

A 5-year retrospective review on mycotic keratitis shows change in fungal trend at tertiary care hospital, Kishanganj, Bihar

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Abstract

Background: Monoocular blindness resulting from corneal ulcer due to *mycotic keratitis* is preventable in all developing countries like India. The early diagnosis by Ophthalmologist and microbiologist is the clue for prevention and recovery. Typical history of corneal injury with plant, use of antibiotics and steroid, injuries cleaning with pond water or stored pipe-line water are significant. The typical feathery ulcers with satellite lesions in one eye of the patients were selected for corneal scrape smear for KOH and Gram stain, fluorescence microscopy followed by fungal culture. **Aims:** To study the incidence and types of fungi involved. **Materials and Methods:** In the above cases corneal scraping was carried out for smear and culture examination under proparacaine local anesthesia. Scraping was done with no. 15 **Bard Parker** knife after removing loose debris with sterile cotton swab. It was immediately inoculated into Sabourauds dextrose agar and KOH mount was also done. Lactophenol cotton blue stain for fungus was used on mycelialisolates. **Results:** From May 2009 to April 2014, seventy eight (78) patients were diagnosed as *Mycotic keratitis* in our Medical College. Sixty-five cases were both culture and KOH preparation positive (fungal hyphae detected). Thirteen cases were only KOH preparation positive. **Conclusion:** From May 2009 to January 2012, the predominant fungi isolated in culture were *Aspergillus* spp., especially *flavus*. But since then the establishment of timed pipe-line water supply in this region most probably was responsible for the change in causative fungal predominance in culture as *Fusarium* Spp. *Fusarium* spp. are capable of producing biofilm in the pipe-line or in stored water container. Further research work is still in progress here in our Medical College.

Keywords: *Mycotic keratitis*, corneal ulcer, *aspergillus* spp., *Fusarium* spp., timed pipe – line water supply

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INTRODUCTION

Corneal infection is the most common cause of profound ocular morbidity leading to blindness worldwide. In both adults and children, infective *keratitis* is of great concern to the treating Ophthalmologist and microbiologist as the

ocular morbidity is more challenging to diagnose and treat. This is borne out by a number of case studies in both adult²² and children²³. In the present study all the patients were examined under the slit lamp biomicroscope to study ulcer details, anterior chamber reaction, scleral involvement and vascularisation^{24, 25}. The ulcer size and location of infiltrates were based upon the position of the ulcer midpoint and was characterised as central, paracentral, peripheral and total. The symptoms namely ocular pain, photophobia, dimness of vision and watering from the affected eye were all enquired into and noted. The signs, as diagnosed by the scheme enumerated above were also noted. The ulcer look (dry or not), presence of satellite lesions and associated hypopyon were noted. The patient's perception of difficulties and the extent of signs were also compared. The predisposing factors which render the immune system very weak are trauma,

administration of topical corticosteroids, antifungal antibiotics and indigenous remedies^{24, 25}. Satellite lesions are formed around main site of involvement. Later in the course of disease process, hyphae may be seen to penetrate descemet's membrane, encased in dense neutrophilic exudates of hypopyon. Leukocytic infiltrate with feathery borders in corneal stroma is characteristic of *mycotic keratitis*. *Fusarium* is more aggressive and less responsive to treatment than *Aspergillus*¹, hence poses theoretic challenges. The phaeoid fungi are of low virulence and produce protracted infections. Stromal keratitis caused by candida species may be more localized in contrast to filamentous fungi and have 'collar – button' configuration often with small ulceration and expanding. Nature.

MATERIAL AND METHODS

From May 2009 to May 2014, 210 patients came with corneal ulcer in eye OPD of MGM Medical College and L.S.K. Hospital, Kishanganj, Bihar. Out of which 78 patient were detected as Mycotic ulcer in our Microbiology Department. 65 patient were fungal culture positive and 13 were culture negative but KOH preparation showed fungal hyphae

KOH smear:

The sample was directly taken on a sterilized glass slide under aseptic conditions with properacaine drops used which disturb the fungus and microbes a leastand labelled as slide A and over that another sterilized glass slide labeled B was placed. Then 10% KOH was poured over the slide B. The slide was covered with a coverslip. The slide was kept in the wet chamber for 1 hour. The same slide was then observed under microscope with low power (10X) and then under high power (40X) objective lens for fungal element.

Fluorescent Microscopy:

Calcofluor White stain was poured on slide B and was examined under fluorescent microscope.

Fungal Culture:

For fungal culture, two sets of SDA (Sabouraud's Dextrose Agar) medium with antibiotics Chloramphenicol and Gentamicin were taken. The slide A was placed in first SDA (Sabouraud's Dextrose Agar) medium for one hour in BOD at 25°C. The same slide was removed from the medium and placed in the other SDA (Sabouraud's Dextrose Agar) medium for one hour in BOD at 25°C over a period of 28 days which were observed for growth. The mycelial isolates were identified by their colony characteristic and microscopic morphology in LCB (Lactophenol Cotton Blue) stain.

Bacterial Culture:

The slide A was removed from (Sabouraud's Dextrose Agar) medium and placed in Blood Agar medium for one

hour in normal incubator at 37° C. After removing the slide from medium, the medium plate was kept in incubator at 37°C overnight to check for growth.

Gram Stain:

The slide A was also stained with gram stain to check under microscope for the presence of any bacteria and / or fungal element.

RESULTS

Out of 210 suspected cases of corneal ulcer (78%) were positive for fungi of which 60 were males and 18 were females. Though cases scattered among all the ages varying from 2nd decade up to 7th decade, maximum cases were found in 5th decade of life and least in 2nd and 7th decade of life. All these 60 males' patients belonged to various occupations like farmers and carpenters mainly. This study showed that *mycotic keratitis* is commonest among farmers. The various risk factors of the positive patients were uses of topical steroid, antibiotics, corneal trauma and unidentified reasons. So from this study it is obvious that corneal trauma is the greatest risk factor for development of *mycotic keratitis*. Out of 78 positive cases 65 cases were positive by KOH and culture 13 was positive by KOH preparation only. Among these 78 *mycotic keratitis* cases upto January 2012, *Aspergillus* spp. were predominantly foruned. After that *Fusarium* spp. were predominantly noticed till May 2014. From all these findings it is obvious that our study showed preponderance of filamentous fungi in compare to yeast. The comparative yearly data in finished below *Table 1*.

Table 1: Comparative studies on mycotic keratitis

Year	No of mycotic keratitis	Predominant organism
2009 – 2010	10	<i>Aspergillus fumigatus</i> .
2010 – 2011	13	<i>Aspergillus flavus</i> .
2011 – 2012	15	<i>Aspergillus flavus</i> .
2012 – 2013	16	<i>Fusarium</i> spp.
2013 - 2014	24	<i>Fusarium</i> spp.

Table 2: Fungal spp. isolated from corneal ulcer 2009 – 2014

Year	Total	<i>Aspergillus</i>	<i>Fusarium</i>	Others
2009 – 2010	10	5	2	3
2010 – 2011	13	7	4	2
2011 – 2012	15	6	5	4
2012 – 2013	16	6	7	3
2013 – 2014	24	8	12	4

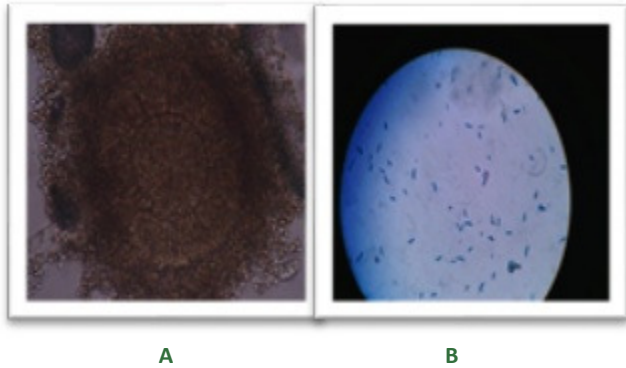


Figure A and B: *Aspergillus* spp. and *Fusarium* spp. From Fungal culture under Microscope

DISCUSSION

The incidence of mycotic keratitis in our study was 37.14%. This higher incidence of positivity in comparison to other Indian study³ could be due to the facts (a) fungal keratitis is expected to be higher in tropical and subtropical climate than temperate climate and (b) the study was done in suburban area where the majority of patients are farmers by profession. Out of the various methods tried during this study, to collect the corneal scrapings and processing of samples, the most successful method that led to minimal contamination and gave consistent result on different media was collecting the corneal scrapings in between two sterile slides, using proparacaine drop which usually least disturbing the microbial colonies. Mycotic keratitis may be reported at any age in our study the age ranged from 11 to 70 years, the mean age being 38.78 ± 10.13 . However majority patients were in 4th and 5th decade, 70.37% (n=38). The above result is in accordance with various Indian^{3,4,5} and abroad⁶ studies. The reason for this major preponderance in 4th and 5th decade is because majority of patients are outdoor workers mainly farmers. In our study majority of patients were men, 74.07% (n=40). The study was done in a suburban area which contributed to the result. The commonest risk factor was corneal trauma (6) and that too with vegetable matters, 70.37% (n=38). The result is more in comparison to various others studies^{7,8,9,10} and¹¹ because our study was done in a suburban area where majority of patients were farmers by profession. Other predisposing risk factors were use of topical steroids and antibiotics because of easy availability of topical steroid eye drop and antibiotics in suburban area. Moreover due to illiteracy patients keep on using topical steroid drops and antibiotics for a longer period. In the present study, history was taken from all the patient about (a) ocular trauma with vegetable matter (b) previous illness (C) use of pipe – line water or pond water following injury (D) use of topical antibiotic or steroid preparation (E) month

of occurrence. A history of ocular trauma particularly with vegetable matter is often present^{23,24}. This is important in both adults and children. A history of previous ocular surgery or systemic illness may be found. Vitamin A deficiency in children with break in epithelium shows increased incidence of infective keratitis. A history of topical corticosteroid or antibiotic use for long time was found to be an important factor. A seasonal variation was although noticed by earlier studies but we didn't find any²³. In this region the harvesting season especially in the month of June to September and November was found noticeable... In our study out of 78 (Seventy eight) diagnosed mycotic keratitis patient 38% were suffering from *Aspergillus* spp. in compare to 41% *Fusarium* infection. The most striking change of infection pattern was, in causative agent. The change was noticed after January 2012 following timed pipe line water supply or may be use of stored water or stagnant pond water after corneal injury by vegetative body.

CONCLUSION

India being agriculture based country and majority of the people being farmers there is a good chance of corneal trauma followed by fungal keratitis during harvesting time. Advancement in management and treatment following early diagnosis is the clue to prevent ocular morbidity. Our study that shows a gradual increase of fungal keratitis in the last five years is epidemiologically important in this region. Moreover, change of causative fungi from *Aspergillus* spp. to more dangerous *Fusarium* spp. is alarming. Significantly we noticed a correlation of timed pipe-line water supply and use of stored water which might be the reason of increase in infection by *Fusarium* spp. More study is required in this region for confirmation of this fact.

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