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"SCREENING OF MICROBIOLOGICAL AND EPIDEMIOLOGICAL PROFILE OF FUNGAL KERATITIS IN PATIENTS ATTENDING A TERTIARY CARE CENTRE AT FARRUKHABAD, UTTAR PRADESH, INDIA".

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ABSTRACT

INTRODUCTION: Infective keratitis is one of the leading causes of monocular blindness in developing countries in Asia and Africa. It is the fourth leading cause of blindness worldwide, and 1.5 to 2.0 million new cases reported every year and approximately there are 6.8 million cases of corneal blindness in India.Definitive diagnosis is by microbiological culture. So, knowledge of local etiological agents and their susceptibility helps to initiate prompt treatment and control the disease.

AIM AND OBJECTIVE: The objective of the study was to identify the fungal pathogenic agents, risk factors and to study epidemiological characteristics of fungal keratitis presenting at a tertiary care centre in Farrukhabad, Uttar Pradesh.

MATERIALS AND METHODS: This was a cross sectional, observational study carried out in the Department of Ophthalmology and Department of Microbiology. The Corneal scrapings were obtained from clinically suspected patients of keratomycosis. Aseptically collected corneal scrapings were subjected to Gram stain, KOH wet mount & Culture. The Further identification was done to study the Colony morphology, staining & biochemical tests according to the CLSI guidelines.

RESULTS: In the present study out of the total 80 suspected cases, 42(52.5%) were positive for fungal etiology. Of these 34(80.95%) were positive on KOH mount, 30(71.4%) and 25(59.5%) were positive in gram stain and culture. The ratio of Males (57.14%) were more commonly affected. In 14 out of 42 patients (33.33%) were of age group 31-40 years. It was observed that the Majority of patients were from agriculture (60%). The most common Risk factor for fungal keratitis was found to be ocular trauma (52.5%) followed by Diabetes (26.25%). In culture, aspergillus spp. (38.09%) were the predominant fungal spp. followed by other fungal spp.

CONCLUSION: Infective keratitis is a significant cause of ocular morbidity in Farrukhabad**.** The knowledge of epidemiology, risk factor, and microbiological profiles of infective keratitis can provide a valuable approach to disease prevention, diagnosis and management. Agricultural activity and

related ocular trauma are principal causes of mycotic keratitis. A potassium hydroxide (KOH) wet mount preparation is a simple, and sensitive, method for diagnosis.

Keywords: Infective keratitis, Corneal scrapings, Risk factors, Epidemiology, CLSI

INTRODUCTION

Keratitis is the term applied for inflammations of the cornea [1] Corneal infections are known to be the second most significant cause of monocular blindness rated after unoperated cataract in some developing nations in particular and in the tropics in general.

Infective keratitis is one of the leading causes of monocular blindness in developing countries in Asia and Africa [1]. Corneal ulcer is infective condition of cornea which is a vision threatening disorder. Corneal blindness is seen worldwide with 1.5 to 2 million new cases reported every year and approximately there are 6.8 million cases of corneal blindness in India [2].

With the overall decline in causes of blindness like trachoma, onchocerciasis and leprosy, the World Health Organization (WHO) has perceived that corneal blindness due to microbial keratitis is emerging as a principal reason for visual inabilityand that it is a "silent epidemic" happening unnoticed around the world [3,4].

Infective keratitis affects both male and females of all age group. Infective keratitis leading to corneal ulcers are caused by various causative agents both bacterial and fungal. The bacterial causes such as *staphylococcus aureus*, *streptococcus pneumoniae*, *streptococcus* viridians and *pseudomonas.* The fungal causes are *Fusarium spp*, *Aspergillus spp*, and unclassified fungi [5].It can be caused by a wide variety of fungi and is usually manifested by severe inflammation, the formation of a corneal ulcer, and hypopyon, with the presence of fungal hyphae within the corneal stroma[6] Fungal corneal ulcers like other fungal infections are commonly present in immunocompromised patients but they have also been reported in healthy humans [7].

Infective keratitis is commonly caused by trauma mainly in people engaged in agricultural, manual labourers usually dealing with plant or vegetative matter that causes infection which ulcerate and leads to corneal blindness if left untreated. Most of the fungal keratitis are associated with various predisposing factors such as trauma, surgical ocular condition, use of contact lens, diabetes and use of steroids[5,8].

The epidemiology of this disease varies considerably in different parts of the world depending on the socio-economic condition and health care facility [9]. Knowledge of the specific regional epidemiological factors underlying the disease is therefore important in diagnosis and treatment of this disease. Only a few studies are available in the literature from eastern India, resulting in under-reporting of the disease [10-15].

The Present study was conducted to systematically determine the microbiological and epidemiological profile of fungal keratitis in this region as early diagnosis and treatment will not only prevent the corneal blindness but also reduce the morbidity associated with it.

MATERIAL AND METHODS

This was a cross sectional, observational study carried out in the Department of Ophthalmology and Department of Microbiology at Major S.D Singh Medical College, Farrukhabad.

The Corneal scrapings were obtained from clinically suspected patients of keratomycosis during the period of 1 year from September 2010 to August 2011. Aseptically collected corneal scrapings were subjected to Gram stain, KOH wet mount & Culture. The Further identification was done to study the Colony morphology, staining & biochemical tests according to the CLSI guidelines 2008.

Study Settings:

Study population: A total of 80 patients with infective keratitis were enrolled during the study period from September 2010 to August 2011.
Inclusion criteria: All patients with clinical findings of infective keratitis of all age groups belonging to both sexes, presenting at hospital during the study period, were included in the study.

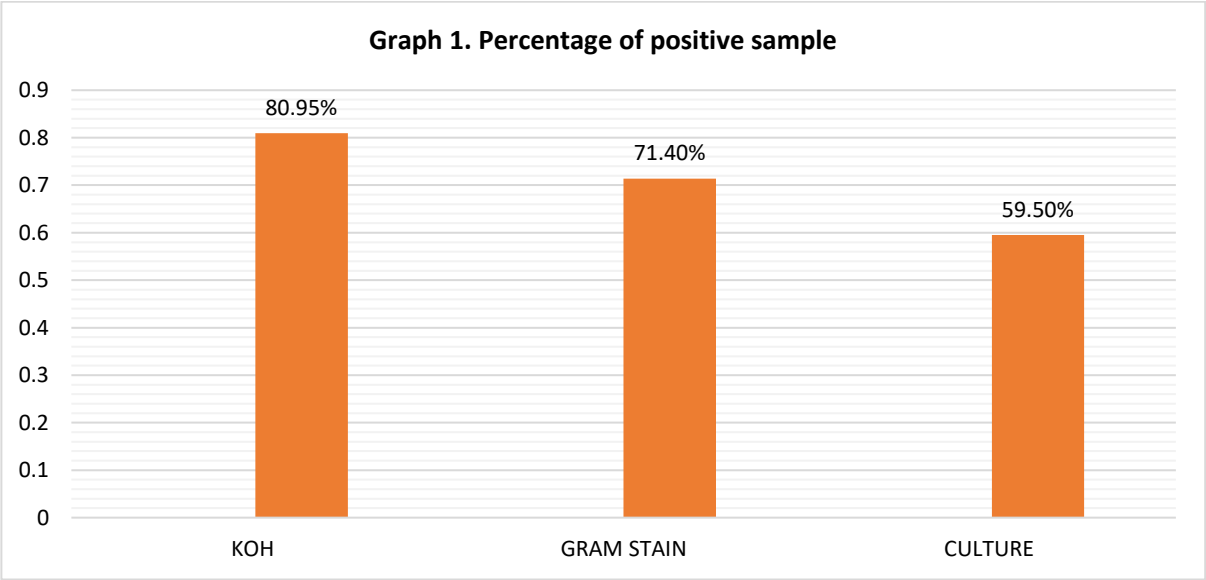
Exclusion criteria: Patients diagnosed as bacterial, viral or protozoal keratitis were not included in the study.

Specimen collection: The scraping material was inoculated onto solid media such as blood agar, chocolate agar, and Sabouraud's dextrose agar (SDA) in multiple rows of C-shaped streaks. The material obtained from next scraping was spread onto glass slides for 10% KOH mount and Gram-staining.
Blood agar and chocolate agar were incubated aerobically at 37°C and were examined daily and discarded after 7 days if there was no growth. Sabouraud's media was incubated at 25°C and examined daily and discarded if no growth was seen after 21 days. Fungal cultures were identified by colony morphology and LPCB mount.

Statistical analysis:
Data recorded on the case report from and structured proforma were subsequently entered into a spreadsheet. Data management and analysis were performed using Microsoft Excel.

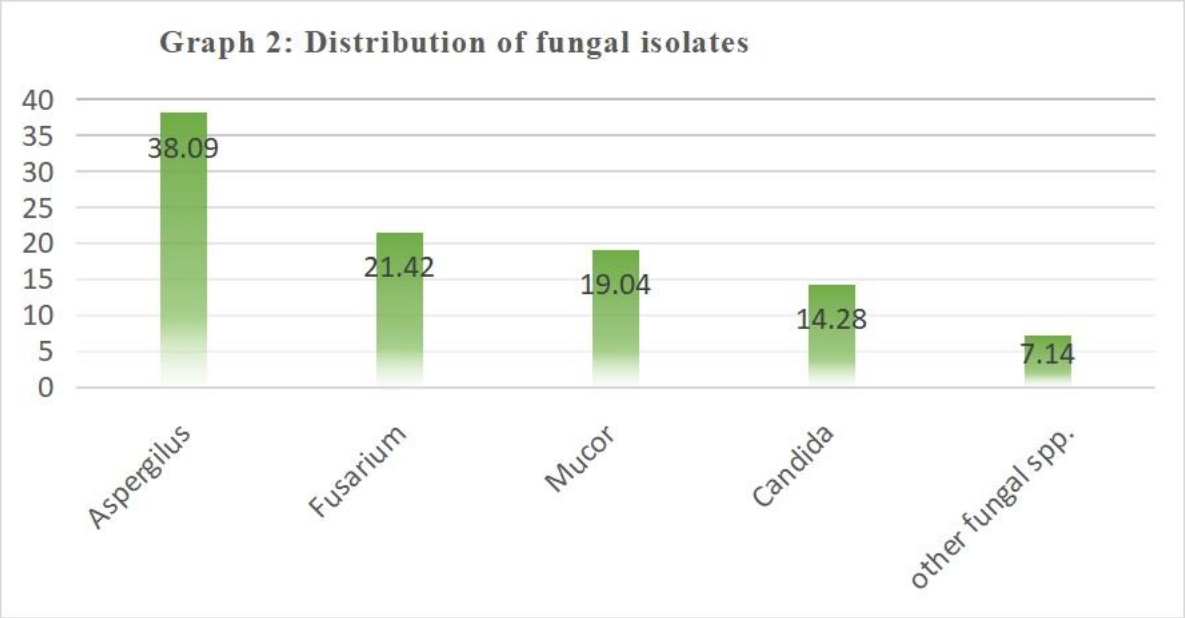
Ethical clearance: The ethical committee clearance certificate was taken before starting of study by Institutional Medical Ethical Committee.

RESULTS
In the present study, a total of 80 patients of suspected fungal corneal ulcers were investigated for fungal etiology at Major S.D Singh Medical College, Farrukhabad over a period of 1 year from September 2010 to August 2011.
Out of total 80 suspected fungal corneal ulcers, 42(52.5%) were positive for fungal etiology, of these 34(80.95%) were positive on KOH mount. 30(71.4%) and 25(59.5%) were positive in gram stain and culture respectively (Graph.1)



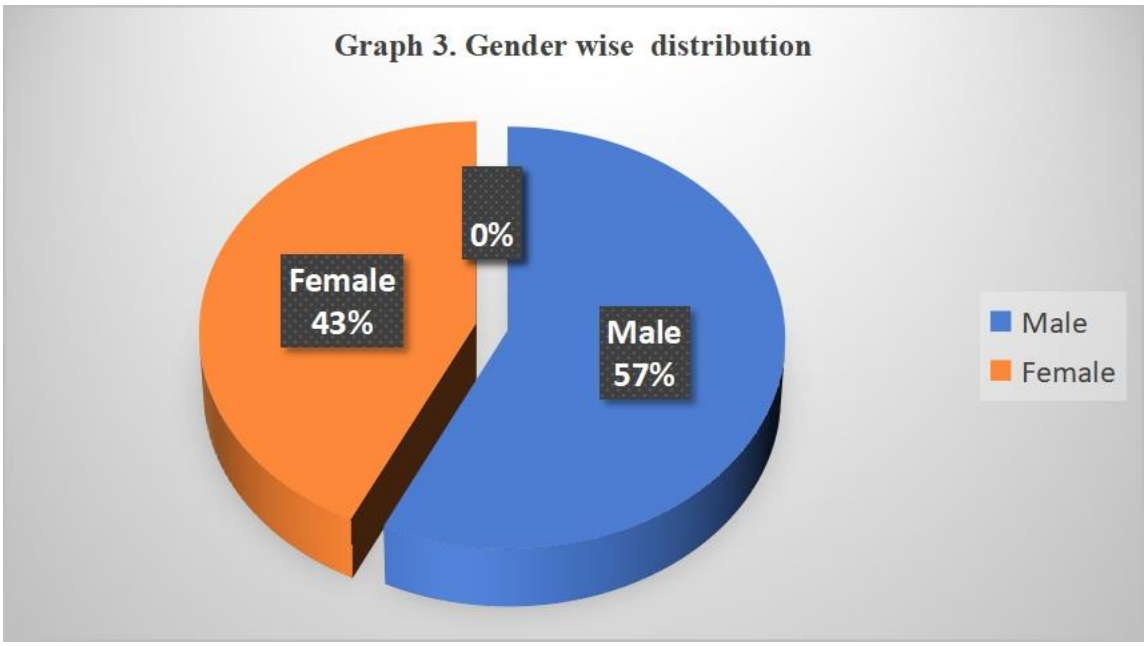
Graph 1: Correlation Between the Findings of Direct Microscopy (KOH And Gram Stain) And Fungal Culture.

It was observed that out of the 42 fungal isolates, Aspergillus spp.(38.9%) was the most common isolate followed by Fusarium spp (21.42%) and other fungal spp. (Graph 2.)



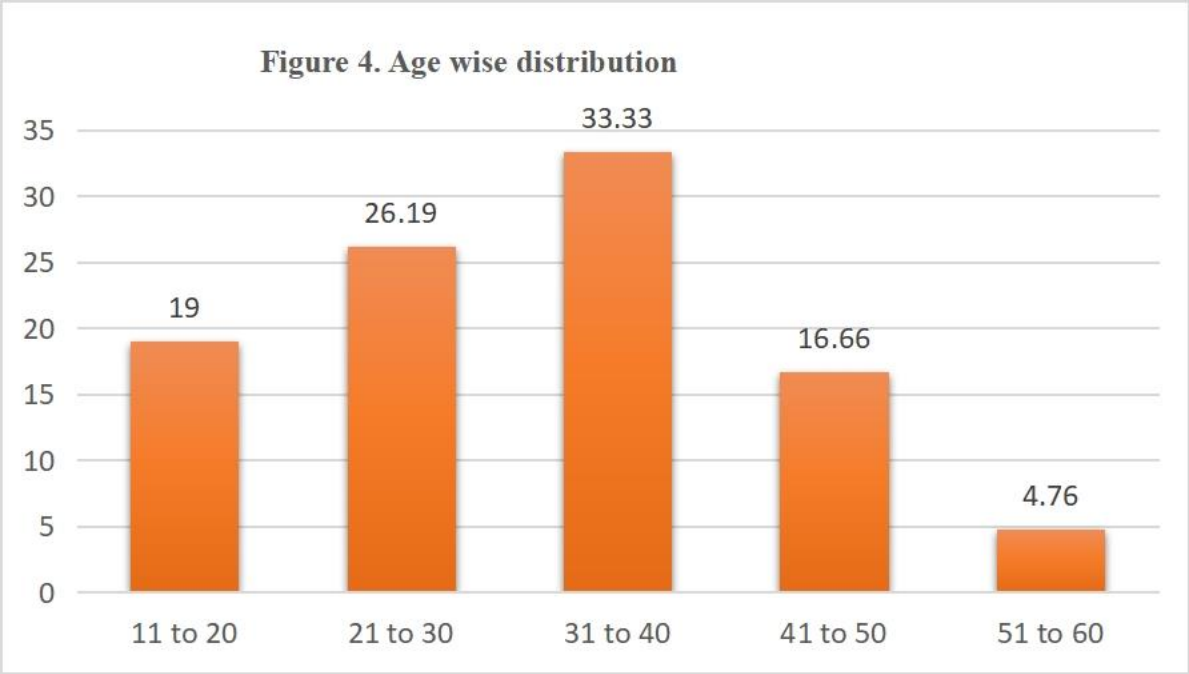
Graph 2: Graphical Representation of Distribution of Fungal Isolates.

Out of 42 cases positive for fungal etiology, 24(57.14%) were males and 18 (42.8%) were females. Infectious keratitis most commonly seen in Males than females with ratio 3:4.



Graph 3: Distribution of cases based on Gender.

In the present study the age wise distribution of infected cases in which maximum number were found was in the age group of 31-40 years followed by 21-30 and least in the age group of 51years and above. (Graph .4)



Graph 4: Age wise distribution of cases.

Agriculturists (60%) are found to be the most commonly affected followed by Manual labour (18.75%), students (7.5%) &others (13.75%). (Table 1)

S.No.	Occupation of patients	No. of cases	Percentage of cases
1.	Agriculture	48	60
2.	Manual labour	15	18.75
3.	Students	6	7.5
4.	Others	11	13.75
	Total	80	100

Table 1. Occupational distribution of cases.

s.no	Risk factors	No. of cases	Percentage of cases
1.	Ocular trauma	42	52.5
2.	Diabetes	21	26.25
3.	Previous ocular disease	9	11.25
4.	Chronic medication	8	10
	Total	80	100

Table 2. Cases distribution based on Risk factors.

The most common risk factor for fungal keratitis was found to be ocular trauma (52.5%%), Diabetes (26.25%), Previous ocular Disease (11.25) followed by Chronic medication (10%). (Table 2).

DISCUSSION

Fungal keratitis continues to be a major cause of visual loss in developing countries. Fungal corneal ulcer is common in India due to the tropical climate and a large agrarian population that is at risk. An understanding of the regional epidemiological features, risk factors, and etiological agents is important in the prevention and appropriate management of this disease entity.

Ocular trauma has been listed as the most common risk factor for mycotic keratitis. In the present study also, the commonest risk factor was ocular trauma 42(52.5%) followed by Diabetes (26.25%). The finding is in accordance with Basak SK et al (2005) [16] Ibrahim YW (2009) [17] .

In the present study, Agriculturists (60%) were found to be the most commonly affected followed by Manual labour (18.75%), students (7.5%) and others (13.75%). The finding was in accordance to the other study where the agriculturists were found to be the most commonly affected with the ratio of 57.6% [16]. There were other studies which were in support to the present study where the agricultural workers and daily wage earners, an occupation profile similar to south Indian study, [18] (66.8% and 79.3% respectively), but in contrast to Ghana, where only 16.1% of the patients were agricultural workers [19].

In our study, the incidence of corneal ulcer was seen most common in age group 31-40 years with 33.33%followed by 21-30 years with 26.19% which is supposed to be comparable with studies conducted by other researchers Basak SK et al (2005) [16].

In our study, out of 42 cases positive for fungal etiology, 57.14% were males and 42.8% were females. The finding was in accordance with Basak SK et al (2005) [16] were the ratio of males (70.6%) was more as compared to the females with (29.4%).

In our study, out of 42 fungal isolates, Aspergillus spp. (38.9%) was the most common isolate followed by Fusarium spp (21.42%). The finding was in accordance with Basak SK et al (2005) [16] where the most commonly isolated fungal pathogens in the current series were Aspergillus spp. Of 623 fungal isolates, 59.8% were Aspergillus spp, 21.2% were Fusarium spp and 10.1% were Penicillium spp.

It was noted that Aspergillus species were predominate in Mumbai, parts of south India, north India, Nepal and Bangladesh [20-25]. but there were Other studies in south India which were in contrast to the present study where Fusarium spp was reported to be more common than Aspergillus spp.[26-28] Fusarium spp have also been found to be the principal fungal pathogen in Florida, Paraguay, Nigeria, Tanzania, Hong Kong and Singapore [29-31] This phenomenon may be explained by differences in climate and the natural environment. Acanthamoeba infections were present in 4 (0.3%) patients. Two of them were contact lens wearers, and the other two patients gave a history of trauma to the eye while takeing bath on pond.

In our study, out of 80 suspected fungal corneal ulcers,42(52.5%) were positive for fungal etiology, Of these 34(80.95%) were positive on KOH mount. 30(71.4%) and 25(59.5%) were positive in gram stain and culture. The finding is in accordance with the other investigators [16-18].

The etiological and epidemiological patterns of corneal ulceration have been found to vary with the patient population, health of the cornea, geographic location and climate, and also tends to vary over time. Hence, an understanding of the epidemiological features, risk factors and etiological agents that occur in a specific region are important in rapid recognition, timely institution of therapy, optimal management and prevention of this disease. In order to start specific therapy, it is necessary to do meticulous laboratory investigations, and this includes microscopy and culture of corneal scrapings for identification of the microbial agent.

Limitation of the Study

Although having a sizable sample size, our study was limited to a single tertiary care facility, thus its findings might not apply to other states or nations with large population sizes and developed healthcare systems. The absence of advanced microbiological analysis tools, such as in vivo confocal microscopy, polymerase chain reaction, and next-generation sequencing, for the identification of organisms that are difficult to cultivate using conventional techniques was another research restriction. A thorough evaluation of susceptibility and resistance profiles was also absent from the study, which is particularly significant in considering the differences in topical antibiotic usage in ophthalmology when compared to other medical specialties. Future research should work with the microbiology department to standardize the screening of in vitro antibiotic susceptibility in order to overcome this issue.

CONCLUSION

Infective keratitis is a significant cause of ocular morbidity in Farrukhabad**.** The knowledge of epidemiology, risk factor, and microbiological profiles of infective keratitis can provide a valuable approach to disease prevention, diagnosis and management. Agricultural activity and related ocular trauma are principal causes of mycotic keratitis. A potassium hydroxide (KOH) wet mount preparation is a simple, and sensitive, method for diagnosis. We hope that the findings of our study will provide valuable insights to guide the implementation of a simple and effective community-based approach, aiming to address these challenges & improve patients outcomes.

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Declarations:
Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: We have consent to participate.
Consent for publication: We have consent for the publication of this paper.
Authors' contributions: All the authors equally contributed the work.

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