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ETIOLOGY AND RISK FACTORS COMMONLY ASSOCIATED WITH INFECTIOUS KERATITIS AMONG PATIENTS ATTENDING TERTIARY CARE OPHTHALMIC HOSPITALS IN CHENNAI

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ABSTRACT
Infectious keratitis is one of the most important causes of preventable blindness in the developing world. Delay in diagnosing the nature of infection is one of the paramount factors, which may leads to inappropriate initial therapy and poor outcomes. Hence this study was aimed to identify the etiological agents and risk factors associated with infectious keratitis among patients attending tertiary care centres located in Chennai. A total of 150 corneal scraping samples from the infectious keratitis patients and data were collected. All bacterial and fungal and parasitic isolates were identified by standard microbiological techniques as recommended by Clinical and Laboratory Standards Institute (CLSI). Of the 150 corneal scrapings, 94 (62.6%) were found to be culture positive for bacterial, fungal and parasitic agents. Of which, fungal agents were found to be predominant 54 (57.4%). The most frequently isolated fungal, bacterial and protozoan agents were *Aspergillus.sp, Staphylococcus aureus* and *Acanthamoeba.spp* respectively. Incidence of infectious keratitis was found to be significantly (p=0.009) higher among male patients and vegetable matter was the commonest traumatic factor, which contributed to 47.8%. Prevalence of infectious keratitis due to trauma was found to be significantly (p=0.005) higher among patients from rural area. In this study, infectious keratitis predominantly associated with fungal etiology and vegetative matter induced ocular trauma as the major predisposing factor, strongly suggests that the diagnosis of nature of infection to establish clinical management is mandatory and create awareness among people especially from rural background with regard to trauma as a major predisposing factor for infectious keratitis.

KEYWORDS: (Infectious keratitis, trauma and corneal scraping).

INTRODUCTION
Microbial keratitis is infection of the cornea that can be caused by a range of non-viral pathogens. It is characterized by an acute or sub-acute onset of pain, conjunctival injection and corneal ulceration with a stromal inflammatory infiltrate, which can cause severe visual loss if not treated at early stage. Importantly, risk factors for infection change over time (e.g. increased popularity of contact lens wear and refractive surgery) and monitoring for changing patterns of disease and its etiology is essential. Specific treatment requires accurate identification of the causative organism. There are large regional differences in the relative prevalence of each of these causative organisms determined by climate and socio-economic factors. In India, the annual incidence is reported to be 11.3 per 10,000 cases.[1] Considering the importance of corneal ulceration and its impact on vision, thus it is necessary to perform laboratory studies including microscopy and culture of a corneal scrapping. The present study was conducted to identify the etiological agents and risk factors among patients attending a tertiary care Regional Institute of Ophthalmology and Rajiv Gandhi Government General hospitals in Chennai.

MATERIALS AND METHODS
A cross sectional study was carried out during a period of one year from October 2011 to September 2012. Corneal scrapings were collected from patients presenting in the outpatient department with the history of trauma and signs and symptoms of infectious corneal ulcer by the Ophthalmologist. The samples were inoculated in a C-streak pattern on culture media, viz., Blood Agar, Potato Dextrose Agar, Chocolate Agar and Non-nutrient Agar. Bacterial isolates were identified by standard microbiological techniques as recommended by Clinical and Laboratory Standards Institute (CLSI).[2] Inoculated SDA slopes were incubated at 25°C and 35°C for a minimum of six weeks before discarding as negative. These slants were inspected daily during the first week and twice weekly during the next three weeks for growth. Identification of filamentous fungi was done

by preparing Lacto Phenol Cotton Blue mount and the morphology of hyphae and conidial arrangement were studied. In ambiguous cases where sporulation was inadequate, Riddle's slide culture technique was performed. Corneal scrapings inoculated on to a non-nutrient agar plate for the cultivation of free living amoeba were observed after 48-72 hours under low power and high power objective of the microscope for the presence of *Acanthaomoeba* cyst. The *Acanthaomoeba* cyst present was further confirmed by calcofluor stain. Antimicrobial susceptibility test was performed for the all bacterial and fungal isolates as per CLSI guidelines.[2]

RESULTS

Of the 150 samples, 94 (62.6%) were found to be culture positive for bacterial, fungal and parasitic agents. Of which majority of culture positivity was due to fungal agents 54 (57.4%) (Table.1). Male patients were found to have significantly high incidence of infectious keratitis (p value =0.009), probably because of their occupation (Table.2). Prevalence of infectious keratitis due to trauma was more common among patient with age group of 51-60 yrs (28.72%). Incidence of infectious keratitis due to trauma was more in rural population compared to urban population which was statistically significant (64.66%) (p value =0.005) (Figure.1). Vegetable matter was the commonest traumatic factor which contributed to 47.87% in majority of the traumatic infectious keratitis patients. *Staphylococcus aureus* was the most common bacterial agent (26.47%), *Aspergillus spp* was the most common fungal agent (63.15%) and *Acanthamoeba spp* was the only parasitic agent isolated in infectious keratitis cases due to trauma (6.38%) (Figure 2, 3 & 4).

Table 1: Culture Positivity in Infectious Keratitis Due to Trauma (n=150).

Total No.of Samples	No.of Culture Positive Samples	Percentage of culture Positivity	Total No. of Bacterial agent	Total No. of Fungal agent	Total No. of Parasitic agent	Total No. of Mixed growth
150	94	62.66%	31	54	6	3

Table 2: Distribution of Trauma Factor in Infectious Keratitis Due To Trauma.

Trauma Factor	Culture +ve Cases			Percentage
	Male	Female	Total	
Vegetable Matter	34	11	45	47.87%
Dust	15	6	21	22.34%
Thorn Prick	4	3	7	7.44%
Cow's tail	4	3	7	7.44%
Stone	3	3	6	6.38%
Insect fall	2	2	4	4.25%
stick	1	1	2	2.12%
Iron Partical	1	1	2	2.12%
Total	**64**	**30**	**94**	**100%**

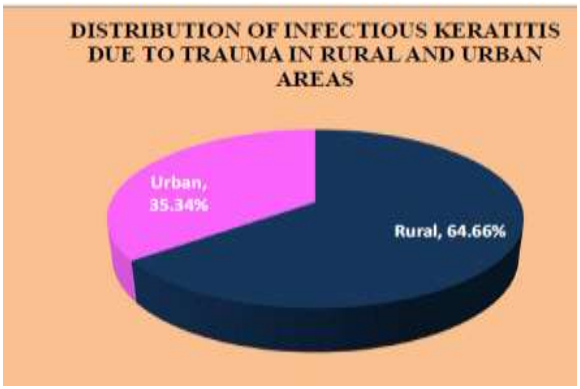

Figure 1:

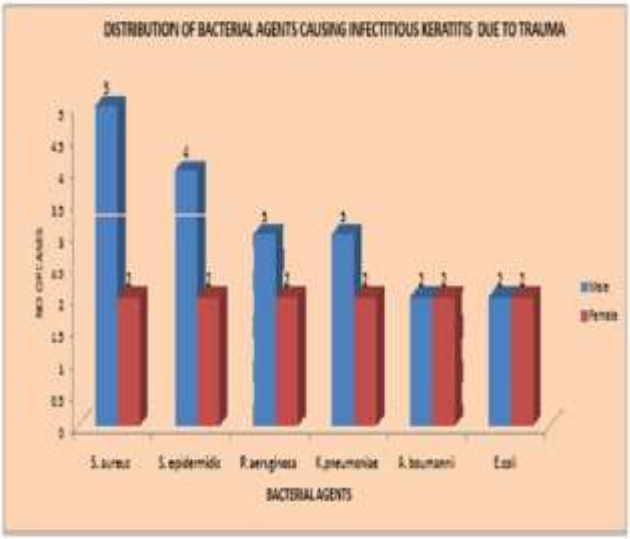

Figure 2:

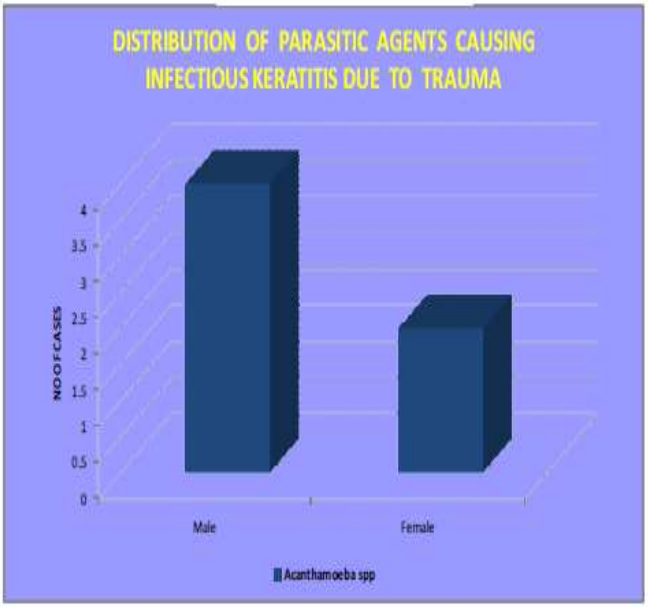

Figure 3:

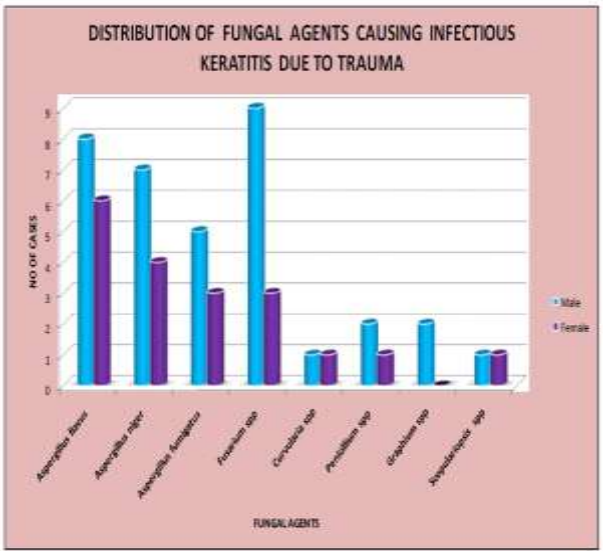

Figure 4:

Plate 1: A Case of Fungal Keratitis.

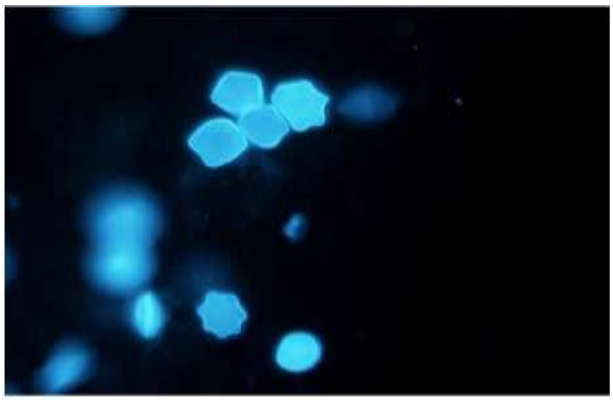

Plate 2: Calco Flour Stain Acanthamoeba Cyst.

DISCUSSION

Corneal disease, especially infectious keratitis, is a major cause of vision loss and blindness second only to cataract.[3] A study from South India reported that the incidence of corneal infections in India is almost 10 times higher than that reported in the United States.[4]

In this study, out of 150 cases of traumatic infectious keratitis studied, 94 cases showed culture positivity which accounts for 62.66%. The culture positivity was similar to a study by Basak *et al*, (2005), which showed 65.4% of corneal injury cases having microbial keratitis.[5] Culture negativity was reported in 44% cases in this study. This is in accordance with Bharathi *et al*., (2003) and Mohapatra *et al*., (2003) have reported 41% and 32.3% respectively.[6,7] Whereas, Gita *et al.*, (2004) reported a higher incidence of culture negativity (62%).[8] Present study showed that male patient (71.1%) were more commonly affected with infectious keratitis than female patient. Male preponderance may be due to occupation, eg. Agricultural workers are more prone to corneal injury and were washing eyes with probably stagnant water.[9],[10] This study was similar to Bharathi *et al*., (2003) from South India reported 56.7% of corneal ulcer in males.[7] Similarly, Gopinath *et al., (*2002) from Hyderabad, have reported 71.1% of corneal ulcer in males.[11] In this study, rural population was found to be more vulnerable to infectious keratitis due to trauma compared to the urban population (64.6%). This present study was similar to Basak *et al* (2005) from West Bengal showed that 78.5% of the patients were from rural areas.[5] In this study corneal injury with vegetable matter was the most common factor casing infectious keratitis (47.8%). This study was similar to Basak *et al*., (2005) West Bengal who reported (59.6%) traumatic cases due to vegetable matter.[5] Laspina *et al.*, (2004) also showed history of ocular trauma in 50% of their patients.[12] In this study, etiological agents were identified and isolated in 94 samples (62.6%). Of these 94 culture positive samples 54 (57%) had pure fungal growth, 31 (32.1%) had pure bacterial growth and 3 (3.19%) had mixed bacterial and fungal growth, 6 (6.38%) had parasitic growth. Fungal agents were commonly isolated in infectious keratitis patients followed by bacterial and parasitic agents. This study was similar to Khanal *et al*, in (2005), conducted in Nepal which showed 42.7% growth positive for fungi present.[13] *Aspergillus spp* was the predominant fungal isolate in this present study (63.15%), which is in concordance with a report of Gopinath *et al*, (2002) from Hyderabad reported *Aspergillus spp* (30.7%) were the predominant isolates in their study.[11] Among the bacterial isolates, *Staphylococcus aureus* 9 (27%) was the most common bacterial agent isolated. This study report was found to be similar with various previous study reports, Alexandrakis *et al* (2000) *(*19.4%); Schaefer *et al*, (2001) (22%) and Gita *et al.*, (2004) (20%) reported that *Staphylococcus aureus* was a predominant bacterial isolate.[14,15,8] Among the parasitic agents protozoa, *Acanthamoeba spp* was isolated in 6 patient (6.38%) and this was the only parasitic agent isolated in infectious keratitis patients due to trauma, this is similar to the study of Bharathi *et al*., (2009). Sharma *et al*, (2000) have reported that 15% of corneal trauma was the predisposing factor for *Acanthamoeba keratitis.*[16]

CONCLUSION

From the present study, vital role of microbiological evaluation in the management of infectious keratitis is clearly evident, diagnostic corneal scraping and culture

are mandatory in order to identify the causative organisms when infectious keratitis is suspected. Since the clinical features alone are not adequate to confirm infection. It is also important to create awareness among people especially from rural background with regard to trauma as a major predisposing factor for infectious keratitis.

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Conflict of interest: None.

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