

Mycological profile of keratomycosis in a tertiary care centre from Pune Maharashtra

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Abstract

Introduction: Microbial keratitis remains a leading cause of corneal ulcer and blindness worldwide. Mycotic keratitis has emerged as a major ophthalmic problem and contributes to 6-53% of all corneal infections worldwide. Thus the present study was undertaken to assess the spectrum of fungal causes of keratitis in BJGMC & SGH, Pune

Aims and Objectives: 1. To establish the microbiological profile of keratitis. 2. To determine the mycological profile of keratitis.

Materials and Methods: The present prospective study was conducted over a period of twelve months in a tertiary care hospital. The study was undertaken to evaluate the etiological profile of keratomycosis with a special reference to mycotic etiology. The corneal scrapings were collected aseptically and processed by direct microscopic methods and standard culture techniques.

Results: Of 90 cases of keratitis screened, culture yielded growth in 50 cases (55.6%). These were included in the study. Out of these 50 cases of growth, fungi were isolated in 32% and bacteria in 64% cases. Mixed growth of bacteria and fungi was found in 4% cases. Most common fungus found were *Fusarium spp.* (44.4%) followed by *Aspergillus spp.* (27.8%), *Alternaria spp.* (11.1%), *Curvulariaspp* (11.1%) and *Acremonium spp* (5.6%).

Conclusion: Routine surveillance of fungal keratitis is necessary to know the existing and emerging pattern of pathogens and to prevent inappropriate use of antimicrobial therapy.

Keywords: Keratitis, Fungus, Aspergillus, Keratomycosis.

Introduction

Microbial keratitis remains a leading cause of corneal ulcer and blindness worldwide. The incidence is relatively high in developing countries whereas it is quite low in developed countries.¹

Keratitis is an inflammation of the cornea and is often caused by an infection. Bacteria, viruses, fungi and parasites all have been implicated in the causation of keratitis.²

Mycotic keratitis has emerged as a major ophthalmic problem and contributes to 6-53% of all corneal infections worldwide. The remarkable increase in the incidence of fungal infection has been attributed to the widespread and indiscriminate use of corticosteroids and broad-spectrum antibiotics.²

In mycotic keratitis, two types have been recognised: Keratitis due to filamentous fungi (especially *Fusarium* and *Aspergillus*), which commonly occurs in tropical and subtropical zones, are associated with corneal trauma and keratitis due to yeast-like and related fungi particularly *Candida*, are associated with corneal disease, local immunosuppression caused by chronic corticosteroid use and systemic disease states that lower host resistance.²

Despite advances in diagnosis and medical treatment of keratitis, many patients require surgical intervention such as keratoplasty, enucleation or evisceration because of either failed medical treatment or advanced disease at presentation.

Materials and Methods

The present prospective study was conducted in the Department of Microbiology at a tertiary care centre over a period of twelve months. Ethical approval was taken from the Institute ethical committee earlier before commencing the study.

Ninety clinically suspected cases of keratitis attending the eye department of tertiary care centre were screened and 50 culture positive cases of keratitis were included in the study detail history of each patient was taken documenting socio-demographic features, history of corneal trauma, traumatising agents, associated ocular conditions, other systemic diseases, therapy received prior to presentation. Informed consent of all patients was taken.

Inclusion Criteria: Patients presenting with corneal ulcer defined as a loss of corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation were included in this study.

Exclusion Criteria: Patients presenting with ulcer having typical feature of viral infection and healing ulcer, Mooren's ulcers, interstitial keratitis, sterile neurotropic ulcers and ulcer associated with autoimmune conditions.

Collection of Specimen: Corneal scraping was collected by an ophthalmologist using a sterile Bard Parker surgical blade no. 15 under magnification of slit-lamp from the active margin and base of ulcer

following debridement of superficial mucus. Inoculation on various media was done bedside.³ For microscopy material was placed on two glass slides for gram stain and 10% KOH.⁴

Processing of specimen: Smear was prepared from the collected sample and subjected to Gram stain using Preston & Morrell's Gram method.⁵

Wet mount with 10% KOH of the corneal scraping was made and observed under the microscope for fungal elements. The material was directly inoculated onto the surface of solid media such as 5% sheep blood agar, chocolate agar and sabouraud's dextrose agar without cycloheximide, row of "C"- shaped inocula were made on the plates. Sabouraud's dextrose agar plate without cycloheximide was incubated at 27°C, examined daily, and discarded after 4 weeks if no growth was seen.⁴

All moulds identified by standard mycological procedures.⁶

Bacteriological media were incubated at 37°C and were evaluated at 24 hours and at 48 hours and later discarded if there was no growth.⁵

Microbial cultures were considered positive only if at least one of the following criteria was met.³

1. The growth of the same organism on two or more solid media on the C-streak; or there was semi-confluent growth at the site of inoculation on one solid medium
2. The same organism was grown from repeated scraping
3. Consistent with clinical signs
4. Smear results were consistent with cultures

Results

In this study out of total 90 patients, 61 (67.8%) were males and 29 (32.2%) were females. The incidence of keratitis was higher in males than females. Male to female ratio was 2.1:1. Maximum numbers of cases were seen in the age group 41 to 50 i.e. 40 (44.4%) followed by 51 to 60 i.e. 18 (20%). (Table 1)

Table 1: Age wise distribution of cases

Age	Male	Female	Number of cases	Percentage (%)
01-10	2		2	2.2
11-20	4	1	5	5.6
21-30	4		6	6.7
31-40	8	3	11	12.2
41-50	26	14	40	44.4
51-60	11	7	18	20
61-70	4	4	6	6.7
71-80	2	0	2	2.2
Total	61	29	90	100

Fifty (55.6%) cases were microbiologically diagnosed. These 50 culture positive patients were taken as our study group and assessed further.

In this study majority of cases i.e 40 (80%) were from rural area and 10 (20%) were from urban area.

Majority of the cases i.e 30 (60%) were farmers followed by labourers 12 (24%), carpenters 3 (6%), housewives 2 (4%), unemployed 2 (4%) and student 1 (2%). (Table 2)

Table 2: Distribution of occupation among urban and rural residency

Occupation	Urban	Rural	Percentage (%)
Farmer(30)	00	30	60
Labourer(12)	07	05	24
Carpenter(3)	02	01	6
Housewives(2)	00	02	4
Unemployed	01	1	5.6
Student(1)	00	01	2
Total	10(20%)	40(80%)	100

History of trauma was documented in 40 (80%) cases followed by history of surgery 4 (8%), history of dacrocystitis 3 (6%), diabetes 1 (2%), contact lens use 1 (2%) and unknown factor 1 (2%). Trauma with vegetative matter was associated with maximum number of cases 30 (75%) in this study. This is

followed by trauma with stone/sand/soil in 6 (15%), trauma with metallic foreign body in 3 (7.5%) and trauma with animal tail in 1 (2.5%) cases. In the present study trauma by sugar cane leaf was found most common cause of injury 22/30 (73.3%) which is

followed by trauma with rice paddy 5/30 (16.7%) and wood stick 3/30 (10%). (Table 3)

Table 3: Predisposing factors associated cases

Predisposing factor		Number of cases	Percentage (%)
Trauma			66.7
	Vegetative matter	8	
	sand/soil	2	
	Metallic foreign body	1	
	Animal tail	1	
Cataract surgery		3	16.7
Dacrocystitis		2	11.1
Diabetes		1	5.5
Total		18	100

In this study out of 50 cases, pure bacterial growth was found in 32 (64%) cases and pure fungal growth was found in 16 (32%) cases. Mixed growth was found in 2(4%) cases.

Out of total 18 fungal isolates, most common species was *Fusarium spp.* (44.4%) followed by *Aspergillus spp.* (27.8%), *Alternaria spp.* (11.1%), *Curvulariaspp* (11.1%) and *Acremonium spp* (5.6%). (Table 4)

Table 4: Total fungal isolates

Type	Number (%age)
<i>Fusarium spp</i>	8 (44.4)
<i>Asergillus spp</i>	5 (27.8)
<i>Alternaria spp.</i>	2 (11.1)
<i>Curvularia spp.</i>	2 (11.1)
<i>Acremonium spp</i>	1(5.6)
Total	18 (100)

For fungal keratitis, topical therapy with 5% natamycin 1 hourly and systemic therapy with fluconazole 150mg twice a day was started for all cases. In addition 1% atropine sulphate eye drop was used in all patients. Patients were followed up after one month. Out of 16 patients who were diagnosed with fungal keratitis, 14 patients recovered. Two patients could not be followed.

In 2 patients where mixed infections were found, 1 patient recovered. Other patient, who had mixed infection with multi drug resistant *Pseudomonas* and *Fusarium*, deteriorated and needed surgery.

Discussion

At birth the eyes are sterile but soon they become invaded by various microorganisms.

A wide spectrum of micro-organisms can produce corneal infection. Accurate knowledge of causative micro-organisms and their clinical presentation helps in appropriate therapy and thus prevent complications.

Shifting trends in the microbiological profile of keratitis have been reported in studies in some parts of

the world.⁷ Hence, it is important to carry out studies periodically to assess local organisms and the sensitivities pattern.

Maximum number of cases in the present study were in the age group between 41-50 years (44.4%) followed by 51-60 years (20%). Similar findings were found in study of Bharathi et al,⁸ Sathyanarayan et al,⁹ Punia et al¹⁰ and Ghosh et al.¹¹ Study by Gopinathan et al¹² and Balagurunathan R et al¹³ showed keratitis was most common in the third decade.

The frequency of keratitis was greater in men than in women in this study. Higher incidence in males might be due to more exposure to outdoor activities, exposing them to trauma, thereby increasing their vulnerability to the disease. Higher incidence in males was also seen in study by Gopinathan et al,¹² Kumar et al,¹³ Basak et al,¹⁴ Tewari et al,¹⁶ Deorukhkar et al¹⁷ and Gaurav et al.¹⁸

Keratitis was more common in people from rural area in this study. Out of 50 cases 40 (80%) were from rural area. Similar finding was also seen in study by Biradar et al.¹⁹ Lower hygiene, farming and more exposure to trauma might be the reason for higher incidence in rural area.

In this study majority of cases 30 (60%) were farmers by occupation followed by labourers 12 (24%), carpenters 3 (6%), housewives 2 (4%), unemployed 2 (4%) and student 1(2%). Farmer was also found to be the most common occupation in studies by Kumar et al,¹⁵ Deorukhkar et al,¹⁷ Gaurav et al¹⁸ and Bharathi et al,²⁰ More exposure to trauma in farmers might be the reason for higher incidence of keratitis in them.

In this study, trauma was the most common predisposing factor found in 40 (80%) cases followed by cataract surgery 4 (8%), dacrocystitis 3 (6%), diabetes 1 (2%), contact lens use 1 (2%) and unknown factor 1 (2%).Trauma was also found to be the major predisposing factor in studies by Basak et al,¹⁴ Kumar et al,¹⁵ Tewari et al,¹⁶ Deorukhkar et al¹⁷ and Srinivasan et al.²¹

Corneal injury with vegetative matter was most common factor found in 30 (75%) cases in this study. Injury with vegetative matter coincides well with other studies by Basak et al.,¹⁴ Bharathi et al.²⁰ and Srinivasan et al.²¹ In this study injury with sugar cane leaf was noted in majority 22 (73.3%) cases followed by injury with rice paddy and wood stick.

Among total 52 isolates, 18 (34.6%) were fungi. Of the 18 fungal isolates, 8 (44.4%) were *Fusarium spp.* followed by *Aspergillus spp.* 5 (27.8%), *Alternaria spp.* 2 (1.1%), *Curvularia spp.* 2 (11.1%) and *Acremonium spp.* 1 (5.6%)

Fusarium was the predominant fungus reported by Gopinathan et al.,¹² Kumar et al.,¹⁵ Gaurav et al.,¹⁸ Bharathi et al.²⁰ and Srinivasan et al.²¹ In contrast, *Aspergillus spp.* was the most common fungus reported by Basak et al.,¹⁴ Tewari et al.,¹⁶ Chander et al.,²² and Krishna et al.²³

No yeast or yeast-like organisms were isolated in this study. The similar finding is also seen in study by Bharathi et al.²⁰ where no yeast or yeast like organism was isolated.

The disease takes a turn for improvement after administration of antifungal agents which promote the ulcers to heal gradually. So it is proved that early diagnosis and timely intervention with antifungal drugs are vital to good therapeutic effects in fungal keratitis.²⁴

Out of 16 pure fungal cases, 14 were recovered and 2 could not be followed up

Out of 2 mixed infection cases 1 patient recovered completely and 1 patient deteriorated where the infection was because of *Pseudomonas* and *Fusarium*. In these 2 patients, surgical management was approached.

Conclusions

Isolation and identification of etiological agents of keratitis helps in initiation of appropriate and effective management. This can prevent complications like endophthalmitis, perforation and finally loss of vision.

Identifying at risk population, screening for predisposing factors and advising people at risk about protective measures such as treating the co-existing ocular diseases and early consultation to an ophthalmologist may reduce the occurrence of microbial keratitis.

The microbial pattern changes according to the regional variation and also periodically in the same region. Hence it is necessary to carry out the study to know the causative organisms.

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