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Fungal Keratitis in North India: Spectrum of Agents, Risk Factors and Treatment

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Abstract To assess the prevalence of fungal keratitis, we conducted a retrospective study over 7 years (2005 through 2011) at a tertiary care center in North India. Effort has been made to analyze the disease burden, spectrum of agents and treatment history. The findings were compared with an earlier study at the same center for any change in the epidemiology of the disease. Microbiology records were screened at the Postgraduate Institute of Medical Education and Research, Chandigarh, India, to identify fungal keratitis cases, and available clinical records of those cases were analyzed. Of 2459 clinically suspected fungal keratitis cases, 765 (31 %) cases were direct microscopy confirmed. Of these microscopy-confirmed cases, fungi were isolated in 393 (51.4 %), with *Aspergillus* spp. ranked top ($n = 187$, 47.6 %), followed by melanized fungi ($n = 86$, 21.9 %) and *Fusarium* spp. ($n = 64$, 16 %). A male predominance of 78.7 % was noted with a peak in the incidence of fungal keratitis during post-monsoon season (September to November). A delay in diagnosis was significantly associated ($p < 0.001$) with keratitis cases due to melanized fungi. In comparison with an earlier study, higher isolation of melanized fungi was noted with a widening of the spectrum of agents identified. Thus, fungal keratitis due to *Aspergillus* spp. remains a serious ocular illness among the active male population in North India with relative rise of keratitis due to melanized fungi. The spectrum of agents causing fungal keratitis has broadened with many rare fungi that are implicated.

Keywords Fungal keratitis · Melanized fungi · Epidemiology · North India

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Introduction

Microbial keratitis has long been acknowledged as an important cause of visual impairment and blindness worldwide, being second only to cataract [1, 2]. Fungi causing keratitis have gained importance in Asian countries [3–7] and contribute nearly half of the world's fungal keratitis cases [8]. The epidemiology of fungal keratitis varies at different geographical locations [9]. The outcome of fungal keratitis is poor due to delay in diagnosis and difficulty in treatment [10].

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In India, high incidence of fungal keratitis is reported from every corner of the country [11–14]. *Fusarium* species are implicated in fungal keratitis cases primarily from South India, while *Aspergillus* species are reported from other parts of the country. A study on the epidemiology of fungal keratitis was conducted two decades back at our center [15]. In the present study, we evaluated the clinical data of the last 7 years (2005–2011) to find any change in the magnitude of the problem and the spectrum of agents causing fungal keratitis.

Materials and Methods

The microbiology records of clinically suspected fungal keratitis patients attending advanced eye center of Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, were evaluated to identify microbiologically confirmed fungal keratitis cases over the period of 2005 through 2011. The microbiological profile of samples in the form of corneal scrapings, corneal button, corneal tissue and corneal exudates was reviewed which included direct microscopy with potassium hydroxide (10 % KOH) and Calcofluor (CFW) stained wet mount and culture. For culture, samples had been inoculated in form of 'C' shape on two Sabouraud's dextrose agar (SDA) plate with chloramphenicol and incubated at 25 °C and 37 °C, respectively, and examined daily for any growth till 15 days. Any fungal growth on the 'C' was subcultured, and identification was performed by phenotypic, macroscopic and microscopic morphology [16]. The isolates that could not be identified by phenotypic methods were identified by sequencing of internal transcribed spacer (ITS) region of ribosomal DNA of the isolates [17, 18]. The rare isolates were deposited at National Culture Collection of Pathogenic Fungi (NCCPF) at PGIMER Chandigarh. Attempts were made to retrieve the clinical records from advanced eye center of microbiology-confirmed fungal keratitis cases.

Statistical Methods

The available clinical records of microbiologically confirmed keratitis were evaluated for demographic details, risk factors and treatment history by using either Chi-square test or Student's *t* test wherever applicable.

Results

A total of 2459 non-duplicate samples from patients with suspected fungal keratitis were received at the microbiology laboratory during the study period. Of these samples, 765 (31 %) were positive on direct microscopy for fungal elements and fungi were isolated from 393 (51 %) of the cases (Table 1). A wide spectrum of fungal agents including 27 genera (25 filamentous, 2 yeast) and 45 different species was isolated. *Aspergillus* species ranked top ($n = 187$, 47.6 %) followed by melanized fungi ($n = 86$, 21.9 %) and *Fusarium* species ($n = 64$, 16 %). The remaining 57 isolates included several hyaline mycelial fungi ($n = 40$, 10 %) and yeasts ($n = 16$, 4 %) (Table 2). The rare molds included *Acremonium*, *Beauvaria*, *Cylindrocarpon*, *Paecilomyces*, *Pseudallescheria*, *Scopulariopsis*, *Sarcinomyces* and *Rhizopus* species, while yeasts included *Candida* and *Trichosporon* species identified as causing fungal keratitis (Table 3). Among all the microscopy positive cases 78.7 % ($n = 602$) were male and majority of them ($n = 505$) belonged to the age group of 21 to 60 years (Supplemental digital content, Table 1).

The analysis of seasonal variation from the 765 fungal keratitis cases indicated a gradual rise in number of cases from March onwards with a peak in the post-monsoon or rice harvesting period (August to November). The *Aspergillus* keratitis cases followed the same seasonal trend, whereas a dip was noted during the same period for melanized fungi keratitis cases. No seasonal variation of *Fusarium* keratitis cases was observed (Fig. 1).

The available clinical data of 197 fungal keratitis cases were analyzed, which included 99 cases of *Aspergillus* infections, 55 cases of melanized fungal infections and 43 cases of *Fusarium* infections. The clinical data of patients with keratitis due to these three major groups of agents were compared. The risk factor for development of fungal keratitis could not be ascertained in 23.2, 23.2 and 10.9 % of the cases of *Aspergillus*, *Fusarium* and melanized fungi, respectively (Table 4). Comparing the risk factors available, trauma from vegetative matter was significantly associated ($p = 0.002$) with keratitis due to melanized fungi compared to *Fusarium* species. No other risk factors were significantly associated with any particular group. Irrespective of the species isolated, the patient had stromal infiltrates >1 mm^2 with associated

Table 1 Prevalence of fungal keratitis positivity during 2005–2011

Year	Sample processed (no)	Microscopy positive (no)	Microscopy positivity (%)	Culture positive (no)	Culture positivity (%)
2005	377	104	27.5	55	52.8
2006	265	89	33.5	60	67.4
2007	408	134	32.8	69	51.4
2008	357	129	36.1	66	51.1
2009	362	105	29	52	49.5
2010	295	89	30.1	27	30.3
2011	395	115	29.1	64	55.6
Total	2459	765	31	393	51

Table 2 Trend of etiologic agents causing fungal keratitis during 2005–2011

Organism isolated	Year-wise culture positivity (figures are in number of isolates)							Total	%
	2005	2006	2007	2008	2009	2010	2011		
Aspergillus group	24	25	34	36	22	13	33	187	47.6
Melanized group	14	17	18	8	14	9	6	86	21.9
Fusarium group	9	11	10	12	5	1	16	64	16
Other molds	6	5	6	8	7	4	4	40	10
Yeasts	2	2	2	2	4	1	3	16	4

signs of inflammation. Comparing the three groups, no significant difference was noted in epithelial defects, presence and size of hypopyon, infiltrates with feathery margins, rough texture, associated endothelial plaque, satellite lesions and heaped-up margins. The duration of illness was significantly longer in case of melanized fungi compared to *Aspergillus* ($p < 0.001$) and *Fusarium* ($p < 0.0001$). Therapeutic penetrating keratoplasty was performed significantly more frequently in *Aspergillus* group (29.3 %) compared to melanized fungal group (11 %, $p = 0.009$).

Ninety-five percent of these patients were treated with either topical preparation of natamycin alone or in combination with other topical antifungals including itraconazole, fluconazole and voriconazole. Few patients (5 %) were treated systemically with either amphotericin B alone or in combination with itraconazole.

Discussion

Fungal keratitis often masquerades as bacterial or parasitic keratitis and is difficult to differentiate clinically. Due to difficulty in diagnosis, a considerable number of patients do not receive targeted medication, which may lead to progression of the disease and ultimately corneal opacification. To estimate the disease burden and spectrum of agents involved, we conducted the present study and compared the results with a previous study from our center.

A significant increase in detection, suggesting an improvement in diagnosis of fungal keratitis, was noted in the present study comparing the earlier one (31 % in the present study compared to 8 % in the earlier study) [15]. The higher efficiency of fungal keratitis detection may be due to increased awareness and improved diagnosis. The prevalence of fungal keratitis of the present study corroborates the findings of Sandhu et al. [19] (32 %) from the same region and other Indian studies (26–45.5 %) [3, 9, 12, 14, 20–22]. However, in our study, we did not include the cases of mixed infection of fungi with bacteria as it would be difficult to implicate either agent in the infection due to the retrospective design of the study. Such mixed growth had been reported up to 12 % of the cases in other studies [3, 23], and the inclusion of such cases would increase the estimate of prevalence. The isolation rate in fungal keratitis is generally low due to the limited amount of sample from cornea. Partially

Table 3 Spectrum of fungal agents isolated during the study period

Name of organism	No. of isolates	Percentage (%)
1. Hyphomycetes		
Aspergillus group		
Aspergillus sp.	13	3.3
A. flavus	129	32.8
A. fumigatus	31	7.9
A. niger	6	1.5
A. nidulans	3	0.8
A. terreus	3	0.8
A. flavipes	1	0.3
A. tamarii	1	0.3
Fusarium group		
Fusarium sp.	9	2.3
F. solani	26	6.6
F. oxysporum	17	4.3
F. chlamydosporum	4	1
F. incarnatum	4	1
F. lichenicola	1	0.3
F. sacchari	2	0.5
F. dimerum	1	0.3
Other Hyphomycetes		
Acremonium sp.	3	0.8
Acremonium kiliense	11	2.8
Acremonium falciforme	4	1
Beauveria bassiana	1	0.3
Cylindrocarpon destructans	1	0.3
Penicillium sp.	10	2.5
Paecilomyces sp.	3	0.8
Pseudallescheria boydii	1	0.3
Scopulariopsis brevicaulis	1	0.3
Scedosporium apiospermum	4	1
Rhizopus arrhizus	1	0.3
2. Phaeohyphomycetes		
Alternaria sp.	4	1
A. alternata	10	2.5
A. chlamydospora	2	0.5
Acrophialophora fusispora	2	0.5
Chaetomium globosum	4	1
Cladophialophora carrioni	1	0.3
Colletorichum truncatum	2	0.5
Curvularia sp.	4	1
C. lunata	27	6.9
C. geniculata	2	0.5
C. brachyspora	1	0.3
C. senegalensis	1	0.3
C. spicifera	4	1

Table 3 continued

Name of organism	No. of isolates	Percentage (%)
Epicoccum nigrum	2	0.5
Exserohilum rostratum	3	0.8
Fonsecaea pedrosoi	3	0.8
Nigrospora sphaerica	1	0.3
Papulaspora equi	1	0.3
Sarcinomyces phaeomuriformis	1	0.3
Scytalidium sp.	2	0.5
Black mycelia (unidentified)	9	2.3
3. Yeasts		
Candida albicans	5	1.3
C. tropicalis	3	0.8
C. krusei	2	0.5
C. parapsilosis	2	0.5
C. glabrata	3	0.8
Trichosporon asahii	1	0.3

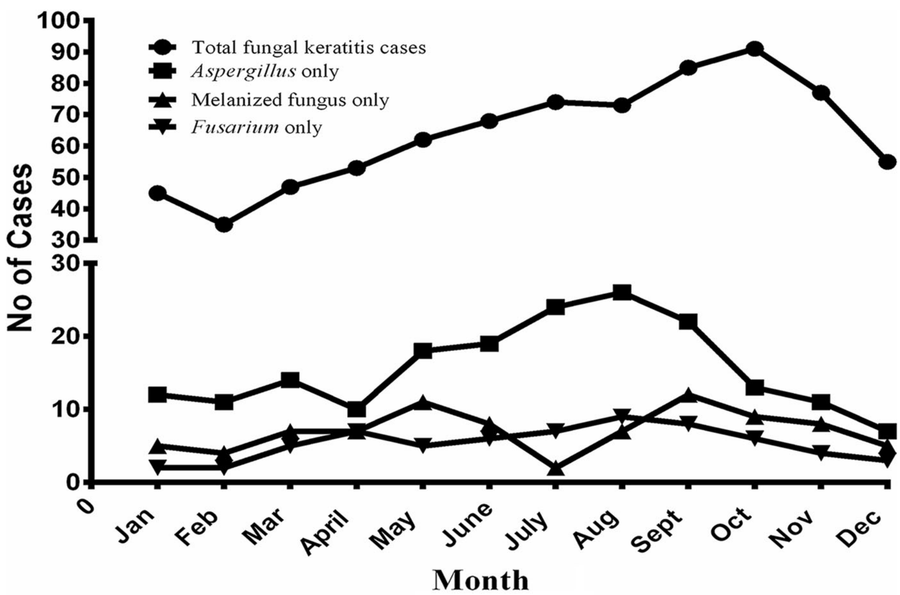

Fig. 1 Seasonal trend and isolation of three different groups of fungi causing keratitis between 2005 and 2011

treated patients referred to our tertiary care might be the other cause of lower isolation.

To date, globally, fungi belonging to 56 genera and from India 21 genera have been reported to cause fungal keratitis [9, 22]. The present study has added a few more species to the list of fungi causing keratitis. The identification of those rare species was possible by molecular techniques only.

Filamentous fungi were the predominant isolates in the present study as was reported in other studies from tropical and subtropical countries, contrary to the predominant yeast isolates in European countries and USA [24]. Microbial pathogens causing fungal keratitis even show geographical variation in their prevalence [25]. *Aspergillus* and *Fusarium* species are the predominant etiological agents for fungal

Table 4 Details of risk factors and clinical features among three major groups of patients with fungal keratitis

Character	*Aspergillus* group ($n = 99$)	Melanized group ($n = 55$)	*Fusarium* group ($n = 43$)
Risk factors			
Trauma with vegetative matter	21 (21.2 %)	23 (41.8 %)*	6 (14 %)
Apparently spontaneous	23 (23.2 %)	6 (10.9 %)	10 (23.3)
Unknown foreign body	23 (23.2 %)	15 (27.3 %)	13 (30.2 %)
Coexisting ocular diseases	12 (12.1 %)	3 (5.5 %)	8 (18.6)
Mud/stone	6 (6.1 %)	2 (3.6 %)	2 (4.7 %)
Blunt trauma	6 (6.1 %)	1 (1.8 %)	0
Insect falling	4 (4 %)	1 (1.8 %)	2 (4.7 %)
Iron particles	2 (2 %)	0	0
Chemical	1 (1 %)	1 (1.8 %)	0
Cow/buffalo tail	0	1 (1.8 %)	1 (2.3 %)
Prior antibiotics/steroids	1 (1 %)	2 (3.6 %)	1 (2.3 %)
Clinical features			
Central location	75 (75.8 %)	45 (81.8 %)	30 (69.8 %)
Hypopyon	53 (53.5 %)	24 (43.6 %)	20 (46.4 %)
Duration of illness (average)	25.71 days	40.03 days**	18.25 days
Illness days range	4–180 days	5–210 days	3–120 days
Therapeutic penetrating keratoplasty	29 (29.3 %)***	6 (11 %)	10 (23.3 %)

* Statistically significant ($p = 0.002$) on comparing melanized fungi with Fusarium group

** Duration of illness was statistically higher ($p < 0.001$) in melanized fungi compared to other groups

*** Statistically higher on comparing *Aspergillus* with melanized fungi $p = 0.009$

keratitis in India with *Fusarium* species predominant in South India and *Aspergillus* species in rest of India [3, 5, 13, 14, 22, 23, 26]. We isolated *Aspergillus* and *Fusarium* species in 47.6 and 16 % of the cases, respectively, maintaining a similar trend. However, our isolation of melanized fungi at 21.9 % indicates a change in the spectrum of agents. Chowdhary and Singh [3] from North India reported similar finding in 2005 by isolating *Curvularia* species followed by *Aspergillus* species. It is not clear whether the high rate of isolation of melanized fungi was due to real emergence of the agents or improved diagnostic competence. A systematic epidemiological study is warranted.

The effect of season on fungal keratitis had been analyzed in the past. It was observed that the fungal keratitis cases peaked during the months of high agricultural activities corresponding to rice sowing (July) and harvesting seasons (January) in India [27]. The number of fungal keratitis cases in the present study peaked during post-monsoon season. High humidity during this period favors fungal growth on agricultural crops [3, 5].

A male predominance (83.9 %) was seen with most of the cases presenting in the 21–60 years age group observed in this study. In contrast to the earlier study from our center along with other centers, it was reported mainly in the 5th decade (51–60 year) [15, 27, 28], a widening of the age group of fungal keratitis patients with involvement of 2nd–5th decade (21–60 years) was observed in this study. Several recent studies reported fungal keratitis in young rural population, who suffer from corneal injury due to vegetative matter during agricultural activities [3, 15, 29]. Similarly in our study, trauma was the predominant risk factor (66.5 %) present in 80, 63.6 and 55.8 % of patients with keratitis due to melanized fungi, *Aspergillus* and *Fusarium* species, respectively. History of trauma due to vegetative matter was reported in 41.8, 21.2, and 14 % of patients with keratitis due to melanized fungi, *Aspergillus* and *Fusarium* species, respectively.

The patients with melanized fungal infection reported late to our hospital compared to infections due to *Aspergillus* and *Fusarium* species. This is probable since melanized fungi are usually of low virulence and the disease due to these agents progresses slowly over weeks [30]. A chronic, slowly progressive and non-healing ulcer should alert the ophthalmologist to consider the possibility of melanized fungal etiology.

The optimal therapy for fungal keratitis is not yet established. In our study, nearly 95 % of the cases were treated by natamycin eye drop with or without other antifungal agents. This is consistent with the worldwide practice of using topical natamycin suspension for suspected fungal keratitis. Natamycin alone is effective in treating *Aspergillus* keratitis and large ulcers, though a combination of oral itraconazole or ketoconazole is used in resistant cases [31]. Natamycin alone or in combination with topical clotrimazole or miconazole was used to treat melanized fungal infections. Topical amphotericin B is used only in few cases as there are no commercially available ocular preparations [10]. The addition of systemic antifungals is preferred whenever deep involvement is suspected [30]. The limitation of the present retrospective study is a lack of follow-up and outcome data.

In conclusion, *Aspergillus* fungal keratitis remains a serious ophthalmic infection among active male population of North India with relative rise of keratitis due to melanized fungi. The spectrum of agents causing fungal keratitis has widened in India with isolation of many rare fungi.

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