

MICROBIOLOGICAL EVALUATION OF KERATOMYCOSIS IN PATIENTS OF A TERTIARY CARE HOSPITAL IN WESTERN UTTAR PRADESH

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ABSTRACT

BACKGROUND

Corneal infections are one of the predominant causes of ocular concern worldwide especially in agriculture based developing countries.

The aim of the study is to evaluate the aetiology and risk factors associated with keratomycosis in Western Region of Uttar Pradesh.

MATERIALS AND METHODS

This cross-sectional study was carried among 62 clinically suspected cases of fungal keratitis from March 2014 to February 2016 in the Department of Microbiology of Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, after approval of Institutional Ethical Committee. The patients were analysed for predisposing factors and clinical characteristics. The corneal scrapings were examined by 10% KOH mount and were cultured on Sabouraud dextrose agar. The fungal aetiologies were identified by colony morphology, lactophenol cotton blue mount, slide culture and other standard tests.

RESULTS

Of 62 clinically suspected cases of keratomycosis, 29 (45.83%) were culture positive and 33 (53.22%) cases were positive on direct microscopy for fungal elements. The most common predisposing factor was found to be traumatic inoculation of vegetative matter to the eye (41.38%), followed by inoculation of dust among brick workers (24.13%), prolonged use of corticosteroid (13.79%), use of contact lenses (13.79%) and severe diabetes mellitus (8.89%). The commonest fungi secluded were *Aspergillus* species (61.5%) followed by *Fusarium* species n=6/29 (20.67%), *Penicillium* species n=4/29 (13.79%), *Candida albicans* n=2/29 (6.90%), *Curvularia* species n=2/29 (6.90%) and *Mucor* species n=2/29 (6.90%).

CONCLUSION

Incidence of keratomycosis is on higher side in this region. It should be considered while diagnosing corneal infection especially in rural area as early diagnosis can help in the management of keratomycosis and hence its complications.

KEYWORDS

Keratomycosis, *Aspergillus*, Corticosteroids, Corneal Scrapings.

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BACKGROUND

Corneal blindness is a major global health problem and keratitis by fungi and bacteria are the predominant causes. Fungal keratitis or keratomycosis results from incursion of the cornea by the fungal organism. Since, its first description from Germany in 1879, it has been recognised as a major public health problem in the tropical areas of various developing nations including India.¹ If not managed early, this condition might lead to total corneal blindness.

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According to the published literatures from various countries, more than 56 genera of fungi and over 100 species have been attributed as the causative agents of keratitis of fungal origin.^{2,3} They have reported *Aspergillus*, *Fusarium*, *Curvularia*, *Helminthosporium*, *Alternaria*, *Penicillium* and *Mucor* species as the most commonly isolated being. However, geological and climatic variations affect the spectrum of aetiology to a great extent. The most common predisposing factor is traumatic inoculation of the aetiological agent in the eye with vegetative matter as observed by recent study.⁴ Seasonal variations are identified to affect the incidence of isolation of fungi. Other factors include prior administration of corticosteroids and long-term use of antibiotics, the use of contact lenses has also been linked to mycotic keratitis.⁵

In a developing country like India with a huge population connected to agriculture, accidental traumatic inoculation of the vegetative matter in cornea is very common. Perplexing

to the problem is the relatively varied and overlying clinical features of fungal keratitis and bacterial keratitis. Early diagnosis and treatment is very important in preventing complications like corneal perforation and endophthalmitis in the patients. However, limited study has been conducted about the aetiology of the condition. The purpose of this study was to establish the aetiology of fungal keratitis and the predisposing factors among the patients attending our hospital.

MATERIALS AND METHODS

Study Type and Design- This was a cross-sectional hospital-based study conducted in the Department of Microbiology from March 2014 to February 2016 after the approval of Institutional Ethical Committee (IEC).

Sample- All the corneal scrapings from patients with clinical indications of corneal infiltration received in Department of Microbiology sent by Department of Ophthalmology from March 2014 to February 2016.

Inclusion Criteria

Patients with clinical signs of keratomycosis.

Exclusion Criteria

Patients with signs other than keratomycosis.

Patients on antifungal treatment within 2 months.

Data Collection- Data regarding age, sex, occupation, patient identification number, detailed clinical history and clinical presentation like the ulcer with a raised, wet, soft, creamy to grayish white infiltrate, ulcer has feathery or hyphae margins and satellite lesions. The symptoms like severe pain, photophobia and redness.

Sample Collection and Processing

Corneal scraping is collected after anaesthetising the cornea with instillation of drops of 0.5% proparacaine and waiting for 2-3 minutes by the ophthalmologist. Scraping is done by applying multiple unidirectional strokes using sterile Kimura spatula under slit lamp illumination. The specimen is collected after retracting the lids and cleaning any discharge or debris from the vicinity both from the base as well as from the border of the ulcer.

The scraped out corneal tissue after is sent for Gram staining, 10% Potassium Hydroxide (KOH) mount and for fungal culture. A portion of corneal scrapings was inoculated

on a set of basal fungal culture media, i.e. Sabouraud's Dextrose Agar (SDA) (HiMedia, Mumbai) with chloramphenicol and cycloheximide and on blood agar. One tube of SDA was incubated at 25°C and another at 37°C for four weeks or till the appearance of fungal growth. The tubes are discarded after 8 weeks if there was no growth.

Fungal Identification

The fungal isolates were identified by studying the obverse and reverse morphology on media, Lactophenol Cotton Blue (LPCB) Mount of the isolate, morphological study after slide culture and other necessary fungal tests. Gram staining and germ tube test were done for yeast like colonies.

Data Analysis- The data are analysed using Microsoft excel for tables and graphs.

RESULTS

Out of total 62 cases of clinically-diagnosed patients of keratomycosis in our hospital, twenty nine n=29/62 (45.83%) cases were confirmed by culture and n=33/62 (53.22%) were positive for fungal elements in 10% KOH mount. Males n=20/29 (68.96%) were significantly more affected ($p<0.001$) as compared to females n=9/29 (31.03%) as depicted in Table 1. The male-to-female ratio was 2.22:1. The majority of the culture confirmed cases of keratomycosis were from rural area, n=22/29 (75.86%) as displayed in Figure 1.

The most common predisposing factor was found to be traumatic inoculation of vegetative matter to the eye n=12/29 (41.38%) as most of the patients were farmers. The other predisposing factors were found to be inoculation of dust among brick workers n=7/29 (24.13%), prolonged use of corticosteroid eye drops n=4/29 (13.79%), use of contact lenses n=4/29 (13.79%) and severe diabetes mellitus n=2/29 (8.89 %) as presented in Figure 2.

The most frequent fungal genera that have been implicated in keratomycosis in our study was *Aspergillus* species n=13/29 (44.83%), followed by *Fusarium* species n=6/29 (20.67%), *Penicillium* species n=4/29 (13.79%), *Candida albicans* n=2/29 (6.90%) and *Curvularia* species n=2/29 (6.90%) and *Mucor* species n=2/29 (6.90%) as tabulated in Table 2. The figure showing the fungal elements in 10% KOH mount and in LPCB is shown in Figure 3 and 4, respectively.

Age Group	Clinically-Suspected Cases of Keratomycosis			Culture Confirmed Cases of Keratomycosis			10% KOH [#] Mount
	Males	Females	Total	Males	Females	Total	Total
10-20 years	7	4	11	3	1	4 (36.36%)	5
21-30 years	11	5	16	4	2	6 (37.5%)	7
31-40 years	11	4	15	3	2	5 (33.33%)	9
41-50 years	10	4	14	6	3	9 (64.28%)	7
51-60 years	3	1	4	2	1	3 (75%)	3
>60 years	2	-	2	2	-	2 (100%)	2
Total	44	18	62	20	9	29 (45.83%)	33

Table 1. Sex and Age Distribution of Culture Confirmed Cases of Keratomycosis

KOH= Potassium Hydroxide.

Sl. No.	Fungal Species	Number of Isolate from Patients	Percentage
1.	Aspergillus species	13	45%
2.	Fusarium species	6	20%
3.	Penicillium species	4	14%
4.	Candida albicans	2	7%
5.	Curvularia species	2	7%
6.	Mucor species	2	7%
	Total	29	100%

Table 2. The Different Genera Isolated from Patients of Keratomycosis

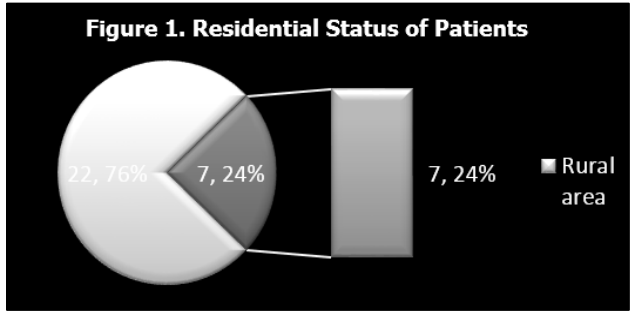


Figure 1. Residential Status of Patients

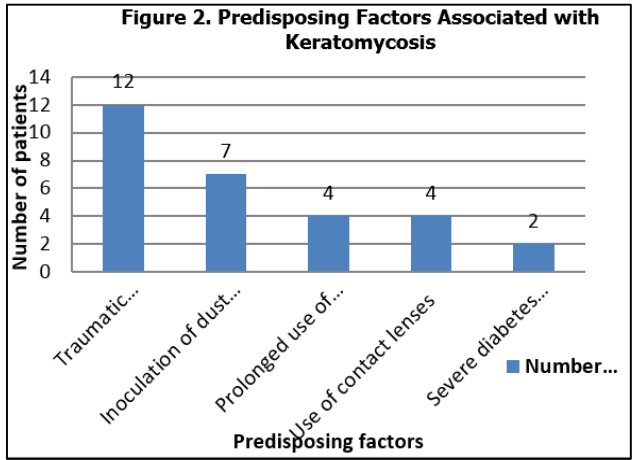


Figure 2. Predisposing Factors Associated with Keratomycosis



Figure 3. The Microscopic Image of 10% KOH Showing Fungal Elements

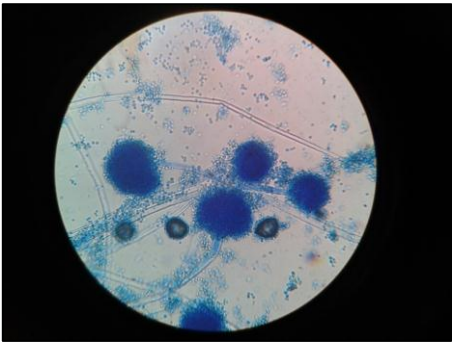


Figure 4. The Image Showing Aspergillus Niger in Lactophenol Cotton Blue Mount

DISCUSSION

Keratomycosis is a very frequent cause of ocular morbidity in developing countries especially in Indian subcontinent despite the advances in the management strategies, although it is not so common in developed western countries. In the present study, the incidence of mycotic corneal involvement, which was culture proven was 45.83%. Different Indian literatures from different parts of the country have reported a varied rate of prevalence (7-40%).¹ Regional distribution of corneal ulcers in India as reported by various studies are 7.3% in northern region, 32% in eastern region, 38.9% in western region and 32%-39.8% in southern region.⁶⁻⁸ Males were more inflicted (68.96%) as compared to females (31.03%). This study result is in accord with the studies of other workers.^{9,10}

In the present study, majority (68.96%) of the cases of keratomycosis were diagnosed in the age group of 21-50 years as this group is the major working group in Indian family. Similarly, a study from north India reported the incidence at 56.67% in the age group of 20-49 years.¹ About 53.22% of clinically-diagnosed patients of keratomycosis were warranted by 10% KOH mount while 45.83% were confirmed by culture. The less culture positivity can be due to use of prior antifungal agents, technical error while inoculating the media. The study available for the sensitivity of 10% KOH mount for the presumptive diagnosis of fungal keratitis varies between 33 to 92%.^{11,12} The majority (75.86%) of the culture established cases of keratomycosis were from rural area. This is due to the agriculture-based population around the study site and the population is predisposed to the major risk factors.

The most common predisposing factor was found to be traumatic inoculation of vegetative matter to the eye (41.38%) as most of the patients were farmers. The other predisposing factors were found to be inoculation of dust among brick workers (24.13%) and prolonged use of corticosteroid eye drops (13.79%). These finding is in line with the result of other studies.^{10,13,14} The prolonged use of corticosteroids alters the corneal metabolism and by changing the local defense mechanism while prolonged antibiotic therapy alter normal local flora leading to increased chances of invasion by fungal pathogen. The other factors that might have culminated to cause keratomycosis were use of contact lenses (13.79%) and severe diabetes mellitus (8.89%) among the culture-proven patients.

It has been established that there is a brawny geographical influence on the incidence of the diverse forms of mycotic keratitis and their aetiology. The fraction of corneal ulcers caused by filamentous fungi has shown a propensity to increase towards tropical latitudes as compared to temperate climates. In the present study, *Aspergillus* species (44.83%) was the most common aetiological agent of keratomycosis, followed by *Fusarium* species (20.67%). Other studies from a 8 year study from northern India and a three year study from western India have reported *Aspergillus* species as most common cause of keratomycosis and their rate was found to be (50.5%) and (40%), respectively.¹⁵ One study has reported in 2013 that the *Fusarium* species are the other common cause of fungal keratitis.¹⁶ The other aetiology of fungal keratitis in our study was found to be *Penicillium* species (13.79%), *Candida albicans* (6.9%), *Curvularia* (6.9%) and *Mucor* (6.9%). Of these, the maximum number of isolates has been recovered from patients on prolonged antibiotic therapy, use of contact lenses and diabetes mellitus. While a study from other part of India have reported incidence of *Penicillium* and *Curvularia* as 9.7%.¹⁴

In conclusion, our study established that mycotic keratitis continues to be an important cause of ocular morbidity in our region especially among the working age group of rural area. The aetiology of keratomycosis was predominantly comprises of filamentous fungi like *Aspergillus* species and *Fusarium* species. In addition of bacterial keratitis, the keratomycosis should also be considered as cause of corneal infection and the early examination of corneal scrapings by direct microscopy and culture can help in the management of keratomycosis and hence its complications among these patients.

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