulting from microbial keratitis as a major cause of visual disability[1]. The incidence of corneal ulcers was 113 per 1 lakh population in 1993, 10 times that of the US. A good understanding of the importance of prevention, risk factors, early recognition, and early initiation of appropriate therapy is important to reduce the morbidity due to infectious keratitis.

2. Aim and Objectives

To study the epidemiologic characteristics, risk factors, etiology, relevance of gram stain and culture, response to treatment and outcome of microbial keratitis in the general adult population

3. Material and Methods

This was a prospective, nonrandomized, analytical clinical Study conducted at the Cornea clinic, Department of Ophthalmology, Tirunelveli Medical College Hospital between August 1, 2011 and July 31, 2013. All patients presenting with suspected microbial keratitis during the study period were examined according to a dedicated corneal ulcer protocol. Inclusion criteria were patients above 16 years of age, the presence of corneal stromal infiltration on slit-lamp examination and microbiological investigation of corneal ulcer scraping. Exclusion criteria were viral keratitis, keratitis of noninfective etiology and children below 16 years of age. History taking included information about the duration of symptoms, prior treatment, predisposing ocular conditions and associated risk factors like trauma and diabetes mellitus. All patients were examined at the slit lamp biomicroscope. Corneal ulceration was defined as loss of the corneal epithelium with clinical evidence of infiltration with or without hypopyon. The size of the ulcer was measured using variable beam on the slitlamp or by using a millimeter ruler. In a similar fashion, the size and depth of stromal infiltration were recorded. Clinical sketches of frontal and cross sectional views and associated hypopyon were noted on the form. Predisposing ocular conditions including dacryocystitis, dry eye, corneal anesthesia and blepharitis were looked for.

The corneal ulcers were sized as small (< 5 mm), medium (5 – 7 mm) and large (above 7 mm). Using standardized techniques, corneal ulcer scrapings were obtained using the back of a sterile 11 blade and inoculated directly onto 5% sheep blood agar and Sabouraud dextrose agar. Scraped material was also subjected to direct microscopy after staining with Gram stain and Lactophenol cotton blue. Growth in culture was deemed significant if the same organism was isolated on atleast two “C” streaks of one culture medium with consistent morphology on direct microscopy. Antibacterial sensitivity testing was performed using Kirby Bauer disk diffusion method. Though sensitivity was tested for 54 antibiotics the commonly used topical gatifloxacin, ofloxacin, ciprofloxacin, tobramycin, fortified

cefazolin, cefotaxime, ceftazidime, amikacin and gentamicin were tabulated.

For bacterial keratitis, ofloxacin (0.3 %) hourly was the first line of therapy. If clinical response was unsatisfactory, antimicrobial therapy was changed either by adding fortified cefazolin (5.0%) and tobramycin (0.3%) or as per culture and sensitivity reports.

For fungal keratitis, natamycin (5%) suspension was the preferred first line of treatment. If clinical response was inadequate fluconazole (0.2 %) or itraconazole (2%) eye drops were added to the treatment regimen. Oral ketoconazole (200 mg bid) was added in cases of severe keratitis and non responders.

Statistical analyses were performed using Excel Worksheets (Microsoft Corporation, Redmond, WA)

4. Results and Discussion

1) Patient Demographics

Of the 100 patients, 85 were below 60 years and 15 were above 60. The age group affected maximum by corneal ulcer was the 40 to 50 years age group. Sixty two were male and 38 female. Most large age–independent series of microbial keratitis report a male preponderance of 61% to 71% [2]. In our study, 62% of ulcers occurred in males. In the younger age group, 54 were males and 31 were females. In the old age group 9 were males and 6 females. Male preponderance was more in the younger age group (63.5%) than in the elderly age group (60%) similar to the study by Pragya Parmar et al[2] who have reported 60%. This is probably due to the increased chances of injury in the male population. However this was not statistically significant (p= 0.794)

The age and sex distribution of the study patients is listed in Table 1 and Figure 1.

Table 1: Age & Sex Distribution

Age distribution	Females	Males
>60	6	9
50-60	7	6
40-50	6	19
30-40	8	15
20-30	8	10
<20	2	3

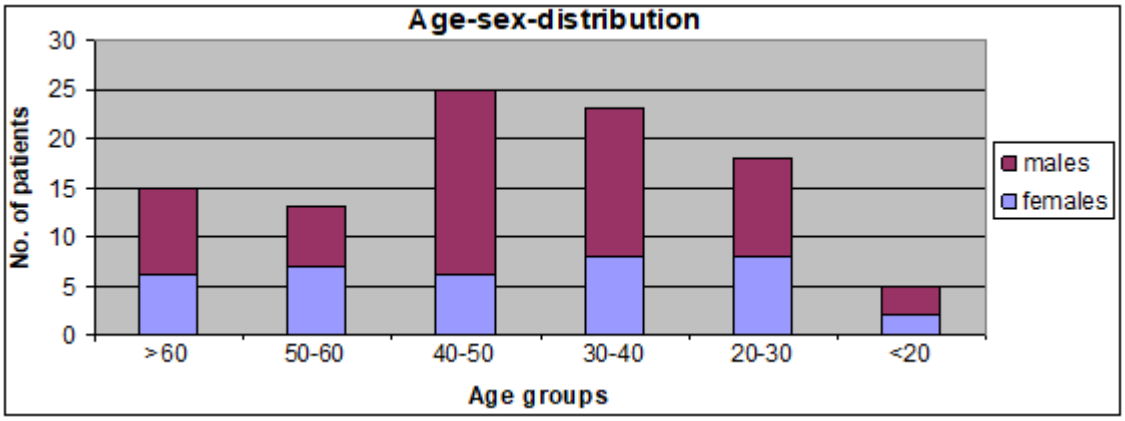

Figure 1

In our study, the incidence of central ulcers was 40%, paracentral ulcers was 40% and peripheral ulcers was 20%. Small ulcers constituted 54%, medium sized ulcers 37% and large ulcers 9%.

The majority of patients (46%) were agricultural labourers, 26% were homemakers, 10% were students, 11% were office workers and 7% were other labourers. This is consistent with reports by Srinivasan M et al [3] and Upadhyay MD et al[4] who have reported farmers and home makers as the most vulnerable to corneal trauma.

2) Predisposing factors

The predominant predisposing factor was trauma in all age groups. The various injuries are listed in table 2. Other age-independent series have also reported trauma to be the major predisposing factor [3,5] The traumatizing agent was organic material in 45%, foreign body 30%, dust 15% and contact lens 6% similar to other large series [3,5] The other predisposing factors were neurotrophic keratitis, viral infection, diabetes(5%) and chronic dacryocystitis 3%. This probably reflects the large proportion of our patients being engaged in farming activity (47%).

Table 2

Injury	Total
-	48
?	1
?neurotrophic	1
Birddropping	1
Cement	1
CL	2
coconut shell	1
cow's tail	2
Dust	7
FB	14
Fly	1
Grass	1
Onion	1
onion peel	1
prior HZO	1
Sand	2
Sandal powder	1
shikakai powder	1

soapnut powder	1
soft CL	1
Stick	5
Stone	1
Straw	1
Sugarcane	1
Thorn	1
Veg	1
wood powder	1
Grand Total	**100**

3) Microbiological Features

Gram staining and KOH preparation of the ulcer scrapings showed 48 gram positive bacteria, 14 gram negative bacteria and 38 fungi. Further culture of the scrapings showed that Gram staining has 95% true positives and 75% true negatives. Hence gram staining of scrapings is 94.7% sensitive and 25% specific. This makes it a reliable screening test rather than a confirmatory test (table 3).

Table 3

Gram stain	Positive	Negative
True	72	18
False	4	6
Total	76	24

Sensitivity = 94.7%; Specificity = 25%

The number of gram stained smears and their positivity and negativity are shown in table 3. Of the total 44 bacterial isolate 29 were gram positive and 15 were gram negative. Fungal elements were demonstrated in direct corneal smears from 20 out of the 37 positive for fungal culture.

Which show the high sensitivity of microscopy of gram stained smears. Hence all positive and negative gram stained samples must be subjected to culture and sensitivity to confirm the etiological organism. The value of gram staining of smears lies in choosing the initial topical antibiotic.

Culture was positive in 77 scrapings out of the 100 corneal ulcers. The incidence of bacterial keratitis was 42% (36/77), fungal keratitis was 39% (22/77) and mixed ulcers was 18% (19/77). This is similar to other reported series from this part of the world [2,5,6,7] There was no significant difference between the incidence of bacterial and fungal keratitis in the different age groups.

The specific organisms isolated from the culture positive cases are detailed in Table 4 and Figure 2.

Table 4

Diagnosis	C&S	Total
Bacterial	Acinetobacter spp	1
	Beta hemolytic streptococcus	1
	Enterobacter spp	2
	Escherichia coli	1
	Escherichia coli+ Staphylococcus aureus	1
	Nil	6
	Non hemolytic Streptococci+ Enterobacter sp	1
	Pseudomonas aeruginosa	3
	Pseudomonas aeruginosa+ nonhemolytic strep	1
	Staphylococcus aureus	18
	Streptococcus pneumoniae	6
	Streptococcus pyogenes	1
Bacterial Total		42
Fungal	Aspergillus flavus	6
	Aspergillus flavus+ Staphylococcus aureus	1
	Aspergillus fumigatus	1
	Candida albicans	1
	Fusarium solani	11
	Nil	17
	Staphylococcus aureus	2
Fungal Total		39
Mixed	Aspergillus fumigatus+Staph.aureus	1
	Coag negative Staph,Klebsiella pneumoniae	1
	Enterococcus fecalis	1
	Escherichia coli	1
	Fusarium solani	1
	Non hemolytic Streptococci	1
	Pseudomonas aeruginosa	1
	Staphylococcus aureus	9
	Staphylococcus aureus, Fusarium solani	1
	Staphylococcus aureus+Aspergillus flavus	1
	Staphylococcus aureus+Escherichia coli	1
Mixed Total		19
(blank)	(blank)	
Total		100

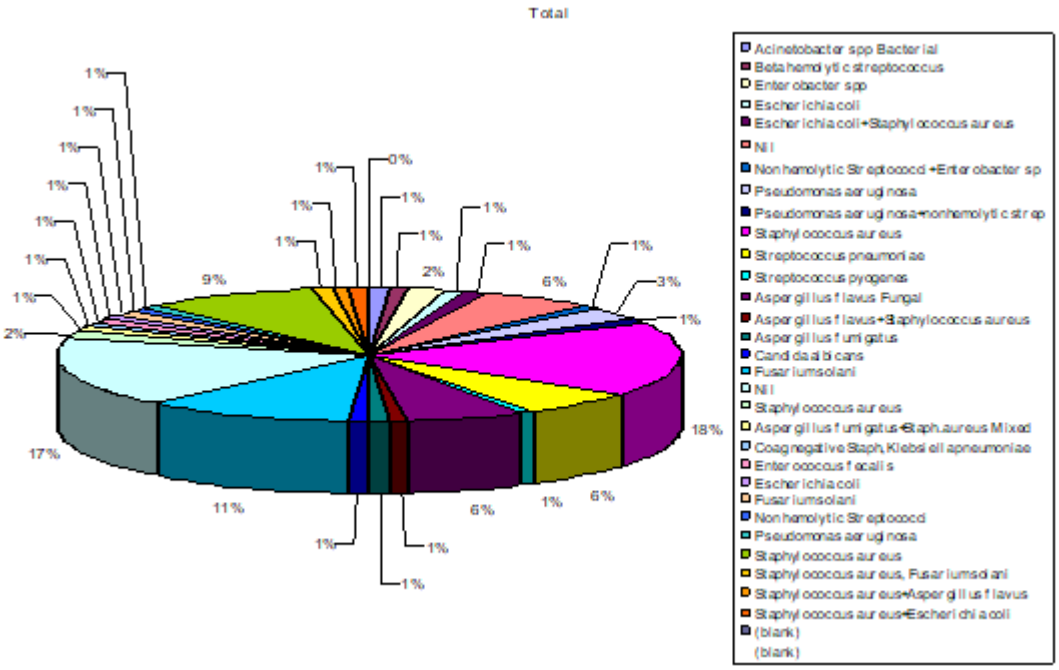

Figure 2

Gram positive cocci accounted for 28% of all microbial keratitis. Staplylococcus aureus was the most commonly isolated bacteria (20%). The next most common gram positive organism was Streptococcus pneumoniae (14%). This was followed by Pseudomonas aeruginosa (6%), Enterobacter (5%) and non hemolytic streptococci (2%).

Pseudomonas aeruginosa was the most frequently isolated gram negative bacterium, accounting for 20%, followed by Enterobacter and E.Coli. Filamentous fungi(Fusarium and Aspergillus) accounted for most of the fungal keratitis. Yeast-like fungi (Candida) accounted for 5% of the total fungal isolates. In a large series in Southern India [6] Staphylococcus epidermidis (31.1%), Pneumococci (13.5%), Pseudomonas (13.5%) and filamentous fungi (15.7%) were isolated. In other series in India, Nepal and Bangladesh, fungi caused 20% to 60% of all infectious keratitis [2,5,6,7]

The sensitivity pattern of the various bacterial isolates to the commonly used topical and fortified antibiotics is shown in Table 5. All Staphylococcus aureus cultures were sensitive to all the antibiotics except gentamicin (95% sensitive) . Of the Pneumococci, 100% were sensitive to gatifloxacin and cefotaxim, 60% to cefazolin and amikacin, 50% to ofloxacin. Of the non hemolytic streptococci, 100% were sensitive to fluoroquinolones, 66% to fortified cefalosporins, 33% to tobramycin and all were resistant to amikacin and gentamicin. Of the Beta hemolytic streptococci, 100% were sensitive to fluoroquinolones and cefazolin and resistant to other antibiotics.

Table 5

Antibiotic Organisim	Gati	Cipro	Oflox	Zolin	Taxim	Zidim	Amik	Tobra	Genta
Staph.aureus (34)	100 (34)	100 (34)	100 (34)	100 (34)	100 (34)	100 (34)	100 (34)	100 (34)	95(27)
B hemolytic Strep. (1)	100 (1)	100 (1)	100 (1)	100 (1)	0	0	0	0	0
Non hemolytic Strep. (3)	100 (3)	100 (3)	100 (3)	66 (2)	66 (2)	66 (2)	0	33 (1)	0
Str. Pneumonia (6)	100 (6)	30 (2)	50 (3)	60 (4)	100 (6)	30 (2)	60 (4)	50 (3)	30 (2)
Pseudo monas (5)	100 (5)	100 (5)	100 (5)	0	60 (3)	100 (5)	100 (5)	100 (5)	100 (5)
E. Coli (4)	50 (2)	25 (1)	50 (2)	50 (2)	0	100 (4)	100 (4)	50 (2)	50 (2)
Enterobacter (3)	100 (3)	100 (3)	100 (3)	0	60 (2)	60 (2)	60 (2)	100 (3)	60 (2)
Kleb. Pneumoniae (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)
Acineto bacter (1)	100 (1)	100 (1)	100 (1)	0	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)
Enterococcus (1)	100 (1)	100 (1)	100 (1)	100 (1)	0	0	0	0	0
Gram +ve	100	82.5	87.5	81.5	66.5	49	40	45.75	31.25
Gram -ve	91.6	87.5	91.6	41.6	53.3	76.6	76.6	75	68.3
Total	95.8	85	89.55	61.5	59.9	62.8	58.3	60.3	49.7

Of the Pseudomonas aeruginosa isolates, all were sensitive to fluoroquinolones, aminoglycosides and ceftazidime and 50% were sensitive to cefotaxim and all were resistant to ceftazidime. Of the E.coli, all were sensitive to ceftazidime and amikacin, 50% to gatifloxacin, ofloxacin, cefazolin, tobramycin and gentamicin, 25% to ciprofloxacin and all were resistant to cefotaxim. The single Enterococcus isolate was sensitive to quinolones and cefazolin and resistant to the other antibiotics.

The overall sensitivity percentage of all bacterial isolates was 95.8% for gatifloxacin, 89.55% to ofloxacin and less for all other antibiotics. Sensitivity of GPC was 100% to gatifloxacin, and less to the other antibiotics. Sensitivity of

GNB was 91.6% to gatifloxacin & ofloxacin, 62.8% to ceftazidime & amikacin and less to other antibiotics.

Thus gatifloxacin (0.3%) is the initial monotherapy of choice as gram positive cocci are the leading cause of microbial keratitis worldwide. When gram negative bacilli are identified it is rational to add fortified amikacin or ceftazidime to gatifloxacin and await culture and sensitivity reports.

Since 33% of patients required change in initial therapy following culture and sensitivity reports (Table 6), it is mandatory to obtain scrapings before the initiation of antibiotic therapy. Culture confirmation provides a gold standard that allows antibacterial comparisons. It also facilitates understanding of the epidemiology of bacterial keratitis, their shifting patterns and emerging resistance.

Table 6

POST C&S Rx	Total
Changed	33
Same	67
Grand Total	100

4) Clinical Outcome

The patients were followed up for a mean of 30 days and visual outcome studied.

The overall visual outcome in this series was good (>6/18) in 59% (59 out of the 100) of eyes, moderate in 15%(15/100) of eyes and poor in 26% (26/100) of eyes.

This is more favourable compared to a study by Parmar et al [2] who have reported 50% of cases with good visual outcome (>6/18), Kunimoto et al [8] who reported 36.4% and Vajpayee et al, who reported 22% [9] The large proportion of non severe ulcers in this series probably accounts the good results.

Out of the 15 patients in the above 60 yrs age group, 6 (40%) had good, 1 (6%) had moderate, and 8 (53%) had poor visual outcome. Out of the 85 patients in the below 60 yrs age group, 54 (63%) had good, 14 (16%) had moderate, and 17 (20%) had poor visual outcome. Hence elderly age was a significant risk factor (p value is 0.021708) for poor visual outcome. this is probably due to higher incidence of severe and central ulcers, poor immunocompetence other and associated ocular pathology such as cataracts in this age group. The relationship between diabetes & visual outcome is represented in Table 7. Diabetes was not a significant (p value is 0.6337) risk factor for poor visual outcome. Tables 8, 9 and 10 represent the relationship of size of the corneal ulcer, location of the ulcer & presence of hypopyon respectively with the visual outcome. Large size (p=0.000032), central location (p=0.00001) and presence of hypopyon (p=0.000012) were significant risk factors for poor visual outcome.

Table 7

DM	Good	Moderate	Poor	Total
+	2	1	2	5
-	58	14	23	95

p = 0.6337

Table 8

Size	Good	Moderate	Poor
Small	42	8	4
Medium	18	7	12
Large	0	0	9

p=0.000032

Table 9

	Good	Moderate	Poor
Central	11	9	20
Paracentral	31	4	5
Peripheral	17	2	1

p =0.00001

Table 10

Hypopyon	Good	Moderate	Poor
+	7	7	15
-	53	8	10

p =0.000012

The relationship between etiological organism and visual outcome is given in Table 11. It was not a significant risk factor for poor visual outcome (p=0.6055).

Table 11

Organism	Good	Moderate	Poor
GPC	27	10	11
GNB	8	1	5
fungi	25	4	9

p= 0.605518

Staphylococcus caused 7 (26.9%), Fusarium 3 (11.5%), Pseudomonas 3 (11.5%), Pneumococcus 2 (7.7%) of the 25 ulcers with poor visual outcome.

A purely empirical therapy of microbial keratitis may achieve a high proportion of success regardless of the identity of the causative organism. Though such a practice would reduce costs in terms of time and money, treatment failures are expected to generate increased costs in terms of patient well being and therapeutic surgical intervention.

5. Conclusion

There is no sex preponderance in microbial keratitis both bacterial and fungal. The most vulnerable age group is 40-50 years of age. The most common risk factor for infectious corneal ulcer is trauma of which the most common is with organic matter and foreign body. Agricultural labourers are the most susceptible, the next common being home makers. The factors that lead to poor visual outcome are age above 60 years, large ulcers, central location, presence of hypopyon, Pneumococcus or Fusarium species are isolated from the ulcer. Pneumococcus is a significant risk factor even when the ulcer is small. Gram staining of the corneal scrapings may be used as a screening test as it is sensitive and to start initial empirical therapy. It is mandatory to subject the scrapings to culture and sensitivity on blood agar plate and Sabouraud's dextrose agar and directly inoculate the media at the time of scraping rather than using transport media. Topical gatifloxacin (0.3%) is the antibiotic of choice for initial monotherapy of bacterial keratitis. Fortified amikacin (20mg/ml) or fortified ceftazidime (50mg/ml) must be added to gatifloxacin 0.3% if gram negative bacilli are identified. Modification of initial antibiotic based on antimicrobial sensitivity is needed only when worsening of

the ulcer occurs clinically. Prevention of corneal ulcers can be achieved by protective eyewear at work and prompt treatment of corneal injury at the primary health care level combined with health education to create awareness regarding early treatment of corneal trauma.

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