PROFILE OF KERATOMYCOSIS IN A TERTIARY CARE TEACHING HOSPITAL IN WESTERN UTTAR PRADESH, INDIA

Microbiology

Vandana Sardana* MD, Associate Professor, Department of Microbiology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly- 243202. *Corresponding Author

Sameer R Verma MD, Professor, Department of Radiodiagnosis, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly-243202.

ABSTRACT

Introduction- Fungal keratitis or keratomycosis refers to an infective process of the cornea caused by fungi capable of invading the ocular surface. **Aims & Objectives**-i) To determine the frequency of fungal keratitis in clinically diagnosed cases ii) To identify the aetiological agents of keratomycosis. iii) To assess the precipitating factors associated with it. **Method**-A retrospective data of 2 years was analyzed to study the number of cases of fungal keratitis, from among the corneal scrapings submitted to the Department of Microbiology, examined for the presence of fungal elements by direct microscopic methods and for the fungal growth in culture. **Results**-The frequency of fungal keratitis in clinically diagnosed cases was 28.6 %. Among the positive cases, males outnumbered females with the ratio of 1.67:1. The maximum number of positive cases were seen in the age group of 21-30 years, followed by the age groups of 31-40 years and 11-20 years. The most common cause of fungal keratitis was *Aspergillus flavus* (31.25 %), followed by *Aspergillus fumigatus* (18.75 %), *Fusarium solani* and *Candida albicans* (12.5 % each), and *Cladophialophora bantiana*, *Curvularia*, *Mucor* and *Candida tropicalis* (6.25 % each). *Aspergillus* species were found to be isolated from 50% of cases of keratomycosis. Filamentous fungi were predominantly associated with mycotic keratitis, accounting for 81.25% of cases, as compared to yeasts which had caused keratitis in 18.75% of cases. Amongst the cases of keratomycosis, all were positive for fungal growth on culture, out of which 81.25% cases were also positive for the presence of fungal elements on direct microscopy. **Discussion**: Keratomycosis is the leading cause of ocular morbidity worldwide, including India. Fungal agents causing keratitis should receive special attention due to their opportunistic behaviour and indolent course of disease. Lack of early diagnosis and treatment prelude the onset of complications, which may compromise the patient's vision and thereby the quality of life. **Conclusion**: Regular surveillance and scrutiny of mycotic keratitis is important and essential to understand the pattern of fungi, existing or emerging, so as to prevent the unnecessary and irrational usage of antibiotics.

KEYWORDS

Fungal keratitis, molds, yeasts, KOH mount, culture, *Aspergillus*, *Fusarium*

INTRODUCTION:

Mycotic keratitis is an invasive fungal infection of the cornea.^{1,2} Corneal ulcer is one of the major causes of ocular morbidity in developing countries and fungi are the main etiological agents of corneal ulceration in India.^{3,4,5} Fungi are ubiquitous organisms, present in environment as saprotrophs.^{1,2,6} Majority of the cases of keratomycosis are caused by hyaline fungi such as *Aspergillus*, *Fusarium*, *Acremonium*, *Penicillium* and *Pseudallescheria*. The phaeoid fungi such as *Alternaria*, *Curvularia*, *Exserohilum* and *Bipolaris* are the relatively uncommon causes of keratomycosis.¹ These fungi usually cause infection when there is a breach in corneal epithelium following trauma but some of them may penetrate the intact cornea.⁶ World Health Organization reported that corneal diseases are the major cause of monocular blindness after cataract. Around 1.5 to 2 million new cases of corneal ulcer are reported annually.⁷ Risk factors for fungal keratitis are male gender, history of ocular trauma, chronic use of corticosteroids and systemic diseases, that impair the host immunity.^{8,9} In India, *Fusarium* and *Aspergillus* species are being frequently isolated from corneal ulcers,^{3,10-14} irrespective of the geographical location. This study has been carried out to determine the frequency of fungal keratitis in clinically diagnosed cases, ii) To identify the aetiological agents of keratomycosis. iii) To assess the precipitating factors associated with it at tertiary care teaching hospital in western Uttar Pradesh.

MATERIALS AND METHOD:

The retrospective study was done for the period of two years after approval from the institutional ethical committee.

Corneal scrapings taken in the ophthalmology department, from the clinically diagnosed cases of infectious keratitis were sent to the Microbiology department for processing. Patient's age, gender, occupation, history of any trauma, use of topical medicines or use of contact lens and ophthalmic ultrasound findings (wherever available) were recorded from the patient clinical records.

The corneal scrapings processed as per standard techniques.^{2,15,16}

1. Direct microscopy was performed for the presence of bacteria, fungus or parasite:

a. Potassium hydroxide (KOH) Mount- Wet mounts of corneal scrapings were prepared in 10% KOH. to observe the presence of fungal filaments

b. Gram's staining was done for the evidence of any bacterial aetiology.

2. Culture:^{2,15,16}

The material from corneal scrapings was inoculated directly onto sheep blood agar and chocolate agar in rows of 'C'- shaped streaks and incubated at 37°C for upto 48 hours. The portion of the sample was inoculated onto Sabouraud's dextrose agar (SDA) without cycloheximide in duplicate tubes and incubated at 25°C and 37°C, respectively, for 4 weeks.

Identification of fungal growth was done as per standard mycological techniques:^{2,15,16}

Molds were identified by their colony characteristics on SDA and sporulation pattern on lactophenol cotton blue mount. Slide cultures were put up for non-sporulating molds.

Yeasts were identified conventionally by their growth on SDA, germ tube test, microscopic findings on cornmeal agar, colony characteristics on Chrom agar sugar fermentation and assimilation tests, and by using Automated Vitek 2 YST identification card (bioMérieux, France), as per manufacturer's instructions.

RESULTS:

In the present study, corneal scrapings were obtained from a total of 56 clinically diagnosed cases of keratitis, out of which 34 were males and 22 were females.

Out of total of 56 samples, 16 (28.6%) were positive for fungal growth, 7 (12.5%), yielded bacterial growth and the remaining 33 (58.9%), samples were culture sterile. Thus the frequency of fungal keratitis in clinically diagnosed cases was 28.6%.

Among the positive cases of fungal keratitis, males (62.5%) outnumbered females (37.5%) with the ratio of 1.67:1 (Table 1). The maximum number of positive cases were seen in age group of 21-30 years, followed by the age groups of 31-40 years and 11-20 years (Table 1).

The most common cause of fungal keratitis was *Aspergillus flavus* (31.25 %), followed by *Aspergillus fumigatus* (18.75 %), *Fusarium solani* and *Candida albicans* (12.5 % each), and *Cladophialophora bantiana*, *Curvularia*, *Mucor* and *Candida tropicalis* (6.25 % each)

(Table 2). *Aspergillus* species were found to be isolated from 50% of cases of keratomycosis. Filamentous fungi were predominantly associated with mycotic keratitis, accounting for 81.25% of cases, as compared to yeasts which had caused keratitis in 18.75% of cases (Table 3).

Amongst the cases of keratomycosis (n=16), all were positive for fungal growth on culture, out of which 81.25% cases were also positive for the presence of fungal elements on direct microscopy (Table 4).

The most common precipitating factor associated with fungal keratitis was prolonged usage of topical medications such as corticosteroids /antibiotics (56.25 %), followed by a trauma with vegetative matter (31.25%), usage of contact lenses and previous corneal surgery (6.25% each) (Table 5).

The ophthalmic ultrasound findings were available from the clinical records in 48 patients out of the 56 patients included in this study and were within normal limits in 42 patients with abnormality detected in six patients (cataract in four patients, and PVD and RD in one patient each). (Table 6)

Table 1: Age and gender wise distribution of cases of fungal keratitis (n=16)

Age (in years)	Total no. of positive cases	Male	Female
0-10	0	0	0
11-20	3	3	0
21-30	5	3	2
31-40	4	2	2
41-50	2	1	1
51-60	1	1	0
≥ 61	1	0	1
Total	16	10	6

Table-2: Distribution of fungal agents isolated from the cases of keratomycosis (n=16)

Fungal agent	Number of cases	Percentage of cases (%)
<i>Aspergillus flavus</i>	5	31.25
<i>Aspergillus fumigatus</i>	3	18.75
<i>Fusarium solani</i>	2	12.5
<i>Cladophialophora bantiana</i>	1	6.25
<i>Curvularia</i>	1	6.25
<i>Mucor</i>	1	6.25
<i>Candida albicans</i>	2	12.5
<i>Candida tropicalis</i>	1	6.25

Table-3: Aetiology of mycotic keratitis :- Molds versus Yeasts (n=16)

Fungus	Number of cases	Percentage of cases (%)
Molds	13	81.25
Yeasts	3	18.75

Table-4: Direct microscopy versus Culture (n=16)

Test	Number of positive cases	Percentage of positive cases (%)
Direct microscopy	13	81.25
Culture	16	100

Table 5: Precipitating factors associated with cases of keratomycosis (n=16)

Risk factor	Positive cases (n=16)	Percentage (%)
Prolonged usage of topical medications	9	56.25
Trauma with vegetative matter	5	31.25
Usage of contact lens	1	6.25
Previous corneal surgery	1	6.25

Table 6: Ophthalmic USG findings in clinically diagnosed cases of infectious keratomycosis (n=48)

Ultrasound findings	No. of patients (%)
Normal	42 (87%)
Cataract	4 (8%)
Posterior vitreous detachment	1(2.5%)
Posterior retinal detachment	1(2.5%)

DISCUSSION:

Despite advancements in the ocular treatment strategies, fungal keratitis remains an important cause of ocular morbidity. In the present study, the frequency of fungal keratitis in clinically diagnosed cases was found to be 28.6%. In India, the prevalence of fungal corneal ulcer is estimated as 44-47%.¹⁰ Keratomycosis comprises of at least 50% of all culture positive cases in India.⁴ The incidences of mycotic keratitis have been documented to be variable across the various states of India: southern and western India with 36.7%¹⁷ and 36.3%^{17,18} northern (7.3%), northeastern (25.6%), and eastern India (26.4%).¹⁹⁻²¹

An increase in occurrence of fungal keratitis, in India, is attributed to tropical climate and farming, as one of the main occupations, which poses a high risk due to vulnerability for occupational trauma, especially by organic materials and sand particles.⁶

Among the positive cases of fungal keratitis, males (62.5%) outnumbered females (37.5%) with the ratio of 1.67:1 (Table 1). Our data is consistent with the study done by Gupta A et al.⁸ in which incidence of keratomycosis was more in males (63.6%) than in females (36.4%). A study done in Chandigarh by Chander J et al.²² also reported the male predominance in cases of fungal keratitis.

We can consider that the higher rate of occurrence of keratitis in males is attributed to their more indulgence in outdoor activities and field work related to agriculture.⁶

In our study, maximum number of positive cases were seen in age group of 21-30 years, followed by the age groups of 31-40 yrs and 11-20 years (Table 1), indicating occurrence of keratomycosis in young to middle aged population, which is the most common actively working age group.²³

Gupta A et al.⁸ found that majority of fungal ulcers were seen in the age group of 21-40 years. Whereas Chander et al.²² from Chandigarh reported that 56.67% cases of fungal keratitis belonged to the age group of 20-49 years, recent study by Rohilla et al.²⁴ in Uttarakhand described 41-60 years was the age group in which most of the keratitis cases were recorded (53%).

In the present study prolonged usage of topical medications such as corticosteroids /antibiotics (56.25 %), and trauma with vegetative matter (31.25%) were the common predisposing factors, followed by usage of contact lenses and previous corneal surgery (6.25% each) (Table 5). Similar observations have also been made by other studies.^{1,6,8,25,26} Antibiotics are known to alter normal local microbial flora and steroids have been the culprit to alter corneal metabolism, and thus changing the defense mechanism.⁸

As far as occupational history is concerned, mycotic keratitis was more common in field workers such as farmers, which coincides with the findings in other studies.²⁶⁻²⁹

Fungal spores are pervasive and ubiquitous in nature. Keratomycosis in farmers /agriculturists is related to the presence of fungal spores on deciduous and senile vegetations.⁸

Amongst the cases of keratomycosis (n=16), all were positive for fungal growth on culture, out of which 81.25% cases were also positive for the presence of fungal elements on direct microscopy, thus indicating that culture is more sensitive than microscopy (Table 4). Similar observations were also made by Gupta A et al.⁸ However, a study done in Chandigarh reported that 46.86% cases were negative for fungal growth on culture despite positive findings on direct microscopy.²⁶⁻²⁹

In our study, the commonest cause of fungal keratitis was *Aspergillus flavus* (31.25 %), followed by *Aspergillus fumigatus* (18.75 %), *Fusarium solani* and *Candida albicans* (12.5 % each), and *Cladophialophora bantiana*, *Curvularia*, *Mucor* and *Candida tropicalis* (6.25 % each). *Aspergillus* species were found to be responsible for 50% of cases of keratomycosis. (Table 2).

Filamentous fungi were predominantly associated with mycotic keratitis, accounting for 81.25% of cases, as compared to yeasts which had caused keratitis in 18.75% of cases (Table 3).

The fungi that are isolated from corneal scrapings are, at many occasions, the part of normal local microbial flora, and also the saprophytes in the soil and environment.⁸

In 2000, a study done in Delhi by Gupta A et al.⁸ found *Aspergillus* species as the most frequent cause of keratitis, followed by *F. solani*. Yeasts were isolated in 14.3% of cases and filamentous fungi in 85.5% of cases⁸, which are consistent with our observations in the present study.

Another study done in north India³⁰, in 2005, also reported *Aspergillus* species as the commonest cause of fungal keratitis accounting for 41% of cases.

In 2012, authors in western India³¹ isolated *Aspergillus* species in 40% of cases.

However, various studies done in south India^{17,32,33} and recently in Northern India²⁴ found *Fusarium* species to be the most common cause of fungal keratitis.

Doczi I et al. reported a case of keratomycosis caused by a mixed infection involving *Fusarium verticillioides* along with *Staphylococcus epidermidis*, an α haemolytic streptococcus and *Propionibacterium acnes*, in Hungary in 2004.¹³ However, Tanure MA et al.³⁴ found *Candida albicans* (46%) as the commonest cause of mycotic keratitis in Philadelphia, in 2000.

Studies³⁵⁻³⁷ have reported *Aureobasidium pullulans* as the cause of keratitis following surgical interventions.

Hyaline fungi (*Aspergillus*, *Fusarium* species) have been reported to be commonly associated with corneal ulcers, followed by dematiaceous fungi (*Curvularia* and *Bipolaris* species). *Candida* species have been found to have a low preponderance in developing countries.^{38,39}

In contrast to non-dermatophyte molds associated with mycotic keratitis, a study done in New Delhi, by Sharma Y et al.²⁶ had reported *Trichophyton* species as a potent fungal agent capable of causing keratitis.

Fungal agents causing keratitis should receive special attention due to their opportunistic behaviour and indolent course of disease. Lack of early diagnosis and treatment prelude the onset of devastating complications, which may compromise the patient's vision and thereby the quality of life.

Figures

Figure 1: Corneal scrapings showing fungal hyphae on 10% KOH wet mount (400x) (arrowed)

Figure 2: Corneal scrapings showing budding yeast cells with pseudohyphae on 10% KOH wet mount (400 x) (arrowed)

Figure 3 : Corneal scrapings showing budding yeast cells on Gram's stain (1000 x) (arrowed)

Figure 4: *Aspergillus flavus* growth on SDA

Figure 5: *Aspergillus fumigatus* growth on SDA

Figure 6: *Fusarium solani* growth on SDA

Figure 7: *Fusarium solani* on LCB mount (400 x)

CONCLUSIONS:

Regular surveillance and scrutiny of mycotic keratitis is important and essential to understand the pattern of fungi, existing or emerging, so as to prevent the unnecessary and irrational usage of antibiotics.

Limitations:

Antifungal susceptibility testing could not be done due to limited resources. Failure of microorganisms to grow in culture would have been amended by the use of molecular methods.

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