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Research Article

Etiological Diagnosis and Epidemiological Characteristics of Microbial Keratitis at Regional Eye Hospital, Visakhapatnam

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Abstract: Keratitis (corneal ulcer) leads to ocular morbidity and blindness worldwide and is second most common cause next to cataract. Etiological agents of microbial keratitis are bacteria, fungi, viruses and acanthamoeba usually after trauma and varies significantly from country to country, and even from region to region. Specific treatment requires quick and accurate identification of the causative micro-organism. The aim of the present study is to isolate & identify the bacterial and fungal etiological agents of corneal ulcer and to study the risk factors & epidemiological characteristics. Corneal scrapings from one hundred clinically diagnosed corneal ulcer cases with or without hypopyon attending Regional eye hospital, Visakhapatnam from September 2011 to February2013 were processed by standard methods of microscopy, bacterial & fungal culture. The clinical and epidemiological details of the patients were noted andanalysed. Economically active age group of 21 – 50 years contributed to 52% of cases. Patients from rural background constitute 62%and 42% attended the hospital within 2 – 3 weeks of their illness. 38% incidence was during harvest season of January to March. Culture positivity was 73% which include 21% bacterial, 49% fungal and 3% mixed growth. Of the 24 bacterial isolates Pseudomonas aeruginosa (29.17%) was predominant followed by Staphylococcus aureus (25%). Of the 52 fungal isolates 19 were Fusarium sps. followed by 17 Aspergillus sps. The study of microbial etiology and regional distribution of corneal ulcer would help in the accurate management of keratitis cases.
Keywords: keratitis, corneal scrapings, bacterial, fungal, microbial etiology..

INTRODUCTION

Keratitis (corneal ulcer) is a leading cause of ocular morbidity and blindness worldwide especially in developing countries second onlyto cataract[1, 2]. Etiological agents may be bacteria, fungi, viruses or acanthamoeba and their incidence vary significantly from region to region [3, 4]. Quick and accurate identification of the causative micro-organism helps in specific treatment [4]. Considering the importance of corneal ulceration many studies have reported the prevalence of microbial pathogens & identified the risk factors predisposing to corneal ulcer in South India.

The objective of the present study was to study the epidemiological characters and risk factors of keratitis and to isolate and identify the bacterial and fungal pathogens in corneal scrapings from keratitis patients.

MATERIALS AND METHODS

One hundred patients with the clinical diagnosis of corneal ulcer with or without hypopyon attending Regional Eye Hospital, Visakhapatnam from September 2011 to February 2013 were included in the study. Age, occupation, residence of the patients and predisposing factors like trauma, systemic diseases were noted. After taking informed consent corneal scrapings were collected under aseptic conditions after thorough slit-lamp biomicroscopic examination from leading edge and base of the ulcer by an ophthalmologist after instillation of 4% lignocaine drops, using a sterile No: 15 Baird Parker blade[5].The specimens were inoculated on to the surface of blood agar, chocolate agar and Sabouraud's dextrose agar in a row of C shaped streaks (Fig – 1). Brain heart infusion broth was also inoculated. All the inoculated media were incubated aerobically at 25° C in BOD incubator.

Gram's staining and 10% KOH mount were examined microscopically for the presence of pus cells, microbes and the presence of fungal hyphae respectively (Fig – 2). The microscopic examination report was immediately informed to the ophtholmologist.

Microbial cultures were considered positive when at least one of the following criteria are met [2, 4, 5].a) The growth of the same organism was demonstrated on two or more solid media on the C-streak; or there was semi confluent growth at the site of inoculation on one solid medium. b) The same organism was grown from repeated scrapings. c) It was consistent with clinical signs. d) Smear results were consistent with cultures. The specific identification of bacterial pathogens was done using standard biochemical identification tests and fungal isolates were identified by macroscopic and microscopic morphology, staining characteristics using standard laboratory criteria [5, 6, 7].

RESULTS

Epidemiological characteristics:

58% of patients were males and 42% were females. Corneal ulcers showed 52% prevalence in the economically active age group of 21-50 yrs. 62% patients were from rural areas. 42% of patients attended the hospital within 2-3 weeks of their illness and 24% of patients reported after 4 weeks. There was a significant increase in corneal ulcer patients (38%) during the period of January to March representing harvest season and second peak incidence was seen during July- September (29%) representing the Monsoon season. Agricultural laborers contributed to 42% followed by daily wage laborers 35%. House hold people (Including house wives, old people) contributed 19% and students 4% (Table – 1).

Table-1: Epidemiological characteristics of patients: (n=100)

Demographics	Indicator	Number (%)
Age (in years)	21-40 41-60 < 20 > 60	52 (52%) 33 (33%) 5 (5%) 10 (10%)
Sex	Male Female	58 (58%) 42 (42%)
Residence	Rural Urban	62 (62%) 38 (38%)
Occupation	Agricultural laborers Daily wage laborers House hold people Students	42 (42%) 35 (35%) 19 (19%) 4 (4%)

Predisposing factors

Culture positivity was obtained in 73 (73%) cases. Out of 73 cases, a history of recent corneal injury was obtained in 72.6% of patients. 23 patients had corneal injury with vegetative matter (43.39%), mostly paddy stalk or grass. Other significant agents were twig of a tree, wood, dirt, mud, sand, etc. Ocular problems predisposing to corneal ulcer were present in 7 cases (Table -2).

Table-2: Predisposing factors of keratitis in culture positive cases (n=73)

Factors associated	Number (%)
Trauma include - Vegetative matter - Sand/ dust - Wood/stick - Others	53 (72.60%) 23 (43.39%) 15(28.3%) 10(18.86%) 5(9.43%)
Systemic diseases	3 (4.10%)
Ocular diseases	7 (9.58%)
Topical steroid usage	5 (6.84%)
Postoperative	2 (2.73%)
Contact lens	1 (1.36%)
No recorded factor	2 (2.73%)

Microbial Diagnosis

Culture positivity was obtained in 73% of cases, among them 21 were pure bacterial growth, 49 were pure fungal growth and 3 showed mixed growth. 27% cases did not show any growth.Of the 24 bacterial isolates 16 (66.67%) were Gram positive bacteria and 8 (33.33%) were Gram negative bacteria. Pseudomonas aeruginosa (29.17%) and Staphylococcus aureus (25%) were the predominant isolates followed by Staphylococcus epidermidis (20.83%), (Table - 3).

Fig-1: Blood agar showing confluent Fungal growth on C shaped streaks

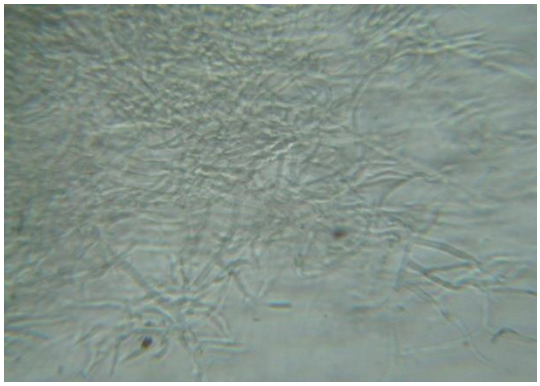

Fig-2: Corneal scrapings in 10% KOH mount showing fungal hyphae

Table-3: Various isolated bacteria (n=24):

Isolates (n=24)	Number	%
Pseudomonas aeruginosa	7	29.17
Staphylococcus aureus	6	25
Staphylococcus epidermidis	5	20.83
Streptococcus pneumoniae	3	12.5
Corynebacterium spp.	2	8.33
Klebsiella pneumoniae	1	4.17
Total	24	100

Of the 52 fungal isolates yeasts constituted only 3 (5.76%) isolates while 49 (94.24%) were moulds. 2 isolates of moulds were unidentified (Fig -3, 4). Fusarium spp. (36.53%) was predominant isolate followed by Aspergillus spp (32.69%),(Table – 4).

Table-4: Distribution of Fungal isolates (n=52)

Isolates	No.	%
Fusarium spp.	19	36.53
Aspergillus spp.	17	32.69
Curvularia spp	3	5.76
Penicillium spp	2	3.85
Candida albicans	2	3.85
Candida tropicalis	1	1.92
Pseudallesheria boydii	1	1.92
Cladosporium spp	1	1.92
Paecilomyces spp	1	1.92
Scopulariopsis spp	1	1.92
Epicoccum spp	1	1.92
Alternaria spp	1	1.92
Un identified	2	3.85
Total	52	99.97

Fig-3: Fusarium species growth on SDA, its LCB mount:

Fig-4: SDA slants with Aspergillus niger and Pencillium sps

DISCUSSION

In the present study at a Government hospital 76% of corneal ulcer cases were from lower socioeconomic group followed by 23% from middle income group and 1% in high socioeconomic status. Higher percentage in low socio economic group may be due to poor nutritional status, their nature of occupation, unhygienic living conditions, low literacy rate, increased use of traditional medication and delay in attending the hospital. Usha Gopinathan et al [3] &AnuradhaM et al [10] also reported that corneal ulceration was more in low socioeconomic status. In the present study 62% were from rural population and studies by Basak SK et al [1] & Reemanath et al [12] reported 78.5% & 87.9% respectively. Anuradha et al [10] and Jyothipadmaja et al [11] from Visakhapatnam also reported high incidence of corneal ulcers in the rural areas. Whereas AartiTiwari etal [2] reported 70%

from urban area. The major predisposing factor in the present study was trauma (74.64%) and the incidence of other factors in various studies was shown in table – 5.

Table-5: Percentage of Predisposing factors among various studies:

Author	Trauma	Systemic diseases	Ocular disease	Chr.antibioti/ steroid use	Post operative	Contact lens use
Basak SK et al, (2005) W. Bengal [1]	82.9	7.6	10.1	19.3	0.6	0.3
AartiTewari et al, (2012),Ahmedabad [2]	90	2.67	0.67	5.33	0.67	-
Bharati et al(2003), Tirunelveli [8]	92.15	16.07	0.67	1.19	1.46	-
Kehinde O et al(2013), Northern Nigeria [13]	51.3	4	17.1	5.7	-	0.4
Nada ALYousuf, (2009),Bahrain [15]	8	2	11	-	3	40
Present study (2013) Visakhapatnam	74.64	4.22	9.85	7.04	2.81	1.40

In the present study, the commonest presenting complaints were poor vision (44%), lacrimation (40%), and ocular pain (38%) and these findings coincides with Kehinde O et al [13], that major complaints were poor vision (47.8%), lacrimation (42.5%) and ocular pain (42.1%). It was observed in the present study that majority of patients (42%) attended the hospital between 15 days to 1 month after onset of symptoms and 24% of patients after 1 month of their illness. Only 13% of patients presented during the first week of their illness. Before their first presentation at the hospital, 74% of patients had consulted health-care provider of some kind, and 32% of them had consulted ophthalmic personnel. It was observed that 30% of patients received some form of topical medication from a chemist before their first consultation. Basak SK et al [1] showed that 51.8% of patients attended the hospital between 2-3 weeks of their illness and 13% of patients after 4 weeks, Rafel Furlanetto etal [16] from Brazil also reported 41.53% of the patients attended after one week.

Agricultural laborers were at highest risk (42%) followed by daily wage laborers (35%) in the present study. Basak SK etal [1] reported 57.6% were farmers/ Agricultural laborers, Bharathi et al [8] study from South India showed 64.75% and Jagadeshchander et al [14] from Chandigarh showed 89.83%Sumansahaetal [17] from Bengal showed that 47.29% were farmers. Anuradha et al [10] from Visakhapatnam reported that 51.21% of bacterial and 63.33% of fungal corneal ulcers in agricultural workers.Male preponderance in the corneal ulceration was reported by Basak SK et al [1] from west Bengal as 70.6%, Geethakumari PV et al [9] from keralaas 65.11%, and the present study coincides showing 58% males.

In the present study, the most common age group affected among bacterial and fungal ulcers was 21-50 years with 79.1% and 55.76% incidence respectively. Fungal ulcers were also common in later age groups, 51- 70 years representing 34.61%. Basak SK et al [1] reported high incidence (49.3%) of corneal ulcers in the age group 21-40 yrs. Usha Gopinathanet al [3] noted in their study that the mean age was 41.20 (±20.36) in bacterial keratitis and 30.90 (± 15.28) in fungal keratitis. These observations may be explained by the fact that they are more involved in outdoor activities and hence having a greater chance of injury & exposure to infections. The peak incidence of corneal ulceration (37.49% in bacterial, 32.68% in fungal) was seen during Jan- March representing Harvest season. Another peak incidence (20.83% in bacterial, 19.23% in fungal) was during July- Sep in the present study representing the Monsoon season. This may be due to the cool, humid atmosphere, offering ideal climate for growth of microbes. Basak SK et al [1] reported significant increase of patients (24.8%) during the months of Nov & Dec (harvest season of that area). Bharathi et al [4] from S. India had reported a high incidence of bacterial ulcers during the harvesting season and fungal ulcers in the monsoon season. Reemanath et al [12] reported that maximum (23.4%) cases were reported during the paddy harvesting season in Assam (January and February).

Bacterial isolation was 28.76% in the present study and it coincides with Basak SK et al [1] and Geeta Kumari et al [9] at 22.7% and 27.41% respectively (Table – 6).

Table-6: Comparison of culture positivity with various authors:

Author	Culture positivity(%)	Bacterial (%)	Fungal (%)	Mixed (%)	Acantha-moeba (%)	No growth (%)
Basak SK et al, (2005), W. Bengal [1]	67.7	22.7	62.7	14.1	0.49	32.3
AartiTewari et al, (2012), Ahmedabad [2]	59.3	65.1	34.9	-	-	40.7
UshaGopinathanet al, (2009), Hyderabad [3]	60.4	51.9	38.2	7.5	2.4	39.6
Bharathi MJ et al, (2002), South India [4]	69.59	34.98	32.26	1.85	0.49	30.41
Geetakumari PV et al, (2011), Kerala [9]	21.36	27.41	69.78	_	_	78.64
Anuradha M etal,(2005), Visakhapatnam [10]	71	53.52	38.02	4.22	_	29
Present study 2013, Visakhapatnam	73	28.76	67.12	4.1	_	27

Jagadesh chander et al [14] and Suman Saha et al [16] showed Aspergillus spp as predominant fungal isolate with 41.18% and 55.4% isolation respectively whereas in the present study the predominant isolate was Fusariumspp with 36.54% followed by Aspergillus spp (32.69%). Curvularia spp were isolated from 5.77% of fungal keratitis cases in the present study and correlates with the observations of Usha Gopinathan et al (5.4%) [3] Jagadesh Chander et al (5.88%)[14].

CONCLUSION

The study of various epidemiological characters of corneal ulcer and their microbial etiology greatly help the clinical ophthalmologist in efficient diagnosis and management of keratitis and to formulate preventive measures.

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